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Full Length Research Paper

Isolation and identification of hydrocarbons degrading bacteria in soil around Shiraz Refinery

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Petroleum hydrocarbons are species of dangerous contaminants in nature. Scientists are looking for many years to find solution for removing contaminants from the soil and water environments. Today, the use of microorganism, for removing crude oil pollution from contaminated sites (bioremediation) is considered by scientists. The purpose of this research is to isolate and identify hydrocarbons degrading bacteria from Shiraz Refinery soil. In this study, nutrient agar (NA) and mineral salt agar (MM2) were used as basic cultures. Using the Techniques Replica plating, we transferred colonies on NA surface to the MM2 agar. For spraying hydrocarbons on the MM2 agar surface, we used spray plating technique. Finally we have identified different bacterial genera by using biochemical tests and morphological study. Upon examination on morphological studies and biochemical tests, it is determined that these strains belong to bacterial genera as follows: *Bacillus, Corynebacterium, Staphylococcus, Streptococcus, Klebsiella, Escherichia, Acinetobacter, Alcaligenes, Shigella. Enterobacter.* It is also discovered that these bacteria can break down Biphenyl, Naphthalene, Camphor and Phenenthrene. The results showed that Biphenyl, Naphthalene, Camphor and Phenanthrene were decomposed by 22, 23, 34 and 23%, respectively. *Bacillus* was 69% of the bacterial population and accounted as the most dominant bacterial genus. Statistical analysis showed that there is a significant relationship with the level of 0.05 among the station, the numbers, and the diversity of Gram-positive bacteria.

Key words: Degrading bacteria, camphor, bioremediation, *Bacillus*, Shiraz Refinery.

INTRODUCTION

Crude oil is composed of complex mixture of Alicyclic, Aliphatic and Aromatic hydrocarbons (Atlas and Richard, 1946). Hydrocarbons are organic compounds whose structures consist of hydrogen and carbon. Hydrocarbons can be seen as linear linked, branched or cyclic molecules. They are observed as aromatic or aliphatic hydrocarbons. The first one has benzene (C_6H_6) in its structure, while the aliphatic one is seen in three forms: Alkanes, Alkenes and Alkynes (McMurry, 2000).

So far 175 kinds of hydrocarbon identified in raw oil that half of them are hydrocarbons with low boiling point while the rest are compound with high boiling point (Khosravi, 2007). Hydrocarbons in nature can be used as gases, liquids, solids, waxes with low melting or polymer. Aromatic hydrocarbons are insoluble in water and are easily soluble in non-polar organic solvents (Survery et al., 2004). Polycyclic aromatic hydrocarbons (PAHs) are dangerous pollutants in nature. They are very poisonous and cancerous. These compound are non polar and very hydrophobic. They hardly dissolve in water (Javid, 2005). Such aromatic hydrocarbons seen in nature, could point to Anthracene, Phenanthren, Biphenyl, Camphor, Benzene (Survery et al., 2004).

Annually, around 6 million tons of crude oil from oily compounds enter the environment in the world which not only cause human health, but also damage our surrounding environment (Maola et al., 2002). Scientists are looking for ways to remove these pollutants from watery and soil environment. Nowadays, the use

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of microorganism or their products for remedy of these pollutants has been taken into consideration by lot of studies in this field which have been carried out inside and outside of the country. Among them, it point out that for their studies, succeeded in isolation of hydrocarbon degrading bacterial strains. Some of these microorganisms have the ability of degrading, transfer or accumulation of hydrocarbons. The operation is done using a series of these enzymes, and this process is called biodegradation.

Kim et al. (2005) points out the effect of pH, temperature, initial concentration of Phenanthrene on its biodegradation. They succeeded in identifying several bacterial genera that had an ability of degradation of Phenanthrene (Kim et al., 2005). Survery et al. (2004) succeeded in isolation and identification of several bacterial genera, they had the ability of degra-dation solid and liquid hydrocarbons. In Turkey, Coral et al. (2005) researched on refinery soil and succeeded in isolating 80 bacterial strains that belong to *Pseudomonas* strain. Those bacteria had ability of degrading Phenanthrene (Coral and Serap, 2005).

