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| 보 문 |

Efficient Clean-up of Oil Spilled Shorelines Using the Compressed Air Jet System and Concomitant Microbial Community Analysis

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압축공기 분사시스템을 이용한 유류오염 해안의 효율적 정화 및 이에 따른 미생물군집분석

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The objectives of this study were to investigate effectiveness of the Compressed Air Jet (CAJ) System for cleaning up shorelines contaminated with crude oils and to examine effects of the system on total petroleum hydrocarbon (TPH) removal and microbial community changes before and after remediation of the oil-contaminated shorelines. These data will lead to better understanding of optimized remediation process. About 66% of TPH reduction was observed when the contaminated site was treated with the CAJ System 2, 3, 4, and 5 times. This treatment system was more efficient than the seawater pumping system under similar treatment conditions (by 40%). By the way, little oil degrader communities were observed despite a potential function of the air jet system to stimulate aerobic oil degraders. The apparent low population density of the oil degraders might be as a result of low concentration of TPH as a carbon source and limiting nutrients such as nitrogen and phosphorus. It was proposed that the CAJ System would contribute significantly to removal of residual oils on the shorelines in combination with addition of these limiting nutrients.

***Keywords*:** compressed air jet, oil spill, sediment remediation, shoreline, total petroleum hydrocarbon (TPH)

The industrial development accompanied by population increase caused rapid consumption of oils. As a result, history observed many oil spill accidents at various levels. In 2007 one of the worst oil spill accidents in Korea happened due to a broken oil tanker, Hebey Spirit, off Taeahn coast, being recorded as one of the biggest international oil spill accidents. Oil spilled in the marine environment typically causes both immediate and long-term environmental damage (Bejarano and Michel, 2010). Once oil is spilled by broken tankers, pipelines or offshore oil rigs, it usually drifts into the shorelines and massively contaminates them and then goes through physicochemical and biological dispersion or degradation (Seo and Song, 1994; Koh *et al*., 1998). While low molecular weight and volatile compounds are dissipated, the residual oil (usually Bunker C oil and asphaltenes, etc.) then can sorb to mudflat, sand, gravel or rock, where they become more difficult to be removed. The oil sorbents and chemical surfactants for oil washing and dispersion have been used to clean up oil spills on the shorelines but they are costly and causing toxicities in the ecosystem (Seo and Song, 1994). Various bioremediation technologies have been effectively used to circumvent these problems (Altas, 1981; Altas and Bartha, 1992; Zhang and Miller, 1994; De Acevedo and McInnerney, 1996; Choi *et al*., 1997; Roenberg and Ron, 1997; Sprocati *et al*., 2012) but these are rather time consuming and selective. Several factors which affect the rate at which indigenous or bioaugmented microbes function in affected sites have been discussed (Fernández-Luqueño *et al*., 2011). In this study an alternative clean up system has been developed to meet a rapid removal of high contaminated oil from various substrates such as sediments, gravels, and rocks as well as to provide air to the polluted environment. The Compressed Air-Jet (CAJ) System developed

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Table 1. Experimental conditions for a field demonstration of the remediation by the Compressed Air Jet (CAJ) System

Parameter	Experimental conditions
Date	8:00 am, Oct. 28, 2010
Temperature	Air 8℃; water 10℃; soil 12℃
Test site:	Taeahn, Choongnam, Korea
Equipments tested and running conditions	High pressure water pump (flow rate: 80 m^3/h)(as a control) Air jet system with compressor (3-5 MPa/kgf/cm^2)
Test runs and time taken	5 runs; 30 min per each run
Test area	High pressure pump: 250 m^2 (5 m×5 m) Air jet system: 300 m^2 (5 m×6 m)
Soil sample collection	Collected after each run (1 kg)

by JH Green, Inc. (Korea) can easily reach, separate and remove spilled oils clinging to rock and grain of sand. The CAJ System generates high pressure bubbles and makes the air bubbles collide one another in aqueous environment to form micro air bubbles ultimately used to effectively separate contaminated materials from the sorbed phase. The micro air bubbles float the precipitated oils, and floated oils can be easily removed by absorbing cloths. The CAJ System is economical, eco-friendly remediation technology, and could be effective for the subsequent bioremediation compared to conventional methods. The objectives of this study were to investigate effectiveness of the CAJ System for cleaning up shorelines contaminated with crude oils and to examine effects of the system on total petroleum hydrocarbon (TPH) removal and microbial community patterns in oil-contaminated shorelines.

