Journal of Chemical Health Risks 3(1): 47-54, 2013
ISSN:2251-6719

Assessment Bioremediation of Contaminated Soils to Petroleum Compounds and Role of Chemical Fertilizers in the Decomposition Process

*H. Parvizi Mosaed[1 *], S. Sobhan Ardakani[2], M. Cheraghi[3]*

[1] Young Researchers & Elites Club, Hamedan Branch, Islamic Azad University, Hamedan, Iran
[2] Department of the Environment, Hamedan Branch, Islamic Azad University, Hamedan, Iran
[3] Department of the Environment, Hamedan Branch, Islamic Azad University, Hamedan, Iran

Abstract: Today oil removal from contaminated soil by new methods such as bioremediation is necessary. In this paper, the effect of chemical fertilizers and aeration on bioremediation of oil-contaminated soil has been investigated. Also the control group, (bioremediation of petroleum hydrocarbons in contaminated soil without treatment by chemical fertilizers and aeration treatment was examined. The condition of experiment is as following: those were treated 70 days in glass columns (30×30×30cm dimensions), ambient temperature (25-30 ^{0}C), relative humidity 70%, aeration operation with flow 0.7 lit/min. The total number of heterotrophic bacteria of break down oil and the total of petroleum hydrocarbons were analyzed using gas chromatography analysis. all experiments were replicated three times. The microbial population results for control soil, treated soil by aeration and treated soil by aeration and chemical fertilizers columns are 2.3×10^5, 1.04×10^{10}, and 1.14×10^{11} CFU/gr, respectively. The concentrations of total petroleum hydrocarbons of remaining are 46965, 38124, and 22187 mg kg^{-1}respectively. The obtained results show that the aeration operation and chemical fertilizers have effective role on degradation of petroleum hydrocarbon by oil degrading bacteria from soil.

Keywords: Bioremediation, Chemical fertilizers, petroleum hydrocarbons, Soil, Microorganisms

INTRODUCTION

Soil purified for natural that addition to provide food, also has treatment effect. But times are that petroleum materials and their derivatives in the effect of the transportation or storage due to soil pollution, however whatsoever petroleum materials penetrate into more deep from soil, their decontamination would be more difficult and costly[17,25]. Physical, chemical and thermal processes are as general methods for the cleanup of contaminated sites to petroleum compounds[6].This techniques, however have some negative effects on the environment and are expensive also[6,12]. Bioremediation is as method that nowadays is used for cleanup contaminated soil and groundwater with petroleum hydrocarbons. Using microorganisms that are found naturally in soil, these pollutants are mineralized or decomposed. Water and carbon dioxide are produced in the end of the mining process [15]. Also bioremediation is a new method and economically effective that can be used to clean up hazardous wastes [3].When purification, most important step is finding microorganisms that have the ability to remove the enzyme and the degradation of petroleum hydrocarbons. These microorganisms are able to metabolize hydrocarbons to gain carbon and energy source. Environmental condition should be optimal for microorganisms to successful biological cleaning. Bacteria, fungi and yeast can gain required energy for reproduction and survival from petroleum [28].

Corresponding Author: H. Parvizi Mosaed, Young Researchers & Elites Club, Hamedan Branch, Islamic Azad University, Hamedan, Iran. E. mail: Hassan.parvizi@iauh.ac.ir

Oil-eating bacteria break down petroleum hydrocarbons to fatty acid or carboxylic acid. These materials also turn into carbon and carbon atoms and to produce energy in the citric acid cycle [14]. If an incident leads to arrive petroleum compounds into the soil, it will due to increase in the number of microorganisms of hydrocarbons degrading in the ecosystem.

