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Bioremediation of Petroleum Contaminated Soils in the Arabian Gulf Region: A Review

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Abstract. While environmental biotechnology is broadly based in variety of environmental protection, restoration, and agriculture industrial practices, for a decade it has been most commonly linked with bioremediation, rehabilitation and waste treatment technology. Most of the Eastern region of Saudi Arabia, including the coastal area bordering Kuwait, became polluted with petroleum during the recent Gulf war (1990). Microorganisms can degrade petroleum as a carbon and energy source and this might provide scope to remediate and rehabilitate petroleum-contaminated soils. There are several problems dealing with petroleum-contaminated soils which effect human health and environmental quality. The present review may give some opportunities in bioremediation of petroleum-contaminated soils for more future studies in the Arabian Gulf region.

Introduction

Preparing for and conducting the second Gulf war resulted in much damage to the environment of the region. The first and most visible effect is related to the damage caused by oil well fires in terms of air pollution as well as the potential damage to the petroleum reservoir. The second detrimental effect has been caused by the oil spill in the Gulf water[1,2]. During the second Gulf war, about 700 km of the western coast of the Arabian Gulf became polluted with crude oil[3-5]. Another consequence was the pollution of large areas of the Kuwaiti desert with crude oil that remained gushing throughout 7 months from about 700 damaged oil wells, producing so called oil lakes. Although most of the crude oil has been pumped out, residual crude oil is still polluting the bottom layers of the lakes to a depth of 20-30 cm or more [3]. Further, most of the oil wells were set on fire. Usually, burning of crude oil produces numerous chemicals and releases various gases. Thus, concentrations of aluminum, cadmium, cobalt, chromium, molybdenum, titanium, nickel and vanadium have been detected in the polluted soil [6]. The burning oil produced vast amounts of carbon particles that formed the soot of the plume. In addition, aliphatic hydrocarbons and polycycellic aromatic hydrocarbons, known to be toxic gases, were identified in the smoke plumes, in addition to sulfur dioxide and hydrogen sulphide [2,7,8].

Microorganisms with a phenomenal array of catabolic activities are widespread and it is clear now that microorganisms can pollute sites and enhance the degradation of crude oil polluting the soil [9]. There are several problems dealing with petroleum contaminated soils which effect human health, marine life, animals, birds and crops, as well as natural desert plants and environmental quality. This study was conducted to screen the microorganism in the soil that could utilize crude oil as a sole source of carbon and energy for growth. Thus, bioremediation of petroleum-contaminated soil is a treatment technology that utilizes naturally occurring microorganisms to degrade hydrocarbons to carbon dioxide, water and humus [10, 11]. There are two techniques for utilizing microorganisms to degrade petroleum in the soil, one method uses the microorganisms that can already be found in the soil, these microorganisms are stimulated to grow by introducing nutrients into the soil and thereby enhancing the biodegradation process, this process is known as biostimulation, the other method involves culturing the microorganisms independently and adding them to the contaminated site, this process is known as bioaugmentation, one advantage of bioremediation is that the process can be done onsite with a minimum amount of space and equipment, by treating onsite, costs and liability are greatly reduced, while extending the life of the current landfills by reducing the amount of waste they would normally receive [12]. Here, we review the published papers concerning biodegradation of crude oil in the Arabian Gulf region.

Bioremediation Technology

Microorganisms including bacteria and fungi can degrade crude oil as a carbon and energy source, converting petroleum products into useful products via the biodegrading activity of the microorganisms [13, 14]. Mckinnon and Vine [15] reviewed earlier bioremediation attempts and showed that the addition of microbial nutrients to the oil associated with the Exxon Valdez spill in Alaska significantly enhanced its biodegradation. In 1990, Texan company, Alpha Environmental, also obtained enhanced bioremediation by spraying oil degrading bacteria over crude oil discharged by the Norwegian tanker Mega

