RESEARCH NOTE

BIOREMEDIATION OF SEDIMENT IN PERSIAN GULF COAST

M. Vossoughi, P. Moslehi and I. Alemzadeh

Department of Chemical and Petroleum Engineering, Sharif University of Technology
Tehran, Iran, Vosoughi@sharif.edu

(Received: August 25, 2004 – Accepted in Revised Form: February 24, 2005)

Abstract The amount of petroleum hydrocarbon and heavy metals in sediment of Persian Gulf's shore at 8 selected stations were determined and showed the maximum of 143.6 and 58.6 mg/kg sediment, respectively. The above 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 (Poly Aromatic Hydrocarbons) 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 the mixed culture due to strength and tolerance of different strains for growth, showed higher degradability compared to pure strains, but due to insufficient mixing under solid state conditions, mass transfer rate of nutrient reduced which caused to reduce cell activity, therefore removal efficiency under slurry conditions was higher.

Key Words Hydrocarbon Pollutant, Heavy Metal, Persian Gulf Coast, Bioremediation, Solid Phase Bioreactor

چکیده میزان آلودگی نفتی و فلزات سنگین موجود در رسوبات ۸ ایستگاه انتخابی در سواحل خلیج فارس اندازه گیری شد. نتایج نشان دادند که میانگین هیدروکربورهای کل بین ۶۴/۳ تا ۱۴۳/۶ میلی گرم بر کیلوگرم است. بالاترین غلظت فلزات سنگین را نیکل با میزان ۵۸/۶ میلی گرم در کیلوگرم در منطقه امام حسن واقع در حدود ۵۰ کیلومتری غرب بندر بوشهر دارا می‌باشد. درمان بیولوژیکی در دو محیط دوغابی و جامد مورد مطالعه قرار گرفت. از ۸ نوع باکتری شناسایی شده در خاک این منطقه ۴ سویه به نام های Em2 ، SH ، GN1 و GN3، بهترین راندمان را برای حذف هیدروکربورهای حلقوی PAH از خود نشان دادند. زمان حذف در محیط دوغابی در حدود ۴۵ روز است که در این مدت نفتالین و فنانترن به ترتیب ۷۳ و ۶۶ درصد حذف می‌شوند. در محیط جامد فعالیت میکروب های مخلوط و خالص مطالعه شد. نتایج نشان داد که میکروب های مخلوط از توانایی بالاتری برخوردارند؛ زیرا گونه های متفاوت قادر به ایجاد شرایط مناسب برای رشد خود در محیط های مختلف هستند. ولی از طرف دیگر به خاطر عدم اختلاط کافی در محیط جامد سرعت انتقال مواد کاهش یافته و در نتیجه فعالیت میکروب ها تحت تأثیر قرار می‌گیرد. لذا بازدهی حذف در محیط دوغابی بیشتر از جامد 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 region during the 1930s and 1940s led to a massive increase in shipping and was principally responsible for the immense economic wealth and geopolitical importance of the region, today. The ecosystem of the Gulf and specially its shore has also been polluted. About 40% of all the tankers traffic in the world are in Persian Gulf and an estimated annual passage of 20,000 to 35,000 tankers pass through the Gulf. About 3 to 8 million tons of ballast is discharged to this region. About 150,000 tons of oil annually enters 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]. Recent studies reported that there are polluted sediments in many parts of Khozestan and Boushehr provinces, which is estimated about 3.5 tons of oil in each hectare of a big parts 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, due to their carcinogenic and mutagenic. These toxic compounds in the nature have a high bioaccumulation potential, and their removal needed long duration of time [6]. Solid treatment technologies are often developed and evaluated in order to confirm with regulatory demands, which may require the residual petroleum hydrocarbons (RPH) concentrations in solid be reduced to lower than 100 mg/kg soil [7].

There are a few technologies available to treat the TPH (Total Petroleum Hydrocarbons) contaminated sites such as 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. These technologies can be integrated to enhance performance.

Bioremediation is a managed or spontaneous process in which biological organisms such as microbial cells act 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 such as 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 microorganisms.

Bioremediation refers to enhancement of the native capability of the microorganisms. The indigenous microorganisms simulated are added to the site to degrade, transform, or attenuate organic and organometallic compounds to nontoxic products.

