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Kinetic Studies on Biostimulation Process for Decontamination of Petroleum Hydrocarbons in Polluted Soil

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Abstract

A microcosm study was constructed with the purpose of investigating the effect of complex co-substrate (corn steep liquor, CSL) addition on the indigenous bacterial community and the resulting rate and extent of total petroleum hydrocarbon (TPH) degradation in an oily soil with TPH content of 63,353mg/kg. TPH degradation was characterized by a rapid phase of degradation that lasted for the first three weeks where 76% removal of TPH occurred, followed by a slower degradation phase, where 83% of the initial TPH was removed by the end of incubation period, 35 days. Branched alkanes are more resistant to microbial degradation than n-alkanes. Furthermore, unresolved complex mixture (UCM) hydrocarbon components are less degradable than n- and iso- alkanes and pristine (Pr) was the most recalcitrant aliphatic compound studied in this work. These results together with the expressive bacterial growth observed (from 10^7 to 10^{10} CFU/g soil) give strong support that the addition of CSL resulted in increased degradation rates. The indigeneuos bacteria grew exponentially during the incubation period of 35 days with a growth rate of 0.26 day^{-1}. In order to investigate the biodegradation process for different petroleum hydrocarbons contaminating the collected soil, kinetic modeling was performed to estimate the rates of biodegradation of each hydrocarbon component in the studied system. Five different error functions (sum of the square of the errors, hybrid fractional error function, Marquardt's percent standard deviation, average relative error and sum of the absolute errors) 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 of $\sum nC_{20}$-nC_{24}, $\sum nC_{35}$-nC_{42} and nC_{18} can be best represented by a second order model, whereas the TPH, TRP, nC_{17}, UCM, $\sum nC_{10}$-nC_{14}, $\sum nC_{15}$-nC_{19}, $\sum nC_{25}$-nC_{29}, $\sum nC_{30}$-nC_{34}, $\sum nC_n$, and $\sum isoC_n$ and isoprenoids Pr and Ph were found to follow first order model.

Key words: Biostimulation, Kinetics, Oily Soil, Corn Steep Liquor

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Introduction:

Oil contamination is one of the most dangerous pollution factors known today. It can cause a threat to the environment. It is very feared by environmentalists and it is very hard to control if it gets out of hand. Oil contamination can be either at sea or in soil.

As petroleum exploration and industrialization are expanded in Egypt, large quantities of petroleum organic wastes are released into the Egyptian environment every year. Soil contamination is a typical side-effect of petroleum industrial activity. Once the soil environment is contaminated with petroleum hydrocarbons, intrinsic bacteria are thought to degrade and utilize them as carbon sources (Leahy and Colwell, 1990 and Ueno et al., 2006).

Oil contamination in soil results in an imbalance in the carbon-nitrogen ratio at the spill site, because crude oil is essentially a mixture of carbon and hydrogen. This causes a nitrogen deficiency in oil soaked soil, which retards the growth of indigenous microorganisms and the utilization of carbon sources. Furthermore, large concentrations of biodegradable organics in the top layer deplete oxygen reserves in soil and slow down the rates of oxygen diffusion into deeper layers (Rosenberg et al., 1992).

Soil contamination can be cured by several ways; biodegradation of organic waste is becoming an increasingly important method of waste treatment in Egypt (Al-Daher et al., 2001; Hussein and Terry, 2002; Abdel-Haleem, 2003; El-Morsy, 2005; Hafez et al., 2006; El-Gendy, 2006 and El-Bestawy et al., 2007). The advantages of this option compared to the conventional physical and chemical options include; inexpensive equipment, environmentally friendly nature of the process and simplicity (Sabate et al., 2004). However, one disadvantage of this process is its relative slow speed in achieving results.

Biostimulation is a method for biodegradation involves nutrients addition to the contaminated field to stimulate the indigenous microflora of the polluted site to enhance and accelerate microbial organic waste breakdown (Olaniran et al., 2006 and Ueno et al., 2006). In contrast, co-substrate addition has not been investigated for the enhancement of petroleum degradation. In part, this because total petroleum hydrocarbons (TPH) are widely considered to be a readily degradable mixture of compounds. However, petroleum is a very complex mixture, and there are many compounds in TPH that may not be readily degraded. In particular, weathered petroleum may be enriched in poorly degradable components (Alexander, 2000 and Admon et al., 2001). Co-substrate additions may therefore be important to the remediation of aged petroleum, where the more readily degradable components are either gone or have already been partially oxidized during weathering.

To warrant a practical application, any bioremediation process should demonstrate that the removal of contaminants is the primary effect of biodegradation, and that the degradation rate is greater than the natural rate of decontamination. One of the difficulties of developing bioremediation strategies lies in achieving as good or better results in the field as in the laboratory (Juhaz et al., 2000).

In this study a kinetic evaluation of biostimulation process for decontamination of an oily soil was performed in a microcosm level study using corn steep liquor as co-substrate.

Materials and methods:

Soil sample:

Soil sample used in this study was collected from beach of Suez Canal Authority (S.C.A) workshop yard for ship maintenance on Temsah Lake, Ismaliya, Egypt.

Microcosm description:

Contaminated soil (1kg) was placed in glass pan with surface area of 400 cm^2 and volume of 4000 cm^3. Soil mixed daily to provide sufficient air and oxygen and kept at room temperature (30°C). The soil was moistened by the addition of 50ml sterile distilled water every week until the end of experiment. The soil was biostimulated by adding 2g/kg corn steep liquor (CSL) as it is composed of protein, lipids and carbohydrates and it also acts as an emulsifier (Kaplan et al., 2003), it was diluted with water prior its addition to the soil to reduce its viscosity and enhance its application. The same conditions provided in the biostimulation microcosms were used but without addition of CSL in the –ve control microcosms (natural attenuation). Incubation period was 35 days. All experiments were preformed in three replicates.

Soil analyses:

The contaminated soils were sampled at 0, 1, 2, 3, 4 and 5 weeks for chemical and microbiological analyses. Composite samples were obtained by mixing 10g of soil collected from five different areas of the microcosm. A portion of the composite soil (1g) was added to 10ml sterile saline (8.5g NaCl per one liter of distilled water) for total viable count (TCFU/g soil), vortexed for 30min, and then serially diluted to 10^{-1}. Serial dilutions of the suspensions were inoculated on Luria Bertani-agar plates (Kirimura et al., 2001) and incubated at 30°C for 48hours to enumerate TCFU. Identification of indigenous bacterial strains was done using Biolog system model; Biolog/Microlog 3420 program (Egyptian Plant Disease Research Institute).

The remainder 9g of contaminated soil was analyzed chemically where the TPH were determined according to the method reported by Viguri et al., (2002). The extracted oil was analyzed by gas chromatograph (Aglient model 6890) equipped with a flame ionization detector (GC-FID) to determine the effect of biotreatment on different components of extracted oil. The column used was; HP-1 (100% poly methyl silicon siloxane, 60m length, 0.25mm ID and 250µl film thickness) with nitrogen as carrier gas at a flow of 2ml/min. the temperature program used was 80-300°C (3°C/min).

