RESEARCH NOTE

SOME INVESTIGATION ON BIOREMEDIATION OF SEDIMENT IN PERSIAN GULF COAST

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Abstract The amount of petroleum hydrocarbon and heavy metals in the sediment of the Persian Gulf's shore, at 8 selected stations were determined and showed the maximum of 143.6 and 58.6 mg/kg sediment, respectively, both maximum amounts were related to Emam Hassan zone which is located at 50 kilometers to west Boushehr port. Bioremediation of contaminated sediment were studied in slurry and solid-state fermentation. 8 bacteria types were isolated. Four species Em2, SH, GN1 and GN3 presented maximum PAH removal efficiency. Biodegradation efficiency under slurry conditions was observed after about 45 days, which during this period, naphthalene and phenanthrene showed 73% and 66% removal efficiency respectively. Under solid-state conditions, microbial activity of mixed and pure culture was studied. The results presented that mixed culture due to high ability of different strains for growth, showed higher degradability compared to pure culture, but insufficient mixing under solid state condition resulted in a low mass transfer rate of nutrient which caused reducing cell activity, therefore removal efficiency decreased under solid state condition.

Key Words hydrocarbon pollutant, heavy metal, Persian Gulf Coast, bioremediation, solid phase bioreactor.

چکیده میزان هیدروکربورهای نفتی و فلزات سنگین در ۸ ایستگاه انتخابی خاک سواحل خلیج فارس اندازه گیری شدند و بالاترین میزان آنها به ترتیب ۱۴۳/۶ و ۵۸/۶ میلی گرم بر کیلو گرم بدست آمدند که هر دوی آنها مربوط به منطقه امام حسن بودند که در ۵۰ کیلومتری غرب بندر بوشهر قرار دارد. درمان بیولوژیکی خاک آلوده در دو فاز جامد دوغابی مورد مطالعه قرار گرفت و ۸ گونه باکتری ایزوله شدند. که ۴ گونه آنها GN_3, GN_1, SH, EM_2 بالاترین راندمان حذف PAH را نشان دادند. راندمان تجزیه بیولوژیکی در فاز دوغابی بعد از ۴۵ روز بررسی گردید که در این دوره نفتالین و فنانترن به ترتیب راندمان حذف ۷۳ و ۶۶ درصد را نشان دادند. تحت شرایط جامد فعالیت میکروبی کشت های مخلوط و خالص مورد بررسی قرار گرفتند نتایج نشان دادند که کشت مخلوط به خاطر قدرت بالای گونه های مختلف برای رشد در مقایسه با کشت خالص قدرت تجزیه بالاتری دارد. اما در این شرایط به خاطر عدم اختلاط کافی شدت انتقال جرم مواد مغذی کمتری می شود و باعث کاهش فعالیت سلولی می گردد و در نتیجه راندمان حذف در این شرایط کمتر است.

1. INTRODUCTION

The Persian Gulf , which lies within the region delineated by latitude 24 - 30 $^{\circ}N$ and longitude 48 – 56 $^{\circ}E$, is a shallow basin with an average depth of 35 m. The volume of water in this area is about 7800 km^3 [1].

The discovery of oil in the Gulf during the 1930s and 1940s led to a massive increase in shipping and was principally responsible for the immerse economic wealth and geopolitical importance of the region today, and also the ecosystem of the Gulf and especially its shore have been polluted. About 40% of all the tankers traffic in the world is in Persian Gulf, and an estimated 20,000 to 35,000 tankers pass through the Gulf annually, and about 3 to 8 million tons of ballast are discharged in this region. About 150,000 tons of oil annually enter into the Gulf from different sources [2]. Golob and Bruss [1984] estimated that the oil pollution in the Gulf area was 47 times the average estimated amount for a marine environment of a similar surface area [3]. The Persian Gulf -war represented the world's largest oil spill which was about 900 million barrels of oil entered the Gulf's environment [4]. Studies reported that there are polluted sediments in many parts of Khozestan and Bousheehr provinces. About 3.5 tons of oil in each hectare are a large part of Iran's sediments [5].