Borg into the Carribean, 100 km off the coast of Texas[16]. Several reports on bioremediation of crude oil have been published by U.S. Congress Office of Technology Assessment[17, 18]. In the Arabian Gulf region little attention has been paid to this subject. Fungal genera isolated from Saudi Arabian soil such as *Aspergillus*, *Cladosporium*, *Penicillium*, *Fusarium*, *Rhizopus*, *Trichoderma* and *Ulocladium*, have been reported to degrade crude oil[19]. Hashem[4] isolated fungal and bacterial flora from contaminated and uncontaminated soils from the Al-Khafji area (Eastern region of Saudi Arabia). These results are summarized in Tables 1 and 2.

Table 1. Fungal flora isolated from petroleum-contaminated and uncontaminated soil.

No. of colonies per gram of soil		
Fungi	**Uncontaminated**	**Contaminated**
Alternaria alternate	36 ± 5	24 ± 4
A. chlamydospora	49 ± 6	39 ± 5
Aspergillus carbonarius	34 ± 7	18 ± 3
A. flavus	52 ± 9	29 ± 5
A. funiculosus	21 ± 3	14 ± 3
A. niger	41 ± 5	21 ± 4
A. thomii	16 ± 3	NF
A. zonatus	12 ± 3	21 ± 4
Chaetomium piluliferum	21 ± 4	26 ± 4
Cladosporium herbarum	34 ± 3	24 ± 3
C. resinae	26 ± 4	59 ± 6
C. sphaerospermum	26 ± 3	16 ± 3
Eurotium sp.	21 ± 5	56 ± 7
Fusarium solani	34 ± 4	18 ± 3
Penicillium chrysogenum	56 ± 3	42 ± 6
P. expansum	18 ± 4	NF
P. notatum	23 ± 4	NF
Trichoderma sp.	26 ± 3	46 ± 5
Ulocladium atrum	39 ± 5	46 ± 5
U. chlamydosporum	32 ± 3	39 ± 4
No. of species/genera	20/9	17/9

NF = Not found[4].

Table 2. Bacterial flora isolated from petroleum-contaminated and uncontaminated soil.

No. of colonies per gram of soil		
Bacteria	**Uncontaminated**	**Contaminated**
Arthrobacter sp.	3539 ± 25	1696 ± 16
Bacillus sp.	6344 ± 26	3439 ± 15
Micrococcus sp.	2691 ± 21	1329 ± 14
Pseudomonas sp.	5648 ± 18	3126 ± 16
Staphylococcus sp.	3636 ± 16	1346 ± 21

Ref. [4].

It is clear from Tables 1 and 2 that the uncontaminated soil supported the growth of fungal and bacterial species. Bokhary & Parvez (19) isolated different fungal flora from soil contaminated with different peteroleum products (Table 3), and observed that *Trichoderma harzianum* exhibited the highest number of colonies per gram of dry soil.

Table 3. Microflora isolated from soil contaminated with petroleum products.

Samples sites*					
Fungi	**A**	**B**	**C**	**D**	**E**
Aspergillus ellipticus	–	–	–	–	15 ± 3
A. flavus	15 ± 4	16 ± 4	21 ± 1	31 ± 3	15 ± 5
A. niger	17 ± 3	7 ± 2	9 ± 3	11 ± 3	16 ± 4
Chaetomium bostrychodes	–	–	7 ± 2	–	–
Mucor sp.	12 ± 4	–	7 ± 2	8 ± 3	14 ± 6
Penicillium chrysogenum	16 ± 4	8 ± 2	17 ± 4	21 ± 2	–
Rhizopus sp.	–	–	–	–	5 ± 3
Trichoderma harzianum	35 ± 2	33 ± 6	25 ± 7	24 ± 6	31 ± 6
Trichoderma sp.	14 ± 2	16 ± 3	18 ± 2	6 ± 1	12 ± 5
Ulocladium atrum	–	–	16 ± 2	–	8 ± 2
Yeast	18 ± 5	19 ± 5	–	–	16 ± 4

Note: Values are number of colonies per gram of dry soil ± SD; n = 5. A dash indicates no growth. A, soil from vehicle service stations; B, soil from petrol filling stations; C, soil from motor oil change places; D, soil from industrial are (vehicle repair garages); E, soil from Ummul Hammam area. (Vehicle repair garages)[19].

