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Some surface properties of carbonate speleothems from Kirillov's, Pionerskaya and Yaschik Pandory caves in the Republic of Khakasia, as well as Yuryevskaya Cave in the Republic of Tatarstan were investigated. The types of the speleothem samples such as crusts, drips, corallites, and moon milk were studied. All the samples were collected in cave aphotic zones at a wall temperature not higher than + 10 ºC. Differently polished marble onyx, gypsum and glass plates were taken as reference surfaces. The surfaces were processed by polishing, heating, etching chemicals, and adding R2A modified growth media. These modes simulated experimentally common natural processes in the system of "calcium carbonate −chemolithotrophic biofilm". The speleothem samples under the cave microbial community, which can exist successfully in the upper soil layer, were considered against the background of the reference mineral surfaces. The captive bubble method, SEM, XRD, EDX, as well as DTG analyses were carried out to determine wettability, roughness, total organic matter content, and elemental and mineral compositions of the samples. The metagenome of the microbial community was estimated using 16S rRNA sequence analysis. The influence of fresh and long-lived biofilms on the carbonate surface properties is assessed. The assumption that biofilm dynamics affects the carbonate surface properties and its toughness is substantiated. Our work hypothesized that the part of organic matter can enter the gaps in growing carbonate crystals, then, is sealed with a new mineral phase, and later, is assimilated by heterotrophic and organotrophic organisms.

Keywords (separated by '-')
Wettability - Organic matter - Surface roughness - Biocement toughness - Biofilm dynamics - Extremophiles - Chemolithotrophs - Biomorphs - Pseudomorphoses

Surface Properties of Carbonate Speleothems in Karst Caves Changing Under Biofilms

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Abstract Some surface properties of carbonate speleothems from Kirillov's, Pionerskaya and Yaschik Pandory caves in the Republic of Khakasia, as well as Yuryevskaya Cave in the Republic of Tatarstan were investigated. The types of the speleothem samples such as crusts, drips, corallites, and moon milk were studied. All the samples were collected in cave aphotic zones at a wall temperature not higher than + 10 ºC. Differently polished marble onyx, gypsum and glass plates were taken as reference surfaces. The surfaces were processed by polishing, heating, etching chemicals, and adding R2A modified growth media. These modes simulated experimentally common natural processes in the system of "calcium carbonate−chemolithotrophic biofilm". The speleothem samples under the cave microbial community, which can exist successfully in the upper soil layer, were considered against the background of the reference mineral surfaces. The captive bubble method, SEM, XRD, EDX, as well as DTG analyses were carried out to determine wettability, roughness, total organic matter content, and elemental and mineral compositions of the samples. The metagenome of the microbial community was estimated using 16S rRNA sequence analysis. The influence of fresh and long-lived biofilms on the carbonate surface properties is assessed. The assumption that biofilm dynamics affects the carbonate surface properties and its toughness is substantiated. Our work hypothesized that the part of organic matter can enter the gaps in growing carbonate crystals, then, is sealed with a new mineral phase, and later, is assimilated by heterotrophic and organotrophic organisms.

Keywords Wettability · Organic matter · Surface roughness · Biocement toughness · Biofilm dynamics · Extremophiles · Chemolithotrophs · Biomorphs · Pseudomorphoses

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1 Introduction

Carbonate formations are promising objects for the design of biocement structures and protective (transport) capsules for microflora, as well as for the refinement of the geologic record (Melim et al. 2016). The surface properties of carbonate formations are closely related to biofilms developing on them (Mouraviev et al. 2006; Banks et al. 2010; Tugarova 2021). Biofilms have a life cycle, at each stage of which they can affect the covered surface differently either destroying or preserving it (Andryukov et al. 2020; Zorina et al. 2019). The interaction between autochthonous or inoculated biofilms and the carbonate leads to the deposition or destruction of minerals and isomorphic replacement in crystal lattices, which should be taken into account when using cements and other carbonate-based materials in geotechnologies (Gray and Engel 2013; Banks et al. 2010; Glazovskaya and Dobrovolskaya 1984; Guvensen et al. 2013; Pronk et al. 2017; Wiseschart et al. 2019; Leonova et al. 2015). Hereby, both lithoautotrophic and organoheterophic modes of microbial metabolism can destroy carbonates. In the first case, this occurs due to biogenic acids production, and in the second case, due to carbon dioxide release (Gray and Engel 2013). Carbonate precipitation requires microbial communities to shift the pH of their environment toward the alkaline side, for example, via alkalis releasing or sulfate reduction (Leonova et al. 2015). Calcium carbonate precipitation passes four stages—amorphous, vaterite, aragonite, and calcite, among which calcite is the only thermodynamically stable phase, while others are metastable. However, the presence of organic matter in the environment is proved to be an inhibitor on the metastable phase transformation and thus, makes them possible to coexist (Myszka et al. 2019).

