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Evaluation of the treatability test for crude oil contaminated sand using CO_2 evolution method

Choi Dong Hyuk, Sim Doo Suep, Oh Young-Sook, Kim Sang-Jin

Microbiology Lab., Marine Biology Div., Korea Ocean Research & Development Institute, P. O. Box 29, Ansan 425-600, Korea

(dhchoi@kordi.re.kr)

요 약

원유로 오염된 모래지역의 생물정화기술 적용을 위하여 인위적으로 오염시킨 해사를 이용한 타당성 연구를 수행함으로써, 오염 환경에 최적 적용방법을 검토하였다. 무기 영양염류, 인공계면활성제, 유류분해미생물의 적용성을 검토한 결과, 영양염류의 첨가가 효과적이며 외부로부터 미생물을 첨가할 경우 토착미생물보다 많은 양을 적용하는 것이 효과적임을 알 수 있었다. 계면활성제는 CMC와 동일 농도로 첨가할 때 높은 효과를 얻을 수 있었다. 원유성분의 무기화에 의해 발생되는 이산화탄소량 측정이 타당성 평가의 유효한 도구임을 알 수 있었으며 미생물의 활성과 원유성분 변화로 이를 검증할 수 있었다.

I. INTRODUCTION

Bioremediation is the act of adding materials to contaminated environments to cause an acceleration of the natural biodegradation process and biodegradation is known to be the principal natural process for the removal of the nonvolatile fraction of oil from the environment [1]. *In situ* bioremediation, which involves the use of indigenous microorganisms to degrade the target compounds, is receiving increasing attention due to its potential cost-effectiveness [2].

Two kinds of *in situ* bioremediation processes are distinguished as natural or intrinsic bioremediation and active or engineered bioremediation [3]. If natural biodegradation process is not being occurred or too slow, the contaminated area has to be manipulated in such a way that the biodegradation process is encouraged and then the reaction rates are increased [4]. This is called engineered *in situ* bioremediation.

One of the major actions in engineered *in situ* bioremediation is supplying the contaminated area with nutrients such as nitrogen and phosphorus, with electron acceptors such as oxygen, and with microorganisms as seeds. However, careful considerations are in order when undertaking a bioremediation project to determine which approach is best for a given contaminant and set of site characteristics. A

proper site evaluation and a bioassessment or treatability investigation should be conducted for this purpose.

Since successful bioremediation programs require application methodologies specifically tailored to the environmental parameters at each contaminated site, treatability studies are required prior to bioremedial action. In the present study, the treatability test, which enables identifying the optimum conditions for degradation of the target compounds, was conducted for sandy area contaminated with crude oil.

II. MATERIALS AND METHOD

Experimental setup

Each 50 g of sea sand in 250 mL Erlenmeyer flask equipped with KOH solution for CO_2 trapping (biometer) was spiked with 3% or 6% of Arabian light crude oil to simulate crude oil contamination at sandy area. All the biometers including unpolluted and untreated controls were incubated at room temperature in the dark, in triplicates. Physico-chemical properties of sea sand used in this study and detailed experimental conditions of biometers are shown in Table 1 and Table 2, respectively.

A slow release inorganic fertilizer (SRIF) was applied to make C:N:P ratio of 100:10:3, in case of nutrients addition. Tween 80 was used as a surfactant at 1 or 10 critical micelle concentration (CMC). A mixture of three oil-degrading microorganisms, *Corynebacterium variabilis* IC10 [5], *Sphingomonas yanoikuyae* KH3-2 [6], and *Yarrowia lipolytica* 180 (CL180) [7] was used as inoculum.

Table 1. Physico-chemical properties of the sea sand used in this study

gravel	sand	mud	water holding capacity	water content[a]
0.14%	88.97%	10.89%	19.1%	0.88%

a) water content was adjusted to 12.6%(w/w) (66% of water holding capacity) for treatability study

Table 2. Experimental conditions for biometers

	treatments																unpolluted controls			
	t1	t2	t3	t4	t5	t6	t7	t8	t9	t10	t11	t12	t13	t14	t15	t16	c1	c2	c3	c4
crude oil(%)	3	3	3	3	3	3	3	3	6	6	6	6	6	6	6	6	0	0	0	0
SRIF[a]		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+			
inoculation[b]			10^6	10^6	10^6	10^8	10^8	10^8			10^6	10^6	10^6	10^8	10^8	10^8		10^8	10^8	10^8
surfactant[c]				1	1		10	10				1	1		10	10			1	10

a) to make the ratio of C:N:P=100:10:3

b) unit; cells/g sand

c) unit; × CMC

Measurements

Microbial activity was investigated by measuring electron transport system (ETS) activity using an artificial electron acceptor, 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT), and superabundance of electron donors, NADH, NADPH, and succinic acid.