In other research, Alquati et al. (2005) researched on Naphthalene degrading bacteria and succeeded in isolating 6 different bacterial population that had an ability of Naphthalene degradation. WU et al. (2003) succeeded in isolating 2 strains of biphenyl degradation bacterial, that belong to *Rhodococcus* strain. Shiraz Refinery with producing Refinery products of 2 million ton per years is an active company. Today pollution in its surrounding soils is one of the biggest problems for environment. Aim of this research is isolation and identification of degrading bacteria in Shiraz Refinery soil which can be used for bioremediation in this region.

MATERIALS AND METHODS

Culture media

Microbial cultures used in this research were nutrient agar, mineral salt agar (MM2).

Sampling

Sampling from Shiraz Refinery soil took place in two seasons, spring and summer of 2010. In each season 3 samples prepared from 5 stations. Samples collected in Microbial culture in sterile vessels and kept in contact with ice. They were then shifted to the laboratory for 3 h after sampling (Table 1).

Dilution

9 cc of physiology serum was added to 10 tubes, and then 1 g of sample soil was added to tubes containing physiology serum. 0.1 cc mixture of physiology serum and soil sample was cultivated on surface of nutrient agar plate and then incubated.

Isolation and identification of hydrocarbons degradation bacteria

After 24 h incubation of nutrient agar plates, bacterial colonies appeared on cultural surface, which were transferred to MM2 agar using Replica plating technique. MM2 agar was incubated for 24 h when colonies appeared on the surface of MM2 agar. Hydrocarbons sprayed on the surface of MM2 agar using spray plating technique. For this purpose, 0.4 g of each Camphor, Biphenyl, Phenanthrene, Naphthalene were dissolved in 20 cc acetone and 0.5 cc of each hydrocarbon sprayed on the surface of MM2 agar, plates were incubated for 7 days. After 7 days, only bacterial colonies which had abilities of degradation remained on the surface of the plates (Atlas and Richard, 1946).

Statistical analysis

Statistical analysis of results was done using chi-square test and analysis of variance (ANOVA) through SPSS software.

RESULTS

In this study, we isolated and identified 80 bacterial strains which belonged to 10 genus as follows: *Bacillus, Corynebacterium, Staphylococcus, Streptococcus, Shigella, Alcaligenes, Acinetobacter, Escherichia, Klebsiella* and *Enterobacter.* Results in this research indicates that percentage of hydrocarbon degrading G^+ bacterial, in summer season has been 82.5% which is compare to spring season (72.5%) show the most number of G^+ bacteria. Statistic analysis indicates that there is meaningful connection between sampling station and number of bacterial diversity in level of 0.05. Percentage of G^+ hydrocarbon degrading bacteria in spring season is 27.5% which is compare to summer showed 17.5% increase (Figure 1).

This research showed that, in spring season Phenanthrene had the most rate of degradation and in summer, Phenanthrene and Naphthalene with 30% showed the highest rate of degradation (Figure 2).

Degradation rate of hydrocarbons in two seasons of sampling; base on number of degradation bacteria to total number of isolation bacteria; showed that camphor with 34% degradation had the highest rate of degradation in both seasons and followed with Phenanthrene and Naphthalene with 30% of degradation (Figure 3). After isolation and identification of hydrocarbons degradation bacteria, the result showed that in spring, *Bacillus* with 60% of degradation was seen as the highest degrading hydrocarbon bacteria (Figure 4) followed by *E. coli* with 17.5%.

In summer, *Bacillus* was identified as the degrading bacteria that had the most frequency rate. In this season, as seen in Figure 5, diversity of bacteria is very few and *Bacillus* was the only dominated bacteria. In Figure 6, percentage of hydrocarbon degrading bacteria in both sampling seasons are shown, in which *Bacillus* with 69%

Table 1. Specification of sampling.

