Chapter 13
Microbiology of Oil-Contaminated Desert Soils and Coastal Areas in the Arabian Gulf Region

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13.1 Introduction

Deserts are of global distribution; they cover considerable areas of all continents, with the exception of Europe. Desert soils are poor in organic substances and water, and are usually subjected to rather high temperature in summer and chilling in winter, and to extensive light. In spite of their extreme character, desert soils usually accommodate communities of micro-organisms including actinomycetes, cyanobacteria and other bacteria, fungi, protozoa, and phototrophic microalgae. Many of such micro-organisms live naturally under stress, and must possess special adaptive mechanisms in order to survive and propagate (see Chapter 2). Desert micro-organisms appear to be limited in their physiological activities due to low availability of certain nutrients, according to Liebig's "law of the minimum" (Liebig 1840).

Sometimes, microbial activities in the desert soil are arrested according to Shelford's "law of tolerance" (Shelford 1913) saying that there are maxima and minima for environmental factors above and below which micro-organisms cannot survive. Nutrient starvation seems to be one of the most serious problems desert soil micro-organisms have to overcome. Apparently, primary producers such as microalgae and cyanobacteria are the major sources of organic materials in the poor desert soils. Roszak and Colwell (1987) identified among bacteria a number of survival approaches against starvation, that fall within two strategies (Jannasch 1967), namely the potential for growth at low nutrient levels, and the potential for entering into dormancy.

Another possible mechanism for survival of starving bacteria is cell size reduction through the phenomenon of multiple division, thus producing the so-called ultramicrobacteria (Novitsky and Morita 1976, 1977, 1978; Morita 1982). The smaller the bacterial cell, the larger is its surface-to-volume ratio, and consequently the greater is its potential for accumulating diluted nutrients from the surroundings.

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Ultramicrobacteria are widely distributed in marine waters (Button et al. 1993). They may be rather dormant and consequently, relatively resistant to environmental stresses (Roszak and Colwell 1987). Some starving bacteria with a depleted amino acid pool exhibit the so-called "stringent response" (Neidhardt et al. 1990) which ultimately reduces the protein synthesis rate by inhibiting rRNA synthesis.

In addition to stresses exerted on desert micro-organisms by factors such as nutrient deficiency, drought, and heat, additional stress may arise due to pollution of the same desert soil areas with crude oil. This is particularly true for oil-producing countries such as the Arabian Gulf countries. Such desert soil areas have been polluted for many ages with small amounts of hydrocarbon vapors naturally volatilizing from the deep oil reservoirs. However, the pollution problems associated with modern and intensive oil production and transport occur on a much expanded scale.

In addition, coastal regions of oil-producing countries are particularly exposed to oil pollution which of course exerts stress on the indigenous coastal microflora.

The objective of this chapter is to shed light on changes in the indigenous microflora of desert and coastal regions in response to pollution. Emphasis is put on the desert and coastal regions of the Arabian Gulf for several reasons. Oil-utilizing micro-organisms of the Gulf area have now been the subject of study in our laboratory for more than 15 years. This area contains ancient oil, and has produced oil intensively for decades. The Gulf oil spill associated with and resulting from the Iraqi occupation of Kuwait, from August 2, 1990, to February 26, 1991, is so far the greatest in the history of mankind.

13.2 The Gulf Oil Spill

Shortly before their withdrawal from Kuwait in February 1991, the Iraqi forces deliberately blew up the Kuwaiti wells, amounting to more than 700 in the desert. It took the Kuwaiti authorities about seven months to get the resulting fires under control. During that period, crude oil kept gushing, and thus about 300 so-called "oil lakes" (Fig. 13.1) formed, covering in total about 50 km^2 of the Kuwaiti Desert. There are estimates (McKinnon and Vine 1991) that such lakes used to contain about 22 million barrels of oil, but 18 million barrels have been recovered and exported, and 3 million remain as pollutants. Oil penetrated between 40 to 60 cm deep into the sand. The total volume of polluted desert soil is estimated to be 20×10^6 m^3, and still contains highly viscous to solid crude.

The Gulf water body also received a share of the oil pollution. The Iraqi forces on January 19, 1990 deliberately released crude oil from the Mina Al-Ahmady oil terminal directly into the water. According to different estimates, the amount of oil released in the course of three successive days ranged between half a million and twelve million barrels (McKinnon and Vine 1991). The slick was 16-km long, that is, several times the size of the famous *Exxon Valdez* spill in Alaska. Most of the oil was transported counterclockwise by the water currents to the south. Most of the crude then became sedimented in the intertidal zone along more than 700 km of the western Gulf coast, leaving the water and the subtidal zone almost free of oil sediments.

Fig. 13.1 One of the small oil lakes in the Kuwaiti desert (February 1993)

13.3 Composition of Crude Oil

Crude oil consists chemically of four major constituents: saturates, aromatics, asphaltenes, and resins (Leahy and Colwell 1990). Saturated hydrocarbons, including normal alkanes with chains of up to 44 carbon atoms, branched alkanes, and cycloalkanes (naphtenes), are the major constituents of the crude, making up between 40 and 60% of the total weight. Aromatic hydrocarbons with from one to six benzene or substituted benzene rings follow the saturates in quantitative importance and amount to roughly 20% of the crude weight. Asphaltenes, which include tar, are very high-molecular weight hydrocarbons, which are used as road paving materials. Resins are crude constituents that contain, in addition to carbon and hydrogen, sulfur and oxygen. The chemistry of asphaltenes and resins is still not yet completely known; both constituents make up 1 to 5% of light oils and up to 25% of heavy oils, which correspondingly contain lower proportions of saturates and aromatics.