Materials and Methods

On-site test of the CAJ System

A field demonstration of the remediation was accomplished under the experimental conditions shown in Table 1. The remediation process using the CAJ System generally followed the procedure described below: geological analysis; determination of oil spill area; set-up of oil boom and fence; piling up of gravels and sands; treatment of contaminated soil and sediment using the CAJ System in the presence of flowing tide; removal of floated oils using sorbents; ground leveling. The CAJ System (Fig. 1) is used to generate air bubbles including micro air bubbles which can float spilled oil in the sediment or on the solid surface. Air bubbles could float precipitated oils along with water and sediments (soils). Some volatile gases and non-volatile compounds appeared to be separated and the floated oils were able to be easily removed by absorbing cloths. Air bubbles could float the precipitated oils along with water and sediments (soils) (Fig. 2A and 2B). The CAJ System would make the subsequent bioremediation more effective as long as limiting nutrients and other environmental conditions are met.

Soil sampling

Sediment soils for the total petroleum hydrocarbon (TPH) and microbial community analysis were taken following the protocol (ES 07130) from the Official Standard Methods for Contaminated Soils (Ministry of Environment, 2009).

Analysis of TPH

TPH analysis was performed based on the protocol (Item 18) from the Official Standard Methods for Contaminated Soils (Ministry of Environment, 2009). The instrument used was GC-FID: Model, QP-5000 (Shimadzu, Japan) with HP-5 capillary column (50 m × 0.2 mm × 0.33 μm) and analytical conditions

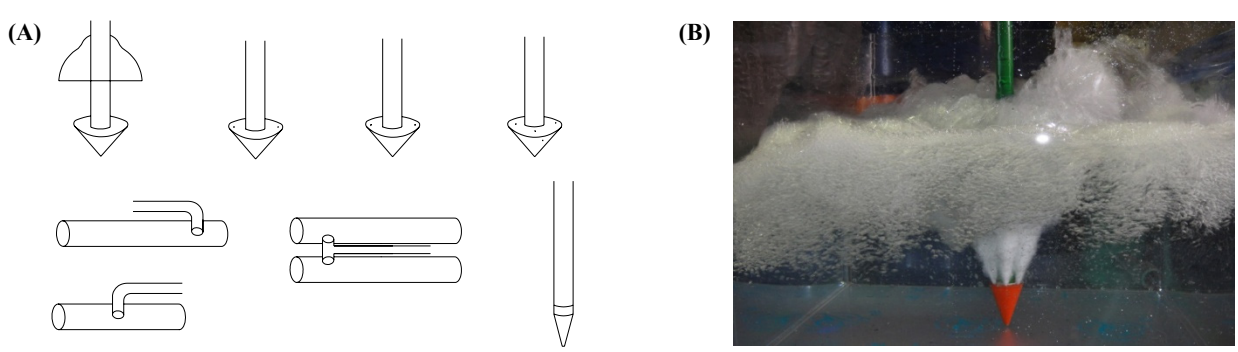

Fig. 1. Various types of compressed air-jet system (A) and operation of the bar type air jet system in water (B).

