Southeast Asian Fisheries Development Center

Oil Pollution in the Vietnamese Waters

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ABSTRACT

Enumeration of hydrocarbon-degrading microorganisms (HDM) and their degradative capacity studies were carried out in area IV of South China Sea (Vietnamese water). Microbial most probable number (MPN) varied from 10^1 to 10^5 cell/ ml of surface seawater or gram of sediment. Some microbial communities and bacterial strains isolated from 97 collected samples show high hydrocarbon degradation and surfactant producing abilities. Preliminary results of our microbial study indicated that oil pollution in IV area was not found in 30 research stations. Slight oil contamination was observed in 28 survey stations.

Key words: hydrocarbon-degrading microorganism, marine microorganisms, oil pollution, polycyclic aromatic hydrocarbon

Introduction

Petroleum-based products are the major source of energy for industry and daily life. Petroleum is also the raw material for many chemical products such as plastics, paints, and cosmetics. The petroleum transport across the world is frequent, and the amounts of petroleum stocks in developed countries are enormous. Consequently, the potential for oil spills is significant, and research on the fate of petroleum in a marine environment is important to evaluate the environmental threat of oil spills, and to develop biotechnology to scope with them since half of world oil production is transported by sea (Shigeaki Harayama, *et al*. 1999) .

The Interdepartmental Collaborative Research Program has been carried out since 1995 with the main objective of collecting and analyzing data and necessary information for the management of fishery resources and the protection of the environment through collaborative research member countries and organization concerned. With the agreement of Vietnamese Government, the Collaborative Research Program in Vietnamese waters, as area IV, has been carried out from 29th April to 30th May 1999.

Our project in the program is to conduct petroleum hydrocarbon pollution study with the following objective:

- *Detection of total petroleum hydrocarbon level.*
- *Investigation of number of HDM.*
- *Study of biodegradative capacity of isolated hydrocarbon-degrading microbial communities in laboratory condition.*

Materials and Methods

Sampling procedures

Surface water and sediment samples for microbial and total oil analysis were collected on

cruise. Enumeration of hydrocarbon degraders was carried out immediately after the sample collection. For evaluation of oil biodegradation capacity and oil concentration, the samples were transported to the laboratory in presterilized glassed bottles. Total oil level was detected by Infra red-spectrophotometry (IRS) method.

Microbial enumeration

Improved most probable number method (MPN) was used for direct count of oil-degrading microorganisms(Ronald Atlas and Rechard Bartha,1974).Serial dilution of samples were inoculated into mineral salt medium (MSM) (Brushnell-Haas medium supplemented with 3% NaCl and adjusted to pH 7.8) in 10 ml tubes with 5% diesel oil as sole carbon and energy source. The results were scored after inoculating from 15 to 20 days at room temperature.

Biodegradative capacity

50 ml of MSM in cornical flasks containing 5% of DO were inoculated with 1 ml of preculture of isolated hydrocarbon-degrading microbial community that collected from sediment of stations No 3, 12, 40, 29, 38, 7, 52, 58, 48, 21 and incubated at 28^0C for 7 days and shaking at 200 rpm.

Aromatic hydrocarbon degradation by purified cultures that isolated from stations No 2 and 57 was also studied. For this experiments, MSM containing 50 ppm/ L phenanthrene as sole carbon and energy sources was used. Shaking culture was incubated at 30^oC for 4 days.

The residual oil was determined by weight and by gas chromatography (GC) method.

Result and Discussion

After finishing the cruise, 97 samples have been collected that including 39 sediment samples and 58 surface water samples. In general, the sediment samples can be divided into four groups such as mud, sandy mud, muddy sands, and sand.

Total hydrocarbon concentration

Only 11 samples (No 1, 2, 3, 4, 8, 10, 13, 27, 40, 43, and 48) from 58 research stations were investigated. Among them only in stations No 1 and No 2 oil concentration 0.095 mg/L and 0.017 mg/ L respectively was detected (Table 1). In other research stations the oil concentration was under detected level of IRS detector (< 0.01 mg/L).

