Role of bacterial membrane components in covellite mineralization and biodegradation in oily hot springs

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Introduction

Technological development and increasing industrial activities in the world have necessitated a vast increase in the use of fuel oil. Over the entire lifecycle including extraction, transportation, refining, storage, usage and ultimate disposal, there is a considerable risk of environmental contamination by these non-aqueous phase liquids composed of a large number of hazardous and toxic constituents. Whereas, biomineralization has been reported in 56 phyla of living organisms including bacteria, protozoans, fungi and plants and has, in a large number of cases, an important role in the remediation of heavy metal.

Hence, The focus of this study is to observe the effect of the green microbial mats on the removal of crude oil. Biomineralization of covellite (CuS) on the surface of crude oil droplets was observed in this study by microscopic techniques, phospholipid analysis, bacterial cultivation and GC-MS/GS techniques. In this integrated program, studies on microbial diversity and activities in response to heavy metals and oil pollution are combined with studies on behavior of micro-organic mineralization attendant on the degradation of hydrocarbon molecules in microbial systems. Knowledge of the biodiversity of microorganisms and the identification of mineral compositions, oil sensitivity, oil tolerance and oil loving species and communities may help to select effective strategies for the bioremediation of heavy metals and oil-polluted areas.

Biomineralization of covellite in Tsukioka hot springs

The biomineralization of covellite in oily hot springs was found at the Tsukioka Hot Springs, Niigata, which formed in green microbial mats. Water quality of hot springs was nearly neutral pH (7.3) and anaerobic condition (Eh -184 mV). The microbial mats contained a large amount of Cu to form Cu-minerals, such as covellite (CuS). The crude oil contained high S with traces of Si and Cu, and emitted the dark orange colored fluorescence light under ultraviolet ray.

Optical and epifluorescence microscopic observations showed that filamentous bacteria and coccus typed bacteria which resided around the oil droplets and formed colonies with the yellow colored mineral particles may be covellite. Oil droplets were covered with filamentous bacteria in the biofilms formed as a result of bacterial cell lysis (Figure 1). TEM observation revealed that filamentous bacteria had double membrane cell wall, suggesting the ability to use oil as a source of energy by degrading enzyme in periplasm space. SEM, TEM observation of ultra thin-section and STEM-EDS analyses showed that oil droplets covered with biofilms were nucleated to form covellite (Figure 2, 3).

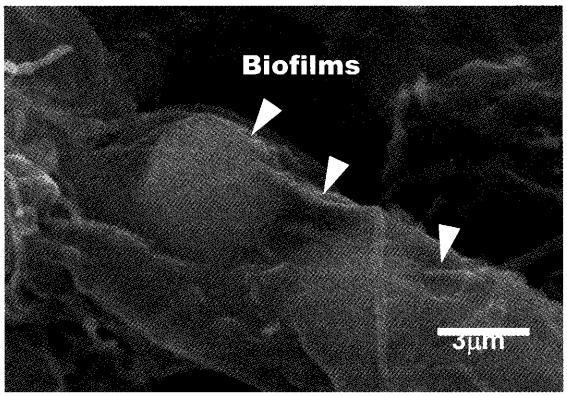

Figure 1. Biofilm covers the mass of filamentous bacteria and oil droplets (arrows)

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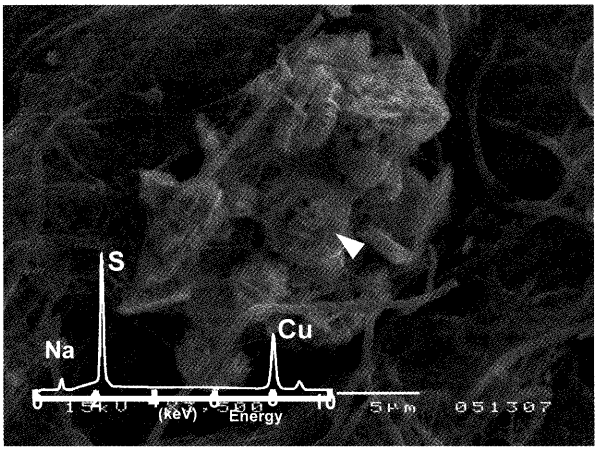

Figure 2. Covellite mineralization has completed and covellite is formed on the surface of oil droplets, is wrapped in biofilms. The crystalline covellite is composed of high S and Cu with trace of Na (an arrow).

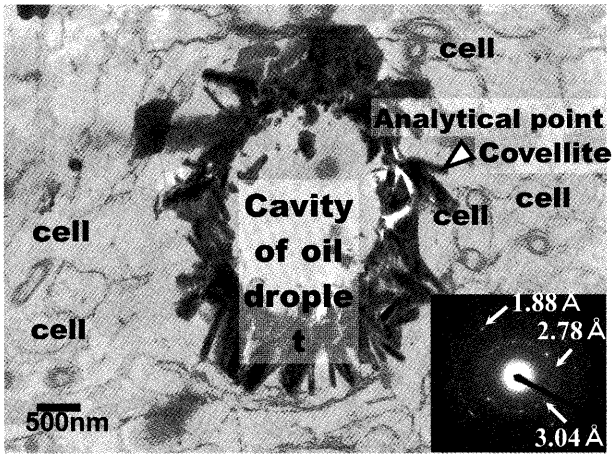

Figure 3. Crystallized covellite is observed around the cavity of oil droplets. Covellite is surrounded by numerous bacterial cells. Electron diffraction pattern with diffraction spots and rings at 3.04, 2.78 and 1.88 Å (white arrows) indicated at the covellite is highly crystallized mineral.

FT-IR analysis and quantitative analysis of phospholipid indicate the presence of lecithin and phosphoric ester which have a surface-active ability in bacterial EPS and cell wall. The bacterial amphipathic ability led to the easy elemental diffusion through the bacterial cell, therefore resulting in the accumulation of Cu-S by the bacterial cell wall which was bounded by phosphoric ester and N-H. The bacterial amphipathic ability and the specific environment under nearly neutral and anaerobic condition in the hot spring water have a great role in the formation of covellite, which is an effective remediation of petroleum hydrocarbons at oil-polluted area and at Cu-contaminated area.

Biodegradation of crude oil in Niigata oil fields area

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 order: 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 C_{30} 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/C_{30} hopane ratio also indicate that heavily biodegradation of crude oil, collected from Tsukioka hot springs, has taken place.

Conclusions

Biomineralization of covellite (CuS) and biodegradation of crude oil at oily hot springs, in Japan were studied. In case of Tsukioka hot springs, Cu ion included crude oil, filamentous bacterial degraded metabolism occurred to the Cu-S mineralization depend on the metal species and surrounded environments. The results indicate that the oil degradation microorganisms have an ability of oil degradation associated with heavy metal fixation.

The biomineralization of covellite and the biodegradation of crude oil by filamentous bacteria in this study could have profound implications for the bioremediation of not only oil-contaminated site but also heavy metal-polluted area. Thereby, these results provide a significant insight into how bioremediation of the crude oil occurs in hot spring water and how heavy metals influence that bioremediation, thereby giving more fruitful information for the bioremediation of the oil and heavy metals-contaminated environments.