

Comparative kinetic study of different bioremediation processes for soil contaminated with petroleum hydrocarbons

LAILA A. FARAHAT and NOUR Sh. EL-GENDY*

Egyptian Petroleum Research Institute, Nasr City, Cairo (Egypt)

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ABSTRACT

Bioremediation of hydrocarbon polluted soil can be achieved by natural attenuation, biostimulation and/or bioaugmentation processes. In this study the three technologies were evaluated to treat hydrocarbons polluted soil of total petroleum hydrocarbon (TPH) content of 42,000mg/kg semi-pilot scale cells, over an incubation period of 120 days at room temperature (25-30°C), moisture content of 45% and pH around neutrality. Bioaugmentation with bacterial consortium *Pseudomonas aeruginosa* I.1.1.6 and *Brevibacterium casei* I.2.1.7 showed the highest degradation potential (76%), followed by biostimulation process with biodegradation efficiency of (62%) and then come the natural attenuation (48%). Kinetic modeling was performed to estimate the rates of TPH biodegradation in the studied systems. Three different error functions (root mean square, sum of the absolute errors and average relative error) were employed in this study to evaluate the goodness of fit of the model equation to the obtained experimental data. This showed that the degradation was found to follow first order model. The highest rate constant (0.012 day^{-1}) was observed in cell augmented with bacterial consortium I.1.1.6 and I.2.1.7, followed by biostimulation cell (0.008 day^{-1}). The lowest rate constant was observed in natural attenuation cell (0.005 day^{-1}). Accumulative evaluation of CO_2 was good qualitative indicator of biodegradation activity in each cell. The CO_2 formations in bioaugmented cells were relatively higher than those in natural attenuation and biostimulation cells.

Keywords: Biostimulation, Bioaugmentation, Natural attenuation, Petroleum hydrocarbons, soil, kinetic modeling.

INTRODUCTION

The development of petroleum industries into new frontiers, the apparent inevitable spillage, which usually occur during routine operations and records of acute accidents during transportations, has called for more studies into oil pollution problems (Okoh, 2003).

Remediation of polluted systems could be achieved by physical, chemical or biological methods. However, the attendant negative

consequences of the physicochemical methods make bioremediation more attractive and as one of the most successful technology for clean up contaminated sites.

Bioremediation use mainly three strategies (Kaplan and Kitts, 2004); natural attenuation, biostimulation and bioaugmentation. The simplest method of bioremediation to implement is natural attenuation, where contaminated sites are only monitored for contaminant concentration to assure regulators that natural processes of contaminant degradation are active. Biostimulation requires adjustments to contaminated soil in order to provide bacterial communities with a favorable environment in which they can effectively degrade contaminants. This includes the addition of nutrients, adjustment

*Corresponding author:
Dr. Nour Sh. El-Gendy
Tel.: (202) 2 274-7917; Fax: (202) 2 274-7433
E-mail: dr.nour@mailcity.com

of pH and moisture content while also making appropriate adjustments for the proliferation of indigenous microorganisms, hence speeding up the bioremediation process. In case where natural communities of degrading bacteria are present in low numbers or even absent, bioaugmentation i.e., the addition of contaminants-degrading microorganisms, can speed up the degradation process.

We have isolated several candidate bacterial strains (El-Gendy, 2006, Farahat et al, 2006 and Rafaat et al, 2007) in our effort in developing an active bacteria consortium that could be of relevance in the bioremediation of crude oil contaminated systems in Egypt. Three of these bacterial isolates, *Pseudomonas synxantha* I.1.1.1, *Pseudomonas aeruginosa* I.1.1.6 and *Brevibacterium casei* I.2.1.7 have been reported to have tremendous potentials for biodegradation of petroleum hydrocarbons (Farahat et al, 2006 and Farahat and El-Gendy, 2007).

This paper reports a comparative kinetic study was performed to provide necessary information for the possible bioremediation of oil polluted soil using these three bacterial isolates compared to biostimulation and natural attenuation processes in a semi-pilot scale.

MATERIAL AND METHODS

Polluted soil

Oil polluted soil of total petroleum hydrocarbon content of (42,000mg/kg) was collected from a drilling site of an oil field in Suez Gulf, Egypt.

