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Ex-situ Bioremediation of Crude Oil in Soil, a Comparative Kinetic Analysis

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Abstract Weathered crude oil (WCO) removals in shoreline sediment samples were monitored for 60 days in bioremediation experimentation. Experimental modeling was carried out using statistical design of experiments. At optimum conditions maximum of 83.13, 78.06 and 69.92% WCO removals were observed for 2, 16 and 30 g/kg initial oil concentrations, respectively. Significant variations in the crude oil degradation pattern were observed with respect to oil, nutrient and microorganism contents. Crude oil bioremediation were successfully described by a first-order kinetic model. The study indicated that the rate of hydrocarbon biodegradation increased with decrease of crude oil concentrations.

Keywords Biodegradation · Petroleum · Nutrients · Kinetic

Marine pollution can cause a variety of impacts and risks to human health and environment (Zahed et al. 2009). Particularly oil spill, whether accidental or due to human activities, is the main pollutant of marine ecosystems. Many petroleum components are toxic, mutagenic and carcinogenic and their clean-up can be very expensive (Mrayyana and Battikhi 2005). Consequently, environmental and socio-economic efficiencies are preferred to identification of remediation and management design (Liu et al. 2004).

Remediation based on pollution metabolism by micro-organisms is a capable approach to clean up polluted environments such as sediments (Diaz 2004). Hydrocarbons are consumed by microorganisms as food source. Therefore addition of nutrients can improve microbial and increase hydrocarbon removal. Accelerating the rate of crude oil bioremediation in contaminated sediment is a challenge to improving the performance and acceptance of soil bioremediation techniques (Zhu et al. 2001).

Bioremediation is an application of various methodologies to increase the rate or extent of the biodegradation process. The success of oil spill bioremediation depends on optimization of various physical, chemical, and biological conditions in the contaminated environment. The most important requirement is the presence of microorganisms with suitable concentrations of nutrients and oxygen and the pH. Some of these factors can be controlled more easily than others. When a major oil spill occurs in freshwater and marine environments, the amount of carbon increases and the availability of nitrogen and phosphorus usually becomes the limiting factor for oil degradation (Leahy and Colwell 1990). Typically, bioremediation starts soon after different concentration of oil and amendments are added to the soil. During bioremediation, the oil degrades through complex biochemical processes, and the changes of the soil microbial population and enzyme activities can be measured (Lin et al. 2009).

Statistical design of experiments can be used to analyze a large number of variables with a small amount of experiments and consequently within a relatively short time. This technique has been successfully used for optimization of process parameters in petroleum hydrocarbons

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Table 1 The condition of each reactor and hydrocarbon removal for all experiments

Run no.	Factors				Hydrocarbon removal (%)			
	Oil (g)	Bacteria	N (g)	P (g)	Observed	Predicted	Cook's Distance	Outlier t
1	2	0	0.2	0.02	47.01	50.59	0.17	−1.48
2	30	0	0.2	0.02	15.62	16.79	0.02	−0.46
3	2	2	0.2	0.02	67.85	62.48	0.37	2.41
4	30	2	0.2	0.02	19.78	19.55	0.00	0.09
5	2	0	3	0.02	54.06	49.98	0.22	1.72
6	30	0	3	0.02	51.25	50.71	0.00	0.21
7	2	2	3	0.02	60.24	61.87	0.03	−0.64
8	30	2	3	0.02	53.57	53.47	0.00	0.04
9	2	0	0.2	0.3	58.96	58.12	0.01	0.33
10	30	0	0.2	0.3	25.63	24.32	0.02	0.52
11	2	2	0.2	0.3	66.33	70.01	0.18	−1.53
12	30	2	0.2	0.3	27.27	27.07	0.00	0.08
13	2	0	3	0.3	63.01	64.75	0.04	−0.69
14	30	0	3	0.3	66.02	65.48	0.00	0.21
15	2	2	3	0.3	77.13	76.64	0.00	0.19
16	30	2	3	0.3	69.87	68.24	0.03	0.65
17	2	1	1.6	0.16	76.58	78.98	0.01	−0.75
18	30	1	1.6	0.16	52.23	57.88	0.05	−1.91
19	16	0	1.6	0.16	68.75	69.56	0.01	−0.31
20	16	2	1.6	0.16	74.17	76.88	0.08	−1.06
21	16	1	0.2	0.16	43.89	43.41	0.00	0.18
22	16	1	3	0.16	59.68	63.69	0.17	−1.62
23	16	1	1.6	0.02	51.85	55.78	0.16	−1.58
24	16	1	1.6	0.3	67.33	66.93	0.00	0.15
25	16	1	1.6	0.16	71.86	68.43	0.01	1.05
26	16	1	1.6	0.16	69.08	68.43	0.00	0.19
27	16	1	1.6	0.16	72.38	68.43	0.01	1.23
28	16	1	1.6	0.16	68.33	68.43	0.00	−0.03
29	16	1	1.6	0.16	70.15	68.43	0.00	0.52
30	16	1	1.6	0.16	73.9	68.43	0.03	1.77