13.4 General Description of Oil-Utilizing Micro-Organisms

Several reports have been published on oil-utilizing micro-organisms, for example, Klug and Markovetz (1971), Levi et al. (1979), Einsele (1983), Radwan and Sorkhoh (1993), Van Hamme et al. (2003), Rosenberg (2006), and Widdel et al. (2006). It is important to note that such micro-organisms are normal indigenous soil and water inhabitants, and that most of them can consume conventional carbon sources. Their defining characteristic, which makes them capable of utilizing hydrocarbons as substrate, is that they possess the so-called mono-oxygenase and/or dioxygenase enzyme systems. Such systems catalyze the introduction of oxygen atoms from molecular oxygen into aliphatic and aromatic hydrocarbon molecules producing the corresponding alcohols that in turn become further oxidized to aldehydes, and ultimately acids. The resulting acids are then biodegraded by β-oxidation, producing acetyl CoA that can be further metabolized (for reviews see Rehm and Reiff 1981; Fukui and Tanaka 1981; Boulton and Ratledge 1984).

The capacity to utilize hydrocarbons is widely distributed among conventional micro-organisms including prokaryotes and eukaryotes. Bacterial genera reported to attack hydrocarbons include *Acinetobacter, Micrococcus, Vibrio*, *Azospirillum* (Roy et al. 1988), *Aeromonas*, *Alcaligenes*, *Chromobacterium*, *Flavobacterium, Klebsiella*, *Pseudomonas* (Klug and Markovetz 1971), *Bacillus* (Loginova et al. 1981; Sorkhoh et al. 1993), *Arthrobacter, Brevibacterium, Corynebacterium*, *Rhodococcus*, *Mycobacterium*, *Nocardia* and other nocardioforms (Egorov et al. 1986), and *Streptomyces* (Barabas et al. 1995). Yeast genera capable of utilizing hydrocarbons include (for review see Radwan and Sorkhoh 1993) *Candida*, *Dabayomyces*, *Endomyces*, *Leucosporidium*, *Lodderomyces*, *Metschnikowia*, *Pichia*, *Rhodosporidium*, *Rhodotorula*, *Saccharomycopsis*, *Schwanniomyces*, *Selenotila*, *Sporidiobalus*, *Sporobolomyces*, *Torulopsis*, *Trichosporon*, and *Wingea*. Filamentous fungi with hydrocarbon utilization potential include *Absidia* (Hoffman and Rehm 1978), *Aspergillus, Aureobasidium*, *Beauveria* (Davies and Westlake 1979), *Botrytis*, *Cephalosporium*, *Cladosporium, Corellospora*, *Canninghamella*, *Dendyphiella* (Kirk and Gordon 1988), *Fusarium, Hormodendrum* (Lin et al. 1971a,b), *Lulworthia* (Kirk and Gordon 1988), *Mortierella*, *Mucor, Penicillium, Phialophora*, *Phoma* (Davies and Westlake, 1979), *Scedosporium* (Ornodera et al. 1989), *Scoleobasidium* (Davies and Westlake 1979), *Sporotrichum*, *Varicosporina*, and *Verticillium* (Kirk and Gordon 1988). In addition, there are reports that phototrophic bacteria such as *Rhodospirillum* and *Rhodopseudomonas* (Cerniglia et al. 1980b); cyanobacteria such as *Oscillatoria* (Cerniglia et al. 1980a), *Microcoleus*, and *Phormidium* (Al-Hasan et al. 1994, 1998); microalgae such as *Chlamydomonas* and *Chlorella* (Ellis 1977); and the phytoflagellate *Euglena* (Ellis 1977) can oxidize aliphatic and/or aromatic hydrocarbons.

None of the micro-organisms listed above can consume all of the crude oil constituents; and each organism has the potential for utilization of only a limited range of compounds. Yet, collectively, all crude constituents from the gaseous low molecular weight (van Ginkel et al. 1987; Ornodera et al. 1989) up to the medium and high molecular weight (Demanova et al. 1980) compounds, including asphaltenes, can be attacked by micro-organisms.

Growth on oil and hydrocarbons is associated in some micro-organisms with certain unique morphological and/or cytological features. One of the most frequent features is the appearance of cytoplasmic hydrocarbon inclusions in actinomycetes (Barabas et al. 1995) and other bacteria (e.g., Scott and Finnerty 1966; Atlas and Heintz 1973; Kennedy and Finnerty 1975), and also in filamentous fungi (Cundell et al. 1976; Koval and Redchitz 1978; Redchitz 1980). The picocyanobacteria *Synechococcus* and *Synechocystis* exhibit much wider interthylakoid spaces in the presence of oil and hydrocarbons than in the absence of these compounds (Al-Hasan et al. 2001). Some micro-organisms produce dense intraplasmic membranes (Kennedy and Finnerty 1975; Ivshina et al. 1982) and volutin inclusions (Redchitz and Koval 1979; Ivshina et al. 1982). *Penicillium* grows in shaken cultures in the presence of hydrocarbons as hollow mycelial balls enclosing hydrocarbon droplets, whereas in hydrocarbon-free media the balls are solid (Cundell et al. 1976).

13.5 Oil-Utilizing Micro-Organisms in the Arabian Gulf Desert Soils

Our group in Kuwait, working for more than 15 years on oil and hydrocarbon-utilizing micro-organisms indigenous to the desert and marine environments of the Arabian Gulf, has collected a wealth of useful information on this subject. This information may help in understanding the composition of oil-utilizing microflora indigenous to other desert and coastal areas similar to those of the Gulf area.

The predominant indigenous oil-utilizing bacteria in the Kuwaiti desert belong to *Micrococcus, Pseudomonas, Bacillus, Arthrobacter*, and the group of nocardioforms, particularly the genus *Rhodococcus* (Sorkhoh et al. 1990, 1995; Radwan et al. 1997). The genus *Streptomyces* is the predominant oil-utilizing actinomycete in the Kuwaiti desert (Barabas et al. 1995, 2000; Radwan et al. 1998b).