In our work, we have simulated experimentally some of the mechanical and chemical effects that can be induced by chemolithotrophic biofilms on the carbonate. From the extended version of the DLVO theory, fineness, roughness, hydration, and the type of contact between particles are known to be the factors significant for particle adhesion strength (Andryukov et al. 2020). Carbonate biofilm can affect each of them. Both the abilities to disperse and to stick particles together are peculiar to microbial communities (Gray and Engel 2013; Banks et al. 2010; Glazovskaya and Dobrovolskaya 1984; Pronk et al. 2017). Particles disintegrate via the dissolution of the cement binding them in aggregates. Particle aggregation begins either by a gel forming in the extracellular polymer substances (EPS) or by a cement precipitating in gaps between particles. Surface roughness is similarly formed by biofilms, however, herewith the latter affect only the aggregates' surfaces but not intra-aggregate bonds. The hydration of biofilm-coated particles most strongly depends on the stage of biofilm development: a fresh biofilm can attach to a particle only if the latter has hydrophobic contact. It produces the hydrophobic substances that promote the strongest attachment to the surface (Zorina et al. 2019; Andryukov et al. 2020). Nevertheless, for sufficient nutrition delivered as an aqueous phase, the biofilm is forced to produce hydrophilic compounds. Thus, fluctuations in the hydrophilic-hydrophobic properties of the substrate surface occur. This leads to the alternation of strengthening and weakening contacts between particles. In the presence of a

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hydrophilic surface, particles form coagulates; and vice versa, in the presence of a hydrophobic one, particles can reversibly aggregate, as well as form direct point contacts, after which the cementation or crystallization contacts are likely.

Based on the dynamic behavior of biofilms concerning the coated mineral phase and considered the previous studies (Melim et al. 2016) it can be hypothesized that a part of the organic matter enters the gaps between growing crystals through selective adsorption and/or capillary condensation. Then, these substances can be sealed with a new mineral phase, and thus preserved. Further, under the acids episodically released by the biofilm and etching a mineral phase, the organic matter is reactivated and can be consumed by heterotrophic and organotrophic organisms. The purpose of this work was to check this hypothesis.

2 Sites and Sampling

Carbonate speleothem samples were purposefully selected from totally different geologic zones (Fig. 1). Karst genesis, the contact of clayey deposits with carbonate rocks, 3–sevenfold excess of the atmospheric partial pressure of CO_2, aphotic zones, and the wall temperatures not higher than + 10 ℃ were the common features of the sampling sites. The samples were taken from the walls and cornices of caves in such a way as to exclude anthropogenic and zoogenic pollution as much as possible.

Yurjevskaya Cave (Fig. 1a) is a horizontal corridor system in Permian sedimentary gypsum-dolomite rocks with a total length of about 1,5 km. It is located on the right

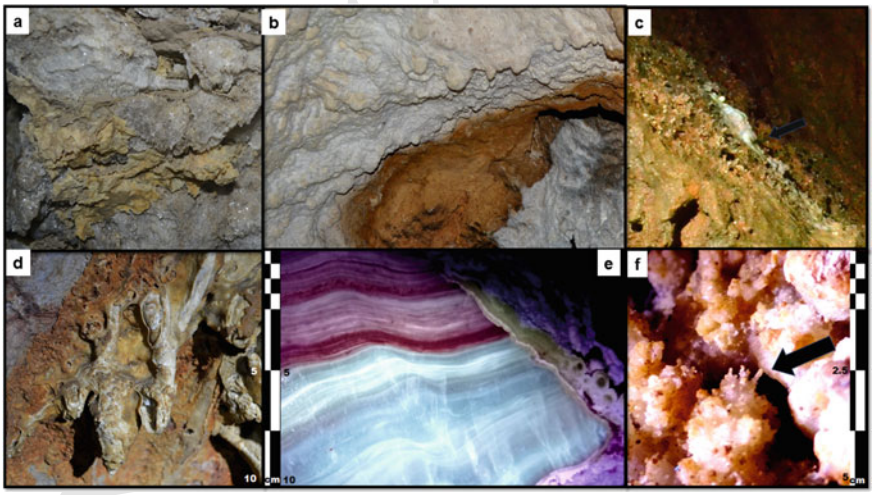

Fig. 1 Some speleothems sampled from caves: **a** Yurjevskaya, crusts; **b** Pionerskaya, dry moon milk; **c** Pionerskaya, fresh moon milk; **d** Yaschik Pandory, drape; **e** Pionerskaya, marble onyx; **f** Yaschik Pandory, corallite with helictites

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bank of the Volga River in the Republic of Tatarstan. Crust-type speleothems were selected from the Hall of Organ Pipes located 190 m far from the entrance. This hall is less attended by tourists compared to the neighboring ones. There was a wall temperature of + 4,0 ºC, a air temperature of + 13,0 ºC, and a relative air humidity of 67%.

Pionerskaya Cave (Fig. 1b, c, e) is a slightly inclined horizontal gallery with a maximum depth of 15 m and a total length of 86 m in Riphean dolomites (Nomokonov 1969) with calcite mines located on the left bank of the Bely Iyus River (the Ob River basin) in the Republic of Khakasia. Sampling points were selected at a distance of 20, 33 and 40 m far from the entrance, which ensured complete coverage of the cave along its length. The speleothem samples were «moon milk» at the different stages of drying, spherulites, and stalactites. There was an average wall temperature of + 0.7 ºC. The air temperature was from + 5,2 ºC to + 8,0 ºC rising from the entrance to the end. A relative air humidity was from 76 to 85%.

Kirillov's Cave is an inclined horizontal gallery with a maximum depth of 36 m and a total length of 320 m in massive Cambrian limestones (Nomokonov 1969) located inside a hill that is 400 m above the right bank of the Bely Iyus River. Somewhere there is an ice floor. Sampling points were selected at a distance of 13, 39 and 68 m far from the entrance of the lower gallery and covered the cave wing, which is the richest in speleothems. The samples collected were small stalactites. A wall temperature increased from −6,0 ºC to −0.8 ºC from the entrance to the end as the gallery rose; an average air temperature was + 5,9 ºC, and a relative air humidity was from 75 to 77%.