Results and discussions:

The physicochemical characteristics of the soil collected at the contaminated site showed that the soil had 10% clay, 75% sand and 15% slit with a sand loamy texture and yellowish brown color, with TPH content of 63,353mg/kg. According to Duarte et al., (2001), the levels of oil in low-polluted soil averaged 710µg/g soil and those in the high-polluted soils averaged 5,500µg/g soil. Based on this classification, the obtained pollutant value for the collected oily soil was extremely above the alarming level and it can be considered as being very highly polluted by oil. This high value may be due to the continuous supply of petroleum inputs in the area. Because of its nearby to S.C.A. workshop yard. Its pH was 7.4, nitrogen and phosphorus content were 0.21 and 0.0(%w/w), respectively. The gas chromatographic (GC) profile of the oil extracted from the collected soil, Fig (1) showed regularly spaced resolved n-alkane peaks ranging from (nC_{10}-nC_{42}) superimposed on an unresolved complex mixture (UCM) containing cycloalkanes, naphthenes and aromatics indicating that the source of contamination is mainly petroleum hydrocarbons. The light components up to nC_{10} were lost due to the natural weathering process; mainly vaporization, dissolution and/or photo-oxidation (Kaplan and Kitts, 2004). The presence of high UCM value 42.89% with total paraffins of 57.11% and Pr/Ph ratio of 2.68 gave an indication of recent and/or continuous

petroleum inputs in this area, confirming the slow weathering rate of the spilled oil and chronic pollution (Garcia de Oteyza et al., 2006). The value of Pr/Ph>1 indicated biogenic addition and/or combination of terrestrial plant waxes and petroleum sources (Mendez et al., 2001).

Chemical analysis and microbial counts are used in the assessment of contaminated soil and in monitoring the effectiveness of bioremediation processes.

The soil microbial properties are perhaps the vital factors determining natural biodegradability. The total viable count on LB-agar plates directly after collection showed a good microbial population of ($5x10^7$ CFU/g soil) but with low biodiversity only four bacterial strains were detected and identified as Gram positive *Bacillus* sp. and *Micrococcus* sp., and Gram negative *Pseudomonas* sp., and *Sphingomonas* sp. which are considered as the mostly reported indigenous hydrocarbon utilizing bacteria in oily soil (Ghazali et al., 2004). This low biodiversity could be attributed to the high concentration of oil (63,353mg/kg) in the soil. According to Huessemann, (1994), high hydrocarbon levels are associated with varying degrees of inhibitory effects on soil microbes for example oxygen and/or nutrients limitations. The terrestrial oil spills are characterized primarily by vertical movement of the oil into the soil rather that the horizontal spreading associated with slick formation over water surface. Infiltration of oil into the soil may prevent evaporative loses of volatile hydrocarbons, which are toxic to microorganisms (Leahy and Colwell, 1990). According to Rahman et al., (2003) the low biodiversity may due to the sandy soil nature of low nutrients and microflora. Accordingly, application of extraneous nutrients is required for developing a feasible bioremediation method in the contaminated soil under study.

Biodegradation experiments were constructed with bulk soil without sieving. Thereby, degradation could take place at "realistic" condition with pores of different sizes being present. Sterilized soil and aseptic conditions were not used in all microcosms to mimic the realistic environmental field as much as it could be and because sterilization will kill indigenous microorganisms, thus natural attenuation and biostimulation could not be studied. More over sterilization can alter considerably the sorption properties of organic matter and then the out come of hydrocarbon biodegradation experiments (Amellal et al., 2001).

Follow up bioremediation process:

pH, moisture content and aeration:

Table (1) and Figure (2) show the trend of mean changes in pH and moisture content allover the incubation period in all microcosms. Generally the recorded pH values (6.8-8.2) allover the incubation period were within or near the optimum pH range for hydrocarbon degradation of 6.5 to 8.5 (Sarkar et al., 2005). The pH increased to a maximum of 8.2 on day 7, then decreased to average of 7.0 at day 14. It increased again to reach 7.5 by 21 days of incubation, then declined to average 6.8 from day 28 until day 35. Decrease recorded may have been due to the formation of low molecular weight organic acids during the degradation of the carbon compounds (Aislabie et al., 2006; Marin et al., 2006). Braddock et al., (1999) reported that addition of nutrients lowered pH of coarse sand from 7.4 to 6.8. Rahman et al., (2003) recorded the increase in pH and attributed this to the release of byproducts during hydrocarbon degradation.

The initial drop in soil moisture content during the first 7 days of incubation was expected because in heavily polluted soils, oil coating the surface of soil particles makes soil more hydrophobic and water droplets adhere to the hydrophobic layer formed, and this prevents wetting of the inner parts of the soil aggregates and reduces the water holding capacity of the soil (Dibble and Bartha, 1979 and Odokuma and Dickson, 2003). Generally

coarse-textured soils, have low water-holding capacity (Aislabie et al., 2006). Water was added weekly to keep the moisture content in all microcosms with an average value of approximately 50%. In soils, water contents of between 20-70% capacities are generally optimal for microbial activity (Morgan and Watkinson, 1989).

Wet conditions may limit oxygen availability; hence, aeration is required to enhance aerobic hydrocarbon biodegradation. Each microcosm was mixed daily to ensure good aeration condition and to distribute the CSL and water throughout the microcosms.

Change in TPH content and microbial population:

The absence of lag phase in TPH removal and microbial growth was observed in biostimulated microcosms, as listed in Table (1) and Figur (3). Sabate et al., (2004) reported that addition of nutrients led to large decrease in TPH and deletion of the lag (adaptation) period. Similar results were reported by Das and Mukhejie, (2007) and attributed this to the supplementation of glucose used as a co-carbon source in that study and enhanced the biodegradation rate. According to Trindade et al., (2005) this can be explained by the previous and prolonged exposure of the native microorganisms to the contaminant, leading to the selection and predominance of the oil-degrading and/or tolerant ones.

At zero time the average value of TPH contaminating the oily soil was ≈ 63,353mg/kg which decreased by ≈ 39% within the first week of incubation. It was further decreased to reach ≈ 76% removal by 21 days of incubation. By the end of experiment within 35 days of incubation ≈ 83% of the initial TPH was removed. This residual TPH was expected (Nocenteni et al., 2000 and Sarkar et al., 2005).

The degradation of TPH had two distinct phases; an initial fast degradation phase (up to 21 days of incubation) which demonstrated that the native soil microbes were capable of degrading hydrocarbons to a large extent, but part of the loss may be attributed to volatilization as well, followed by a slow degradation phase. It was a breakthrough to obtain fast degradation rate of TPH, inspite of the addition of the readily utilizable co-substrate CSL. According to Kaplan et al., (2003) this might be explained by the fact that CSL acts as an emulsifier which consequently increases the initial proportion of bioavailable TPH. The most likely explanation for the observed change in degradation rate is the petroleum sequestration in soil particles, which renders it unavailable to bacteria. The rate of degradation during the slow phase would thus be limited by the desorption rate of petroleum from soil particles rather than bacterial activity (Kaplan et al., 2003).

During the first 21 days (rapid phase of degradation), microorganisms might have been stimulated by labile hydrocarbon sources (probably linear and open-chain hydrocarbons) that induced a high percentage of degradation. As those forms decreased, microbial populations had to use the more recalcitrant hydrocarbons (probably aromatic hydrocarbons with higher molecular weight) less efficiently. With the decrease in labile carbon sources, nutrients were most likely limited in supporting microbial growth which might explain the stationery phase in microbial growth started from 21 days of incubation. It is also possible that degradation of higher molecular weight hydrocarbons may produce toxic intermediates that can inhibit microbial activity (Bento et al., 2005).