Oily hydrocarbons and various heavy metals are in the soil of these areas which are harmful for human and all biota's because these compounds are carcinogenic and mutagenic in nature and have a high bioaccumulation potential, and naturally removal of them needs a long time [6]. Solid treatment technologies are often developed and evaluated in order to confirm with regulatory demands, which may require or suggest that residual petroleum hydrocarbons (RPH) concentrations in solid be reduced below 100 mg/kg soil [7].

Their total petroleum hydrocarbons are many technologies available for treating sites contaminated with hydrocarbons like thermal treatments, incineration, air stripping, oxidation and reduction, flotation and bioremediation. However the treatment selected depends upon contaminant and site characteristics, regulatory requirement, costs, and time constrains. Commonly used technologies can be integrated to enhance performance.

As reported by many investigators, the bioremediation is a managed or spontaneous process in which biological organisms such as microbial cells, as the catalysis acts on pollutant compounds, there by remedying or eliminating environmental contamination [8,10].

Degradation of polluted soil and ground waters may be influenced by environmental constrains like dissolved oxygen, pH, temperature, hazardous compounds, oxidation and reduction potential and availability of inorganic nutrients like hydrogen and phosphate, salinity, nature of organic compounds, quantity and kinds of organisms which are in polluted sites also have important roles.

Bioremediation refers to enhancement of the native capability of the microorganisms. The indigenous microorganisms could be simulated or specially developed to be added to the site to degrade, transform, or attenuate organic and organo metallic compounds to low levels and nontoxic products.

Unlike other techniques that temporarily vary and displace the problem or transfer the contaminants to another medium, bioremediation attempts to render the contaminants into harmless substances. Wang reported that by employing suitable microorganisms PAH compounds of diesel oil were completely eliminated in 12 weeks [9]. Many researchers reported that the bacteria has better ability than fungi for degradation [10,11]. Yaghmaei et al. showed that the fungi can also remove hydrocarbons compounds like naphthalene, phenanthrene and antheracen and the maximum removal was 75, 65, 59 percent respectively[10,11]. In another study it was proved that the *Phanerochaete chrysosporium* is able to degrade aromatic hydrocarbons. *Phanerochaete chrysosporium* could remove 90% of phenanthrene after 6 days in a rotating biological contactor. [12]

The objectives of this study were to determine the concentration of the most important heavy metals and petroleum contaminants in the sediments of the Persian Gulf coast, and the investigation of some important factors effected by the bioremediation of petroleum contaminants in the sediment.

2. MATERIALS AND METHODS

2.1. Sampling

Samples from pollutant sites, sediment and water were taken during two seasons, autumn and summer 2002, according to the standard methods[14].

8 stations, which seemed to be more polluted than the others were selected, the average distance between the station is about 80-50 kilometers. The stations, which located from the west to the east of the Persian Gulf, were named:

Daylam (DA), EmamHasan (EM), Gonaveh (GN), Shaghab (SH), Dayear(DR), Kangan (KN), BahmanPort (BA), and Asalouih (AS), soil and sea water samples were taken from these stations according to standard methods [14].

2.2. Analysis

Heavy metals were determined with the Atomic Absorption spectrophotometer Analytic, Jena, GmbH 26000 – 126 Ziess AAS5EA Germany. For determinatiing of polycyclic aromatic hydrocarbons (PAH) high performance liquid chromatography HPLC was used. The column was Odssil 25 cm length and 4.6 mm intra diameter and wave length 254 nm, which was coupled to a flurecesence detector with 280 nm wave length and emission bigger than 389 nm. The solvent was Dichloromethane + hexane with equal volume ratio.

TOC and TPH were determined by TOC Analyzer (CAIO made by skaler company) and suxhelet respectively. All analysis were performed in dublicate.