It should be noted that a microorganism is able to degradation of certain kind of oil compounds, but a combination of microbial communities able to degrade hydrocarbons in greater levels. In the natural conditions, present of microorganisms that are used from products of due to decomposition of petroleum compounds, have specific importance. Speed, efficiency and rate of biodegradation in oil-contaminated soils and petroleum compounds depend on number of microorganisms of breaking down of hydrocarbons in soil and type of soil [20, 25]. However, biodegradation of hydrocarbons in soil can be limited by factors such as microorganisms, nutrients, pH, temperature, moisture, oxygen, soil characteristics and pollutants concentration. Research has showed that crude soil can be disappeared from the sea by adding nutrients such as nitrogen or phosphorous and/or both [3]. Studies have shown that the use of microorganisms can be effective for bioremediation of oil- contaminated soils. For example, species of microorganisms that capable to treatment of oil-contaminated soils are included:Pseudomonas pseudoalcaligenes, Bacillus firmus, Bacillus alvei ،Penicillium funiculosum ،Aspergillus sydowii ، Rhizopus sp, that able to degrade 79,80,68,86,81 and 67 percent of the total petroleum hydrocarbons in oil [23]. In study on the effects of bioremediation in oil-contaminated soils in Nigeria had been concluded that the treatment of chemical fertilizers, increased biodegradation of oil-contaminated soils and is achieved 50-95% reduction in pollutants hydrocarbons [27]. In the study conducted by Facundo et al(2000) found that 13 days after inoculation of bacteria and application of ammonium nitrate fertilizers, soil pollution into gas oil decrease to 90% . In the study conducted by Chorom et al (2010) found that application of fertilizers at the rate 2 tons in per acre on oil-contaminated soils lead to increased rate of biodegradation after 5 weeks, that is indicating facilitating in biodegradation process. In the study conducted by Milic(2009) in investigation of the composition of microbial for biodegradation heavily oil-contaminated soils and products indicated that with passing 5.5 months of testing, by stimulating and bioremediation with the participation of gram-positive bacteria and actinomycetes group (Nocardia), the concentration of petroleum hydrocarbons (TPH) reduced from 29.8 to 3.29 g/kg that it is equivalent to 89% decrease. So that the microorganisms that degrade hydrocarbons, in this process had major role about 80% [16]. Therefore, in this study, we tried to investigate the bioremediation of soil hydrocarbons and chemical fertilizers role and soil aeration operation in facilitating of the process of biodegradation.

MATERIAL AND METHODS

Preparation of soil samples

Samples had collected from oil- contaminated soils around Tehran. Then soil was passed through a sieve 2 mm until remove large lumps of soil and conditions be uniform for each of the three columns of soil. In order to bioremediation of petroleum pollutants was used from 3 glass columns with a diameter of 4 mm and with dimensions 30×30×30 in 3 replications. Column 1 including contaminated soil into petroleum hydrocarbons without chemical fertilizers treatment and without aeration treatment was as control sample. Column 2 was including

contaminated soil into petroleum hydrocarbons without chemical fertilizers treatment and with aeration treatment. Column 3 was including contaminated soil into petroleum hydrocarbons with chemical fertilizers and aeration treatment.

For preparation these columns, in the first time columns floor must was filled up to height 2.5 cm by stons that made of silica.

Then soil was poured onto to a height 18 cm and over soil columns was set sands that made of silica and glass wool[18].

To column of number 3 was added 5 gram from chemical fertilizers NPK that include urea nitrogen fertilizer with 54% nitrogen, di ammonium phosphate with 25% nitrogen and phosphorus with 57% and potassium sulfate with 46% potassium. In order to supply soil nutrients include nitrogen, phosphorous and potasieum was used from nutrients respect to 10:10:20 for N:P:K in the mixed mood (equivalent with 1 ton per hectare). Then vertical steel pipes with height 16 cm were placed for transmission of air from aeration pumps with rate 0.7 lit/min in the soil. Aeration was conducted in 70 day and 12 hours in every day and then aeration was removed for 12 hours. Treated soils were placed under conditions of light and controlled temperature[21]. Temperature and humidity in the columns were documented Simultaneous in the daily and measurement pH was recorded in intervals ten day after of starting of aeration.

METHODS

Some of physical and chemical properties of studied soils include soil texture were measured by method hydrometery, the electrical conductivity was measured by saturation mud extraction, pH was analyzed by a glass electrode (Jenway 3015, United Kingdom) using a 1:1 soil: water ratio, Phosphorus was measured by Olsen P extracting solution, total nitrogen was measured by Kjeldahl digestion [26] for measuring soil sodium and potassium was used from extraction procedure with ammonium acetate and reading with flame detector [1] and for measuring soil-lime was used from titration method by NaOH [11].