bioremediation (Mohajeri et al. 2010; Zahed et al. (2010a, b); Vieira et al. 2009; Huang et al. 2008).

This work aims to improve the bioremediation of sediments artificially contaminated with weathered crude oil using concentrations of oil, nutrients and indigenous bacterial consortia as independent variables and to apply kinetic model for oil bioremediation.

Materials and Methods

Sediment was collected from the upper of 20 cm of the intertidal zone of Butterworth Beach, Penang, Malaysia. Sediment (1 kg) was transferred to the reactors (length, 30 cm, diameter, 15 cm). Light crude oil obtained from the Shell Refining Company, Port Dickson, Malaysia was artificially weathered (Mills et al. 2004). The sediment in the reactors was thoroughly mixed with weathered crude oil. Inorganic nutrients were added after oil addition at a C:N:P ratio of 100:10:1. Seawater was added periodically to reactors to balance losses due to evaporation. The seawater had a pH of 7.5 and total N and P concentrations of 2 and 0.04 mg/L, respectively. The acclimatized sample was stirred and aerated and maintained at room temperature under natural light conditions and pH 7.0. The reactors were monitored for 60 days. Detail of bacteria used in this research (characteristic, adaptation and enumeration) has been described by Mohajeri et al. (2010).

Total petroleum hydrocarbons (TPHs) were determined using (US-EPA 1999). The results were confirmed by gas chromatography. Hydrocarbon was extracted with dichloromethane (CH_2Cl_2) by sonication according to USEPA

Table 2 WCO removal at optimum condition for 2, 16 and 30 g/kg crude oil

Oil (g)	Bacteria (CFU)	N (g)	P (g)	WCO removal (%)					
				0 day	6 days	14 days	25 days	40 days	60 days
2	4.8×10^6	0.680	0.140	0	15.50	28.50	48.50	74.00	83.13
16	4.8×10^6	2.164	0.231	0	14.63	29.88	51.75	72.56	78.06
30	4.8×10^6	2.520	0.250	0	13.67	26.33	44.07	63.23	69.92

protocol 3550 (US-EPA 1991). Then it was passed through column chromatography using n-hexane as a solvent. After that GC 2000 Series gas chromatograph equipped with a flame ionization detector (Fisons Instruments, Milan, Italy) was used for hydrocarbon determination. The method detection limit (MDL) was 5 mg/kg soil. Extraction, analysis and QA/QC were carried out according to (APHA 2005) and (US-EPA 1991).

DESIGN EXPERT® 6.0.7 (Stat-Ease Inc., Minneapolis, USA) was used for statistical analysis of data and central composite design (CCD) under RSM was employed for design and modeling. Each of the independent variables, lie weathered crude oil concentration (A), bacteria concentration (B), nitrogen concentration (C), phosphorus concentration (D), was studied at three different levels as per CCD in four variables with 30 experiments. The minimum, maximum and mean values for weathered oil concentration were set at 0.2, 3 and 1.6 g/kg (sediment dry weight), the variables optimized were nutrient concentration (0.2, 3.0 and 1.6 g and 0.02–0.3 and 0.16 g nitrogen and phosphorus, respectively) and bacteria inoculum (2.4–4.8 $\times$ 10^6 CFU).

