Bioremediation of oil contaminated soils -ultivation experoments using an oil adsorption-resolution item "Sponge"-

BIOREMEDIATION OF OIL CONTAMINATED SOILS

– CULTIVATION EXPERIMENTS USING AN OIL ADSORPTION-RESOLUTION ITEM "SPONGE"-

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ABSTRACT

Laboratory scale experiments using a commercial oil adsorption-resolution item "sponge" were carried out for the potential bioremediation of soils and factory drainage water polluted by oil. Optical microscopic observation revealed that the "sponge" contains various microorganisms. High activity of microorganisms might resolve oil adhering to "sponge" under certain conditions. Changes of qualities of the cultivation solutions showed that microbial activities depend on cultivation systems in the ratio of "sponge" and solution. In addition, the use of "sponge" in the field must be paid attention to prevent secondary effects to the surrounding environments.

INTRODUCTION

Oil spill pollution of soil and water continues to be a significant environmental problem. Most of the large oil spills that have received much attention have occurred in marine environments. Parallel concern for the freshwater environment has lagged behind. However, oil spills do occur in freshwaters as a consequence of the many oil-related activities in this environment (Bhattacharyya et al., 2003). For example, many industrial activities related to the exploration of natural resources cause heavy disturbances of the ecosystems due to the relocation of natural materials (Liu and Sulfita,

1993).

One of the recent examples of oil spill accidents is that of the *Nakhodka* Russian oil tanker which discharged approximately 6,240 kl of C-heavy oil into the Japan Sea 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 from Mikuni, Fukui Prefecture to Noto Peninsula, Ishikawa Prefecture (Tazaki et al. 1997a; Tazaki et al. 1997b; Tazaki et al. 1997c; Tazaki 1998). Since 1997 a careful bioremediational study by our group has been performed to clean up oil spill and accelerate the rate of hydrocarbon degradation such as 1) degradation of oil by ultraviolet-rays, 2) separation of oil, water and sand by warmed up seawater, 3) isolation and characterization of indigenous hydrocarbon-degrading bacteria, 4) the use of clay minerals such as smectite and kaolinite as adsorbents and microbial growth-support media (Tazaki et al., 1997; Chaerun et al., 2002a, 2002b, 2002c, 2002d, 2002e).

Exploitation of microorganisms capable of degrading hydrocarbons has become a promising approach to restore contaminated sites. Bacteria are considered to represent the predominant agents of hydrocarbon degradation in the environment (Leahy and Colwell, 1990). In addition, microorganisms are able to accumulate specific elements from environments and fix them (Tazaki et al., 2003). Hence, it is most likely to use such materials mentioned above in combination with microorganisms to remediate the sites polluted by oil spill. Their use for bioremediation process is generally low cost, accessible, and results in the prolonged microbial degradation of oil (Chaerun et al., 2002d). Nonetheless, care must be taken in employing such materials, because their incorrect application would be harmful to the surrounding ecosystems.

The major objective of this paper is to describe the effect of utilizing a commercial oil adsorption-resolution item "sponge" in laboratory microcosms for the possibility of bioremediation of soils and factory drainage water contaminated by oil.

SAMPLES AND METHODS

Samples, quality of cultivation solutions and optical microscopy

The laboratory cultivation systems were designed in a static batch culture .There was

eighteen (18) cultivation systems, including controls (Table 1): Cultivation systems (No. 1-6) contained seawater, underground water, oil contaminated sea sand (collected from Shioya Seashore, Japan), 2 kinds of oil contaminated soils (type 1 and type 2), and "sponge" that is a commercial oil adsorption-resolution item. These cultivation systems were incubated aerobically at room temperature for 10 days under intermittent illumination. Cultivation systems (No. 7-15) contained "sponge", surfactant, and 2 kinds of painting factory drainage water (type 1 and type 2). The controls (No. 16-18) contained "sponge", underground water, and distilled water. These cultivation systems were incubated aerobically at room temperature for 30 days under intermittent illumination. The samples were removed and cultures were further monitored during the experimental period, and the water parameters such as pH, Eh (electrical potential versus the standard hydrogen electrode), EC (electrical conductivity), DO (dissolved oxygen) and WT (water temperature) were measured for experimental water samples using a portable inspection meter made by HORIBA. The distribution, color, form, and texture of microbial films formed on glass sides that was set up in all cultivation vessels were also intermittently observed using a differential interference microscope (Nikon NTF2).

Energy dispersive X-ray fluorescence spectroscopy (ED-XRF)

After the cultivated solution and "sponge" were air-dried at room temperature, the powder samples were mounted on Mylar films and the chemical compositions were analyzed by an energy dispersive X-ray fluorescence spectroscopy (ED-XRF; JEOL JSX-3201), using Rh-Kα generated at 30 kV under vacuum condition. A mixture consisting of Al, Mn, and Mo were used for correction of energy. Conversions of intensities into concentrations were accomplished using a standard less FP-Bulk program set in a computer. In case of cultivated solutions, the chemical compositions were analyzed before and after cultivation.