Bacterial strains

Pseudomonas synxantha I.1.1.1, *Pseudomonas aeruginosa* I.1.1.6 and *Brevibacterium casei* I.2.1.7 previously isolated from hydrocarbon polluted soil were used in this study. Luria Bertani (LB) medium (Kirimura et al., 2001) was used to obtain biomass for augmenting the polluted soil.

Bioremediation treatments

The remediation study took place over a period of four months at room temperature (25-30°C), pH around neutrality and moisture content of the soil was about 45%. The three biotreatment processes are illustrated as follows:

Biostimulation

10 kg of oil polluted soil were placed in plexi-glass cell (cell 1) of dimensions (60 X 60 X 20 cm) supplied with an air sparger connected to an air compressor of capacity (200-300 L/min), as illustrated in Fig 1. The cell was incubated at room

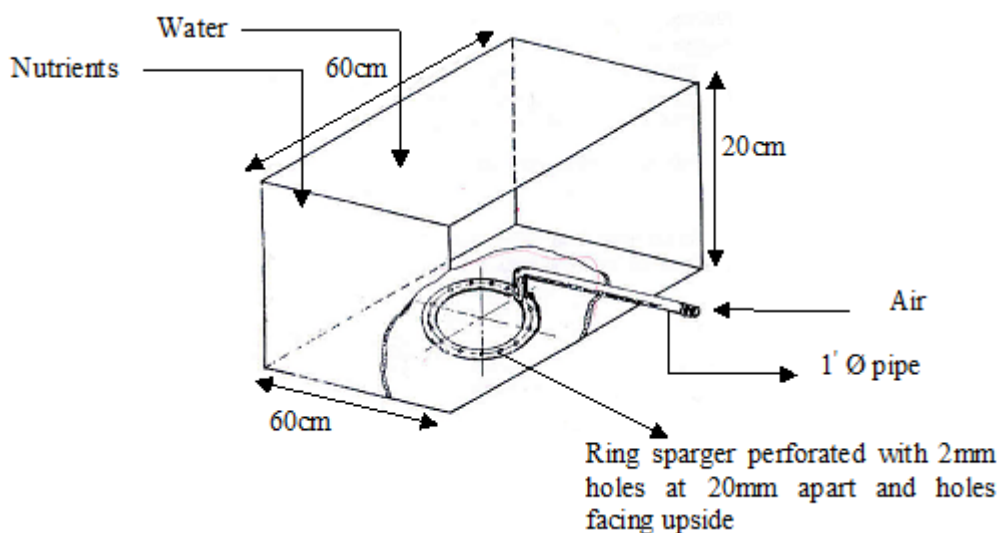


Fig. 1: Bioremediation cell

temperature (25-30°C) in a green house. At zero time and after 20 days of incubation the following were added:

1. Nutrients: NH_4Cl and K_2HPO_4 were added to keep the ratio of C:N:P (100:10:1).
2. Co-Carbon source: Molasses (2.5g/kg soil).
3. Surfactant: Tween 80 (30 μL in 20mL distilled water).

Periodical tilling and addition of water were done day after day to ensure good aeration and to keep the moisture content around 45% in addition to assuring good dispersal of microbial population, nutrients and surfactants.

Bioaugmentation

This treatment was equivalent to biostimulation treatment with the exception of adding bacterial isolates in the following criteria:

Cell 2 : Bioaugmented with bacterial isolate, *Pseudomonas aeruginosa* I.1.1.6.

Cell 3 : Bioaugmented with bacterial consortium, *Pseudomonas synxantha* I.1.1.1 and *Pseudomonas aeruginosa* I.1.1.6.

Cell 4 : Bioaugmented with bacterial consortium, *Pseudomonas aeruginosa* I.1.1.6 and *Brevibacterium casei* I.2.1.7.

Cell 5 : Bioaugmented with bacterial consortium, *Pseudomonas synxantha* I.1.1.1, *Pseudomonas aeruginosa* I.1.1.6 and *Brevibacterium casei* I.2.1.7.

Natural attenuation

A plexi-glass cell (cell 6) containing oil polluted soil without addition of nutrients or bacteria.

