FIELD TEST ON CLEANING OF OIL POLLUTION ON NHATRANG BEACH OF VIETNAM

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Abstract

Vietnam has long beach (3620 km) with many tourist places. We isolated and selected 6 bacteria strains, which capable of crude oil degradation from Danang, Nhatrang, and Vungtau beaches. We carried out the application of selected and indigenous microorganisms for cleaning oil pollution on Nhatrang beach from April to May 1999 and April to May 2000. The result showed that six selected strains degraded crude oil from 34 to 64% of total oil. Mix of selected strains cooperated with indigenous hydrocarbon-degrading microorganisms degraded 95.15% of total oil, 97.68% of saturated hydrocarbon and nearly 100% aromatic hydrocarbon after 30 days at the field tests. Mix selected strains may be applied for cleaning up crude oil pollution, especially on the south coast of Vietnam.

Key words: Bioremediation, Cleaning of oil pollution, Indigenous and seeding oil-degrading bacteria, Oil polluted sites.

Introduction

In recent years, large accidental oil spills received much attention and considerable public concern. When the incidents occur, they can cause significant contamination of ocean and shoreline environments. For example, Amoco Cadiz discharged 0.2 megatons of crude oil into the water along the Brittany coast in 1978. Exxon Valdez discharged 0.04 megatons into the Prince William Sound in 1989. Heaven caught fire and sank off the coast of Italy in 1991 with 0.14 megatons on board. Braer released 0.08 megatons into coastal water of Shetland, Islands in 1993. During the Gulf war in 1991, 0.82 megatons of oil were released in Kuwait, threatening the desalination plants and coastal ecosystem of the Gulf.

These incidents have prompted the development and refinement of techniques for dealing with oil pollution. These include physical, chemical and biological technologies may fall into the category of biological method:

- Use of straw or plant material as an absorbent for oil.
- Biosurfactants to clean oil surfaces.
- Biopolymers to coat surfaces to prevent oil adhesion.
- Addition of materials to encourage microbiological biodegradation of oil.
- Bioremediation has received the most attention, notably after the Exxon Valdez incident.

Vietnam has long beach with 3620 km. At present, almost activities of oil recovery are carried out on offshore platform. The oil production has been improved intensively from 1985. Out put of crude oil and natural gas are about 15 million tones and 11 billion m^3 per year, respectively. During production, transportation and using, leaking oil into the sea is unavoidable. The Lecla (Sip) sank at Quynhon, 200 tones of crude oil were released in the sea in 1989. Transco-01 (Vietnam) rushed into Unihumanity (Taiwan) spilled 138 tones of FO in Hochiminh City in 1994. Most dangerous spill up to now in Vietnam happened at Catlai. Neptune Aries (Singapore) released 1668 tones of oil damaged 115000 ha of cultivate land. In the other hand, there are many tourist places on the beaches of Vietnam. Therefore, it is necessary to research on treatment of oil pollution on coastal area.

Material and methods

Sampling: Bacterial strains taken from seawater, oil polluted sand in Danang, Nhatrang, and Vungtau. A single composite soil sample (10-20 cm depth) was taken from each site using sterile trowel.

Cultivation and store: Bacteria were incubated in mineral medium with crude oil as carbon source, shaking at 180 rpm. Crude oil was from White tiger and Rangdong oil fields. Strains were stored in medium Gost-902374 with oil as substrate or dry freezing or in glycerin 50%.

Characterization: Cell morphology of bacteria was observed by photomicroscope Laboval 4 and electron microscope JEM 1010.

Oil-degrading ability of bacteria was estimated by three methods: weight of total oil, gas chromatography and microbial analysis.

Field tests were carried out on Nhatrang beach with three sites: oil and sand (Control - 1); oil - sand and nutrient (2); oil - sand - nutrient and bacteria (3). Before starting the experiment, 3 sand samples for control enumeration at least 30 meters from contaminated areas was collected (10^1 CFU/g).