Microbial enumeration

Hydrocarbon-degrading and heterotrophic bacteria were enumerated using a MPN (Most-Protable Number) method adapted from Wrenn and Venosa (1996). Bushnell Haas medium (composition: magnesium soleplate 0.2 g g/L; calcium chloride 0.02 g/L ; monopotassium phosphate 1 g/L; ammonium phosphate dibasic 1 g/L; potassium nitrate 1 g L^{-1} and ferric chloride 0.05 g/L) supplemented with 2% (w/w) NaCl was used as the growth medium in 96 well microstate plates. Hydrocarbon sources were added to stimulate the growth of hydrocarbon–degraders. A sample of 3 g of soil samples was placed in a vial containing 10 mL of Bushnell Haas medium supplemented with 2% (w/w) Nail and mixed to form slurry. One mL of this slurry was added to a vial containing 9 ml of Bushnell Haas medium supplemented with 2% (w/w) NaCl. A dilution series was prepared from this sample, from 10^{-1} to 10^{-12}, and used to inoculate plates. Each rarity has three repetitions, after diluting Risasorin identifier in a rate of 90 µl added to pipes and then sterilized crude oil in a rate of 0.2 mL should be added to each pipe and plates for enumeration of hydrocarbon-degrading bacteria were incubated at room temperature (26-27°C) for 2 weeks. After incubation, all plates were read using a MPN chart to determine positive or negative growth of bacteria, it is necessary to mention that all the needed instruments were sterilized [3,7].

Determination of petroleum hydrocarbons in soils

In order to determine the concentration of total petroleum hydrocarbons (TPHs) and some of polycyclic hydrocarbons (PAHs) in soil, in the first time, extraction performed by sox let method with

proportion same of n-hexane and dichloromethane (EPA 8100 method) Christopher et al (1998). Then using of gas chromatograph set and by EPA 831 method determined the concentration of total petroleum hydrocarbons (TPHs) and some of polycyclic hydrocarbons (PAHs) in soil.

RESULTS

Results of physical and chemical analysis of studied soils are showed in table 1. The pH of studied soils is 6.8, it shows that oil- contaminated soils were the neutral. Furthermore, the results of measurement pH soil on three performed treatments during the biodegradation process in 10 weeks was identified that pH optimal for biodegradation is in range 6-8.

Table 1: Some of physical and chemical characteristics in studied soils

Factor	amount	unit
PH	6.8	-
EC	10	ds m^{-1}
Clay	25	%
Lime	23	%
Organic matter	11.2	%
Total nitrogen	1.4	%
Phosphorous	148	mg/kg
Potassium	147.6	mg/kg
Sodium	32	mg/kg

Concentration of total of petroleum hydrocarbons and some of polycyclic hydrocarbons in studied soils were shown in table 2. As it was showed, the sample soils had pollution more than standard level of TPH in soil (2000 mg/kg) [9, 10, 2].

Table 2: Concentration of petroleum total hydrocarbons and some of polycyclic hydrocarbons in contaminated soils

Hydrocarbons	Concentration ($mgkg^{-1}$)
TPH	100678
Naphthalene	43
Phenanthrene	33.5
Anthracene	2.7
Phelorantene	28.2
payrn	17.8

Table 3 was showed results of the enumeration of soil heterotrophic bacteria in temperature of 25-30 $^{\circ}C$ and humidity of 70% at during ten weeks in three soil type, control soil, treated soil with aeration operation and treated soil with chemical fertilizer and aeration operation. As was seen from the first weeks until fifth weeks increased quickly the enumeration of heterotrophic bacteria and from fifth weeks to ten weeks will begin to decline at the lower rate. Growth intensify of heterotrophic bacteria of oil degradation in treated soils with chemical fertilizers and aeration operation be more than control soils and treated soils alone with aeration operation that identified interaction effect of chemical fertilizers treatment and aeration treatment on the growth of bacteria.

Table 3: Mean of number of heterotrophic bacteria ($CFUgr^{-1}$) in studied soil samples

Week	Number of total of heterotrophic bacteria (CFU/gr)		
	Contaminated soils without treatment	Contaminated soils with aeration treatment	Contaminated soils with aeration and chemical fertilizers treatment
1	9×10^{3}	8×10^{3}	8.7×10^{3}
2	7.2×10^{5}	8.8×10^{8}	9.1×10^{9}
3	4.3×10^{6}	2×10^{9}	2×10^{10}
4	5×10^{7}	3.3×10^{10}	1.2×10^{11}
5	6×10^{7}	5×10^{10}	3.3×10^{11}
6	5.1×10^{6}	4×10^{10}	3.1×10^{11}
7	7×10^{5}	3.4×10^{10}	2.6×10^{11}
8	6.5×10^{5}	3×10^{10}	2×10^{11}
9	3×10^{5}	2.2×10^{10}	1.5×10^{11}
10	2.3×10^{5}	1.04×10^{10}	1.14×10^{11}

Table 4 shows results of the mean of concentration of total petroleum hydrocarbons of soil in temperature of 25-30 ^{0}C and humidity of 70% at during ten weeks in three soil types, control soil, treated soil with aeration operation and treated soil with chemical fertilizer and aeration operation. As it was shown with passing time, decreased concentration of total petroleum hydrocarbons of soil from first week to tenth weeks so that until fifth weeks quickly increased degradation rate and from fifth weeks until tenth weeks decreased biodegradation rate. Further amount of reduction of concentration of total petroleum hydrocarbons in treated soils with chemical fertilizers and aeration be more than control soils and treated soils only with aeration operation. Figure 1 shows concentration of total petroleum hydrocarbons in three soil treatments in during ten weeks.

