MICROORGANISMS LIVING IN EMULSIFIED HEAVY OIL

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ABSTRACT

Environmental pollution on the coast of Hokuriku district caused by the heavy oil spill from the wreaked Russian tanker Nakhodka, showed borderless marine pollution by foreign bottoms at the regional level. The SEM-EDX observation of local microorganisms living in emulsified heavy oil found that the microbial communities are developed with the increase of species of microorganisms in a short period. This study totally explained the organization of food chain in the microbial communities is fundamental for maintaining microbial activities, which contributes to environmental purification..

INTRODUCTION

Environmental pollution on the coast of Hokuriku district caused by the heavy oil spill from the wreaked Russian tanker Nakhodka, showed borderless marine pollution by foreign bottoms at the regional level.

Every marine pollution by crude petroleum and heavy oil increases the public awareness to environmental purification methods. Bioremediation using microorganisms was really introduced into the coast polluted by the Exxon Valdez oil spill off Alaska in 1989 (Harayama 1995). Afterwards, the assessment was carried out, that intentional supplement of nutritious elements into the environment promotes the activities of oil decomposing bacteria, which results in effective purification of the marine environment (Bence et al. 1997).

On the contrary this study focuses on the environmental purification with any unintentional management. Accordingly cultural experiments using the heavy oil and seawater collected from the polluted area were attempted to observe microbial activities by a scanning electron microscope.

METHOD

Sample description

The samples for this study were arranged from the drift heavy oil collected at Akasaki in Togi Machi, Noto Peninsula of Ishikawa Prefecture on 15 January, 1997 (SAMPLE-1), and the oil balls collected at the Aramiko-jima Island of the Nanatsu-jima Islands off the shore of Wajima city in Ishikawa Prefecture on 14 March, 1997 (SAMPLE-2).

Using SAMPLE 1 and the local seawater, cultural experiments were started in the condition of pH 8.5, Eh 110mV, EC 48.6mS/cm, and 20.6℃ of seawater temperature. Three days later, various oil balls 3mm or below in diameter appeared, and exudation of light brown colored oil slick was observed in the vicinity of the oil balls. The observation materials were obtained from this oil slick. Similarly, cultural experiments were attempted, using the SAMPLE-2 in which oil slick had already been overspreaded. Seawater temperature, pH, Eh and EC at the sample collection were 10.8℃, 8.3, 270mV, 51.5mS/cm, respectively.

Experimental method

For micro-morphological traits of the oil balls from both the SAMPLE-1 and the SAMPLE-2 were observed by a scanning electron microscope (SEM; JEOL-JSM-5200) at accelerating voltage of 10 and 15 kV. The oil slick and the seawater in the SAMPLE-1 were removed on a cover glass stuck on a sample stub by a pipette. The low vacuum mode of the SEM was initially introduced into the observations of the SAMPLE-1. Following observations in the high vacuum mode (normal mode) of the SEM were carried out after applying gold coating to the sample. The samples of oil slick in the SAMPLE-2 were observed in the high vacuum mode of SEM, after applying carbon coating to the sample.

Qualitative and semi-quantitative analyses were carried out by an energy-dispersive X-ray analyzer (EDX; Philips-EDAX PV9800 STD) equipped with the SEM.

RESULTS

Observation results of the SAMPLE-1

Observation by the SEM in the low vacuum mode showed that numerous globular oil particles 5-100 μm in large are suspended in the local seawater. Additionally oil slick was seen around the circumference of the globular oil particles, and the microbial communities were observed on the surface of the particles.

The SEM observation in the high vacuum mode found rod-shaped microorganisms 1-2 μ m long and 0.5-1 μ m wide, and spherical microorganisms 0.5-1 μ m in diameter (Figure 1). Further observation showed that these microorganisms inhabit the oil slick exuding from the globular oil particles, and develop the communities on the edge of the oil particles (Figure 2A). EDX spectra were obtained from the center of the globular oil particle (I), microorganisms (II) (III), and a portion of the oil particle where the microbes concentrate (IV). EDX pattern of the globular oil particle indicated strong peaks of Na, S and Cl. Furthermore S and Ca are derived from the microorganisms, while Si, K and Ti are the major components of cover glass.

Observation results of the SAMPLE-2

The SEM observation of the oil slick exuding from the oil ball found that the prominent species of microorganisms, in comparison with the microbial communities shown in Figure 1, make large communities on the surface of the oil slick. SEM images showed that the microorganisms in the communities are varied in size, and *Vibrio*-like microbes propagate in the communities (Figure 3A).

EDX pattern showed strong peaks of Si, Cl and Ca in addition to S, with high background indicating organic matter. Cu and Zn are the main component of the sample stub (Figure 3B).

