Bioremediation of Crude Oil-Contaminated Water and Sediments from the Shatt Al-Arab River (SAR)

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Abstract

In the SAR, the oil-degrading bacteria (O-DB) are widespread, forming 8 % to 14 % of heterotrophic bacteria (HB). The O-DB numbers and biodegradability of petroleum hydrocarbons (PHY) are important in summer (SU) and sediments (SE) compared to winter (WI) and water (WA). The common O-DB retrieved from the SAR are *Pseudomonas* sp. (*PS*), *Pseudomonas putida* (*PP*), *Pseudomonas fluorescens* (*PF*), *Pseudomonas aeruginosa* (*PA*), *Pseudomonas cepacia* (*PC*), *Corynebactrium* sp. (*CO*), *Bacillus* sp. (*BA*), *Bacillus cereus* (*BC*), *Bacillus subtilis* (*BS*), *Flavobacterium* sp. (*FL*), *Aeromonas* sp. (*AE*), *Arthrobacter* sp. (*AR*), *Vibrio* sp. (*VI*), *Nocardia* sp. (*NO*), *Acintobacter* sp. (*AC*), *Micrococcus* sp. (*MI*), and *Staphylococcus* sp (*ST*). The most effective O-DB utilized more than 52 % of oil in 21-days. A mixed culture made of mutant *PP+AR* utilized 93 % of oil during the same period. The oil biodegradation rates in the SAR have been restricted by biotic and abiotic factors. By providing these factors to the oil-contaminated sites would improve the degradation rates. The biodegradation of n-alkanes was much faster than polycyclic aromatic compounds (PAC).

Keywords: *Bacteria, Crude oil, Hydrocarbons, Bioremediation*

Introduction

Petroleum production leads to hydrocarbons (HY) pollution, which has become one of the most important problems facing the environment today. Oils may contaminate WA, SE, soil and air, menacing human health and other organisms, and causing the deterioration of the ecosystem[1]. Nevertheless, most of the oil released into the environment is largely harbored as a result of the ability of microorganisms to degrade HY[2]. These HY degraders are ubiquitous in the ecosystem, and their PHY degradation rates depend on the environmental factors. The stability of oil pollutants in the environment relies on the HY quality and quantity that make up the crude oil and on the influencing environmental properties[1].

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The current research will demonstrate the SAR ability to biodegrade the PHY. The SAR is one of the economic, social and environmental rivers in Iraq and the prime fresh WA source in its southern part and Arabian Gulf (AG). The WA of SAR has been diagnosed as containing oil pollutants due to various industrial processes and aqueous activities[3]. PHY can be introduced to the

SAR from transportation operations, fixed structures, naturalistic inputs etc. A few is known on the HY biodegradation in the SAR. Most of the former studies on this topic focused on HY degraders isolation.

There is a necessity to comprehend the PHY behavior and fate in the SAR. Therefore, this study was accomplished. The aim of study is to estimate the O-DB and HB numbers and isolate O-DB from SAR, determine the crude oil biodegradability in SAR WA, SE and bacterial isolates, know the seasons, nutrients and external O-DB effects on the bioremediation, and

create a mutation in O-DB and make a high ability mixed bacterial cultures to degrade oil.

Materials & Methods

Three sites on SAR were selected for the study (Abu-Al-Kahaseeb (30°27'44.5'' N-48°00'06.0'' E), Basrah (30°33'00.0" N-47°47'10.0" E) and Karmat-Ali (30°48'10.6'' N-47°45'03.8'' E)). The WA and SE samples were taken from the sites during the SU and WI of 2019. The samples were transported to the laboratory and their temperature (TE), salinity (SA) and pH were determined.

The HB were counted by mixing WA (1 mL) or SE (1 g) with normal saline (9 ml). The suspension was then left to 10 minutes. Six dilutions were intended and 0.1 ml of suitable dilution was spread over the nutrient agar (Difco), incubated at 37 ° C for 48 hours.