To understand this low plateau of bioremediation activity, we examined the residual hydrocarbon percentage of TRP and UCM during the time course of the experiment. The residual concentration of TRP decreased while that of UCM was significantly enriched. Therefore, the residual contaminants are more recalcitrant. Sabate et al., (2004) reported similar results with a residual fraction in a soil after bioremediation experiment using glucose as a co-substrate.

Although there was a good microbial population by the end of the experiment ($9x10^{10}$ CFU/g soil), the average residual TPH in all biostimulated microcosms was ≈ 10,629mg/kg. Margesin and Schinner (1998) reported that between 10-30% of the initial soil pollution remains in soil after bioremediation techniques have been applied. Complete hydrocarbon reduction cannot occur due to their low bioavailability, especially after the labile compounds are being used by the microorganisms and the accumulation of recalcitrant compounds occurred. Sabate et al., (2006) reported that the accumulation of toxic intermediate metabolites has been postulated a cause for the lack of complete contaminant biodegradation in soil.

In –ve control microcosm without addition of nutrients (natural attenuation), showed a low reduction in TPH concentration reaching 12.15% by the end of incubation period (35 days), which shows that the indigenous population can biodegrade the petroleum hydrocarbons in the oily soil but the process is very slow. Margesin and Shinner, (2001) reported that natural attenuation process was slow, but nevertheless it was effective over a long period of time. Ghazali et al., (2004) reported similar results and attributed removal to combined actions of indigenous microbial population of the polluted soil as well as abiotic weathering. Abiotic weathering processes in polluted soils include evaporation, photochemical oxidation and adsorption onto particulate material.

There was a rapid bacterial growth within the first 21 days followed by period of stationary phase that corresponded to the breakpoint between the two degradation rates observed during the study, as illustrated in Table (1). This implies that the addition of co-substrate CSL on day 14 has not the same efficiency of increasing the amount of bioavailable TPH beyond what might have already been emulsified in the first application (at zero time). The increase in the bacterial populations in all biostimulated microcosms indicated that the indigenous bacteria are well adjusted to their environment and well adapted to the contaminated soil. According to Mishra et al., (2001) and Bento et al., (2005), this rapid growth could ensure rapid degradation of the pollutant. In –ve control microcosms although the culturable bacterial count is relatively high ($8.8x10^{8}$ CFU/g soil) at the end of incubation period 35 days, the rate of biodegradation is relatively low (table.1) this might be attributed to the lack of adequate nitrogen and phosphorus nutrients for supporting optimal microbial activities (Bento et al., 2005).

Change in different hydrocarbon components:

Figure (1) represents the chromatographic patterns of the biodegradation experiment. The chromatogram shows clearly a significant enhancement of the biodegradation of n- and iso- alkanes.

Table (1) and Figures (3 and 4) show the change of seven biodegradation indicators within the whole process. These indicators were selected as appropriate for estimation of the effective biodegradation on hydrocarbon destruction in polluted soils (Seklemova et al., 2001) depending on the concentration of total resolvable peaks (TRP; n- and iso- alkanes), unresolved complex mixture (UCM; naphthenes, aromatics and cycloalkanes), Pristane (Pr; 2,6,10,14-tetramethylpentadecane, iso-$C_{19}H_{40}$), Phytane (Ph; 2,6,10,14-tetramethylhexadecane, iso-$C_{20}H_{42}$). Additionally, the changes in the following parameters $\sum nC_{10}$-nC_{14}, $\sum nC_{15}$-nC_{19}, $\sum nC_{20}$-nC_{24}, $\sum nC_{25}$-nC_{29}, $\sum nC_{30}$-nC_{34} and $\sum nC_{35}$-nC_{42} were monitored.

It is obvious that the amount of n-alkanes with length up to nC_{14} was significantly reduced up to 85% within the first two weeks and approximately complete degradation ≈ 99% occurred by the end of incubation period as result of abiotic losses comprising evaporation of lower alkanes in addition to the biotic losses through biodegradation. The evaporation is the

major process that takes place after spillage and its duration is quite short-from some hours up to several days (Oudot, 1994; Seklemova et al., 2001).

The nC_{15}-nC_{24} fraction was the most abundant at day zero (19,840mg/kg) and fell to 3,183mg/kg by day 35, with ≈ 84% removal. The nC_{25}-nC_{42} fraction was reduced from 4,953mg/kg at day zero to 461mg/kg with ≈ 91% removal. Overall, there was ≈ 88% degradation of the n-alkanes and ≈ 85% removal of the iso-alkanes and ≈ 77% removal of the UCM. Intriguingly, the nC_{15}-nC_{19} fraction increased in biostimulated microcosms by the end of the study (35 days). This may reflect the accumulation of partial degradation products from longer chain hydrocarbons and high molecular weight compounds of UCM.

Table (1) and figure (3) represent the changes in nC_{18}/nC_{16} and nC_{18}/nC_{20} ratios during the biotreatment process. The increase of the ratio nC_{18}/nC_{16} upon reduction of the TPH concentration indicates higher level of degradation of nC_{16} compared with nC_{18}. The decrease of the ratio of nC_{18}/nC_{20} is evidence that destruction of nC_{18} is preferential to nC_{20}. Based on the results obtained, it could be assumed that chain length of n-alkanes is of great importance and degree of degradation of n-alkanes with shorter chain was higher than that of ones with longer chains. Similar observations were reported by (Seklemova et al., 2001).

Table (1) and figure (4) represent the changes in nC_{17}/Pr and nC_{18}/Ph ratios during the biotreatment process. nC_{17}/Pr and nC_{18}/Ph ratios decreased up to 21 days of incubation then increased again within the 28 days of incubation. nC_{18}/Ph continued in its increment but nC_{17}/Pr decreased again within the end of incubation period. The continuous decreases during the first 21 days of incubation proving that the indicated n-alkanes were preferentially degraded compared with their respective isomers. The increase in the ratios indicates the biodegradation of Pr and Ph and their rate of degradation might be close to that of n-alkanes. The evaluation of the dynamics of the above mentioned parameters could be applied for measurement of the biodegradation process, proving the observations of other authors (Pritchard and Costa, 1999; Seklemova et al., 2001, Ghazali et al., 2004 and Chaineau et al., 2005), due to the fact that Pr and Ph are relatively persistent isoprenoids. The established trend outlines that the pollutant reduction is a result of microbial metabolism, showing impact of biotic factors.

Statistically significant and continuous decrease of $nC_n/isoC_n$ ratio up to the end of incubation period, as illustrated in Table (1) and Figure (4) indicated that the rate of biodegradation of n-alkanes is much higher than that of iso-alkanes. One reason for that could be the molecular structure of iso-alkanes is more stable to microbial destruction compared with n-alkanes. It is well known that bacteria degrade n- alkanes readily whereas the isoprenoidal alkanes are relatively resistant to microbial degradation (Atlas, 1984 and Diaz et al., 2002).

Statistically significant decrease of TRP/UCM ratio was observed within the first two weeks after the beginning of the experiment, illustrated in Table (1) and Figure (4) proving that the indicated n- and iso-alkanes were preferentially degraded compared to the UCM components aromatics, naphthenes and cycloalkanes). But this ratio increased again and remained nearly constant up to 28 days of incubation indicating the rates of TRP and UCM degradation were close. But TRP/UCM ratio decreased again thereafter to the end of incubation period (35 days).

The general increase in Pr/Ph ratio indicates that the rate of biodegradation of Ph is higher than that of Pr. Similar observation was reported by Diaz et al., (2002).

