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EXPERIMENTAL ARTICLES

Interaction between Anoxygenic Phototrophic Bacteria of the Genus *Rhodovulum* and Volcanic Ash

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Abstract—Volcanic ash (Karymskii Volcano, Kamchatka) stimulated growth of the bacterium *Rhodovulum* sp. A-20s. The interaction between ash, water, media, and bacteria resulted in changes in the chemical composition of the solutions and ash. The ash–water interaction resulted in release of calcium to the solution, as well as in an increase in the proportions of sodium and calcium among the exchange cations of ash. As a result of the ash–medium interaction, calcium and copper were released to the solution; the exchange sodium cations were substituted by calcium and potassium. As a result of the ash–bacteria interaction, the content of copper in the solution decreased, and the exchange cations of calcium and sodium were actively substituted by potassium and magnesium. An increase in the magnesium content among the exchange cations of ash was especially apparent. The products of bacterial metabolism formed mineral–organic complexes with the ash substrate. The data obtained indicate the biogenic transformation of ash, which may lead to the initial phase of formation of clay minerals from volcanic ashes.

Key words: volcanic ash, anoxygenic phototrophic bacteria, haloalkaliphiles, anaerobic conditions, exchange cations.

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Microbial colonization of igneous rocks is one of the strongest weathering factors [1]. The biogenic effect results in the geochemical evolution of vulcanite, as well as in the ore and soil formation (accumulation of organic matter and synthesis of clay minerals). Bacteria are ubiquitous and have been found in rocks formed at the earliest stages of the planetary evolution, 3.5–3.34 billion years ago [2]. However, in spite of the importance and scale of the bioweathering-related problems, we have as yet no clear understanding both of the mechanisms and conditions of biomineral reactions and of the involvement of bacteria in the global transformation of minerals in the course of diagenesis.

Most studies on bioweathering address aerobic processes. It was shown that, under aerobic conditions, bacterial weathering is quite intense. For instance, in the presence of aerobic bacteria, the leaching of various silicon-containing rocks intensified by an order of magnitude [3, 4]. The following mechanisms of bioweathering are presently known: dissolution of minerals affected by organic acids, sugars, or sulfuric acid [1, 5, 6]; concentration and crystallization of minerals on bacterial surfaces [7]; active cation extraction by the cell glycocalyx and siderophores [8, 9]; and local pH changes.

Experimental data on bioweathering in anaerobic biotopes are rather scarce [10, 11]. At the same time, anaerobic transformation of rocks is a widespread natural process typical of deeper soil horizons, oceanic sediments, subsurface waters, and deeper crust layers, as well as of some terrestrial ecosystems. Moreover, presently accepted reconstructions indicate reductive properties of the Archaean and Early Proterozoic atmosphere (ca. 2.4 billion years ago), when bacteria already existed [12]. Thus, 1/3 of the time taken by the evolution of the surface of the Earth's lithosphere most probably proceeded under anaerobic conditions and with the active involvement of bacteria.

The purpose of this work was to study the interactions between bacteria and volcanic ash under anaerobic conditions. The haloalkaliphilic purple nonsulfur bacterium *Rhodovulum* sp. A-20s was used as a model subject. According to modern theories, the inhabitants of alkaline environments were probably among the first living organisms on Earth [6]. In addition, lability of the metabolism of purple nonsulfur bacteria makes these organisms promising models for studies of the specific traits of biogeochemical processes occurring under various ambient conditions.

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Table 1. Bulk element composition of volcanic ashes

Macroelements, %			Trace elements, μg/kg		
	Specimen 1	Specimen 2		Specimen 1	Specimen 2
MgO	0.787	0.878	Ni	9	434
Al_2O_3	15.355	15.467	Cu	38	95
SiO_2	59.950	63.133	Zn	81	102
P_2O_5	0.052	0.059	Ga	23	20
SO_3	0.027	0.036	As	0	0
Cl	0.034	0.051	Br	0	0
K_2O	1.431	1.527	Pb	14	5
CaO	6.315	6.412	Rb	26	26
TiO_2	0.851	0.960	Sr	432	415
Cr_2O_3	0.005	0.103	Y	30	31
MnO_2	0.125	0.136	Zr	165	176
Fe_2O_3	6.531	7.665	Nb	0	6

MATERIALS AND METHODS

The main object of microbiological investigations was the haloalkaliphilic purple nonsulfur bacterium *Rhodovulum* sp. A-20s isolated from the saline soda Lake Khilganta (Transbaikal Region, Russia) [13]. The interactions between the strain *Rhodovulum* sp. A-20s and volcanic ash (Karymskii Volcano, Kamchatka) were studied. We used two ash samples (specimens 1 and 2) with different total elemental compositions (Table 1). The bacteria were cultivated under anaerobic conditions in the light with an organic electron donor and acceptor in the presence or absence of ash. Bacteria-free ash incubated with water or medium was used as a control.