Unlike other techniques that temporarily vary, displace the problem or transfer the contaminants to another medium and bioremediation attempts to render the contaminants into harmless substances. Wang has reported that by employing Pseudomonas spp. strain BT, PAH compounds of diesel oil were completely eliminated in 12 weeks [9]. It has been reported that the bacteria have better ability than fungi for degradation polyaromatic compounds [10,11]. Yaghmaei, et al. [11] showed that fungi can also remove hydrocarbon compounds and the maximum removal of naphthalene, phenanthrene and antheracen were 75, 65, 59 percent, respectively [10,11]. In another case study, it was stated that Phanerochaete chrysosporium is able to degrade aromatic hydrocarbons. Phanerochaete chrysosporium was able to remove 90% of phenanthrene after 6 days of operation in a Rotating Biological Contactors [12].

The objectives of this study were to determine the concentrations of heavy metals (nickel, chrome and cadmium) and petroleum contaminants in sediments of Persian Gulf coast, and investigation on the effect of suitable strains of bacteria on bioremediation of petroleum contaminated sediments.

2. MATERIALS AND METHODS

2.1. Sampling Samples of sediment and water were taken during two seasons, autumn (temperature between 15 to 25 $^{\circ}$C) and summer 2002 (temperature between 30 to 45 ^{0}C) from polluted sites, based on instruction given in standard methods [14,17]. The initial pH of sediment and water were between 7.6 and 8.2.

Eight stations, which seemed to be more polluted than the others were selected. The average distance between stations was about 50 to 80 kilometers. The stations were located in the west to east coast of the Persian Gulf were

Daylam(DA), EmamHasan(EM), Genaveh(GN), Shaghab(SH), Dayear(DR), Kangan(KN), BahmanPort(BA) and Asalouieh(AS). The samples were taken by sterile shovel from 10 cm depth and about 200 to 300 meter far from the beach, one quart sterile bottles were filled by samples and were kept bellow 4°C and transferred to the laboratory which was located about 152 km far from stations [14,17].

2.2. Analysis Heavy metals were determined by Atomic Absorption Spectrophotometer Analytic, Jena, GmbH 26000 – 126 Ziess AAS5EA Germany. For determination of polycyclic aromatic hydrocarbons (PAH), high performance liquid chromatography (HPLC) was used. The column was Odssil 25 cm length and 4.6 mm internal diameter and wave length of 254 nm, which was coupled to a florescence detector at wave length of 280 nm and emission greater than 389 nm. The solvent was mixture of dichloromethane and hexane with equal volumetric ratio.

TOC (Total Organic Carbon) and TPH were determined by TOC Analyzer (CAIO made by Skaler Company) and suxhelet, respectively. All analyses were performed in duplicate.

Microorganisms were isolated and characterized by the standard methods of microbiology [15,16]. Slurry phase consisted of nutrient solution. Table 1 shows the composition of the culture media.

For solid phase treatment a solid phase bioreactor were employed. Figure 1 shows schematic diagram of the bioreactor. The bioreactor employed was constructed from Plexiglas with 20cm diameter and 40cm height. Aeration of the bioreactor was performed by vacuum condition in the bioreactor and substrate was fed from the storage tank and distributed on top of the bed. A condenser was used to condense the humid air released from the bioreactor.

TABLE 1. Composition of Inorganic Compounds for Nutrient Solution [10, 11].

Inorganic Compounds	Concentration (g/l)
Na_2HPO_4	1.34
NH_4Cl	0.67
$MgSO_4 \cdot 7H_2O$	2.5
$CaCl_2 \cdot H_2O$	0.025
$FeSO_4 \cdot H_2O$	0.1
$MnSO_4 \cdot H_2O$	0.004