Generally, degradation of branched-chain alkanes is repressed by the presence of n-alkanes. However, some reports have shown that many of these compounds are more degradable than it had previously been thought (Del Arco and de Franca, 2001). Our data confirm these observations,

The change of nC_{17}/Pr, nC_{18}/Ph, Pr/Ph and TRP/UCM ratios have been long recognized and used as indicators of biodegradation (Wang and Fingas, 2003).

By following up the residual percentage of each petroleum hydrocarbon components as listed in table (1), it was observed that different petroleum components are differentially degraded: branched alkanes are more resistant to microbial degradation than n-alkanes due to their molecular structure. Furthermore, UCM hydrocarbon components are considered to be less degradable than n- and iso- alkanes and Pr was the most recalcitrant aliphatic compound studied in this work.

Growth kinetics of indigenous microbial population:

The exponential growth equation (Bailey and Ollis, 1986) is usually used to describe growth of microbial populations, which can be expressed as

$$R_x = K_g X \qquad (1)$$

Where R_x is the rate of cell growth (number of cells/kg day), X is the cell concentration (number of cells/kg) and K_g is the kinetic growth constant (1/day). For batch system, this equivalent to

$$\frac{dX}{dt} = K_g X \qquad (2)$$

Separation and integrating Eq.(2)

$$\int_{X_0}^{X} \frac{dX}{X} = K_g \int_0^t dt \qquad (3)$$

Thus

$$\ln(\frac{X}{X_0}) = K_g t \qquad (4)$$

The kinetic growth constant can be obtained as the slope of the line if $\ln(X/X_o)$ is plotted versus time. Figure (5) shows that the indigenous bacteria grow exponentially during the 35 days of incubation period. The kinetic growth constant, K was found to be 0.2572 day^{-1} and the correlation coefficient, R = 0.95.

Kinetic modeling for the degradation of the pollutants:

In order to investigate the biodegradation process for different petroleum hydrocarbons contaminating the collected soil, Kinetic modeling was performed to estimate the rates of biodegradation of each hydrocarbon component in the studied system. Kinetics of the biodegradation reaction can be described in terms of its order (Snoeyink, and Lenkins, 1980). The frequently used kinetic models such as the first order and second order models were investigated in this work to determine the mechanism of the process.

First order model:

The first order equation is given as

$$\frac{-dC}{dt} = K_1 C \qquad (5)$$

Where C is the concentration of the compound (mg/kg), t expresses time (day), k_1 is the first order rate constant (day^{-1}). Separation and integrating Eq.(5) with respect to limits $C = C_o$ at t=0 and C=C at any time:

$$-\int_{C_o}^{C}\frac{dC}{C} = K_1\int_{0}^{t}dt \qquad (6)$$

$$\ln C = -K_1 t + A \qquad (7)$$

Where A is a constant (mg/kg), at t= 0 then $C = C_o$, then $A = \ln C_0$

Second order model:

The second order equation can be written as

$$\frac{-dC}{dt} = K_2 C^2 \qquad (8)$$

Where C is the concentration of the compound (mg/kg), t expresses time (day) and k_2 is the second order rate constant (kg/mg day).Integrating Eq. (8) with the previous limit

$$-\int_{C_0}^{C}\frac{dC}{C^2} = K_2\int_{o}^{t}t \qquad (9)$$

$$\frac{1}{C} = K_2 t + B \qquad (10)$$

Where B is a constant (kg/mg) at t = 0 then $C = C_o$ and $B = 1/C_o$

Figures 6 and 7 show the curve-fitting plots of the first order and second order models, respectively, and the parameters obtained for the two models are presented in Tables 2 and 3. The goodness of fit of the models was at first expressed by the linear regression coefficients of determination R, the obtained R values for the plots of all the studied petroleum hydrocarbon components were in the range 0.906-0.992 for first order and 0.908-0.995 for second order models, except $\sum nC_{10}$-nC_{14} (0.817). A relatively high R value indicated that the models successfully described the kinetics of the degradation of petroleum hydrocarbon components.

Based on the results listed in table (2), it could be assumed by comparing the $t_{1/2}$ of the biodegradation of each petroleum hydrocarbon component it was found that $t_{1/2}$ of biodegradation of $nC_n < isoC_n \approx UCM$, proving that the biodegradation of iso- alkanes and UCM components (aromatics, naphthenes and cycloalkanes) are much difficult than that of n-alkanes.

$t_{1/2}$ of the biodegradation of $nC_{17} < nC_{18}$, proving that chain length of n-alkanes is of great importance and degree of degradation of n-alkanes with shorter chains was higher than that of ones with longer chains.

$t_{1/2}$ of the biodegradation of Pr > Ph, proving the difficulty of biodegradation of Pr and it was the longest $t_{1/2}$ which confirmed that Pr was the most recalcitrant compound studied in this work.

The shortest $t_{1/2}$ of biodegradation was observed for the nC_{10}-nC_{14} fraction due to the abiotic losses comprising evaporation of lower alkanes in addition to biotic losses which accelerated the removal of this fraction.

But the $t_{1/2}$ of biodegradation of other studied fractions as listed in Table (2) didn't follow a certain trend. Similar observation was reported by Seklemova et al., (2001). De Jonge et al., (1997) reported that at high concentration of oil pollution (12,000-4,000mg/kg) due to the different mechanism of oil distribution in the soil matrix, all n-alkanes were degraded at the

same rate, regardless that chain length of n-alkanes. Our results prove that the scheme of microbial hydrocarbon utilization is not universal.

Error analysis for the studied kinetics:

The classical method to find out the most suitable kinetic model to represent the experimental data was the use of the correlation coefficient (R), which measures the difference between the experimental and theoretical data in linear plots only, but not the errors in kinetics curves.

Due to the inherent bias resulting from linearization, five different error functions were employed in this study to enable the optimization process to determine and evaluate the fit of the model equation to the obtained experimental data. The error functions employed were as follows

- The sum of the square of the errors (ERRSQ):

$$\sum_{i=1}^{p}(C_{exp}-C_{cal})_i^2 \qquad (11)$$

This widely used error function has one major drawback. Parameters derived using this error function will provide a better fit as the magnitude of the errors (and thus the squares of the errors) increase biasing the fit towards data obtained (Rengaraj et al., 2007).

- The hybrid fractional error function (HYBRID):

This error function was developed by Porter et al., (1999) in order to improve the fit of the ERRSQ method.

$$\frac{100}{p-n}\sum_{i=1}^{p}\left[\frac{(C_{exp}-C_{cal})^2}{C_{exp}}\right]_i \qquad (12)$$

- Marquardt's percent standard deviation (MPSD):

$$100\left[\sqrt{\frac{1}{p-n}\sum_{i=1}^{p}\left(\frac{C_{exp}-C_{cal}}{C_{exp}}\right)_i^2}\right] \qquad (13)$$

This is similar in some respects to a geometric mean error distribution modified according to the number of degrees of freedom of the system (Marquardt et al., 1963).

- The average relative error (ARE) which minimizes the fractional error distribution (Kapoor et al., 1989).

$$\frac{100}{p}\sum_{i=1}^{p}\left(\frac{(C_{exp}-C_{cal})}{C_{exp}}\right)_i \qquad (14)$$

- The sum of the absolute errors (SAE)(Mall, et al., 2005)

$$\sum_{i=1}^{p}\left(C_{cal}-C_{exp}\right)_i \qquad (15)$$

Where p is the number of data points and n the number of parameters within the equation, C_{cal}, is the calculated data with the kinetic models and C_{exp}, is the experimental data.