Table 1. List of 18 cultivation systems.

No.	Samples (g or ml)	Sponge (g)	Sea water (ml)	Underground water (ml)	Distilled water (ml)
1	Oil contaminated sea sand (5g)	5	150	-	-
2	Oil contaminated sea sand (5g)	-	150	-	-
3	Oil contaminated soil (type 1; 5g)	5	-	150	-
4	Oil contaminated soil (type 1; 5g)	-	-	150	-
5	Oil contaminated soil (type 2; 5g)	5	-	150	-
6	Oil contaminated soil (type 2; 5g)	-	-	150	-
7	Painting factory drainage water (type 1; 150ml)	15	-	-	-
8	Painting factory drainage water (type 1; 150ml)	5	-	-	-
9	Painting factory drainage water (type 1; 150ml)	-	-	-	-
10	Painting factory drainage water (type 2; 150ml)	15	-	-	-
11	Painting factory drainage water (type 2; 150ml)	5	-	-	-
12	Painting factory drainage water (type 2; 150ml)	-	-	-	-
13	Surfactant (150ml)	15	-	-	-
14	Surfactant (150ml)	5	-	-	-
15	Surfactant (150ml)	-	-	-	-
16	Control	15	-	150	-
17	Control	5	-	150	-
18	Control	5	-	-	150

RESULTS AND DISCUSSION

Qualities of cultivated solutions

pH

The presence of "sponge" apparently resulted in a decrease of the pH of the solution, suggesting that "sponge" had a profound effect of acidification (Fig. 1, 2, 3 and 4). In case of cultivation systems from No. 7 to No. 18, the solution pH fluctuated more or less at the first 2 weeks, expect for No. 18 of cultivation system (Fig. 3 and 4). After 2 weeks, the solution pH of all cultivation systems took steady state. The results indicated

that activities of microorganisms in cultivation vessels were high during the initial 2 weeks. Microbial growth generally decreased the pH of the solution. Presumably, the presence of "sponge" was favorable for microorganisms to grow well, eventually resulting in lowering the pH.

Eh

In cultivation systems using sea water, underground water and distilled water (No. 1 - 6, and No. 16-18), solutions containing "sponge" showed high Eh in comparison with the other solutions, suggesting that "sponge" bring about an increase of Eh in the solution (Fig. 1, 2 and 4). In case of cultivation systems that were incubated 30 days for (No. 7 - 18), the Eh fluctuated more or less at the initial 2 weeks (Fig. 3 and 4). After 2 weeks, the Eh relatively took steady state. The results indicated that activities of microorganisms in cultivation vessels were high during initial 2 weeks.