Station	Sampling location	Intensity of pollution	Geographic situation	
			Latitude eastern	Longitude northern
1	Refine industry unit	Very intense	29°11`	52° 20`
2	Bandval tank 2010	Intense	31° 51`	52° 40`
3	Refinery operation location	Medium	29° 50`	52° 47`
4	Bandval tank 2040	Little	30° 29`	52° 22`
5	Bandval tank 2039	Little	30° 29`	52° 22`

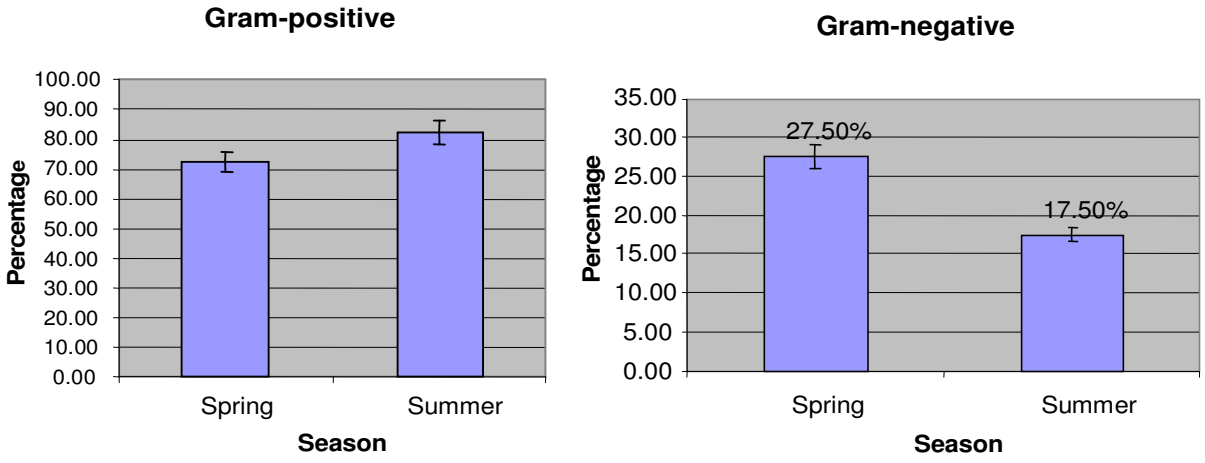

Figure 1. Percentage of Gr+ and Gr- bacteria.

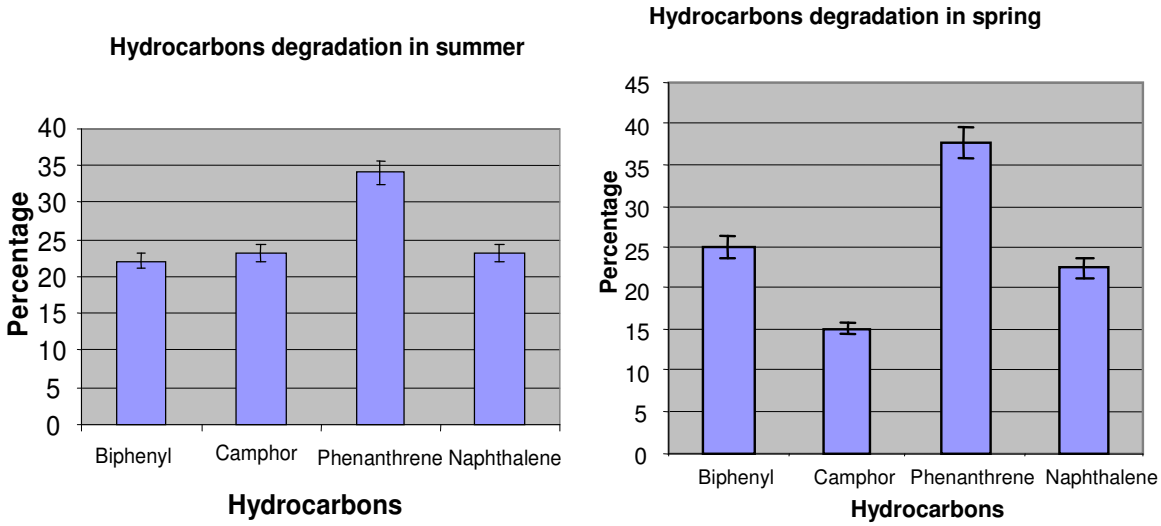

Figure 2. Percentage of degrading hydrocarbon.

