

EXPERIMENTAL  
ARTICLES

## Transformation of Clay Minerals Caused by an Alkaliphilic Cyanobacterial Community

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**Abstract**—Transformation of clay minerals (smectite–zeolite, illite, kaolinite, and bentonite) and admixtures of iron oxides (hydroxides) under the action of an alkaline cyanobacterial community was studied. The results demonstrate that the processes of transformation of clay minerals such as intensification of removal of exchange bases and dissolution of silicates and iron oxides occurred in the presence of the alkaliphilic cyanobacterial community. The main factor that determines resistance of a mineral to biochemical weathering is its composition. Transformations of clay minerals in the course of active cyanobacterial photosynthesis (up to 14 days) and at decomposition of organic matter (OM) (28–60 days) are different. For smectite–zeolite and illite, these processes are dissolution of silicates and oxides (removal of Si and Fe) and removal of exchange bases (K), which were observed at both the of biomass production and OM destruction stages. For two other clays, the processes of neosynthesis are more typical: formation of carbonates (most probably siderite for bentonite clay and Mg–calcite for kaolin clay) and transformation of ferrihydrite into the more thermodynamically stable goethite.

**Key words:** clay minerals, alkaliphilic cyano-bacterial community, cyanobacteria.

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Clay minerals, i.e., layered aluminum silicates, are the most widespread minerals of sedimentation basins (both marine and continental), weathering crusts, and soils. Frequent occurrence in their structure of the ions with variable valence (Fe, Mn, etc.) makes clay minerals extremely sensitive to ambient conditions and, first of all, to variation in the ambient pH and redox conditions, so that they can be used as indicators of the state of the environment [1].

The recent studies revealed an enormous role of bacteria, including cyanobacteria, in the processes of ancient sedimentogenesis. Discovery of the fossilized residues of microorganisms confirms the important role of microbial communities in sedimentation on the Earth surface starting from the Archaean time [2]. Ancient microbial communities were involved in both formation and transformation of many minerals. It is known that many representatives of the microbiota (as a rule, bacteria and fungi) can be sorbed on the surface of clay minerals and the products of their metabolism interact with the minerals [3]. The works concerned with the influence of cyanobacteria on clay minerals are scarce [4], and there are no data on the effect of alka-

liphilic cyanobacteria and their communities on clay minerals.

The goal of this work was to study the changes in the mineral composition of different clays resulting from productive and destructive processes of an alkaliphilic cyanobacterial community.

### MATERIALS AND METHODS

The test object was an alkaliphilic cyanobacterial community from the Hilganta soda lake (Buryatiya), domineered by the cyanobacterium *Microcoleus chthonoplastes*. The community was grown for 4 days at an illumination of 3000–4000 lx and at 28°C on an S alkaline medium [5] containing the following (g/l): KCl, 1.0; K<sub>2</sub>SO<sub>4</sub>, 1.0; NaHCO<sub>3</sub>, 16.8; K<sub>2</sub>HPO<sub>4</sub>, 0.5; NaNO<sub>3</sub>, 2.5; NaCl, 30.0; MgSO<sub>4</sub> · 7 H<sub>2</sub>O, 0.2; CaCl<sub>2</sub>, 0.04; FeSO<sub>4</sub>, 0.01; and a solution of trace elements (A<sub>2</sub>), 1.0 ml/l (g/l: H<sub>3</sub>BO<sub>3</sub>, 2.86; MnCl<sub>2</sub> · 4H<sub>2</sub>O, 1.81; ZnSO<sub>4</sub> · 5H<sub>2</sub>O, 0.222; MoO<sub>3</sub>, 0.176; and CuSO<sub>4</sub> · 5H<sub>2</sub>O, 0.079); pH 8.5. Then, the biomass was washed three times with a salt solution (NaCl, 2%; NaHCO<sub>3</sub>, 1.2%), dispersed with a brush, filtered through a filter with a pore diameter of 0.5 mm, and introduced by 4 ml into a vessel with the following mixture: ground clay without preliminary purification and separation, 0.5 g; NaCl,

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Some properties of the clays under study

Sample	Mineralogical composition	Chemical composition, %		Exchange bases, mg-eq/100 g					Magnetic susceptibility, $\text{SI U} \times 10^{-8} \text{ m}^3 \text{ kg}^{-1}$	$C_{\text{org}}$ , %
		Fe	K	Ca	Mg	K	Na	Total		
Kaolin clay (Vimianzo, Spain)	Kaolinite, muscovite, quartz	0.48	0.76	1.2	0.8	0.07	0.18	2.25	0.9	0.24
Smectite–zeolite (Cretaceous deposits, the Don River bank, Volgograd oblast)	Montmorillonite, clinoptilolite, quartz, muscovite, and calcite	1.07	1.15	23.2	2.0	5.10	4.30	34.6	2.73	0.04
Illite (Fuzerradvany, Hungary)	Illite, quartz, microcline	0.42	5.12	10.0	1.9	0.84	0.10	12.84	0	0.15
Bentonite (Wyoming, United States)	Montmorillonite, quartz, feldspars, and calcite	2.17	0.41	34.2	3.0	1.25	56.08	94.53	10.47	0.86