The values of all five error analysis were presented in Table 4. while other authors Nocentini et al, (2000) adequately described their hydrocarbon degradation data using a first-order kinetic model, in the present study by comparing the results of the values of error

functions, it is found that the second order kinetic model best fits the degradation of $\sum nC_{20}$-nC_{24}, $\sum nC_{35}$-nC_{42} and nC_{18}, whereas the first order best fits all the other studied components.

Figures (8) and (9) represent examples for the comparison between the experimental data and predicted first and second order models, respectively. They confirm the results obtained from the error analysis.

Conclusion:

Bioremediation can be a viable and effective response to soil contamination by petroleum hydrocarbons. This investigation compared monitored natural attenuation with a method of biostimulation, where the addition of commercial complex co-substrates during biotreatment of oil polluted soil appears to be effective when added at process initiation. In the early phase of operations when bacterial abundance and physiology are important, the amendment was able to increase TPH-degradation rates by altering bacterial community physiology. It is also possible that CSL served as an emulsifier, increasing the amount of bioavailable TPH. An incidental benefit of CSL was that only one nutrient addition was necessary to maintain N and P levels during degradation. This is important in the treatment of oil polluted soil where TPH or sludge is regularly added in oil fields where the objective is the removal of large amounts of TPH and not complete remediation of the soil, an increased initial degradation rate is desirable because it would result in less TPH sequestered in soil particles after each application and results in better degradation efficiency over several TPH or sludge applications.

The results of this study in the investigated oily soil type, by means of TPH, n-alkanes, isoprenoids and UCM determination and application of some diagnostic parameters and relations, the oil pollutant reduction resulting from combined occurrence of abiotic and biotic processes were determined. Applying such approach is suitable for the purposes of measurement of bioremediation activities in field pollution. The proved biodegradation capabilities of indigenous microorganisms within the investigated range of contamination by applying commercial nutrients demonstrated the opportunity to carry out effective treatment of polluted soils.

Kinetic modeling studies and error analysis have been investigated for all petroleum hydrocarbon components in the oily soil through the incubation period of the biostimulation process. Reaction rates of degradation for every group have been calculated and the error analysis show that the degradation of $\sum nC_{20}$-nC_{24}, $\sum nC_{35}$-nC_{42} and nC_{18} can be best represented by a second order model, whereas the TPH, TRP, nC_{17}, UCM, $\sum nC_{10}$-nC_{14}, $\sum nC_{15}$-nC_{19}, $\sum nC_{25}$-nC_{29}, $\sum nC_{30}$-nC_{34}, $\sum nC_n$, and $\sum isoC_n$ and isoprenoids Pr and Ph were found to follow first order model.

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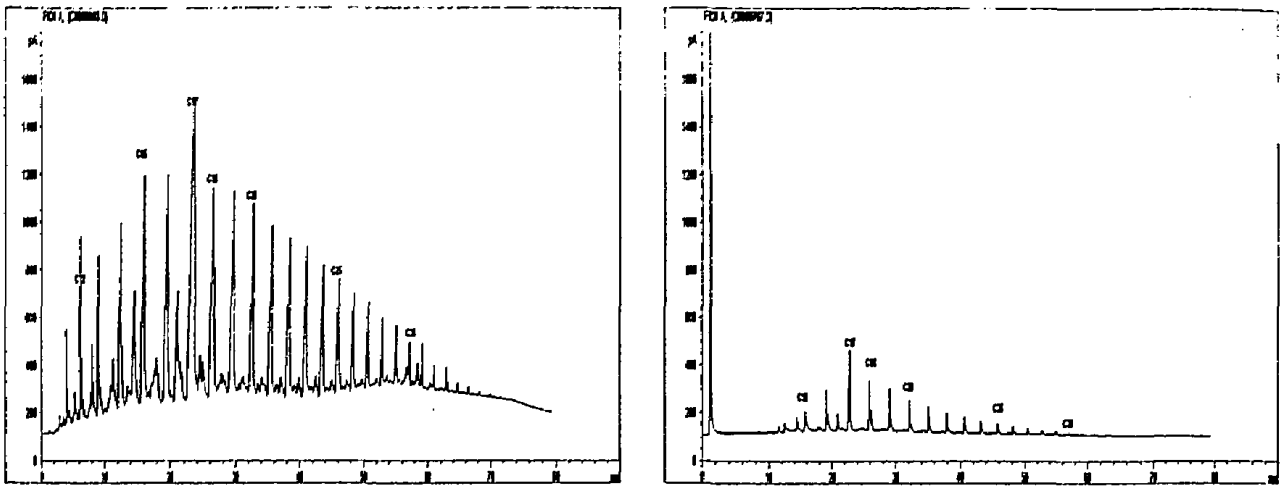


Zero time 35 days

Figure. 1. Gas chromatographic profile of oil extracted from oily soil before and after biotreatment

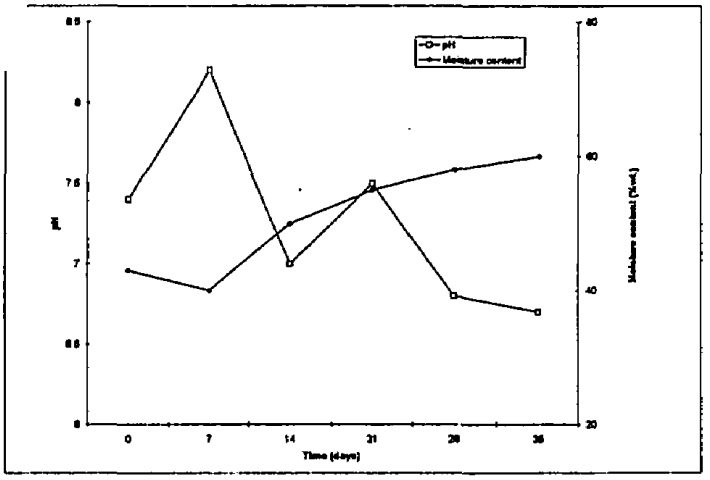


Figure. 2. Change in pH and moisture content during biotreatment of oily soil

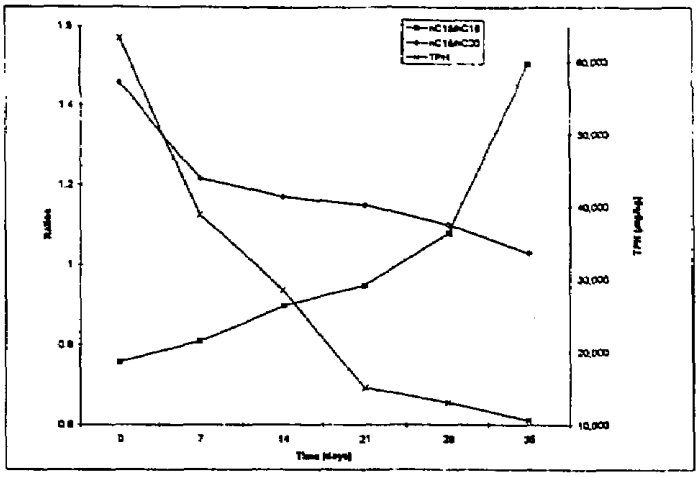


Figure. 3. Change in nC_{18}/nC_{16}, nC_{18}/nC_{20} and TPH concentration during biotreatment of oily soil