Yaschik Pandory Cave (Fig. 1d, f) is a system of vertical and inclined wells with a total depth of 180 m and a total length of about 11 km in the Cambrian rocks, which are represented by argillaceous limestones, sandstones, siltstones, gravelites up to a depth of 40 m, and there appear marls, diabases, porphyrites in the deeper layers (Nomokonov 1969). In some areas there the carbonate rock transition into marble occurs, probably due to the contact metamorphism in the zone of geologic faults. The cave is situated on the left bank of the Bely Iyus River, and is connected hydraulically with it on the lowermost floor. The samples were collected from depths of 0, 100, and 180 m below the entrance. There were spherulites, small stalactites, and corallites. The wall and air temperatures decreased from + 7 ºC to + 4 ºC and from + 18 ºC to + 4 ºC, respectively, and a relative air humidity increased from 68 to 90% as descending to the bottom.

Experimental impact on the samples. Freshly sampled speleothems were placed into strong aluminum foils at an initial humidity, a temperature of + 12(±2) °C, and total darkness to preserve the active autochthonous biofilm. These samples participated in our experiment in the following modes: untreated slices, variously polished plates, ones powdered to sizes < 250 microns, heat-treated powders at 105 and 525 ºC, as well as chemically treated powders.

The mechanical treatment of a few samples was carried out in order to examine the effect of roughness and surface layer removal on the properties of natural carbonate formations. Marble onyx plates were processed in the following ways:

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- *zero polishing*–untreated slices;
- *polishing No. 1*–with a grinding disc grit of 75 microns and the creation of surface roughness at a level of 20−25 microns with oriented hatching;
- *polishing No. 2*–with a grinding disk grit of 20 microns and the creation of surface roughness at a level of 8−12 microns with oriented hatching;
- *polishing No. 3*–with free abrasive SiC M 14 and a uniformly rough surface at a level of 5−8 microns;
- *polishing No. 4*–with free abrasive SiC M 10 and a uniformly rough surface at a level of 2−4 microns.

The samples were heated in order to dehydrate the surface (at 105 ºC) and accelerate the removal of chemically bound water and organic matter (at 525 ºC).

The chemical treatment of the samples was aimed at the release of the hypothetical organic matter from mineral chambers in two ways: by mineral etching and by the extraction of the organic matter with a solvent. The minerals were etched using oxalic, citric, acetic, hydrochloric, and sulfuric acids, commonly secreted by lithotrophic microorganisms (Glazovskaya and Dobrovolskaya 1984). The organic matter was extracted from the samples into NaOH and ethanol solutions (Golovanova 2022).

The fresh chemolithotrophic biofilm was stimulated by the modified R2-based growth medium intended for psychrophilic oligotrophs (Wiseschart et al. 2019; D'Angeli et al. 2017). The difference from the base R2 medium layed in the addition of an alkaline humus extract and a triple compared to the base medium $CaCO_3$ content. The increased content of $CaCO_3$ was applied to establish the ionic equilibrium between the studied surfaces and the washing solution, as well as to selectively stimulate the organisms adapted to carbonate metabolism. The humus extract was applied as a pH buffer for its natural level of 7.68−8.02, as well as a model for the contact of carbonate formations with the upper soil layer. Thus, the stimulation was aimed at those members of the cave community that can successfully exist in surface soil conditions.

3 Methods

The main surface investigation methods included contact angle measurement, SEM, EDX, TG-DSC, and XRD analyses. The contact angle is an indicator of both hydrophilic-hydrophobic properties and surface roughness. It was determined using the captive bubble method in the author's modification for clay fraction powder specimens (Sofinskaya et al. 2022). The powders were stuck onto flat glass using adhesive tape at a pressure of 30 kPa, after which they were placed into an atmosphere with a relative air humidity of >96% for several minutes to saturate the pores with capillary-condensed moisture. Next, the specimens were immersed into a thermostatic bath of an optical tensiometer with deionized water, and an air bubble was placed and photographed on the powder/water interface. The images obtained were adjusted using the ImageJ program and then contact angles were calculated by the

Contact Angle Option. The representation of the measurements consisted of 30–100 per a specimen depending on its surface stability.

Most of the samples were examined with a FEI XL-30ESEM scanning electron microscope. The SEM was carried out at a low vacuum mode under an accelerating voltage of 20 keV. Elemental analysis was performed using an EDAX energy dispersive spectrometer operating in combination with the SEM, based on which the mineral composition was calculated from the ratio of the weight of elements on the surface.

X-ray diffraction analysis was carried out with a D2 Phaser diffractometer (Bruker, Germany) in order to detail the mineral composition. Analysis mode was an X-ray tube voltage of 30 kV, a current of 30 mA, and the scanning step of 0.02° at a speed of 1 deg/min. The range of scanning angles in the Bragg–Brentano geometry was from 3 to 40°. Standard powder preparations were used. The qualitative and quantitative mineral composition was determined using the DIFFRAC.EVA and TOPAS software.

Thermal analysis was carried out with an STA 449 JupiterF3 device to determine the boiling point of the structural components of the samples. The burning interval was from 30 to 1000 °C, the heating step was 10 deg/min. with continuous air purging. There the reference Al_2O_3, which did not give a thermal effect within the temperature range from 20 to 1200°, was applied.

Taxonomic profiling of the bacterial communities was performed using S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 primers (Klindworth et al. 2013) targeting V3 and V4 regions of the 16S rRNA gene. The libraries were sequenced in the "Regulatory genomics" lab of Kazan Federal University (Kazan, Russia) on the MiSeq (Illumina, USA) platform using the 2 × 300 bp paired-end MiSeq Reagent Kit v3. To process the sequencing data, we used the dada2 microbiome data denoising pipeline (version 1.14.0). The dada2 pipeline is based on sequencing error correction algorithms and generates exact amplicon sequence variants (ASVs). The dada2 pipeline implements the Naive Bayes classifier method for taxonomy assignment (Callahan et al. 2016). This classifier compares sequence variants with a training set of classified sequences, the Silva (version 138) rRNA gene database (silva_nr99_v138.1_train_set.fa), which is currently considered the most comprehensive 16S rRNA database of all microorganisms. To visualize and filter the processed data, a package written in the R programming language - phyloseq (McMurdie and Holmes 2013) was used. Data filtering was carried out to remove artifacts generated during the analysis. A set of samples passed through a bioinformatics pipeline with the same parameters.