The growth of some fungal flora assayed at different concentrations of petroleum products[19] (Table 4). It is clear from Table 4 that *Trichoderma harzianum* was the best degrader of petroleum products among the isolated mycoflora. *Aspergillus* and *Penicillium* species have been frequently reported as degraders of various complex hydrocarbons, including petroleum products[20-25].

Table 4. Mycelium dry weight of the most common fungi isolated after 7 days of incubation at room temperature (20-25ºC) in different concentration of petroleum oil.

Mycelium dry weight (mg ± SD)													
	Type of contaminant	Crude oil			Gasoline			LLO			LHO		
Fungi	% concentration	0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2
Aspergillus flavus		207 ± 12	272* ± 6	301 ± 7	95 ± 12	136* ± 21	49 ± 8	56* ± 12	22 ± 4	15 ± 4	69* ± 6	76* ± 12	16 ± 3
A. niger		210 ± 11	240* ± 23	198 ± 5	163 ± 9	109 ± 13	66* ± 7	78* ± 14	95 ± 6	106 ± 18	59* ± 7	32 ± 4	18* ± 8
Tricoderma harzianum		257 ± 16	277 ± 14	275* ± 19	132 ± 16	169 ± 11	170* ± 3	69* ± 8	87 ± 6	113* ± 24	64* ± 11	42* ± 13	16* ± 8
Trichoderma sp.		300* ± 33	190* ± 26	–	–	–	–	66 ± 7	23* ± 3	–	76 ± 4	109* ± 17	106* ± 17

Note: LLO, lubrication light oil; LHO, lubrication heavy oil. A dash indicates no growth. *$P < 0.095$ level significance[19].

Alternaria alternate, *Aspergillus flavus*, *Curvularia lunata*, *Fusarium solani*, *Mucor racemosum*, *Penicillium notatum* and *Ulocladium atrum* were isolated from the soil of the industrial Yanbu city, Saudi Arabia. Their growth was assayed in liquid medium amended with 1% crude oil for 10 days to identify the fungal species potentially useful in degrading the crude oil. *A. flavus* and *notatum* registered significant growth and caused a shift in pH toward acidity (Table 5).

Table 5. Mycelial growth weight with loss in the oil used of the tested fungi after 10 days of incubation (35ºC) in 40 ml of Czapek medium amended with 1% crude oil with the shift in the pH of the medium (n = 5, ± standard deviation, start pH = 6.0).

Fungi	Mycelium dry wt mg/10 days	Rate/mg	pH
Alternaria alternate	52 ± 0.11	1.3	5.3
Aspergillus flavus	170 ± 1.13	4.25	4.1
Curvularia lunata	48 ± 0.92	1.2	5.5
Fusarium solani	33 ± 0.66	0.82	5.6
Mucor racemosum	26 ± 0.11	0.65	5.4
Penicillium notatum	218 ± 2.86	5.45	4.6
Ulocladium atrum	63 ± 0.32	1.57	5.3

Ref. [25].

It is clear from Table 5 that *A. flavus* and *P. notatum* are capable of growth and utilize the crude oil more than the other tested fungi. Crude oil consists of saturated and aromatic hydrocarbons and asphaltic compounds of varying molecular weights, weights, complexity, and degree of susceptibility to microbial oxidation [26]. Mycelial organisms can penetrate insoluble substances such as crude oil and this increases the surface are available for microbial attack [21]. *A. flavus* isolated from contaminated soil of the industrial Al-Jubail city, Saudi Arabia can grow on liquid media amended with different concentrations of gasoline (Table 6).