The oil-utilizing fungal flora of the Kuwaiti desert comprises predominantly the genera *Aspergillus, Penicillium, Fusarium*, and *Mucor*. Members of other genera are also found (Sorkhoh et al. 1990). The Gulf region is characterized by a rather long, dry, and very hot summer. Therefore, it may be expected that the desert in this region may accommodate thermophilic hydrocarbon-utilizing micro-organisms. The analysis of 38 Kuwaiti Desert soil samples polluted with crude oil revealed the occurrence of 3.7×10^3 to 1.1×10^7 cells of thermophilic (with an optimal temperature of 55°C) oil-utilizing bacteria per g of soil, all of which were identified as *Bacillus stearothermophilus* (now *Geobacillus stearothermophilus*) (Sorkhoh et al. 1993). The isolation of hydrocarbon-utilizing thermophiles is not unexpected inasmuch as Loginova et al. (1981) observed the growth of obligate thermophilic bacteria in a medium with paraffin. Similarly, Zarilla and Perry (1984) reported on *Thermoleophilum album* as a novel bacterium obligate for thermophily and utilizing *n*-alkane substrates.

In the course of our studies on oil-utilizing micro-organisms in the Kuwaiti Desert soils, we noticed that oil-polluted areas generally contained higher numbers of such organisms than pristine areas. However, pristine desert soil was never free of oil-utilizing bacteria. In particular, in both pristine and contaminated Kuwaiti Desert areas, the soil fraction in direct contact with the desert plant roots (or rhizosphere) represented microenvironments enriched in oil-utilizing micro-organisms. The rhizospheres of desert plants growing in the Kuwaiti Desert were found to contain more hydrocarbon-utilizing micro-organisms than the soil farther away from the roots (Radwan et al. 1995b, 1998a). These plants included *Senecio glaucus*, *Cyperus cenglomeratus*, *Launaea mucronata*, *Picris babylonica*, and *Salsola imbricata*. It was observed that some plants, although growing in black, oil-polluted, desert soil areas, possessed white clean roots rich in oil-utilizing bacteria (Radwan et al. 1995b). Not only the rhizospheres of wild plants, but also those of legume crops (e.g., *Vicia faba* and *Lupinus albus*) were richer in hydrocarbon-utilizing bacteria than nonrhizosphere soil. The rhizosphere effect (which is measured as the ratio of the number of micro-organisms in the rhizosphere soil to the number of micro-organisms in nonrhizosphere soil (Anderson et al. 1993)) was much more pronounced for plants growing in oil-polluted than in pristine soils. The most prevalent hydrocarbon-utilizing bacteria in the rhizospheres of the above plants were *Cellulomonas flavigena*, *Rhodococcus erythropolis*, and *Arthrobacter* spp.

There is experimental evidence for self-cleaning of oily desert soil in Kuwait through the activities of the indigenous hydrocarbon-utilizing microflora. The total amounts of extractable alkanes from heavily polluted soil cores in a Kuwaiti oil field exposed to the open air were quantitatively determined once every two weeks through a whole year (Radwan et al. 1995a). It was found that the total amount of extractable alkanes remained fairly constant during the dry hot summer months, but decreased during the rainy months reaching, after one year, slightly more than one half of the amount at zero time. This result demonstrates the self-cleaning capacity of the Kuwaiti Desert soil and the essential role of moisture in this process. The loss of alkanes could not be attributed to simple physical volatilization, because loss occurred at a slower pace during the hot summer. On the contrary, self-cleaning was faster during the rainy period of the year, suggesting that it was mainly occurring through biological processes.

13.6 Micro-Organisms in the Gulf Coastal Areas

One of the interesting observations our group made during trips to the oily coastal regions in the early 1990s was the appearance of mats of an intense blue-green color at the top of oil sediments in the Saudi Arabian Gulf coasts, about 300 km south of Kuwait (Sorkhoh et al. 1992). Those mats are frequent in the Gulf coasts, even in the nonpolluted areas (Golubic 1992). Later, we made similar observations along the oily Kuwaiti coasts (Fig. 13.2). Strikingly, the mats were tightly associated with oil, and oil-free coastal areas were also free of those mats. It was also noted that all forms of higher life on the oily coasts were dramatically inhibited by the oil sediments. Animal inhabitants were absent or dead, and their coastal underground tunnels were full of crude. The mats appeared to be at that time the only, or one of the few living things in the oily coasts. The microbiological analysis of mat samples we collected from the Saudi research station of Jubail revealed that they consisted mainly of photosynthetic and heterotrophic prokaryotes. The phototrophs included filamentous cyanobacteria, such as *Microcoleus, Phormidium*, *Spirulina*, and others, in addition to some eucaryotes, mainly diatoms. The filamentous

Fig. 13.2 Microbial mats on the top of oil sediments along the Kuwaiti Coast of the Arabian Gulf

cyanobacteria adhered together through excreted mucilage forming the mat matrix. The heterotrophic bacteria associated with those mats included millions of oil-utilizing bacteria per g fresh mat.

In this context, blue-green mats, also comprising the cyanobacterium *Microcoleus*, have also been recorded in the pristine coast of Abu Dhabi (Golubic 1992). The hydrocarbon-utilizing bacteria associated with the mats (Sorkhoh et al. 1995) consisted of nocardioforms (63%), belonging mostly to the genus *Rhodococcus*, the genera *Bacillus* (21%), and *Arthrobacter* (13%), in addition to *Pseudomonas* as a minor bacterium. Actinomycetes belonging to the genus *Streptomyces* and filamentous fungi belonging to the genera *Aspergillus* and *Penicillium* were also identified, but were much less frequent than the other heterotrophs. *Microcoleus* consortia comprising heterotrophic bacteria capable of biodegrading oil have also been described by Garcia De Oteyza et al. (2004). The microbial consortium in the blue-green mats is active in self-cleaning of the oily coasts of the Gulf. Successive visits to the lightly polluted coasts along Kuwait revealed that the oil sediments gradually vanished. Today, those coasts have become absolutely oil-free, and their mats have disappeared.