Table 4: Mean of concentration of total petroleum hydrocarbons in studied soils

week	Mean of concentration of total petroleum hydrocarbons (mg/kg)		
	Contaminated soils without treatment	**Contaminated soils with aeration treatment**	**Contaminated soils with aeration and chemical fertilizers treatment**
1	100178	99567	95904
2	88108	78021	65234
3	76324	62134	58209
4	67456	57890	50932
5	62901	54234	42834
6	54679	45679	35734
7	53213	43214	30217
8	51234	40236	28632
9	50954	39765	25601
10	46965	38124	22187

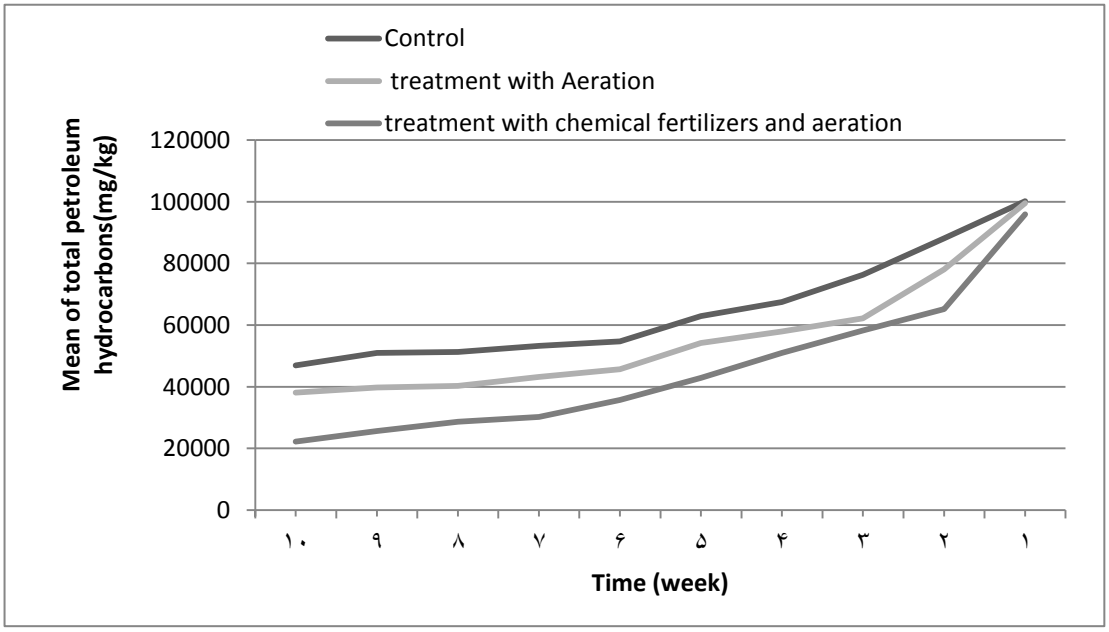

Fig 1: The mean of concentration of total petroleum hydrocarbons in during ten weeks in between control, treatment with aeration and treatment with chemical fertilizers and aeration

DISCUSSION

The results showed that there is a significant difference at 5 percent statistical level in petroleum degradation between treated soil samples by chemical fertilizers and aeration and untreated soil samples by chemical fertilizers and aeration. This is due to application of chemical fertilizer NPK and aeration treatment during degradation process that was resulted to increase in growth of oil –degradation bacteria that eventually have been led to the degradation of petroleum hydrocarbons. In the control soil (no chemical fertilizers and aeration treatment) after of

