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EXPERIMENTAL ARTICLES

Interaction of Anoxygenic Phototrophic Bacteria *Rhodopseudomonas* sp. with Kaolinite

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Abstract—The interaction between freshwater nonsulfur purple bacteria *Rhodopseudomonas* sp. UZ-25p (Uzon caldera, Kamchatka, Russia) and two kaolinite samples (Zhuravlinyi Log, Chelyabinsk oblast) was investigated. Alterations in the chemical composition of the minerals and solutions, the parameters of bacterial growth, and crystal morphology and mineralogy of the kaolinite samples indicated the interactions between all components of the system (minerals, water, growth medium, and bacteria). Bacteria removed some elements from the medium, used them for growth, and promoted their transition into the mineral exchange pool. In the presence of bacteria, kaolinite cation exchange capacity increased and saturation of kaolinites with bases occured. Partial biodegradation of kaolinites, accompanied by ordering of the crystalline structure of their lamellar phase, was the main factor responsible for the increase in cation exchange capacity. For the first time anoxygenic phototrophic bacteria were found to degrade kaolinite with formation of gibbsite. The theoretical and applied significance of the experimental results is discussed.

Keywords: kaolinite, anoxygenic phototrophic bacteria, anaerobic conditions, exchange cations, differential thermal analysis, electron diffraction analysis, bioleaching, gibbsite

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We have previously discussed the importance of investigation of the interactions between living organisms and dead matter, as well as the present state of the problem [1–4]. The present article continues the line of research on the interaction between anaerobic bacteria and aluminosilicate minerals, which comprise 95% of the Earth crust. Previous works on the interaction of anoxygenic phototrophic bacteria with volcanic ash [1] and various layered aluminosilicates [2] revealed a significant effect of bacteria on the chemical composition of the minerals, resulting in their increased cation exchange capacity and pool of exchangeable bases. The methods used in those works (e.g., X-ray diffractometry) were, however, insufficiently sensitive for monitoring the alterations in the structure and mineral composition of aluminosilicates in order to achieve better understanding of the processes occurring.

Kaolinite, a widespread secondary mineral which is formed as a result of weathering or hydrothermal modification of the mica–feldspar rocks [5], was chosen as the mineral subject of investigation. Kaolinite $Al_4[Si_4O_{10}](OH)_8$ belongs to layered aluminosilicates and is one of the major clay minerals. Kaolinite crystals are formed by two-layered packets of a layer of aluminum hydroxide octahedrons and a layer of silicon oxide tetrahedrons. The packets are densely bound and are closely adjacent, so that water molecules and metal cations can not penetrate into the interpacket space. Kaolinite therefore does not swell in water and has the lowest cation exchange capacity among clay minerals (3 to 15 mg-eq/100 g). For comparison, the cation exchange capacity of clay minerals of the montmorillonite group, which have loose interpacket bonds and swell significantly, is 80 to 150 mg-eq/100 g [5].

Kaolinite is the main component of kaolins, which may be, depending on their chemical and mineral composition, normal (alkaliless) and alkaline (alkali-containing). Alkaline kaolins, in which kaolinite is associated with potassium feldspar and/or muscovite, have high K_2O content (1.7 to 4–6%), while normal kaolins contain not more than 0.3–0.5% K_2O [6]. As the main component of kaolins, kaolinite has numerous practical applications. It is used in the manufacturing of paper (~50% of all kaolin mined), rubber,

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porcelain, earthenware, and refractory materials, in medicine, perfumery, textile industry, etc. It is also a potential source of aluminum [6].

Unlike our previous works, where haloalkaliphilic bacteria were used as microbiological objects, neutrophilic freshwater bacteria with the natural habitats corresponding to the conditions of kaolin occurrence were used.

The goal of the present work was to apply various techniques in order to determine the effect of bacteria on the chemical, mineralogical, and crystal morphological characteristics of aluminosilicates.

MATERIALS AND METHODS

Nonsulfur purple bacteria *Rhodopseudomonas* sp. UZ-25p isolated from the Termofil'nyi freshwater thermal sulfur spring (caldera of the Uzon volcano, Kamchatka, Russia) were the microbiological object of the study. Kaolinite samples from the Zhuravlinyi Log deposit (Southern Urals, Russia), the largest kaolin deposit in Russia, were kindly provided by B.F. Gorbachev (Central Research Institute of Geology of Nonmetallic Minerals, Kazan, Russia). Kaolinite concentrates with particle size <0.02 mm obtained by sedimentation from two kaolin samples, the normal (no. 08015-16) and alkaline one (no. 08028-29) will be further referred to as kaolinite 1 (K_1) and kaolinite 2 (K_2), respectively.

Bacteria were grown in anaerobic conditions under light with organic electron donor, in the presence and absence of the minerals. Kaolinites incubated with water and sterile medium were used as the controls. Inoculated medium incubated in the dark was used as the control for biomass increment and the content of photosynthetic pigments. The growth medium contained the following (g/L): KH_2PO_4, 0.33; NH_4Cl, 0.33; $MgCl_2 \cdot 6H_2O$, 0.33; KCl, 0.33; Na_2SO_4, 0.33; $NaHCO_3$, 1.0; $CaCl_2 \cdot 2H_2O$, 0.1; trace elements solution, 1 mL. The trace elements solution contained the following (g/L): EDTA, 5; $FeSO_4 \cdot 7H_2O$, 2; $ZnSO_4 \cdot 7H_2O$, 0.1; $MnCl_2$, 0.03; H_3BO_3, 0.3; $CoCl_2 \cdot 6H_2O$, 0.2; $CuCl_2$, 0.01; $NiCl_2 \cdot 2H_2O$, 0.02; $Na_2MoO_4 \cdot 2H_2O$, 0.02. The mineral medium was supplemented (g/L) with sodium acetate, 1.5; fructose, 0.5; and yeast extract, 0.1; pH was adjusted to 7.0.