2.0 g;  $\text{NaHCO}_3$ , 1.2 g; and sterile distilled water, 100 ml. Since heating is known to cause various endo- and exothermic effects [3], clays were not presterilized to avoid possible changes in their structure at sterilization. Cultivation on nonsterile clays implies the introduction of foreign bacterial microflora into the community. However, since the photosynthetic component was constant, variation of the species composition of destructors was unlikely in the presence of the same metabolic products of cyanobacteria. After addition of the cells, the suspensions were thoroughly stirred and incubated in the light for up to 60 days. Sterile distilled water was added periodically, as the solution dried out (with an interval of approximately 10–14 days). Clays without microorganisms were used as a control. The duration of the experiments was 7, 14, 28, and 60 days. Biomass increase was determined spectrophotometrically by the concentration of chlorophyll *a* after its extraction with ethanol ( $\lambda = 665 \text{ nm}$ ) [5]. The rate of photosynthetic oxygen evolution by cell suspensions was measured polarographically in a 1-ml cell with a Clark electrode [6].

The following clays were used in the work: smectite-containing clays (Wyoming bentonite SWy-2 and smectite–zeolite) of marine origin, illite of hydrothermal–metamorphic origin, and kaolinite from the weathering crust. The main properties of the clays are presented in the table. The mineralogical composition of bentonite SWy-2 is as follows: montmorillonite is predominant; quartz, feldspars, and calcite are minor. In the mineralogical composition of smectite–zeolite,

montmorillonite is predominant; clinoptilolite is present in considerable amount, while muscovite, quartz, and calcite are minor. Illite is a muscovite mica containing 17–26% of swelling (smectite type) layers. Besides illite, this sample also contains quartz and K-feldspars (microcline).

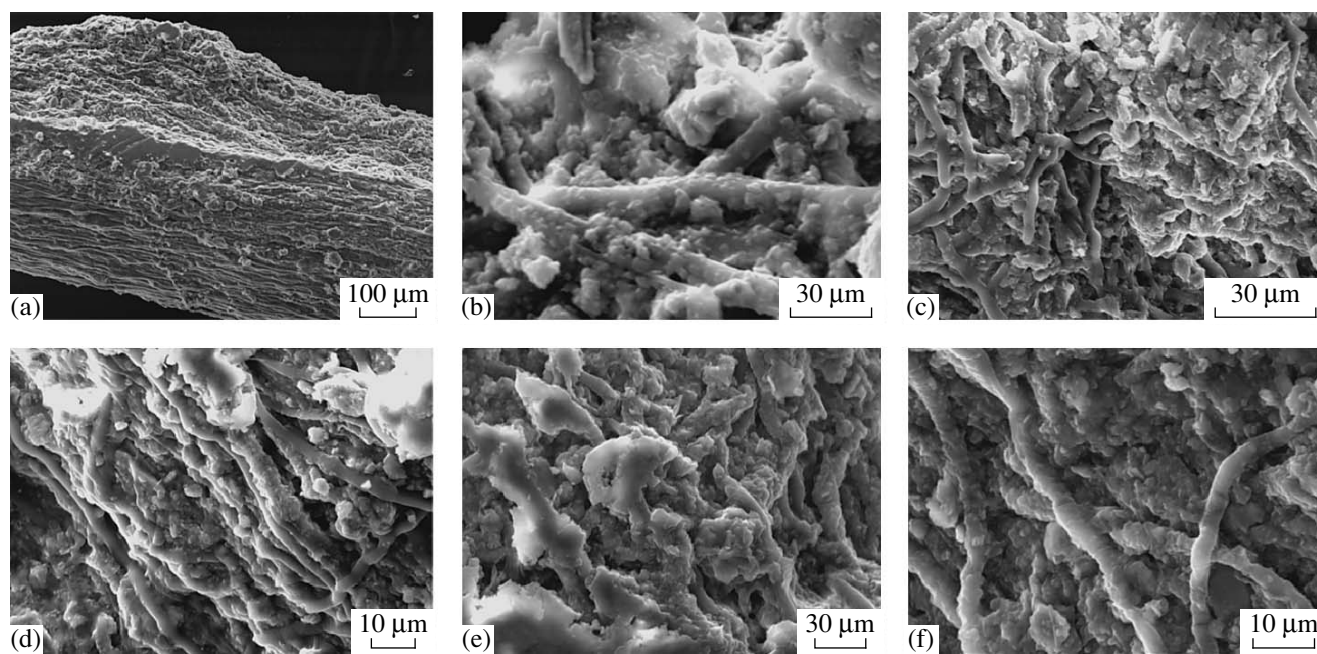
Kaolin clay contains, apart from kaolinite, muscovite and quartz as impurities.

The composition of solutions and solid phase components, i.e., organic carbon content, mineralogical composition, and magnetic properties, were determined for each point of the experiment (days 7, 14, 28, and 60).

The organic carbon content ( $C_{\text{org}}$ ) in the samples was measured according to Tyurin [7]; the composition of exchange bases was determined by the acetic–ammonium method; the atomic adsorption method was used for the content of Si, Fe, and Al in the solutions; and the flame photometric method was used for the K content.

