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Remediation of crude oil by microorganisms in oily hot springs : The study of microbial degradation and covellite biomineralization

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Abstract

The biomineralization of covellite and the biodegradation of crude oil were found in microbial mats which formed at Tsukioka hot springs and Toyotomi hot springs. Bacterial mineral formation of covellite (CuS) associated with crude oil were found in Tsukioka hot springs, Niigata, Japan. The hot springs is located in Tsukioka, Niigata, Japan. The hot spring water (pH 7.3, Eh -184 mV and 49.2 ° C) that was produced from a 280 m in depth originated from fossil seawater. The inner wall of the Tsukioka hot spring well was covered with green microbial mats which contained black crude oil. The aim of this study was to investigate of covellite biomineralization in the green microbial mats with crude oil from the Tsukioka hot spring water. Observations by optical and electron microscopy, phospholipid analysis and bacterial cultivation showed that the biomineralization of covellite (CuS) was found on the surface of crude oil droplets. ED-XRF analysis showed that the crude oil contained SO3 (67.6 wt%), CaO (24.8 wt%) and Cu2O (2.6 wt%). Observations by optical and epifluorescence microscopy as well as scanning electron microscopy (SEM) showed that two morphological types of bacteria (i.e., filamentous and coccus typed bacteria) were found on crude oil droplets. Transmission electron microscopic (TEM) observations further revealed that the cell wall of the filamentous bacteria had a double membrane and was surrounded by Extracellular Polymeric Substance (EPS), which might provide the favorable nucleation sites for CuS. X-ray powder diffraction and electron diffraction analyses of the microbial mats identified that the materials precipitated on the surface of the oil droplets were highly crystallized covellite (CuS). FT-IR spectra of the microbial mats that contained covellite showed the appearance of C-N-H, P=O, P-O-C and N-H bands, indicating the presence of phospholipids (lecithin and phosphoric ester) in the microbial mats. Cultivation experiments, furthermore, demonstrated that the cultivated bacteria were identified as sulfate reducing bacteria (for coccus-typed bacteria) and oil degrading bacteria (for filamentous bacteria). A schematic formation model is here suggested to better understand the interaction between bacterial cells and oil droplets under anaerobic conditions in the Tsukioka hot spring water.

GC, GC-MS and GC-MS/MS analyses determined the crude oil degradation level

concerned with oil degrading bacteria in Tsukioka hot springs. The susceptibility of oil to microbial degradation is in the following processes: n-alkanes > branched alkanes > low-molecular-weight aromatics > cyclic alkanes with lower concentration of normal alkanes, with unresolved complex mixture (UCM). The increasing relative quantities of C30 hopane and the absence of pristine, phytane and trepan indicate the evaporation, thus leading to biodegradation. Moreover, diasteran and regular sterane ratio, Ts/Tm and oleanane/C30 hopane ratio also indicate that the high biodegradation of crude oil, collected from Tsukioka hot springs, has taken place.

In contrast, the accumulation of oil by diatom cells was found in the reddish brown microbial mats at the Toyotomi hot springs, Hokkaido, Japan. The oily hot spring water and nearby river water showed a neutral pH (pH 7.7 and 6.4), anaerobic condition (Eh 37 mV and -14 mV) with methane gas. The crude oil in hot spring water contained Fe, S, and Si elements except petroleum hydrocarbons. The reddish brown microbial mats were composed mainly of Fe with several elements (e.g., Si, Al, S, K, Ba, Ca and Mn), to form ferrihydrite. The organic carbon gleamed light-yellow in color under the fluorescence ultraviolet-ray. Optical and epifluorescence microscopic observations showed two types of oil accumulation; Pinnularia spp. and Frustulia rabenhorst were encrusted with oil on the surface of cells, whereas Achnanthes sp., Navicula sp. and/or Gomphonema sp. accumulated oil in their internal cells associated with coccus-typed bacteria, iron bacteria and green algae. The results suggest that diatoms have the ability to remediate petroleum hydrocarbons in oil-contaminated areas.

The biomineralization of covellite and the biodegradation of crude oil as described in this study could have profound implications for bioremediation of not only hot springs but also oil-contaminated site but also heavy metal-polluted area. Bioremediation is a proven alternative treatment tool that can be used to treat certain anaerobic oil-contaminated environments.