Study Role of Bacterial Consortium *Rhodococcus* and *Gordona* in Clean up Contaminated Soil with Naphthalene and Anthracene in the Natural Environment

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Abstract: Carcinogenic polycyclic aromatic compounds are common and dangerous pollutants of water, air, and soil. Bioremediation is a major solution for decomposing these pollutants, inexpensively and efficiently without any harmful environmental side effects. This study aims to examine the bioremediation of the polycyclic aromatic compounds naphthalene and anthracene through a consortium of Gordona and Rhodococcus bacteria, the bacteria previously isolated at Iran Industrial Microorganism Collection Center. In the first stage, the ability of the bacteria consortium to eliminate naphthalene and anthracene (100 ppm in the liquid phase in the Erlenmeyer flask) was studied. In the second stage of the study, the effects of temperature and pH on liquid phase elimination of the pollutants were examined. In the third stage, a bioreactor was designed for bioremediation of the existing naphthalene and anthracene in the oil-polluted soil in Isfahan Refinery using the studied bacterial consortium. To provide suitable conditions for bioremediation, we adjusted the three parameters of air flowrate, moisture, and mixing of materials inside the bioreactor. In the last stage, the naphthalene and anthracene elimination levels in the polluted soil at the designed bioreactor in Isfahan Refinery were measured. This stage of bioexcitation took 120 days, during which bioaugmentation and bioventilation techniques were implemented. The obtained results showed that the maximum elimination of naphthalene in the liquid phase (96% reduction in the chromatograph peak) occurred within 60 days under the following conditions: temperature=30C, pH=7.0 and shaker rpm=150. The reduction of anthracene obtained under similar conditions was 83%. The peak naphthalene and anthracene concentrations in the 0.9 L/min bioreactor within a 120 day period were reduced by 100% and 89% respectively.
[Shadi Shahrokhi Moghaddam, Abbas Akhavan Sepahi. **Study Role of Bacterial Consortium Rhodococcus and Gordona in Clean up Contaminated Soil with Naphthalene and Anthracene in the Natural Environment.** *Nat Sci* 2016;14(6):79-83]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 10. doi:10.7537/marsnsj14061610.

Keywords: Rhodococcus, Gordona, Clean up contaminated soil with naphthalene and anthracene

1. Introduction

One of the biggest problems raised in today's world, pollution is due to the oil pollution in different ways (leakage of oil caches, pipelines, etc.) cause pollution of soil, water and the air. Biological treatment is a suitable method to clean up petroleum hydrocarbons; because of easy maintenance, applicable in wide areas, reasonable prices and perfect will lead to a deterioration of pollutants another advantage of this method does not produce waste and byproducts. If contamination is biodegradation microorganisms as a carbon source or electron acceptor or both that this process may be aerobic or anaerobic. When fully bio-degradable done by oxidation of pollutants combine carbon dioxide and water remain, a process called mineralization (Danielle, 2006). Our bio-stimulation method in this study to get a better result, we have increased biological and biological conditioning. The aim of this study naphthalene and anthracene biological therapy polycyclic aromatic compounds by bacteria Rhodococcus and Gordona been Consortium, the bacteria were taken from the collection center of Iran's industrial microorganisms.

Analysis method:

Bacteria Rhodococcus and Gordona were prepared from the collection of industrial microorganisms in Iran. The growth of these bacteria, turbidity standard was half McFarland. The medium used to remove naphthalene and anthracene medium has been MSM. Available in medium and concentration of each material was shown in Table 1.

Table 1: culture medium used to remove anthracene in the liquid phase in the flask

Substance	Concentration (g/L)
Ammonium sulfate	0.5
Potassium hydrogen phosphate	0.5
Potassium chloride	0.2
Calcium chloride	0.1
Magnesium sulfate	0.1

Due to the fact that the test is more appropriate in a batch process, batch mode large numbers of tests were carried out. The process of optimizing the

number of parameters such as the amount of aeration, mixing rate, temperature and pH have an important role in the analysis of biological were studied. General description of the flask batch experiments that were conducted, as follows: The optimum temperature and pH was growing. Then strain of bacteria to adapt naphthalene and anthracene as the sole source of carbon and energy was growing in optimal conditions, naphthalene and anthracene after the process of elimination in the liquid phase in the flask were discussed.