The mats harbor a number of advantages as valuable biological systems in self-cleaning of oily coasts. Heterotrophic oil-utilizing bacteria are naturally immobilized within the mats, thus avoiding being washed out into the open sea. Furthermore, such bacteria are adequately aerated with oxygen produced by the photosynthetic partners in the mats. It is known that oxygen could be a limiting factor in microbial biodegradation of hydrocarbons (for a review, see Radwan and Sorkhoh 1993). In addition, some of the cyanobacterial partners in the mats are probably nitrogen fixers (see also Steppe et al. 1996); nitrogen fertilization is known to enhance microbial degradation of hydrocarbons (Radwan et al. 1995c). In addition, there is experimental evidence for direct hydrocarbon oxidation by cyanobacteria and algae (Cerniglia et al. 1980a,b; Al-Hasan et al. 1994, 1998; Raghukumar et al. 2001; Todd et al. 2002).

Along the Gulf coast and also probably elsewhere, littoral materials from intertidal zones are associated with much higher numbers of oil-degrading micro-organisms than inshore and offshore water samples (Radwan et al. 1999). This may indicate that the coasts have a better potential for oil biodegradation than the water body. Oil-utilizing bacteria found associated with coastal materials along the Arabian Gulf belonged to the genera *Acinetobacter*, *Micrococcus*, and the group of nocardioforms (Radwan et al. 1999). Interestingly, those bacteria are more frequent in association with littoral animate materials, such as microbial mats and epilithic biomass, than in association with inanimate materials, such as sand, stonelets, and gravel particles. However, gravel particles coated with blue-green biofilms are also rich in oil-utilizing bacteria (Radwan and Al-Hasan 2001).

In coastal and offshore waters of the Gulf, oil-utilizing bacteria are found preferentially associated with macroalgae (Radwan et al. 2002), fish (Radwan et al. 2007a), and picoplankton (Radwan et al. 2005a), rather than free-living. Thus, microbial consortia in biofilms along the coasts and various associations in the water body seem to play a major role in self-cleaning of the oily marine ecosystems.

13.7 Extremophilic Oil-Utilizing Micro-Organisms

There is an information gap regarding the extremophilic oil-utilizing micro-organisms in desert, coastal, and marine environments. Mention has already been made of the few studies on thermophilic hydrocarbon-utilizing bacteria (Loginova et al. 1981

Zarilla and Perry 1984; Sorkhoh et al. 1990; see Section 13.5). However, there are no studies on hyperthermophilic hydrocarbon-utilizing bacteria.

A significant number of oil-polluted ecosystems are characterized by rather high alkalinities and/or salinities. These include estuaries, beaches, salt marshes, inland lakes, rockpools, desert rain pools, and others. Furthermore, billions of gallons of wastewaters with high contents of salts and waste organics are generated by industry, and disposed of in the environment. An example of such industrial activities is the production, transport, and refining of crude oil, normally associated with the generation of a large volume of oily salt water that displays a wide range of alkalinities and/or salinities (Flynn et al. 1996; Roe et al. 1996). This makes oil pollution difficult to treat using conventional microbial strains, whose cell membranes may be disrupted and enzymes denatured (Kargi and Dincer 2000; Woolard and Irvine 1994). To bioremediate oily alkaline and saline environments without costly pretreatment, alkaliphilic and halophilic micro-organisms should be used (Díaz et al. 2002).

With these facts in mind, recent work performed in our laboratory (Sulaiman, 2006; Al-Awadhi et al. 2007) concerned the isolation and identification of alkaliphilic and halophilic oil-utilizing bacteria from the Arabian Gulf coasts of Kuwait. The results showed that animate coastal materials such as epilithic biomass and cyanobacterial mats were associated with considerable numbers of alkaliphilic and halophilic oil-utilizing bacteria. Inanimate material, such as coastal sand and gravel particles, as well as coastal waters contained much fewer numbers, if any, of these bacteria. The alkaliphilic oil-utilizing bacteria were found to belong to the genera *Marinobacter*, *Micrococcus*, *Dietzia*, *Bacillus*, *Oceanobacillus*, and *Citricoccus*. The halophilic oil-utilizing bacteria were found to belong to the genera *Marinobacter*, *Georgenia*, *Microbacterium*, *Stappia*, *Bacillus*, *Isoptericola*, and *Cellulomonas*. All isolates had a good potential for hydrocarbon degradation, and consequently may be suitable tools for self-cleaning and bioremediation for oily alkaline and salty regions.

13.8 Microbial Consortia and Associations

In previous sections of this review, mention has been made of the frequent occurrence of microbial associations probably involved in oil biodegradation in the Gulf environments (see also Grötzschel et al. 2002; Abed et al. 2002; Abed and Köster 2005; Sanchez et al. 2005). Such associations were recorded in the desert and coastal environments as well as in the water body of the Gulf. In the present section, more light is shed on those associations. Although it should be expected that oil-biodegradation in oily desert soil is mediated by "hypothetical" microbial consortia comprising bacteria and fungi, with the possibility of establishment of cometabolic and syntrophic strategies, there is only little information on the role of individual partners in such consortia.

The rhizospheric microflora associated with roots of desert plants may offer unique opportunities in this respect, as in this case the benefits of the association to the oil-utilizing microbes are obvious. In the rhizosphere, oil-utilizing micro-organisms find proper conditions for propagation and activity. These micro-organisms can cover their vitamin requirements from exudates excreted by the root tissues. Indeed, more than 90% of oil-utilizing bacteria need vitamins for optimal growth and activity (Radwan and Al-Muteirie 2001). Furthermore, plants are known to aerate their rhizospheres by pumping air down into the soil. Oxygen was reported to be a limiting factor for the microbial attack on hydrocarbon molecules (Rehm and Reiff 1981; Fukui and Tanaka 1981). The roots, particularly of legumes, may also provide oil-utilizing bacteria with nitrogen fixation potential, thus enriching soil with compounds reported to enhance microbial hydrocarbon biodegradation (Atlas 1981; Leahy and Colwell 1990).