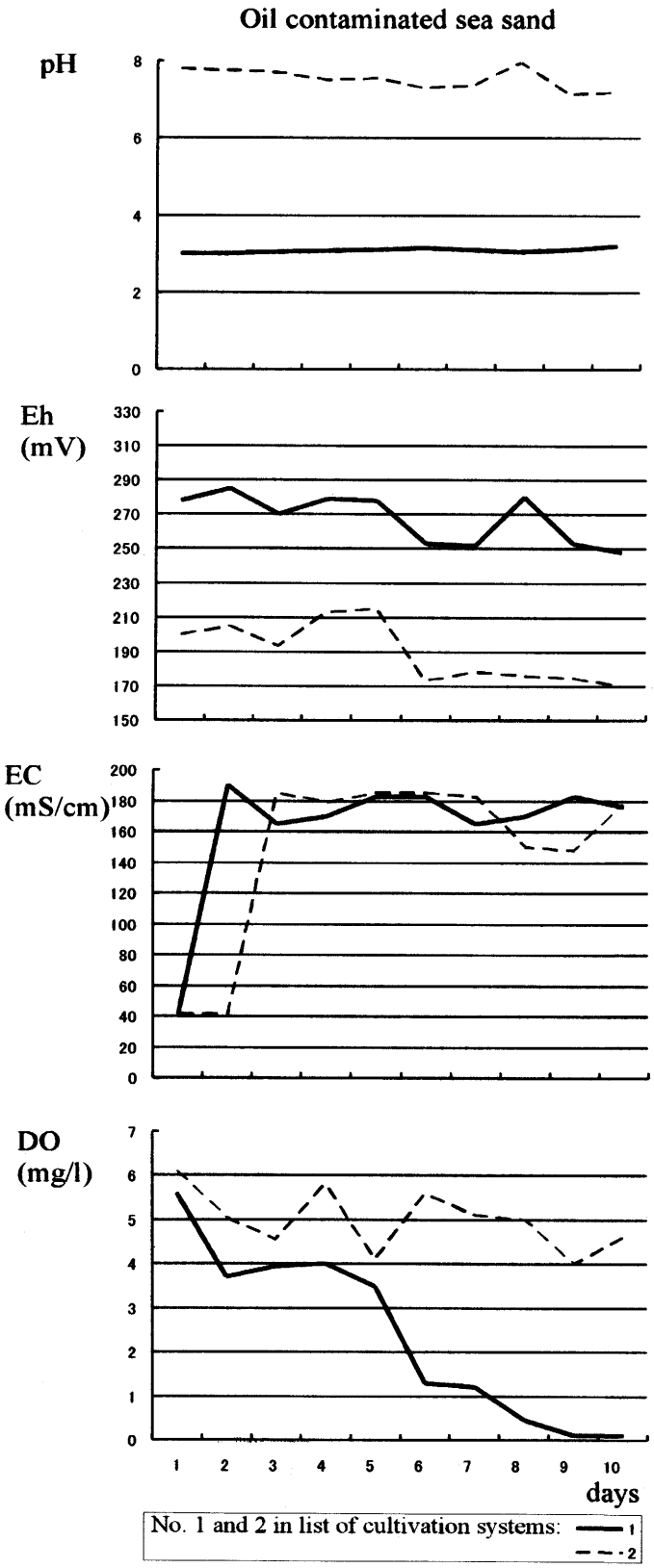
EC

The solution of all cultivation systems containing "sponge" showed high EC at the first 3 days in comparison with the other solutions, expect for cultivation systems using sea water (No. 1 and 2), suggesting that "sponge" bring about an increase of EC (Fig. 2, 3 and 4).

DO

In cultivation systems using sea water and underground water (No. 1 - 6), DO of solutions containing "sponge" decreased at the first several days, (Fig. 1 and 2). In the other cultivation systems, DO of all solutions decreased at the initial several days (Fig. 3 and 4), indicating that microorganisms in cultivation vessels consumed dissolved oxygen.

In cultivation systems containing oil contaminated soil, DO of both types showed similar tendency (Fig. 2). In systems of painting industrial wastewater, DO of both types showed different tendency each other (Fig. 3). In the type 1 of painting factory drainage water, DO of No. 7 containing 15g of "sponge" increased at the initial several days, and DO of No. 8 containing 5g decreased. In the type 2, DO of No. 10 containing 15g of



"sponge" decreased at the initial several days, while DO of No. 11 containing 5g increased. The results suggested that the activities of different type of microorganisms were very sensitive to the ratio of "sponge" and solution. In addition, the solution water temperature in all cultivation systems ranged between 15 and 25 °C.

Figure 1 Quality of cultivated solutions using oil contaminated sea sand (pH, Eh, EC, DO) for 10 days aging. Note that solutions containing "sponge" showed low pH. DO of solutions containing "sponge" decreased at the initial several days.