Prior to the experiment, the minerals were placed into glass vials (1 g per vial) with a small amount of water and autoclaved for 20 min at 121°C. The cultures and the controls were incubated under the same conditions: in the light (~2000 lx, except for the dark growth control), in 60-mL vials filled to capacity and hermetically sealed, at 25–30°C. All experimental variants (in two repeats) were incubated simultaneously.

Bacterial biomass, chemical composition of the liquid phase, and content of exchangeable cations in the kaolinites were determined immediately after the onset of the experiment and after 3 months of incubation. At the same time points, the structure of the minerals was studied by differential thermal analysis, electron diffraction analysis, and electron microscopy.

Protein content in the cell suspension (determined by the Lowry method [7]) was used as the criterion of biomass increment. Kaolinite-containing water was used as the control in order to exclude the effect of minerals on the results of analysis. A calibration curve was built for determination of dry biomass. The criterion of bacteriochlorophyll *a* content was the optical density of the acetone extract from the cells in 5 mL of the cell suspension. The measurements were carried out on a KFK-3 spectrophotometer at 770 nm in a 0.5-cm cuvette.

After sampling, the suspension was centrifuged and used for analyses of the solid and liquid phases. The composition of exchangeable cations was determined by the Pfeffer method as modified by Molodtsova and Ignatova [8]. The concentrations of Fe, Ca, Mg, Mn, Cu, Ni, and Zn were determined by atomic absorption analysis on an AAS-3 spectrometer; P content was determined colorimetrically by Merfy–Raily on a SPECOL-221 spectrophotometer [9]; K and Na were determined on a FlaFO-4 flame photometer [10].

The mineralogical properties and crystal morphology of kaolinites were investigated by differential thermal analysis (DTA), electron diffraction analysis, and transmission electron microscopy. DTA was carried out on a STA 449 F1 Jupiter synchronous thermal analyzer (Netzsch, Germany) which combined differential scanning calorimetry (DSC) and differential thermal gravimetry (DTG). The measurements were taken at 30–1050°C and heating rate of 10 K/min in the air atmosphere. Electron diffraction patterns of oblique structures were obtained on an EMR-102 electron diffractometer (Ukraine) at accelerating voltage of 100 kV and 60° tilt angle of the textured samples. Diffraction pictures corresponded to 2.5 mm of the sample. Crystal morphology was studied under a JEM-2100 transmission electron microscope at 200 kV accelerating voltage.

RESULTS

Effect of kaolinites on bacterial growth. The increment of bacterial biomass (measured as protein content) in the presence and absence of the minerals was similar (Table 1). Bacteriochlorphyll *a* content in the cultures grown with kaolinites was, however, more than 1.5 times higher than in the control without kaolinite (Table 1). This probably resulted from the lack of light caused by shading of bacteria with mineral particles. Since bacteria growing under lower illumination in the presence of kaolinites had to synthesize additional photosynthetic pigments, the biomass increment in the presence of kaolinites should have been higher than in the control under equal illumina-

Table 1. Growth of the phototrophic bacteria *Rhodopeudomonas* sp. UZ-25p in the presence and absence of kaolinites

	Without kaolinite		With kaolinite in the light	
	Dark	Light	Kaolinite 1	Kaolinite 2
Protein, mg/L	6*	275	273	277
Dry biomass, g/L	0.01	0.42	0.41	0.43
OD_{770}**	0.01	0.75	1.28	1.17

Notes: * All values are averages of four measurements.

** The indicator of bacteriochlorophyll *a* content was optical density of the acetone extract from bacterial cells contained in 5 mL of the cell suspension, measured in a 0.5-cm cuvette at 770 nm.

tion conditions. Thus, the minerals studied had probably a beneficent effect on bacterial growth.

The morphology of the cells of *Rhodopseudomonas* sp. UZ-25p grown with and without kaolinites was similar and typical of the strain (short budding motile rods, 0.6–1.0 × 1.8–2.2 μm, Fig. 3c).

Alterations in the chemical composition of the solutions. Analyses revealed significant differences in the chemical composition of both kaolinites and the surrounding solutions.

Existence of two types of ion exchange in minerals should be kept in mind. The first, the extramicellar one, is associated with instantaneous adsorption of the cations by the outer surface of the crystal lattice. The second, intramicellar, type is associated with much slower adsorption of the cations from the spaces within the crystal lattice (interpacket spaces). In the present work the initial concentrations of the elements in the solution and in the exchangeable cation pool of the minerals were determined immediately after the onset, so that the alterations associated with extramicellar exchange were primarily observed. The final values obtained after 3 months of incubation reflected all the alterations related to exchange processes of both types.

In the course of kaolinite incubation with water (Table 2), small amounts of potassium, magnesium, and calcium ions (1 to 3 mg/L) were detected in the solution just after the onset, and decreased by the end of the experiment.