The O-DB were counted by adding WA (1 mL) or SE (1 g) to a Erlenmeyer flasks (250 ml) containing liquid mineral media (LMM) of Chaineau et al[4]. Samples were not added to some flasks (control). The flasks were incubated for 30 days at 37 °C in shaker incubator (155 rpm). Bacterial growth in liquid cultures was observed at 7, 14, 21, and 28 days by planting 0.1 mL of suitable dilution on the nutrient agar (Difco), incubated for 48 hours at 37 °C. Each type of colonies on the media were registered and selected. The colonies were purified, counted and examined. Stock cultures were made. The bacteria were identified based on morphological and biochemical parameters.

Various methods have been utilized to estimate the bacterial degradation ability in samples. These included subjecting the WA and SE to crude oil and then determining the O_2 consumption[5], the production of CO_2[6], and the alternation that occur in the PHY weight and concentration, every 7 days, up to 21 days. The PHY weight alterations were determined by gravimetric method[7]. To determine the PHY concentration, the HY were extracted from the liquid cultures according to Pourbabaee et al[8]. The extract was then analyzed by a spectrofluro-meter **(**Shimadzu RF-540).

The ability of each bacterial isolate was examined for PHY degradation in LMM. The flasks placed in shaker incubator (155 rpm) at 37 °C for 21 days. Bacterial growth were observed and the oil concentration percentages loss were determined (spectrofluro-meter).

To mutate the bacteria (*PA*, *PP*, *PC*, *PF*, *BC* and *BS*) were subcultured on the nutrient agar, incubated for 8 or 14 hours, and suspended in the phosphate buffer (20 mL), and exposed to ultraviolet light (UVL) for 6, 35 or 65 minutes. The various radiation rates and stages were destined to involve different exponential bacterial growth phases to stimulate an effective mutation. A germicidal-lamp (254 nm) was the source of UVL with output was 10 erg/mm/second. Irradiation was only practiced while the lamp fluency was at a constant maximum. The morphology of each bacteria growing on the nutrient agar for phenotypic expression was observed, and its ability to degrade PHY was examined. After that, mixed cultures were made from mutated and non-mutated bacteria and their ability to biodegrade PHY in LMM was examined.

In situ, biological remediation was studied by designing four experimental WA ponds (2×2 ×1 m) in site 2 within the SU. The first pond was equipped with 1L of crude oil. The second pond was supplied with crude oil, nutrients (350 g of $(NH_4)_2SO_4$ and K_2HPO_4) and mixed O-DB (*MI*, *CO*, *BA*, *PS*, *AR*, and mutant *PP*). The third one was supplied with crude oil and O-DB. The fourth pond was without treatment (control). The WA samples were collected from ponds every 7 days to 21 days. The O-DB were counted on a solid mineral medium (it is LMM with 20 g of agar), incubated in 37 °C. The PHY were extracted and fractionated[9] (n-alkanes and PAC) from samples, and the PHY concentration percentages loss were estimated by analyzing them in gas chromatography (GC) (Allegent, USA). The data were statistically analyzed by ANOVA. The means were compared through Duncan-test.

Results

Average TE, pH, and SA of SAR WA were 16 °C, 7.5 and 4.0 ‰, in WI, and 37 °C, 7.6 and 5.4 ‰, in SU, respectively.

The O-DB makes up from 8 % to 14 % of HB. The SE have more bacteria than WA. The bacteria in SU samples were high than WI. The highest bacterial numbers and percentages were in site 2 than others (Table 1).

The O-DB growth in SE was higher than WA and in SU was more than WI in LMM. The highest O-DB growth was in site 2 samples. The growth generally increased with the incubation period. The O-DB numbers in WA and SE on 21-days were high than 7-days of incubation. The O-DB growth declined on 28-days of incubation (Table 1).