Periodical tilling and addition of water were done day after day to ensure good aeration and to keep the moisture content around 45%.

Monitoring of bioremediation

TPH concentration

Gravimetric determination of TPH concentration was done according to the method described by Viguri *et al.*, (2002).

$$\ln C = -K_1 t + A$$

CO₂ evaluation

Accumulative production of CO₂ as a measurement of soil heterotrophic activity was done according to the method described by Isermeyer (1952).

Kinetic modeling

This was preformed to estimate the rates of TPH biodegradation in the studied systems. Kinetics of reaction can be described in terms of its order (Sarkar *et al.*, 2005).

First order kinetic model:

$$\ln C = -K_1 t + A \quad \dots(1)$$

Second order kinetic model:

$$\frac{1}{C} = K_2 t + B \quad \dots(2)$$

where C is the TPH concentration (mg/kg), t expresses time, K₁ and K₂ are the first and second order rate constants, respectively where A and B are constants.

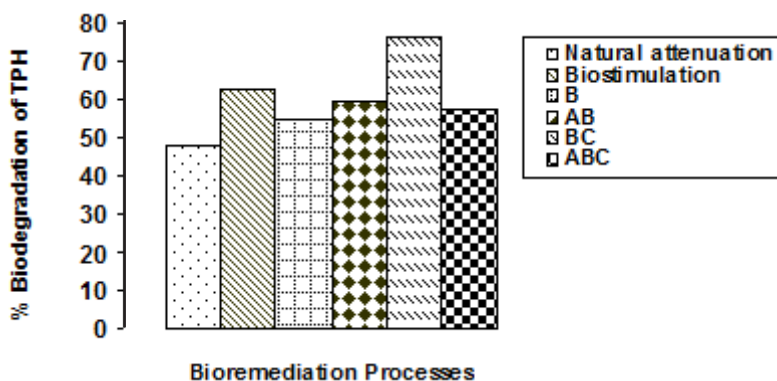


Fig. 2: Biodegradation percentage of TPH in each cell during the biotreatment process

RESULTS AND DISCUSSION

TPH removal

The most direct way to measure bioremediation efficiency is to monitor hydrocarbon disappearance rates (Margesin and Shinner, 2001).

Fig. 2, shows the biodegradation percentage at the end of incubation period of different biotreatment processes in all cells. Biotreatment processes showed different degrees in biodegradation efficiencies.

Natural attenuation cell showed the lowest biodegradation efficiency of $\approx 48\%$, which indicates that the indigenous population can biodegrade the petroleum hydrocarbons in the polluted soil. The highest biodegradation efficiency $\approx 76\%$ was observed in cell (4), bioaugmented with bacterial consortium, *Pseudomonas aeruginosa* I.1.1.6 and *Brevibacterium casei* I.2.1.7. The biostimulation cell (1) showed biodegradation efficiency of $\approx 62\%$, followed by cell (3) bioaugmented with bacterial consortium *Pseudomonas synxantha* I.1.1.1 and I.1.1.6, where biodegradation reached $\approx 60\%$. While cell (2) bioaugmented with I.1.1.6 and cell (5) bioaugmented with bacterial consortium I.1.1.1, I.1.1.6 and I.2.1.7 showed nearly the same biodegradation efficiency of 55% and 57%, respectively.

In this study, bioaugmentation with different bacterial consortia produced a significant

impact on the removal of TPH. The biodegradation efficiencies in cells augmented with bacterial consortia were better than that augmented with individual bacterial isolate in the following order cell 4 > cell 3 > cell 5 $\approx$ cell 2.

The advantage of employing mixed cultures as opposed to pure cultures in bioremediation has also been widely demonstrated (Ghazali *et al.*, 2004). It could be attributed to the effects of synergistic interactions among members of the association. Mechanisms through which bacteria benefit from synergistic interactions are complex. It is possible that one species removes the toxic metabolites (that otherwise may hinder microbial activities) of the species preceding it. It is also possible that the second species are able to degrade compounds that the first are not able to degrade or partially degrade them (Alexander, 1999).

Degradation rates of TPH

Kinetic modeling was performed to estimate the biodegradation rates in the studied systems.