4 Results

Wetting contact angle (CA). Finely polished marble onyx (*polishing No. 2, 3, 4*) is a hydrophilic matter with a fairly small standard deviation (StDev) of CA fluctuating within the observed range of 28º to 38º (Table 1, see *polishing No. 3*). It is noteworthy that the finest *polishing No. 4* caused a StDev rising (i.e., surface heterogeneity) due

to an increase in the range of CA observed at both ends of their distribution, which is probably followed the artifacts introduced after polishing at dimension hatching less than 5 μm.

The *polishing No. 2* and *No. 1* had a larger range of CA variation than finely processed ones, i.e., here macro-roughness was set by irregularities larger than 8 μm. The coarse polishing led to the splitting of one mode of the quasi-Gaussian CA distribution into two, resulting in a bimodal distribution. Herewith, the low-angle mode remained close to surfaces after fine polishing, while the high-angle mode exceeded the maximum CA observed on these surfaces. The *zero polishing* sample also had two CA distribution modes, of which the low-angle one was close to the high-angle mode for a roughly polished surface (*polishing No. 1*), and the high-angle one belonged to the hydrophobic range. That is, hydrophobic properties were set by heterogeneities with a size of more than 25 μm corresponded to the order of biofilm thickness (Wagner and Horn 2017)

The speleothem powders had practically the same CA distribution parameters as the *zero polishing* sample. Nevertheless, after dehydration at 105 ºC the hydrophobic heterogeneities and bimodal CA distribution disappeared and the distribution parameters approached surfaces with *polishing No. 1, 2, 3*. Heat treatment at 525 ºC led to a strong increase in surface heterogeneity (according to an increase in StDev), but unless the CA distribution mode splitting.

The polished plates treated with the R2 medium demonstrated a decrease in the hydrophilicity and homogeneity of the surfaces as the StDev had risen and the second mode of CA distribution had appeared. At the same time, the first distribution mode

Table 1 Distribution parameters for wetting contact angle on reference and model surfaces (degrees)

	St.dev.est.*	Minimum of CA observed	Mode 1*	Mode 2*	Maximum of CA observed
	Initial/under R2, 1 month / under R2, 2 months				
Glass	6/4	25/23	43/32	–	54/46
Onyx polishing No 3	3/7/7	28/24/19	32/27/27	–/40/46	38/53/52
Onyx polishing No 4	4	23	32	–	42
Onyx polishing No 2	6	23	32	–	51
Onyx polishing No 1	12	22	35	54	67
Onyx zero polishing	15	33	56	102	120
Gypsum smooth slice	7/4	27/30	40/45	–/72	57/81

* estimated by Tikhonov and von Mises

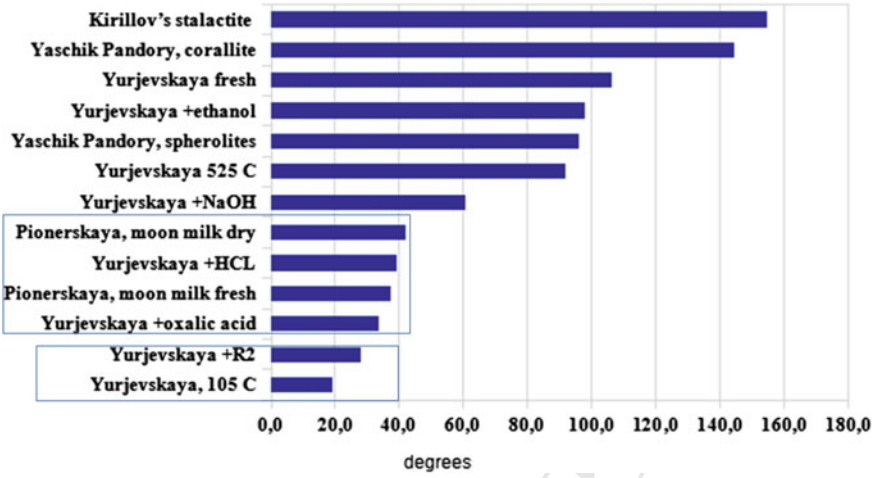

Fig. 2 Euclidean distance between the reference cluster and the samples calculated for wetting contact angle characteristics (min., max., mode 1, mode 2, St.dev.)

and the minimum CA observed shifted toward a more hydrophilic range. This observation is also valid for a gypsum crystal surface. On the contrary, the surface heterogeneity of the calcite speleothem powders under the R2 medium declined, which was indicated by a decrease in the StDev and the disappearance of the hydrophobic CA distribution mode. A similar trend was demonstrated for the silicate glass plate in the homogeneity of the CA distribution and the hydrophilicity of the surface risen under the R2-stimulated biofilm.

CA data was ranged compared to a reference class consisting of flat smooth surface specimens: onyx plates of the *polishing No. 2*, *3*, *4* and a glass plate treated with the R2 medium (Fig. 2). The reference class showed CA fluctuations in the range of 24º to 44º around an average direction of 32º (by Tikhonov and von Mises) with a StDev estimated as a class average of 4º.