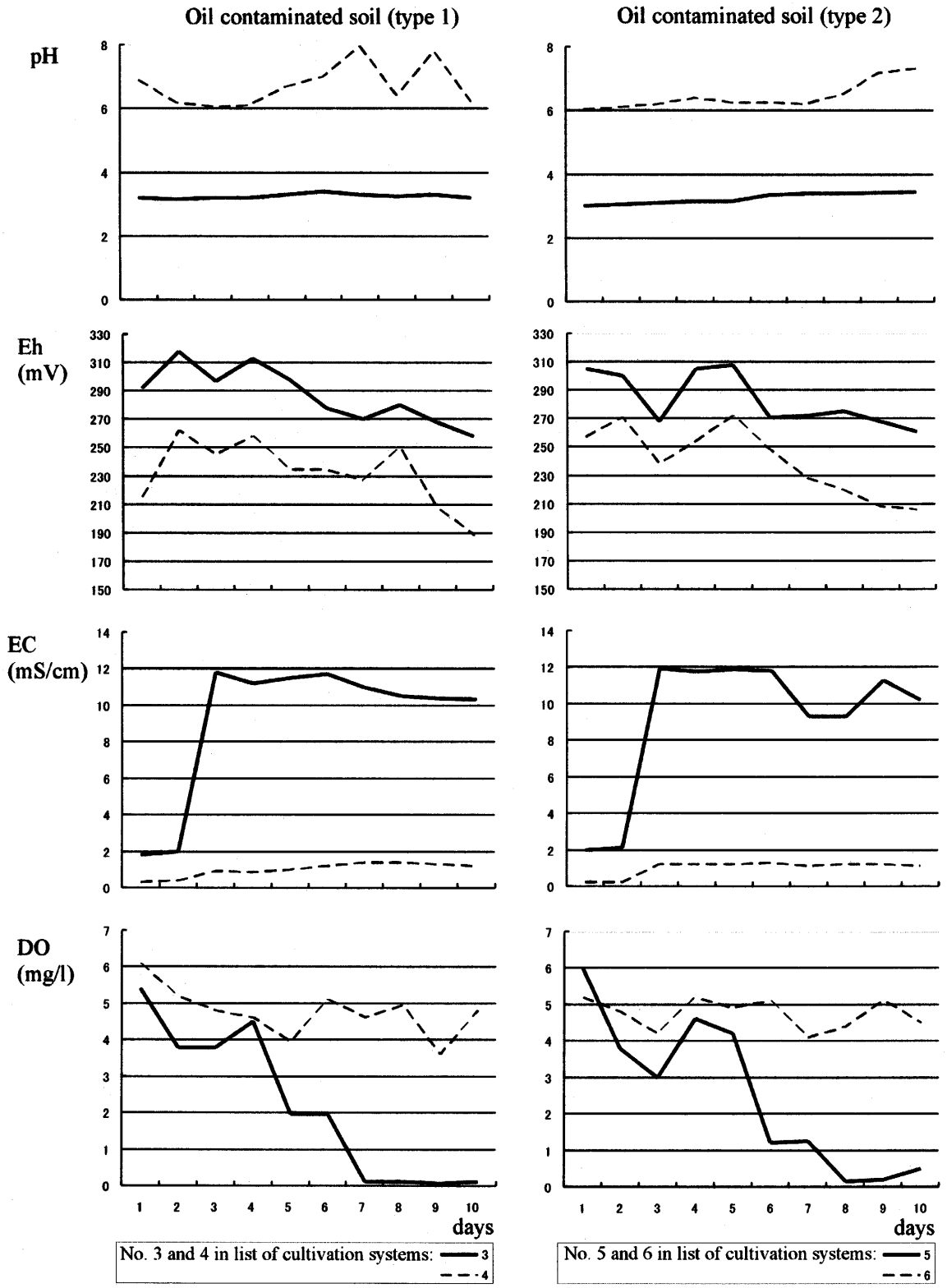


Figure 2 Quality of cultivated solutions using oil contaminated soil (pH, Eh, EC, DO) for 10 days aging. Note that solutions containing "sponge" showed low pH. DO of solutions containing "sponge" decreased at the initial several days.