These results indicated that in these samples no oil contamination was observed. This data can not be used for the conclusion of oil pollution level in seawater because of several technical reasons that concerning to the method used for these chemical analysis.

Microbial investigation

The enumeration result of hydrocarbon-degrading microorganisms was illustrated in Table 2 and Table 3. According to obtained results HDM number is in the range from 10^1 to 10^5 cell/ml or cell/ g of sample. Number of microorganisms in sediment samples was normally higher than in surface water samples. Number of HDM in the surface water samples was higher than in the sediment samples, it was found only in two stations No 2 and 38. There are 12 stations in which the number of hydrocarbon-degraders was equal in both sediment and surface(Fig. 1) water samples such as stations No 3, 4, 5, 12, 13 (10^5 cell/ml); stations No 47, 50, 56 (10^4 cell/ml); stations No 8, 9 (10^3 cell/ml), and stations No 29, 40 (10^2 cell/ml)

In 18 samples (6 from surface and 12 from sediment) number of HDM was 10^5 cell/ml, in 22 samples (8 from surface and 14 from sediment) bacterial number was 10^4 cell/ml, in 32 samples (23 from surface and 9 from sediment) number of HDM was 10^3 cell/ml and in the last 23 sample (19 from surface and 4 from sediment) number of HDM 10^2 cell/ml was detected only (Table 2). According to colony morphology of all isolated bacteria, they can be belonged to about 20 groups. In almost far from shore samples, including stations No 2, 6, 10, 15, 16, 17, 24, 25, 32, 33, 34, 41, number of HDM was lowest, except in stations No 42, 45, 46, 51, 52, 55.

According to the number of HDM (indirect indication), oil contamination was not detected in 30 stations, and light oil contamination was detected in other 28 stations. In almost stations in the South Sea (from No 43 to 58) light oil contamination was observed (Table 2).

Microbial community of hydrocarbon- degraders in different research stations showed different oil degradative capacity (Table 4). The strongest community in diesel oil degradation was found in two stations No 21 and 48, about 94 % oil was reduced during 7 day shaking cultivation. The weakest communities in oil degradation study detected in stations No 3, 12, 43, 29, only 15.8 – 26.4 % oil was reduced. In stations No 38, 37, 52, 58 the microbial communities were able to degrade 31.6 – 47.4 % oil.

Surfactant producing bacteria were isolated from 8 stations (Fig. 2). Isolated bacteria and their products play an important role in the process of cleaning up oil contamination (Oberbremer A. et al., 1990).

Study of polycyclic aromatic hydrocarbon degradative capacity by purified culture was also carried out. The result showed that some bacterial strains isolated from different stations degraded rapidly phenanthrene. For example, after 4 day cultivation, strain I-572 that isolated from sediment of research station No 57 (Fig. 3) could degrade 99 % of added phenanthrene in MSM (Fig. 4).

Table 1. Hydrocarbon concentration in sea water at some research stations.

Research stations	Oil concentration (mg/L)
1	0.095
2	0.017
3	< 0.01
4	< 0.01
8	< 0.01
10	< 0.01
13	< 0.01
27	< 0.01
40	< 0.01
43	< 0.01
48	< 0.01

Table 2. Number of hydrocarbon- degrading microorganisms in survey stations.

Station No	Number of HDM (cell per ml)		Station No	Number of HDM (cell per ml)	
	Surface water	Sediment		Surface water	Sediment
1	10^4	10^3	30	ND	10^3
2	10^2	10^3	31	ND	10^3
3	10^5	10^5	32	ND	10^3
4	10^5	10^5	33	ND	10^1
5	10^5	10^5	34	ND	10^1
6	10^3	10^2	35	10^3	10^2
7	10^3	10^2	36	10^3	10^2
8	10^3	10^3	37	10^3	10^2
9	10^3	10^3	38	10^2	10^3
10	10^3	10^2	39	10^3	10^2
11	ND	10^5	40	10^2	10^2
12	10^5	10^5	41	ND	10^2
13	10^5	10^5	42	ND	10^4
14	10^4	10^2	43	10^5	10^4
15	ND	10^2	44	10^4	10^3
16	ND	10^2	45	10^5	10^3
17	ND	10^3	46	10^5	10^3
18	ND	10^3	47	10^4	10^4
19	ND	10^3	48	10^4	10^3
20	10^4	10^2	49	10^4	10^3
21	10^4	10^2	50	10^4	10^4
22	ND	10^3	51	10^4	10^3
23	ND	10^3	52	10^5	10^4
24	ND	10^3	53	10^5	10^4
25	ND	10^3	54	10^4	10^2
26	ND	10^2	55	10^4	10^3
27	ND	10^2	56	10^4	10^4
28	10^5	10^2	57	10^4	10^3
29	10^2	10^2	58	10^5	10^4