Next, cluster analysis was performed using the K-means algorithm for the independent parameters of the empirical CA distribution as a factor space. This resulted in the CA dataset being combined into 3 subgroups with several members and 4 single values. The first combined subgroup included marble onyx plates after treatment with R2 medium, *polishing No. 1*, specimens of Yuryevskaya Cave dehydrated at 105 ºC, treated with oxalic and hydrochloric acids, R2 medium, as well as fresh and dry moon milk from Pionerskaya Cave (Fig. 1c and b). The common features of this subgroup included the unimodal CA distribution, the mode of which was higher than in the reference group, hydrophilicity, and roughness. The surface of the specimens from Yuryevskaya Cave treated with NaOH was distinguished by the transition through the threshold of hydrophobicity. The next subgroup included the specimens from Yuryevskaya Cave treated with ethanol and burning at 525 ºC, as well as spherulite formations from the entrance grotto of Yaschik Pandory Cave. That subgroup was characterized by an average CA direction of 58º and CA maximum

lying in the hydrophobic area. The last combined subgroup included an untreated powder specimen from Yuryevskaya Cave and a *zero polishing* slice of marble onyx. There was the CA distribution split into two modes, a hydrophilic one about 45º and a hydrophobic one about 100º in this group. The differences between the rest powder specimens belonging to Yaschik Pandory Cave (corallites with helictites, Fig. 1f) and Kirillov's Cave (stalactite) did not allow them to be combined into any group, however, all of them were characterized by the low-angle CA mode lied in the hydrophilic area whereas the high-angle CA mode had a superhydrophobic range and individual CA reached 140º–150º.

SEM, EDX, XRD and DTG analyses. In most cases, the images show a film gained with frequent biomorphs and carbon compared to calcite alone (Fig. 3). On crusts filling cracks this film had some differences between the inner smoothed surface adjacent to the host rock (dolomite) and the outer subaerial surface regarding the ratio of elements (Table 2). There was less carbon on the inner side.

When the crusts developed on the bat bones (Fig. 4a) they had the same C/Ca ratio as the outer surface of the crack filler, but a higher Ca/O ratio. A peculiar tubular structure constituted with crystals elongated along one axis allows us to assume confidently aragonite pseudomorphs formed on the initial bone substances. Such an aragonite structure may include the "rose" grown on the gypsum surface (Fig. 4b) structures of micro-rods with notches of moon milk (Fig. 4d), and helictite needles in corallites (Fig. 4c).

Figure 5d shows two synchronously recorded thermal analysis curves, namely, TG−thermogravimetric curve and DSC−differential scanning calorimetry curve. The first indicates the mass loss of the moon milk sample, and the latter shows the thermal effects that appear during its burning. The most noticeable changes in

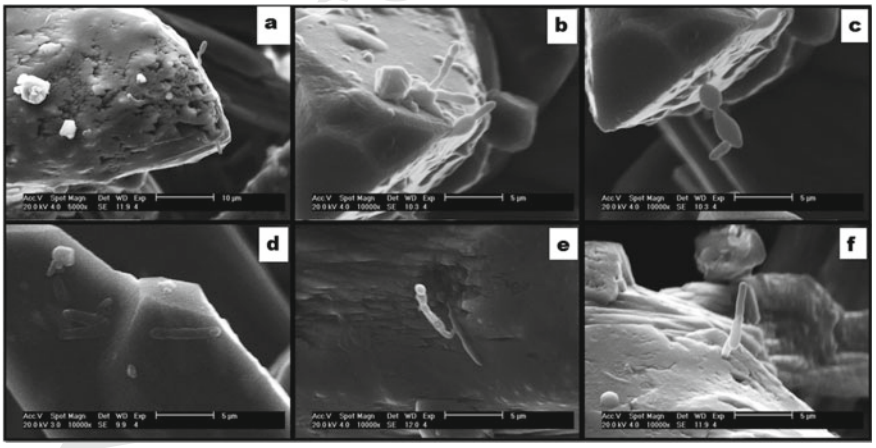

Fig. 3 Biomorphs on calcite speleothems, Yurjevskaya cave: **a**–a continuous biofilm with prominent biomorphs; **b**, **c**, **e**, **f**–cells penetrated the biofilm surface; **d**–monads on the speleothem surface

Table 2 Relative content of elements on the surfaces of speleothems and host rocks

Element, %wt	Speleothem crusts				Other speleothems			Gypsum			Dolomite	
	Inner	Extern	Bat bone	+ R2	Lateral	Apex	Apex	"rose"	Initial	+ R2	Initial	+ R2
C	16	18	22	20	12	12	18	–	–	45	13	18
O	42	50	39	53	38	54	58	60	64	41	48	50
Mg	0,4	0,5	0,3	6,0			0,2			0,5	8,9	0,2
Al	0,3	0,6	0,7	0,8	0,7	0,3		0,3	0,2	0,8	1,1	0,2
Si	0,4	0,5	0,3	1,2	0,5	0,3	0,2	0,4	0,1	0,7	5,3	0,3
P				0,3	0,7	0,5	0,4			4,1		1,5
S	0,7	0,4	0,4	0,3				0,9	16,0	0,9	0,3	0,3
K				0,3						0,8	0,4	
Ca	41	30	37	17	47	32	23	38	19	5	21	30
Mn								0,4			0,2	
Fe		0,3		0,3	0,4	0,3	0,4	0,6	0,5		0,6	
C/Ca	0,4	0,6	0,6	1,2	0,3	0,4	0,8	–	–	9,2	0,6	0,6
Ca/O	1,0	0,6	0,9	0,3	1,2	0,6	0,4	0,6	0,3	0,1	0,4	0,6
Ca/P	–	–	–	54,6	65,4	63,1	58,9	–	–	1,2	–	20,7