The coastal and aquatic marine environments appear to contain more interesting oil-degrading microbial consortia than those of the desert soil. In most of those associations there are phototrophic partners and heterotrophic oil-utilizing partners. This is true for coastal epilithic biomass (Radwan et.al. 1999), coastal cyanobacterial mats (Radwan et al. 1999), coastal gravel particles coated with picocyanobacteria and other phototrophs (Radwan and Al Hasan 2001), macroalgae coated with bacterial biofilms (Radwan et al. 2002), and picocyanobacteria associated with oil-utilizing bacteria at the water surface (Radwan et al. 2005a). In all these consortia, the conditions are suitable for growth and activity of oil-utilizing bacteria. Thus, oxygen becomes available as a byproduct of photosynthesis, and nitrogenous compounds as a result of nitrogen fixation by some cyanobacteria. Furthermore, as mentioned above, in many of these associations the bacteria are protected against dispersal in the open sea.

Another interesting association in coastal and offshore waters is that of oil-utilizing bacteria in biofilms coating fish surfaces and gills and gut linings (Radwan et al. 2007a). All test samples of ten types of the Arabian Gulf fish and two of farm fish were found to accommodate rather high numbers of oil-utilizing bacteria, with 10^5 to 10^7 cells being found per cm^2 of fish surface and per gram of gills and guts. Such numbers were much higher than in the surrounding Gulf water which contained only 10^2–10^3 bacteria per ml. Such bacteria belonged to the genera *Acinetobacter*, *Micrococcus*, *Bacillus*, *Rhodococcus*, and other nocardioforms. It should be expected that ships, boats, and other vehicles navigating in the Gulf (and other open waters) are also probably coated by biofilms rich in oil-utilizing micro-organisms which apparently play a role in self-cleaning of the polluted aquatic environments.

13.9 Responses of Micro-Organisms to Environmental Variables

The numbers and activities of oil-utilizing micro-organisms in oily desert soil and coastal regions are affected by a number of environmental factors.

13.9.1 Organic Matter Content

There is a lack of information on the effect of desert soil organic matter on oil-utilizing micro-organisms. Desert soils are characterized by their very low content of organic substances. Therefore, it may be assumed that the organic oil pollutants would specifically favor oil-utilizing micro-organisms. They certainly do, and we have observed this through our studies on the Gulf desert microflora. How oil-utilizing micro-organisms would be affected, should an oily desert soil sample receive conventional organic substances, should also be examined. This question is relevant, because most of the oil-utilizing micro-organisms also have the potential for utilizing conventional organic carbon sources. A partial answer to this question lies in the fact that hydrocarbon-utilizing micro-organisms are more frequent in the rhizospheric than in the nonrhizospheric soils (Radwan et al. 1998a). It is well established that plant roots permanently excrete into the soil organic exudates, for example, sugars, vitamins, and amino acids. Such exudates probably stimulate the oil-utilizing micro-organisms in the rhizospheric soils.

A more direct answer to the interesting question of conventional substrate utilization is provided by the results of one of our studies (Radwan et al. 2000). It was found that fertilizing an oily sample of desert soil with a mixture of glucose and peptone resulted in enhanced oil attenuation in that sample. The magnitude of the stimulation effect was too great to be attributed solely to nitrogen fertilization by the added peptone. Soil fertilization with KNO_3 containing an equivalent amount of nitrogen to that in peptone brought about a much lower oil attenuation value than that obtained with peptone. Glucose/peptone addition to a clean desert soil sample resulted in a dramatic increase of the total numbers of oil-utilizing micro-organisms in that sample. After 13 days, the micro-organisms had depleted all the added glucose and peptone and their numbers decreased. In the oily desert soil sample, glucose/peptone addition also increased the numbers of oil-utilizing micro-organisms. Yet, after the depletion of glucose and peptone, the numbers of oil-utilizing micro-organisms remained high and enhanced oil attenuation was recorded. It was thus concluded that easily utilizable conventional carbon and nitrogen sources in desert soil favor the oil-utilizing microflora, and consequently oil attenuation in such soils.

A question may be raised here regarding the origin of easily utilizable organic matter in the poor desert and coastal soils. It is well known that the primary producers in the various ecosystems of our planet are the phototrophic (and chemolithotrophic) organisms. In this respect, coastal areas, being wet all or most of the time, benefit from supporting algae and cyanobacteria. The latter produce and liberate easily utilizable organic matter via photosynthesis. On the other hand, dry desert soils seem to support such phototrophs only temporarily, following rainy periods. Therefore, their organic substance contents should be low, and dependent on the frequency of precipitation.

Certain organic compounds, such as carboxylic acids, alcohols, and aldehydes, inhibit or even kill most micro-organisms. Interestingly, hydrocarbon-utilizing micro-organisms permanently produce such compounds from their hydrocarbon substrates as metabolic intermediates and release some of them into the environment (for a review see Radwan and Sorkhoh 1993).

13.9.2 Temperature

Micro-organisms in the Gulf desert soils are naturally subjected to a rather wide range of temperatures. During daytime the surface desert soil in the Arabian Gulf environment may reach or exceed 70°C. In winter nights the temperature may fall below the freezing point. Although there are a few reports in the literature on thermophilic hydrocarbon-utilizing bacteria (Loginova et al. 1981; Zarrilla and Perry 1984; Sorkhoh et al. 1993), our experience indicates that hydrocarbon-utilizing micro-organisms in desert soils and coastal regions of the Gulf are predominantly mesophilic with optima at 30–35°C. We have isolated only one thermophilic species, *Bacillus stearothermophilus*, from desert soils, and this organism was not markedly predominant in any of the numerous desert soil samples analyzed (Sorkhoh et al. 1993). We also failed to isolate obligate psychrophilic hydrocarbon-utilizing micro-organisms from desert soil samples.