Obtain optimum temperature for growth

To find the optimum temperature, a series of independent experiments naphthalene and anthracene at a concentration of 100 ppm at temperatures ranging between 15-40 degrees Celsius and pH was seven. For this study of 500 ml Erlenmeyer flask, containing 200 ml of mineral medium autoclave was used. Naphthalene and anthracene good after sterilization due to the toxic mineral medium were cultured under sterile conditions. Erlenmeyer flask with 10 ml (10%) was inoculated bacterial suspension in sterile conditions and in a shaker incubated 160 rpm and temperature was different. During the growing season 600 OD culture media inoculated against non-inoculated culture medium (control) was called a spectrophotometer.

Obtaining pH growth optimal

To obtain optimum pH bacteria cultured in flasks of 500 ml initial concentration of 100 ppm naphthalene and anthracene growth at optimum temperature (30 °C) with different pH (5-6-7-8-9) was carried out. To this end mineral medium pH using hydrochloric acid or caustic soda, and the autoclave was set to the required value and add naphthalene and anthracene bacterial suspension in sterile conditions, were placed in an incubator shaker. The bacterial growth in each pH was compared by measuring 600 OD.

Design of Experiments in the liquid phase in the flask

In order to assess the ability of the consortium to remove bacteria Rhodococcus and Gordona anthracene and naphthalene in the liquid phase inside the flask, initially naphthalene and anthracene were dissolved in acetone. Then the necessary amount of liquid in the flask of 500 mL final concentration of naphthalene and anthracene spilled so that each is 100 ppm. After evaporation of the solvent under the hood, in each of the flasks were poured 200 ml mineral medium and, finally, after sterilization, the flasks are prepared inoculants before the inoculation bacterial suspension amounting to 10% was considered. Prepared flask with cotton and foil and incubated at 30°C in a shaking away 150 rpm were killed. It is worth noting that all tests in order to check the repeatability and also reduce errors, with a repeater were done.

Bioreactor design and calculations for the necessary aeration, adjusting the humidity and the mixing in bioreactor

In order to design a pilot bioreactor, the bioreactor volumes of 5 liters are optional, and we do design calculations on it. To provide living conditions, sources of energy supply and to the needs of carbon and oxygen is vital (Akhavan Sepahi, 2009). In this phase of the research naphthalene and anthracene calculations for the design of bioreactors for biodegradation in contaminated soil by a consortium of oil refineries bacteria Rhodococcus and Gordona was done. Due to the size and type of pollutants bioreactor to establish the amount of oxygen required to decompose the computational aromatic compounds was performed and the air pump to deliver oxygen to be used for analysis, moisture analysis process was controlled. As well as for mixing material inside the bioreactor and speed up the biodegradation process conditions to bringing oxygen and pollutants properly naphthalene and anthracene degrading bacteria are provided.