Two series of experiments were conducted. In the first experiment, our main goal was to determine the effect of ash on bacterial growth. For this purpose, *Rhodovulum* sp. A-20s was grown in a standard medium (see below) supplemented with different amounts of ash (0.5 and 1.5 g per 60-ml vial). Specimen 1 of volcanic ash was used in this experiment. The duration of the experiment was 1 month. At the onset of the experiment, as well as after 2 and 4 weeks of incubation, the biomass yield was assessed; X-ray diffraction analysis and microscopic investigations were carried out.

In the second experiment, bacteria were grown in the presence of equivalent amounts (1.5 g per vial) of ash (Specimen 2), but in different media: a poor (P-, K-, Mg-, and S-free medium without microelements) and a standard one. The aim of this experiment was to elucidate whether mineral-rich volcanic ash is able to compensate for the deficiency in biogenic elements and trace elements in the media, as well as whether bacteria are capable of extracting the missing elements from the ash substrate. Medium 1 (poor) contained the following compounds (g/l): NH_4Cl, 0.33; NaCl, 10.0; and $NaHCO_3$, 5.0. Medium 2 (standard) contained the following compounds (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; $NaHCO_3$, 5.0; NaCl, 10.0; and trace element solution, 1 ml/l. The trace element solution contained the following (g/l): EDTA, 5.0; $FeSO_4 \cdot 7H_2O$, 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; and $Na_2MoO_4 \cdot 2H_2O$, 0.02. In addition to the mineral compounds, both media (pH 7.8) were supplemented with 2.0 g/l of Na acetate and 0.1 g/l of yeast extract. The duration of this experiment was 2 months. At the onset of the experiment, as well as after 2, 4, and 8 weeks of incubation, the biomass yield was assessed; X-ray diffraction analysis and microscopic investigations were carried out; the chemical composition of the liquid phase and the content of exchange cations in the ash were determined.

For both experiments, ash (0.5 or 1.5 g per vial) was placed in the vials prior to inoculation, supplemented with a small amount of water, and sterilized at 121°C for 20 min. The cultures and controls were incubated under the same conditions (in 60-ml vial filled to the top and sealed, at 25–30°C, and in the presence of light, under illuminance of about 2000 lx). The contents of the vials were agitated two times a day. In all variants of this experiment, performed in duplicates, the vials were incubated simultaneously.

Since, in the presence of ash, it is not possible to determine the biomass yield from the optical density of the cell suspension, the growth rate of the cultures was determined by the percentage of bacteriochlorophyll *a* which was extracted with acetone. The optical density of the extract was measured with a KFK-3 spectrophotometer at 770 nm. To convert the results obtained into other parameters of biomass increase (optical density of the cell suspension, protein content, and dry weight), the calibration curves were obtained. The protein content was determined by the Lowry method [14].

The cell morphology was examined under an Olympus BX-41 phase-contrast microscope and a CamScan-4 scanning electron microscope.

The mineral composition of ash was determined using a DRON-5 X-ray diffractometer. X-ray diffraction analysis yields a series of reflexes (peaks) in the diffraction pattern. The quantitative parameters of the reflexes designate more or less unambiguously the positions of atoms in the crystal lattice and can serve as unique descriptors of minerals. In this case, the mineral composition of the initial and experimental substrates was assayed from the combinations of the reflexes and changes in their properties.

After sampling, the remaining suspension was centrifuged and used for chemical analyses of water and ash. The methods of chemical analysis were as follows. The total elemental composition of ash specimens was determined by the X-ray fluorescence method. The composition of exchange cations was analyzed by the

Table 2. Growth dynamics of the phototrophic bacterium *Rhodovulum* sp. A-20s in the presence of different amounts of volcanic ash (Specimen 1)

Incubation time	0			2 weeks			4 weeks		
Experimental conditions	pH	Dry biomass, g/l	Protein concentration, mg/l	pH	Dry biomass, g/l	Protein concentration, mg/l	pH	Dry biomass, g/l	Protein concentration, mg/l
Without ash	7.8* ± 0.05	0.01 ± 0.001	7 ± 0.1	8.3 ± 0.05	0.06 ± 0.005	40 ± 1	8.3 ± 0.05	0.17 ± 0.01	113 ± 3
With ash (0.5 g/vial)	7.8 ± 0.05	0.01 ± 0.001	7 ± 0.1	8.4 ± 0.05	0.06 ± 0.005	41 ± 2	8.6 ± 0.05	0.26 ± 0.01	173 ± 3
With ash (1.5 g/vial)	7.8 ± 0.05	0.01 ± 0.001	7 ± 0.1	8.4 ± 0.05	0.10 ± 0.007	67 ± 3	8.6 ± 0.05	0.31 ± 0.01	207 ± 4

* All the results presented are an average of four measurements.