The mineralogical composition of clays was studied by X-ray diffractometry in a DRON-3 diffractometer. The samples were studied in the original form and in monoionic (Mg- and K-) forms air-dried, saturated with ethylene glycol, and calcined to 350 and 550°C.

Magnetic characteristics of the solid phase of clays were determined from the measurements of a complex of magnetic parameters including full magnetization curves (in the interval from +1 to –1 T) named the hysteresis loops. The shape of the loop is determined by the



**Fig. 1.** Interaction of cyanobacteria and clays (exemplified by bentonite clay): (a) layered mat formation (28 days); (b) clay structuring, formation of distinct layering, and mineralized and living cyanobacteria; (c) cyanobacteria twining of round clay particles; (d) cyanobacteria of the upper mat layer with clay minerals on the sheath surface; (e) cyanobacterial band mineralized by clay minerals; (f) cyanobacteria fossilized by calcium carbonate. Scale bar: (a) 100 µm; (b, c, e) 30 µm; and (d, f) 10 µm.

magnetic properties of the material. Complete magnetization curves (hysteresis loops) were obtained in a VSM Molspin vibration magnetometer. Magnetic hysteresis loops were analyzed by the HYSTERAX software (Dobenec, 1998) in order to separate the paramagnetic and ferromagnetic components in the samples [8, 9].

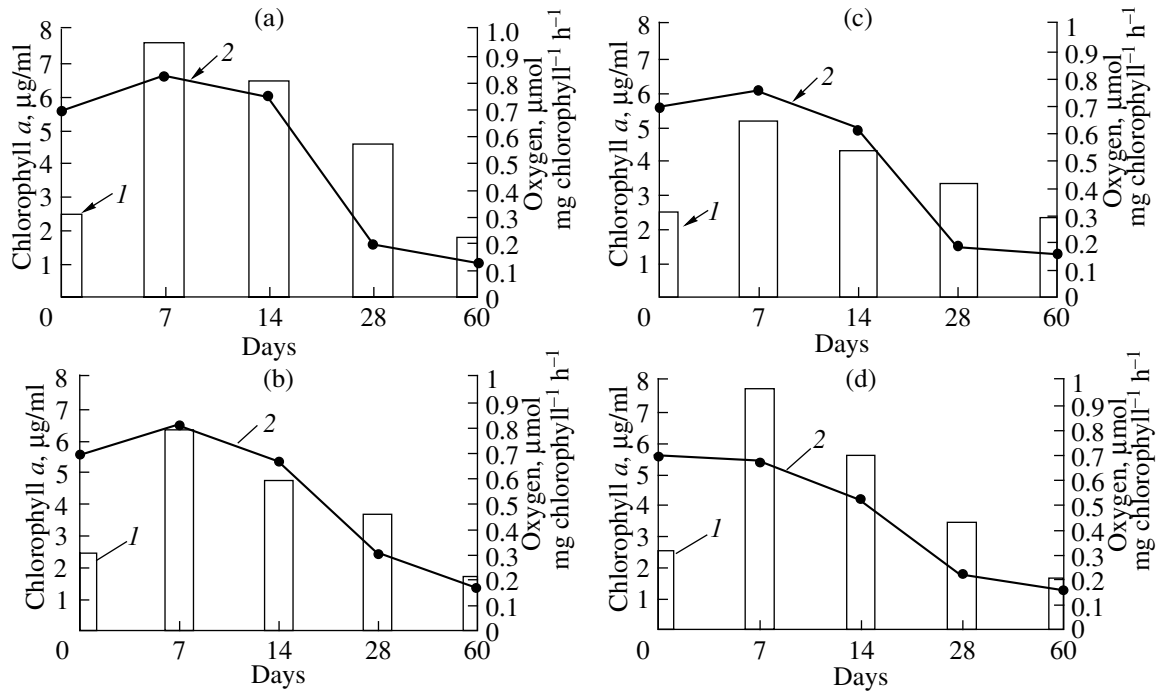
The character of interaction of cyanobacteria with clay minerals at different stages of the experiment was studied by the methods of optical and electron microscopy using a CamScan scanning electron microscope with a Link-860 microanalyzer at the Paleontological Institute, Russian Academy of Sciences.

## RESULTS AND DISCUSSION

**Development of the cyanobacterial community grown on clays.** The behavior of cyanobacteria in the presence of bentonite, illite, and smectite–zeolite was identical: during the first 14 days, the filaments of cyanobacteria actively developed on the clay surface and twined round the clay lumps maintaining normal morphology. After 2 weeks, a dense layered mat was formed (Fig. 1a), consisting of the layers of cyanobacteria with normal morphology and the layers of clay particles with fragmented cyanobacterial filaments (Fig. 1b). The interaction of cyanobacteria with clay minerals was accompanied by abundant glycocalix formation, which explains good structural integrity of the formed mat. On day 28, the filaments on the mat surface were covered with the particles of clay minerals

(Fig. 1d). By the end of the experiment, the formation of mineralized cyanobacterial bands was observed inside the mat (Fig. 1e), while fossilization of cyanobacterial filaments with calcium carbonate occurred on the surface (Fig. 1f). On kaolinite, unlike other clays, cyanobacteria did not produce glycocalix.