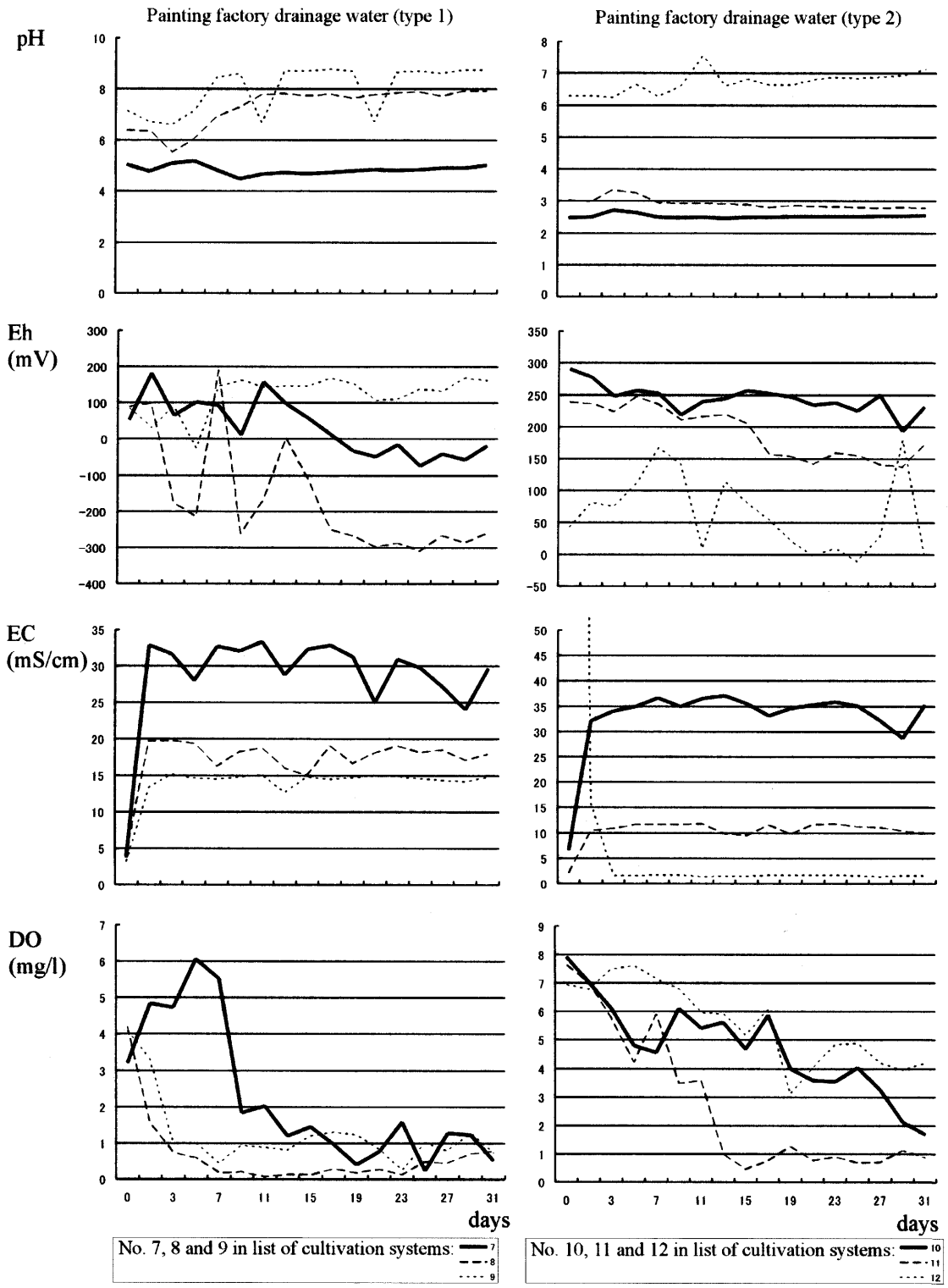


Figure 3 Quality of cultivated solutions using painting factory drainage water (pH, Eh, EC, DO) for 30 days aging. Note that DO of all cultivation solutions decreased at the initial several days, indicating that microorganisms in cultivation vessels consumed dissolved oxygen. DO of both types showed different tendency each other. The results suggested that the activities of different type of microorganisms were very sensitive to the ratio of "sponge" and solution.