DISCUSSION

In the comparison with the result of EDX analyses of commercial heavy oil, significant peaks of Na, S and Cl in the globular oil particles in the SAMPLE-1 indicate that the seawater enters the interior of the floating oil to diffuse and emulsify the oil, which results in the development of the oil balls. This causes the exuding of oil slick in the vicinity of the oil balls, where microorganisms develop the communities, while concentrating sulfur. SEM-EDX analyses suggest that the microorganisms absorb sulfur component from the oil for yielding energy.

It is reported that decomposition of petroleum in the marine environment proceeds in the processes of collaborative management of the communities by a variety of microorganisms (Swannell et al. 1996). Actually cultural experiments using oil decomposing bacteria ascertained that mixed culture with a variety of bacterial species is more effective in oil decomposition than isolated culture (Swannell et at. 1997). Additionally an on-site study reported that microbial communities are developed in one or two weeks after appearance of oil slick on the surface of the sea, and remove it within a few months (Swannell et al. 1997).

SEM image of the SAMPLE-1 showed the microbial community which is thought to be developed in three days after the cultural experiment started. Meanwhile, the cultural experiment for the SAMPLE-2 was arranged in condition that oil slick had already been exuded, and SEM observation of the SAMPLE-2 after a month, found the communities are composed of more various species of microorganisms, including comparatively large-sized microorganisms. The different SEM image of the microbial communities between the SAMPLE-1 and the SAMPLE-2, is thought to explain a process of the organization of food chain at the micro scale. From the result of the SEM observation of the SAMPLE-2, the organization of food chain is thought to be fundamental for maintaining microbial activities, which contributes to environmental purification.

CONCLUSION

The SEM-EDX observation of local microorganisms living in emulsified heavy oil found that the microbial communities are developed with the increase of species of microorganisms in a short period. This study totally explained the organization of food chain in the microbial communities is fundamental for maintaining microbial activities, which contributes to environmental purification..

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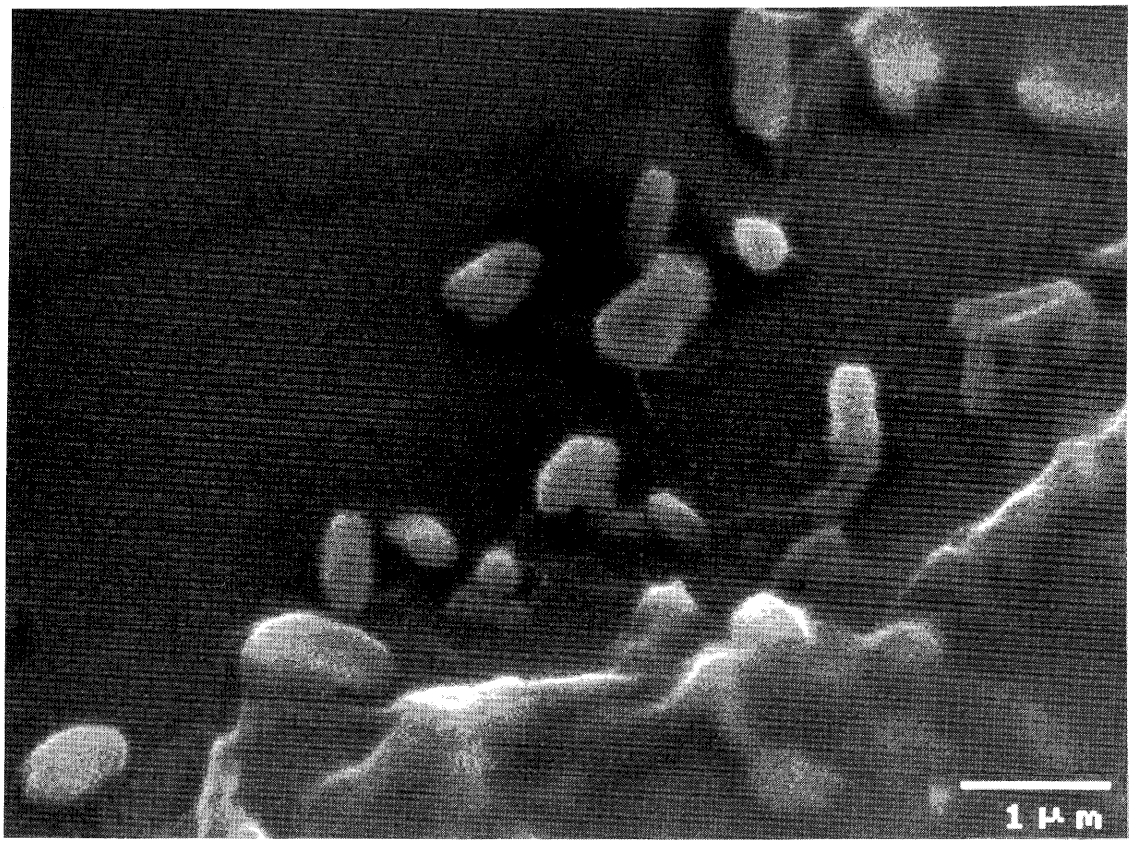

Figure 1 SEM image of a microbial community in the vicinity of the micro globular oil particle in the sample collected from Akasaki in Togi Machi, Noto Peninsula of Ishikawa Prefecture. Microbial communities were observed in adhesion to the oil particles.