**Photosynthetic activity and the content of chlorophyll *a*.** The pH values of the solutions varied during the experiment from 8.6 to 9.2–9.5 in all clays. Figure 2 shows the concentration of chlorophyll *a* and the rate of oxygen evolution for *Microcoleus* in the presence of clays. During the first week, the growth of cyanobacteria was observed on all clays and their biomass increased threefold on average. Photosynthetic activity was approximately the same on all clays ( $\approx 0.8 \mu\text{mol O}_2 \text{ mg chl}^{-1} \text{ h}^{-1}$ ), except for kaolinite. During further cultivation, these values decreased, smoothly for chlorophyll and abruptly for oxygen. On day 14, photosynthetic activity was still rather high:  $0.7\text{--}0.8 \mu\text{mol O}_2 \text{ mg chl}^{-1} \text{ h}^{-1}$  for smectite–zeolite and bentonite, respectively, and  $0.5\text{--}0.6 \mu\text{mol O}_2 \text{ mg chl}^{-1} \text{ h}^{-1}$  for kaolinite and illite. On day 28, the drop of photosynthetic activity was especially abrupt. Thus, the maximum rate of oxygen evolution was observed at incubation in the presence of bentonite and smectite–zeolite; the highest content of chlorophyll *a* was found for bentonite and kaolinite, while its lowest content was revealed in the presence of illite. The cells remained viable on all clays for the first 14 days and then began to die off.



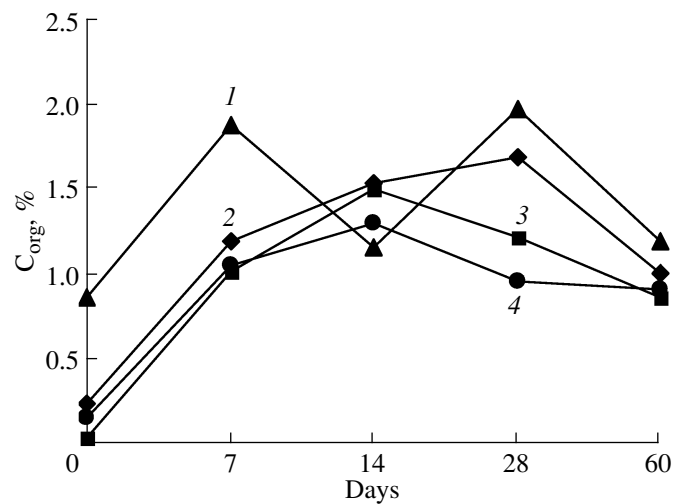
**Fig. 2.** Dynamics of chlorophyll *a* concentration (1) and oxygen evolution rate (2) for *Microcoleus chthonoplastes* in the presence of clays: (a) bentonite; (b) smectite-zeolite; (c) illite; (d) kaolinite.

The studied clay minerals initially differed in the content of  $C_{\text{org}}$ , which was minimal (0.04%) for smectite-zeolite and reached 0.9% for bentonite (see the table). On addition of *Microcoleus*, the increase in  $C_{\text{org}}$ -content was observed for illite and smectite-zeolite during the first 14 days of the experiment, although the decrease of biological activity was noted already by day 14 of incubation (Fig. 3). For kaolinite, the  $C_{\text{org}}$  content increased right up to day 28 of the experiment and then began to decrease. On day 60, the total increase in  $C_{\text{org}}$  for these three clays was about 0.8%. The situation was different in the experiment with bentonite. On day 14, the content of  $C_{\text{org}}$  decreased by 0.7% at high values of biological activity, and then, increased again by day 28 of the experiment. By the end of the experiment, the  $C_{\text{org}}$  content was the same as in other clays. Thus, it may be concluded that the increase of organic matter in clays was determined by the metabolic products of cyanobacteria during the first 2 weeks and by biomass destruction in the subsequent period. The decrease of organic matter content observed in the period from day 28 to day 60 may be due to its utilization during the growth of heterotrophic microflora.