degradation was detected to have the highest degrading rate. The results of the statistical analysis indicated that between the station and the number of bacteria diversity, there is a meaningful connection in the 0.05 level.

DISCUSSION

Many bacteria have the ability of biodegradation of oily pollutants (Prince and Lessard, 2003). These organisms

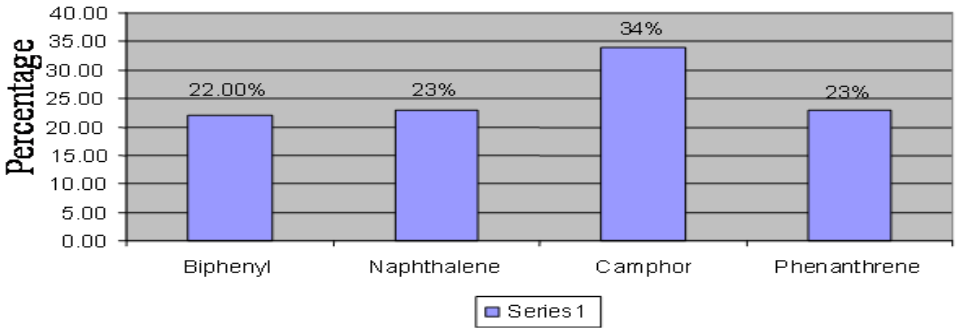

Figure 3. Percentage of degrading of hydrocarbon in two sampling season.

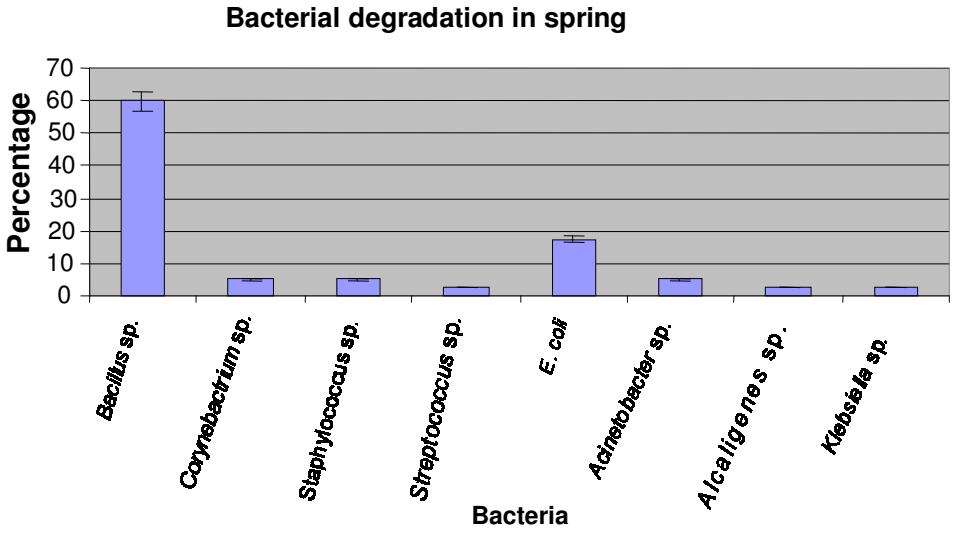

Figure 4. Percentage of degrading bacteria in spring.

are widely found in soil or watery ecosystem (Barathi and Vasudevan, 2001). Number and diversity of hydrocarbon degrading bacteria are affected by environmental, physical and chemical factors. Effective environmental factor on biodegradation could be: Temperature, oxygen, nutrients, pH and humidity (Leahy and Rita, 1990). Seasonal and climate changes are of the effective factors in changing the number and diversity of hydrocarbons degradation bacteria. In this research sampling season are spring and summer. Due to the moderate climate and rain observed in winter, the winter is an ideal condition for growth of all bacterial genus in spring. Summer due to dryness and severe heat condition for growth of bacterial has limitation.

