Arabian-Gulf coastal materials loaded with oil-utilizing bacteria, an overview

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Abstract

As a consequence of the 1990-1991 Iraqi occupation of Kuwait, during which crude oil was deliberately released from the Al-Ahmadi Terminal into the Gulf, coastal areas have become heavily polluted with oil sediments. This paper presents an overview of a decade of research on indigenous oil-utilizing microorganisms that we found associated with inanimate and animate materials along the Arabian Gulf coasts, and their roles in the self-cleaning of the oily areas. Animate materials *viz.* cyanobacterial mats, epilithic algal biomass and macroalgae collected from intertidal zones of oil-polluted and clean coasts were found heavily loaded with oil-utilizing bacteria. Each gram of those coastal materials was associated with up to hundred millions of oil-utilizing bacterial cells. Nearby coastal waters on the other hand, were much poorer in such bacteria, containing only hundreds to thousands of cells per ml. Inanimate materials, e.g. coastal sand were also found associated with oil-utilizing bacteria, but the numbers were lower than those associated with animate materials, yet much higher than those in coastal waters. Coastal gravel particles were found coated with blue-green biofilms also rich in oil-utilizers. Oil utilizing bacteria associated with coastal materials belonged predominantly to *Acinetobacter*, *Rhodococcus* and other nocardioforms, with several other less frequent genera, e.g. *Bacillus, Pseudomonas*, and others. These bacteria could utilize a wide scope of individual aliphatic and aromatic hydrocarbons as sole-sources of carbon and energy. It was concluded that coastal materials, especially animate ones, contribute to the self-cleaning of the oily Gulf coasts and the polluted Gulf waters during tidal movements.

1 Introduction

The Arabian Gulf reaches about 1000 km in length, 300 km in width and 30 m in depth. This water body is rather rich in nutrients and planktonic microorganisms.

It covers the fish requirements of the Gulf inhabitants, and in part the water requirements after desalination. Meanwhile, this water body is a rather fragile and vulnerable ecosystem due to several reasons. Most important are its continuous pollution with crude oil due to transport activity in this oil-producing area, and its low tidal displacement, i.e. limited water discharge into the Indian Ocean, meaning a limited chance to flush out its pollutants. It has been estimated that the Arabian Gulf is discharged yearly with about 160,000 tons of crude oil [1], arising from legal and illegal activities. Yet, the oil spill associated with the Iraqi invasion and occupation of Kuwait (August 2, 1990 to February 26, 1991) is the greatest ever recorded. More than a decade after this environmental catastrophy, oil sediments can still be observed in the Kuwaiti deserts as well as along the western Gulf coast. This is true although the situation has improved considerably due to self-cleaning activities in these environments [2]. The catastrophy started in January 19, 1990 when the Iraqi forces released according to various estimates between half a million and 12 million barrels of crude oil from Mina Al-Ahmady Terminal directly into the water body [3, for review see ref. 4]. Most of the crude drifted with the water currents southward and through tidal movements became sedimented in the intertidal zone along about 700 km of the Saudi-Arabian Coast. The Kuwaiti coasts were also slightly polluted, but due to self-cleaning activities, are now quite clean.

At the end of the war, about 700 Kuwaiti oil wells were blown up (February 1991), and about seven months elapsed before the fire was completely killed. During that time about 300 oil-lakes, covering a desert area of about 50 km^2 formed and containing a total of about 22 million barrels of oil, 18 million of which were recovered by the Kuwaiti authority. But the polluted desert areas still contain oil sediments to depths reaching 50 cm.

The major objective of this paper is to give an overview of results of one decade research in our laboratory on the microbiology of the oil-polluted Gulf environments. The paper also includes some of the most recent results that have not been published, so far.