Table 3. Growth dynamics of the phototrophic bacterium *Rhodovulum* sp. A-20s in different media in the presence of volcanic ash (Specimen 2)

Incubation time		0			2 weeks			4 weeks			8 weeks		
Experimental conditions		pH	Dry biomass, g/l	Protein concentration, mg/l	pH	Dry biomass, g/l	Protein concentration, mg/l	pH	Dry biomass, g/l	Protein concentration, mg/l	pH	Dry biomass, g/l	Protein concentration, mg/l
Medium 1	With ash	8.1* ± 0.05	0.01 ± 0.001	7 ± 0.1	9.1 ± 0.05	0.17 ± 0.01	117 ± 2	9.2 ± 0.05	0.19 ± 0.01	124 ± 2	9.1 ± 0.05	0.20 ± 0.01	134 ± 2
	Without ash	8.1 ± 0.05	0.01 ± 0.001	7 ± 0.1	9.1 ± 0.05	0.16 ± 0.01	110 ± 2	9.1 ± 0.05	0.18 ± 0.01	116 ± 1	9.1 ± 0.05	0.16 ± 0.01	108 ± 1
Medium 2	With ash	8.1 ± 0.05	0.01 ± 0.001	7 ± 0.1	9.1 ± 0.05	0.30 ± 0.02	200 ± 4	9.1 ± 0.05	0.31 ± 0.01	203 ± 3	9.1 ± 0.05	0.28 ± 0.01	183 ± 3
	Without ash	8.1 ± 0.05	0.01 ± 0.001	7 ± 0.1	9.1 ± 0.05	0.25 ± 0.01	167 ± 3	9.1 ± 0.05	0.26 ± 0.01	172 ± 2	9.1 ± 0.05	0.23 ± 0.01	152 ± 2

* All the results presented are an average of four measurements.

Pfeffer's method modified by Molodtsov and Ignatova [15]. The concentrations of Fe, Ca, Mg, Mn, Cu, Ni, and Zn were determined by the atomic adsorption method using an AAS-3 spectrometer; the content of P was determined colorimetrically using the Murphy–Riley method with a Specol-221 spectrophotometer [16]; and the concentrations of K and Na were determined with a FLAFO-4 flame photometer [17].

RESULTS AND DISCUSSION

Effects of Ash on Bacterial Growth

Cultivation of *Rhodovulum* sp. A-20s in the presence of different amounts of volcanic ash (specimen 1) revealed a positive effect of ash on the biomass yield (Table 2). At both ash concentrations, bacterial growth was higher than in the controls. The stimulatory effect was more pronounced at higher ash concentrations in the medium.

Cultivation of bacteria in different media yielded similar results (Table 3). The presence of ash (specimen 2) stimulated bacterial growth in both media. The most pronounced effect was observed during growth on the standard medium 2. As was expected, biomass yield in the poor medium 1 was lower; however, growth was still stimulated by ash. Moreover, the composition of the media affected the dynamics of bacterial growth. On medium 1, the growth rate was low; the maximum biomass yield was obtained after 8-week incubation. On medium 2, growth ceased at a much earlier point; by week 8 of incubation, the biomass yield was somewhat lower due to lysis of some cells.

Importantly, although the stimulatory effect of ash on bacterial growth was detected in both experiments, it was more pronounced in the first case, when specimen 1 of volcanic ash was used. This was perhaps due to the differences in the elemental composition of the ash specimens (Table 1), in particular, due to the higher contents of nickel, chrome, and copper in specimen 2.

The cells of *Rhodovulum* sp. A-20s grown in the presence or absence of ash have similar morphologies, typical of this strain, and were represented by straight rods, 0.5–0.7 ± 0.8–1.4 μm. However, bacteria grown in the ash-containing medium were somewhat more

