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Differential regulation of phenanthrene biodegradation process by kaolinite and quartz and the underlying mechanism

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ABSTRACT

Natural and cost-effective materials such as minerals can serve as supportive matrices to enhance biodegradation of polycyclic aromatic hydrocarbons (PAHs). In this study we evaluated and compared the regulatory role of two common soil minerals, i.e. kaolinite and quartz in phenanthrene (a model PAH) degradation by a PAH degrader *Sphingomonas* sp. GY2B and investigated the underlying mechanism. Overall kaolinite was more effective than quartz in promoting phenanthrene degradation and bacterial growth. And it was revealed that a more intimate association was established between GY2B and kaolinite. Si and O atoms on mineral surface were demonstrated to be involved in GY2B-mineral interaction. There was an higher polysaccharide/lipid content in the EPS (extracellular polymeric substances) secreted by GY2B on kaolinite than on quartz. Altogether, these results showed that differential bacterial growth, enzymatic activity, EPS composition as well as the interface interaction may explain the effects minerals have on PAH biodegradation. It was implicated that different interface interaction between different minerals and bacteria can affect microbial behavior, which ultimately results in different biodegradation efficiency.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic, fused benzene ring containing pollutants that have been increasingly accumulated in the environment, especially in the soil due to anthropogenic activity. Phenanthrene ($C_{14}H_{10}$) is one of the priority pollutant PAHs and has long been a model PAH for the study of PAH degradation in the environment. Biodegradation is demonstrated to be an effective strategy in dealing with PAH contamination and a great number of bacteria species with degradative ability has been isolated from PAH contaminated soils and sediments, among which members of the sphingomonads are well adapted to PAH contaminated environment and show great promise in PAH bioremediation [1].

Clay minerals and other minerals in soil are readily available supportive matrices and habitats for microorganisms. Minerals are basic materials in the earth crust with which microbes interact at all spatial and temporal scales. Minerals with a large surface area are ideal materials for the attachment of bacteria because of their environmental-friendliness and cost-effectiveness to modify bacterial growth and degradation. Compared with other supportive matrices like polymeric hydrogels [2], minerals are much more endurable and recyclable. The extensive interplay between bacteria and minerals is crucial for bioremediation enhancement [3,4]. Yet, detailed analysis and in-depth elucidation of this process is still lacking.

Kaolinite is one of the most abundant and widespread clay minerals with typical layered structure. Kaolinite ($Al_2Si_2O_5(OH)_4$) is a 1:1 type non-expandable clay mineral, with basic units consisting of one silicon tetrahedral sheet and one aluminum octahedral sheet [5]. Quartz is another common mineral and a major constituent of most soils. The quartz crystal is comprised of SiO_4 tetrahedrons linked together in a three-dimensional framework, the surface of which is full of silanol groups (Si—OH) [6]. Quartz and kaolinite are both chemically stable, however, there is clearly a wide structural difference between clay mineral kaolinite and non-clay mineral quartz [7,8].

Although a number of literatures have documented positive effects of microorganism/clay minerals complexes on the biodegradation of

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hydrocarbon organic pollutants [9–13], the mechanism underlying the regulatory role of clay minerals in biodegradation of organic pollutants is often inconclusive. The factors determining the degradation performance of microorganism/clay mineral complexes include the species of microorganisms, the mineral type, and the pollutant type [14]. Comparison of the interface interaction mode of microorganism/clay mineral complexes and microorganism/non-clay mineral complexes as well as the biological behavior of the microbial species involved should be helpful in exploring the regulation mechanism of different mineral types. Therefore, in this study, we examined and compare the impacts of kaolinite and quartz on phenanthrene degradation by *Sphingomonas* sp. GY2B previously isolated from PAH contaminated soil [15] and investigated the underlying mechanism including bacterial growth, interface interaction characteristics and EPS production.

2. Materials and methods

Detailed information and procedure are presented in Appendix A in Supplementary material.

2.1. Biodegradation of phenanthrene by free Sphingomonas sp. GY2B or GY2B attached on minerals

Biodegradation of phenanthrene was carried out in flasks containing MSM and 100 mg/L of phenanthrene by diluting phenanthrene stock solution of 1.0 g/L in MSM and allowing hexane to evaporate. The enriched *Sphingomonas* sp. strain GY2B was pre-cultured in a 250 mL flask containing 50 mL of MSM and 100 mg/l phenanthrene for 48 h at 30 ℃ and 150 rpm. After that, aliquots of 0.5 mL of the culture solution were added to 50 mL flasks containing 10 mL of MSM and 100 mg/L phenanthrene with or without 0.02 g of kaolinite or quartz powder (2.0 g/L). The minerals were passed through a 200 mesh sieve, and meshed mineral powders were mixed with MSM and were autoclaved together prior to use. The adsorption of bacteria on both kaolinite and quartz should reach an equilibrium within 1 h, leading to formation of GY2B/kaolinite and GY2B/quartz complexes [16,17]. The flasks were agitated in an aphotic shaker at 30 ℃ and 150 rpm. At different time intervals, the flasks were retrieved and the amount of phenanthrene left was measured by HPLC (Hitachi, L-2000) equipped with a UV/Vis detector and Athena C18-WP column (4.6 mm × 250 mm, 5 μm) at 30 ℃, using methanol-water (v/v, 90/10) as the mobile phase at a flow rate of 1.0 mL/min. The injection volume was 20 μl and the UV wavelength for phenanthrene detection was 254 nm.