ND: not detected

Table 3. Distribution of samples according to the number of HDM.

Samples	Number of HDM (cells per ml)					Total of samples
	10^1	10^2	10^3	10^4	10^5	
Surface	2	19	23	8	6	58
Sediment	0	4	9	14	12	39

Table 4. Oil degradative capacity of microbial communities isolated from sediment in some research stations.

Station	Residual oil (mg/L)	Degraded oil (%)
Control	38	0
3	32	15.8
12	30	21.1
40	28	26.4
29	28	26.4
38	26	31.6
7	26	31.6
52	24	33.9
58	20	47.4
48	2.4	93.7
21	2.2	94.2

A B

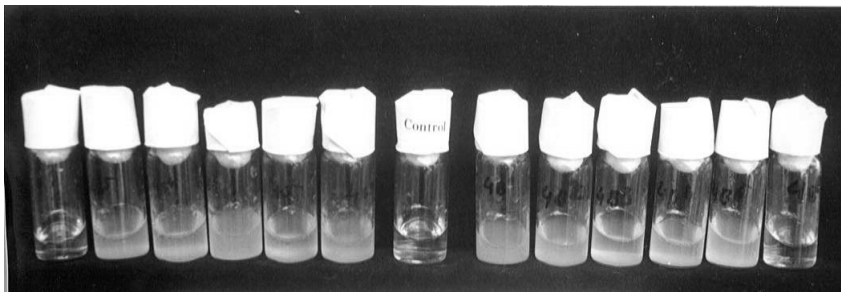

Fig.1. Hydrocarbon degrading microorganism from (A) surface and (B) sediment samples determined by improved MPN method.

A B

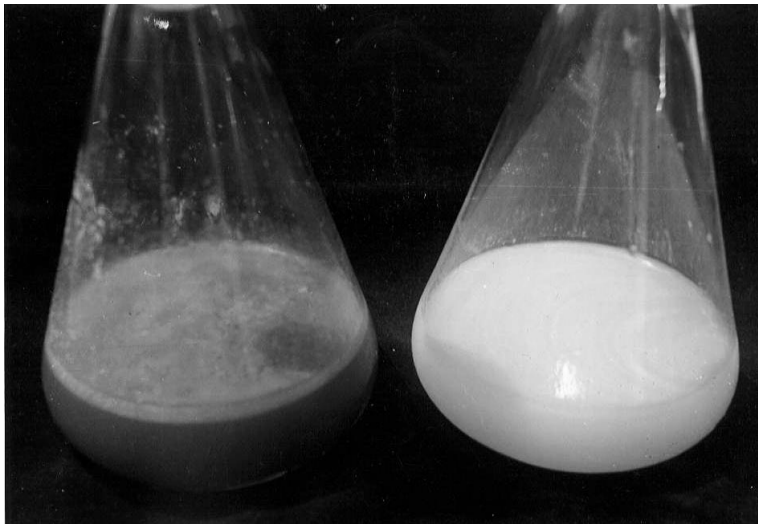

Fig. 2. Biosurfectant produced by two different isolates from stations No.13 (A) and No. 21 (B).

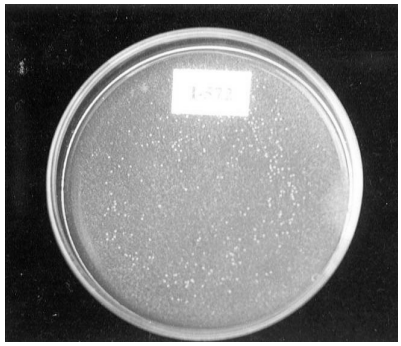

Fig. 3. Polycyclic aromatic hydrocarbon degrading microorganism strain isolated from research station No. 57.