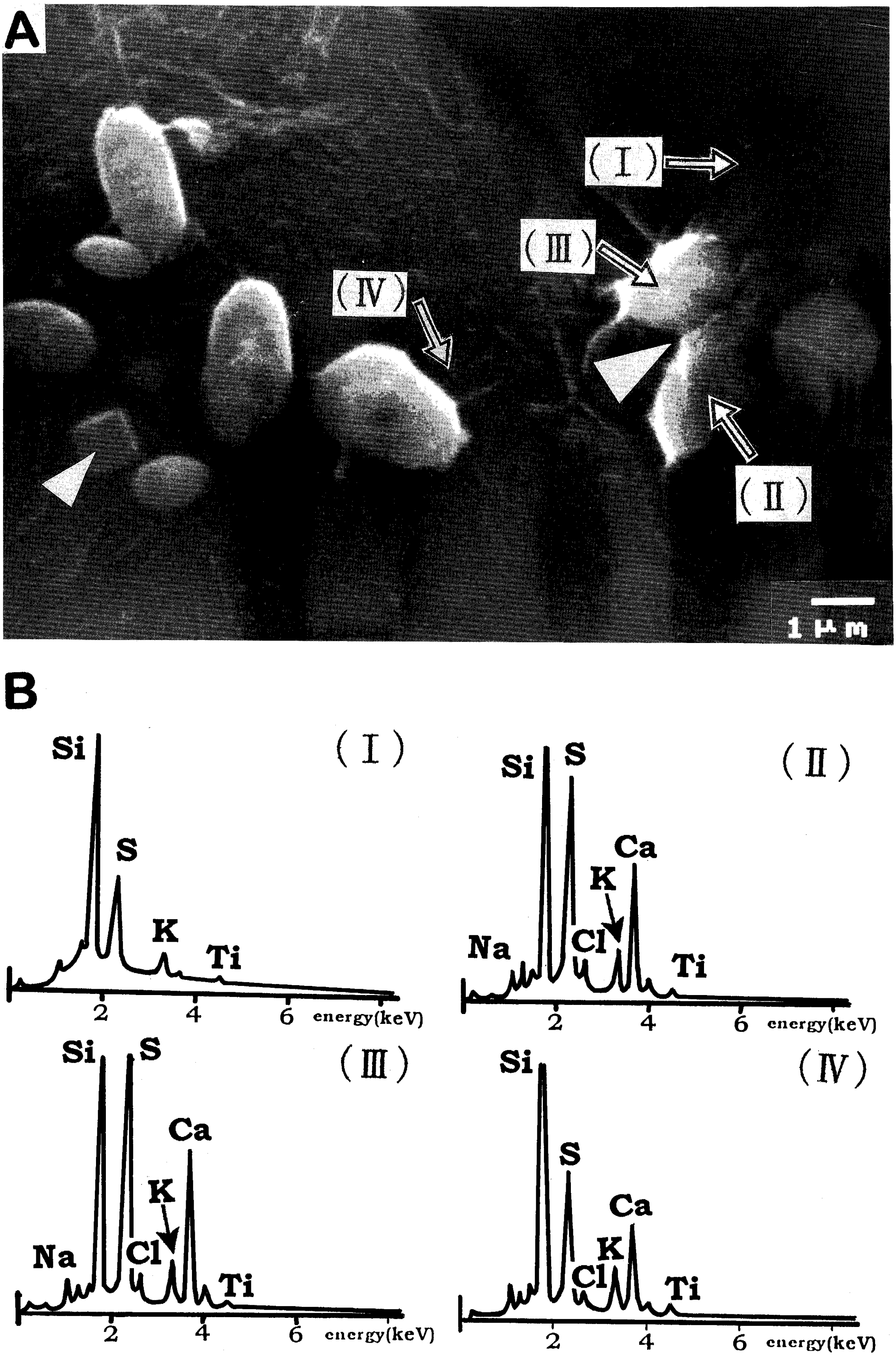

Figure 2 SEM image of microorganisms living in the vicinity of the globular oil particles in the sample collected from Akasaki in Togi Machi, Noto Peninsula of Ishikawa Prefecture (A), and EDX spectra (B).