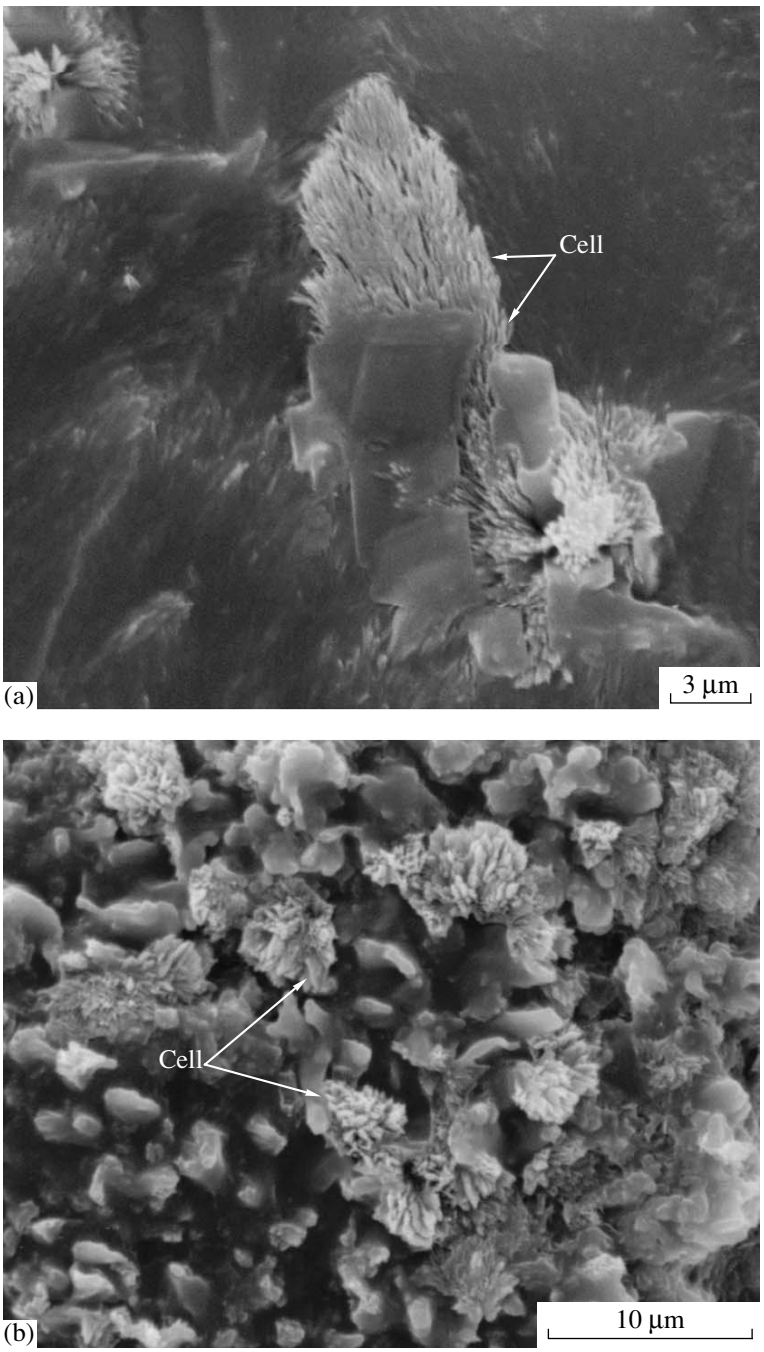

Fig. 1. Microcolonies of the phototrophic bacterium *Rhodovulum* sp. A-20s on ash particles (specimen 1). Scanning microscope. Scale bar, 3 μm (a) and 10 μm (b); Cell, bacterial cells.

elongated. In addition, in the presence of ash, bacterial cells were often found attached to mineral particles, forming dense microcolonies (Fig. 1).

Mineralogical Analysis of Ash

The results of the X-ray diffraction analysis of the two ash specimens revealed the similarity of their composition: a mixture of volcanic glass and basic crystalline plagioclase was detected.

After a 2-week incubation of volcanic ash (1.5 g/60 ml) with bacterial cells, a new reflex (18 Å) was discovered in the X-ray diffraction patterns (Fig. 2b). During the following 2 weeks, this reflex shifted to 15 Å (Fig. 2c). In the experiments with small amounts of ash (0.5 g), a new reflex (20.3 Å; Fig. 2d)