It appears that desert micro-organisms are provided with survival mechanisms at both high and low temperatures. Our knowledge of such mechanisms is still far from clear. The production of "dormant" units such as endospores, cysts, and others, is one of such mechanisms; yet it is limited only to a few species of bacteria and fungi. However, diminishing metabolic activity in vegetative cells to a minimum level may probably be an effective strategy for survival at unsuitable temperatures. This strategy might be more easily applied at suboptimal than at superoptimal temperatures. The moisture content may be an important interfering factor here. Moist heat is known to be more effective in killing micro-organisms than dry heat. Dry proteins need higher temperatures for denaturation than wet proteins. In view of the fact that the highest temperatures in the desert environment are reached during the long "dry" summer, a preliminary understanding of probable surviving mechanisms of mesophilic micro-organisms at temperatures much above their optima could be gained through examination of dry heat effects on proteins.

Wet coastal regions appear to be more protected against the high summer temperatures than the dry desert areas.

13.9.3 Hydrogen Ion Concentration

Our routine measurements revealed that pristine and oily desert soils of the Gulf were rather neutral. This was also true for coastal soil samples except for a tendency of these soils to become slightly alkaline. It is known that soil pH affects the dissociation of the carboxyl and amino groups of proteins and thus the microbial enzymatic activities. For optimal activity, enzymes have to be in a certain state of dissociation. Furthermore, the pH affects the solubility and consequently the availability of many nutrients, for example, phosphate and ammonium (see Atlas and Bartha 1998). It has been mentioned (see Section 13.4) that hydrocarbon-utilizing micro-organisms in the Gulf desert soils and coastal areas comprise bacteria and fungi. Bacteria predominantly prefer neutral environments and are usually sensitive to acidity. Most fungi also prefer a neutral pH value, but are tolerant of acidity.

These facts mean that the microbiological attack on hydrocarbons in the Gulf desert soils and coastal areas can occur at a wide range of hydrogen ion concentrations, yet with an optimum at pH 7. In view of the well-known fact that fungi are commonly slower in growth and activity than bacteria, it should be expected that acidity would slow down the hydrocarbon biodegradation in the polluted soils. On the other hand, the coastal areas of the Gulf contain limited numbers of alkaliphilic hydrocarbon-utilizing bacteria (Al-Awadhi et al. 2007). This also means that extremely alkaline areas polluted with oil could be enriched with alkaliphilic hydrocarbon-utilizing bacteria that would sustain the oil biodegradation process.

Hydrocarbon biodegradation in oily soils proceeds in the pH range of 4.5 to 11.5 with optima between pH 6.5 and 8 (Daylan et al. 1990). Our group found that the irrigation of oil desert soil samples with sewage-effluent as source of water and nitrogen inhibited alkane-biodegradation due to increasing soil acidity (Radwan et al. 1995c). However, liming relieved this inhibition.

13.9.4 Moisture and Aeration

Desert soils are characterized by extremely low moisture content most of the year. In the Arabian Gulf region, precipitation is rather rare and occurs only during the short winter. The dry period may exceed nine months in the year. On the other hand, coastal areas are submerged with sea water during tidal and wave movements, and suffer from drought only temporarily. There is an inverse proportion between the soil moisture content and the soil degree of aeration. Thus, the Gulf desert soils are well aerated most of the time. Microbial activities in dry soils are substantially enhanced by increasing the water content, but only until the latter starts to fill the soil air space. When water logging occurs, growth and metabolism of aerobic micro-organisms become inhibited. Moisture content of soil is optimal for residing micro-organisms at 50–75% of its water holding capacity.

Oil-utilizing micro-organisms are predominantly aerobic, and the first step in the microbial attack on hydrocarbon substrates involves the introduction of one (for alkanes) or two (for aromatic rings) oxygen atoms into the substrate molecule (Rehm and Reiff 1981; Boulton and Ratledge 1984; Buehler and Schindler 1984; Singer and Finnerty 1984). There are reports on anaerobic biodegradation of hydrocarbons, but such a process is so slow that it is considered negligible in nature (Ratledge 1978; Atlas 1981; Aeckersberg et al. 1991). Experimental results showed that the microbiological degradation of hydrocarbons in Kuwaiti Desert soil samples was insignificant during the long dry period of the year, but was resumed actively during rainy months (Radwan et al. 1995c). This result demonstrates that water is a major limiting factor controlling the microbiological hydrocarbon degradation in desert soils. Water in soil is also needed for microbial motility, microbial spacial proliferation and substrate transport (Smiles 1988).

Salinity reduces water activity and thus, water availability to micro-organisms and other living beings. However, oil-utilizing bacteria in the desert soils and coastal areas seem to be adapted to the range of salinities common in their natural habitats. We have noticed that terrestrial oil-degrading bacteria operate optimally in the absence of any added sodium chloride, whereas coastal and sea water isolates needed about 3.5% (w/v) sodium chloride for optimal activity.

13.9.5 Inorganic Nutrients

Desert soils are commonly quite poor in organic matter but usually contain adequate amounts of most of the inorganic nutrients needed by micro-organisms. Under certain conditions, however, soil micro-organisms may show increased requirements for some specific inorganic nutrients. Oil pollution is a typical example of such a condition. Hydrocarbons are consumed by micro-organisms as carbon and energy sources. In order for hydrocarbon-utilizing micro-organisms to synthesize proteins, nucleic acids, and other organic nitrogenous compounds, and to enhance their energy metabolism, they require additional amounts of nitrogen and phosphorus (Atlas and Bartha 1972; Gibbs 1975; Gibbs et al. 1975). It has been estimated that 60 mg N and 6 mg P are needed for the metabolism of one gram of hydrocarbons (Kant et al. 1985).

Experimental results in our laboratory showed that the microbiological hydrocarbon degradation in oily Kuwaiti Desert soil samples and coastal areas was enhanced by nitrate but not by phosphate fertilization (Radwan et al. 1995c). This result indicates that such soil samples contain insufficient concentrations of nitrogen, but adequate concentrations of phosphorus. Some authors suggested the use of oleophilic nitrogen and phosphorus fertilizers for enhancing oil biodegradation especially in the oily marine ecosystem (Atlas and Bartha 1972; Atlas 1977). Hydrocarbon-utilizing bacteria predominantly have the potential for using both inorganic and organic nitrogen sources (Radwan et al. 1995a,b, 2000). Unpublished results in our laboratory indicate that some of such bacteria, such as *Bacillus* spp., are capable of atmospheric nitrogen fixation, and that the symbiotic nitrogen-fixing nodule bacteria (*Rhizobium* and *Bradyrhizobium* spp.) can utilize hydrocarbons as the sole sources of carbon and energy (see also Prantera et al. 2002).