Interaction between the minerals and the medium (Table 2) was ascertained as the differences in the concentrations of the elements in sterile medium with and without minerals (LM–L). Alterations in the chemical composition of kaolinite-containing sterile medium were observed both immediately after the onset (due to the extramicellar cation exchange) and after prolonged incubation (due to the intramicellar exchange). Immediately after the start of the experiment, the concentrations of potassium (by 16–

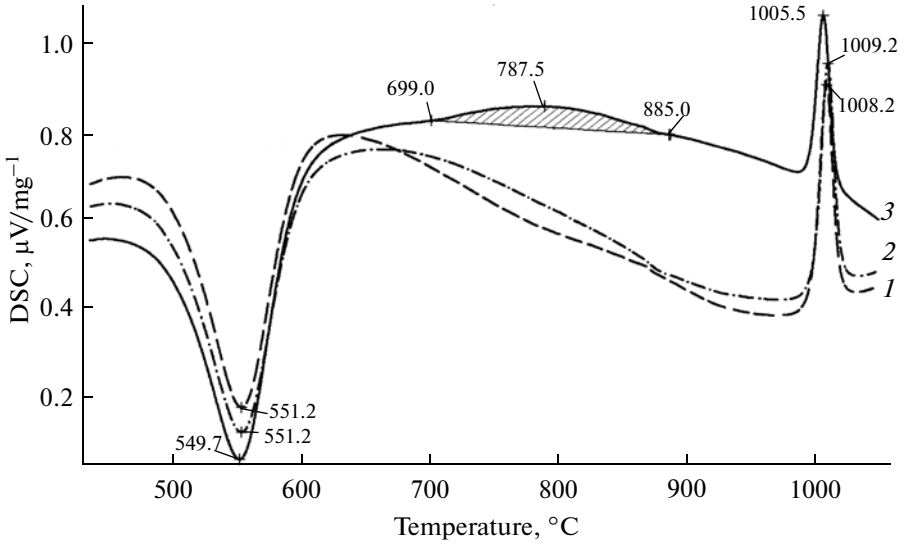

Fig. 1. Curves of the differential scanning calorimetry of kaolinite 1 before (*1*) and after incubation in sterile (*2*) and inoculated (*3*) medium.

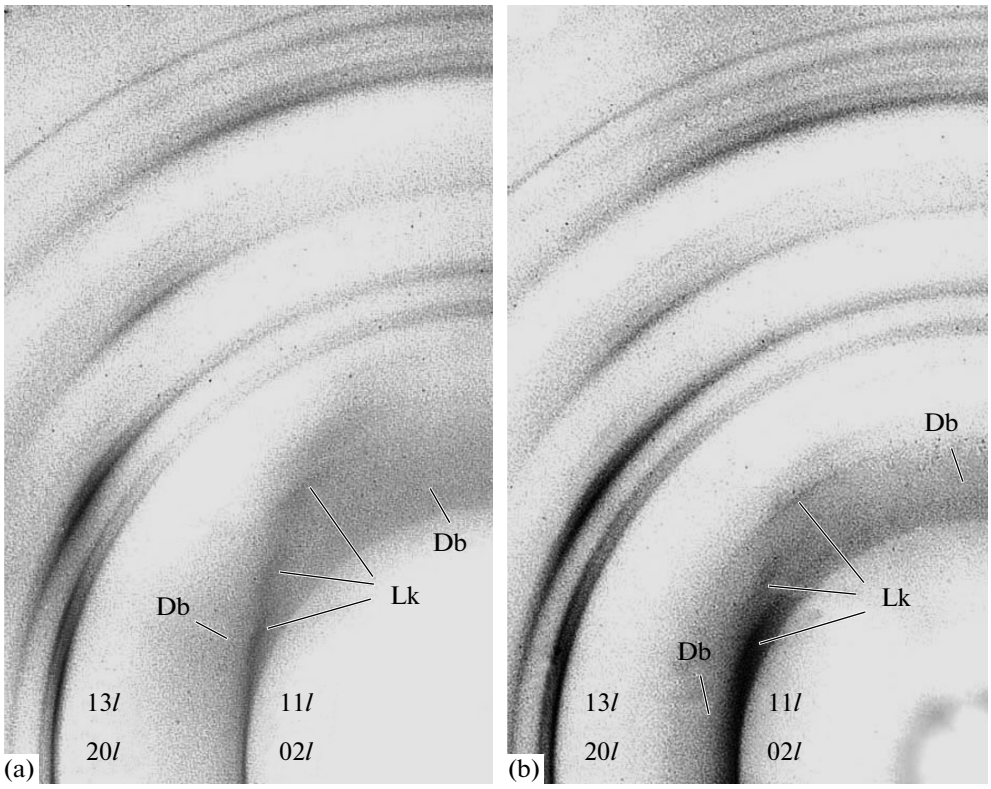

Fig. 2. Electron diffraction patterns of oblique textures of kaolinite 1 before (a) and after (b) incubation in inoculated medium. Db indicates diffusion bands, Lk indicates reflexes of the lamellar kaolinite.

18 mg/L) and iron (by 0.2 mg/L) decreased, while calcium concentration increased by 2.4–2.8 mg/L. Magnesium concentration remained practically unchanged. Opposite processes took place during the incubation: an increase in potassium concentration by 9–10 mg/L and a decrease in calcium (by 1.8–2 mg/L) and magnesium (by 2 mg/L). Iron concentration in the medium decreased to zero in the presence of kaolinite 1 (K_1) and increased by 3.4 mg/L in the presence of kaolinite 2 (K_2). Phosphorus concentration in the medium with kaolinite did not change significantly throughout the experiment.

Interaction of bacteria and the medium was ascertained as the differences in the concentrations of the elements in inoculated and sterile medium without minerals (LB–L), while the interaction between bacteria and the mineral–medium system, as the differences in the concentrations of the elements in inoculated and sterile medium with the minerals (LMB–LM). Since the effect of bacteria was associated with bacterial growth, it was observed only at the end of incubation. The changes observed at the beginning of the experiment resulted from the extramicellar interaction between kaolinites and the medium.