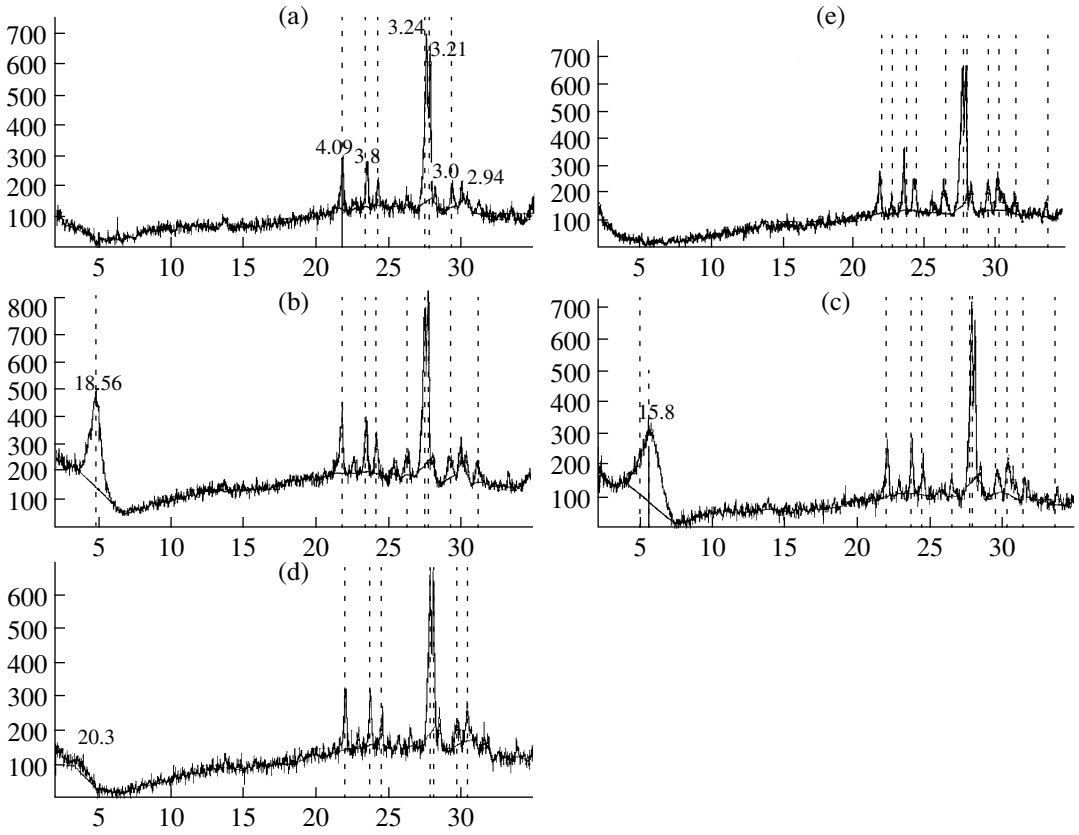

Fig. 2. X-ray diffractograms of the ash from Karymskii Volcano (specimen 1) after incubation under anaerobic conditions in the light with a growing culture of the purple nonsulfur bacterium *Rhodovulum* sp. A-20s. The amounts of ash in 60-ml vials were 0.5 (d) and 1.5 g (b, c, e): dry ash (a); after 2-week incubation (b); after 4-week incubation (c, d); burnt ash after 1-month incubation (e).

was detected only after 1 month of incubation. In the latter case, the reflex was of small amplitude, which may be due to lower rates of bacterial growth in the presence of low amounts of ash and, consequently, to the low content of organic matter involved in the biomineral reactions. After calcination of the specimens, new reflexes completely disappeared (Fig. 2e), which indicates their mineral-organic origin. Soluble elements were removed from the ash samples with distilled water, which ruled out the possibility of registering organic residues unbound to minerals.

The appearance of a new reflex was recorded two times, but only during the experiments with one ash specimen. The bulk chemical composition of specimen 1 differed from that of specimen 2 (Table 1): the concentrations of chrome, nickel, and copper in specimen 1 were 50, 48, and 2.5 times lower, respectively, than those in specimen 2.

Hence, it was established that colonization of volcanic ash by anaerobic bacteria stimulated the production of mineral-organic complexes which remained in the substrate and were not released into the solution. As a consequence, organics accumulated in the mineral phase; this phenomenon may be one of the mechanisms of the initial stage of soil formation.

Changes in the Chemical Compositions of the Solutions and Ash

In the experiments with various media, the rate of bacterial growth was measured and X-ray diffraction and microscopic analyses were performed; in addition, the chemical composition of the liquid phase and the exchange's cation composition of volcanic ash (specimen 2) were analyzed.

The purpose of these experiments was to study the interactions between bacterial cells and volcanic ash under anaerobic conditions. However, since nutrients and liquid media were required for bacterial growth, the contents of each test vial represented a multicomponent system, in which ash interacted both with bacteria and water, as well as with dissolved compounds. To determine the effects of the individual components of this system, various combinations of ash, water, medium, and bacterial cells were used; these combinations ruled out the influence of one or two of the constituents and were used as controls relative to each other. As a result, we determined the changes in the composition of the

Table 4. Concentration dynamics of the solution (mg/l) of chemical elements during the incubation of volcanic ash (specimen 2) in water and culture media, in the presence or absence of the cells of the phototrophic bacterium *Rhodovulum* sp. A-20s

	Duration of the experiment	WA*	M1A	M1AM	M1M	M2A	M2AM	M2M
Phosphorus	0	0.44	1.05	3.71	3.65	93.50	92.30	94.90
	2 weeks	0.46	0.96	1.28	1.02	96.25	86.80	89.50
	4 weeks	0.45	1.05	1.00	0.87	91.88	78.13	70.33
	8 weeks	0.44	1.15	0.89	0.85	90.40	76.21	76.50
Potassium	0	0.05	65.70	88.00	78.50	445.00	445.00	440.00
	2 weeks	0.10	68.00	83.50	76.20	435.00	435.00	435.00
	4 weeks	0.34	67.50	79.90	72.50	435.00	425.00	400.00
	8 weeks	0.36	65.80	31.50	28.00	445.00	430.00	415.00
Magnesium	0	0.47	0.93	3.96	3.27	28.00	30.50	29.40
	2 weeks	0.58	1.06	3.86	1.87	28.27	27.50	24.17
	4 weeks	0.43	1.10	1.83	1.15	27.00	20.30	22.70
	8 weeks	0.42	1.10	1.83	1.14	28.8	23.43	25.80
Calcium	0	1.01	0.98	1.19	0.53	0.84	0.84	0.46
	2 weeks	0.94	0.95	1.21	0.35	0.57	0.56	0.27
	8 weeks	0.95	0.99	0.88	0.35	0.63	0.72	0.38
Iron	0	0.06	0.12	0.31	0.37	0.12	0.32	0.30
	2 weeks	0.07	0.12	0.35	0.22	0.13	0.29	0.37
	8 weeks	0.04	0.11	0.33	0.17	0.08	0.37	0.31
Nickel	0	0.06	0.16	0.10	0.13	0.15	0.14	0.10
	2 weeks	0.02	0.16	0.14	0.13	0.15	0.14	0.12
	8 weeks	0.03	0.18	0.17	0.15	0.15	0.14	0.13
Manganese	0	0.03	0.04	0.04	0.03	0.05	0.04	0.04
	2 weeks	0.01	0.03	0.02	0.00	0.02	0.02	0.00
	8 weeks	0.03	0.06	0.05	0.03	0.06	0.06	0.04
Zinc	0	0.01	0.04	0.16	0.13	0.32	0.22	0.30
	2 weeks	0.01	0.10	0.13	0.11	0.43	0.29	0.22
	8 weeks	0.03	0.14	0.16	0.15	0.39	0.28	0.33
Copper	0	0.01	0.27	0.25	0.04	0.30	0.28	0.05
	2 weeks	0.02	0.44	0.13	0.03	0.37	0.06	0.04
	8 weeks	0.03	0.44	0.13	0.08	0.53	0.16	0.09