The mineralization of crude oil was monitored by measuring the conductivity of KOH solution in each biometer in which the evolved CO_2, a final product of mineralization, was trapped. Crude oil constituents were analyzed using Fused Silica capillary column (30 m × 0.32 mm × 0.25 μm, SPB^{TM-1}, Supelco Inc.) gas chromatography (GC) equipped with flame ionization detector (FID) after extracting whole samples in biometer with chloroform.

III. RESULTS AND DISCUSSION

Fig. 1 shows the cumulative amount of CO_2 evolution as a result of mineralization of crude oil hydrocarbons during incubation. The large amounts of CO_2 evolutions were observed in the biometers inoculated with high concentrations of microorganisms (solid legends), 10^8 cells/g sand, in both of 3% and 6% polluted ones. The inoculum size of 10^6 cells/g sand, expected as the same cell concentration with indigenous microorganisms, was found to have no effect on enhancing the mineralization of crude oil hydrocarbons compared to the results of SRIF treated biometers without inoculation. This result suggested that the addition of SRIF could increase the degradation potential of indigenous microorganisms and the concentration of cells for seeding should be larger than that of indigenous ones for the effective enhancement of crude oil mineralization. The 1 CMC of surfactant addition was considered to be sufficient in enhancing the mineralization of crude oil and was found to be more effective in case of large inoculum size

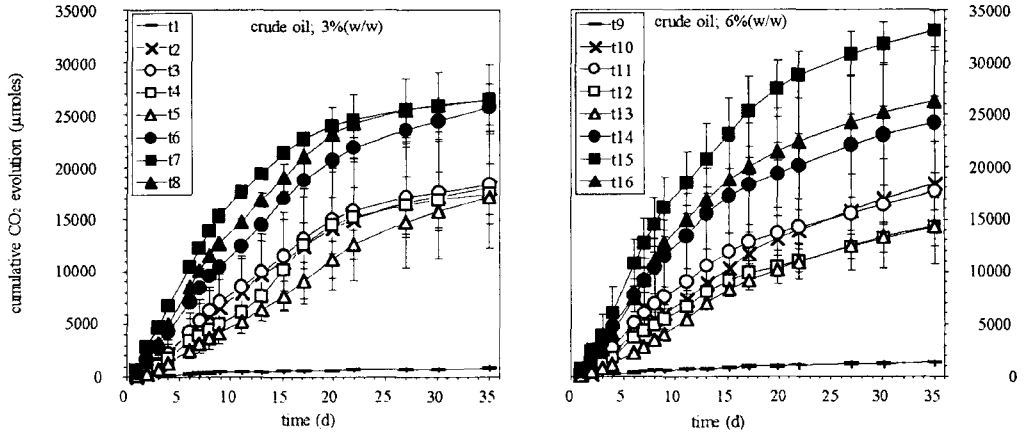

Fig. 1. The cumulative amount of CO_2 evolution as a result of mineralization of crude oil hydrocarbons during incubation.

application.

The evolution of CO_2 was almost the same in both of 3% and 6% polluted sands until 10 d of incubation. The results of microbial activity (Fig. 2) also supported those of CO_2 evolution, where almost all the same activities were observed in both concentrations of crude oil contamination at 10 d. The evolution of CO_2 in 3% contamination reached its plateau at 20 d of incubation, while it lasted in 6% contamination. This was also the case in microbial activities as the high activities were observed in 6% contamination after 35 d of incubation. On the basis of the results, the 3% of contamination appeared to reach the saturation point of substrate concentration or to have the substrate transport limitation. This might cause no significant difference in CO_2 evolution rate between 3% and 6% contamination within 20 days, though the total amount of CO_2 evolution of 6%

