Role of Hydrocarbon-Degrading Bacteria in the Bioremediation of Heavy Oil Polluted Coastal Area

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The laboratory and field studies have been carried out on hydrocarbon-degrading bacteria which play an important role as degrader in the bioremediation for cleanup of heavy oil polluted shorelines for 5 year since1997. This study is the case study of the Russian tanker (Nakhodka) spilled approximately 6,240 kl of C-type-heavy oil into the Japan Sea occurring on January 2, 1997. The heavy oil spill led to a serious impact to the surrounding environment, particularly the heavy oil pollution of the shoreline. In addition, we have performed laboratory examination for bioremediation process of heavy oil with the two different treatments, namely (1) the inside building examination and (2) the outside building one to investigate the significantly role of hydrocarbon-degrading bacteria.

In this research, samples of heavy oil, seawater, and polluted beach sand were firstly collected from (Nakhodka) tanker on February 21, 1997, and were secondly collected on November 21, 2001 in Ozawa and Atake at Wajima seashore in Ishikawa. All samples were stored at 4 oC until required for analysis. Hydrocarbon-degrading bacteria were cultured on nutrient agar plate. Heavy oil samples were extracted by using n-hexane to separate heavy oil from others. The 1 ml of the heavy oil solution was added to 9.0 ml of sterile 0.85% NaCl (w/v). In addition to sand samples, bacteria were extracted by adding 1.0 g of the sand to 9.0 ml of sterile 0.85% NaCl (w/v) and vigorously shaking the mixture. All agar plates were incubated at 27 oC for 2 to 3 days. Observation of hydrocarbon-degrading bacteria was employed with using optical microscope with DAPI staining and SEM for natural and cultured samples. In addition, measurement of pH, dissolved-oxygen (DO), temperature, electrode potential vs the standard hydrogen electrode (Eh) and electrical conductivity (EC) was performed to investigate the bioremediation process taken place. While, the organic chemical composition of heavy oil was estimated by using NCS analyzer.

The results revealed that after 5 years bioremediation, a large number of hydrocarbon-degrading bacteria still exist in the sites consisting of a variety of morphological form of bacteria such as coccus (streptococcus and staphylococcus), bacillus (streptobacillus and staphylobacillus), spirilla, and filamentous (Gaudy and Gaudy 1981). The cells of bacteria tend to remain together in cluster. Hydrocarbon-degrading bacteria develop rapidly on oil films and slicks, are able to attach to insoluble oil droplets, and can often be seen there in large number. Accordingly, the value of pH tends to alkaline condition (7.3 - 8.6) and the value of dissolved-oxygen (DO) indicated to aerobic condition (4.8 - 11.1 mg/l). These results confirm those of previous reports that significant aliphatic hydrocarbon oxidation occurs only in the presence of O2. Even in aerobic environments, hydrocarbon-degrading bacteria can act only if other environmental condition, such as temperature, pH and inorganic nutrients, are adequate. In this case, bacteria participate in oil spill cleanups by oxidizing the oil to CO2. Moreover, microbial utilization of oil is desirable and may even be enhanced by adding inorganic nutrients such as phosphorous and nitrogen to oil spill areas can increase bioremediation rates significantly shown by decreasing of C content at all sampling sites. Compared with the content of carbon, the content of nitrogen (N) and Sulphur (S) are relatively low (0 - 0.22% N and 0 - 2.44% S). This study also confirms that a diverse microbial community exists capable of utilizing hydrocarbon as an electron donor and carbon source. Thereby, hydrocarbon-degrading bacteria obviously play a key role in the bioremediation process.