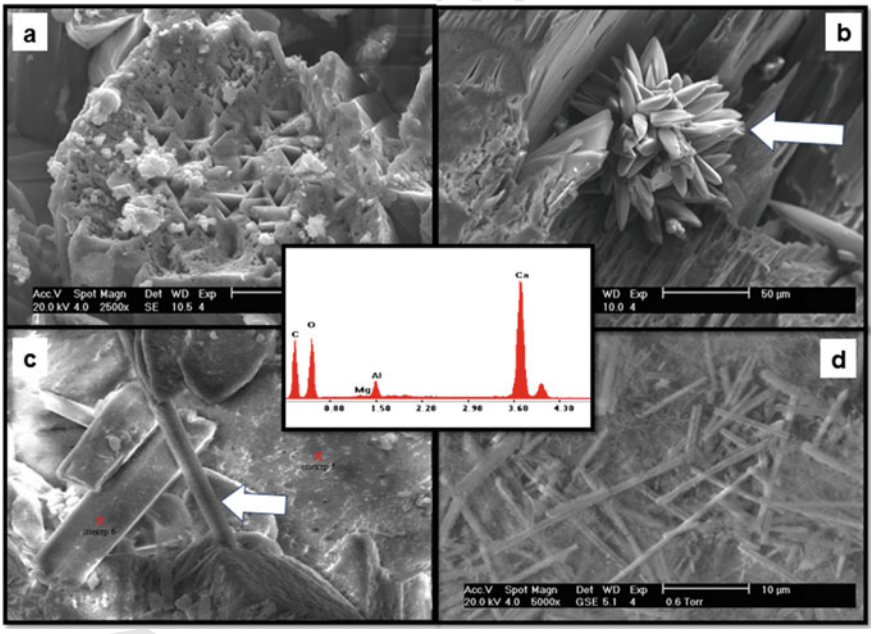

Fig. 4 Pseudomorphoses after aragonite: **a** bat bone from Yurjevskaya cave; **b** "rose" form on gypsum surface from Yurjevskaya cave; **c** micro-rod in corallite from Yaschik Pandory cave; **d** micro-rods in moon milk from Pionerskaya cave

the sample are noted in a burning temperature range of 180–500 ºC. In this range, there two exo-effects caused by the thermal dissociation of organic matter are highlighted. These two exo-effects manifested at low temperatures and higher temperatures correspond to the dissociation of low and high molecular weight organic matter, respectively. The total weight loss in this temperature range is 3.5%. The next clear endo-effect is observed at a temperature of 680–780 ºC. Its presence is due to the thermal dissociation of calcite according to the reaction.

$$CaCO_3 \rightarrow CaO + CO_2 \uparrow$$

Then, one can suppose the formation of calcite pseudomorphs after aragonite according to XRD and TG-DSC data, since there is present the calcite predominant phase (91–98%) with the admixture of dolomite, celestite, and quartz in these tubes (Fig. 5).

Based on the results of EDX analysis, the lateral part of the tubular crystals reproducibly differed in elemental composition from the apical part and approached organic compounds (Table 2). The presence of phosphorus, which is not common for the speleothems, also indicates organic inclusions in the crystals.

The treatment of calcite speleothems with the R2 medium caused the fixation of Mg, P, and K elements on the surfaces and a threefold increase in the C/Ca ratio (Table 2). The appearance of new threads and films over caverns was visually observed (Fig. 6b, c). The gypsum surface after such treatment was strongly colonized by hyphae-forming microorganisms (Fig. 6f). Herewith high C/Ca ratio, and precipitation of a new phase containing Mg, P, and K clearly increasing the surface roughness were observed (Fig. 6d, g, h). The latter is consistent with the previous investigations that found the phosphate groups released by biofilms to improve their adhesion to the solid (Andryukov et al. 2020). On the of dolomite surface, the R2 environment caused the appearance of a calcite crust (Table 2).

4.1 Taxonomic Diversity

The clone libraries belonged to 6 major phyla that comprised 10 and 17 OTUs in the initial speleothems and in the treated with R2 media one, respectively. Proteobacteria dominated all clone libraries, ranging from 57 to 81% of the clones depending on the option. About one sixth of the OTUs comprised clones from both options. Phyla Actinobacteriota and Nitrospirota being quite abundant in the initial speleothems were suppressed by the R2 medium. Stimulation with the R2 medium was manifested in the phyla Bacteroidota, Firmicutes, Bdellovibrionota (Table 3).

Genera Kribella, Gaiella, and Brevundimonas found in the initial speleothems can be assigned to the typical extremophiles, and genera Pseudoarthrobacter and Nitrospira are obligate chemolithotrophs. R2 media stimulated the most genus Brevundimonas, whose representatives are aerobic consumers of organic matter, inhabit aquatic environments, and have a wide range of resistance to desiccation.