2 Indigenous oil utilizing microorganisms

We already started working on the indigenous oil utilizing microflora in the Gulf environments a couple of years before the Iraqi invasion in August 2, 1990, i.e. before the catastrophy. The results of these studies appeared in a number of publications [5-8]. The predominant indigenous bacteria in aquatic and terrestrial habitats belonged to the group of nocardioforms, in addition to the genera *Pseudomonas, Bacillus* and *Streptomyces*. The most dominant fungi belonged to the genera *Aspergillus, Penicillium, Mucor* and *Fusarium*. The numbers of oil utilizing bacteria in clean desert soil and costal sand was in the magnitude of 10^7 to 10^8 cells/g, and in the coastal waters only 10 to 10^2 cells/ml. Similarly the numbers of fungi in desert soil were in the magnitude of 10^4 propogules/g and in water in the magnitude of 10 propogules/ml water. Those results allow to conclude that the Gulf desert soils and coastal sand have a much better potential for oil biodegradation than the water body.

After the liberation of Kuwait in 1991 we restarted our research programs by a number of field trips to oil-polluted desert and coastal areas. We made interesting observations and analyzed a great many of samples microbiology. In view of the long hot summer in this region of the world (April to October with maximum temperature exceeding 40°C) we made several attempts to isolate thermophilic oil-utilizing bacteria. To our surprise, those were not frequent, and only *Bacillus Stearothermophilus* was isolated from desert soil, but not from the Gulf water body [9]. This result indicates that the oil-biodegradation potential of the Kuwaiti environments at high temperatures is rather limited.

One of the interesting observations we made during early field trips was the appearance of intensive blue green mats at the top of oil sediments along the polluted coasts [2]. Those mats were microbial consortia of filamentous cyanobacteria, predominantly *Microcoleus* sp and *Phormedium* sp and others, together with hydrocarbon utilizing bacteria, predominantly nocardioforms. It was calculated that each gram mat contained about one to several millions of hydrocarbon utilizing bacteria. This microbial consortium is quite efficient in bioremediating the oily coasts. Oil-utilizing bacteria are immobilized in the mats and protected from being washed out to the open sea. Further, those bacteria are provided with the oxygen produced by the cyanobacteria during phorosynthesis. Oxygen is known to be a limiting factor for microbial attack on hydrocarbons [for review see ref. 10]. As a matter of fact those mats mediated complete cleaning of the lightly polluted coasts of Kuwait during the past decade. The much more heavily polluted coasts of Saudi Arabia still contain sediments, yet the situation visually improved a lot, thanks to those natural microbial consortia, mainly.

The above result attracted our interest to the probable role of filamentous cyanobacteria in oxidizing hydrocarbons. There are reports on aromatic hydrocarbon oxidation by unicellular and filamentous cyanobacteria and algae [11, 12]. Our studies on filamentous cyanobacteria brought some tentative evidence that they may also oxidize aliphatic hydrocarbons [2, 13, 14]. However, their important role in this process in apparently the immobilization of oil-degrading microorganisms and providing them with oxygen and fixed nitrogen [15, 16].

One of the most interesting findings of our studies is that the hydrocarbon utilizing microorganisms prefer to occur attached to substrates rather than free in the ecosystems. Thus, the results in Table 1 show that littoral materials were associated with much more hydrocarbon utilizing bacteria than the water body. Therefore, bioremediation strategies should be refocused on attenuating oil pollutants along the coast rather than in the water body [17].

3 Self-cleaning and Bioremediation

We have collected evidence that the process of self-cleaning, mediated by the indigenous microflora is naturally operating in the oily marine and terrestrial Gulf ecosystems [2, 4, 15, 16]. Further, we attempted to accelerate the self-cleaning process by particular management [20]. We subjected oily desert samples to specific types of management, once every 2 weeks, through a year; control samples were not treated. The total amounts of extractable hydrocarbons from the control samples remained constant during the dry hot months, but decreased during the rainy months, reaching after 1 year about 50% of the amount at zero time. This result demonstrated the self-cleaning of the Kuwaiti desert and the essential need for moisture in this process. Out of the studied types of management, the repeated fertilization of oily soil with KNO_3 was most efficient, reducing the extractable hydrocarbons after 1 year to about one-third of the zero reading. We also showed that fertilization of oily soil with organic carbons, e.g. glucose and peptone [21] and vitamins [22] enhanced hydrocarbon biodegradation, apparently by promoting bacterial growth and consequently enlarging the size of the hydrocarbon-degrading microflora. In another experiment we succeeded in establishing the indigenous oil-utilizing bacteria associated with coastal cyanobacterial mats into oily desert soil samples [23]. Those microorganisms biodegraded 50% of the oil within up to 20 weeks. On the other hand, we brought experimental evidence that hydrocarbon degradation in oily soil samples is primarily due to the indigenous strains; seeding with local or foreign hydrocarbon utilizing strains did not lead to any enhancement of hydrocarbon degradation, and led to dramatic decreases in the numbers of the indigenous strains [24]. These results indicate that the seeding practice could be harmful to the bioremediation for oily soils, or at least useless.