The SE showed the highest rates of O_2 consumption and CO_2 production and crude oil weight and concentration percentages loss

Table 1: Bacterial numbers CFU/ml WA or g SE in SAR, LMM and ponds

Sample	**Bacteria**	**WI**			**SU**			
		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	
WA	HB	7.73x104	7.96x104	7.25x104	5.12x106	7.65x106	2.68x106	
	O-DB	6.44x103	8.34x103	5.83x103	4.14x105	8.31x105	2.47x105	
	O-DB/HB%	8	10	8	8	10	9	
		LMM						Control
	O-DB 1st week	2.74×102	3.28×102	2.01×102	1.23×104	1.96×104	2.14×103	0
	O-DB 2nd week	5.25×102	7.69×102	4.03×102	2.89×104	4.17×104	4.13×103	0
	O-DB 3rd week	6.26×102	8.88×102	4.28×102	4.97×104	6.32×104	4.16×103	0
	O-DB 4th week	4.62×102	6.75×102	2.84×102	2.36×104	3.19×104	0.55×103	0
		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	
SE	HB	2.86x105	3.89x105	2.24x105	5.29x107	5.46x107	2.87x107	
	O-DB	2.42x104	5.72x104	2.17x104	4.74x106	6.23x106	2.79x106	
	O-DB/HB%	8	14	9	8	11	9	
		LMM						Control
	O-DB, 1st week	6.57×102	9.15×102	5.08×102	7.16×104	9.76×104	4.94×104	0
	O-DB, 2nd week	1.32×103	2.85×103	1.09×103	1.47×105	2.11×105	1.12×105	0
	O-DB, 3rd week	2.47×103	3.04×103	2.07×103	2.35×105	3.15x105	1.89×105	0
	O-DB, 4th week	0.86×103	1.22×103	0.54×103	1.44×105	2.17×105	1.23×105	0
					Pond-1	Pond-2	Pond-3	Pond-4
WA	O-DB, 1st week				3.1x104	7.3x109	2.3x106	3.8x102
	O-DB, 2nd week				4.3x104	8.2x109	4.4X106	4.3x102
	O-DB, 3rd week				6.2X104	9.9x109	5.4x106	4.6X102

compared to WA. These rates and percentages were generally higher in the SU than WI. In site 2 samples, the O_2 consumption and CO_2 production rates and oil weight and concentration percentages loss were higher than another sites. An increase in the rates and percentages were observed with an increase in the incubation period up to 21-days (Fig. 1, 2, and 3).

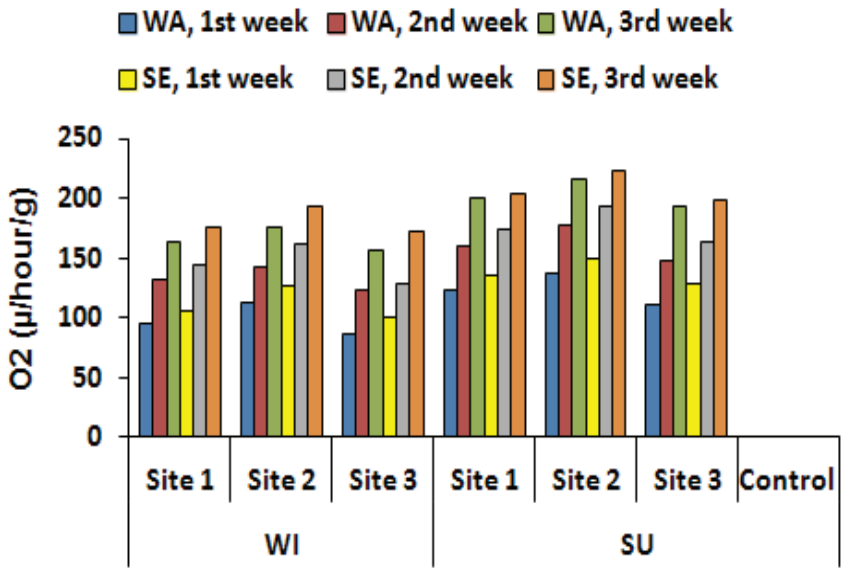

Fig. 1: O_2 consumption rates in oil-exposed SAR samples

Various species of O-DB were identified from WA and SE of the SAR, including *PS*, *PP*, *PF*, *PA*, *PC*, *CO*, *BA*, *BC*, *BS*, *FL*, *AE*, *AR*, *VI*, *NO*, *AC*, *MI* and *ST*. Elements of the same bacteria were observed in WA and SE.