* WA, water + ash; MA, medium + ash; MAM, medium + ash + microorganisms; MM, medium + microorganisms; M1, poor medium; M2, standard medium.

solutions and exchange cations associated with each interaction. Tables 4 and 5 show the results of these analyses.

Chemical composition of the solutions. First of all, the data presented in Table 4 revealed two patterns of differences between the concentrations of dissolved elements, not associated with the interactions between ash, media, and microorganisms (Table 4). First, although medium 1 did not contain all the studied elements, their concentrations in the ash + medium 1 variant were higher than in the ash + water variant. It is obvious that it results from the presence of impurities in the constituents of the media. Thus, we failed to obtain a medium completely devoid of some elements. The concentrations of trace elements in medium 1 were relatively high; in the majority of cases, the difference between the media was virtually nonexistent, since the amounts of trace elements added to medium 2 were smaller than those added to the media in the form of impurities contained in its other components. The content of potassium in medium 1 was high enough as well (about 70 mg/l). Another pattern not associated with the interactions between ash, medium, and bacterial cells was a higher content of elements in the ash + medium 1 + bacteria variant than in the ash + medium 1 variant (from the beginning of the experiment). This

difference may be due to the addition of certain amounts of the elements with the inoculum grown in the standard medium 2.

In all other cases, the observed changes in the concentrations were element-specific and resulted from the interactions of the elements with each other, as well as with ash and microorganisms. For instance, the dynamics of changes in the contents of three biogenic elements (phosphorus, potassium, and magnesium) in the solution was similar (Table 4). A decrease in the concentrations of these elements in the vials (with or without ash) inoculated with bacterial cells was observed, while their concentrations in the sterile controls remained constant. These changes are associated mainly with the active absorption and utilization of these elements by bacteria. The other possible mechanisms of the elimination of elements from the solutions may be reversible adsorption and precipitation due to increasing the values of the medium pH [18]. After 8-week incubation in medium 2, the element concentration in the medium increased slightly, most probably due to the cell lysis and release of cell contents, since growth in medium 2 ceased by this time and a slight decrease in the biomass was observed.

For dissolved calcium, a different pattern was observed (Table 4). In all variants of ash-containing media (both sterile and inoculated), the calcium concentration was higher than in ash-free cultures. This means that calcium is easily solubilized irrespective of the presence of bacteria, simply as a result of mineral–water interactions. The effect was more pronounced in water and medium 1 and less pronounced in medium 2, probably due to its high phosphate content.

As to the trace elements, the amounts of most of them (iron, nickel, manganese, and zinc) in the media remained virtually the same throughout the experiment; alternatively, the quantitative assessment methods were probably not sensitive enough to reveal significant dynamics (Table 4). Only the copper concentration varied significantly as a result of two differently directed processes caused by the medium–ash and medium–microorganism interactions, respectively. The content of copper increased in ash-containing sterile media (but not in water) and decreased in the presence of bacteria. This indicates that copper was washed out from ash by both media (but not by water) and removed from the solution by bacteria. In the latter case, the most probable mechanism of the binding of copper ions is reversible adsorption of cations on the cell surface [18].

Composition of the exchange cations of ash. The experiments demonstrated large changes in the amount of exchange cations of ash (Table 5, Fig. 3). The content of exchange sodium in ash decreased during the incubation in both media, whether in the presence or absence of bacteria, and increased, although to a lesser extent, during the incubation in water. The concentration of the exchange calcium cations in ash increased in all experimental variants (both in media and water) after 8-week incubation. However, in the presence of bacteria, this increase was less pronounced. It should be remembered that an increase in the calcium concentration in the solution was observed as well.

Table 5. Contents of the exchange cations (mg-eq/ 100 g ash) in ash (specimen 2)

	Duration of the experiment	WA*	M1A	M1AM	M2A	M2AM
Na	4 weeks	0.16	12.37	13.31	10.00	12.60
	8 weeks	0.59	9.31	9.80	6.65	10.64
K	4 weeks	0.16	0.15	0.35	0.36	0.72
	8 weeks	0.39	0.38	0.32	0.67	0.70
Ca	0	0.12	0.14	0.15	0.14	0.15
	4 weeks	0.17	0.10	0.17	0.10	0.18
	8 weeks	0.35	0.33	0.24	0.25	0.18
Mg	0	0.05	0.01	0.01	0.40	0.51
	4 weeks	0.05	0.03	0.26	0.39	3.00
	8 weeks	0.05	0.05	0.24	0.42	6.21

* See Table 4 for designations.

