Applied Clay Science 119 (2016) 141–145

Contents lists available at ScienceDirect

Applied Clay Science

journal homepage: www.elsevier.com/locate/clay

Effect of *Bacillus mucilaginosus* D4B1 on the structure and soil-conservation-related properties of montmorillonite

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ARTICLE INFO

Article history:
Received 30 January 2015
Received in revised form 22 August 2015
Accepted 24 August 2015
Available online 5 September 2015

Keywords:
Bacillus mucilaginosus
Montmorillonite
Soil clays
Microbe–mineral interaction

ABSTRACT

Bacillus mucilaginosus is one of the common soil bacteria and is widely used as a microbial fertilizer to release potassium from K-rich soil minerals. But few researchers have discussed possible effects that the bacteria may have on montmorillonite. This study investigated the effect of *B. mucilaginosus* strain on the capability of montmorillonite to retain water/cations. After 20 days of interaction with *B. mucilaginosus* D4B1, the expansion ratios of montmorillonite samples decreased from 83.2% to 76.7%, and the cation exchange capacities (CEC) decreased from 80.8 mmol/100 g to 67.8 mmol/100 g, suggesting the durative interaction with the bacteria may reduce the capabilities of incorporating water molecules and nutrient into the interlayer space of montmorillonite. Morphological changes of discrete layers and etch pits were observed with scanning electron microscope (SEM). In the synchrotron radiation X-ray diffraction (SR-XRD) profiles, the full-width at half maximum (FWHM) of 001 reflection increased to 0.24 nm as compared to the abiotic sample (0.19 nm), indicating a decreased structural order in bacteria-treated montmorillonite. Also, a new band at 1540 cm^{-1} assigned to asymmetric stretching vibrations of carboxylate was detected by micro fourier-transform infrared spectroscopy (FTIR) in bacteria-treated samples, suggesting the alteration in the mineral structure of montmorillonite was possibly triggered by the organic acids produced by bacteria. Moreover, in bacteria involved dispersion, a significant decrease of the pH value was coupled with a consecutive increase of total protein concentration, indicating the continuous activities of the bacteria during 20 days of interaction. Above all, the decreased water-holding or cations-exchange capacity of montmorillonite was related to the partial alteration in the mineral structure of montmorillonite by *B. mucilaginosus* D4B1. Such a durable interaction on structural alteration of soil clays would have detrimental impact on long-term sustainability of soil resources.

1. Introduction

Interactions between clay minerals and microbes are ubiquitous in natural environments (Dong, 2010). Among various kinds of clay minerals that may interact with microorganisms, smectite minerals have drawn much interest because they are widespread in soils and sediments where microbes thrive. It has been reported that microbes may greatly influence the decomposition and transformation of smectite minerals (Dong, 2012). For example, after interaction with *Shewanella oneidensis* strain MR-1 for only 2 weeks at room temperature and 1 atm, nontronite transformed to illite through dissolution-recrystallization as a result of microbial reduction of structural Fe(III) (Kim et al., 2004). Other bacteria, such as *Thermoanaerobacter ethanolicus* (Zhang et al., 2007) and *Desulfovibrio sp.* (Li et al., 2004), have shown similar abilities in promoting this reaction (Dong, 2012). Moreover, microbial activities may also lead to significant changes in physical and chemical properties of smectite minerals (Kostka et al., 1999; Stucki et al., 2002; Stucki and Kostka, 2006; Kim et al., 2005; Jaisi, 2007).

Bacillus mucilaginosus, known as a silicate bacterium, is very common in soils. It is often used as a model strain to study the role bacteria plays in the weathering processes of silicates (Liu et al., 2006; Hu et al., 2006; Basak and Biswas, 2009). Because of its capability to dissolve potassium from K-rich soil minerals and to extract phosphorous from apatite (Boyle and Voigt, 1973; Lian, 1998; Liu et al., 2006), extensive researches have focused on its application for bio-fertilization (Basak and Biswas, 2009). It is also widely applied for wastewater treatment as a bio-sorbent (Lian et al., 2004; Lian et al., 2008; Cao et al., 2010). These researches have focused on metabolic products produced by the bacteria and bacteria-promoted dissolution of silicates (Mo and Lian, 2010). Few researches have discussed specific changes in mineralogical characteristics and property of montmorillonite after interaction with *B. mucilaginosus*.