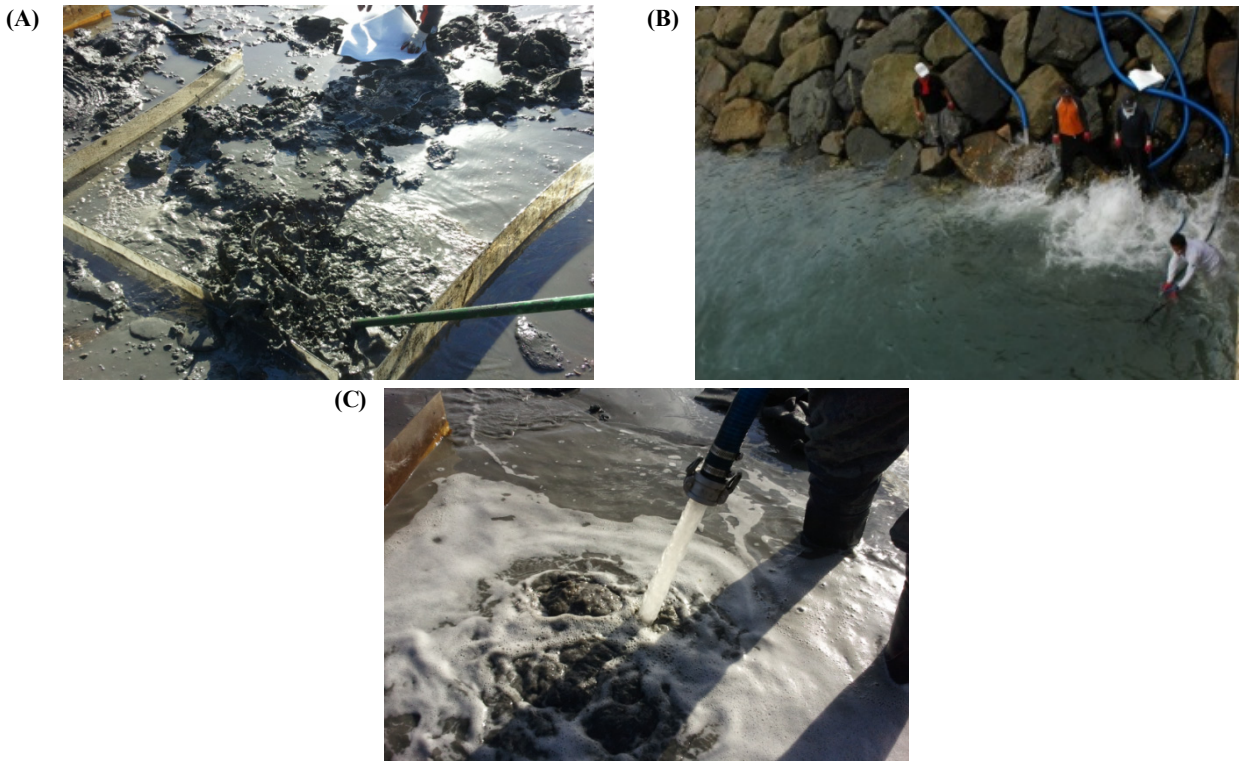

Fig. 2. Clean up of field precipitated oils in the sediments (A) and on the rock surfaces by the air jet system (B) and in the sediments by water pumping (C).

were: temperature gradient 2 min at 80℃, temperature increase 8℃/min, and 10min at final temperature (320℃); temperatures at inlet and detector were 300℃ and 320℃, respectively; nitrogen gas as a carrier gas; sample injection 1 μl.

Microbial community analysis

Total DNA extraction was made using Fast DNA extraction Kit (Bio101, USA) and PCR-DGGE was conducted for the microbial community analysis following the previous report (Ekpeghere *et al*., 2009).

Results and Discussion

Clean-up of the contaminated shorelines using the CAJ System

In the on-site demonstration test, after treating the site with the CAJ System once, TPH was reduced by 26% compared to that before the treatment (control: bt) (Fig. 3). About 66% of TPH reduction was observed when the contaminated site was treated with the CAJ System 2, 3, 4, and 5 times (a-2, a-3, a-4, and a-5). After treating the site with the CAJ System 2 times, no further decrease of TPH was observed, indicating the TPH removal from the site was quite effective. In case of seawater pumping, however, reduction of TPH was observed about 60% after treating the site once and twice, and the reduction of TPH was about 40% and 25% after treating the site 3 times and 4 times, respectively. This indicates that in the seawater pumping, removal of sorbed oil was not complete resulting in sorbed oil release at more than 2 times of seawater washing. Overall, the seawater pumping showed less oil removal efficiency compared to the CAJ System.