Table 6. Mycelial growth weight in mg after 7,14,21 day and shift in pH for 6.0 of *A. flavus* at different concentrations of gasoline. Values of weight are means of 3 replicates (n = 3, ± standard deviation).

Harvest (days)	Gasoline concentration (ml)									
	0		1.0		2.0		3.0		4.0	
	W	pH	W	pH	W	pH	W	pH	W	pH
7	20 ± 0.3	5.5	23 ± 0.1	5.2	31 ± 0.5	5.6	28 ± 0.3	5.0	34 ± 0.9	4.1
14	33 ± 0.5	5.8	27 ± 0.6	5.5	37 ± 0.8	5.8	35 ± 0.4	5.3	44 ± 1.3	4.3
21	48 ± 1.1	5.3	34 ± 0.9	5.9	47 ± 1.7	5.5	46 ± 1.5	5.1	51 ± 0.16	4.2

Ref. [27].

It is clear from Table 6 that at all gasoline concentrations, *A. flavus* was able to grow. It is also clear that this fungus was capable of growth and utilizing the gasoline. It was observed that *A. flavus shifts* the pH towards acidity, and this agrees with earlier studies [24,25].

Sandy soil from Riyadh Refinery, Saudi Arabia was sterilized and contaminated with crude oil and incubated for five weeks under laboratory conditions. *Aspergillus flavus*. *A. niger*, *Curvularia lunata*, *Rhizopus* sp. and *Trichoderma* sp. were isolated from this soil, and the fungal flora were then retested for growth on media containing different concentrations of crude oil *Trichoderma* sp. exhibited an increasing mycelium dry weight with increase in crude oil concentrations, while *Rhizopus* sp. was the lowest degrader of crude oil [10] (Table 7).

Table 7. Mycelium dry weight (mg) of the tested fungi after 35 days of incubation (35ºC) at different concentrations (ml) of the crude oil (n = 5, ± standard deviation).

Crude oil (ml) Fungi	Mycelium dry weights (mg)				
	0	0.5	1.0	1.5	2.0
A. flavus	156 ± 1.35	213 ± 1.96	223 ± 2.16	235 ± 2.35	269 ± 2.68
A. niger	162 ± 1.81	189 ± 1.36	201 ± 2.01	227 ± 1.96	275 ± 2.81
C. lunata	180 ± 1.85	230 ± 1.91	263 ± 2.83	271 ± 2.65	283 ± 3.0
Rhizopus sp.	136 ± 2.11	151 ± 1.31	179 ± 1.31	211 ± 1.83	221 ± 2.11
Trichoderma sp.	201 ± 2.11	235 ± 2.12	278 ± 2.9	282 ± 2.61	301 ± 3.1

Ref. [10].

Less attention has been paid to the bacterial utilization of petroleum products in the Arabian Gulf region. Sorkhoh *et al*. [3] found that bacteria were the most dominant group of oil-utilizing microflora throughout the study period of 22 weeks. Filamentous fungi, actinomycetes and yeasts appeared from week 4 to 16 after incubation, reaching highest proportions from week 6 to 12 (Table 8). Diagnostic features of oil utilizing bacteria are given in Table (9).

Conclusion

The application of microorganisms activities dominates current biotechnology. There are different economically and environmentally important uses for microorganisms, such as remediation and rehabilitation of petroleum-contaminated soils. Little attention has been paid to the role of microorganisms in the environmental biotechnology in the Arabian Gulf region.

Table 8. Composition of the oil-utilizing microflora in oil-polluted sand* inoculated with a suspension of Kuwaiti mats.

% of total			
Incubation period (weeks)	**Bacteria**	**Filamentous actinomycetes**	**Filamentous fungi + yeasts**
0	100.0	0.0	0.0
2	100.0	0.0	0.0
4	90.0	4.0	6.0
6	61.0	26.0	13.0
8	68.0	13.0	19.0
10	73.0	4.0	23.0
12	68.0	9.0	23.0
16	92.0	3.0	3.0
18	100.0	0.0	0.0

*100 g oil per 100 g sand[3].