2.3. Bioremediation

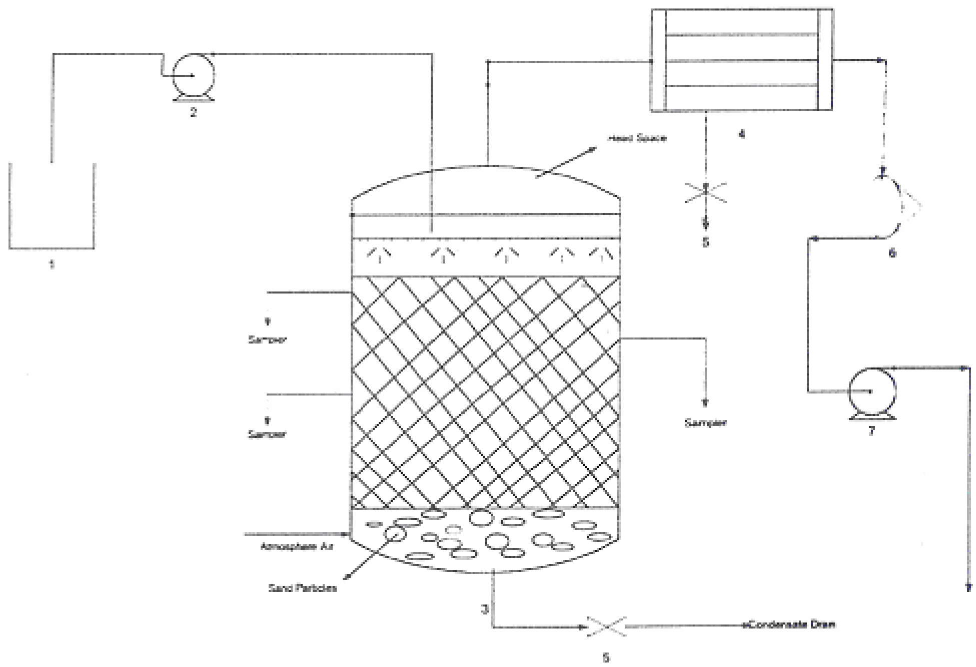
Bioremediation was done in three steps.

1. Assessment of the microbes
2. Treatments in slurry phase
3. Treatments in solid phase

Table 1. Composition of inorganic compounds for nutrient solution

Inorganic compounds	**Concentration (g/l)**
Na2HPO4	1.3375
NH4Cl	0.6675
MgSO4, 7H2O	2.5000
CaCl2, H2O	0.0250
FeSO4, H2O	0.1000
MnSO4, H2O	0.0037

Microorganisms were isolated and characterized by the standard methods of microbiology [15, 16].



1. nurient storage tank 2. pump 3. bioreactor 4. condensor
5. drain control valve 6. rotameter 7. suction pump

Figure 1. Solid phase bioreactor

Slurry phase consisted of nutrient solution. Table 1 shows the composition of culture media.

For solid phase treatment a solid phase bioreactor was employed. Figure 1 shows schematic diagram of the bioreactor.

3. RESULTS AND DISCUSSION

Heavy metals in sediment and water environments are mainly due to oily hydrocarbons. The most important of them are cadmium, nickel and chrome. Table 2 shows that the concentration of heavy metals in all areas are high and the maximum of them is in Emam Hasan station with 58.6 mg Ni/kg sediment. Many of light hydrocarbons and alkanes are volatile and sediments are free of them, therefore the most important parts of hydrocarbons in soils are due to mono, di and hetroaromatics, polar and colloidal compounds of oil. Table 3 shows the TPH, TOC and PAHS contamination in Persian Gulf sediments.

3.1. Isolation and characterization of microorganisms

For determination the ability of the native microbes in removing hydrocarbons, various types of microorganisms were isolated from oily contaminated sediments, and the growth test were performed. The growth duration for microorganisms in suitable environments are about 4 to 6 days and during this time various types of colonies appeared. Among them 8 species which had higher purity were selected and separated.

Comparing the ability of the isolated species by cell growth, showed that four species EM2, SH, GN1, GN3, had the best growth under specific and contaminated environments and they showed high cell concentration.

Figure. 2 shows the comparison of the cell concentration with respect to bacteria species. As could be seen, four species isolated from the soil of stations have shown better growth, therefore, these species were selected for contaminant removal studies. The biological treatment was performed by these four type of bacteria.