Table 1. Numbers of hydrocarbon utilizing bacteria free in the Gulf water and associated with substrates.

Free in	Numbers/g	References
The water body:		
Coastal water	2-48	[4]
Coastal water	10-300	[17]
Offshore water	10-300	[17]
Littoral materials:		
Sand	$1.7- 4.2 \times 10^5$	[17]
Rock pieces	$0.4- 3.5 \times 10^5$	[17]
Epilithic biomass	$2.8- 6.0 \times 10^7$	[17]
Cyanobacterial mats	$2.3- 4.1 \times 10^6$	[17]
Plant roots	$3.4- 6.6 \times 10^6$	[17]
Plant roots	$2.0- 3.4 \times 10^8$	[18]
Macroalgae	$1.6- 30.2 \times 10^6$	[19]

In another group of experiments we studied the role of rhizospheric hydrocarbon-utilizing bacteria in the attenuation of hydrocarbons in oily soils. We found that roots of desert plants and crop plants growing in clean and oily soils were densely colonized with hydrocarbon-utilizing bacteria [25, 26]. The most dominant were *Cellulomonas flavigena, Rhodococcus erythropolis* and *Arthrobacter* sp. The rhizospheric soils contained more hydrocarbon-utilizing bacteria than the nonrhizopheric soils. We confirmed that cropping is a feasible phytoremediation practice for oily desert soil [27]. We selected broad beans (*Vicia faba*) because it could tolerate up to 10% crude oil in soil, and its roots carry nodules that fix nitrogen gas, thus representing a natural source for nitrogen fertilization. The amounts of hydrocarbons recovered from oily soil samples supporting this plant were less than the amounts extracted from uncultivated soil samples. We also found that excised fresh roots of this plant with their intact rhizospheres resulted in the attenuation of crude oil and pure hydrocarbons, when shaken in sterile soil extract or sea water containing these compounds.

4 Bioremediation via biofilms

We worked on biofilms containing hydrocarbon-utilizing bacteria, and their role in attenuation of hydrocarbons especially in the marine Gulf environment [28]. We reported on hydrocarbon-degrading consortia of microorganisms in biofilms naturally coating gravel particles in the intertidal zone of the Arabian Gulf. Each particle was coated with about 100 mg biofilm of blue-green biomass. The predominant phototrophs were the cyanobacteria *Dermocarpella* and *Lyngbya*. The predominant hydrocarbon-degrading bacterium was *Acinetobacter calcoaceticus*. In 5 successive cycles of cleaning of oily sea water using the biomass-coated gravel particles it was found that these biofilms were quite efficient in attenuating the hydrocarbons. In another study [29] we collected various coastal materials from the intertidal zone of the Arabian Gulf, that were coated with biofilms containing hydrocarbon-utilizing microorganisms. We used those materials as inocula and investigated their potential for attenuating hydrocarbons in sea water in batch cultures. All materials were efficient in hydrocarbon attenuation, especially when KNO_3 was added as a nitrogen source (Table 2).

Our laboratory succeeded in “artificially” establishing oil-degrading biofilms on gravel particles and glass plates, and used them for attenuating hydrocarbons in sea water [30]. These biofilms could potentially be used in bioreactors for cleaning oily water and oily waste.