Results and discussion

Crude oil - degrading ability of isolates

Table 1. Oil-degrading ability of isolated strains

Strains	Places	Residual oil (mg/l)	Degraded oil (%)
Control		59657	00.00
BT2	Vungtau	31342	47.46
BT5	Vungtau	23577	60.48
No.1	Nhatrang	21346	64.22
No.5	Nhatrang	39530	33.74
No.10	Danang	39639	33.56
Ps2	Danang	34991	41.35

All of 6 selected strains degraded crude oil 33.56 - 64.22% after 7 days (table 1). No.1 strain degraded 70% saturated hydrocarbon, 77.2% aromatic hydrocarbon, 30.5% resin and 70% asphalten, strongest in isolates (fig. 1).

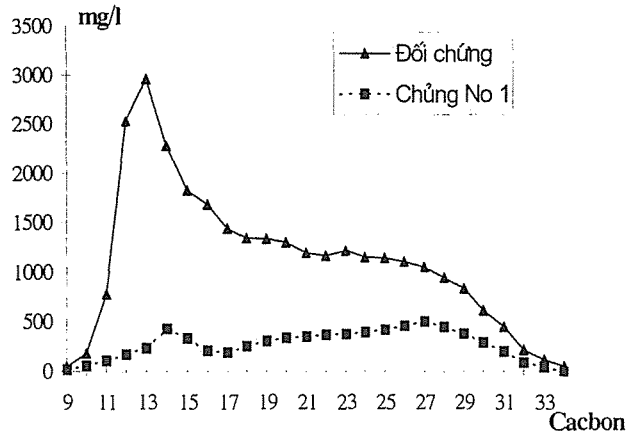

Fig 1. Saturated hydrocarbon degrading of No.1 strain

The above result shows that No.1 and BT.5 degraded strongly saturated and aromatic hydrocarbon, especially they degraded both resin and asphalten. These strains could be applied for cleaning of oil pollution.

Crude oil- degrading ability of mix selected strains at field experiment

Field tests on cleaning of oil pollution were carried out on Nhatrang beach from April to May in 1999 and in 2000. The first sample was contained oil. The second sample was added only nutrient. The third sample was added nutrient and mix strains No.1 and BT5 in oil contaminated sand on Nhatrang beach. Crude oil-degrading ability of mix-selected strains was assessed by two criteria: number of hydrocarbon - degrading bacteria and hydrocarbon component of crude oil before and after experiment.

Table 2. Distribution of aerobic bacteria amount

Date	Sample 1		Sample 2		Sample 3	
	Aerobic bacteria	Fungi	Aerobic bacteria	Fungi	Aerobic bacteria	Fungi
4.19.00	$7x10^3$	$3x10^3$	$7x10^4$	10^3	$1.4x10^4$	10^3
4.21.00	$7x10^4$	10^4	10^5	$4x10^3$	10^6	$2x10^4$
4.23.00	$6x10^6$	$4x10^3$	$2x10^6$	10^3	$3x10^7$	-
4.27.00	10^7	-	10^7	-	10^9	-
5.01.00	$4x10^7$	-	10^8	-	$1.6x10^9$	-
5.5.00	10^8	-	$3x10^9$	-	10^{10}	-
5.9.00	10^9	-	10^{10}	-	$6x10^{10}$	-
5.13.00	10^9	-	10^9	-	10^{10}	-
5.19.00	10^8	-	10^9	-	10^{10}	-

At the beginning of the experiment, number of hydrocarbon-degrading bacteria was 10^3 CFU/gram of sand (included mix strains adding and indigenous bacteria). Maximum quantity of aerobic bacteria reached up 10^{10} CFU/gram sand after 22 days in the case of adding both nutrient and bacteria. But fungi number was decreased and disappeared at third day of experiment (table 2). The highest number of oil degrades also observed at the 22 days (table 3).

Table 3. Distribution of hydrocarbon-degrading bacteria amount

Samples	4.19.00	4.21.00	4.23.00	4.27.00	5.01.00	5.5.00	5.9.00	5.13.00	5.19.00
1	10^2	10^3	10^5	10^6	10^7	10^7	10^8	10^8	10^7
2	10^2	10^3	10^5	10^6	10^7	10^8	10^9	10^8	10^7
3	10^3	10^5	10^6	10^7	10^8	10^9	$2x10^9$	10^9	10^8

Oil analyzed result showed that, after 15 days, 68% of saturated and 70% of aromatic hydrocarbons were degraded. After 30 days, 95.15% of total oil, 97.68% saturated and nearly 100% aromatic hydrocarbon were degraded.