70 days, the total number of heterotrophic bacteria reach to 2.3×10^5 $CFUgr^{-1}$ and concentration of total petroleum hydrocarbons reach to 46965 mg/kg. While in treated soils with chemical fertilizers and aeration after of 70 days, the total number of heterotrophic bacteria reaches to 1.14×10^{11} CFU/gr and concentration of total petroleum hydrocarbons reach to 22187 mg/kg. It showed 54% reduction in the concentration of total petroleum hydrocarbons in control soils and 78% reduction in the concentration of total petroleum hydrocarbons in treated soils with chemical fertilizers and aeration. Gogoi and his colleagues (2003) in study on bioremediation of crude oil-contaminated soils in arrival place of crude oil showed that aeration operation, application fertilizers containing nitrogen and phosphorous and microbial inoculation led to degradation 75% of crude oil. Margesin (2000) saw that biological stimulation of oil –contaminated soils, with mineral nutrient example chemical fertilizer containing NPK led to increase in biodegradation and hydrocarbons degradation in amount 27-53%. In treated soils with aeration and without chemical fertilizer, after of 70 days, the total number of heterotrophic bacteria reaches to 1.04×10^{10} CFU/gr and concentration of total petroleum hydrocarbons reach to 38124 mg/kg that showed 62% reduction in the concentration of total petroleum hydrocarbons in treated soils with aeration. It was due to regular aeration with discharge 70 lit min^{-1} and temperature conditions (25-30 $^{\circ}C$) and available humidity (70%) for bacteria. In addition, there was significant difference between the mean of petroleum degradation in soil in different weeks and the mean of petroleum degradation has been in the first weeks more than the last weeks. This indicates the significant effect of time factor on petroleum degradation in the statistical level 5% in treated soils. As it was shown that with passing of time decreased petroleum degradation process and was petroleum degradation in the first weeks of testing more than the last weeks. This observation is fully consistent with the growth of heterotrophic bacteria and the most of bacteria growth was observed in the first weeks. In the first weeks of treatment, it is probably due to the presence of normal alkanes of biodegradable, abundant of mineral nutrients, favorable temperature and humidity conditions and regular aeration operation, bacteria activities and petroleum degradation was been more, but with passing time, remain petroleum compounds with the low ability of degradation, long chain and containing the low nitrogen and in the result increased bacteria growth and petroleum degradation [22]. Also Ouyang and his colleagues (2005) observed that coincide with the rise of microbial population in the soil, petroleum degradation is got more and this rise was been rapidly during the first weeks of treatment and with passing time, this process will reduce. In addition, the results Katsivela and his colleagues (2005) in study of bioremediation in situ of contaminated agricultural soils with engine oil was showed that normal alkane degradation during four months of firth treating occur rapidly due to existence and life of active bacteria population.

REFERENCES

1. Norris R. D., Hinchee R. E., Brown R. A., McCarty P. L., Semprini L., Wilson J. T., Kampbell D. H., Reinhard M., Bower E. J., Borden R. C., Vogel T. M., Thomas J. M., Ward C. H., 1994. Handbook of Bioremediation. Boca Raton, FL:CRC Press.
2. U.S. Environmental Protection Agency (EPA).,1991. Soil Vapor Extraction Technology: Reference Handbook. Cincinnati, OH: Office of Research and Development. EPA/540/2-9.