5 Oil-degrading bacteria associated with picocyanobacteria

Oil-degrading bacteria in the Arabian Gulf are not only associated with coastal materials, e.g. cyanobacterial mats, epilithic biomass and macroalgae, but we also found them in association with picocyanobacteria isolated from the water body. It is well known that picoplanktonic cyanobacteria e.g. *Synechococcus* and *Synechocystis* are of cosmopolitan occurrence in seas and oceans. This fact highlights the importance of our recent finding that also such phototrophs are associated with unusually high numbers of oil-utilizing bacteria (Table 3). Cultures of 17 picocyanobacterial strains isolated from the Arabian Gulf water body were predominantly associated with bacterial numbers of the magnitude of 10^9 to 10^{10} cells per g cyanobacterial biomass. The lowest numbers we found were in the magnitude of 10^7 cells/g biomass, and the highest numbers in the magnitude of 10^{13} cells per g biomass. Although rather frequent, the oil-utilizing bacteria associated with the 17 picocyanobacterial cultures from the Arabian Gulf appear to belong to only a limited number of taxa, namely the group of nocardioforms and the genera *Pseudomonas, Acinetobacter* and *Bacillus*. These organisms are typical indigenous oil-utilizing bacteria to the Gulf. We have repeatedly found that indigenous hydrocarbon utilizing bacteria can utilize a wide scope of hydrocarbons as sole sources of carbon and energy (Table 4).

Table 2. Potential of materials loaded with hydrocarbon utilizing bacteria for hydrocarbon attenuation in sea water in bach culture.

Materials	Days of incubation	Hydrocarbon	% Consumed	Reference
Blue-green mats	16	Crude oil	47.8	[4]
Broad beens roots	3	Crude oil	14.6	[27]
Broad beens roots	1	*n*-Octadecane	1.6	[27]
Broad beens roots	3	Phenanthrene	5.0	[27]
Coastal gravel	10	Crude oil	28.0	[28]
Enteromorpha (alga)	14	*n*-Octadecane	64.5	[19]
Enteromorpha	14	Phenanthrene	47.5	[19]
Colpomenia (alga)	14	*n*-Octadecane	86.7	[19]
Colpomenia	14	Phenanthrene	55.8	[19]
Iyengania (alga)	14	*n*-Octadecane	92.5	[19]
Iyengania	14	Phenanthrene	38.1	[19]
Padina (alga)	14	*n*-Octadecane	98.1	[19]
Padina	14	Phenanthrene	39.9	[19]
Sea water	20	*n*-Octadecane	45.1	[29]
Sea water	20	Phenanthrene	21.3	[29]
Sea water	20	Crude oil	45.3	[29]
Coastal sand	20	*n*-Octadecane	49.0	[29]
Coastal sand	20	Phenanthrene	15.3	[29]
Coastal sand	20	Crude oil	41.7	[29]
Blue-green mats	20	*n*-Octadecane	45.2	[29]
Blue-green mats	20	Phenanthrene	21.6	[29]
Blue-green mats	20	Crude oil	42.5	[29]
Epilithic biomass	20	*n*-Octadecane	49.0	[29]
Epilithic biomass	20	Phenanthrene	24.0	[29]
Epilithic biomass	20	Crude oil	49.3	[29]

The materials were loaded with different numbers of hydrocarbon utilizing bacteria; yet in batch cultures fertilized with KNO_3 - which was the case in all the above studies, the inoculune size did not play any significant role in the magnitude of hydrocarbon attenuation [See ref. 29].

The picocyanobacteria isolated from the water body of the Arabian Gulf could grow well in an inorganic medium containing up to 0.1% crude oil and could survive in the presence of up to 1% crude oil [13]. Hydrocarbon analysis showed that the picocyanobacteria had the potential for the accumulation of hydrocarbons (the aliphatic *n.* hexadecane, aromatic phenanthrene and crude oil hydrocarbons) from aqueous media. Electron microscopy showed that the cells of those phototrophs appeared to store hydrocarbons in their interthylakoid spaces. There was no evidence however that the picocyanobacterial cells could oxidize hydrocarbons. We assume that hydrocarbon-utilizing bacteria associated in rather huge numbers with the picocyanobacteria in nature may carry out the biodegradation of these compounds. Currently we are attempting to collect experimental evidence for this assumption.