To determine the order of the reactions in each of the soil treatments, the data were plotted in a scatter diagram. Fig. 3 and 4 represent the data plots of first order and second order kinetics models, respectively. All the parameters obtained for the two models are presented in Tables 1 and 2, respectively.

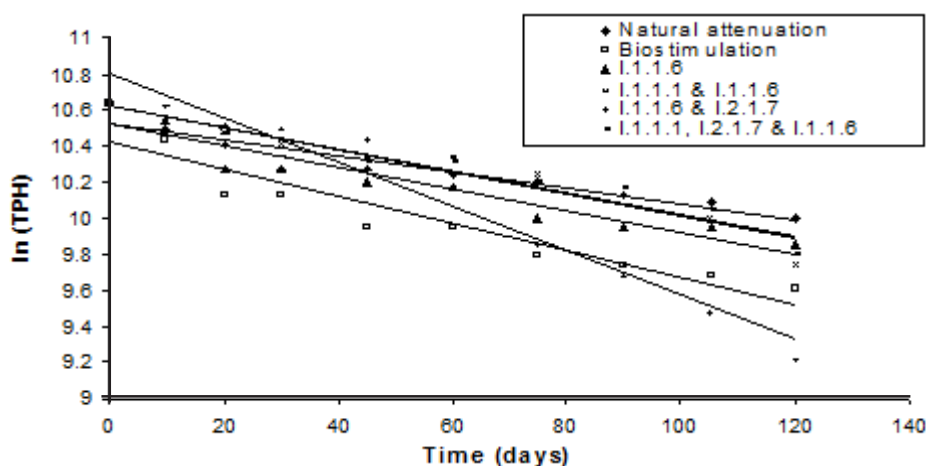


Fig. 3: Plots of first order kinetics of TPH degradation

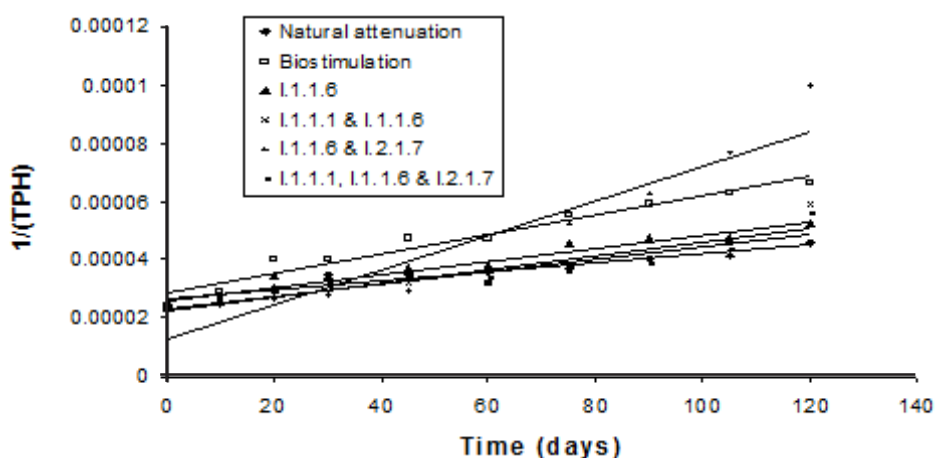


Fig. 4: Plots of second order kinetics of total petroleum hydrocarbon degradation

Table 1: The first order parameters for the degradation of TPH during different bioremediation processes

Bioremediation processes		$K_1(\text{day}^{-1})$	$t_{1/2}(\text{day})$	Correlation coefficient(R)
Cell 1	Biostimulation	0.008	91.20	0.940
Cell 2	I.1.1.6	0.006	115.52	0.906
Cell 3	I.1.1.1.&I.1.1.6	0.006	111.80	0.964
Cell 4	I.1.1.6&I.2.1.7	0.012	56.35	0.967
Cell 5	I.1.1.1.I.1.1.6&I.2.1.7	0.006	115.52	0.964
Cell 6	Natural attenuation	0.005	154.03	0.904

 $t_{1/2}$ the half life of TPH biodegradation

Table 2: The second order parameters for the degradation of TPH during different bioremediation processes