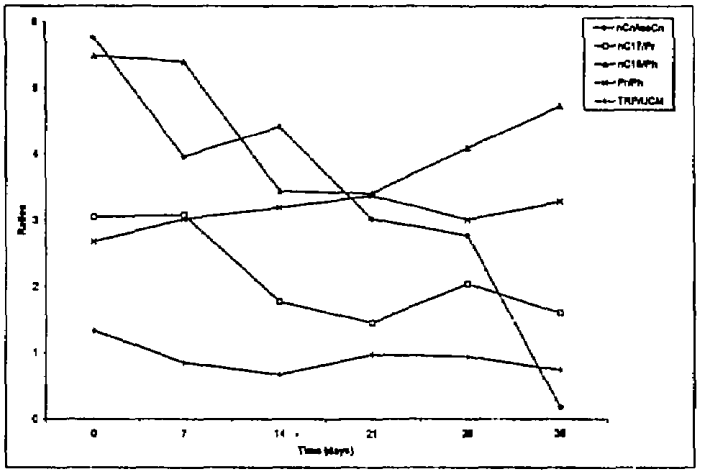


Figure. 4. Change in $nC_n/isoC_n$, TRP/UCM, Pr/Ph, nC_{17}/Pr and nC_{18}/Ph ratios during biotreatment of oily soil

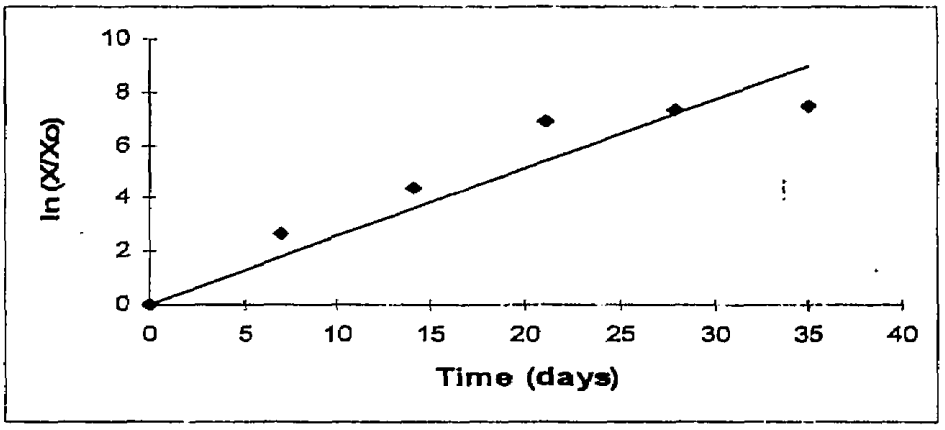


Figure. 5. Plots of exponential growth equation for indigenous microbial growth

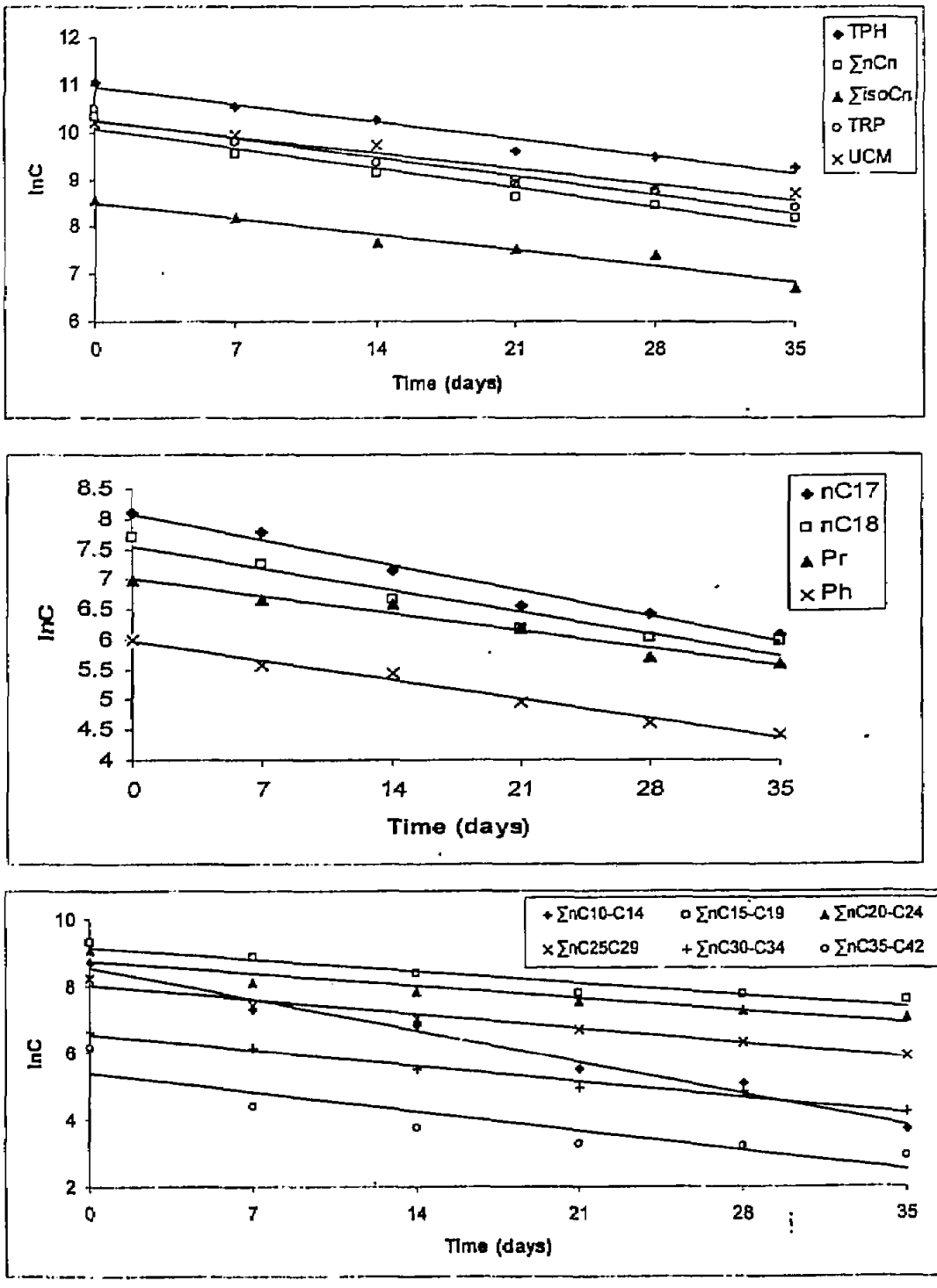
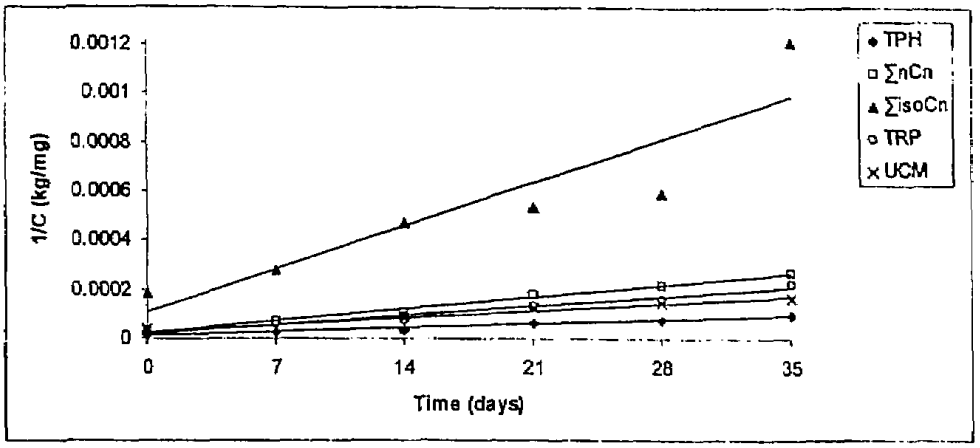
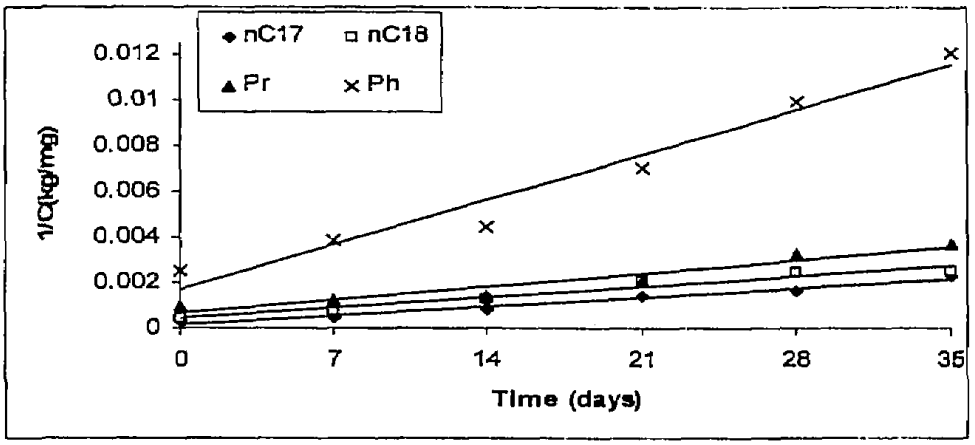