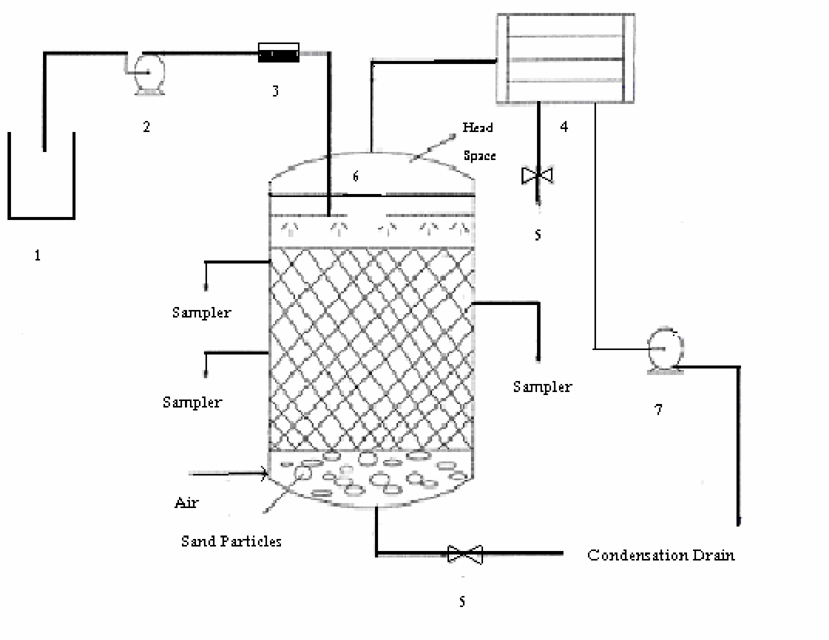
3. RESULTS AND DISCUSSION

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 samples from Persian Gulf is high and the maximum of them is in Emam Hasan station with 58.6 mg Ni/kg sediment.

Most of light hydrocarbons and alkanes are volatile and sediments are free of them, therefore the most important parts of hydrocarbons in sediments are due to mono, di and heteroaromatics, 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 was about 4 to 6 days and during this period various types of colonies were developed. Among them there were 8 species, which had higher purity, all were selected and separated.

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



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

Figure 1. Solid phase bioreactor.

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

In Figure 2 the cell concentrations of the 8 isolated strains of microbial species are compared. From 8 species, only four isolated ones from the sediment of stations have shown better growth. Therefore, these species were selected for further contaminant removal studies. Biological treatment was performed also by the mixed culture of four isolated strains.

More investigations on microbial and morphological characteristics showed that they are related to pseudomonas group (see Table 4). In the literature, the ability of pseudomonas spp. in hydrocarbons removal had been frequently reported. Eriksson, et al. isolated two species capable of growing on pyrene, both strains of

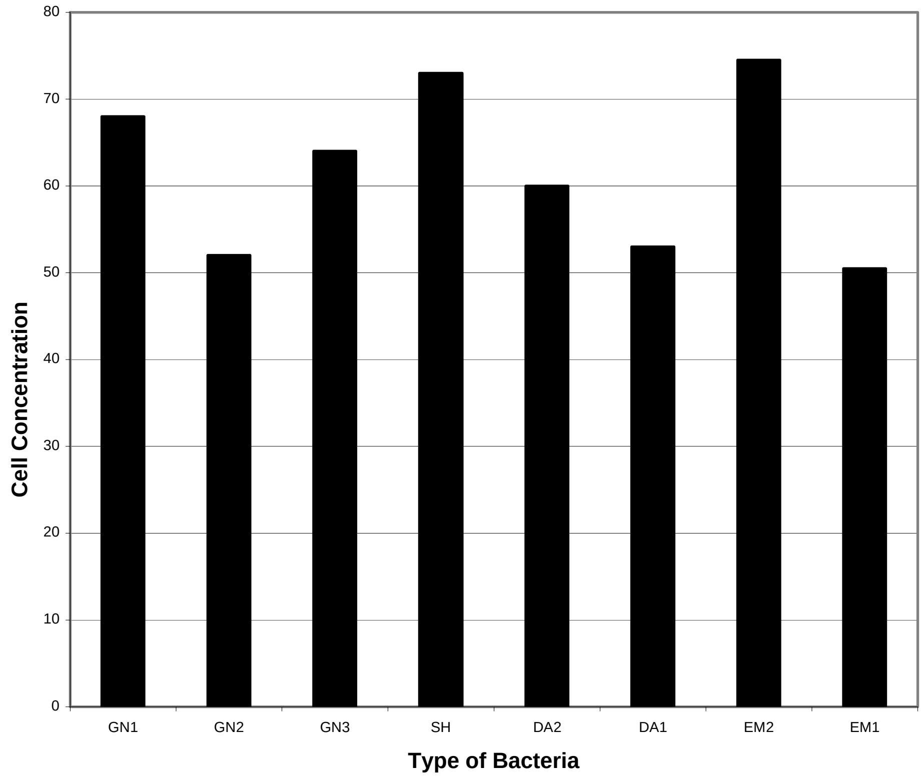


Figure 2. Column diagram of cell concentration.

TABLE 4. Some characterization of four species bacteria.