Table 9. Diagnostic features of oil-utilizing bacterial genera.

Genus	Diagnostic features
Rhodococcus	Gram-positive filaments subdividing into irregular rods, ultimately into cocci, nonmotile, aerobic, catalase-positive, lipids contain tuberculostearic acid.
Pseudomonas	Gram-negative small rods, actively motile, polar flagella, aerobic, oxidative metabolism.
Bacillus	Gram-positive, rods, producing oval central, slightly swollen endospores, aerobic, motile, peritrichous flagella.
Arthrobacter	Gram-positive rods, subdividing into perfect cocci in regular cycles, aerobic, nonmotile.

Ref. [3].

Serious risks to public health and the environment can occur when crude oil pollutes the soil. Biodegradation can result in the complete degradation of hazardous substances, while technologies, such as carbon adsorption and air-stopping simply transfer the pollutant to different media. Remediation and rehabilitation in Kuwait after the second Gulf war was a good example of applied biotechnology.

Certain criteria must be met for bioremediation to be seriously considered as a practical means for crude oil treatment, including the existence of microorganisms that have (1) the required catabolic activity, (2) the capacity to transform the pollutants at reasonable rates and (3) bring the concentrations to levels that meet regulatory standards. In addition, the organisms must not generate products that are toxic at concentrations likely to be achieved during the remediation. The site must not contain concentrations or combination of chemicals that are markedly inhibitory to the biodegrading microorganisms. The target compounds must be available to the microorganisms, conditions at the site or in a bioreactor must be made conductive to microbial growth or activity, and the cost of the technology must be less or no greater than other technologies that can also destroy the pollutants [28].

When crude oil burns numerous chemicals and toxic gases are produced, which affected the growth and microbial density [4]. Several species of oil-degrading fungi and bacteria have been detected in the sand soils of the Arabian Gulf [4, 29], which need to be examined for their role in the natural bio-degradation of petroleum in contaminated soils. Addition of microbial nutrients such as carbon, nitrogen, calcium, potassium and magnesium and micronutrient elements such as copper, zinc, cobalt and nickel enhanced biodegradation activities [28]. Seeding practice my be a useful method for bioremediation [30]. Biological degradation is a very effective treatment technology for remediating petroleum contaminated soils. Significant improvements in treatment capacity can be achieved with specialized aeration equipment, additional research is presently underway to improve degradation rates.

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الاستصلاح الحيوي للتربة الملوثة بالنفط في منطقة الخليج العربي: مراجعة

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المستخلص. تستند التقنية الحيوية بتوسع على مختلف أوجه الحماية البيئية مثل التطبيقات الصناعية والزراعية والبتروكيميائية والهندسة البيئية، وفي العقد الأخير فإن هناك ارتباط وثيق مع الاستصلاح الحيوي وتقنية معالجة التلوث النفطي.

تلوثت معظم شواطئ المنطقة الشرقية للمملكة العربية السعودية بالإضافة إلى السواحل المتاخمة للحدود الكويتية بالنفط الخام خلال حرب الخليج الأخيرة عام ١٩٩٠م.

وجد أن الكائنات الحية الدقيقة تستطيع هدم وتفكيك النفط واستخدامه كمصدر للكربون والطاقة وهذا ربما يقدم العديد من الأهداف لمعالجة وتأهيل التربة الملوثة بالنفط ومشتقاته، والتي تؤثر على صحة الإنسان وجودة البيئة.

الاستعراض الحالي للعديد من الأبحاث العلمية ربما يعطي بعض الفرص في مجال المعالجة والتأهيل الحيوي للتربة الملوثة بالنفط للمزيد من الدراسات التطبيقية المستقبلية في منطقة الخليج العربي.