Bioremediation processes		$K_2(\text{day}^{-1})$	Correlation co efficient(R)
Cell 1	Biostimulation	3×10^{-7}	0.977
Cell 2	I.1.1.6	2×10^{-7}	0.974
Cell 3	I.1.1.1. & I.1.1.6	2×10^{-7}	0.929
Cell 4	I.1.1.6 & I.2.1.7	6×10^{-7}	0.932
Cell 5	I.1.1.1 I.1.1.6&I.2.1.7	2×10^{-7}	0.944
Cell 6	Natural attenuation	2×10^{-7}	0.970

The R value represents the correlation coefficient of the data, the nearer the value of R to 1, the stronger the correlation of the data (Everitt, 2002). The obtained R values for the plots in all the studied systems were in the range 0.904-0.967 for first order and 0.929-0.977 for second order models. A relatively high and close R values indicated that both models successfully described the kinetics of the degradation of petroleum hydrocarbon components.

Error analysis for the studied kinetics

Since both first order and second order rate equations gave close R values, error analysis were necessary to differentiate between the two

models. Three different error functions were used to determine how well models represent the experimental data. The error functions employed were as follows:

- 1 Root mean square, RMS (Arslanoglu *et al.*, 2005):

$$RMS = (\sum ((C_{exp} - C_{cal}) / C_{exp})^2 / N)^{1/2} \quad \dots(3)$$

- 2 Sum of the absolute errors, EABS (Perez-Marin *et al.*, 2007):

$$EABS = \sum_{i=1}^N (C_{exp} - C_{cal}) \quad \dots(4)$$

- 3 Average relative error, ARE (Han *et al.*, 2007):

Table 3: Values of three different error analysis functions of kinetic models for degradation of TPH during different bioremediation processes

Bioremediation processes	RMS	EABS	ARE
Cell 1			
Biostimulation			
First order	0.10596	2582.22	0.012
Second order	0.09012	5119.83	0.095
Cell 2			
I.1.1.6			
First order	0.07615	1170.16	0.080
Second order	0.09634	21894.67	0.101
Cell 3			
I.1.1.1 & I.1.1.6			
First order	0.06857	136.14	0.072
Second order	0.1735	42620.3	0.183
Cell 4			
I.1.1.6 & I.2.1.7			
First order	0.12018	80.51	0.127
Second order	0.4918	73135.6	0.518
Cell 5			
I.1.1.1, I.1.1.6 & I.2.1.7			
First order	0.06528	540.51	0.069
Second order	0.1557	40320.3	0.164
Cell 6			
Natural attenuation			
First order	0.05725	1044.36	0.060
Second order	0.15067	42594.67	0.159