The SEM observation found the microorganisms adhering to the oil particle (a right white arrow in the Figure 2A). A left white arrow in Figure 2A indicates a crystalline salt. Note the differential intensity of S in the EDX spectrum between the oil particle and the microorganisms. Each analytical point is indicated by a Roman figure in Figure 2A.

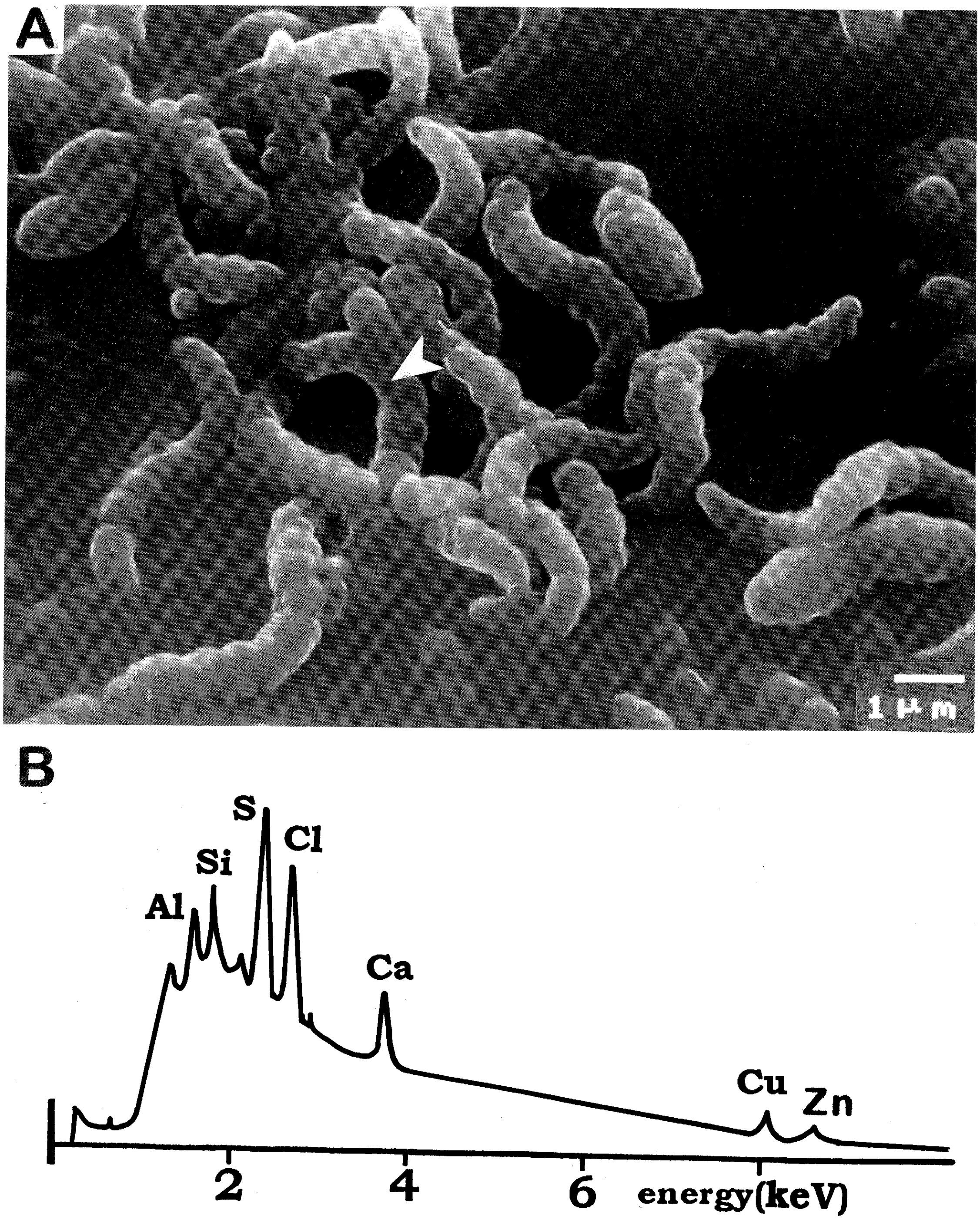

Figure 3 SEM image of the microbial community in the oil slick exuding from the oil ball collected at Aramiko-jima Island, off shore of Wajima city, Ishikawa Prefecture. In comparison with the microbial communities in the SAMPLE 1 as shown in Figure 1, the prominent species of microorganisms make large communities on the surface of the oil slick. (A). The EDX spectrum of the microorganism shows the significant peak of S. The analytical point is indicated by a white arrow in Figure 3A.