On the other hand, clay minerals have attracted increasing interest in the field of soil research because of their capability of water retention and controlled release of nutrients (e.g., Al-Mukhtar et al., 1999; Gaiser et al., 2000; Wanyika, 2014). As one of the most common eogenetic

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http://dx.doi.org/10.1016/j.clay.2015.08.033
0169-1317/

smectite minerals, montmorillonite makes considerable contributions to retention and persistence of organic and inorganic compounds in soil, which benefit from its expandable interlayer space and large cation exchange capacities (Dudek et al., 2006). Furthermore, modifications of montmorillonite in order to remove excessive pesticides or herbicide in soils has been a research focus. Modification of montmorillonite was considered to help develop soil remediation capability and ensure long-term sustainability of soil resources (Boyd et al., 1988; Bojemueller et al., 2001; Cruz-Guzmán et al., 2004; Cornejo et al., 2008; Ghaemi et al., 2011). However, if soil clay minerals undergo durative modifications in their structure during the interactions with indigenous microbes in soil or microbes introduced by bio-fertilizer, their fertility and water-holding capacity may be changed.

The previous study (Zhu et al., 2011) evidenced that *B. mucilaginosus* could cause incomplete dissolution of bentonite-forming montmorillonite and a release of major elements from montmorillonite after 7 days of interaction. But we did not investigate whether the interaction could further lead to possible changes in properties of bentonite-forming montmorillonite that related to soil conservation. The present study aims to investigate on potential effects of *B. mucilaginosus* on soil-conservation properties and structural changes of montmorillonite after a comparatively long-term interaction.

2. Material and methods

2.1. Mineral preparation

The initial bentonite sample was the same as used in the preliminary research (Zhu et al., 2011), which was collected from Jianping District of Liaoning Province, China. It consists of 65% montmorillonite, 10% quartz, 10% calcite, 10% feldspar, and ~5% dolomite. The initial bentonite powder was centrifuged at 6000 rpm (r/min) for 20 min in a HC-3018R high speed refrigerated centrifuge (ZONIKA, China), and was washed with secondary deionized water. Only the suspended particles were collected. The above steps were repeated for a couple of times until the supernatant was clean. Then the collected samples were dried in an incubator chamber at 60 °C for 24 h. The samples ready for use were dominated by montmorillonite and cristobalite.

2.2. Bacterial culture

The bacterial strain (*B. mucilaginosus* D4B1, cryopreserved in glycerol media at -20 °C) was kindly provided by the Soil and Fertilizer Institute, Chinese Academy of Agricultural Sciences, which is a different strain of *B. mucilaginosus* from what was used in previous research (Zhu et al., 2011). The media applied in the present study was modified from the media used in the preliminary research (Zhu et al., 2011). It contained 1.0 g/L of yeast extract, 5.0 g/L of sucrose, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 0.2 g/L of K_2HPO_4, 5 mg/L of $FeCl_3 \cdot 6H_2O$, and 10 mg/L of $CaCl_2$ in secondary deionized water (pH adjusted to 7.20 with 0.1 mol/L of HCl, autoclaved at 121 °C and 103 kPa for 20 min). It was used as the media for both bacterial culture and bacteria-mineral interaction experiment. The strain was inoculated into 50 mL sterile media and cultured in an oscillating incubator maintained at 35 ± 1 °C and at the speed of 150 r/min for about 36 h to collect enriched bacteria in the exponential phase. Optical density of bacterial solutions was monitored to ensure the equal amount of bacteria for parallel experiments.