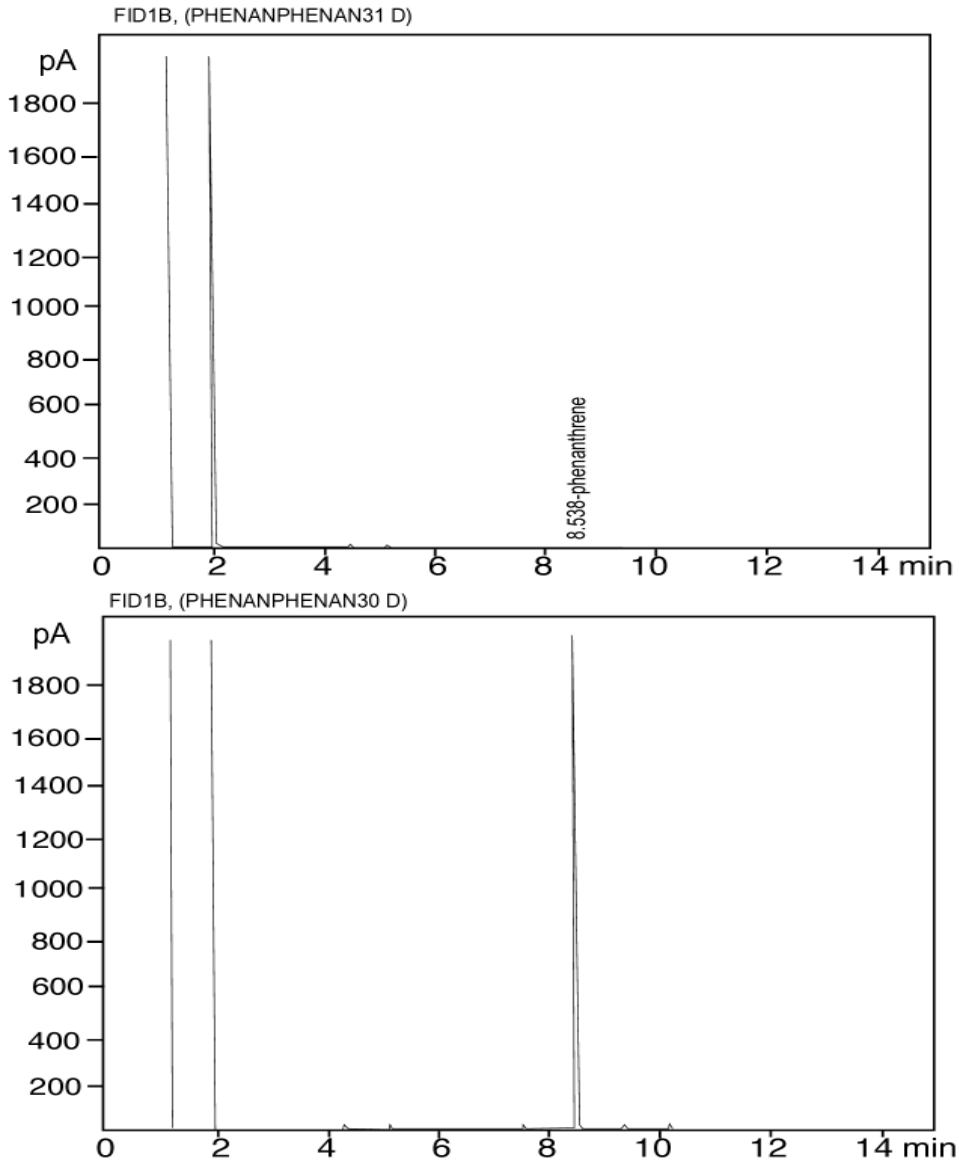

Fig. 4. Gas chromatograms of residual phenanthrene in shaking culture inoculated with I-572 (A) and without (B) after 4 days.

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