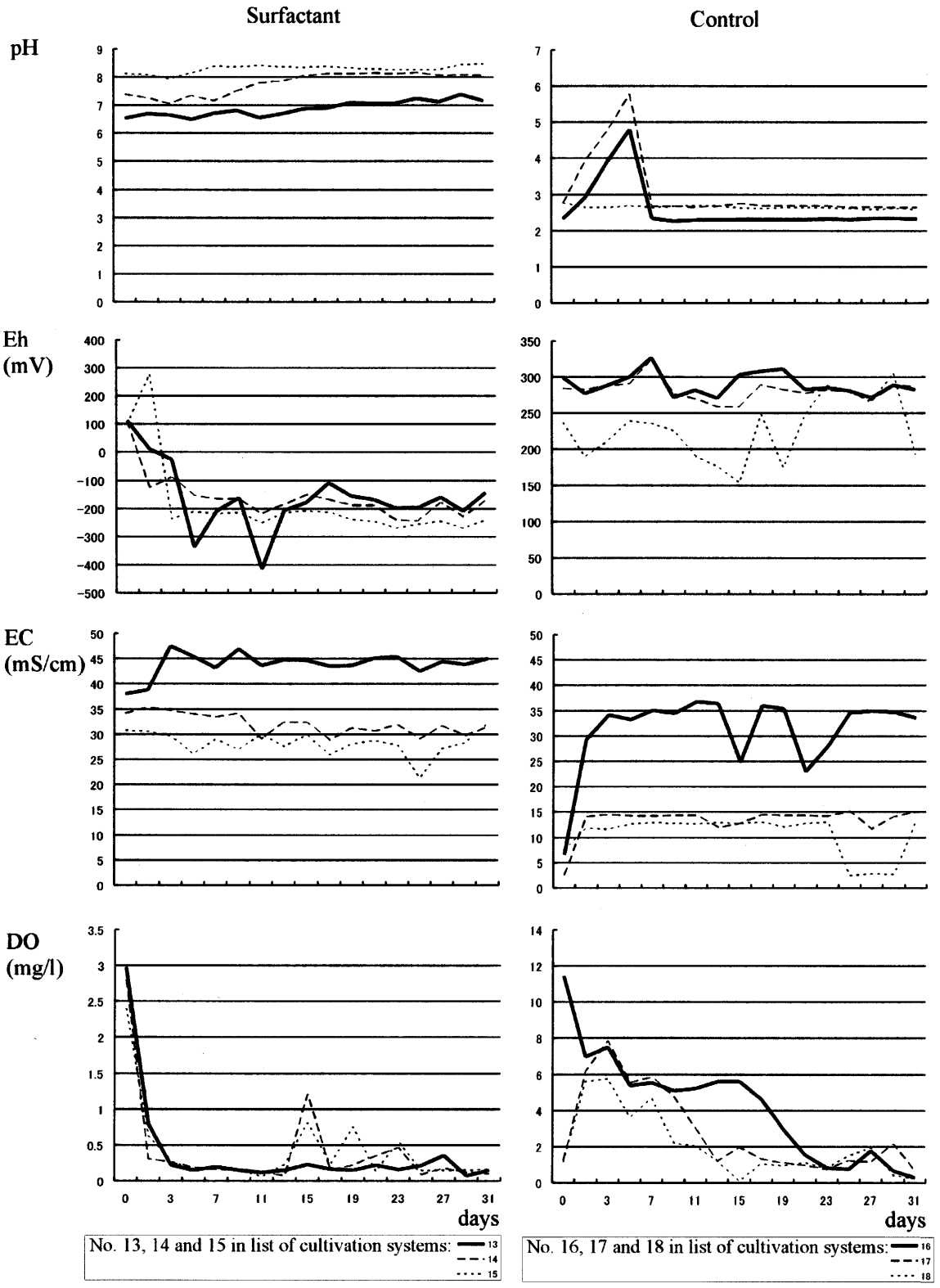
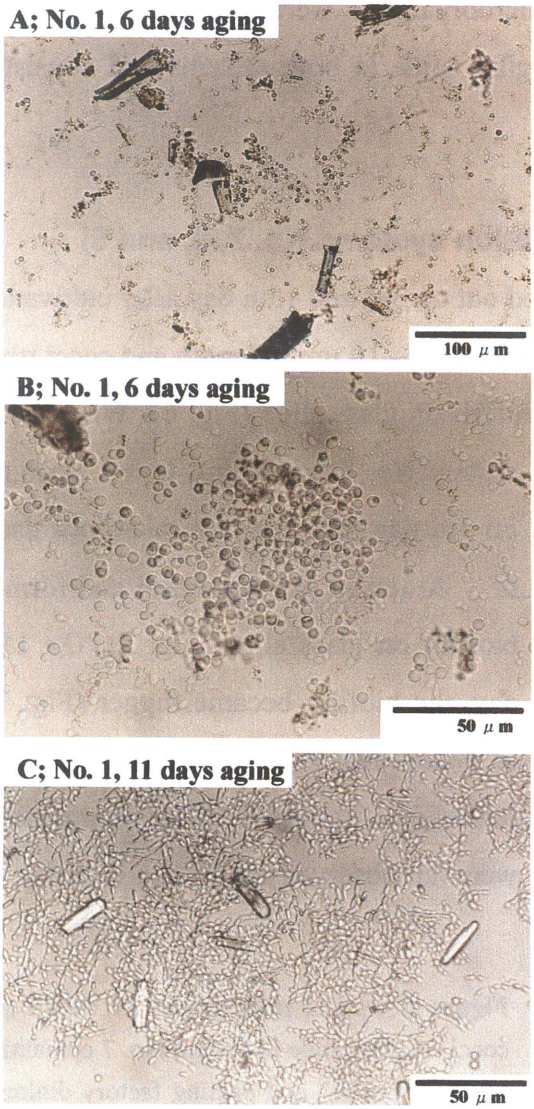


Figure 4 Quality of cultivated solutions using surfactant, and underground water, distilled waster as control (pH, Eh, EC, DO) for 30 days aging. Note that DO of all solutions containing surfactant decreased at the initial a few days.

Optical microscopic observation

Oil contaminated sea sand (cultivation systems No. 1 and 2)



Optical microscopic observation was carried out on 6th and 11th day after cultivation. In system No. 1 containing "sponge", cocci of ~ 5 μm in diameter were dominant in 6 days aging (Fig. 5A and B). The cocci were found around sawdust-like materials in "sponge". Bacilli of ~ 5 μm in length were dominant in 11th days aging (Fig. 5C). The bacilli link together about 30 μm in length. Inversely, microorganisms were rarely found in system No. 2 on 6th and 11th day aging.

Figure 5 Optical micrographs of microorganisms on glass slide in system No. 1 containing "sponge" and oil contaminated sea sand. A: Cocci around sawdust-like materials in "sponge". B: Cocci of ~ 5 μm in diameter in 6 days aging. C: bacilli of ~ 5 μm in length were dominant in 11th days aging.

Oil contaminated Soil (cultivation systems No. 3 and 4)

Optical microscopic observation was carried out on 6th and 11th day after cultivation. In system No. 3 containing "sponge", cocci of ~ 5 μm in diameter were dominant in 6 days aging, colony of bacilli were also partially found. On 11th day, biofilm was found on glass slide and consisted mainly of cocci. Bacilli were also found. In system No. 4 not containing "sponge", bacilli and filamentous bacteria were found, indicating that these bacteria live originally in oil contaminated soil or underground water. These bacteria also gathered around drops of oil on 11th day aging.