The oil biodegradation rates of O-DB were varied (Table 2). The oil concentration percentages loss ranged from 65% (*PS*) to 33% (*VI*). The most potent bacteria in the oil degradation was *PS*, *PP*, *PA*, *AR*, *CO* and *MI* (% concentration loss < 52%).

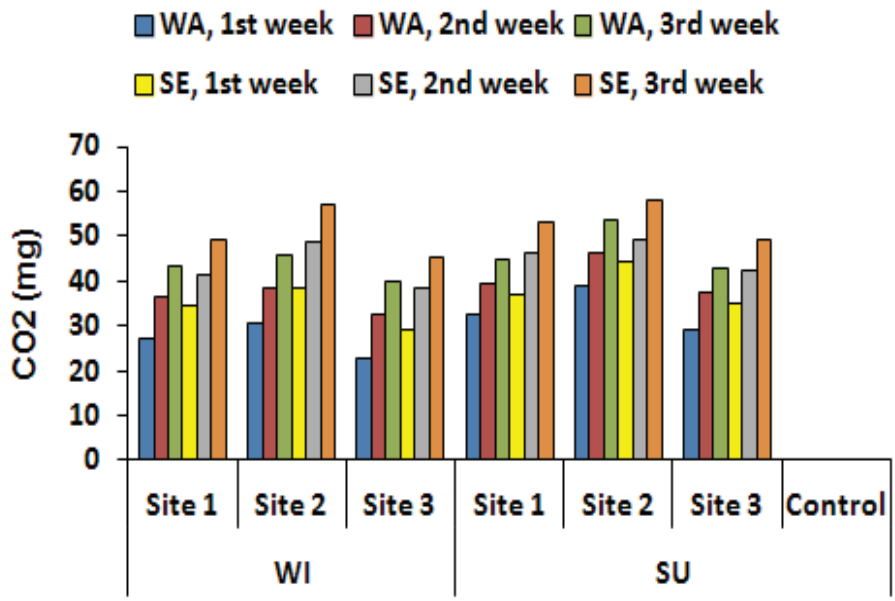

Fig. 2: CO_2 emancipated from SAR oiled samples

Mutation experiments have shown that only two bacteria (*PP* and *PA*) have mutated among six bacteria (*PP*, *PA*,

PF, *BS* and *BC*) exposed to UVL. The *PP* demonstrated a high ability to break down crude oil. The oil concentration percentage loss by mutant bacteria was 67 %. The mixed culture (mutant *PP+AR*) utilized the oil (93 % during 21-days) best than the other mixed cultures produced (Table 2).

