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

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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 CO₂ was good qualitative indicator of biodegradation activity in each cell. The CO₂ 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
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...
temperature (25-30°C) in a green house. At zero time and after 20 days of incubation the following were added:
1. Nutrients: NH₄Cl and K₂HPO₄ 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 (30µL 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:

\[ \ln C = -K_1 t + A \]  \text{...(1)}

Second order kinetic model:

\[ \frac{1}{C} = K_2 t + B \]  \text{...(2)}

where C is the TPH concentration (mg/kg), \( t \) expresses time, \( K_1 \) and \( K_2 \) 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](image-url)
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.
Table 1: The first order parameters for the degradation of TPH during different bioremediation processes

| Bioremediation processes | $K_1$(day$^{-1}$) | $t_{1/2}$(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 | $K_2$(day$^{-1}$) | Correlation coefficient( $R$ ) |
|-------------------------|------------------|-------------------------------|
| Cell 1                  | Biostimulation   | 3x10$^{-7}$                  | 0.977 |
| Cell 2                  | I.1.1.6          | 2x10$^{-7}$                  | 0.974 |
| Cell 3                  | I.1.1.1 & I.1.1.6| 2x10$^{-7}$                  | 0.929 |
| Cell 4                  | I.1.1.6 & I.2.1.7| 6x10$^{-7}$                  | 0.932 |
| Cell 5                  | I.1.1.1.1 & I.1.1.6 & I.2.1.7 | 2x10$^{-7}$ | 0.944 |
| Cell 6                  | Natural attenuation | 2x10$^{-7}$                  | 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):
   \[ RMS = \left( \frac{1}{N} \sum_{i=1}^{N} (C_{ap} - C_{ap})^2 \right)^{1/2} \]  

2. Sum of the absolute errors, EABS (Perez-Marin et al., 2007):
   \[ EABS = \sum_{i=1}^{N} |C_{ap} - C_{ap}| \]

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

| 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.
\[ ARE = \frac{1}{N} \sum_{i=1}^{N} \left| \frac{C_{\text{cal}} - C_{\text{exp}}}{C_{\text{exp}}} \right| \]  

(5)

Where \( N \) is the number of data points, \( C_{\text{cal}} \) is the calculated data from the kinetic models and \( C_{\text{exp}} \) 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. Nocenteni 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 (\( \approx 154 \) 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 (\( \approx 56 \) 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 (\( \approx 91 \) 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 (\( \approx 116, 112 \) 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 species.
microorganisms.

Fig. 5 represents 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, bioaugmentation 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* l.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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