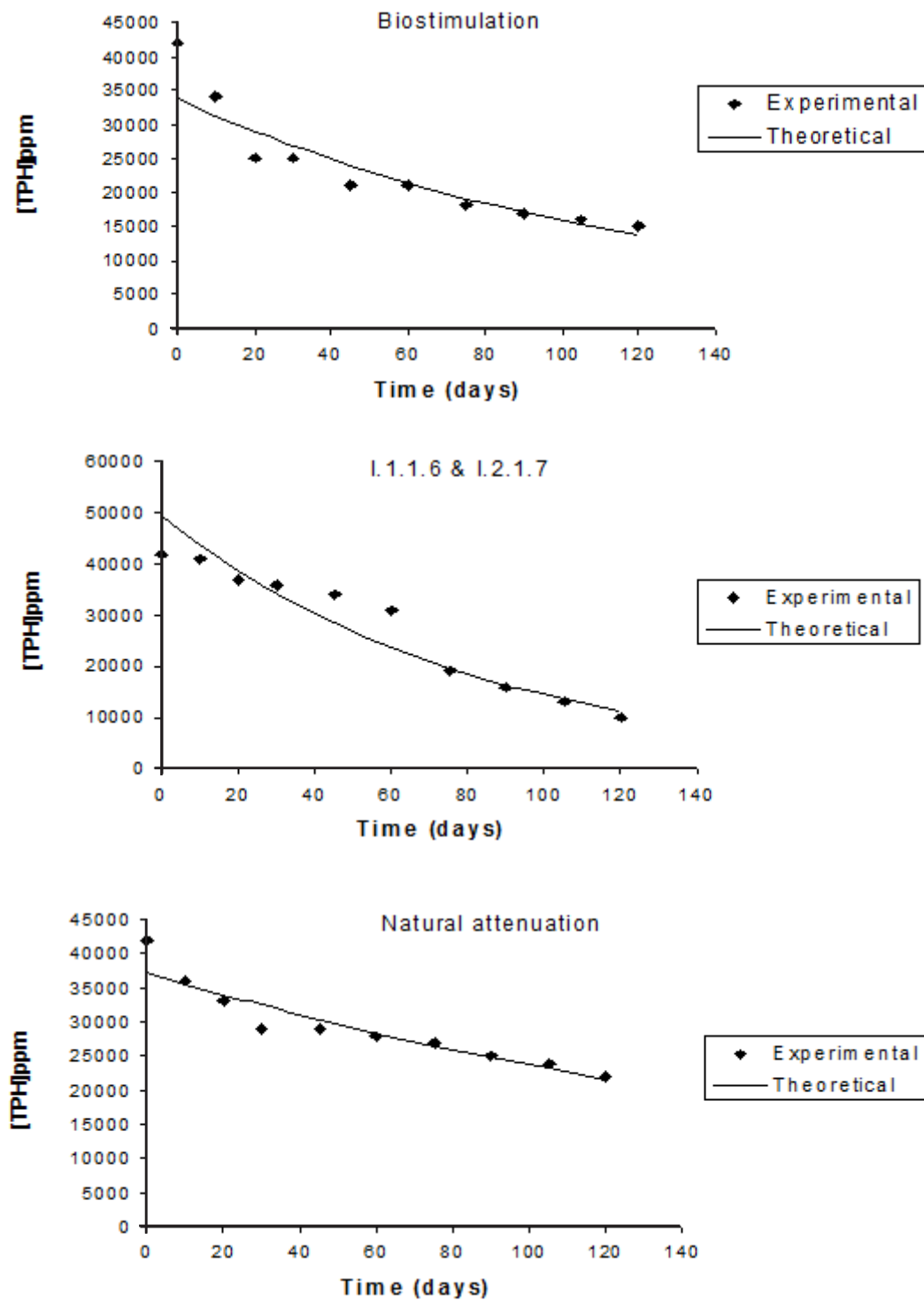


Fig. 5: Representative examples for comparison between the experimental data and predicted first order model

$$ARE = \frac{1}{N} \sum_{i=1}^N \left| \frac{C_{cal} - C_{exp}}{C_{exp}} \right| \quad \dots(5)$$

Where N is the number of data points, C_{cal} is the calculated data from the kinetic models and C_{exp} is the experimental data.

The values of all three error analysis were presented in Table 3; the lower the values of error analysis the better will be the goodness of fit. In the present study, experimental results were better described using a first-order kinetic model. Nocentini *et al.*, (2000) and Namkong *et al.*, (2002) were adequately described their hydrocarbon degradation data using a first-order kinetic model.

Rate constant and $t_{1/2}$

The rate constants are reflective of the relative effects of various treatments on TPH degradation in contaminated soils (Sarkar *et al.*, 2005). The rate constants K_1 obtained from our experimental results of all the treated systems are listed in Table 1.

The highest rate was observed in cell(4) augmented with bacterial consortium I.1.1.6 and I.2.1.7, followed by biostimulation cell(1). Then came cells (2, 3 and 5) which showed the same degradation rate, they were augmented with

bacterial isolate I.1.1.6, bacterial consortia I.1.1.1 and I.1.1.6 and bacterial consortia I.1.1.1, I.1.1.6 and I.2.1.7, respectively. The lowest rate was observed in natural attenuation cell.