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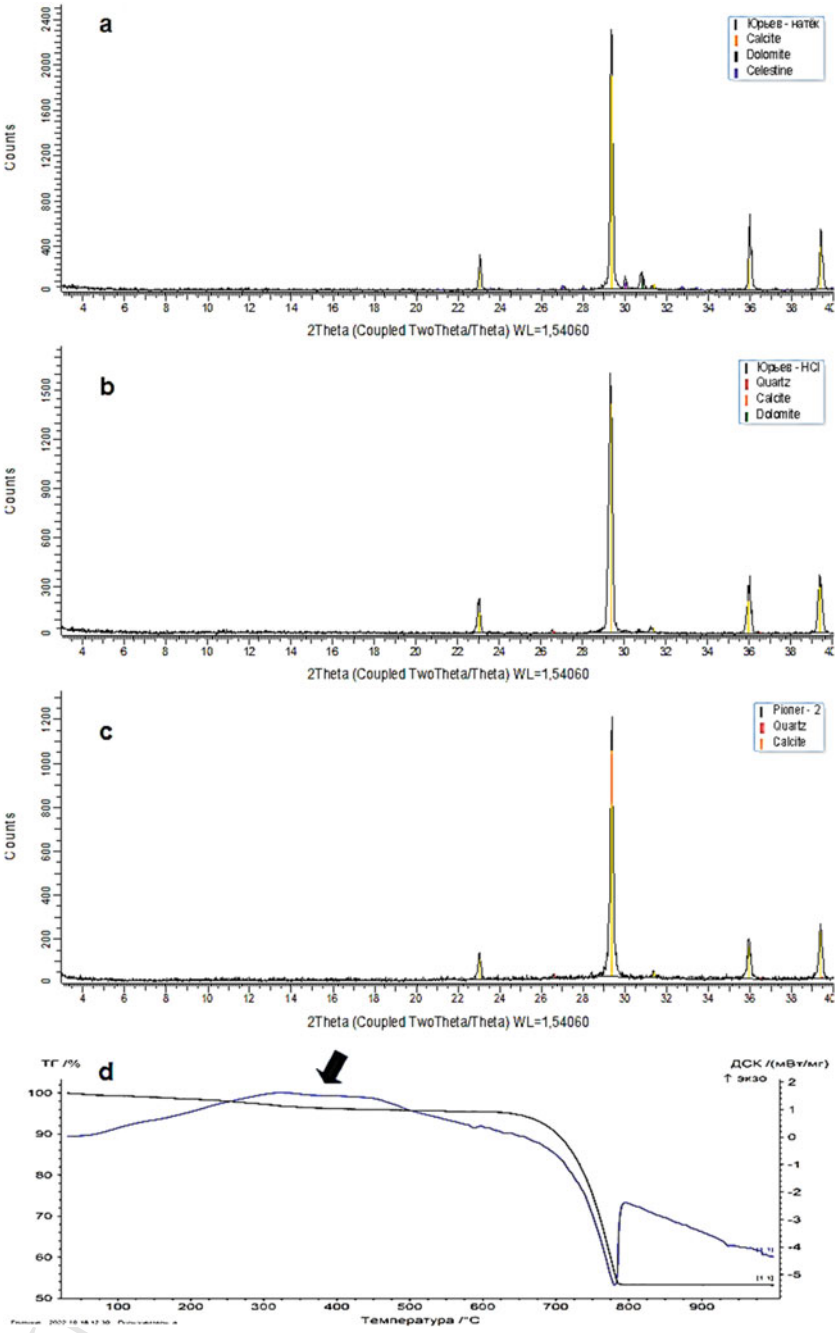

Fig. 5 The analyses of speleothems: **a** XRD of crusts from Yurjevskaya cave; **b** XRD of crusts etched with HCl from Yurjevskaya cave; **c** XRD of moon milk from Pionerskaya cave; **d** DTG and DSC of moon milk from Pionerskaya cave

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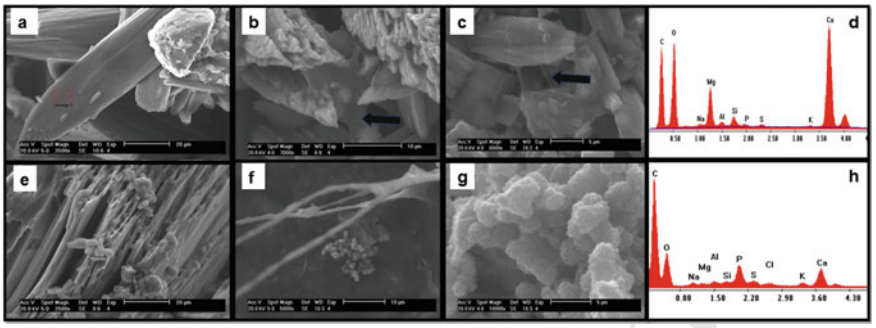

Fig. 6 The surfaces of calcite crusts (**a**-**d**) and gypsum (**e**–**h**) from Yurjevskaya Cave before (**a** and **e**) and after the stimulation of biofilm with R2 media: **b** films; **c** threads; **d** EDX analysis of the crusts after the stimulation; **f** hyphae on gypsum slice after the stimulation; **g** precipitants on gypsum slice after the stimulation; **h** EDX analysis of gypsum slice after the stimulation

Table 3 The abundance of bacterial phylla in the crusts of Yurjevskaya cave, %

Option	Initial	+ R2 modified
Proteobacteria	57–77	65–81
Actinobacteriota	16–29	1–1
Nitrospirota	0–4	0
Bacteroidota	0	4–5
Firmicutes	0	2–3
Bdellovibrionota	0	6–7
Total sequence identity	92	96

5 Discussion

The CA distributions demonstrate that the speleothem intact surfaces are covered with a partly hydrophobic film. This film was spread as hydrophobic spots with a size of at least 25 μm. Based on infrequent clear individual biomorphs and a high C/Ca ratio, we can confidently attribute the main part of the film to the EPS.

The ratio of the biofilm elements is approximately the same both on the untreated speleothems and speleothems treated with the R2 medium. The difference is manifested only as more clearly defined threads and films covered cavities in crystal surfaces in the latter case (Fig. 6). Samples of both fresh and hardened moon milk were exceptions not forming hydrophobic spots, but they included more than 3% organic matter in their mass too (Fig. 5). Simultaneously, the experiment showed that the development of a biofilm along with hydrophobization can hydrophilize and smooth the surface, i.e. our observations do not contradict each other.