More investigations on microbial and morphological characteristics showed that they can

Table 2. Heavy metals mg/kg sediment in different stations

Code of sampling area mg/kg sediment	SH	DA	EM	GN	DR	KN	BA	AS
Ni	43.2	48.2	58.6	42.0	50.6	26.1	40.6	44.6
Cr	37.4	21.7	11.9	32.6	34.7	23.2	32.7	39.6
Cd	6.7	5.6	7.9	9.27	10.6	7.2	6.3	7.6

Table 3. TPH, TOC, PAHs contaminations in Persian Gulf sediments

Code of sampling area mg/kg sediment	SH	DA	EM	GN	DR	KN	BA	AS
TPH	20.4	54.9	143.6	14.3	23.7	43.4	24.3	21.4
TOC	25.2	56.8	159.2	16.8	29.2	59.6	30.6	27.2
PAH	15.05	35.5	89.6	11.2	18.25	34.65	18.43	17.43

belong to the *pseudomonas group*. (see Table 4).
In many references the ability of this group of microorganisms in hydrocarbons removal were reported [10,11].

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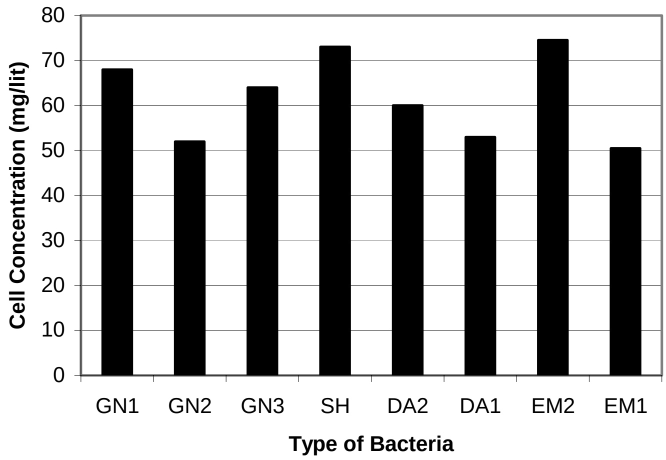

Figure 2. Column diagram of cell concentration

Table 4. Some characterization of four species bacteria

Site collected samples	Strain named	Morphology	Gram test
Shagab	SH	Greenish Yellow, Rod--shaped colonies	Gram-negative
Emam Hassan	EM2	Cream, Cocci-shaped	Gram-positive
Ganaveh	GN1	Cream, chained cocci-shaped colonies	Gram-negative
Deilam	GN3	Yellow, rod-shaped	Gram-positive

More investigations on microbial and morphological characteristics showed that they can belong to the *pseudomonas group*. (see Table 4).
In many references the ability of this group of microorganisms in hydrocarbons removal were reported [10,11].

3.2. PAH degradation in slurry conditions

Figure 3 illustrates the percent removal of phenanthrerne by four types of bacteria.
As it is presented, the maximum removal belongs to EM2 species isolated from the Emam Hasan area, and it must be related to the pollution in this place, which was higher than the other sites. In figure 3, the slow sharpness at the end of the plot is due to reduction of nutrients in the culture. Phenanthrene has a maximum removal which had reached from.140 to 68 mg/kg sediment after 45 days. Phenantherene has more ring and shows lower degradation compared to naphthalene. In this condition the suspended solids concentration in the slurry was near 40000 mg/l which contained near 10% of MLVSS. Figure 4, shows the percent removal of naphthalene, phenantherene and TOC by four selected species under slurry conditions. In slurry conditions with a controlled temperature (35 $°C$) and PH (near 7.6), EM2 species have the maximum removal efficiency, which were about 73 and 66% for naphthalene and phenanathrene respectively.

