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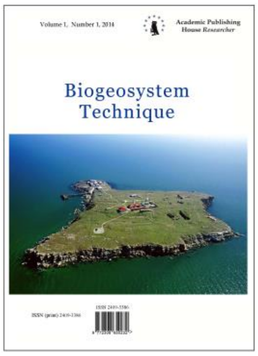

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Microbiological Modification of Kaolinite and Montmorillonite Surface: Changes in Physical and Chemical Parameters (Model Experiment)

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Abstract

The interaction of microorganisms with mineral part of the soil solid phase determines the number of processes, such as weathering of primary minerals, their destruction or transformation due to the production of organic ligands and acids. The article describes the modification of the surface of the clay minerals kaolinite and montmorillonite by the pure culture of *Bacillus circulans*, capable of removing silicon and potassium from the minerals. After two months of culturing the bacteria in a clay medium, where the clay minerals were the source of potassium, the surfaces properties (contact angle, surface area) and microaggregate composition of clays were changed. The composition of the microbial community was reconstructed according to the microbial markers (fatty acids and their derivatives—fatty hydroxyacids and aldehydes) that were determined after the acid methanolysis of the samples using the molecular method of gas chromatography–mass-spectrometry. It is shown that the result of adsorption of bacteria and their metabolites and the formation of tightly bound microbial biofilm on the surface of a solid mineral phase was the clay surface hydrophobization. Sorption of bacterial metabolic products on the clay surface causes a decrease in the specific surface area, contact angle and C/N ratio. The formation of stable microaggregates with a diameter of about 30 mkm was noted. The role and importance of microorganisms in the changing mineral surface and in the initial stage of formation of aggregate waterproof structure are highlights.

Keywords: microorganisms-mineral interactions, contact angle, microaggregate formation, clay specific surface.

1. Introduction

Physical and chemical properties of the surface of the soil solid phase largely determine the transport and sorption/desorption of the substances in the soil solution. It depends on the properties of the surface of the spatial distribution of solutes and gases in the soil and their accessibility to the soil biota, in particular for plants and microorganisms. The role of microorganisms emphasized primarily in the formation of soil structure and nutrient availability to plants. In 90-ies of the last century there were even the first mathematical models for the effects of

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microorganisms in the processes of decomposition of organic matter and the formation of soil structure (Van Veen and Paul, 1981; Van Veen et al., 1985; Van Veen and Kuikman, 1990). However, the conventional physically-based mechanisms of microorganisms influence on the creation of water stable soil 1959structure, the formation of microaggregates so not offered to date. The interaction of microorganisms with mineral part of the soil determines the number of processes in the soil, such as weathering of primary minerals, their destruction or transformation due to the production of organic ligands and acids, which allows them to produce so weathering products for its operation, such as potassium from biotite (Shelobolina et al., 2012; Hazen et al, 2013; Simon et al, 2014) and demonstrate that biofilms can exert an effective control on clay mineralogy (Cuadros et al, 2013). Microorganisms themselves are undergoing the morphological changes. Since silicate bacterium *Bacillus mucilaginosus* forms flagella in the presence of bentonite mineral (Yun Zhu et al., 2011) from which they free Si and Al in the soil solution, reducing communication Al-O-H and Si-O-Si in the mineral. It describes in detail the mechanism of transformation of basalt for chemolitoautotrophic microorganisms that oxidize Fe (II) species as a source of energy, and using rocks NO_3 as electron acceptor (Shrenk et al., 2011). Microbial transformation of the surface of the soil solid phase affects many processes occurring in the soil, including infiltration, preferential flows and runoff, which depends on the wettability of solids with water, numerically characterized by the contact angle (CA) (Shein, 2010).