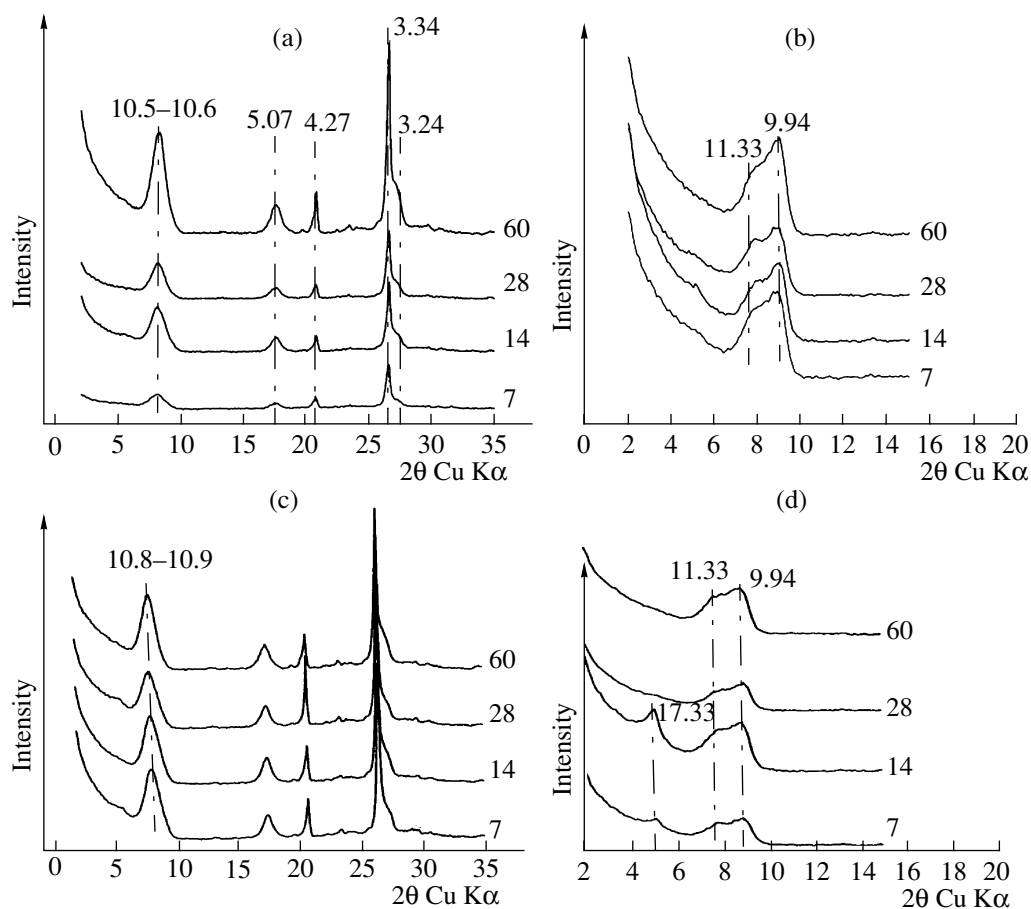
**The transformation of clay minerals under the influence of the cyanobacterial community.** The characteristics of clays after incubation in the presence of the cyanobacterial community revealed that kaolin and bentonite clays exhibited the maximum stability, showing insignificant variations in their mineralogical composition. In the case of kaolinite, synthesis of Mg-

calcite was registered on diffractograms (peak 2.85 Å) in the presence of cells on day 28 of the experiment.

Transformation of smectite-zeolite consists in the formation of a defect "island" layer in the interlayer space of montmorillonite on day 28 of incubation and is probably associated with potassium substitution by the molecules of hydroxides (e.g., Al) or their complexes with organic anions. The defect layer was not



**Fig. 3.** Dynamics of organic carbon content in clays in the presence of *M. chthonoplastes*: bentonite (1); kaolinite (2); smectite-zeolite (3); and illite (4).



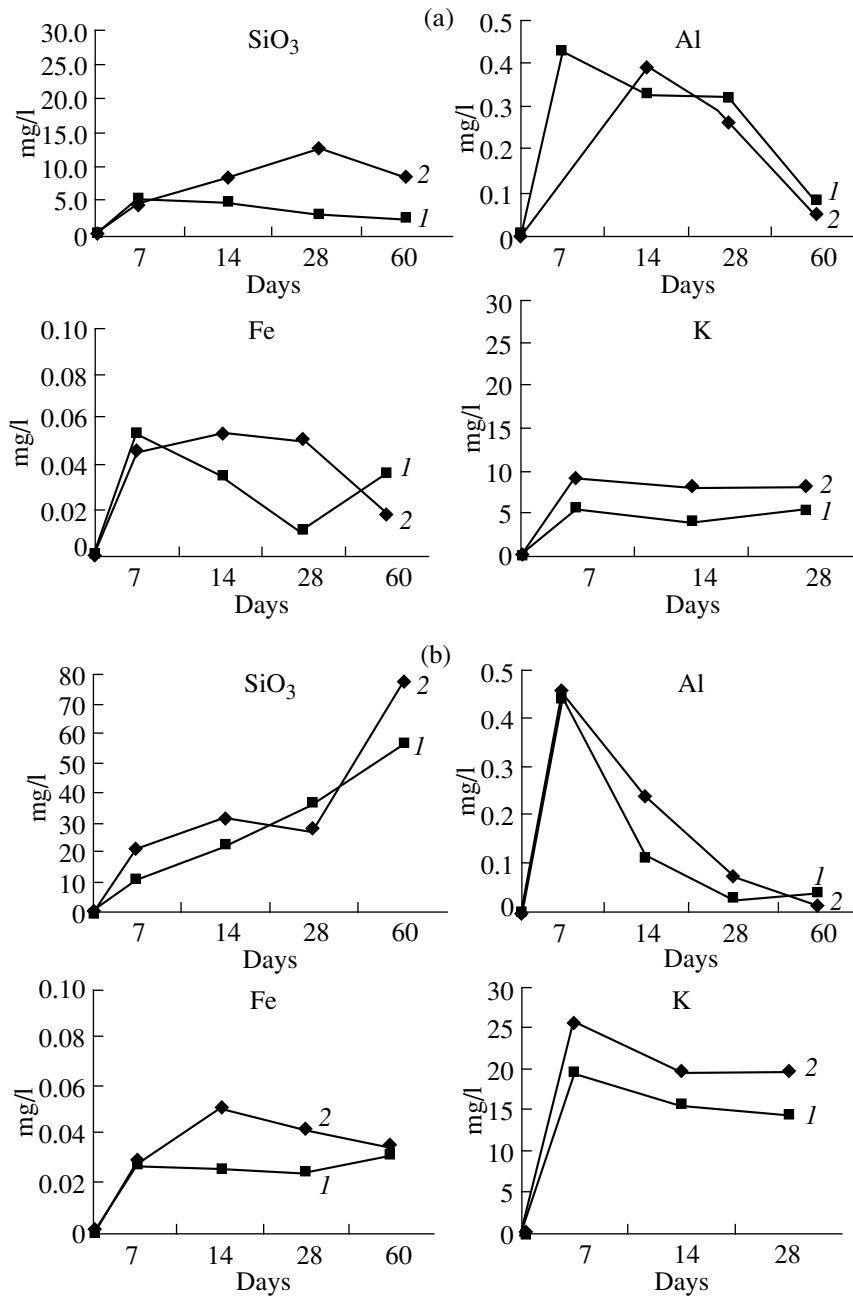
**Fig. 4.** XRD patterns of illite after day 7, 14, 28, and 60 of the experiment: (a) control samples Mg-saturated; (b) control samples saturated with ethylene glycol; (c) samples with cyanobacteria, Mg-saturated; (d) samples with cyanobacteria, saturated with ethylene glycol; d- spacing values are given in Å.