Site collected samples	Strain named	Morphology	Gram test	pH	Temp©
Shagab	SH	Greenish Yellow, Rod- -shaped colonies	Gram-negative	8.1	32-35
Emam Hassan	EM_2	Cream, Cocci-shaped	Gram-positive	7.8	32-35
Genaveh	GN_1	Cream, chained cocci-shaped colonies	Gram-negative	7.8	32-35
Deilam	GN_3	Yellow, rod-shaped	Gram-positive	7.6	32-35

pseudomonas spp., were obtained from the pyrene enrichment culture [18].

3.2 PAH Degradation in Slurry Conditions

Figure 3 illustrates the percent removal of phenanthrerne by four strains of bacteria.

The maximum removal was associated with EM2 species isolated from the Emam Hasan area, and it must be related to the pollution exists in this site 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, reduced from 140 to 68 mg/kg sediment after 45 days.

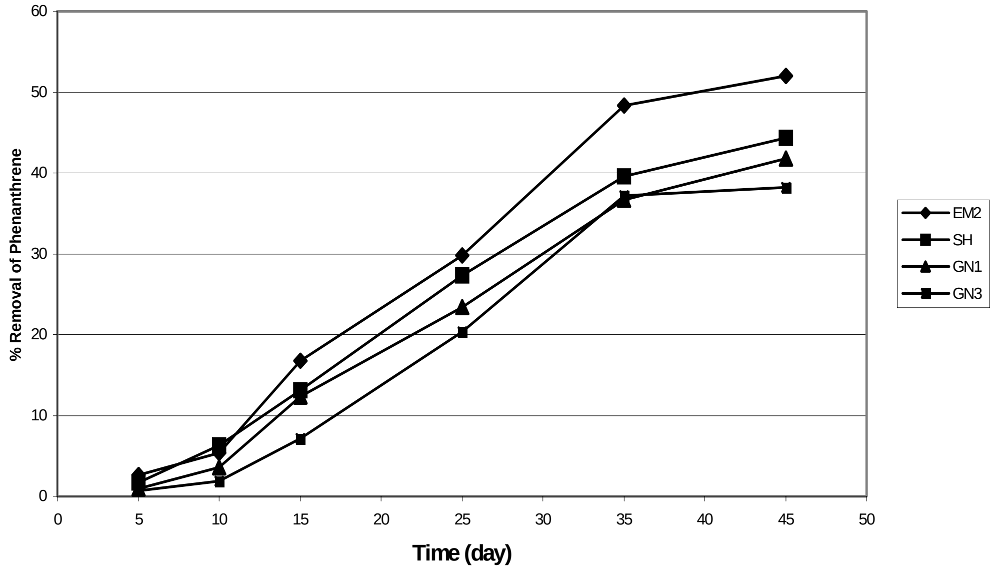


Figure 3. A comparison of phenantherene percent removal by isolated bacteria.

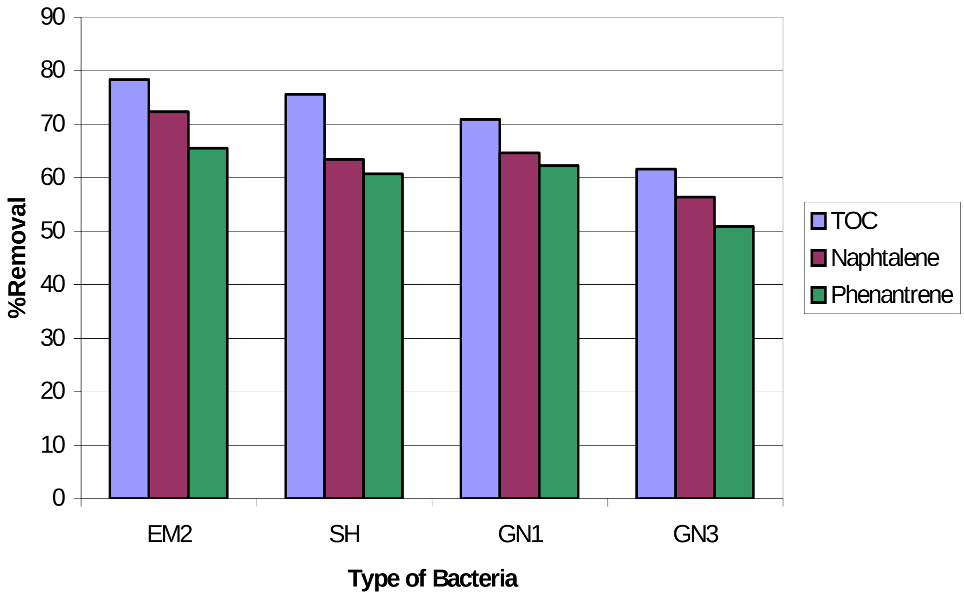


Figure 4. Comparison of percent removal of TOC, phenanthrene and naphthalene by selected species after 50 days.