Changing the wettability of the solid phase from the soil depends on the hydrophilic-hydrophobic properties of soil organic matter, including the specific organic substances produced by microorganisms, as shown in numerous experimental studies middle of the last century (Vershinin et al., 1959). So in the thesis (Lyapshina, 1952, cited by Basic agrofiziki, 1959) on the role of microorganisms in the formation of soil structure, the conclusion is made that the creation of water-stable soil structure due to the activity of fungi and anaerobic bacteria – butyric, denitrifying and anaerobic cellulolytic and facultative anaerobic ammonifiers. According to current data, using molecular techniques refined their species Mass spectrometry of microbial markers (MSMM data) confirm that anaerobes of *Clostridium* and *Propionibacterium*, and aerobe actinobacteria of *Rhodococcus* and *Mycobacterium* are dominant in soil. Such species as *Acetobacter diazotroficus*, *Bacillus* sp., *Bifidobacterium* sp., *Lactobacillus rhamnosus* and *Clostridium* spp. are always present in the microbial community of soils. Questions concerning the potential effects on plant health (the oxygen content, redox potential, water activity, concentration of nutritious elements) and soil biofertilization (formation of water-stable aggregates, humus preservation) in agricultural systems are considered (Verkhovtseva et al., 2014). The favorable structure of the pore space in heavy textured soils with the low humus content is suggested to be stipulated by the activity of the numerous and diverse representatives of soil biota. Four phyla predominate in the microbiological composition of the soils studied; among them, *Actinobacteria* is the dominant. The composition of this phylum is dominated by the elevated number of both higher (*Streptomyces*) and lower (three species of *Rhodococcus*) actinobacteria (Shein et al., 2014).

When the content of soil organic matter (SOM) > 2 % there was a direct linear relationship between the SOM and the boundary wetting angle. In the range 0 < SOM <1 % of the variability observed extremal value of the contact angle of 0 ° to 90 ° (Bachmann et al, 2008). The reason for varying the degree of surface hydrophobicity authors relate to the spatial organization of organic compounds on the surface of the mineral particles. At low levels of SOC hydrophilic groups organic molecules are directed to the active centers of the mineral particles surfaces. In the "flat" molecular layer, most of the hydrophobic molecules are oriented outwardly zones, hydrophobicity of mineral surface increases. With the increase in the organic load, the spatial structure of molecules of SOM rarefied plane orientation is changed to a vertical tight. Here again is an increase in surface wettability. At a high RH/mineral, the outer surface of a separate "molecular layer" can interact "with excess" SOM molecules, forming a second molecular layer. The outer surface again becomes hydrophobic. This process can also be linked to formation of biofilms of microorganisms on mineral soil. As it was shown (Fucamizo et al., 2005), some strains of *Bacillus circulans*, who possessed chitinase activity in communication with the substrate through lysine residues of amino acids (mostly) and tyrosine (less), which provides a special kind of stacking cells or hydrogen bond. Physical modeling transformation surface of the individual minerals pure cultures of microorganisms, allows us to study this process in more detail.

The purpose of this study was to examine changes in the physical characteristics of the surface of the two primary minerals solid soil phase (kaolinite and montmorillonite) under the influence of silicate bacterium *Bacillus circulans*. The objectives were to (1) create a model system controlled the mineral-microorganisms with known initial properties, (2) control of microorganisms in terms of a model system, and (3) to register the change in the surface state of the mineral solids (the contact wetting angle).

2. Materials and methods

Objects of study. We used highlighted with river sand surface strain *Bacillus circulans*, which had the ability to interact with silicates in accordance with its habitat isolation and possessed the ability to chitinase activity. Kaolinite and montmorillonite – clay minerals that brought in nutrient medium for a bacterium. At the same time, there was a transformation of their surface.

Culture media and culture conditions. In the model experiment, the culture medium for the isolation of silicate bacteria (breeding ground by Aleksandrov, 1953), where as potassium silicate source was added kaolinite and montmorillonite, the following composition, (g/l): starch – 20.0, kaolinite/montmorillonite – 2.0, $CaCO_3$ - 2.0, $Ca_3 (PO_4)_2$ – 1.5, $MgSO_4 \times 7H_2O$ – 0.15, NaCl – 0.15, $MnSO_4$ – 0.05, $FeSO_4$ – 0,05, water. The medium was sterilized for 20 minutes in an autoclave at 1 atm, pH ≈ 7.0. The sterile medium was added nystatin (1 g/liter) to inhibit fungal growth. The test was conducted 3 times repeatedly.

