Formation of microaggregates in kaolinite suspension inoculated by *Bacillus velezensis* (SEM-investigations)

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Abstract. Kaolinite suspensions were inoculated by microorganisms (*Bacillus velezensis*) for 2 months. During the period of inoculation the formation of microaggregates of a certain shape and size was traced by electron microscopy. During the experiment the initially predominant planar structure was partially transformed. The newly-formed large (up to 250 µm in diameter) organo-mineral aggregate-like particles were registered. We suppose that during the 2-month period of incubation *B. velezensis* microorganisms partially destroyed the crystal structure of minerals to obtain the vitally necessary potassium. The protein compounds produced by bacteria hydrophobized the surface of the minerals which resulted in the formation of organo-mineral aggregates by bonds of hydrophobic-hydrophilic interaction.

1. Introduction

Investigations of aggregate formation in soils are relevant and topical in modern soil science with problems of the physical degradation of soils, the necessity to maintain the aggregates water and mechanical stability and related to the properties of aggregates soil functions in the biosphere. For this reason, much attention is focused on the processes of aggregates’ formation and the role of soil biota in these processes [1-5]. In recent years many researchers relate the aggregate formation to the role of amphiphilic compounds formed by soil microbiota. These amphiphilic compounds consist of hydrophilic components and components of biological origin, affecting the connection of mineral particles to stable microaggregates. In most cases the mechanism of this connection can be described as follows: in aqueous medium hydrophilic compounds of soil organic matter can make bonds with hydrophilic surface of clay mineral particles. Hydrophobic compounds create bonds of hydrophobic interaction, connecting hydrophilic organomineral complexes. This is the way of formation of aggregated organomineral compounds, “glued” by amphiphilic organic matter of microbiological origin [3, 4, 6]. There are some data, which can indirectly proof described mechanism, using various analysis and experiments on different agricultural and native soils [1, 3, 4, 6]. In this study an attempt to trace the formation of microaggregates in the model experiment with pure kaolinite particles and the certain species of microorganisms was made. The idea of direct model experiment was that *Bacillus velezensis* bacteria in suspension with kaolinite, consuming required for their vital activity the potassium ion from a clay mineral, would produce special protein compounds in the form of their bodies and specific secretions of glycopeptide and polysaccharide nature. These amphiphilic compounds would lead to aggregation of kaolinite particles resulting in the increase of coarse fractions percentage in
microaggregate composition. The objective of the research was to trace the dynamics of formation of aggregate-like structures in the suspension of kaolinite with bacteria *Bacillus velezensis*, using scanning electron microscopy (SEM). The plan of this work was: (1) to conduct a long-time (60 days) model experiment with kaolinite suspension and *B. velezensis* bacteria; (2) to take samples of inoculated and control kaolinite suspensions to study in dynamic the presence, form and size of aggregate-like formations and (3) to show the morphological changes of the initial kaolinite and newly formed organomineral aggregate-like structures using SEM method.

2. Objects and methods

The pure *B. velezensis* culture, isolated from the surface of river sand was cultivated on solid growth medium (peptic digest of animal tissue – 5 g/l; beef extract – 1.5 g/l; yeast extract 1.5 g/l; sodium chloride – 5 g/l; agar – 15 g/l) for 3-5 days in a thermostat at 28 °C. Colonies were washed off with the sterile saline solution into the flack with liquid growth medium. Prepared suspensions were used for the experiment. During the experiment microorganisms were cultivated in the liquid growth medium for cultivating silicate bacteria described in [7], where the only source of potassium was the clay mineral. Half of the flasks were inoculated with microbial suspension with a concentration of $2.7 \times 10^7$ CFU/ml. The another half of flasks was not inoculated and served as the control; pure kaolinite mineral served as the control too. All flasks were deposited at the 27 °C and shaken once a day.

Samples to study the particle size distribution and microaggregate composition were regularly taken during the course of experiment. The investigations were performed using the Microtrac Bluwave (Microtrac, United States) laser diffractometer [8]. Morphology of mineral was studied by scanning electron microscopy (SEM) method on a JSM-6380LA device (JEOL, Japan) [5, 9]. Samples were sifted through a sieve with a mesh diameter of 1 mm. After that samples were glued on aluminum support tables and dried in high vacuum in the chamber of the coating system. The samples were coated by a mixture of metals (Ag and Au) to prevent the formation of excessive charge on their surface in the SEM chamber. Samples were scanned at 20 kV, WD = 10, SpotSize = 30 [5], at magnifications from x30 to x20000.