Phenantherene has more ring and show lower degradation compared to naphthalene. At this condition the suspended solids concentration in the slurry was nearly 40000 mg/l which contained about 10% of MLVSS (Mass Liquor Volatile Suspended Solids). Figure 4 shows the percent removal of naphthalene, phenantherene and TOC by four selected species under slurry conditions. The slurry temperature was controlled at $35\,°C$ and PH value of 7.6, EM2 species has the maximum removal efficiency, which were about 73 and 66% for naphthalene and phenanathrene, respectively.

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 therefore mass transfer 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 result showed that percent removal of aromatic hydrocarbons by pure culture under solid phase is lower than slurry condition, and also mixed culture show higher degradation ability under this condition.

4. CONCLUSION

The amount of heavy metals such as Ni, Cr, Cd and hydrocarbon in the sediment of 8 station (SH, DA, EM, GN, DR, KN, BA and AS) near by Boushehr Port were determined. 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.

Various types of bacteria were isolated from oily contaminated sediments, the ability of the isolated bacteria showed that four species had significant growth under specific and contaminated environment. In slurry condition the maximum

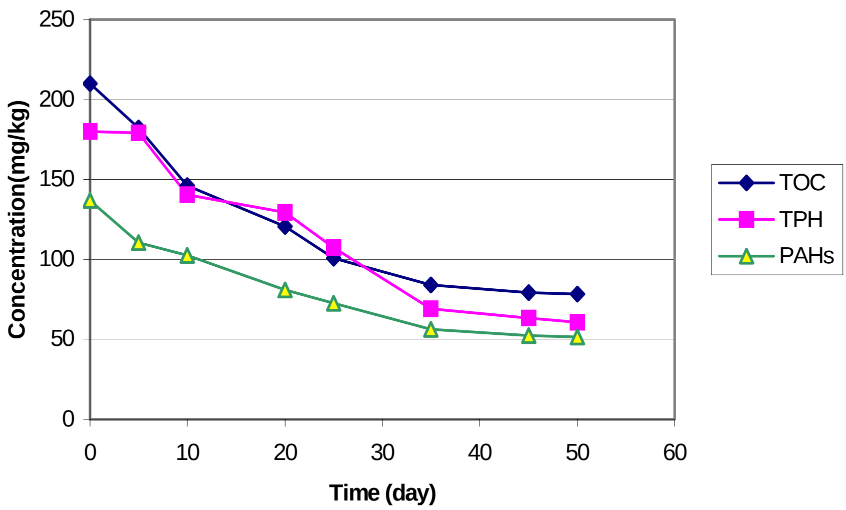


Figure 5. Concentration changes of TOC, TPH, PAHs with time in solid phase bioreactor.

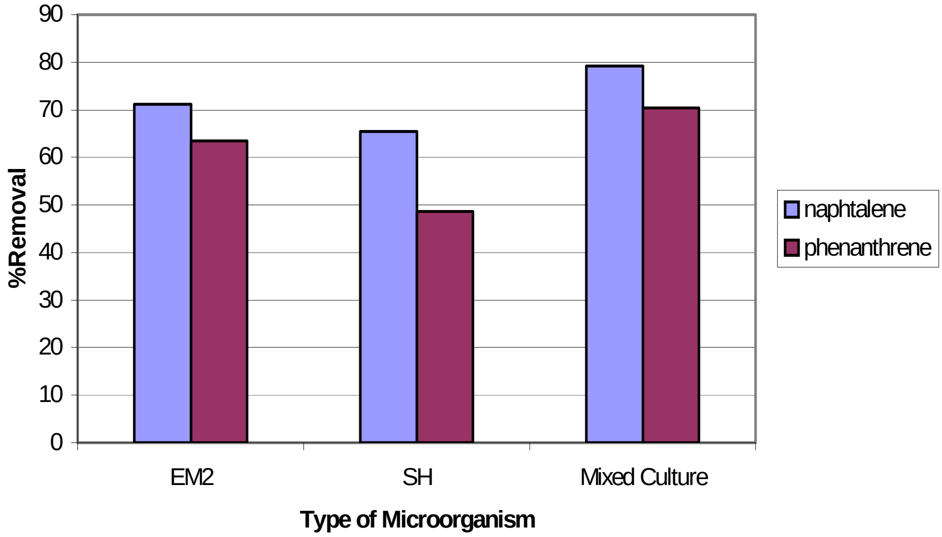


Figure 6. Removal percent changes of naphthalene and phenantherene after 50 days in solid phase.