Experimental Set. Microbial suspension *Bacillus circulans* (1 % vol) was inoculated in flasks V = 1000 ml where 200 ml of breeding ground were placed for silicate bacteria. This ratio (volume flask/volume ground) provided a weak aeration mode when this culture was performed without forced aeration. Flask was equipped with replaceable tool receiving sterile test tubes for sampling the culture medium and its analysis (after centrifugation to separate the clay mineral) for protein content, and the construction of growth curves. Cultured at 28 °C for 2 months. According to the growth curve of the culture of that time it remained on the stationary phase. Minerals after culturing were separated by centrifugation, washed with distilled water several times and dried. Next, they were used for determining the wetting contact angle (CA), the specific surface area by nitrogen desorption (m^2/g), as well as nitrogen content, organic carbon (C_{org}, %) and the ratio (C/N), on the surface of clay minerals.

Contact angles determination was performed by static sedentary drops on digital protractor (Analysis System drop shape, DSA100, Krüss, Germany) equipped with a video camera and software.

The particle size composition was determined using the laser diffraction method and a FRITSCH Analysette 22 device with the preliminary treatment of the samples with ultrasound in pure water. The contents of C and N were measured using a CNHS analyzer (Vario EL III Elementar) for solid samples. The composition of the microbial community was reconstructed according to the microbial markers (fatty acids and their derivatives-fatty hydroxyacids and aldehydes) that were determined after the acid methanolysis of the soil samples using the molecular method of gas chromatography-mass-spectrometry (GC–MS). The analysis was performed using a GC-MS system (HP-5973 Agilent Technologies, USA). This methodology is described in detail in (Osipov, Turova, 1997; Shekhovtsova et al., 2003). The organic carbon content was determined on an automatic analyzer (AN-7529) at the temperature of 900-1000 °C in the flow of purified oxygen (Shein et al, 2009). The specific surface was also determined by the method of the heat desorption of gases (nitrogen) with the help of a SORBTOMETER_M device and particle size distribution in the fraction <0.25 mm was determined on a FRITSCH Analysette-22 laser diffraction-meter after the ultrasonic pretreatment (Shein et al., 2009).

3. Results and discussion

Sorption on the clay surface of the bacteria metabolic products causes a decrease in the specific surface area and eliminates the differences in the surface area of CA the original minerals. The measured values of CA of the initial forms of clay minerals indicate a better wettability of the surface of kaolinite (Table 1).

Table 1. Some analytical characteristics of the clay minerals in their modification in the culture medium after cultivation of *Bacillus circulans*

Minerals	Sample	C, %	C/N	Surface area, m^2/g	Contact angles, ^{o}
Kaolinite	initial forms	0.39	19.5	11.01	30.8
	after incubation	7.82	6.6	6.02	54.5
Montmorillonite	initial forms	0.42	21.0	49.29	48.4
	after incubation	6.33	4.9	14.03	67.7

According to the literature, the contact angle wettability of the kaolinite is 27.8 ^{o}, montmorillonite – 55.7 ^{o}. Thus, *Bacillus circulans*, was sorbed on the surface of kaolinite and montmorillonite and thanks to the ability to extract silicon from minerals led to modification of their surface that increased their hydrophobicity. The organic matter sobbed on mineral surface was characterized by C/N ratio, which is characteristic of the organic matter of bacteria (Zavarzin, 2003) – about five, which confirms our assumption about the formation of tightly bound microbial biofilm on the surface of a solid mineral phase.

Integral due to a decrease of the specific surface of clay minerals during the growth of their hydrophobic properties is the formation of a very stable microaggregtes fractions with a diameter of about 30 mkm, which is absent in samples of kaolinite and montmorillonite from mild to incubation experiment (Fig. 1).