Table 3. Numbers of oil-utilizing bacteria associated with cultures of 17 picocyanobacterial strains. [unpublished data by the authors]

Cyanobacterial srains no.	Nocardioforms	*Bacillus*	*Pseudomonas*	*Acinetobacter*	Total
Single cell Forms:					
Synechococcus					
3001	$1.1\text{x}10^{10}$	$4.5\text{x}10^{9}$			$1.5\text{x}10^{7}$
3002	$1.5\text{x}10^{7}$				$1.5\text{x}10^{7}$
3003		$2.9\text{x}10^{7}$	$3.7\text{x}10^{5}$		$2.9\text{x}10^{7}$
3004	$1.8\text{x}10^{10}$	$7.7\text{x}10^{9}$			$2.6\text{x}10^{10}$
3005	$3.8\text{x}10^{9}$				$3.8\text{x}10^{9}$
3006	$1.0\text{x}10^{10}$			$7.5\text{x}10^{9}$	$1.8\text{x}10^{10}$
3007	$4.6\text{x}10^{12}$	$7.2\text{x}10^{12}$			$1.2\text{x}10^{13}$
Synechocystis					
3009	$1.4\text{x}10^{7}$			$2.1\text{x}10^{6}$	$1.7\text{x}10^{7}$
3010	$7.8\text{x}10^{6}$			$3.2\text{x}10^{6}$	$8.1\text{x}10^{7}$
Colonial forms:					
Pleurocapsa					
3011	$2.6\text{x}10^{8}$			$10\text{x}10^{6}$	$2.7\text{x}10^{8}$
3012				$1.9\text{x}10^{7}$	$1.9\text{x}10^{7}$
3013	$1.8\text{x}10^{8}$		$2.1\text{x}10^{8}$		$3.9\text{x}10^{8}$
Dermocarpella					
3014	$2.4\text{x}10^{9}$			6.9x109	$9.3\text{x}10^{9}$
3015	$1.9\text{x}10^{9}$			1.2x1010	$1.4\text{x}10^{10}$
3016	$7.1\text{x}10^{9}$			3.5x109	$1.1\text{x}10^{10}$
3017				3.5x107	$3.5\text{x}10^{7}$
3018	$1.4\text{x}10^{8}$			1.5x109	$1.7\text{x}10^{8}$

Data are expressed in numbers per gram cyanobacterial biomass.

6 Conclusions

The indigenous microflora in the Gulf environments is naturally active in cleaning the oily areas. The activity occurs best at interfaces, especially of animate coastal materials. Consortia of heterotrophic and phototrophic marine microorganisms seem to possess a number of advantages making them ideal for hydrocarbon biodegradation.

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Table 4. Potential of coastal and aquatic bacteria for growth on hydrocarbons as sole sources of carbon and energy [Results from Ref. 17].

Bacteria	Isolated from	Substrate		Growth
Acinetobacter calcoaceticus	Epilithic biomass	*n*-Alkanes:	C_9- C_{11}, C_{24}- C_{30}	Good
			C_{12}- C_{23}	Excellent
			C_{32}- C_{36}, C_{40}	None
		Aromatics		Good
Acinetobacter calcoaceticus	Water	*n*-Alkanes:	C_9- C_{15}, C_{19}, C_{30}	Weak
			C_{16}- C_{18}	Excellent
			C_{32}- C_{36}, C_{40}	None
		Aromatics		Good
Micrococcus sp	Epilithic biomass	*n*-Alkanes:	C_9- C_{11}, C_{20}- C_{30}	Weak
			C_{12}- C_{23}	Good
			C_{32}- C_{36}, C_{40}	Weak
		Aromatics		Excellent
Micrococcus sp	Water	*n*-Alkanes:	C_9- C_{15}, C_{19}- C_{30}	Weak
			C_{16}- C_{18}	Good
			C_{32}- C_{36}, C_{40}	Weak
		Aromatics		Good
Nocardioform	Epilithic Biomass	*n*-Alkanes:	C_9	Good
			C_{10}- C_{30}, C_{32}, C_{36}, C_{40}	Excellent
		Aromatics		Excellent
Nocardioform	Water	*n*-Alkanes:	C_9- C_{12}	Good
			C_{13}- C_{30}, C_{32}, C_{36}, C_{40}	Excellent
		Aromatics		Good

The aromatics tested were benzene, phenanthrene, xylene, tolune, pyrene and biphenyl.

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