The contents of the exchange potassium cations in ash (Table 5, Fig. 3a) in the sterile variants (both with water and media) increased approximately twofold during the 2nd month of incubation (unfortunately, the initial content was unknown); however, in the experimental variants with bacteria, it was already high by the end of the 1st month, reaching the saturation level (0.36 and 0.7 mg-eq/100 g ash for medium 1 and 2, respectively), and remained subsequently at the same level. The content of the exchange potassium cations in ash was always two times higher in the variants with medium 2 than in those with medium 1 and water; this may probably be attributed to the higher potassium concentration in medium 2 (440 mg/l) than in medium 1 (70–80 mg/l). Thus, an increase in the proportion of potassium in the pool of exchange cations resulted both from abiogenic processes of ash–water or ash–medium interaction and from the interaction between ash and bacteria; the latter process significantly accelerated the increase.

The content of the exchange magnesium cations (Table 5, Fig. 3b) at the zero point (immediately after the beginning of the experiment) was ten times higher in the ash added to medium 2 (with or without bacteria) than in the variants with medium 1 or water. This increase was abiogenous and occurred immediately after the contact of ash with medium 2 which contained approximately 30 mg/l of magnesium. During the subsequent 2-month incubation, the content of the exchange magnesium cations in the ash increased more than tenfold in both media, but only in the presence of bacteria. This increase in magnesium content among the exchange cations was due to biogenic processes, since the amount of the exchange magnesium cations

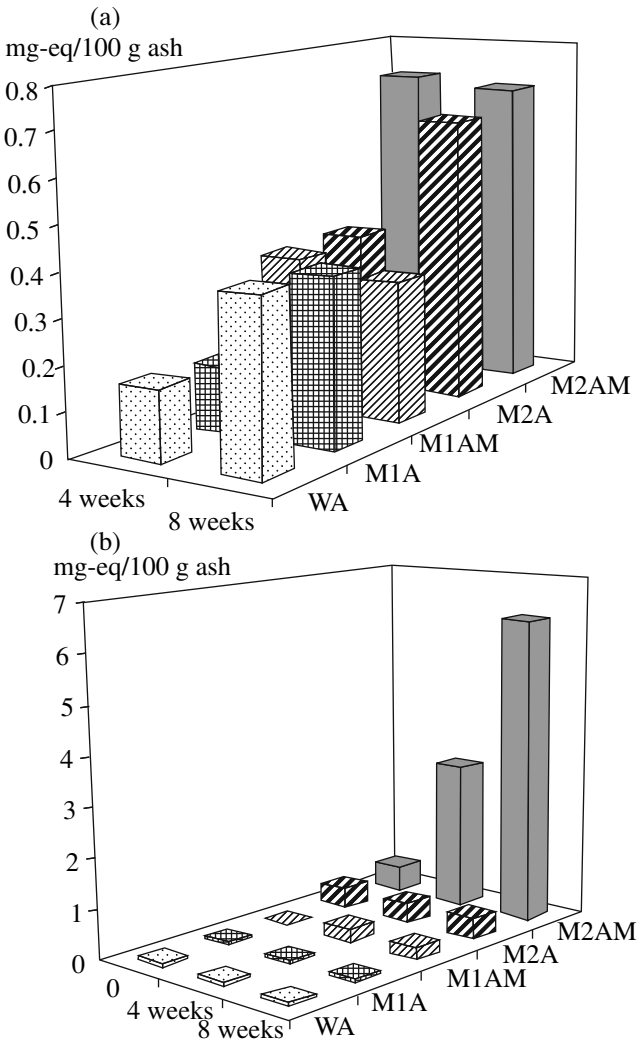

Fig. 3. The amounts of exchange cations of potassium (a) and magnesium (b) (mg-eq/100 g ash) in ash (specimen 2) after its incubation with water, culture media, and bacterial cells. WA, water + ash; M1A, medium 1 + ash; M1AM, medium 1 + ash + microorganisms; M1M, medium 1 + microorganisms; M2A, medium 2 + ash M2AM, medium 2 + ash + microorganisms; M2M, medium 2 + microorganisms.

remained unchanged in the sterile variants. Hence, by the end of the experiment, the content of the exchange magnesium cations in the ash + medium 2 + microorganisms variant was 100 times higher than that in the bacteria-free variants with water and medium 1. These data indicate a pronounced effect of the cells of the anoxygenic phototrophic bacterium *Rhodovulum* sp. A-20s on the chemical properties of ash minerals, resulting in the substitution of magnesium for other exchange cations.