In a similar research by Kim et al. (2005) in Korea Refinery the effect of pH, temperature and initial concentration of Phenanthrene on rate of biodegradation of Phenanthrene was evaluated. They found 30°C and 7 as the best temperature and optimum pH respectively. They also succeed in isolating and identifying some bacterial genus which could degrade the above mention hydrocarbons as follows: *Pseudomonas, Alcaligenes, Mycobacter, Rhodococcus.*

In this research performed in Shiraz Refinery we succeed in isolating and identifying 10 of bacterial strains which all had the ability of degradation of the mentioned hydrocarbons. In this research it was specified that in spring season there was much bacterial diversity in the soil and more G^+ bacterial were identified in this season in comparing to summer season. They belonged to bacterial genus as follows: *Escherichia, Acinetobacter, Alcaligenes, Klebsiella, Shigella, Enterobacter.*

In similar research made by Survery et al. (2000) on soil near different petrol pumps of Karachi, the following bacteria genera were isolated: *Staphylococcus, Corynebacterium, Bacillus, Proteus, Pseudomonas, Klebsiella, Escherichia,* while *Proteus* and *E. coli* had the highest rate of degradation (Survery et al., 2004). It is obvious that isolated bacteria in Survery research are the same as bacteria which were isolated in Shiraz Refinery

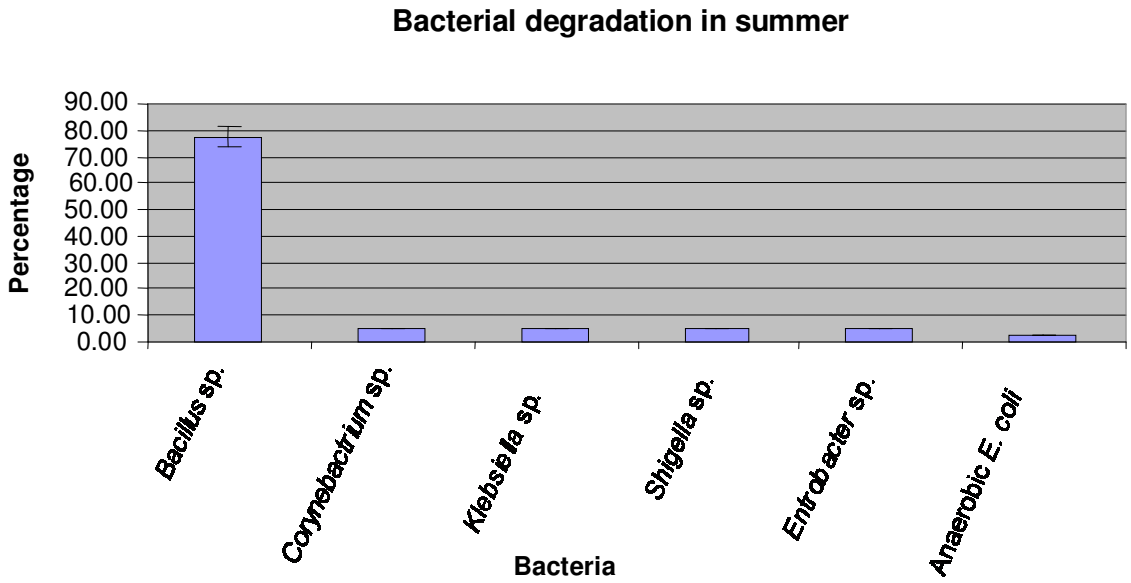

Figure 5. Percentage of degrading bacteria in summer.