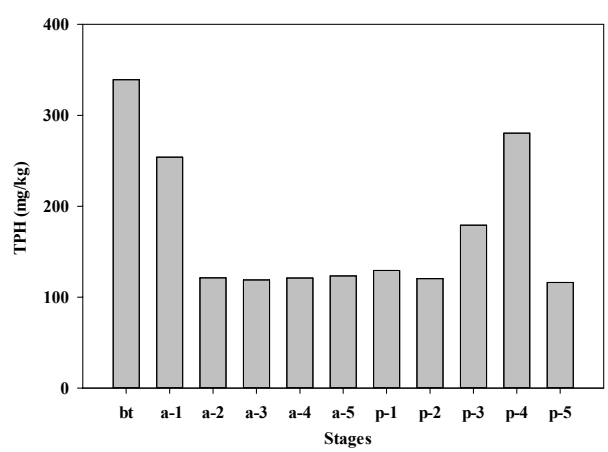

Fig. 3. Effects of the air jet and pumping treatments on TPH removal in a demonstrated site; bt, before treatment (control); a-1, 2, 3, 4 and 5, air jet system treatment; p-1, 2, 3, 4 and 5, water pumping system; all data were the average value of duplicate samplings .

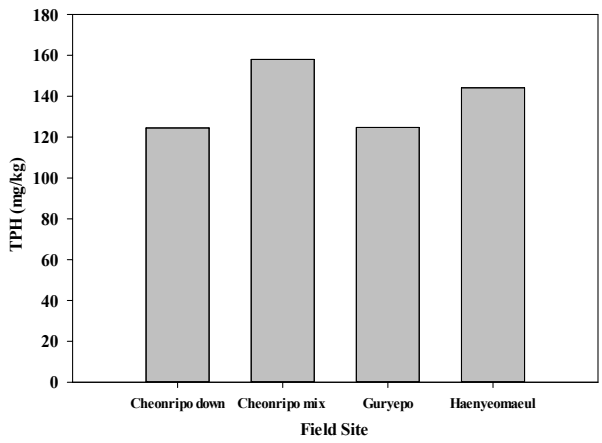

Fig. 4. Effects of the air jet treatment on TPH removal in the field sites (Haenyeomaeul: pumping as control); all data were the average value of triplicate samplings.

Treatments with the CAJ System were also examined in other contaminated field sites (Cheonripo down, Cheonripo mix, and Guryepo) with Haenyeomaeul as a control (Water pumping). After the treatment, the concentrations of TPH were about 14% lower in Cheonripo down and Guryepo than Haenyeomaeul (control) but the levels of TPH in Cheonripo-mix were about 7% higher that the control. This indicates TPH treatment effects might be variable depending on the treated sites (Fig. 4).

Microbial community analysis of the treated sites (demonstration and other field sites)

Microbial community analysis based on PCR-DGGE of the mudflats (demonstration sites) after remediation was shown in Fig. 5. The community identification analysis results were shown in Table 2. Although most of these microbial communities have not been identified as oil degraders, most of them have been confirmed to be of sea and sediment origin. Even though the air jet system was supposed to stimulate oil degraders, the identified communities appeared to include little oil degraders. This may indicate that there were oil degrader populations with relatively low density if any. The low density might be due to the low concentration of TPH as a carbon source and limiting nutrients (nitrogen and phosphorus, etc.) and other limiting factors (temperature, etc.). The microbial community profiles of all samples including control (before treatment) appeared to be quite similar except bands 15 and 16 which were uniquely present in the control. The gradual community change was observed over a week period as bands 17 and 18 were unique in the air jet treatment and the bands 19, 20, 21, and 22 in the pumping treatment. The fate of these communities will be contingent upon environmental conditions ahead in the sites (e.g., available sources of substrates such as C, N, and P, and competing microbial communities and grazers). Epsilon proteobacterium (bands 1, 2, 3, and 14) was dominant species