In the course of growth (both in the presence and in the absence of kaolinites), bacteria consumed phosphorus, potassium, as well as small amounts of calcium and iron (Table 2). The concentrations of dissolved nickel, manganese, zinc, copper, cobalt, and molybdenum remained practically unchanged in all experimental variants or the changes were below the sensitivity level of the analytical procedures.

Importantly, the alteration of the concentrations of some ions in inoculated media with and without the minerals were different (Table 2). For example, the decrease in the concentrations of potassium, magnesium, and calcium in the presence of kaolinites (MLB–ML) significantly exceeded the consumption of these elements during growth in kaolinite-free medium (LB–L). This additional, not growth-associated decrease in the concentrations [(MLB–ML)–(LB–L)] for potassium, magnesium, and calcium was ~8, 6–7, and ~3 mg/L, respectively. Since the biomass increment was almost the same in the presence and absence of kaolinites, some of the potassium, magnesium, and calcium ions removed from the medium evidently increased the exchangeable cation pool of the minerals. Apart from this, a certain increase in Mg^{2+} consumption was probably associated with the synthesis of additional bacteriochlorophyll *a* in the presence of kaolinites (Table 1).

Alterations in the exchangeable cation pool of kaolinites. The content of exchangeable bases (sodium, potassium, magnesium, and calcium ions) was determined in all mineral samples incubated under experimental conditions. Significant changes in

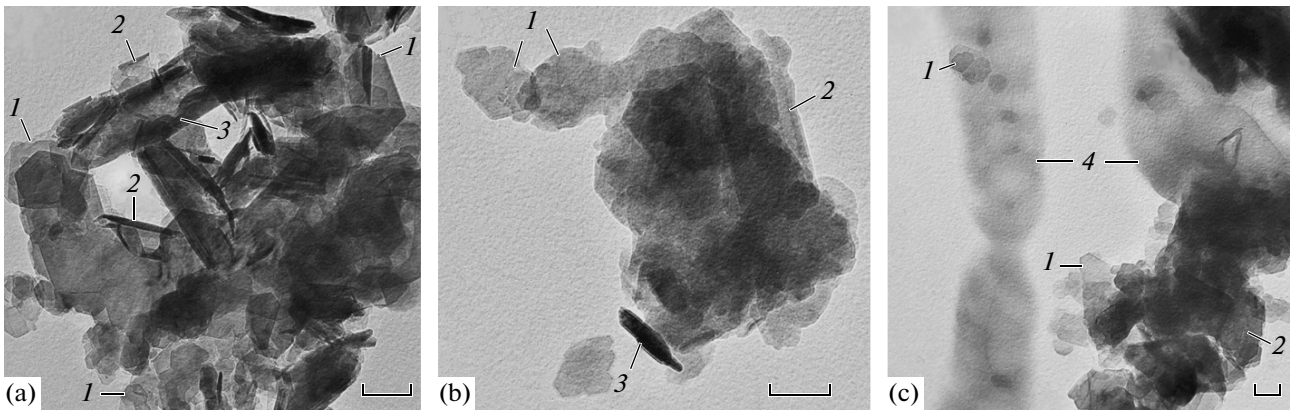

Fig. 3. Kaolinite 1 before (a) and after (b), (c) incubation in inoculated medium. Electron microscopy. Scale bar, 0.2 μm. Lamellar kaolinite (*1*), deformed kaolinite (*2*), halloysite (*3*), *Rhodopseudomonas* sp. UZ-25p cells (*4*).

the composition of exchangeable cations were found to occur during the experiment.

The total content of exchangeable bases changed (Table 3); with this change more pronounced in the presence of bacteria. A certain increase in the content of exchangeable bases (~2 mg-eq/100 g) due mainly to calcium was observed in kaolinites incubated with water. Small amounts of cations were present in the water at the onset of the experiment (Table 2). The presence of poorly soluble calcium compounds in the minerals, which were gradually solubilized during long-term incubation, providing the cations for ion exchange, can not be ruled out. A more pronounced increase in the exchangeable bases pool was detected for kaolinites incubated in sterile medium. This increase partially occurred (1.2–1.4 mg-eq/100 g) immediately after the onset of the experiment due to immediate sorption of the cations by the outer layers of the crystalline lattice, and partially (2.2–3 mg-eq/100 g) by the end of incubation due to the slower process of intramicellar adsorption. The total increase of the exchangeable bases pool for the minerals incubated in sterile medium was 4.4–5.0 mg-eq/100 g. The increase caused by the medium proper (i.e., excluding the effect of water) for K_1 and K_2 was 2.2 and 3.0 mg-eq/100 g, respectively. Incubation of kaolinites in inoculated media resulted in a still more significant increase in the total content of exchangeable bases. The short-term extramicellar sorption was 1.2–1.4 mg-eq/L, similar to the sterile medium, and was not associated with the presence of bacteria. However, subsequent incubation resulted in the exchangeable cation content increasing by 5.7–7.3 mg-eq/100 g. Thus, the increase in the exchangeable bases pool caused by the presence of bacteria (i.e., excluding the effect of the medium) was 2.7 and 3.7 mg-eq/100 g for K_1 and K_2, respectively.