2.2. Determination of bacterial total protein content

At different time intervals during degradation, the bacteria cells or GY2B/mineral complexes were harvested and centrifuged. The pellets were resuspended in 1.0 mL of PBS and then subjected to ultrasonication. After centrifugation, the protein content in the supernatants was measured by Bradford method [18] with a UV–vis spectrophotometer detected at 595 nm (Shimadzu model no. UV-2101PC, Shimadzu Europe Ltd., UK). Albumin was used as standard.

2.3. Extraction of EPS secreted by GY2B/mineral complexes

After 48 h of degradation, EPS secreted by GY2B/mineral complexes was obtained by centrifugation at 8000 rpm for 20 min, the supernatant was collected and EPS was precipitated by adding 3 volumes of isopropanol into the supernatant and keeping it overnight at 4 ℃. The precipitates formed was recovered by filtration through a 0.22 μm membrane filter. The purified EPS was washed with 70% ethanol several times and then freeze-dried. The obtained EPS was subjected to FTIR analysis.

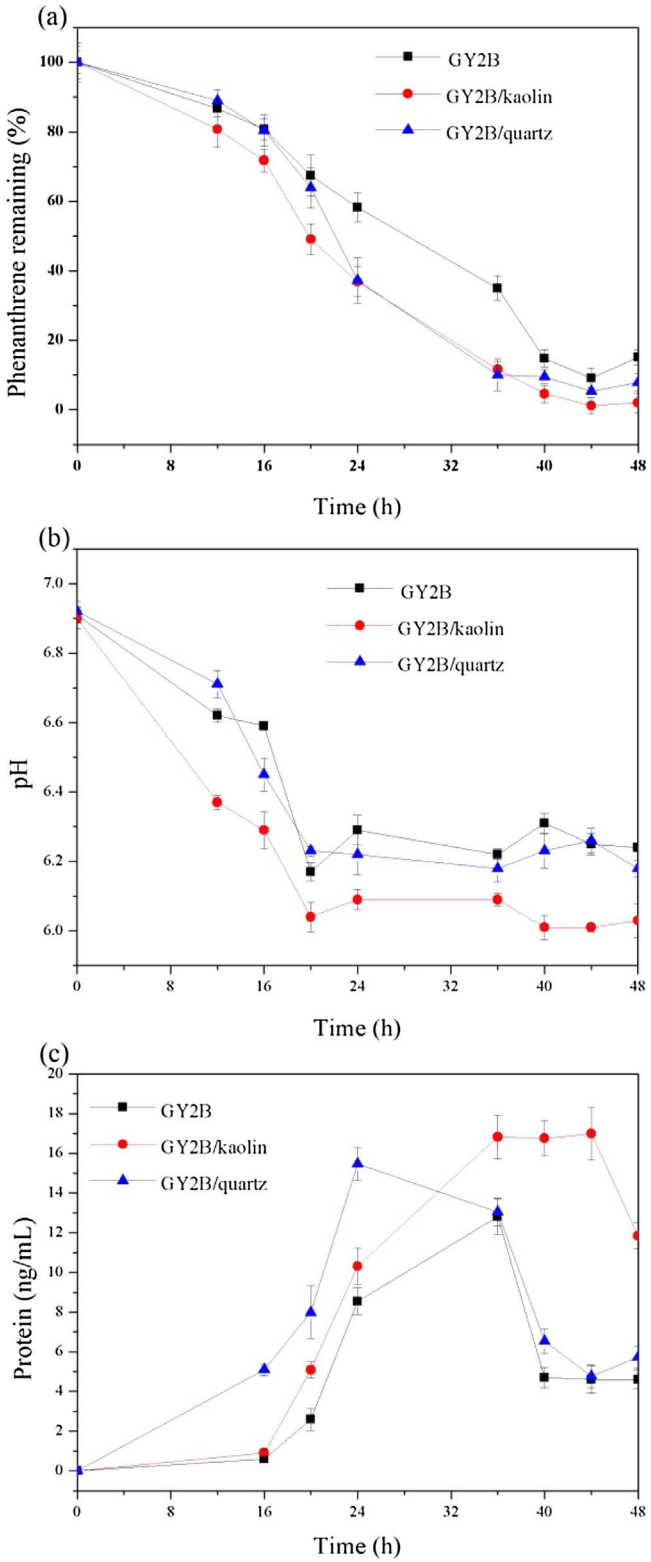

Fig. 1. Phenanthrene degradation efficiency of free GY2B or GY2B/mineral complexes (a) as well as the pH variation (b) and growth patterns of free GY2B or GY2B in GY2B/mineral complexes (c) during degradation.

2.4. Statistical analysis

All the experiments were carried out in triplicate. The mean values and standard deviations were determined by excel.