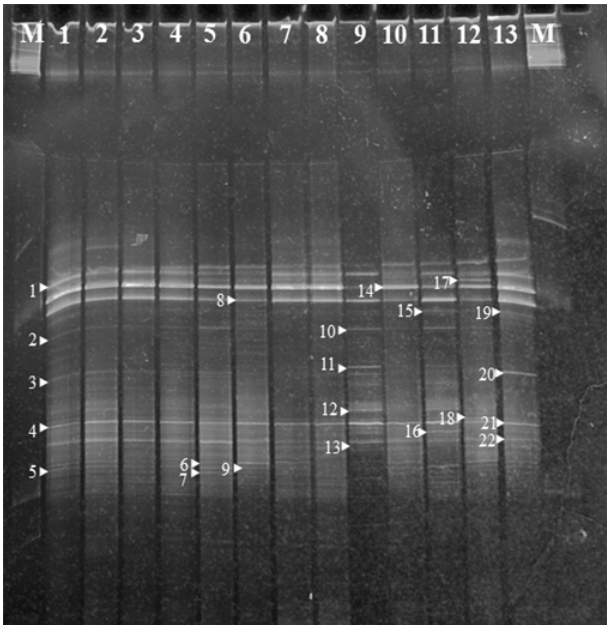

Fig. 5. PCR-DGGE based microbial community analysis of mudflat (demonstration site) after remediation using the CAJ System and water pumping system. Lanes: M, marker; 1, 2, 3, 4 and 5, a-1, a-2, a-3, a-4 and a-5; 6, 7, 8, 9 and 10, p-1, p-2, p-3, p-4 and p-5; 11, before treatment; 12, 7 days after air jet treatment; 13, 7 days after water pumping treatment.

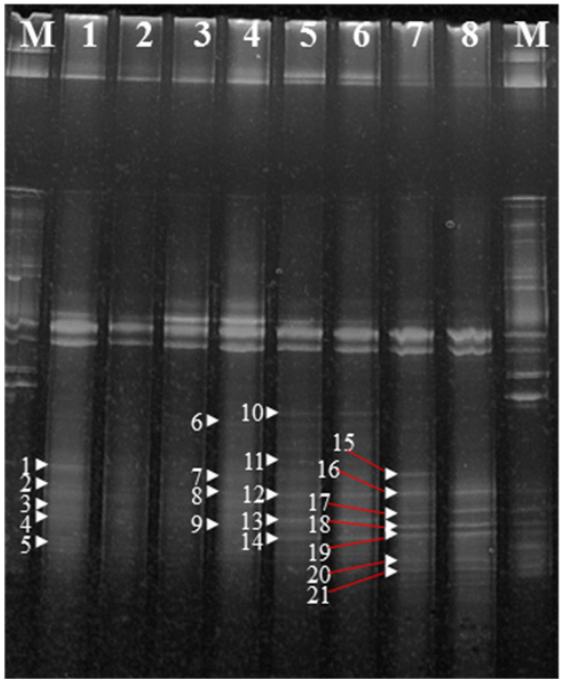

Fig. 6. PCR-DGGE based microbial community analysis of mudflat (field sites) after remediation using the CAJ System; Lanes: 1, 2, Cheonripo down; 3, 4, Cheonripo mix; 5, 6, Guryepo; and 7, 8, Haenyumaeul (pumping as control); all samples were run in duplicate.

Table 2. Identification of microbial communities based on sequence analysis of 16S DNA amplified from DNA fragments derived from PCR-DGGE (Fig. 5)