The ratio of exchangeable bases also changed during the experiment (Table 3). Incubation of kaolinites with water resulted in a marked increase of exchangeable calcium (almost twofold, by 1.6–1.7 mg-eq/100 g), while the content of other cations did not change significantly. In the presence of the medium, rapid extramicellar replacement of calcium by sodium and potassium occurred at the onset of the experiment, while the level of exchangeable magnesium remained almost unchanged. Further incubation of kaolinites with the medium resulted in an increase of exchangeable calcium, which was, however, not as significant as in the mineral + water variant. The content of sodium, calcium, and magnesium increased, while the share of potassium decreased somewhat. The effects of bacteria and of the medium were not always unidirectional. For example, bacteria suppressed the process of potassium substitution by sodium, which occurred in the presence of the medium. On the other hand, bacteria, similar to the medium, stimulated the saturation of the minerals by the Ca^{2+} and Mg^{2+} exchangeable cations. In the presence of bacteria, the content of these cations increased by 5–6 mg-eq/100 g, which was two to four times greater than the increase observed in the presence of the sterile medium.

Alterations in crystal morphology and mineralogical characteristics of the minerals. Crystal morphology and the mineralogical parameters of kaolinite samples were investigated by differential thermal analysis, electron diffraction analysis, and transmission electron microscopy.

DTA records the temperature-induced alterations in the properties of materials compared to the etalon, which do not change within a given temperature range. The equipment for synchronous thermal analysis used in the present work, combining the methods of differential scanning calorimetry (DSC, heat measurement) and differential thermal gravimetry (DTG, mass measurement), made it possible to obtain a number of quantitative thermal characteristics, to calculate the content of kaolinite and admixture minerals, and to monitor the changes in crystal morphology.

Table 2. Content of the chemical elements in solutions, mg/L

Element	Experimental variant	Value	MW*	ML	MLB	L	LB	Effect of kaolinite on the medium (ML–L)	Effect of bacteria on the medium without kaolinite (LB–L)	Effect of bacteria on the medium with kaolinite (MLB–ML)	Effect of bacteria on the medium with kaolinite not associated with biomass increment (MLB–ML)–(LB–L)
P	Without kaolinite	Initial				58.6	58.7		**+0.1**		
		Final				58.6	41.4		**–17.1**		
	Kaolinite 1	Initial	0.1	58.5	58.6			**–0.1**		**+0.1**	**0.0**
		Final	0.1	58.5	41.7			**–0.1**		**–16.8**	**+0.3**
		Change	0.0	0.0	–16.9			**0.0**		**–16.9**	**+0.2**
	Kaolinite 2	Initial	0.1	58.6	58.7			**0.0**		**+0.1**	**0.0**
		Final	0.1	58.4	41.1			**–0.2**		**–17.3**	**–0.2**
		Change	0.0	–0.2	–17.6			**0.0**		**–17.6**	**–0.5**
K	Without kaolinite	Initial				291.6	291.7		**+0.1**		
		Final				291.5	279.8		**–11.7**		
	Kaolinite 1	Initial	1.8	273.9	273.9			**–17.7**		**0.0**	**–0.1**
		Final	1.1	282.5	263.4			**–9.0**		**–19.1**	**–7.4**
		Change	–0.7	+8.6	–10.5			**+8.7**		**–19.1**	**–7.4**
	Kaolinite 2	Initial	2.8	275.9	276.1			**–15.7**		**+0.2**	**+0.1**
		Final	1.5	285.3	265.1			**–6.2**		**–20.2**	**–8.5**
		Change	–1.3	+9.4	–11.0			**+9.5**		**–20.4**	**–8.7**
Mg	Without kaolinite	Initial				47.6	47.6		**0.0**		
		Final				47.7	39.4		**–8.3**		
	Kaolinite 1	Initial	1.0	47.6	47.9			**0.0**		**+0.3**	**+0.3**
		Final	0.4	45.4	31.4			**–2.3**		**–14.0**	**–5.7**
		Change	–0.6	–2.2	–16.5			**–2.1**		**–14.3**	**–6.0**
	Kaolinite 2	Initial	1.2	47.5	47.8			**–0.1**		**+0.3**	**+0.3**
		Final	0.7	45.2	30.1			**–2.5**		**–15.1**	**–6.8**
		Change	–0.5	–2.3	–17.7			**–2.2**		**–15.4**	**–7.1**
Ca	Without kaolinite	Initial				9.4	9.4		**0.0**		
		Final				9.4	9.3		**–0.1**		
	Kaolinite 1	Initial	2.6	11.8	11.9			**+2.4**		**+0.1**	**+0.1**
		Final	0.2	10.0	7.3			**+0.6**		**–2.7**	**–2.6**
		Change	–2.4	–1.8	–4.6			**–1.8**		**–2.8**	**–2.7**
	Kaolinite 2	Initial	2.8	12.2	12.3			**+2.8**		**+0.1**	**+0.1**
		Final	0.4	10.2	7.1			**+0.8**		**–3.1**	**–3.0**
		Change	–2.4	–2.0	–5.2			**–2.0**		**–3.2**	**–3.1**
Fe	Without kaolinite	Initial				0.5	0.5		**0.0**		
		Final				0.5	0.4		**–0.1**		
	Kaolinite 1	Initial	0.0	0.3	0.3			**–0.2**		**0.0**	**0.0**
		Final	0.0	0.0	0.0			**–0.5**		**0.0**	**+0.1**
		Change	0.0	–0.3	–0.3			**–0.3**		**0.0**	**+0.1**
	Kaolinite 2	Initial	0.0	0.3	0.3			**–0.2**		**0.0**	**0.0**
		Final	0.1	3.7	0.0			**+3.2**		**–3.7**	**–3.6**
		Change	+0.1	+3.4	–0.3			**+3.4**		**–3.7**	**–3.6**

Note: M, W, L, and B stand for mineral, water, medium, and bacteria, respectively.