Results and Discussion

Regression model and analysis were described in accordance to Eq. 1 (Montgomery 2008):

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i \neq j=1}^{n} \beta_{ij} x_i x_{ij} + \varepsilon_i \tag{1}$$

The significance of each coefficient was checked by statistical tests including Student t-test and p-values. F-test indicated that all the factors and interactions considered in the experimental design are statistically significant ($p < 0.05$) at the 95% confidence level.

To evaluate the results, the data were subjected to regression analysis and the following quadratic equation was obtained (Mohajeri et al. 2010):

$$\begin{aligned} Y = {} & 68.43 - 10.55\,A + 3.66\,B + 10.14\,C + 5.57\,D \\ & + 4.79\,B^2 - 14.89\,C^2 - 7.08\,D^2 - 2.28\,AB + 8.63\,AC \\ & + 1.81\,CD \end{aligned} \tag{2}$$

where Y is the percent weathered crude oil removal, A is weathered oil concentration, B refer to bacteria inoculums, C is nitrogen concentration and D is phosphorus concentration. In this case A, B, C, D, B^2, C^2, D^2, AB, AC and CD are significant model terms. The condition of each reactor and hydrocarbon removal for all experiments is tabulated in Table 1. The highest removal of 77.13% was observed for 2 g/kg crude oil concentration.

WCO removal at optimum condition for 2, 16 and 30 g/kg crude oil are presented in Table 2. A significant correlation

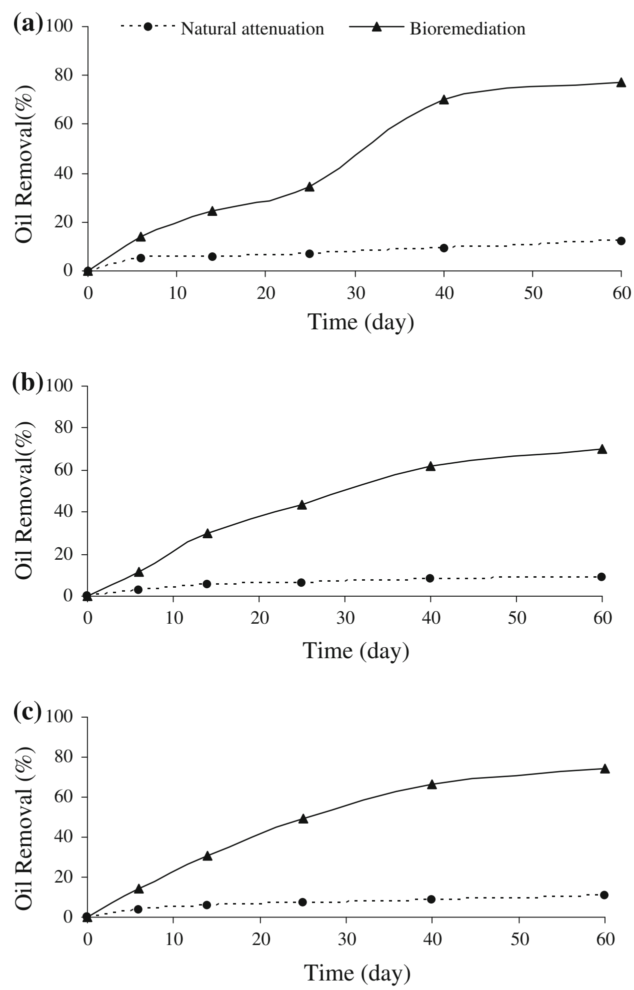

Fig. 1 Comparison natural attenuation and bioremediation for **a** 2, **b** 16, **c** 30 g oil/kg soil