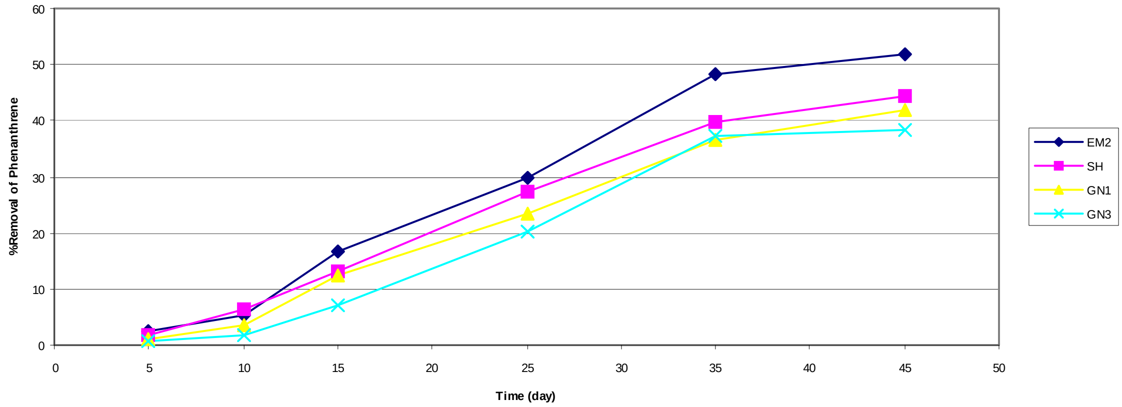

Figure 3. A comparison of phenantherene percent removal by isolated bacteria

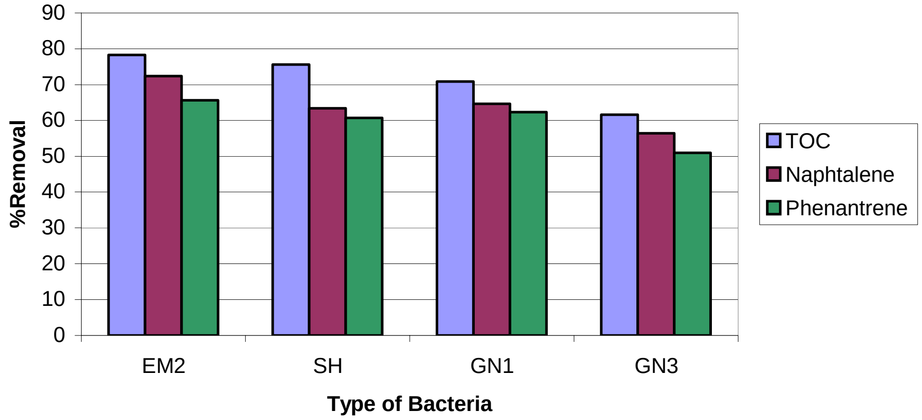

Figure 4. Comparison of percent removal of TOC, phenanthrene and naphthalene by selected species after 50 days.

3.3. Bioremediation in solid phase bioreactor(microcosm)

On the next step, the oily contaminated sediments were taken in a solid phase bioreactor and suspension of mixed bacteria were added (7%). Figure 5 shows the concentration changes of total organic carbon TOC, total petroleum Hydrocarbons (TPH) and Poly Aromatic Hydrocarbons (PAHs) under solid Phase conditions.

As it is presented, the microbial activity is reduced, which is due to the insufficient mixing and therfore the mass transfer of coefficients decreased. Mixed culture due to high ability of different strains for growth show higher degradability compared to pure culture. Degradability of pure culture at non suitable conditions is low. Figure 6 shows the removal percent of naphthalene and phenanthrene after 50 days in solid condition (microcosm) for mixed culture and two pure species SH and EM2. Comparison of the results showed that percent removal of aromatic hydrocarbons by pure culture under solid phase is lower than slurry the condition, and also a mixed culture showed higher degradation ability under this condition.