Band No.	Accession No.	Description	Source	Identity (%)
1	AY280396	Uncultured epsilon proteobacterium clone CH-B30 16S ribosomal RNA	Active Deep-Sea Vent Chimney Juan de Fuca Ridge, Pacific Ocean	95
2	DQ112521	Uncultured epsilon proteobacterium clone KorMud-V8C120 16S	Intertidal mudflat sediment Ganghwa Island, Korea	99
3	DQ112521	Uncultured epsilon proteobacterium clone KorMud-V8C120 16S	Intertidal mudflat sediment Ganghwa Island, Korea	99
4	AM911409	Uncultured bacterium partial 16S rRNA, clone D05_CW03_full	Cold-water coral, *Lophelia pertusa,* S. C Neulinger, Norway	99
5	HM368279	Alpha proteobacterium HA-mar-Is18-14 16S ribosomal RNA gene	Estuarine and marine bacteria, Wadden Sea, German	99
6	GQ143753	Uncultured bacterium clone 069C79 16S ribosomal RNA gene	Yellow Sea continental shelf sediment	99
7	EU652622	Uncultured bacterium clone D8S-50 16S ribosomal RNA gene	Yellow Sea sediment, Korea	98
8	HM366476	Uncultured bacterium clone ADB-16 16S ribosomal RNA gene	Urban aerosols Seoul, Korea	99
9	AB530225	Uncultured bacterium gene for 16S rRNA	Marine sediment, Tokyo Bay	99
10	NR_025817	*Erythrobacter seohaensis* strain SW-135 16S ribosomal RNA	Yellow sea continental shelf sediment	99
11	AY161043	*Brevibacillus* sp. PLC-3 16S ribosomal RNA gene	*Brevibacillus* sp. PLC-3	97
12	DQ341414	Pseudomonas sp. Antarctic IS02 16S ribosomal RNA gene	Antarctic ice and snow	99
14	AY580419	Uncultured epsilon proteobacterium clone PI_4t12b 16S ribosomal RNA gene gene	Plum Island Sound Estuary, northeastern Massachusetts	100
15	AB441527	Uncultured bacterium gene for 16S rRNA, partial sequence, clone; CC35	Solid waste compost, Japan	99
16	AB530205	Uncultured bacterium gene for 16S rRNA, partial sequence	Marine sediment, Tokyo Bay	98
17	DQ521175	Uncultured bacterium clone C53 16S ribosomal RNA gene, partial sequence	Sediment of cold hypersaline (15% salt) sulfidic spring, Arctic	98
18	AB441527	Uncultured bacterium gene for 16S rRNA, partial sequence, clone; CC35	Solid waste compost, Japan	100
19	AY580419	Uncultured epsilon proteobacterium clone PI_4t12b 16S ribosomal RNA gene	Coastal bacterioplankton sample of Plum Island Sound Estuary, northeastern Massachusetts	100
20	GU553835	Uncultured bacterium clone ORI-860-27-P_W_27WB08 16S ribosomal RNA gene, partial sequence	Methane seep of YungAn ridge, SW Taiwan	100
21	U85879	Psychrobacter glacincola ICP9 16S ribosomal RNA gene, partial sequence	Psychrophilic bacteria in Antarctic sea ice	100
22	AM040115	Uncultured delta proteobacterium	Sandy intertidal sediments in the North Sea, Sylt-Romo Basin, Wadden Sea	100

almost for all the samples that was widely observed in the ocean and terrestrial environments (Campbell *et al*., 2006).

In the other on-site tests, the levels of TPH after treating with the CAJ System were not generally much lower than the control (water pumping treatment) (Fig. 4). Microbial community changes were also monitored using PCR-DGGE depending on the different field site treatments. Dominant species in Cheonripo down and Cheonripo mix after treating with the CAJ System were different from dominant species in the control (seawater pumping; Haenyeomaeul). Even in sites treated with the same CAJ System, different microbial communities were shown. Most of these microorganisms were identified as marine bacteria originated from the marine environment (seawater or marine sediment) while the densities of the oil

Table 3. Identification of microbial communities based on sequence analysis of 16S DNA amplified from DNA fragments derived from PCR-DGGE (Fig. 6)