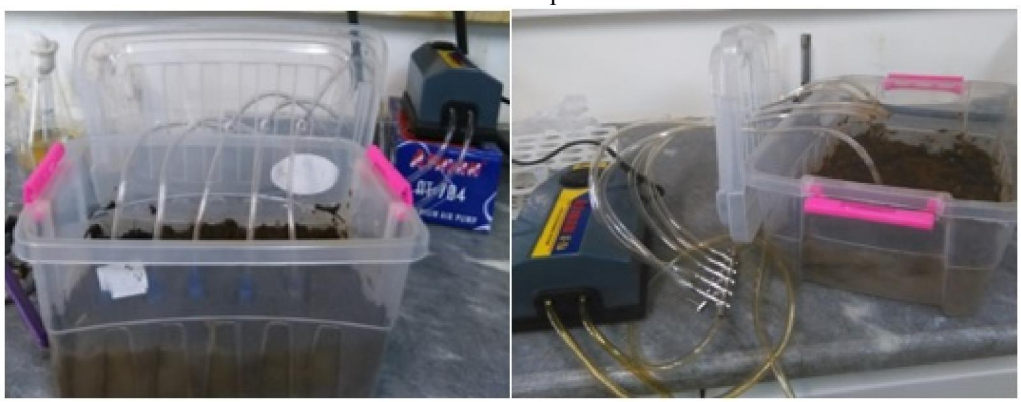


Figure 1: Bioreactor image

Table 2: Bioreactor physical specifications

Height of soil in the reactor	5 cm
Weight of soil in the reactor	2 kg
Length of Bioreactor	26 cm
Width of Bioreactor	14 cm
Height of Bioreactor	12 cm

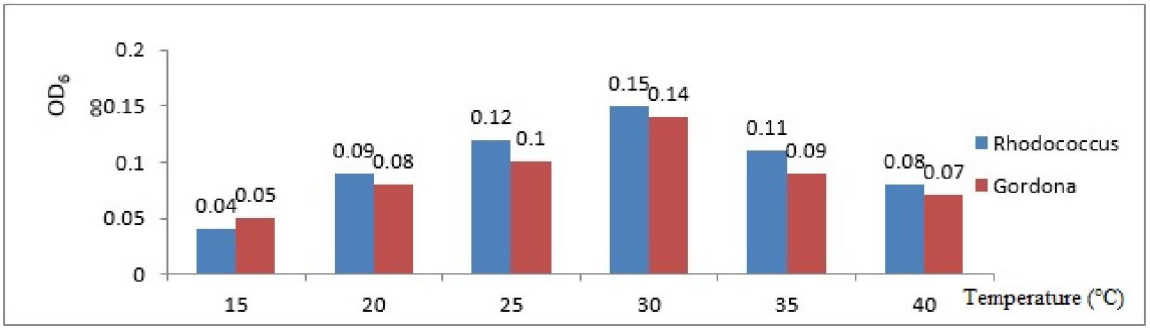
Working conditions of designed bioreactor

This will be the first bioreactor bioreactor working conditions in order to be sterilized in an autoclave for 20 minutes at 121°C And then 2 kg of refined petroleum contaminated soil was removed from the bioreactor was shed, the mineral base culture of the substances contained in it and their concentration in Table 1 are given, taken and were cast into the bioreactor, finally, after cooling and humidity adjustment of pH, and temperature optimum, bioreactors by bacteria Rhodococcus and Gordona flask containing inoculant was prepared previously, was inoculated in the vicinity of the flame.

Findings

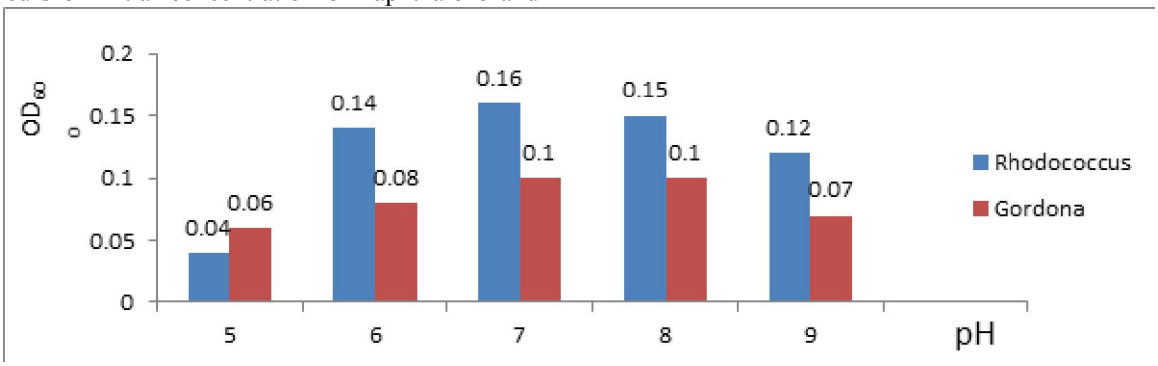
Determine conditions for optimal growth temperature of bacteria

In Chart 1, bacteria Rhodococcus and Gordona at different temperatures between 15-40°C after 24 hours is shown. As the figure suggests, this bacteria can grow at temperatures ranging from 25-35 degrees centigrade. The optimum growth temperature of 30 °C was observed.