Table 3. Exchangeable cations (mg-eq/100 g^{-1}) in kaolinite samples

Cations	Kaolinite	Value	MW	ML	MLB	Effect of		
						medium (ML–MW)	bacteria (MLB–ML)	medium and bacteria (MLB–MW)
Na	Kaolinite 1	Initial	0.2	1.1	1.1	**+0.9**	**+0.0**	**+0.9**
		Final	0.4	2.4	1.9	**+2.0**	**–0.5**	**+1.5**
		Change	+0.2	+1.3	+0.8			
	Kaolinite 2	Initial	0.0	1.0	0.9	**+1.0**	**–0.1**	**+0.9**
		Final	0.2	2.2	1.5	**+2.0**	**–0.7**	**+1.3**
		Change	+0.2	+1.2	+0.6			
K	Kaolinite 1	Initial	0.1	2.1	2.2	**+2.0**	**+0.1**	**+2.1**
		Final	0.1	1.2	1.8	**+1.1**	**+0.6**	**+1.7**
		Change	0.0	–0.9	–0.4			
	Kaolinite 2	Initial	0.2	2.1	2.1	**+1.9**	**+0.0**	**+1.9**
		Final	0.2	1.1	1.9	**+0.9**	**+0.8**	**+1.7**
		Change	0.0	–1.0	–0.2			
Ca	Kaolinite 1	Initial	2.0	0.4	0.4	**–1.6**	**0**	**–1.6**
		Final	3.7	2.6	4.0	**–1.1**	**+1.4**	**+0.3**
		Change	+1.7	+2.2	+3.6			
	Kaolinite 2	Initial	1.8	0.3	0.3	**–1.5**	**0**	**–1.5**
		Final	3.4	2.5	4.3	**–0.9**	**+1.8**	**+0.9**
		Change	+1.6	+2.2	+4.0			
Mg	Kaolinite 1	Initial	1.1	1.0	1.1	**–0.1**	**+0.1**	**0**
		Final	1.2	1.6	2.8	**+0.4**	**+1.2**	**+1.6**
		Change	+0.1	+0.6	+1.7			
	Kaolinite 2	Initial	0.9	0.9	1.0	**0**	**0**	**0.1**
		Final	1.1	1.4	3.9	**+0.3**	**+1.5**	**+1.8**
		Change	+0.2	+0.5	+1.9			
Total	Kaolinite 1	Initial	3.4	4.6	4.8	**+1.2**	**+0.2**	**+1.4**
		Final	5.6	7.8	10.5	**+2.2**	**+2.7**	**+4.9**
		Change	+2.2	+3.2	+5.7			
	Kaolinite 2	Initial	2.9	4.3	4.3	**+1.4**	**0.0**	**+1.4**
		Final	4.9	7.9	11.6	**+3.0**	**+3.7**	**+6.7**
		Change	+2.0	+3.6	+7.3			

Note: The designations are as in Table 2.

DTA revealed that kaolinite samples differed in their initial composition and therefore behaved differently during the experiment (Table 4, Fig. 1). The original K_1 sample had high kaolinite content (94%), a small admixture of hydromica (6%), and close-to-perfect structure of the crystal lattice, as was evident from the 1.38 value of the asymmetry index of the main endoeffect on the DSC curve (the index for perfect kaolinite is 1.2–1.3). According to DTG, the K_2 sample contained, apart from kaolinite (65%), 5% hydromica and 30% of other admixtures. The high asymmetry index (2.0) of the main endoeffect on the DSC curve also indicated its heterogeneity.

During the incubation of K_1 in sterile medium, the hydromica content decreased somewhat (by 1.2%), while no new products were detected. In the presence of bacteria, the changes were much more pronounced (Table 4, Fig. 1). The contents of kaolinite and hydromica decreased by 1.7 and 1.9%, respectively, compared to the mineral + medium variant. Gibbsite (1.2%) (an additional endoeffect at 337°C on the DSC curve) and an organo-mineral phase (2.4%, an addi-

Table 4. Differential thermal analysis of kaolinites

	Kaolinite 1			Kaolinite 2	
	Initial	ML	MLB	Initial	MLB
Kaolinite, %	94.0	95.2	93.5	65.0	58.4
Hydromica, %	6.0	4.8	2.9	5.0	5.0
Admixtures, %	0.0	0.0	0.0	30.0	33.1
Gibbsite, %	0.0	0.0	1.2	0.0	3.5
Organo-mineral phase, %	0.0	0.0	2.4	0.0	0.0
Adsorption water, %	14.5	13.8	14.7	7.3	10.3
Temperature of the main/additional endoeffects, °C	551.2/105	551.2/105	549.7/105; 337	525.5/105	530.9/105; 310
Asymmetry index of the main endoeffect	1.38	1.38	1.66	2.0	1.8
Temperature of the main/additional exoeffects, °C	1008.2	1009.2	1005.5/787.5	1001.0	1001.4

Note: The designations are as in Table 2.

tional endoeffect at 787.5°C) were detected. A decrease (by 2°C) of the temperature of the main endoeffect on the DSC curve indicated a decrease in the average size of the mineral particles, i.e., their partial degradation. An increase of the asymmetry index by 0.3 suggested a certain general deterioration of the structure of the sample, resulting probably from the preferential dissolution of more perfect kaolinite crystals and an increased share of the particles with less perfect structure.

Unfortunately, the DTA data are not available for the sample K_2 incubated in the sterile medium. The sample incubated in the presence of bacteria showed, however, significant changes (Table 4). After 3 months of incubation, kaolinite content decreased by 6.6%, while the share of admixtures increased by 3.1% and a significant amount of gibbsite appeared (3.5%). The high content of admixtures made it impossible to use the DSC data to assess the degree of perfection of the structure of kaolinite particles in the K_2 sample.