formed in the absence of cyanobacteria. Previously it has been shown [10] that the swelling minerals of the smectite type in biological systems can act as a “catch pit” for accumulation of ammonium and other metabolic products.

The most significant changes in the mineralogical composition were found for the effect of cells on illite (Fig. 4). Dioctahedral illite from the control samples was characterized by the following series of peaks: 10.5–10.6; 5.07; and 3.34 Å (Fig. 4a). After treatment with ethylene glycol, some part of the smectite layers swelled to 11.33 Å (Fig. 4b), which corresponds to the content of the smectite phase in illite within the range of 17–26%. Other reflexes on diffractograms presented in Figure 4a belong to quartz (4.27 and 3.34 Å) and feldspars (3.24 Å). The effect of cyanobacteria contributed to the release of some part of potassium from the mica lattice and increase of the number of swelling smectite layers, which was registered on days 7 and 14 by appearance of an additional peak at 17.33 Å after ethylene glycol saturation (Fig. 4d).

Intensification of removal of dissolved elements in the experimental variants (Fig. 5) confirmed the data on

transformations of illite and smectite–zeolite clays occurring under the influence of a microbial community. The removal of elements due to biological processes exceeded the removal of elements in the controls without cyanobacteria. Illite and smectite–zeolite showed an increase in the content of dissolved K, Si, Fe, and Al (Figs. 5a, 5b). At the same time, the elevated content of K and Si persisted until the end of the experiment; the content of Fe increased until day 14 and decreased after day 28, while the control shows again its increase. Aluminum content dropped to zero on both clays. High concentrations of K in the experiment with smectite–zeolite, which significantly exceed its concentrations in the experiment with illite, demonstrate that initially the exchange K was removed; its content in smectite–zeolite is the highest among the clays investigated (see the table). The substitution of sodium for exchange potassium was typical also of the control experiments on these two clays, but the presence of *Microcoleus* intensified this process. The composition of solutions at cultivation on bentonite and kaolinite remained practically constant (Figs. 5c, 5d), except for Si removal on day 60 on kaolinite (Fig. 5d). The notice-



**Fig. 5.** The content of elements in solution after the interaction of clays with cyanobacteria: (a) illite; (b) smectite-zeolite; (c) bentonite; (d) kaolinite. Control (1); experiment (2).

able difference in the composition of solutions in the experiments with different clays confirms that the composition of a mineral is the main factor determining its resistance to biochemical weathering.

As a rule, clay minerals have paramagnetic properties. The most widespread rock-forming minerals of sedimentary rocks, i.e., quartz, calcite, and feldspars, are diamagnetics or weak paramagnetics and do not make any noticeable contribution to the value of magnetic susceptibility. Thus, the magnetic properties of clays are determined mainly by admixtures of iron

oxides. The bulk content of iron in the studied clays was 0.42–2.17% (see the table), being minimal for illite and maximal for bentonite. The magnetic parameters of the clays under study demonstrated the presence of admixtures possessing mainly paramagnetic and partially ferromagnetic properties, the carriers of which were dispersed forms of magnetite (maghemite), hematite, and hydroxide films. The maximum content of ferromagnetic minerals was shown for bentonite.