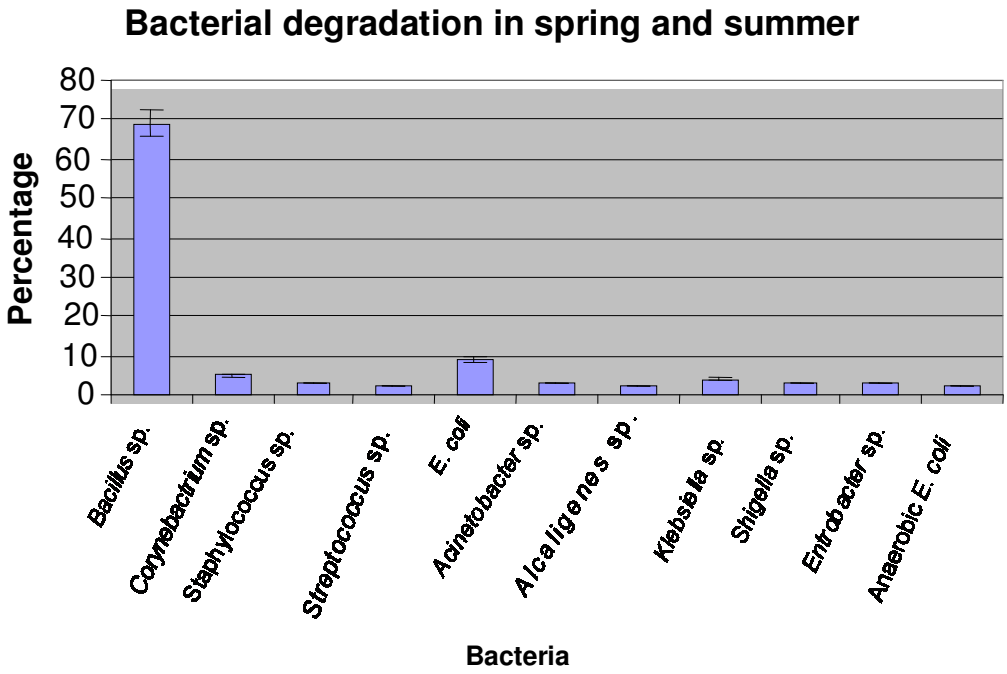

Figure 6. Percentage of degrading bacteria in both sampling season.

soil project. In Fars province, summer is hot and dry, hence Shiraz Refinery is under heat, dryness condition in summer season. In this condition diversity of bacteria is low and just a few bacterial genus hare the ability to grow in this situation. They would categorize under G^+ group including: *Bacillus, Corynebacterium, Staphylococcus* and *Streptococcus. Bacilli* with 77.5% degradation were the most dominated bacteria in this population. Having endospore they can easily live continually in difficult condition.

The existence of *Bacillus* with high percent in soil regions around refineries cause Iranian researcher to have made more research on this bacterial genus. Yosefi et al. (2009) studied on crude oil compound degrading bacteria. They isolated and identified native *Bacillus* in Tehran Refinery soil that had the ability of diesel fuels

degradation (Yousefi et al., 2009). Used hydrocarbons in this research would be under solid hydrocarbons group which belong to polycyclic aromatic hydrocarbons (PAHs). Among these hydrocarbons, Phenanthrene is one of the 16 PAHs listed as priority pollutants by US Environmental protection Agency (Coral and Serap, 2005).

Many researches have been done on Phenanthrene and Naphthalene in the world and scientists have succeeded in isolation and identification of bacterial genus which degrade hydrocarbons. Such could be pointed out to a research done by Coral (2005) that were succeeded in isolation of 50 strains which belonged to *Pseudomonas* (ARP). Also a joint Co-operation took place between Qatar and Egypt scientist that Alquati et al. (2005) performed this project and succeeded in identifying *Pseudomonas geniculate, Achromobacter xylosoxidase*. In our research, Phenanthrene in spring with 37.5% had the most rate of degradation. In summer both Phenanthrene and Naphtalene showed most rate of degradation (30%).