3. Frick C. M., Farrell R. E., Germida J. J., 1999. Assessment of Phytoremediation as an in situ technique for cleaning oil-contaminated sites, Petroleum Technology Alliance Canada, Calgary.
4. Lundstedt S., 2003. Analysis of PAHs and their transformation products in contaminated soil and remedial processes, Solfjodern Offset AB, Umea, Sweden, pp: 55.
5. Mills M. A., Bonner J. S., McDonald T. J., Paqe C. A., Autenrieth R. L., 2003. Intrinsic bioremediation of a petroleum impacted wetland. Marine Pollutin Bulletin, 46, pp 887-899.
6. Chorom M., Sharifi H. S., Motamedi H., 2010. Bioremediation of a crude oil-polluted soil by application of fertilizers, *Iran. J. Environ. Health. Sci. En, Vol. 7, No. 4, pp: 319-326.*
7. Prince R. C., Lessard R. R., Clark R. ,2003. Bioremediation of marine oil spills, Oil & Gas and Technology-Rev, 58 (4), 463-468.
8. Mendelssohn I. A., Lin Q., 2003. Development of bioremediation for oil spill cleanup in costal wetland. OCS Study, MMS 2002-2048.
9. Rittmann B. E., McCarty P. L., 2001. Enviromental Biotechnology: Principles and Applications, McGraw-Hill, New York, p. 695.
10. Tang J., Niu X., Sun Q., Wang R., 2010. Bioremediation of Petroleum Polluted Soil by Combination of Ryegrass with Effective Microorganisms, Journal of Environmental Technology and Engineering, 3(2) : pp 80-86.
11. Walworth J. L., Reynolds C. M., 1995. Bioremediation of a petroleum-contaminated cryic soil: effects of Phosphorous, nitrogen, and temperature. Journal of Soil Contam, 4:pp 299 - 310.
12. Facundo J., Marquez R., Hernandez V., Teresa Lamela M. A., 2000. Biodegraradation of diesel oil in soil by a microbial consortium, pp: 313-320.
13. Milic J. S., Beskoski V. P., Ilic M.V., Ali S. M., GojgicCvijovic G. D., Vrvic M. M., 2009.Bioremediation of soil heavily contaminated with crude oil and its products: composition of the microbial consortium, J. Serb. Chem. Soc. 74 (4): pp 455–460.
14. Osterreicher-Cunha P., Do Amaral Vargas Jr. E., Remy Davee Guimaraes J., Mauro Pereira de Campos T., Maria Ferreira Nunes C., Costa A., Dos Santos Antunes F., Isabel Pais da Silva M., Maria Mano D., 2004. Evaluation of bioventing on a gasoline–ethanol contaminated undisturbed residual soil, Journal of Hazardous Materials, Vol. 110, pp 64-66.
15. Schaefer M., Juliane F., 2007. The influence of earthworms and organic additives on the biodegradation of oil contaminated soil. Applied Soil Ecoogy, 36, pp: 53-62.
16. USDA., 1993. "Soil Survey Manual, United States Department of Agriculture", USA.
17. AOAC (Association of Official Analytical Chemists), 2005. Methods of Analysis., Washington D.C.
18. Klute A. (ed.)., 1986. Methods of Soil Analysis.Part 1, 2nd ed. American Society of Agronomy and Soil Science Society of America, Madison, WI.
19. Wrenn B. A., Venosa A. D., 1996. "Selective enumeration of aromatic and aliphatic hydrocarbon degrading bacteria by a most-probable-number procedure". Canadian Journal of Microbiology: 42 (2) :pp252–258.
20. Gogoi B. K., Dutta N. N., Goswami P., Krishna Mohan T. R., 2003. A case study of bioremediation of petroleumhydrocarbon contaminated soil at a crude oil spill site. Advances in Environmental Research, 7: pp 767-782.
21. Christopher S., Hein P., Marsden J., Shurleff A. S., 1988. Evaluation of methods 3540 (soxhlet) and 3550(Sonication) for evaluation of appendix IX analyses from solid samples. S-CUBED, Report for EPA contract 68-03-33-75, work assignment No.03, Document No. SSS-R-88-9436.

22. U. S. EPA., 1984. Interalaboratory Comparison Stunt: Methods for volatile and semi–volatile compounds, Environmental monitoring systems laboratory. Office of Research and Development, Las Vegas, NV, EPA. 600/4- 84- 027.
23. Kang W., Cheong J., Kim K., Chang J., 2011a. Remediation of TPH-Contaminated Soils in the Railroad Area by Using Soil Washing. Proc. Of Korea Society of Environmental Engineers., pp: 102-103.
24. Kang W., Cheong J., Kim K., Chang J. Kim D., 2011b. Remediation of Petroleum-Contaminated Railroad Soils by Soil Washing. Proc. Of International Symposium on Mine reclamation., pp: 160-161.
25. Chungnam University, 2011.Verification report of soil remediation., pp: 8-9, 24-27.
26. Margesin R., 2000. Potentiol of cold-adapted microorganisms for bioremediation of oil-polluted Alpine soils. International Biodeterioration & Biodegradation, 46:pp 3-10.
27. Tajik M., 2004. Assessment of geoenvironmental effect of petroleum pollution on coastal sediments of Bushehr province. Iran.M.Sc. Thesis, tarbiat modares university, Tehran-Iran(in Persian) 97p.
28. Ouyang W., Liu H., Murygina V., Yongyong Y., Xiu Z., Kalyuzhnyi S. ,2005. Comparison of bio-augmentation and composting for remediation of oily sludge : A field-scale study in China. Process Biochemistry, 40: pp 3763-3768Prince,R.C. et al., 2003. Bioremediationof oil spill. Oil & Gas and Technology-Rev, 58 (4), pp 463-468.
29. Katsivela E., Moore E. R. B., Maroukli D., Strompl C., Pieper D., Kalogerakis N., 2005. Bacterial community dynamics during in-situ bioremediation of petroleum waste sludge in landfarming sites. Biodegradation, 16: pp 169-180.