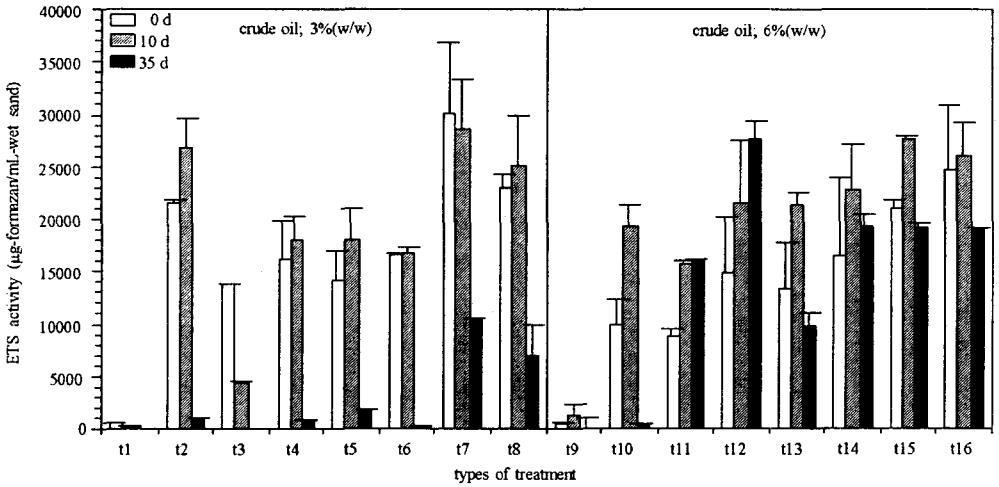

Fig. 2. Microbial activity evaluated by electron transport system (ETS) activity during incubation. ETS activity of each treatment was expressed as the amount of formazan by measuring the absorbance of reduced INT-formazan at 480 nm.

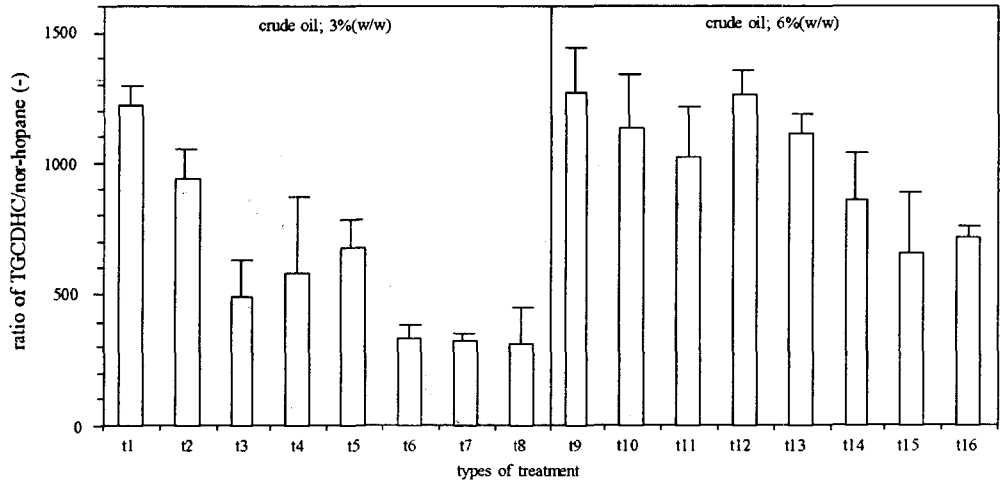

Fig. 3. The ratio of total GC detectable hydrocarbons (TGCDHC) to nor-hopane as an index of biological degradation of crude oil hydrocarbons after incubation.

contamination was higher than that of 3% contamination. The CO_2 evolution and microbial activity of unpolluted controls were found to be negligible (data not shown).

Fig. 3 indicates the biodegradation of crude oil hydrocarbons evaluated from the ratio of total GC-detectable hydrocarbons to nor-hopane, one of the conservative internal biomarkers [8]. The low ratios thus the high biodegradation efficiencies were observed in the biometers supplied with microorganisms or surfactants, especially in those supplied with microorganisms and surfactants together, in both of 3% and 6% contaminations. The addition of surfactants was considered to increase the bioavailability of hydrocarbons in some ways by altering water solubility, emulsifiable effect, and surface tension of the substrates.

From the results in the present study it could be concluded that the addition of inorganic nutrients was essential and the application of higher concentration of oil-degrading microorganisms than that of indigenous ones was important in the bioremediation of sandy area contaminated with crude oil. And the application of surfactant in the concentration of 1 CMC was sufficient in enhancing the mineralization of crude oil. The measurement of CO_2 evolution as a result of crude oil mineralization was studied and found to be a simple and useful tool for the evaluation of treatability study.

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