Oil-polluted desert soils and coastal areas become simultaneously enriched with heavy metals which are known to inhibit micro-organisms at rather low concentrations (Gadd 1990). However, micro-organisms are provided with defense mechanisms against heavy metal toxicity. These mechanisms include the reduction of transport of such metals across the cell envelope, their complexing and subsequent precipitation outside the cell, and compartmentalization inside the cell (Atlas and Bartha 1998).

13.9.6 Surfactants

Many, but not all, hydrocarbon-utilizing micro-organisms produce surfactants extracellularly in order to emulsify or pseudosolubilize these water-insoluble substrates prior to their uptake (Desai and Banat 1997; Cameotra and Makkar 1998; Makkar and Cameotra 1998, 2002; Banat et al. 2000). Such biosurfactants comprise low molecular weight compounds such as trehalose lipids, rhamnolipids, surfactin, polyol lipids, and fatty acids, and high molecular weight compounds such as emulsan, liposan, mannan, and lipoproteins. Apparently, biosurfactants and chemical surfactants change the physical nature of oil, but do not eliminate it from the environment.

There are contradicting reports on the effect of such compounds on the microbiological degradation of oil substrates, as both enhancing and inhibitory effects were recorded (Tumeo et al. 1994; Bai et al. 1997; Lang and Wullbrandt 1999). The inhibitory effect was attributed to surfactant toxicity, preferential metabolism of the surfactants over the hydrocarbons, and/or interference with the membrane uptake process (Efroymson and Alexander 1991; Rouse et al. 1994; Mulligan et al. 2001). Several authors emphasize the need for more study before surfactant-enhanced bioremediation approaches may be suggested (Leavitt and Brown 1994; Van Eyk 1994; Korda et al. 1997; Bolba et al. 1998).

13.10 Bioremediation Strategies

Bioremediation has been defined as the technology in which microbial activities are implemented to mineralize and remove xenobiotic pollutants from the environment (Atlas and Pramer 1990). During the 1990s a number of books and review articles were published on this subject (e.g., Hinchee and Olfenbuttel 1991a,b; Riser-Roberts 1992; Rosenberg 1993, Alexander 1994; Stoner 1994; Atlas 1995; Radwan et al. 1995c). Out of the xenobiotic pollutants, the hydrocarbon contaminants were among the first to receive close attention (Mueller et al. 1989; Song et al. 1990; Hinchee and Olfenbuttel 1991a,b). Bioremediation technology involves two basic approaches: biostimulation, or enhancing the activity of indigenous micro-organisms capable of degrading the pollutants, and bioaugmentation, or seeding the environment with pollutant-degrading micro-organisms.

The current section of this review deals with bioremediation strategies for cleaning oily desert and coastal areas of the Arabian Gulf region. However, before examining such strategies, it may be useful to refer to earlier bioremediation attempts from other parts of the world. One such attempt was the partial bioremediation of about 800,000 gallons of oily wastewater in the bilge tanks of the *Queen Mary* moored in Long Beach Harbor, CA (Applied Biotreatment Association 1989). There were also attempts to clean oily terrestrial environments in refineries and tank farms using bioremediation technology (Zitrides 1990). Even oil spills in the open sea were subjected to bioremediation attempts. In the summer of 1990, the Norwegian tanker *Mega Borg* discharged 100,000 barrels of crude oil into the Caribbean, and the Texan Company Alpha Environmental applied a mixture of oil-degrading micro-organisms in an attempt to clean up the resulting spill (McKinnon and Vine 1991). In the spring of 1989, the *Exxon Valdez* tanker released about 11 million gallons of crude oil into Prince William Sound in Alaska, thus heavily contaminating more than 1,000 miles of the Alaskan coasts. Because physical removal of oil by simple washing of the coasts was of only limited effectiveness, it was suggested to fertilize contaminated areas with nutrient that might enhance the potential of the indigenous microbial flora for biodegrading the oil (Pritchard and Costa 1991; Bragg et al. 1992, 1994). Reportedly, the rates of oil biodegradation were enhanced three to five times when contaminated coasts were fertilized with 4–8 kg nitrogen per $10\,m^2$, and the time required for oil removal was thus reduced from 10–20 years to only 2–3 years.

There are no large-scale attempts to bioremediate in situ oily desert soils and coastal areas in the Gulf. Yet, results from basic studies make it possible to design cleaning protocols. As mentioned above, bioremediation involves two major approaches, seeding with oil-degrading micro-organisms and fertilization with nutrients enhancing the indigenous microflora. As far as seeding is concerned, there are commercial mixtures of micro-organisms available in the market for in situ application (Applied Biotreatment Association 1989, 1990). However, experience in our laboratory indicates that seeding should not be the approach of choice for bioremediating oily environments in the Gulf (Radwan 1990; Radwan et al. 1997). Bioremediation should depend on indigenous strains, which are adapted to the prevailing environmental conditions. Oil spills create specific conditions for enhancing indigenous oil-degrading strains. In one of our studies (Radwan et al. 1997), we found that after a simulated oil spill, and for 28 weeks, desert soil samples became steadily enriched with one specific, indigenous, oil-degrading *Arthrobacter* strain, KUCC201. Other indigenous oil-utilizing bacteria, including other *Arthrobacter* strains either remained unchanged at low numbers or steadily disappeared. Conversely, seeding the 24-week-old polluted samples with local or foreign oil-degrading isolates resulted in dramatic decreases in the numbers of the predominant, indigenous, oil-degrading *Arthrobacter* strain, KUCC201. We concluded that seeding is probably a useless, or even harmful approach for bioremediation. In this context, genetically engineered oil-degrading micro-organisms have been created for decades (Hartmann et al. 1979; Reineke and Knachmuss 1979). However, the introduction of such strains into the environment is obviously hazardous and requires governmental regulation (Halvorson et al. 1985).