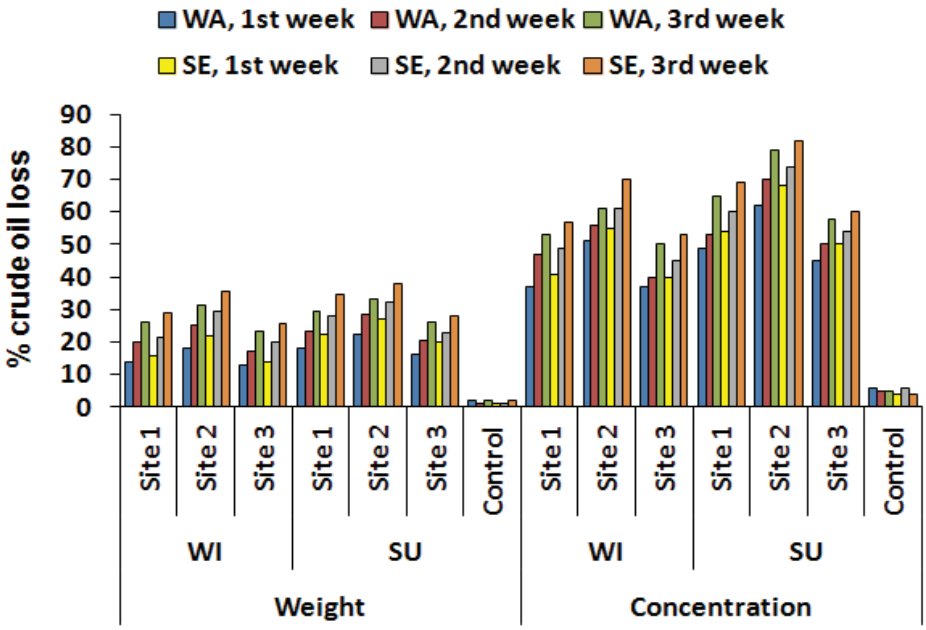

Fig. 3: Oil weight and concentration percentage loss in SAR samples

Table 2: Bacterial degradability on 21-days of incubation

O-DB	Oil concentration loss %
MI	58
VI	33
FL	36
CO	61
PS	65
PP	55
PA	56
PC	47
PF	38
AR	60
BA	52
BS	40
BC	44
ST	37
NO	47
AC	37
AE	41
PP (mutant)	67
PP (mutant)+AR	93
PP+AR	67
PS+AR	77
PS+CO	77
AR+CO	71
PS+BC	67

Table 4 shows that the bacteria numbers in the WA of four pond were significantly different. The largest number was

in the oiled pond, equipped with nutrients and external bacteria. Fig. 4 illustrate changes in the petroleum composition in oiled ponds. GC analysis evaluated the n-alkanes of C_{14} to C_{30} in addition to pristane (PI) and phytane (PH). Low molecular weight n-alkanes (C_{14} to C_{16}) were almost completely utilized by O-DB. Other n-alkanes (C_{17} to C_{30}) were less biodegraded. Higher molecular weight PAC (floranthene (FE), pyrene (PE), benzo [a] anthracene (BAE), chrysene (CE), benzo [b] fluoranthene (BBF), benzo [k] fluoranthene (BKF), benzo [a] pyrene (BAP), benzo [ghi] perylene (BGP) and indino [1, 2, 3 ed] pyrene (IP)) were more resistant to biodegradation than lower molecular weight PAC (fluorine (FO) and anthracene (AE)). An increase in the HY degradation rates was observed in the pond with nutrients and exogenous bacteria. The n-and branched-alkanes degraded faster than PAC.