Oil contaminated Soil (cultivation systems No. 5 and 6)

Optical microscopic observation was carried out on 6th and 11th day after cultivation. In both systems No. 5 and 6 on 6th day, microorganisms were rarely found on glass slide. On 11th days, biofilm was found on glass slide in both systems and consisted mainly of bacilli of ~ 5 μm in length.

Painting factory drainage water (cultivation systems No. 7, 8, and 9)

Optical microscopic observation was carried out on 3rd and 15th day after cultivation. In system No. 7 containing 15g of "sponge", bacilli of ~ 10 μm in length and cocci were found on 15th day. These bacteria formed biofilm on glass slide (Fig. 6). Whereas, in system No. 8 containing 5g of "sponge" on 3rd day, hanging-like microorganisms of ~ 40 μm in size increased and formed biofilm on glass slide (Fig. 7). On 15th day, the biofilm became bigger (Fig. 7). In system No. 9 not containing "sponge", remarkable activities of microorganisms were not observed on 3rd and 15th day.

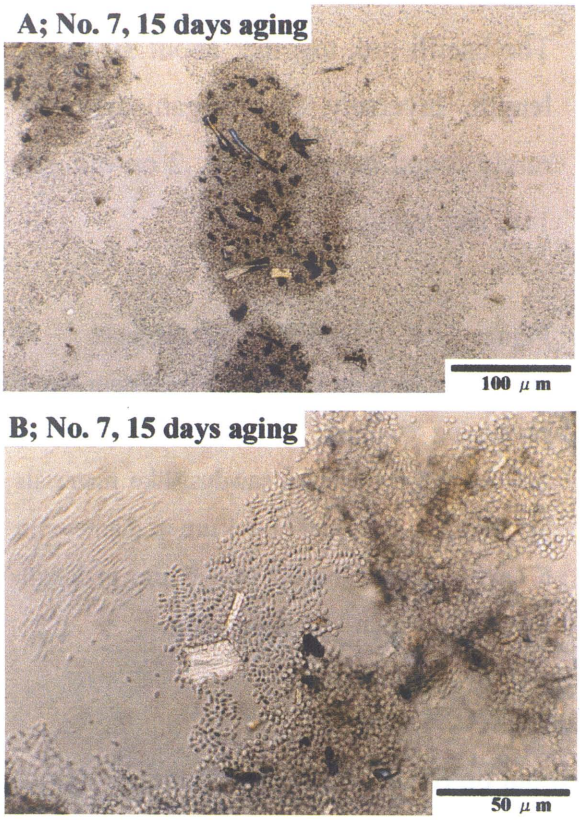
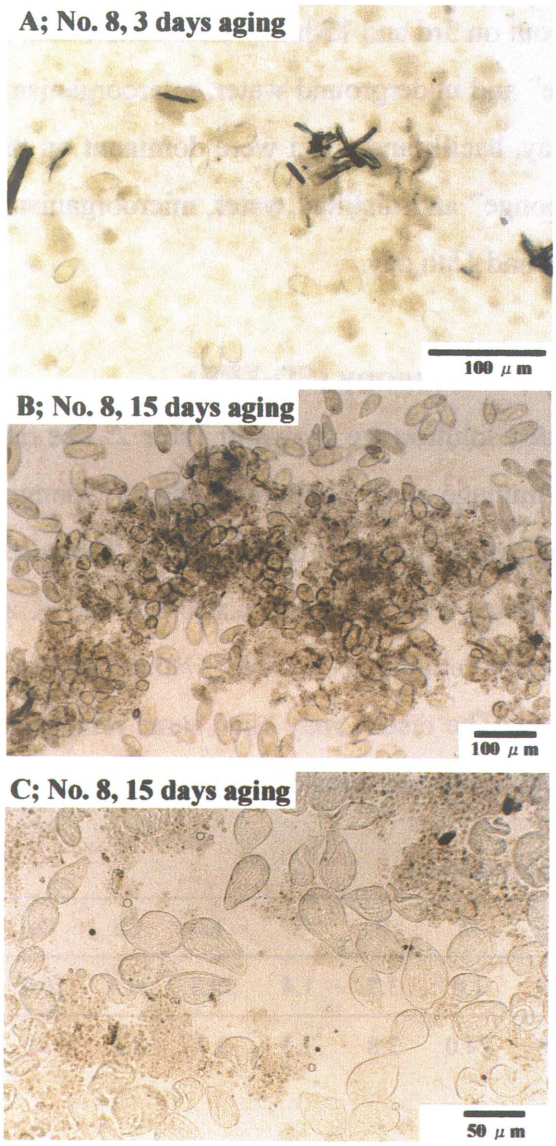


Figure 6 Optical micrographs of bacilli and cocci on glass slide in system No. 7 containing 15g of "sponge" and panting factory drainage water. A: biofilm on glass slide on 15th day. B: Close-up photo of a part of the biofilm showing a colony of bacilli and cocci.