Determining the optimal growth conditions for bacteria in medium pH

In the diagram (2) the growth of bacteria Rhodococcus and Gordona in different pH of 9-5 after 24 hours of initial concentration of naphthalene and anthracene were 100 ppm and optimal growth temperature of 30°C is shown. According to the chart, pH range tolerated by the bacteria is 6-9. The optimal pH of 7 was set.



Results of air flow to the pump inlet in the bioreactor designed to speed up the process of biodegradation of naphthalene and anthracene by a consortium of soil contaminated with bacteria Rhodococcus and Gordona:

According to the investigation and the information contained in the research and development of refineries in the amount of 19.7 mg per kilogram of soil contaminated with oil is naphthalene. And because we are biodegradable to clean up soil contaminated with naphthalene and anthracene 2kg we have examined, the amount of naphthalene in the soil of the study is:

×19.7 2 = 39.4 mgr naphthalene

As a result, 39.4 mg of naphthalene in two kilograms of contaminated soil have studied the equivalent of 0.151 grams of oxygen. (Density of oxygen in the ambient temperature is 0.00133 grams per cubic centimeter):

$\rho_{o2} = 0.00133 \text{ gr} / \text{cm}^3$

0.151 gr o_2 / 0.00133 gr/cm^3 = 113.7 cm^3O_2

Including 21% by volume of oxygen in the air (113.7 divided by 0.21), total amount of air required is

obtained. The total amount of air required for aeration in the bioreactor designed:

113.5 cm^3O_2 / 0.21 = 541.5 cm^3 air

541.5 cm^3air / 1000 cm^3/lit = 0.540 lit air

Also per kilogram of soil contaminated with oil refinery is anthracene amount of 26.6 mg, and because we are bio-degradable cleaning up the 2 kg of soil contaminated with anthracene we have examined, the amount of anthracene studied soil is:

2× 26.6 = 53.2 mgr antheracene

As a result, 53.2 mg kg of anthracene in soil that is equivalent to 0.27 grams of oxygen. (Density of oxygen in the ambient temperature is 0.00133 grams per cubic centimeter):

ρ_{o2} = 0.00133 gr / cm^3

0.27 gro_2 / 0.00133 gr /cm^3 = 203 cm^3O_2

Including 21% by volume of oxygen in the air (203 divided by 0.21) cubic centimeters of air required is calculated equal to 0.26 liters of air.

203 cm^3O_2 / 0.21 = 966.6 cm^3air

966.6 cm^3/ 1000cm^3/lit = 0.966 lit air

Results of soil moisture content in the reactor design

Measuring the moisture in the soil contains water and the medium was measured every 10 days. The results were accepted in order to have the desired moisture content, therefore with regular 100 ml of distilled water (2 times per week) in the bioreactor contaminated soil moisture needed to grow soil microorganisms and biological degradation by microorganisms in the microbial treatment for the act has been provided. Table 3 shows the results of these experiments. The results of the measurement of moisture content during operation indicate that there is no significant change in the range of 50-40 percent and average moisture content of the columns chamber, according to studies (Juwarkar, 2010) is an acceptable amount to biodegradation.

Table 3: Average moisture in the soil inside the designed bioreactor

Date of sampling	1	10	20	30	40	50	60
Amount of humidity (%)	36	40	48	57	50	50	54

Naphthalene and anthracene analysis to compare the results of analysis of soil contaminated with oil in the liquid phase in the reactor flask lab and designed by means of GC-MS

According to the results of the biodegradation of aromatic compounds naphthalene and anthracene as pollutants by the consortium bacteria Rhodococcus and Gordona flask and soil contaminated with oil refinery in the liquid phase of the designed reactor, according to Table 4 and Chart 3, it can be concluded that analysis of soil contaminated with oil and the reactor is designed after 120 days from the decomposition in the liquid phase in a laboratory flask was after 60 days. The reason for this was the existence of native bacteria found in soil contaminated with oil described.