Bacterial degradation of kaolinite was observed in both samples. Its calculated decrement correlated with the amount of gibbsite (aluminum hydroxide) produced. According to the chemical formula, degraded kaolinite of the samples K_1 (1.7%) and K_2 (6.6%) contained approximately 0.7 and 2.6% of aluminum oxide, while gibbsite (1.2% from K_1 and 3.5% from K_2) contained approximately 0.8 and 2.4% Al_2O_3, respectively. These values coincide within the limits of accuracy of the calculation. This result implies that practically all Al_2O_3 formed in the course of kaolinite degradation was converted to gibbsite. This was confirmed by the fact that gibbsite formation did not correlate with the degradation of hydromica. For example, in K_1 incubated in sterile medium, hydromica degradation was not accompanied by gibbsite formation, while in K_2 gibbsite formation was not associated with hydromica degradation (Table 4). Moreover, gibbsite can not be formed directly from hydromica, since clay minerals or transitional forms of more complex composition are the products of hydromica leaching.

The DTA data correlated with the results of chemical analysis presented above and indicating an increased exchangeable cation pool in kaolinites after their interaction with bacteria, since biodegradation of mineral particles resulted in their increased specific surface and therefore to higher cation exchange capacity.

Electron diffraction investigation also confirmed partial modification of kaolinites upon their interaction with bacteria. The method is based on electron diffraction and reveals the atomic structure of a substance. An electron diffraction pattern produced by the electrons scattered by an object is a set of spots; their mutual position and density provides information concerning the structure of the object. Three crystallographic indices, *h, k*, and *l*, characterize the position of facets and atomic planes. Electron diffraction analysis of oblique textures was used in the present work. On the patterns obtained by this method, reflexes with the same *h* and *k* indices and different *l* indices formed ellipses, making it possible to separately analyze the reflexes bearing different information concerning the structural characteristics of the minerals [11]. Unfortunately, printing of the electron diffraction patterns results in the picture quality (its clearness and the nuances of the picture) significantly inferior to computer-generated images. Fig. 2 presents two most demonstrable diffraction patterns of the initial K_1 sample and of the same sample incubated in the medium with bacteria.

According to the results of electron diffraction analysis, kaolinite samples contained two phases differing in the degree of perfection of the crystalline structure and in particle morphology [12]. As can be seen from the relatively short reflex arcs of the first ellipse (02*l*, 11*l*), the predominant ordered phase consisted of lamellar particles (Fig. 2a). The fuzziness of these reflexes resulted from the defects of stacking of the layers [12, 13]. The second phase, with low-

ordered structure, was visible as a background of diffuse scattering on the first ellipse; its distribution was close to annular (Fig. 2a). The sample K_2 had a higher content of the ordered lamellar phase and a more perfect crystal structure than K_1. Apart from kaolinite phases, the samples contained small amounts of the finely disperse micaceous phase.

After incubation of the minerals in sterile medium, kaolinite reflexes on the diffraction patterns remained almost unchanged. However, the reflex of the micaceous phase disappeared from the pattern of K_1, probably due to its partial dissolution confirmed by the DTA data (Table 4).

The electron diffraction patterns changed significantly after incubation of kaolinites in the medium with bacteria (Fig. 2b). Reflexes of the ordered lamellar phase became more pronounced (02*l* and 11*l*) and the background level decreased, so that the annular band of diffuse scattering corresponding to the low-ordered phase became clearer. Since reflexes of the lamellar kaolinite on the first ellipse (02*l*, 11*l*; Fig. 2) were especially sensitive to defects of stacking of the layers, their increased contrast and clearness were an unequivocal indication of a decreased number of such defects (dislocations and interlayer space failures) and of an increased degree of perfection of lamellar kaolinite. The decreased background level indicated was also an indication of the partial dissolution of amorphous admixtures of the sample. Thus, improved quality of the electron diffraction patterns after incubation in the medium with bacteria was explained primarily by improved structure of the lamellar kaolinite and, to a lesser degree, by the dissolution of the amorphous phase.

Transmission electron microscopy (Fig. 3) showed that the samples consisted mainly of the lamellar and deformed kaolinite. The K_1 sample also contained the particles of halloysite (a mineral of the kaolinite group with tubular particle morphology containing interlayer water and possessing a less ordered structure). In the initial samples, the lamellar kaolinite was mainly represented by particles of clearly hexagonal shape, 0.01–0.5 μm (Fig. 3a). The particles of deformed kaolinite exhibited bends of different degrees, marginal and/or longitudinal; thin layers were separated from some particles. The presence of the bends, which were visible as darker areas, was confirmed by electron diffraction analysis: the patterns obtained from deformed particles under an electron microscope contained, apart from the reflexes peculiar for the lamellar kaolinite, the specific basal reflexes 00*l*. Halloysite was mostly present as partially degraded or longitudinally split tubes. Under vacuum in an electron microscope, halloysite becomes anhydrous, and its shrinkage and sometimes splitting and turning of the crystalline grains occur. Transmission electron microscopy revealed both the tubular (Fig. 3b) elongated particles and uncoiled (Fig. 3a) halloysite particles. Halloysite was also identified by electron diffraction analysis (data not shown). The results of microscopy correlated with the results of electron diffraction analysis, which revealed the presence of two kaolinite phases. The structurally ordered phase was represented by lamellar kaolinite, while the low-ordered consisted of deformed kaolinite and halloysite.