Figure. 6. Plots of first order kinetics of petroleum hydrocarbon degradation





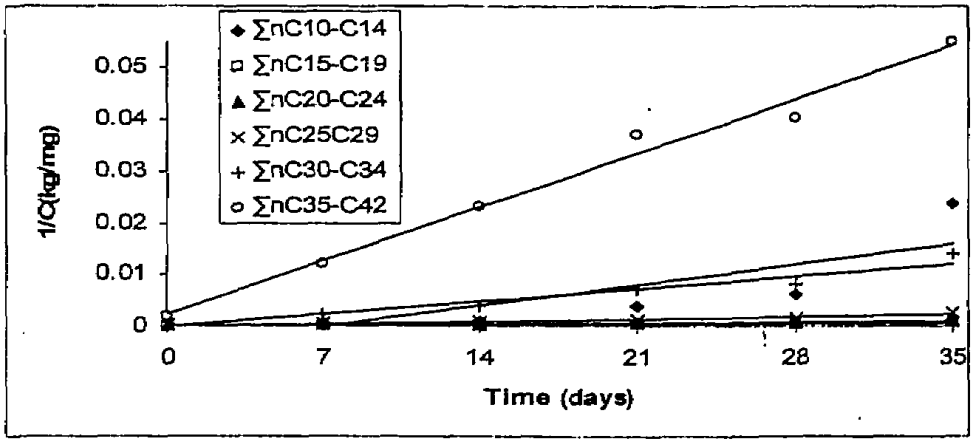


Figure. 7. Plots of second order kinetics of petroleum hydrocarbon degradation

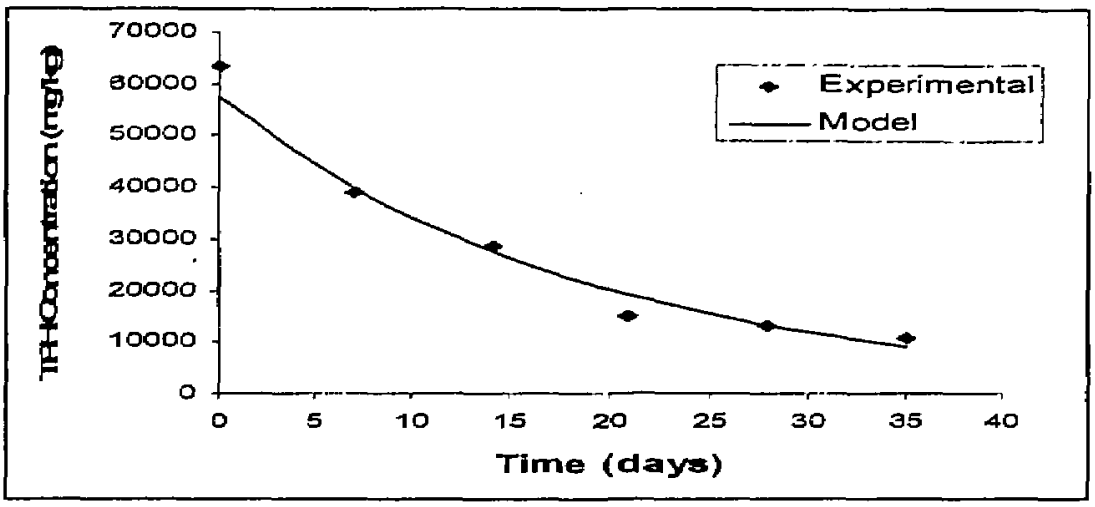


Figure. 8. A representative example for comparison between the experimental data and predicted first order model

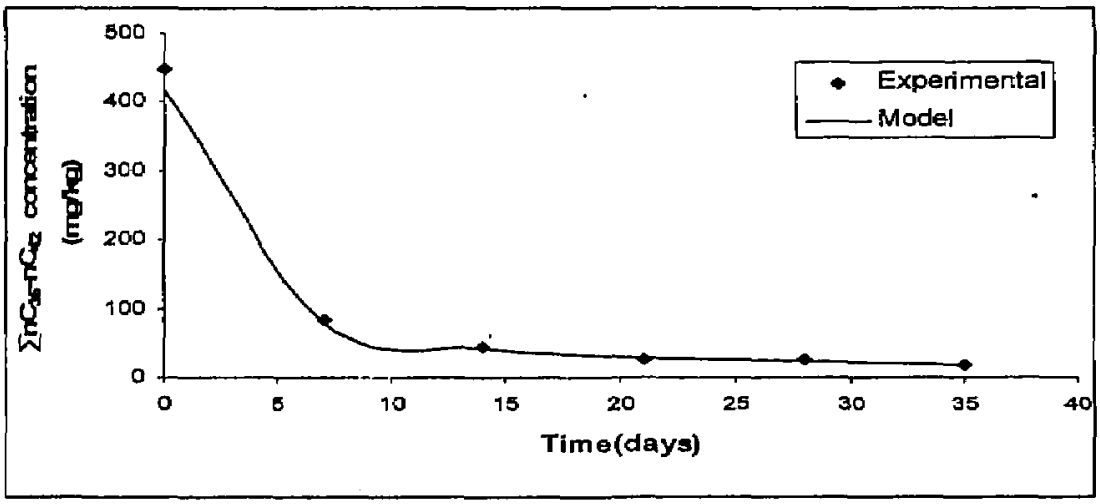


Figure. 9. A representative example for comparison between the experimental data and predicted second order model