In spite of insignificant content of bulk iron and its oxides in clays under study, its transformations involv-

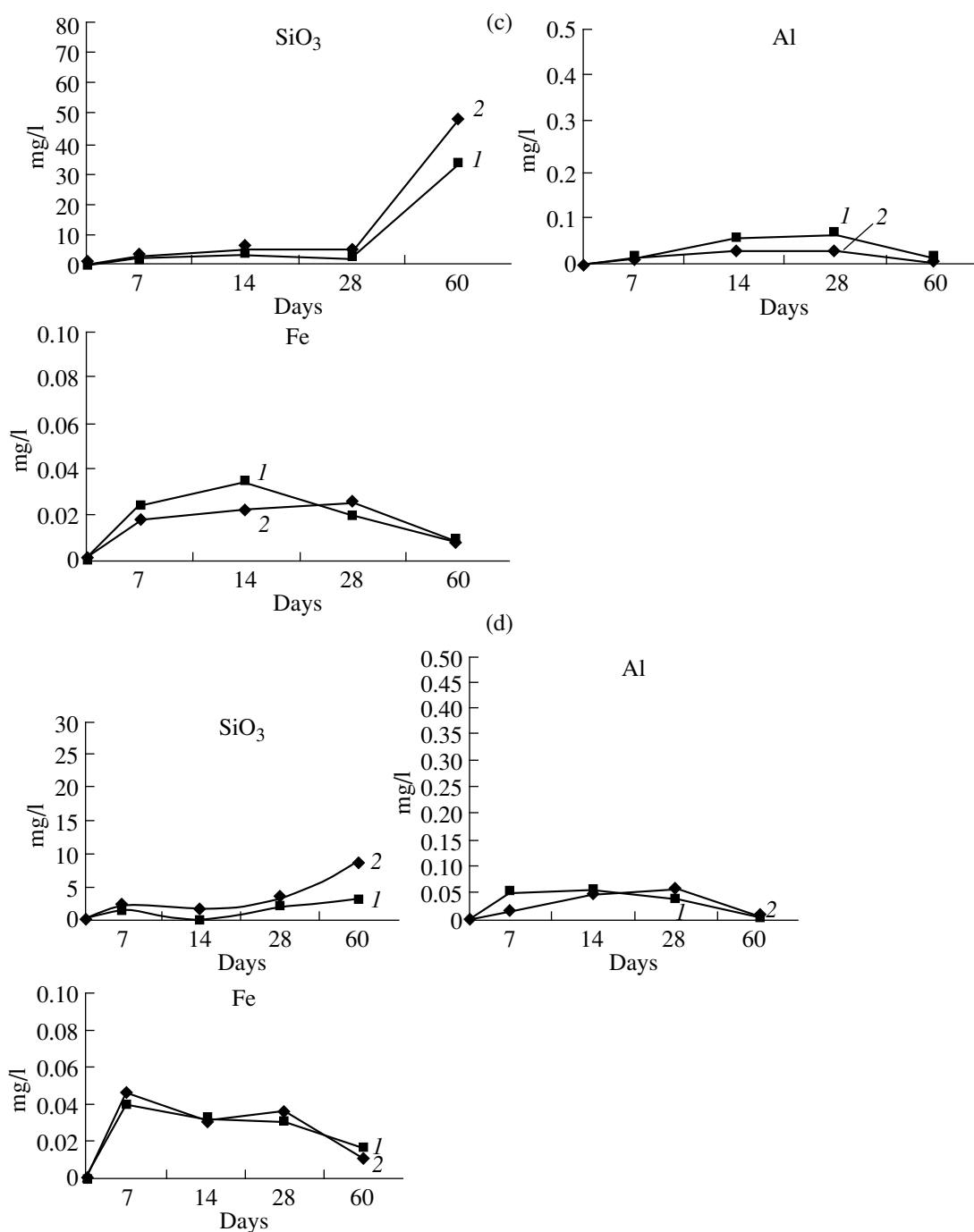
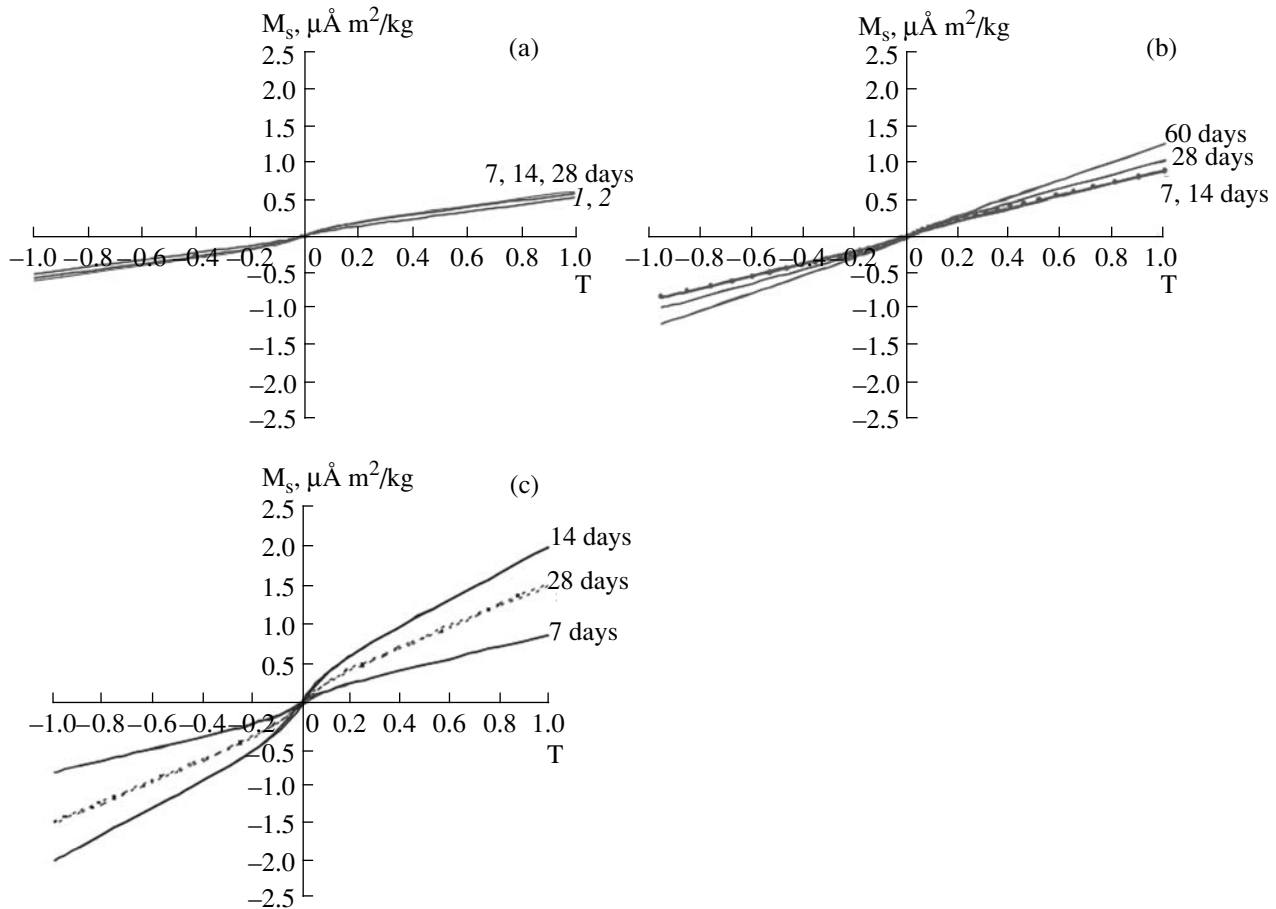