By the end of the experiment, the morphological characteristics of the samples incubated in the inoculated medium changed significantly (Figs. 3b, 3c). Lamellar kaolinite particles of clearly hexagonal shape became less common. They became mostly of irregular shape with smoothed edges. The surface of the particles of deformed kaolinite also became smoother; removable thin layers typical for initial sample were almost never found. The ends of the tubes and of the split tubes of halloysite, which exhibited sharp projections in the original sample, obtained smooth faces. Thus, the effects of biodegradation were observed at peripheral parts of the crystals: along the edges of the lamellar kaolinite particles, on the thin layers removing from the particles of deformed kaolinite, and at the ends of halloysite particles.

DISCUSSION

In multicomponent systems (experimental variants), all components (minerals, water, medium, and bacteria) interacted with each other (Tables 1–4). These interactions resulted in the changes in the chemical composition of the minerals and solutions, the parameters of bacterial growth, and in the mineralogy and crystal morphology of the kaolinites. Bacteria removed some elements from the medium, either using them during growth or promoting their transfer to the exchangeable cation pool of the minerals. The minerals probably had a positive effect on bacterial growth, since while the biomass increment was the same with and without the minerals, the amount of bacteriochlorophyll *a* was 1.5 times higher in the presence of kaolinites. The medium and bacteria facilitated the increase in the pool of exchangeable bases in kaolinites. While the instantaneous extramicellar sorption of cations from the medium did not depend on the presence of bacteria, the subsequent development of bacteria resulted in the bases saturation of the minerals and in their increased adsorbing capacity. By the end of the experiment, the content of exchangeable bases in kaolinites incubated with bacteria was 1.4–1.5 times higher than in those incubated in sterile medium.

We have previously obtained similar results investigating the interaction of haloalkaliphilic purple bacteria *Rhodovulum steppense* A-20s^T with volcanic ash [1] and a number of lamellar aluminosilicates [2]. Similar to our experiment, the cation exchange capacity and saturation of the minerals with bases were higher in the presence of bacteria, although the rates of these processes and the cation ratios were somewhat different

due to the differences in the structure of the minerals and in the cultivation conditions. Haloalkaliphilic bacteria had generally a more pronounced effect on the chemical composition of minerals, probably due to higher pH and salinity values in their habitats.

The methods used in our previous works were insufficient for elucidation of the reasons for enhanced adsorbing capacity of the minerals in the presence of bacteria. In the present work, the application of DTA, electron diffraction analysis, and electron microscopy enabled to reveal that bacterially mediated increase in the cation exchange capacity of kaolinites resulted from significant changes in their crystal morphology. The results obtained by all the methods used confirmed a decrease in size and partial dissolution of the kaolinite particles upon their interaction with bacteria and indicated increased orderliness of the crystalline structure of the lamellar kaolinite due to decrease number of defects of the stacking of the layers of the crystal lattice. The edges of the crystals were the first to undergo biodegradation. This was the cause for increased cation exchange capacity of kaolinites. Bacterially induced processes of degradation resulted in an increase in the specific surface of the mineral particles and therefore in an increase in the capacity for extramicellar sorption. This type of cation exchange is the only one possible for the perfect kaolinite, since its packets are close to each other, not allowing the cations to penetrate into the interpacket space. Real mineral samples contain admixtures and defective particles, which provide for a certain increase in the exchange pool due to the intramicellar sorption. This effect was observed by the end of the experiments for kaolinites incubated in sterile medium. The increase in cation exchange capacity of kaolinites in the presence of bacteria (apart from that caused by the medium) was associated mainly with biodegradation of the minerals, resulting in increased capacity for extramicellar exchange.

Biodegradation affected not only the crystal morphology, but also the mineralogical composition of the samples. After incubation with bacteria, both samples exhibited a decrease in kaolinite content and formation of gibbsite. Moreover, in the K_1 sample partial decomposition of the micaceous component and formation of an organo–mineral complex were observed. We have previously detected formation of organo–mineral complexes only during the interaction between bacteria and one of the volcanic ash samples [1].

Obtained data concerning bacterially mediated alterations in the structure and composition of the minerals are of both theoretical and applied importance. We have previously discussed the processes of saturation of the minerals with exchangeable bases and formation of organo–mineral complexes as initial stages of the mineral diagenesis and soil formation [1, 2]. Degradation of aluminosilicates with formation of gibbsite (aluminum hydroxide) is of special interest due to its role in aluminum production. Due to the limited store and growing prices of bauxites, the major aluminum ore, new techniques for processing of non-bauxite aluminum ores, including kaolinites (the major concentrator of aluminum in exogenous processes) are under development. The biotechnological approach is among the most promising ones. Silica removal is its main objective. Enrichment of aluminum ores by various microorganisms (bacteria, fungi, and algae) has been studied in a number of works and has already been practically applied [14–17]. Acidolysis and complexation are considered the major pathways for aluminosilicate biodegradation [14, 16, 17]. Anoxygenic phototrophic bacteria have not been previously investigated in this respect. The present work, however, showed their ability to increase aluminum content in kaolins. Importantly, gibbsite formation was observed only in the presence of bacteria. It was the result of degradation of kaolin, but not of admixture minerals, and occurred at neutral pH and at room temperature. In our experiment, gibbsite yield did not exceed several per cent. However, the diversity of the physiological capacities of anoxygenic phototrophic bacteria, including their ability to grow in a broad range of conditions, makes them a promising group for the search for strains efficiently enriching the non-bauxite aluminum ores.

Thus, the results of the present work showed that the interaction between kaolinite and anoxygenic phototrophic bacteria *Rhodopseudomonas* sp. UZ-25p resulted in significant modifications in the chemistry, crystal morphology, and mineralogy of kaolinite minerals. Some of these modifications may be of both theoretical and applied interest.

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