Based on the comparison of surface microanalysis data, one can see that carbon compounds exceed the classical content for calcium carbonate alone, phosphates sometimes appear, and manganese is almost absent, which in total is characteristic

of biogenic deposits. The proportions of elements are closest to calcium oxalate in all samples, but sometimes one can assume the presence of other organic compounds (malates, succinates, etc.).

According to the data presented, the following path of organic matter into the crystalline matrix can be assumed. Biofilms inhabit fresh carbonate formations (along the bone, cave milk, feeding channels of corallites and helictites), which are initially represented by tubular structures of aragonite. Somewhat part of their EPS gets inside the tubes (but not cells because of the tube diameter of fewer than 2 microns), and at the same time, the solution is supersaturated with ions in the tubes (due to selective sorption), which causes precipitation and filling of the inner cavities. Mixing of the organic and crystallizing phases increases the defectiveness of the crystal lattices formed and declines their energy; thus, aragonite is replaced by biochemogenic calcite. This process also occurs without the participation of organic matter (Myszka et al. 2019), but the aragonite structure preservation observed indicates its acceleration under the biofilm.

The roughness could partially reduce the wettability and cause the appearance of a fuzzy bimodality of the CA distribution, but in no case caused the transition of the surface properties through the hydrophobicity threshold by itself. Highly likely, the increase in geometric heterogeneity induced the appearance of preferential inclination angles of the landing sites for air bubbles.

A clear bimodal CA distribution occurs when phases with different surface properties form clusters and spots, due to which there are both hydrophobic and hydrophilic areas on the surface, but few areas with intermediate CA values. We observed that the bimodal distribution of CA appeared only in the following situations: either the samples were not subjected to any processing other than dispersion or the treated surface was repopulated with biofilm. Hereby, when fresh biomass developed, smooth wavy mineral surfaces (calcite and gypsum) able to easily release cations demonstrated an increase in roughness due to the precipitation of a new mineral phase and the formation of hyphae, as well as the appearance of hydrophobized spots. When cations were difficult to release (e.g., glass), the surface could be smoothed (healed) by a biofilm.

More or less, the hydrophobicity of the speleothem specimens was preserved after the treatment, decreasing in the set: ethanol, burning, and alkali. However, at the same time, their bimodality disappeared, i.e., spots of hydrophobic substances spread over the surface. Other modes of treatment formed the following sequence with increasing hydrophilicity: hydrochloric acid, oxalic acid, R2 medium, drying at a temperature of 105 ºC, and polishing. Thus, the organic matter extracted from the mineral matrix partially remains as a film sorbed on the upper layer. This residual film can be removed due to the etching of minerals, evaporation, and scraping.

Genus Kribella, Gaiella, and Brevundimonas found in the original speleothems are typical extremophiles, while genera Pseudoarthrobacter and Nitrospira are obligate chemolithotrophs. The latter genera has the potential for the variable consumption of organic matter and/or CO_2, as well as participation in a nitrogen cycle. The modified R2 media contained organic compounds, therefore it stimulated chemoorganotrophic growth (e.g., Brevundimonas) that suppressed other members of the cave community,

which most likely have a low chance of adapting to near-surface soil. In this study, just like in the metagenomes studied earlier, many microbial types were partly able to diverge in metabolic pathways, but most of them were heterotrophic (Turrini et al. 2020). The biofilm autotrophic basis could be the genera Nitrospira possessed the potential for variable consumption of organic matter and/or CO_2, as well as participation in a nitrogen cycle. However, the abundance of this genera is not high, therefore, its ability to provide nutrients for the heterotrophic biomass in a cave environment is doubtful. In the stimulated community, the functions of the genera Nitrospira as a nitrifier in an environment depleted in organic matter (Daims et al. 2015) were probably transferred to Pseudomonas as a heterotrophic nitrifier (Trung et al. 2019).

The hyphae-forming members of phylum Actinobacteria are most likely responsible for an increase in surface roughness. The appearance of hydrophobic groups is probably the result of the total biofilm secretions, since both their synthesis during the metabolism of the chemolithoautotrophs and the reverse process of hydrophilization caused by microbial lytics are possible.

6 Conclusion

The biological changes in minerals depended on metabolic precipitants that either smoothed or scratched their surfaces. All the fresh speleothems showed higher carbon in EDX than expected for carbonate alone. Simultaneously, the surface hydrophobicity was detected for these samples in a solid state only, which indicated the hydrophobization of speleothems under long-life biofilms. The spots of a low wettability were removed from the surfaces by the thermal treatment and washing with solvents, whereas acid etching and burning of minerals induced the appearance of such spots. These facts are interpreted as the organic matter is distributed as both spread on the surface and enclosed in mineral channels. The consumption of the enclosed organic carbon compounds by biofilms is possible, since we have reason to believe that the observed cave biofilm community is mostly heterotrophic, with Nitrospira being the only obligate autotrophic among other autochthonous organisms. Nevertheless, there is a lack of organic matter for a heterotrophic nutrition in all the samples, except fresh moon milk. We can conclude that heterotrophs consume the organic matter, which can be released and detected only after the destruction of the crystalline matrix.

Then, the storage of nutrients during the periods of their excess, their uptake and preservation during the crystallization, and the consumption after the mineral destruction by metabolites can be hypothesized. Such a cycle is possible not only in caves, but also in subsoils, as well as technogeneous carbonates. If this dynamic is confirmed, the surface properties of alike formations should be considered as the function of organic matter releasing and preserving cycles. The collection of more complete evidence for this hypothesis requires further research.

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