According to the result obtained in both seasons; on the basis of degradated number of bacterial to the total of isolated strain; it was detected that *Bacillus* with 69% was the best hydrocarbon degrading bacteria and Camphor with 34% showed the highest rate of degradation.

Conclusion

With regard to obtained results, it could be interpreted that Shiraz Refinery soil has low density of bacterial diversity especially in summer season. Shiraz Refinery soil especially around tanks of Refinery products is surrounded with lots of pollutants that have threat for plant and animal ecosystem. These pollutants would be transferred through water and Kor River which is next to Refinery and cause pollution of waters. According to the results of this research, it is possible to use this *Bacillus* as a native and dominated bacterial of this soil for bioremediation of region's soil and resolve the Refinery soil polluted problem.

REFERENCES

Aiquati C, Maddalena P, Canmela R, Sergio S, Giuseppina B (2005). Diversity of naphthalene–degrading bacteria from a petroleum contaminated soil. Ann. Microbiol., 55(4): 242-237.

Atlas MR, Richard B (1946) Microbiology ecology, fundamentals and application. Microbiol. Interaction Xenobiotic Inorganic Pollut., 13(511): 517-529.

Barathi S, Vasudevan N (2001). Utilization of petroleum hydrocarbons by *Pseudomonas fluorescens* isolation from petroleum contaminated soil. Environ. Int., 26: 413-416.

Brochures (2002). Public relations. Shiraz Oil Refining Company. Http://www.sorc.ir.

Coral G, Serap K (2005). Isolation and characterization of phenanthrene – degrading bacteria from a petroleum refiney soil. Ann. Microbiol., 5(4): 225-259.

Javid H (2005). Isolation and identification of halophyl microorganisms from Lake Bakhtegan and their impact on bioremediation. M.Sc. Thesis, Islamic Azad University Jahrom, Jahrom, Iran, pp. 1-17.

Khosravi M (2007). Petrochemical, refinery products and refined techniques, 1386. Tehran University, Institute of Publishing and Printing, pp. 1-17.

Kim J.D, Su-Hyeun S, Choul G (2005). Degradation of phenanthrene by bacterial strains isolated from soil in oil refinery fields in Korea. J. Microbial., Biotechnol., 15(2): 337-345.

Leahy GJ, Rita RC (1990). Microbial degradation of hydrocarbons in the Environment, Microbiol. Rev., pp. 305-315.

Maola D, Taher A, Elham E (2002). Review of the feasibility of inoculation *situ* biological therapy techniques to reduce pollution at the Shiraz refinery lands using Taguchi Method. University of Chemical Engineering, Oil and Gas, Shiraz University, pp. 637-627.

Marinescu M, Dumiru M, Anca L (2009). Biodegradation of petroleum hydrocarbons in artifical polluted soil. Res. J. Agric. Sci., 41(2): 157-162.

McMurry J (2000). In Aromatic hydrocarbons. Organic Chmistry.5th ed brooks/cole: Thomas learning. New York, pp. 120-180.

Prince RC, Lessard R (2003). Oil and Gas Science and Technology-re IFP, 58(4): 463-468.

Seo J, Young SK, Qing XL (2003). Bacterial Degradation and Bioremediation of polycyclic Aromatic hydrocarbons. Pol. J. Environ. Stud., 12(1): 15-25.

Survery S, Samia A, Syed AA (2004). Hydrocarbon Degrading Bacteria from Pakestani soil: Isolation, Identification, screening and Genetical Studies. Pak. J. Biol. Sci., 7(9): 1518–1522.

Wu G, Fery S, Boyed A (2003). Characterization of Bacteria capable of degrading soil–sorbed biphenyl. Bull. Environ. Contam. Toxicol., 71: 768-775.

Yousefi K, Khodadadi A, Ganjidust H, Badkoubi A, Amoozegar M (2009). Isolation and characterization of a natine novel *Bacillus* strain capable of degrading diesel fuel. Int. Environ. Sci. Technol., pp. 435-442.