3. Results and discussion

Differential curves of particle size distribution and microaggregate composition of studied samples are presented in figure 1. All of these curves have one distinct peak. In particle size distribution of all samples the fraction of 10-50 µm prevailed, constituting about 60%. The percentage of fractions 5-10 µm and 1-5 µm was 20 and 13%, respectively, being quite notable. In the microaggregate composition the fraction of 10-50 µm was also predominant (more than 60%), the content of fractions 5-10 µm and 1-5 µm was reduced by an average of 5%, while the content of fractions larger than 50 µm was increasing (the content of the microaggregate fraction of 50-250 µm was higher than the granulometric one by 10%).

The particle size distribution and microaggregate composition data have shown, that during the experiment with the inoculated by the microorganisms kaolinite suspension there was the increasing of content of microaggregates with diameter 50-250 µm and 250-500 µm by 5.4 and 1.5%. There were no significant changes in the control samples during incubation. The formation of microaggregates with diameter 250-500 µm by the end of experiment was noted (figure 1,c), which have formed visible local peak in the microaggregate size distribution.

The results of SEM investigations are presented in figure 2. Even in a small magnification (x30) it can be seen that by the end of the experiment rather big microaggregates were formed. In these aggregates particles, most likely, were cemented by organic matter, which was manifested by a clearly visible contrast white glow in the SEM-images.
Figure 1. Particle size distribution (solid line) and microaggregate composition (dotted line) of kaolinite in the course of incubation with the microorganisms (a - the original kaolinite; b - the 10-th day of incubation, c - the 60-th day of incubation).

Figure 2. SEM images (30x magnification) of the initial mineral (a) and of the mineral after two months of incubation with microorganisms (b).
At the following image (figure 3) one of the formed after two months of incubation aggregates is shown. We can see, that it consists of small plain scaly particles of mineral, adhered together by the organic matter – microorganisms’ metabolites.

![Figure 3. SEM image of the microaggregate, formed after two months of incubation with microorganisms (350x magnification).](image)

Let’s trace the dynamics of transformation of initial mineral and the formation of aggregate-like structures. We compared morphology of the initial mineral and the microbiologically-modified one (figure 4) after different periods of incubation and at different magnification. Initial mineral was presented by separated particles of different sizes, covered by small flecks. After ten days of incubation these flecks become more prominent and turned out to be partially detached from the mineral surface. We believe that by the end of the experiment many of these flecks were completely detached and were included into newly-formed microaggregates.

We can conclude that microbiological modification of mineral surface during two months of activity *B. velezensis* resulted in the increase in percentage of 50-250 μm and 250-500 μm fractions in microaggregate composition by 5.4 and 1.5%. Extracting the necessary nutrients, microorganisms have broken down the mineral, at the same time, the metabolites have adhered together small particles in proaggregates. Simultaneously, the strength of structural bonds in the mineral suspension have increased significantly. After 50 days of the experiment, the specific surface area of microbiologically modified mineral has increased. There were significant changes in micromorphology of kaolinite. These first results support the idea that microorganisms modify the surface of a clay mineral and connect individual particles of the mineral into aggregate-like structures, i.e. facilitate the bonding of small mineral particles in proaggregates.
4. Conclusions
This study was focused on the electron microscopic observations of the formation of microaggregates in kaolinite suspension due to inoculation of *B. velezensis* microorganisms. We are not ready to give the explanation of the physical mechanism of formation of the mentioned microaggregates. Only the fact of the formation of microaggregates of a certain shape and certain size during 2 month incubation period in the kaolinite suspension with *B. velezensis* is emphasized. However, the authors proceed from the assumption of the following mechanism of formation of microaggregates: microorganisms in a kaolinite suspension, in a medium containing no potassium ions, during their life partially destroyed the crystal lattice of the mineral to obtain potassium for their vital activity. They formed protein compounds in the form of their bodies and specific secretions of protein nature. Highly likely these compounds were hydrophobized the surface of the minerals, as the result, the formation of organo-mineral aggregates by bonds of hydrophobic-hydrophilic interaction was observed.

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