Fig. 5. Contd.

ing the biota are of apparent interest. It is known that iron compounds, e.g., oxides–hydroxides, sulfides, sulfates, and phosphates, take the third place in occurrence among the minerals formed with participation of living organisms, after carbonates and silica minerals [11]. Extensive participation of iron reducers in the iron cycle is well known.

Our results demonstrated that *Microcoleus* affected the composition of iron oxides–hydroxides impurities.

The experiments on kaolinite and smectite–zeolite (Fig. 6a) revealed an increase of magnetization and magnetic susceptibility values, as can be seen from the hysteresis loops on Figure 6b. It may be associated with iron precipitation as ferrihydrite ( $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ ) followed by formation of the thermodynamically more stable goethite ( $\alpha\text{-FeOOH}$ ) [12]. Formation of a poorly ordered phase (ferrihydrite) at the first stage may be determined by the aerobic medium enriched in free



**Fig. 6.** Dependence of magnetization on the value of external magnetic field intensity (hysteresis loops) ( $M_s$ ): (a) control variants for smectite-zeolite (1) and bentonite (2); (b, c) experimental variants: smectite-zeolite (b); bentonite (c).  $\mu\text{A m}^2/\text{kg}$  is the measuring unit for magnetization.

oxygen, quick iron oxidation, and the presence of a crystallization inhibitor (organic matter). Its transformation into goethite at the end of the experiment (day 28–60) correlates with the activity of destructors and mineralization of organic carbon..

The iron content in bentonite is two- to fivefold higher than in other clays, and its compounds are partially represented by ferromagnetics. On day 14 of the experiment, against a decrease of the ferromagnetic signal, there was a jump of the paramagnetic signal (Fig. 6c) accompanied by loss of  $C_{\text{org}}$  (see Fig. 3). It may be supposed that oxidation of the organic matter (OM) initially contained in bentonite to  $\text{CO}_2$  is accompanied by partial reduction of Fe(III) and formation of a paramagnetic phase, probably siderite. The possibility of such a scheme in the Fe cycle has been considered for a soil system in the work of Zavarzina et al. [13]. An increase of the paramagnetic signal observed throughout the experiment both in the control variants and in the variants with cells is associated with partial dissolution of ferromagnetics and precipitation of hydroxides (ferrihydrite and goethite).

Thus, our findings demonstrate that the following processes of clay mineral's transformation occur in the presence of alkaliphilic cyanobacterial communities: intensification of the removal of exchange bases and dissolution of silicates and iron oxides. The main factor determining the resistance of a mineral to biochemical weathering is its composition. Transformations of clay minerals occurring under active photosynthesis of cyanobacteria (up to day 14) and under decomposition of organic matter (days 28–60) are different. For smectite-zeolite and illite, such processes are dissolution of silicates and oxides (removal of Si and Fe) and removal of exchange bases (K), which were observed at the stages of both biomass production and OM destruction. For two other clays, the processes of neosynthesis are more typical: formation of carbonates (most probably siderite for bentonite clay and Mg-calcite for kaolin clay) and transformation of ferrihydrite into the thermodynamically more stable goethite.

The special feature of the registered processes of biota-induced transformations of clays and iron-containing admixtures is the metastable nature of the intermediate products (smectite phase in illite, formation of



ferrihydrate, and probably, siderite). Ferrihydrate most likely acts as a key mineral in the biogeochemical cycle of iron.

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