Painting factory drainage water (cultivation systems No. 10, 11, and 12)

Optical microscopic observation was carried out on 3rd and 15th day after cultivation. In system No. 10 containing 15g of "sponge", bacilli of ~ 5 μm in length were found on 15th day. The microbial activities were very high. In system No. 11 containing 5g of



"sponge", bacilli of ~ 10 μm in length and cocci were found on 15th day. Bacteria formed biofilm on glass slide. In system No. 12 not containing "sponge", microorganisms were rarely observed on 3rd and 15th day.

Figure 7 Optical micrographs of hanging-like microorganisms of ~ 40 μm in size on glass slide in system No. 8 containing 5g of "sponge" and panting factory drainage water. A: biofilm formed by hanging-like microorganisms on glass slide on 3rd day. B: biofilm formed by hanging-like microorganisms on glass slide on 15th day. C: Close-up photo of a part of the biofilm showing a colony of hanging-like microorganisms.

Surfactant (cultivation systems No. 13, 14, and 15)

Optical microscopic observation was carried out on 3rd and 15th day after cultivation. In system No. 13 and 14 containing "sponge", oil drops of 1 μm to 40 μm in diameter were dotted on glass slides. Although bacteria were also found on the both slide, the amounts are small in comparison with that of painting factory drainage water. While, in system No. 15 not containing "sponge", microorganisms were not observed on 3rd and 15th day.

Control systems (Underground water: No. 16 and 17, Distilled water: No. 18)

Optical microscopic observation was carried out on 3rd and 15th day after cultivation. In system No. 16 and 17 containing "sponge" and underground water, microorganisms were rarely observed on 3rd day. On 15th day, bacilli and cocci were dominant on the glass slide. In system No. 18 containing "sponge" and distilled water, microorganisms were rarely observed on the glass slide on 3rd and 15th day.

Energy dispersive X-ray fluorescence spectroscopy (ED-XRF)

ED-XRF analyses of "sponge" and cultivated solution are shown in Table 2. The rate of P and K in two kinds of painting factory drainage water containing 15g of "sponge" (No. 7 and 10) increased after cultivation. The results indicated that the P and K resulted from "sponge". It may be that "sponge" contains a lot of easily soluble P and K as nutrient for microorganisms. In case of surfactant containing "sponge" (No. 13 and 14), the rate of P and Ca increased after cultivation. The results may have some relation to the fact from optical microscopic observation that the activities of microorganisms were

Table 2. ED-XRF analyses of "sponge" and cultivation solutions.

	Na	Mg	Si	P	S	K	Ca	Fe
Sponge (solid)	-	2.0	1.6	3.7	1.4	39.3	34.4	16.9
Solution using in systems No. 7 and 8	47.9	5.0	24.0	3.8	7.4	3.7	8.2	-
After cultivation								
Solution of system No. 7	5.8	8.2	9.2	14.2	16.3	34.0	11.6	0.2
Solution of system No. 8	38.4	4.3	12.8	2.9	11.7	29.8	-	-
Solution using in systems No. 10 and 11	17.4	11.5	16.2	12.0	13.0	1.3	24.9	2.0
After cultivation								
Solution of system No. 10	-	13.1	3.4	22.4	17.3	27.9	12.8	1.8
Solution of system No. 11	-	17.8	6.2	7.1	11.0	24.4	30.8	2.7
Solution using in systems No. 13 and 14	-	-	4.6	30.1	-	45.1	17.5	2.6
After cultivation								
Solution of system No. 13	-	-	-	55.0	-	-	45.0	-
Solution of system No. 14	-	-	-	51.1	-	11.0	37.9	-

Values shows rate of elements in samples heavier than oxygen. -: not detected.

low in comparison with that in two kinds of painting factory drainage water.

CONCLUSIONS

Optical microscopic observation revealed that a commercial oil adsorption-resolution item "sponge" used in this study contains various microorganisms. High activity of microorganisms might resolve oil adhering to "sponge". The laboratory microcosm experiments showed that microbial activities depend on cultivation systems in the ratio of "sponge" and solution. The use of oil adsorption-resolution item "sponge" may be very likely to remediate most of the oil contaminated areas in an environmentally friendly, relatively nontoxic way with an optimum "sponge" amendment level, since its use significantly decreased pH of the solution.

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