The results from field experiment were significant. Mix strain of No.1 and BT5 corporated with indigenous microorganisms seems to be able to clean oil contaminated sites of Nhatrang beach.

In the control plot (not add bacteria), saturated hydrocarbon in crude oil at the beginning was 105 mg/kg, decreased to 20.8 mg/kg at the end of experiment. That means indigenous microorganisms degraded 80% saturated hydrocarbon. Aromatic hydrocarbon was not extracted after treatment. Resin decreased 46,5%.

On the seeding plot (*Bacillus* and *Pseudomonas*), at the end of the test, saturated hydrocarbon and resin were discharged 97,2% saturated hydrocarbon and 93,47% resin, respectively. The result showed that adding bacteria and nutrients (NH_4NO_3, $Ca_3(PO_4)_2$) strongly stimulated oil degradation (fig. 2 & 3). This result confirmed by Swannell {14]

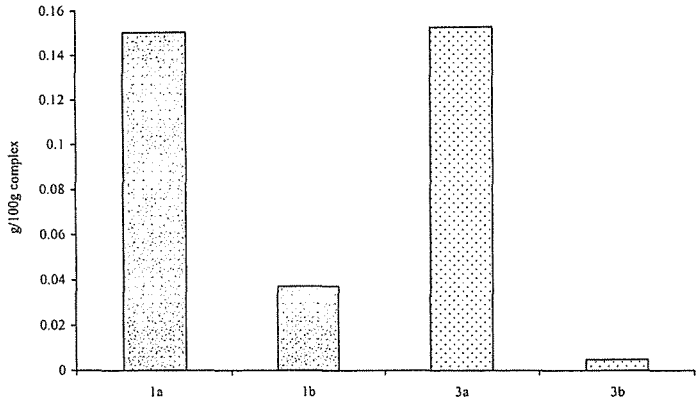

Fig2. Total oil

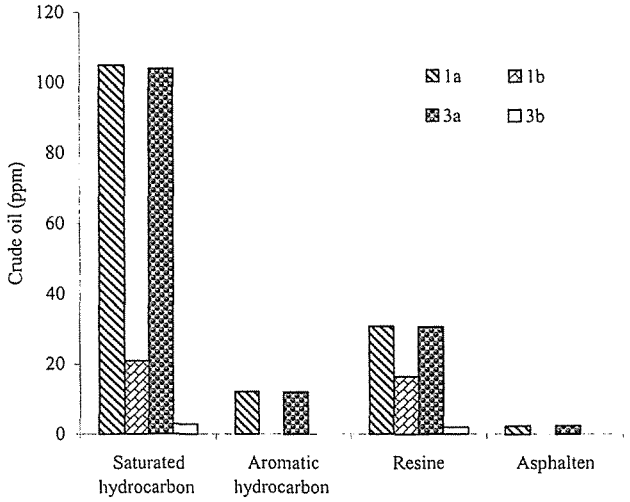

Fig3. Component of residual oil

1a - Before experiment supplementing crude oil without seeding bacteria
1b - After experiment supplementing crude oil without seeding bacteria
3a - Before experiment supplementing crude oil and seeding bacteria
3b - After experiment supplementing crude oil and seeding bacteria

Conclusion

- From difference places and petroleum products, we isolated about 500 microorganism strains. Six of them were capable of crude oil degrading were selected and analyzed. All of six selected strains degraded crude oil from 34 to 64% of total oil.
- Mix of selected strains corporated with indigenous hydrocarbon-degrading microorganisms degraded 95.15% of total oil, 97.68% of saturated hydrocarbon and nearly 100% aromatic hydrocarbon after 30 days at the field tests.
- Mix selected strains may be applied for cleaning up crude oil pollution, especially on the south coast of Vietnam.

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