2.3. Batch experiments of mineral-bacteria interaction

The interaction experiments were conducted in 250 mL glass culture flasks with vented caps (~0.3 μm polytetrafluoroethylene membrane). 1.0 g of sample powder was mixed with 50 mL media and then sterilized. For the experimental group, 10 mL solutions of living bacteria (D4B1) and 40 mL of sterile medium were inoculated into the dispersion. Abiotic controls with mineral dispersion and 50 mL sterile medium (Blank) were also conducted to check the possible effects of the medium solution. All the dispersions were allowed to equilibrate in an oscillating incubator at 35 ± 1 °C and at the speed of 150 r/min for 20 days.

During the interaction process, aliquots (3.5 mL) were removed from evenly mixed dispersions with sterilized pipettes at fixed time intervals under sterile conditions. The 3.5 mL of aliquot was immediately processed as follows: 200 μL dispersion was unfiltered for total protein concentration measurement, the remaining dispersion was unfiltered for pH analysis. The 200 μL unfiltered aliquot was boiled for 10 min after addition of 0.2 mol/L NaOH and 0.9% m/V saline, and then centrifuged to harvest total protein from the supernatant for measurement.

At the end of the experiment, the remaining solid minerals were collected by centrifugation at 9000 r/min for 15 min and washed with secondary deionized water. Both steps were repeated for 3 times. All solid products were dried at 60 °C for 24 h and then ground in an agate mortar. The particles less than 150 μm were selected and stored in tubes at room temperature before analyses.

2.4. Analysis techniques

2.4.1. Liquid phase measurement

Acidity change of aliquot was measured with a FastEasy-50 pH meter (Mettler-Toledo). Total protein concentration was measured to approximately estimate the change of biomass during the experiment process because it was difficult to enumerate bacterial cells mixed with mineral particles. It was quantified with the linearized Bradford Coomassie brilliant blue assay (Bradford, 1976; Zor and Selinger, 1996) using a Cary8453 ultraviolet/visible (UV/Vis) spectrophotometer (Agilent Technologies). Bovine serum albumin (BSA) was used as the standard. Optical density of bacterial solution was monitored at 600 nm using a Cary8453 ultraviolet/visible (UV/Vis) spectrophotometer (Agilent Technologies).

2.4.2. Solid product characterization

Expansion ratio and cation exchange capacities (CEC) of the final solid products were measured to estimate the variations in their capabilities to retain water or nutrient elements. Scanning electron microscopy (SEM) was applied to observe morphological changes of the minerals. Final solid products were also analyzed by Synchrotron radiation 2 Dimension X-ray Diffraction (SR-2D-XRD), Micro Fourier-transform infrared spectroscopy (Micro-FTIR) and X-ray photoelectron spectroscopy (XPS) to investigate the changes in mineral constitutions and crystal structures.

For measurement of the expansion ratio via centrifugation, the solid products were ground into 75–150 μm powder. 0.4 g of samples from each group were mixed with 8 mL of secondary deionized water in a 10 mL quantified tube. Another 0.4 g of samples were mixed with 8 mL of kerosene. After standing for 24 h, all tubes were centrifuged at 1500 r/min in a swing-out rotor for 15 min. Volumes of samples in water and in kerosene were recorded as V_1 and V_0, respectively. The expansion ratio (P) of each sample was calculated as follows $P = (V_1 - V_0) / V_0 * 100\%$.

Determination of CEC was carried out with Orion StarTM and Star Plus Meter (Thermo Scientific) by the method of Conductometric Titration according to Wu et al. (2002).

For SEM observations, about 0.5 mg of mineral powder was dispersed in ethanol and treated with ultrasonic for 10 min. One drop of the dispersion was dripped onto a clean silicon slice. Scanning electron micrographs were obtained using a Quanta 650 FEG (FEI) under a high voltage of 15 kV and a pressure on 0.90 Torr (~120 Pa).

XRD analyses were performed at Shanghai Synchrotron Radiation Facilities (SSRF) during dedicated storage ring operation with Beam Line 15 U (18 KeV, λ = 0.688889 Å, beamsize 150 μm × 100 μm). XRD data were collected using a 2D area detector. About 1.0 mg of sample powder was mounted into the interspace of two pieces of 3 M film before testing. Collecting time for each sample was 30 s. CeO_2 was used