Effect of the medium composition. As regards the differences in the effect of two different media on ash and bacteria, they were mostly quantitative. As has been stated above, we failed to obtain a medium completely devoid of some elements due to the presence of impurities in the reagents. As a consequence, medium 1 (poor) contained high concentrations of trace elements (even higher than in medium 2) and potassium. Medium 1 was only deficient in phosphorus and magnesium. Ash-induced stimulation of cell growth was observed in both media, although, on the whole, the biomass yield was lower in medium 1 than in medium 2 (Table 3), obviously due to phosphorus limitation. Changes in the chemical composition of the liquid phase, as well as in the amount of exchange cations of ash, were similar in both media; however, in the case of medium 2, these changes were more pronounced (Tables 4, 5). For instance, the amounts of the exchange potassium and magnesium cations were two and ten times higher, respectively, by the end of the experiment with bacteria-free medium 2, and 25 times higher in the bacteria-containing medium 2 than in medium 1 (Table 5, Fig. 3). Therefore, in most cases, highly mineralized media may cause more substantial changes in the chemical composition of ash.

Results of the interaction between the components of the systems under study. On the whole, the results obtained revealed active interactions between all components (ash, water, medium, and bacteria) of the studied systems. These interactions caused changes in the chemical composition of the solutions and ash due to the release into and/or removal from the solution of some elements, as well as due to changes in the contents of exchange cations of the ash minerals. Table 6 shows the significant effects of each interaction revealed in the course of the experiment.

The ash–water interaction resulted in the release of calcium to the solution and an increase in the proportion of sodium and calcium exchange cations. As a result of the ash–medium interaction, calcium and copper were released to the solution; the sodium exchange cations were substituted by calcium and potassium. The presence of bacterial cells in the media also affected the composition of the solution and exchange cations. Sometimes, this effect coincided with the effects of the media and enhanced the latter; sometimes it had an adverse effect; and sometimes it manifested itself in the absence of the effect exerted by the media. Similar to the medium, bacteria increased the proportion of potassium in the exchange cations in the same manner as the media; this process was accelerated in their presence. Unlike the media, bacteria reduced the copper content in the solution and calcium contents among the exchange cations. Finally, the content of the exchange magnesium cations in ash increased more than tenfold in the presence of bacteria, whereas in the sterile variants it remained unchanged. It is obvious that, in the presence of bacteria, calcium and sodium cations were actively replaced by potassium and magnesium. In addition to the above-described effects, bacteria reduced the contents of phosphorus, potassium, and magnesium in the solution; however these processes did not depend on the presence of ash in the media, i.e.,

Table 6. Changes in the compositions of the solutions surrounding the ash material, as well as in the content of exchange cations of ash minerals induced by the interactions between ash (specimen 2) and water, culture media, and bacterial cells under anaerobic conditions in the presence of light

Element	Ash–water		Ash–medium		Ash–bacteria	
	Solution	Exchange cations	Solution	Exchange cations	Solution	Exchange cations
Phosphorus	≈	ND	≈	ND	↓*	ND
Sodium	≈	↑	≈	↓	≈	v
Potassium	≈	≈	≈	↑	↓*	↑
Magnesium	≈	≈	≈	≈	↓*	↑
Calcium	↑	↑	↑	↑	≈	↓
Iron	≈	ND	≈	ND	≈	ND
Nickel	≈	ND	≈	ND	≈	ND
Manganese	≈	ND	≈	ND	≈	ND
Zinc	≈	ND	≈	ND	≈	ND
Copper	≈	ND	↑	ND	↓	ND

Designations: ≈, no changes; "↑", increasing concentration; "↓", decreasing concentration; ND, not determined; *, changes occur both in the presence and absence of ash.

of the ash–microorganism interaction. However, the possibility that bacteria derived from ash some elements required for growth cannot be excluded.

The changes in the composition of ash minerals revealed in the course of the experiments indicate possible geochemical consequences of the ash–microorganisms interactions. For instance, under the influence of an alkaline medium, copper was released into the solution from the ash minerals, and from there, it was removed by bacteria, probably as a result of reversible adsorption. The biogenic removal of copper and other heavy metals from the solution and concentration of these metals on the surface of bacterial cells is one of the mechanisms responsible for the formation of ore bodies. In this case, the binding of copper may suggest a possible pathway of the formation of copper-sulfide deposits based on the vulcanite transformation [19]. In addition, the experiments demonstrated significant changes in the amount of exchange cations of ash induced by bacteria. For example, the mineral phase became saturated with magnesium in the presence of bacterial cells. This suggests the possible involvement of anaerobic biota in the formation of magnesium-containing silicates (e.g., chlorites, chlorite-smectites, and vermiculites).

In summary, several principal conclusions can be made from the results of this investigation. Volcanic ash stimulated the growth of the anoxygenic phototrophic bacterium *Rhodovulum* sp. A-20s. The fact that an interaction exists between ash and bacterial cells was confirmed by the results of chemical and X-ray diffraction analyses. The changes in the chemical composition of the liquid phase occurred owing to the release to and/or removal from the solution of some elements, as well as due to changes in the amounts of the exchange cations of ash minerals. Microbial products and the ash substrate formed mineral–organic complexes.

The data obtained indicate the biogenic transformation of ash, which may lead to the initial phase of the formation of clay minerals from volcanic ashes.

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