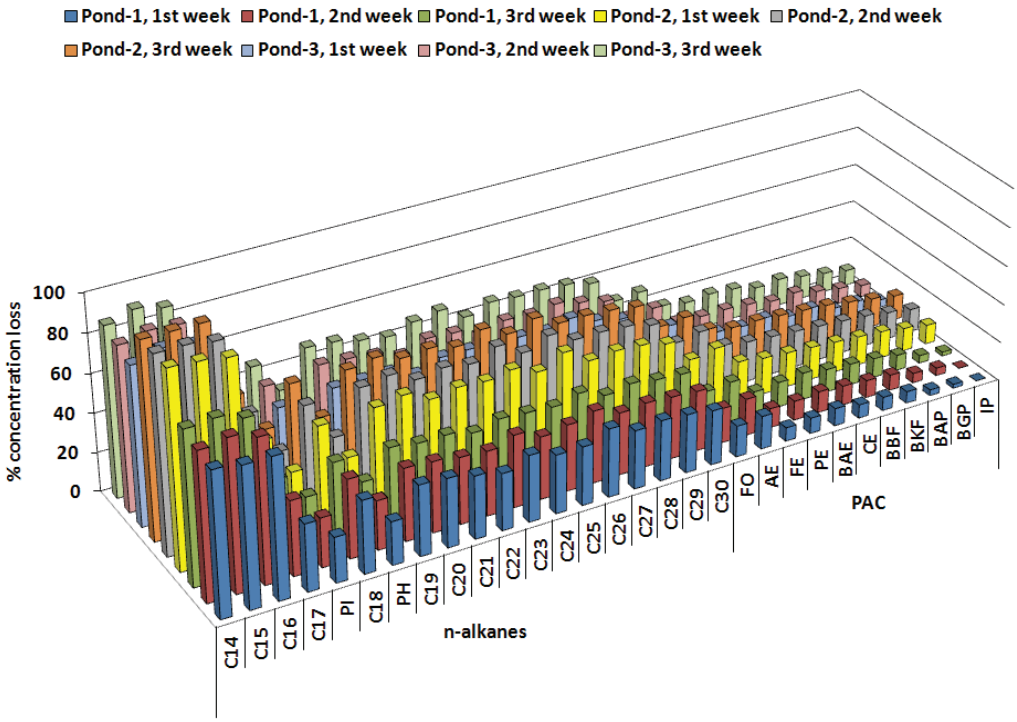

Fig. 4: n-alkanes and aromatics bioremediation in oiled-pond at 21-days

Discussion

The SAR has the potential for biodegradation of PHY due to the wide distribution of O-DB in SAR. Atlas et al[5] showed that the oil degraders numbers in a given ecosystem partially determine the system's ability to degrade oil pollutants. The O-DB numbers in the SAR vary according to TE. At high TE during the SU, O-DB numbers were much higher than in low TE during WI. Chaineau et al[4] previously reported the relationship between the change in O-DB numbers and the environment TE. The difference in O-DB numbers in the SAR sites depend on the differences in nutrients available and oil pollutants level[10]. A large numbers and percentages of ODB at site 2 may indicate that this site may has previously been exposed to PHY[5].

A significant changes in O-DB numbers of oil-exposed samples in LMM within 21-days of incubation indicates the rapid adaptation of O-DB to the PHY degradation[11]. However, on 28-days, O-DB numbers start to decline. The O-DB response to the oil was immediate without time delay associated with a prolonged period of enzymatic adaptation. After the depletion of biodegradable HY, the residual PHY

resist the degradation, thus O-DB numbers decreased. Accumulation of some microbial metabolites may also inhibit the PHY degradation in media[10]. This may partly explain the decrease occurred in the O-DB numbers after a certain period.

The WA and SE samples are able to metabolize PHY. A rapid increase in the O_2 consumption rates when adding oil indicates that samples are effective in PHY degradation when collected and are able to immediately initiate the degradation[1]. The CO_2 production suggests that samples are able to convert PHY to CO_2 and WA as a result of biodegradation[5]. A significant decrease in the oil weight and concentration in the samples refers that these samples suffer from biodegradation. The loss of oil weight also indicates that non-biological factors may impact on the biodegradation[11].

The degradation effectiveness in SE was higher than WA[5]. Biodegradation rates in SU were generally greater than WI, possibly due to differences in oil degraders levels and non-biological factors especially TE[2]. The site 2 shows higher biodegradation than other sites due to the fact that this location is characterized by a large O-DB numbers able to utilize PHY.

Some current bacterial isolates were Gram-negative (*PS*, *PP*, *PF*, *PA*, *PC*, *AE*, *VI*, *FL*, and *AC*) and others were Gram-positive (*AR*, *BA*, *BC*, *BS*, *ST*, *CO*, and *MI*). The bacteria were able to utilize oil at different rates. These bacteria were also isolated and characterized as O-DB by other authers[4 and 11].

The UVL are often used to produce mutant in microorganisms. DNA and amino acids prefer to absorb the wavelength of this beam, and therefore this radiation has great genetic and biological effects[12]. The mutated bacteria (*PP*) and the mixed culture (mutant *PP+AR*) showed high oil degradation capacity.

The biological degradation of oil in the ponds was limited by the nutrients availability (N and P). By providing nutrients and oil degraders to the oily ponds, biodegradation is enhanced[2]. The crude oil really biodegrade in the ponds. The biodegradation of n-alkanes and PAC is inversely proportional to the increase in molecular weight. The PI and PH (isoprenoids) and PAC were resistant to degradation, due to their molecular construction[10].

Conclusions

The ability of the SAR to biodegrade the petroleum is largely due to its ownership of the oil degraders, The spatial and seasonal changes of the SAR affect the biodegradation. By adding nutrients and oil degraders to environment, the petroleum biodegradation rates stimulated. Some n-alkanes are completely degraded, while others are slowly utilized. Branched alkanes are relatively more resistant to degradation than linear alkanes. PAC are the most rebellious to biodegradation.

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Ethical Clearance- From the College

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