The above discussion indicates that the approach of choice for bioremediating oily environments should rely on the enhancement of activity of indigenous hydrocarbon-utilizing micro-organisms. This may be achieved, in particular, by fertilization with nutrients such as nitrogen.

As far as the oily desert soil areas are concerned, management should primarily include irrigation and nitrogen fertilization. The Gulf desert soils receive intermittent precipitation only during the short winter, therefore self-cleaning of oily areas by indigenous micro-organisms ceases most of the year (Radwan et al. 1995). Mere irrigation with water activates the micro-organisms in the summer, and fertilization with KNO_3 enhances the cleanup process considerably (Radwan et al. 1995). In view of these facts, a phytoremediation approach depending on rhizosphere technology may be suggested as the biotechnology of choice for bioremediating oily deserts.

Our group studied the role of rhizospheric bacteria in the attenuation of hydrocarbons in oily desert soils. As mentioned before, roots of desert plants and crop plants growing in pristine and oily soils were found to be densely colonized with oil-utilizing bacteria (Radwan et al. 1995a, 1998). Our group offered experimental evidence that cropping is a successful practice for cleaning up oily desert soils (Radwan et al. 2000). The crop we used was broad beans (*Vicia faba*), because the plants were able to tolerate up to 10% crude oil in soil. Common cropping practices such as irrigation and mechanical managements improve soil moisture content and aeration. Obviously, such practices provide better conditions for the growth and activities of soil micro-organisms. The roots of legume plants, including *Vicia faba*, carry nodules that fix molecular nitrogen, thus providing a natural and economical route for nitrogen fertilization. We found that the amounts of extractable hydrocarbons recovered from oily desert soils supporting *V. faba*, were less than those recovered from the uncultivated soil controls. A group of rhizospheric bacteria known as plant growth-promoting rhizobacteria (PGPR), predominantly pseudomonads, has been described to enhance plant growth when inoculated into the root area (Polonenko et al. 1987; Zhang et al. 1996, 1997).

We have found that inoculation of *V. faba* roots with PGPR enhanced the growth of this plant in oily desert soil samples, and increased the phytoremediation potential of this crop for the oily soil (Radwan et al. 2005b). In this context, we also found that nodule bacteria and PGPR have the potential for biodegradation of hydrocarbons (Radwan et al. 2007b). Thus, the rhizosphere microenvironments provide conditions optimal for activity of hydrocarbon-degrading bacteria. The rhizospheres are well aerated by the oxygen pumped down through the roots which may be an important factor because oxygen is a limiting factor for hydrocarbon biodegradation. The rhizospheres of legume crops are enriched with compound nitrogen fixed by the nodule bacteria, and assimilable nitrogen is also a limiting factor for hydrocarbon biodegradation. In addition, the rhizospheres are enriched with exuded vitamins and organic substances that have been recorded to enhance growth and activities of oil-utilizing bacteria in culture (Radwan et al. 2000; Radwan and Al-Muteirie 2001).

The main technical problem involved in bioremediation of oily coastal regions is that any added microbial inocula or nutrient fertilizers can potentially be washed out into the open sea during wave and tidal movements. On the other hand, such environments do not seem to suffer much from drought. In view of these facts, we believe that the most promising approach for bioremediating oily coastal areas in the Gulf region is self-cleaning via oil-utilizing micro-organisms in biofilms (see Section 13.6) along the coasts. Those biofilms occur in cyanobacterial mats and epilithic algal growth, and coat gravel particles, sand grains, and other animate and inanimate coastal materials. In such biofilms, micro-organisms are firmly immobilized, and thus are not readily washed out into the open water. Furthermore, in cyanobacterial mats and epilithic biomass, such micro-organisms are provided with oxygen and other products of photosynthesis such as organic nutrients including nitrogenous compounds and vitamins. Our regular field trips along the oil-polluted Kuwaiti coasts after the war revealed that the coastal oil sediments were predominantly covered with cyanobacterial mats. Month after month, we noticed that the oil sediment layers became steadily thinner until they completely disappeared together with their mat covers three to five years after the spill. Thus, it became obvious that such natural phenomena are efficient in self-cleaning of the coasts in the Gulf region.

13.11 Conclusions

Desert soils and coastal areas of the Arabian Gulf contain indigenous hydrocarbon-utilizing micro-organisms. In desert soils, those micro-organisms are found in the sand and the rhizospheres of desert plants, whereas in coastal areas they occur mainly in association with inanimate (e.g., sand, gravel) and animate (e.g., blue-green mats, epilithic algal growth) materials. The predominant desert soil bacteria belong to the genera *Micrococcus*, *Pseudomonas*, *Bacillus*, *Arthrobacter*, and to the group of nocardioforms. The predominant fungi belong to the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Mucor*. Predominant micro-organisms in the rhizospheres are the genera *Cellulomonas*, *Rhodococcus*, and *Arthrobacter*. The major actinomycete in the desert soils is *Streptomyces*.

In general, organisms from the same genera occur in coastal areas, where they are mainly associated with blue-green mats in which the predominant cyanobacterial partners are the genera *Microcoleus* and *Phormedium*. Oil-pollution results in dramatic increases of the frequencies of these organisms. Micro-organisms in the desert soils suffer mainly from drought and lack of nutrients, particularly fixed nitrogen. Therefore, bioremediation of oily desert soils should involve activating indigenous micro-organisms via watering and fertilization with nitrogenous and easily utilizable carbon compounds. Phytoremediation, particularly by planting oily areas with legume crops whose roots are loaded with nodule bacteria, may be the approach of choice for cleaning oily desert soil. In coastal areas, which are naturally rich in blue-green mats, self-cleaning occurs at a satisfactory rate.

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