PAHs removal was belong to EM2 species which isolated from Emam Hasan area. In solid phase bioreactor the microbial activity was reduced that can attributed to the insufficient mixing.

Comparison of the results showed that PAHs removal by pure culture under solid phase was lower than slurry condition also mixed culture showed higher degradation rate at solid condition.

5. REFERENCES

1. Renolds, R. M., "Physical Oceanography of the Persian Gulf Strait of Hormoz and Gulf of Oman Results from the Expedition", ***Marine Pollution***, Vol. 27, (1993), 32–60.
2. "IMCOS Hand Book of Weather in the Gulf. General Eliminate Data", London, IMCOS Marine LTD, (1984).
3. Golob, R. and Bruss, E., "Marine Pollution Bulletin", Vol. 27, (1984), 305–314.
4. Bamaby, F., "The Environment Impact of the Persian Gulf War", ***The Ecologist***, Vol. 21, (4), (1991), 166–172.
5. Lang, W. J. H. and Puhakka, J. H., "In Site Biological Remediation of Contaminated Ground Water a Review" ***Env. Pollution***, Vol. 107, (2000), 187–197.
6. EverIsen, R., "Remediation of Petroleum Contaminated Soil", Lewis publisher, Canada, (1998).
7. Escantin, E. P., "Assessment of PAH Pollution in Coastal Areas from the NW Mediterranean through the Analysis of Fish Bile" ***Marine Pollution Buletin***, Vol 38, No.12, (1999).
8. Hakstage, A. L. and Vangelder Malsem L. A. "Pilot Remediation of Sediment from the Petroleum Harbor on Amsterdam Water", ***Sci. Tech.***, Vol. 37, No. 6-7, (1998), 403–409.
9. Wang, X., Yu, X. and Bartha, R., "Effect of Bioremediation of Polycycle Aromatic Hydrocarbons Residue in Soil", ***Environ. Sci. Technol***., Vol. 24, (7), (1990), 1086–1089.
10. Yaghmaei, S., Vossoughi, M. and Safekordi, A., "Biodegradation of Polycyclic Aromatic Hydrocarbons by Fungi Isolated from tar Contaminated Soil", ***Proceedings of Chisa (2000)***, Praha Czech Repnblic, (August 2000), 27–31.
11. Yaghmaei, S., Vossoughi, M., Safekordi, A. and Alemzadeh, I., "Modeling and Simulation on Bioremediation Process", ***Proceeding of 4th Nation. Chem. Eng. Congress***, Tehran, Iran, (Feb. 1999), 265-270.
12. Zhongming, Zeng, Jeffery and Obbard, "Removal of Surfactant Solubilized Polycyclic Aromatic Hydrocarbons by Phanerochaete Chrysosporium in a Rotating Biological Contactor Reactor", ***J of Biotech*** Vol. 46, (2000), 241–279.
13. Lee, M. D. and Ward, C. H., "Environmental and Biological Methods for the Restoration of Contaminated Aquifers", ***Envi. Toxical. Chem***., Vol. 4, (1985), 743–750.
14. APHA/ AWWA/ WEF, "Standard Methods for the Examination of Water and Wastewater", 20th Ed. Washing DC, USA, (1998).
15. Noel, R., Krieg. and John G. Holt., "Bergey's Manual of Systematic Bacteriology", Vol. 3, Williams and Wilkins Press, (1994).
16. Ronald, M., Atlas, "Handbook of Microbiological Media", 2nd Ed., CRC Press, (1996).
17. Soil VOC Sampling for EPA Method 50 35A Analysis, (2000).
18. Eriksson. M., Dalhammer, G. and Mohn, W. W, "Bacterial Growth and Biofilm Production on Pyrene", ***FEMS Microbioloy Ecology***, Vol. 40, (2002), 21–27.