The half-life of TPH biodegradation listed in Table 1 confirmed the experimental results illustrated in Fig. 1. The natural attenuation cell represented the longest half life time (≈ 154 days) which shows that the indigenous population can biodegrade the petroleum hydrocarbons in the polluted soil but the process is very slow and it would be effective over a long period of time. The shortest half life time (≈ 56 days) was recorded in cell bioaugmented with bacterial consortium I.1.1.6 and I.2.1.7, which shows that augmenting the contaminated site with an appropriate bacterial inoculum is a promising technique to enhance the biodegradation of hydrocarbons. Biostimulated cell showed an efficient half life time (≈ 91 days). The significant increase in the biodegradation rate by the addition of nutrients and bacteria, suggests that a combination of biostimulation and bioaugmentation may be required for the efficient biodegradation in contaminated soil. While the other bioaugmented cell2, cell3 and cell5 showed nearly the same half life time (≈ 116 , 112 and 116 days, respectively) and longer than biostimulation cell which indicates that bioaugmentation may not necessarily manifest oil-degrading activity under conditions of competition with indigenous

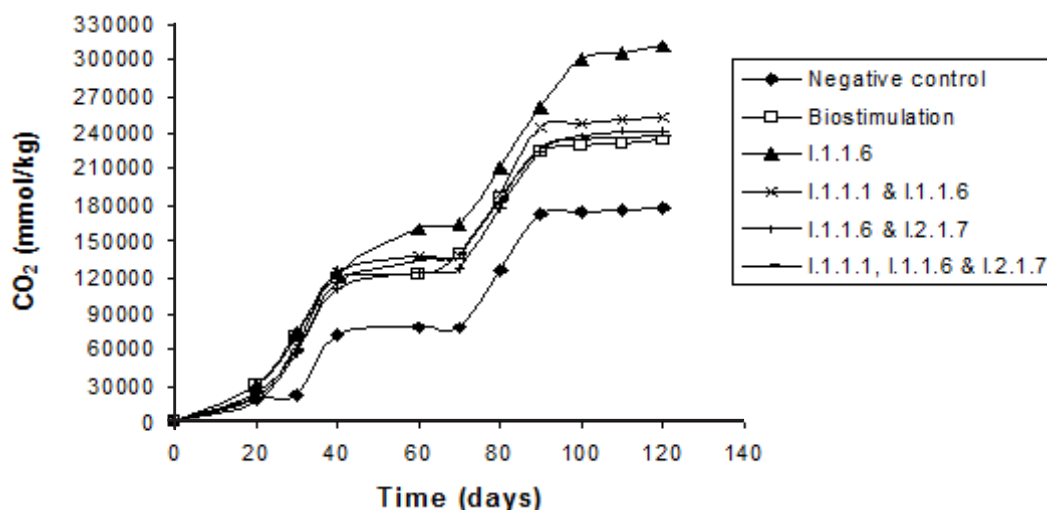


Fig. 6: CO₂ evolution of different biotreatment cells

microorganisms.

Fig. 5 represent examples for the comparison between the experimental data and predicted first order model.

Heterotrophic activity (CO₂)

CO₂ evolution was used as a measure of soil heterotrophic activity. In Fig. 6 the profile of CO₂ evaluation of the six experimental sets are shown: Overall there were considerable variations in CO₂ evolution trends between studied systems. These variations indicated that CO₂ measurements were good qualitative indicator of biodegradation activity in each cell. It can be observed that natural attenuation showed the lowest CO₂ production (178003 mmol/kg soil) after 120 days of incubation. Generally biostimulation and bioaugmentation processes caused increase in the heterotrophic activity of the treated cells. Similar observations were reported by Nocentini *et al.*, (2000).

The highest CO₂ production was observed in cell 3 bioaugmented with *Pseudomonas aeruginosa* I.1.1.6, where (312470 mmol/kg soil) of

CO₂ was recorded after 120 days of incubation. Other cells showed intermediate production of CO₂ in the following order cell 3 > cell 4 ≈ cell 5 ≈ cell 1, where 252520, 242460, 236490 and 233050 mmol/kg soil of CO₂ were recorded, respectively after 120 days of incubation.

In all cells CO₂ production rate was high during the first 40 days of incubation followed by a stationary phase up to 70 days of incubation then the production rate increased again up to 90 days of incubation then followed by another stationary phase up to 120 days of incubation.

According to Sabate *et al.*, (2004), the differences in CO₂ evolution could be attributed to the absence of assemble sources of carbon and energy or to a presence of toxic compounds.

The CO₂ formation rates in bioaugmented cells were relatively high than natural attenuation and biostimulation cells. This fact would suggest that some positive effects of our augmented bacterial isolates in reclamation of oil polluted soil.

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