Table 4: results of studies in the laboratory and in the liquid phase flask in designed bioreactor

Biodegradation time (days)					
Naphthalene and anthracene degradation rate of the liquid in laboratory flask after 60 days	Naphthalene 46% Anthracene 40%	Naphthalene 77% Anthracene 68%	Naphthalene 86% Anthracene 79%	Naphthalene 90% Anthracene 93%	
Naphthalene and anthracene oil contaminated soil decomposition rate at 120 days				Naphthalene 83.4% Anthracene 47.5%	Naphthalene 100% Anthracene 89%

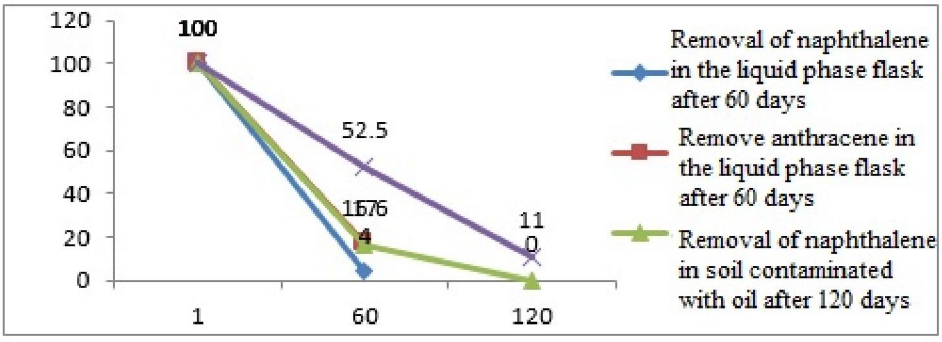


Figure 4: Comparison of naphthalene and anthracene decline in the liquid phase and contaminated soil flask of oil refinery

Discussion and conclusion:

We naphthalene and anthracene biological research polycyclic aromatic combination therapy by a consortium of two bacteria Rhodococcus and Gordona was taken to the collection center of Iran's industrial microorganisms were checked. The concentration of polycyclic aromatic hydrocarbons in soil contaminated with oil refineries and even several times a lot of international standards (0.5 mg kg) were reported.

The results of this study are that using bacteria consortium studied petroleum-contaminated soil can contain polycyclic aromatic hydrocarbons and biological methods with acceptable results eliminated. Naphthalene percent as contaminants in the liquid phase flask after 60 days in the laboratory and in optimum conditions of pH and temperature was 96% and the reduction of anthracene with the same conditions close to 10% higher. In the process of biodegradation in soil contaminated with oil refinery in the bioreactor, air flow rate of 0.9 liters per minute was calculated according to the type of pollutants and continuously aerated soil contaminated by air pump was 120 days. Moreover, humidity is controlled between 40 and 50%. The results of the analysis in this case has been that of naphthalene after 120 days in the bioreactor designed completely removed in the same conditions anthracene 89% peak intensity was low. In research conducted by researchers in the past can be concluded that the consortium of bacteria Rhodococcus and Gordona can be acceptable breaks polycyclic hydrocarbons live. Amini et al., in a study in 1391 that the oil contaminated soil samples around Isfahan refinery, bacteria and bio-degradable was identified nocardia, anthracene bacteria with a concentration of 50 mg per liter decreased 36.6% after 9 days.

Mehrasbi et al. in 2005 in a study of aromatic hydrocarbons in contaminated soil was removed, after 4 months of naphthalene nearly 80 percent. Similar results were reported in 2003 by the Kalantari et al. that soil samples contaminated with oil refinery in Tehran after 70 days of biodegradation, naphthalene at a concentration of 0.65 mg completely removed and anthracene fell 87.6% in the same condition.

Acknowledgements

I appreciate cooperation and compassionate assistance of Dr. Abbas Akhavan Sepahi and also my father and mother.

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4/19/2016