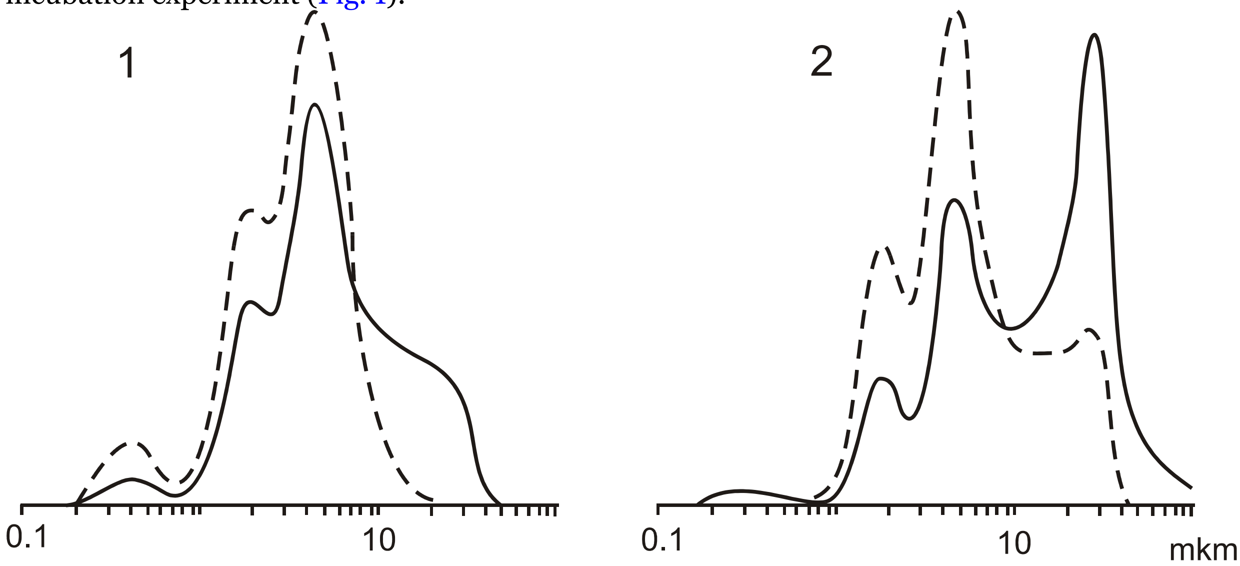

Fig. 1. Size distribution of kaolinite (1) and montmorillonite (2) particles: original (dashed line) and after incubation with the bacteria (solid line).

Processes of interparticlal bonds formation, ensuring the stability of the fractions under intense ultrasonic dispersion, require further research. At this stage, it can be assumed adhesion contacts between the organic matter sorbed on the surface of minerals. Microaggregates formation should lead to the development of the pore structure in the clay minerals and this pores will be the main channels for the root exudates, water and matter transport in soil (Ashman et al., 2003). These conditions favor the specific functioning of soil biota; microorganisms may participate in the transport of various ions (Ca, Fe, etc.), and new interparticle bonds of crystallization type are formed; in turn, these bonds strengthen the first aggregates and contribute to the development of specific water stable structure. Assumptions about the nature of the relationship of microbial organic matter with the mineral surface can be based on the research of Japanese scientists (Fucamizo et al., 2005), which showed that some strains of *Bacillus circulans*, who possessed chitinase activity in communication with the substrate through lysine amino acid residues (mainly) and tyrosine (less), which was provided by a special type of cell stacking or stacking hydrogen bond.

4. Conclusions

In special laboratory experiments, special suspensions of kaolinite and montmorillonite were inoculated by silicate *Bacillus circulans* and then cultured at 28 °C for 2 months. According to the growth curve of the culture of that time it remained on the stationary phase. Minerals after culturing were separated by centrifugation, washed with distilled water several times and dried. After inoculation phase minerals have been investigated by the value of the contact angle, the specific surface area by nitrogen desorption, and the concentration of the fractions of microaggregates. It was shown that due to the bacteria, the formation of microaggregates was noted and the most intense for montmorillonite in suspension, which revealed a maximum, content of 20–30 mkm microaggregates. The suspension of kaolinite also found an increase in this fraction, however, is not so noticeable. There was a significant increase in the contact angle and the reduction of the specific surface minerals after cultivation of silicate *Bacillus*. The formation of stable microaggregates may be associated with an increase in adhesion contacts between the organic matters, sorbed on the surface of minerals. The role and importance of microorganisms in the changing mineral surface and in the initial stage of formation of aggregate waterproof structure is highlights. It is assumed that the formation of macroaggregates should lead to the development of the pore structure. On the next stage soil pores will be the main channels by which plant roots, root exudates and largely determine the transport and sorption / desorption of the substances in ecosystems.

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