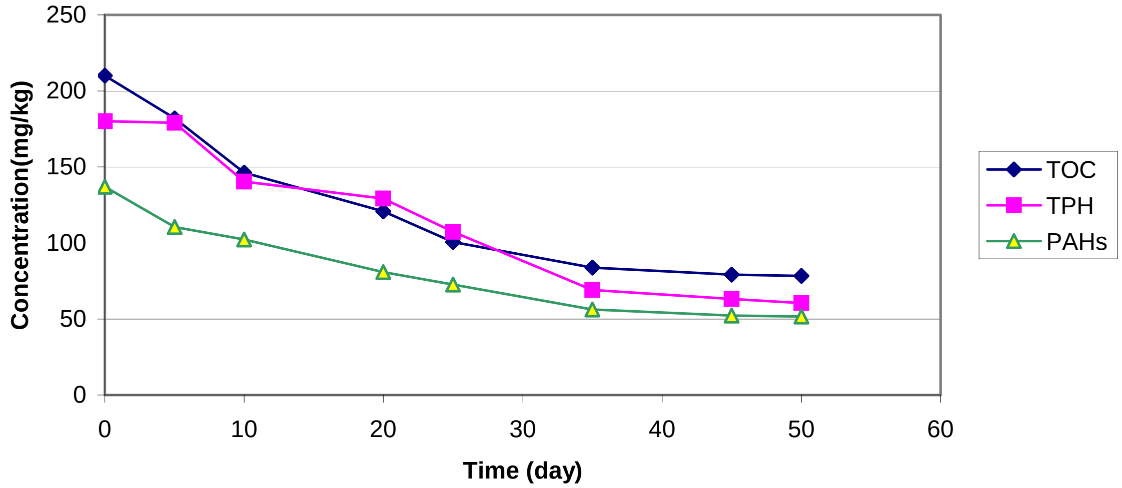

Figure 5. Concentration changes of TOC, TPH, PAHs with time in solid phase bioreactor

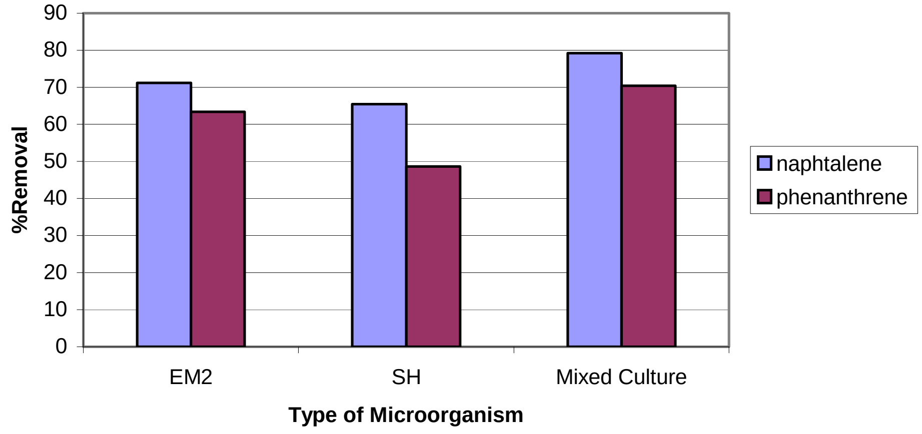

Figure 6. Removal percent changes of naphthalene and phenantherene after 50 days in solid phase.

4. CONCLUSION

The amount of heavy metals such as Ni, Cr. Cd and hydrocarbon in the sediment of 8 stations (SH, DA, EM, GN, DR, KN, BA and AS) around Bousher Port were determined. The maximum amount of heavy metals was observed for Ni in the area of EM with 58-6 mgNi/kg sediment.

Total petroleum hydrocarbon, (TPH), total organic carbon (TOC) and polyaromatic hydrocarbon (PAH) were determined for these stations. EM (Emam Hasan) was the most infected station with 143.6, 159.2 and 89.6 mg/kg sediment for TPH, TOC & PAH respectively.

Varous types of bacteria were isolated from oily contaminated sediments, the ability of the isolated bacteria showed that four species had the best growth under specific and contaminated environments. In slurry conditions the maximum PAHs removal was belong to EM2 species which isulated from Emam Hasan area. In solid phase bioreactor the microbial activity was reduced that can attributed to the unsufficient mixing.

Comparisson of the results showed that PAHs removal by pure culture under solid phase is lower than slurry conditions also mixed culture showed higher degradation ability during solid condition.

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