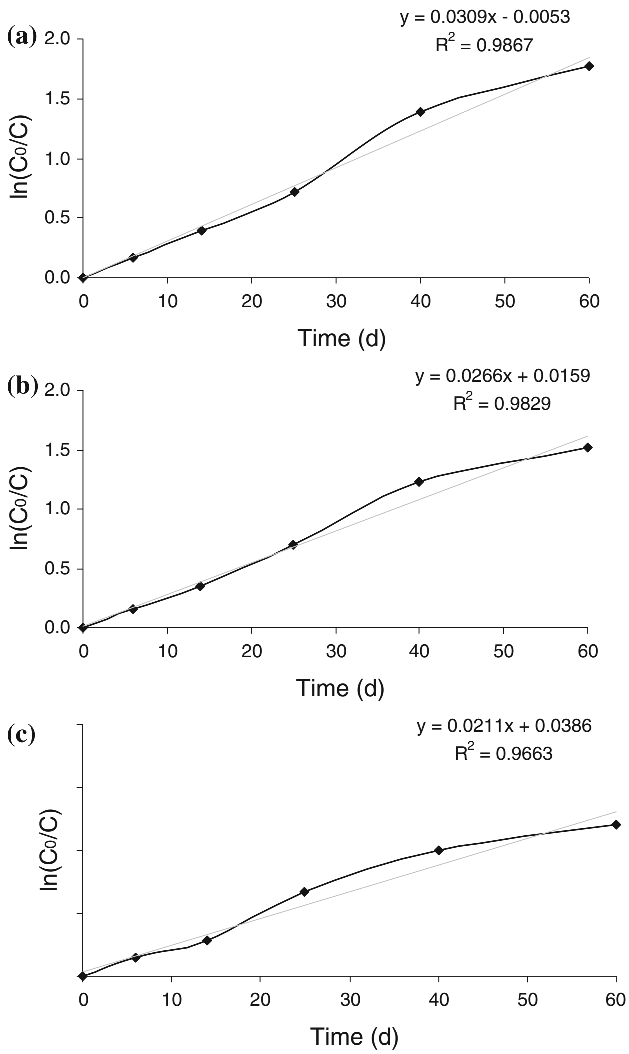

Fig. 2 Kinetic analysis of WCO Bioremediation for **a** 2 g oil/kg soil, **b** 16 g oil/kg soil, **c** 30 g oil/kg soil

between initial oil concentration and amount of reduction was observed: lower initial oil concentrations exhibited better removal in all experiments. The best hydrocarbon removal (87.13%) was observed for initial crude oil concentrations of 2 g/kg.

A comparison of natural attenuation and bioremediation is represented in Fig. 1a–c. Figure 1 demonstrated that WCO removal was faster in the reactor with 2 g oil/kg soil. The highest natural attenuation was also observed in this concentration. Two months monitoring of crude oil biodegradation rate shows that the process started slow and the rate became faster after 2 weeks. At the final stage, bioremediation was slower because bioavailability of organic mater and nutrients decreased during bioremediation experimentation.

First order kinetics is commonly used to describe petroleum hydrocarbons biodegradation (Venosa and Holder 2007; Roncevic et al. 2005). The following equation was used for kinetic study:

$$C = C_0 e^{-kt} \tag{3}$$

where C denotes concentration of hydrocarbons (g/kg), t is bioremediation time (day), C_0 is initial concentration of hydrocarbons (g/kg) and k is the rate constant for the change in hydrocarbon content of the soil (day^{-1}).

In order to clarify effect of initial oil concentration on biodegradation rate of WCO, three oil concentrations were compared. As presented in Fig. 2a, for 2 g/kg soil, $\ln(C_0/C)$ of 1.77 were observed in day 60 of bioremediation trial, while at the same time for 16 and 30 g/kg soil it was 1.52 and 1.20 (Fig. 2b and c, respectively). In general lower doses of oil shows more efficient degradation than high oil levels. Same effects were obtained by other researchers (Kim et al. 2005; Lepo et al. 2003; Zhu et al. 2001). Hence the application of bioremediation is not recommended for sediments highly contaminated with oil. The present experiments confirm that usage of optimum amount of nutrients as well as acclimatized microorganisms improved the rate of biodegradation in bioreactors simulating coastal environments contaminated with crude oil.

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