Table. 1. Effect of biotreatment on oil extracted from oily soil

parameters	Zero time	-ve control	7 days	14 days	21 days	28 days	35 days
TPH mg/kg	63,353	55,657	38,854	28,514	15,107	13,070	10,629
TCFU cells/g	$5x10^7$	$8.8x10^8$	$7x10^8$	$4x10^9$	$5x10^{10}$	$7.8x10^{10}$	$9x10^{10}$
pH	7.4	7.0	8.2	7.0	7.5	6.8	6.8
Moisture content (%)	45	48	40	50	55	58	60
$nC_n/isoC_n$	5.76	4.85	3.96	4.42	3.02	2.77	0.18
TRP/UCM	1.33	1.05	0.85	0.67	0.97	0.94	0.74
Pr/Ph	2.68	2.66	3.02	3.19	3.37	3.01	3.28
nC_{17}/Pr	3.05	2.98	3.08	1.77	1.45	2.04	1.60
nC_{18}/Ph	5.49	5.30	5.40	3.44	3.41	4.09	4.73
nC_{18}/nC_{16}	0.757	0.905	0.809	0.897	0.949	1.080	1.504
nC_{18}/nC_{20}	1.456	1.284	1.216	1.170	1.150	1.101	1.030
	Residual hydrocarbon %						
$\sum nC_{10}$-nC_{14}	9.53	3.26	3.87	3.24	1.67	1.21	0.40
$\sum nC_{15}$-nC_{19}	17.27	16.97	18.29	15.56	16.12	17.86	18.67
$\sum nC_{20}$-nC_{24}	14.04	14.26	8.68	8.97	12.70	11.08	11.28
$\sum nC_{25}$-nC_{29}	5.87	5.97	4.33	3.99	5.45	4.30	3.50
$\sum nC_{30}$-nC_{34}	1.19	1.22	1.20	0.89	0.93	0.95	0.66
$\sum nC_{35}$-nC_{42}	0.75	0.77	0.21	0.15	0.18	0.19	0.17
$\sum nC_n$	48.67	42.45	36.57	32.80	37.06	35.59	34.68
$\sum isoC_n$	8.45	8.76	9.23	7.43	12.29	12.87	7.73
TRP	57.11	51.20	45.80	40.23	49.35	48.45	42.41
UCM	42.89	48.80	54.20	59.77	50.65	51.55	57.59
nC_{17}	5.19	5.57	6.23	4.48	4.64	4.74	4.08
nC_{18}	3.49	3.73	3.61	2.74	3.23	3.16	3.70
Pr	1.70	1.87	2.02	2.54	3.19	2.33	2.56
Ph	0.64	0.70	0.67	0.80	0.95	0.77	0.78

Table. 2. The first order parameters for the degradation of different petroleum hydrocarbons components during biostimulation process

Parameters / Components	K_1 (day^{-1})	$t_{1/2}$ (day)	Correlation coefficient (R)
TPH	0.0524	13.23	0.982
∑nCn	0.0591	11.28	0.972
∑isoCn	0.048	14.44	0.971
TRP	0.0569	12.18	0.978
UCM	0.0476	14.56	0.964
nC_{17}	0.0604	11.48	0.984
nC_{18}	0.0522	13.28	0.962
Pr	0.0414	16.74	0.984
Ph	0.0457	15.17	0.992
$\sum nC_{10}\text{-}nC_{14}$	0.1295	5.35	0.986
$\sum nC_{15}\text{-}nC_{19}$	0.0509	13.62	0.966
$\sum nC_{20}\text{-}nC_{24}$	0.0524	13.23	0.950
$\sum nC_{25}\text{-}nC_{29}$	0.0617	11.23	0.987
$\sum nC_{30}\text{-}nC_{34}$	0.067	10.34	0.990
$\sum nC_{35}\text{-}nC_{42}$	0.0833	8.32	0.906

Table. 3. The second order parameters for the degradation of different petroleum hydrocarbons components during biostimulation process

Parameters / Components	K_2 kg/(mg day)	Correlation coefficient (R)
TPH	$2x10^{-6}$	0.986
∑nCn	$7x10^{-6}$	0.995
∑isoCn	$3x10^{-5}$	0.908
TRP	$5x10^{-6}$	0.995
UCM	$4x10^{-6}$	0.960
nC_{17}	$6x10^{-5}$	0.980
nC_{18}	$7x10^{-5}$	0.981
Pr	$8x10^{-5}$	0.962
Ph	$3x10^{-4}$	0.979
$\sum nC_{10}$-nC_{14}	$6x10^{-4}$	0.817
$\sum nC_{15}$-nC_{19}	$1x10^{-5}$	0.977
$\sum nC_{20}$-nC_{24}	$2x10^{-5}$	0.997
$\sum nC_{25}$-nC_{29}	$7x10^{-5}$	0.970
$\sum nC_{30}$-nC_{34}	$3x10^{-4}$	0.957
$\sum nC_{35}$-nC_{42}	$15x10^{-4}$	0.993

Table.4. : Values of five different error analyses of kinetic models for degradation of different petroleum hydrocarbons components during biostimulation process

Error function / Components	ERRSQ	HYBRID	MPSD	ARE	SAE
TPH					
First order	54652516	46671.77	15.78	-0.72	-3085.04
Second order	1380584983	583958.2	34.52	-19.70	45337.8
$\sum nC_n$					
First order	5219973	56907.55	20.48	-1.41	-3204.84
Second order	420156686	551139	76.73	23.47	8516.07
$\sum isoC_n$					
First order	757366	5327.18	17.11	-1.06	-139.4
Second order	84399272	1996834.5	481.50	-160.42	10863.6
TRP					
First order	57238585	53116.75	17.41	-1.07	-3007.13
Second order	191876421	135553.3	20.71	-9.49	14925.12
UCM					
First order	17166627	39187.9	20.63	-1.33	-1116.52
Second order	83288057	99943	22.93	1.99	-1883.6
nC_{17}					
First order	159978	2885.66	17.33	-0.97	-167.3
Second order	45374140	346587	103.17	-27.88	6001.3
nC_{18}					
First order	156968	3454.86	22.09	-1.53	-197.9
Second order	168251	2664.22	14.43	2.13	-34.4
Pr					
First order	15704	631.96	10.97	-0.39	-4
Second order	376090	9220.17	31.00	-9.91	549.16
Ph					
First order	1289	138.61	8.31	-0.71	-4.6
Second order	37869	2546	26.99	-0.99	109.5
$\sum nC_{10}$-nC_{14}					
First order	1495843	10165.11	28.87	-6.43	-687.75
Second order	41509717	203150.7	86.31	54.04	-8366.53
$\sum nC_{15}$-nC_{19}					
First order	2732624	14238.15	21.47	-1.42	-782.02
Second order	3966256	20375.4	25.64	-14.71	3023.74
$\sum nC_{10}$-nC_{24}					
First order	8006736	29613.1	24.52	-2.09	1132.22
Second order	1862368	8300.19	13.77	-8.00	20.95.36
$\sum nC_{25}$-nC_{29}					
First order	562648	4868.04	14.57	-0.78	-259.45
Second order	39523999	267365	85.98	-20.03	5838.34
$\sum nC_{30}$-nC_{34}					
First order	4725	395.34	14.54	-0.62	-35.44
Second order	3042756	100873.6	116.83	-44.23	1762.5
$\sum nC_{35}$-nC_{42}					
First order	66061	4485.1	56.08	-10.23	-178.73
Second order	3681	210.83	9.66	3.29	-65.84