Band No.	Accession No.	Description	Source	Identity (%)
1	AY711463	Uncultured Chromatiales bacterium clone SIMO-2097 16S ribosomal RNA	Dean creek marsh sediment, Sapelo Island, USA	98
2	EU050805	Uncultured gamma proteobacterium clone SS1_B_04_19 16S ribosomal	sediment from the Kings Bay, Svalbard	98
3	AY568822	Uncultured bacterium isolate JH10_C70 16S ribosomal RNA	Intertidal mudflate sediment, Ganhwa, Korea	99
4	FJ227884	Uncultured bacterium clone ESSM071205_802H3AOB 16S ribosomal RNA	marine sediment California, Elkhorn Slough, South Marsh.	95
5	HM437482	Uncultured marine bacterium clone B-Alg14 16S ribosomal RNA	Surface of senescence algea, Jiaozhou Bay, China	99
6	DQ112521	Uncultured epsilon proteobacterium clone KorMud-V8C120 16S	Intertidal mudflate sediment, Ganhwa, Korea	99
7	FJ717246	Uncultured bacterium clone G6_10.4_2 16S ribosomal RNA gene	Marine sediment from Cullercoats, UK	98
8	AY771946	Uncultured delta proteobacterium clone SK11 16S ribosomal RNA gene	Marine surface sediment; Waden, Germany	99
9	EU287287	Uncultured bacterium clone S11-104 16S ribosomal RNA gene	Artic surface sediment pacific ocean	98
10	GQ143753	Uncultured candidate division TM6 bacterium clone JJB103 16S	Yellow sea continental shelf sediment	90
11	AB530235	Uncultured bacterium gene for 16S rRNA, partial sequence	Marine sediment, Tokyo Bay, Japan	97
12	AJ557849	Marine arctic deep-sea bacterium HG1	50 m above sediment surface, arctic current from North Pole	99
13	DQ357036	*Loktanella* sp. K0-28-006 16S ribosomal RNA gene, partial sequence	Deep sea sediment core DMO5-2907, Okinawa, Japan	99
14	AY592607	Uncultured bacterium clone Napoli-1B-52 16S ribosomal RNA gene	Napoli mud volcano; isolated from sediment layer	99
15	HM030990	Marine bacterium KS-9-10-4 16S ribosomal RNA gene, partial	Marine bacterium *Ruditpes philinarum* isolated from South Korea	99
16	GQ245896	*Marinobacterium rhizophilum* strain UDC307 16S ribosomal RNA gene	Sea water, Dokdo, South Korea	99
17	AM998269	Uncultured deep-sea bacteriumpartial 16S rRNA gene, clone	Deep-sea surface sediments, Guinea Basin, Atlantic Ocean	99
18	FJ264673	Uncultured bacterium clone OrigSedB32 16S ribosomal RNA gene	Methane seep sediment. Eel River Basin, CA,USA	97
19	GU475276	Uncultured bacterium clone DSH1B28 16S ribosomal RNA gene	Cold seep sediment, south China sea	97
20	FJ175562	Uncultured bacterium clone B5-8 16S ribosomal RNA gene	Hydrocarbon seep sediment in Timor Sea, Asyralis	99
21	GQ263399	Uncultured bacterium clone FW1_b8316S ribosomal RNA gene	Simulated low level waste site Idaho National Labs, USA	97

degraders seemed to be relatively low. Therefore, distribution of the microbial communities under the current condition having relatively very low oil contamination (less than 160 ppm of TPH) was attributed to differences in environmental characteristics rather than the oil treatment measures.

The conclusions were drawn as follows: The compressed air jet technology appeared to be highly effective to remove TPH from soil matrices contaminated with crude oil. The air jet system showed different microbial communities from the conventional water pumping treatment system. However, oil degraders were not observed mainly because of lower amount of oil as a carbon source and depleted nutrients (particularly N and P) in the environment. Mechanisms associated with the removal of oil or contaminants using the air jet system need to

be elucidated in the future study. It would be possible to apply the air jet system to removal of other pollutants as one of the economical and eco-friendly remediation technologies.

적 요

본 연구의 목표는 압축공기분사시스템을 이용하여 원유로 유출이 된 해안을 정화함에 있어서 그 정화효율성과 정화 전후의 총석유탄화수소(total petroleum hydrocarbon; TPH) 농도 및 미생물군집변화를 관찰함으로써 그 최적 정화과정을 이해하기 위한 기초자료를 얻고자 하는 것이다. 압축공기제트시스템을 2–5회 연속적용 시 오염지의 TPH가 약 66%까지 저감이 된 반면에 대조구인 해수를 펌핑한 경우에는 40% 정도의 저감효과가 관찰이 되었다. 압축공기의 분사 후 PCR-DGGE에 의한 미생물군집 분석 결과에서는 유류분해미생물의 군집은 확인이 되지 않았다. 이는 정화에 의한 낮은 TPH 농도(약 100 mg/kg 수준, 탄소원), 처리환경에 내재적인 제한적인 질소 및 인의 농도에 기이한 것으로 판단된다. 따라서 잔여분의 유류는 에어제트시스템을 적용 시 제한적 영양염류(질소 및 인 등)를 적절한 방식과 농도로 투여할 경우 거의 완전하게 제거가 가능할 것으로 사료된다. 향후 본 기술은 고농도의 유류 및 유기물로 오염된 다양한 수질환경 및 토양환경의 효율적이고 환경친화적인 정화에 활용이 될 것으로 기대된다.

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