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Kinetic modeling and half life study on bioremediation of crude oil dispersed by Corexit 9500

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ABSTRACT

Hydrocarbon pollution in marine ecosystems occurs mainly by accidental oil spills, deliberate discharge of ballast waters from oil tankers and bilge waste discharges; causing site pollution and serious adverse effects on aquatic environments as well as human health. A large number of petroleum hydrocarbons are biodegradable, thus bioremediation has become an important method for the restoration of oil polluted areas. In this research, a series of natural attenuation, crude oil (CO) and dispersed crude oil (DCO) bioremediation experiments of artificially crude oil contaminated seawater was carried out. Bacterial consortiums were identified as *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Pseudomonas* and *Vibrio*. First order kinetics described the biodegradation of crude oil. Under abiotic conditions, oil removal was 19.9% while a maximum of 31.8% total petroleum hydrocarbons (TPH) removal was obtained in natural attenuation experiment. All DCO bioreactors demonstrated higher and faster removal than CO bioreactors. Half life times were 28, 32, 38 and 58 days for DCO and 31, 40, 50 and 75 days for CO with oil concentrations of 100, 500, 1000 and 2000 mg/L, respectively. The effectiveness of Corexit 9500 dispersant was monitored in the 45 day study; the results indicated that it improved the crude oil biodegradation rate.

1. Introduction

Crude oil spillage can cause dramatic damage to the oceans and coastal areas. It may persist in shorelines for years and have catastrophic effects on the marine environment. Some fractions of oil can cause chronic sub-acute toxicological effect (reduced growth and reproduction, poor health, low recruitment rates), which can alter population dynamics and disrupt tropic interactions and the structure of natural communities within ecosystems [1]. After weathering of the surface oil layer by evaporation, dispersion, and dissolution and by sedimentation of heavier slicks or after physical removal of the major part of oil slicks, dissolved hydrocarbons in the water may still be toxic for organisms [2]. It is estimated that more than 2 million tons of oil enters marine environments from ships and other sea-based activities annually. Table 1 summarizes total hydrocarbon pollution of marine environments worldwide [3].

Physical/mechanical methods are the primary response options for oil spill clean up but the crude oil recovery is only about 10–15% [4]. Physical strategies include the use of booms, skimmers, washing, cutting vegetation and burning [5]. Chemical methods, including the application of dispersants, demulsifiers, biosurfactants, surface film chemicals and cleaners, have been developed in the past two decades. Often, the bioavailability of crude oil increased after application of chemical methods [6,7]. However it is not a sustainable technique since it does not remove oil from the sea and just transfers oil on water layer and/or sediments. Biological treatment is based on the fact that a large percentage of petroleum compounds are biodegradable. In fact, the most significant environmental recovery mechanism is biodegradation. Bioremediation is the use of some techniques to accelerate contaminant biodegradation.

Several factors may affect hydrocarbon degradation and, in particular, the oil concentration is an important consideration in determining whether bioremediation is a viable option [8].

Hydrocarbons are hydrophobic compounds with low water solubility; therefore one of the major factors limiting the degradation of hydrocarbons is their low availability to the microbial cells. Microorganisms employ several strategies to enhance availability of hydrophobic pollutants, such as biofilm formation and biosurfactant production [9]. Dispersant application to accelerate crude oil bioavailability has been developed and described in numerous reports [10–13].

The dispersant Corexit 9500® was formulated in 1992. This dispersant effectively extends the "window of opportunity" because it is effective on crude with viscosities up to 20,000 cp and it is also lower in toxicity than other dispersants for most species [14]. US-

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Table 1
Annual hydrocarbon contamination of marine environments worldwide [3].

Source	Amount (1000 tons/year)	Percent
Land-based	1200	45.45
Oil transportation and shipping	457	17.31
Offshore production discharge	20	0.76
Small craft activity	53	2.01
Atmospheric fallout	300	11.36
Natural seeps	600	22.73
Other	10	0.38
Total	2640	100.00

EPA and Nalco (Nalco Company, Naperville, IL) released the list of the ingredients in Corexit 9500, revealing constituents including sorbitan, butanedioic acid, and petroleum distillates. The identity of the sulfonate used in the dispersant was disclosed to the US-EPA in June 2010, as dioctyl sodium sulfosuccinate [15].

Kinetics of bioremediation process can be evaluated in two ways: (1) the first concerns with the factors influencing the amount of transformed compounds with time and (2) the other approach seeks the types of curves describing the transformation and determines which of them fits the degradation of the given compounds by the microbial culture [16]. Studies of biodegradation kinetics in a natural environment are often empiric, reflecting only the basic level of knowledge about the microbial population and its activity in the given environment [17]. Lighter crude oils (higher API gravity) normally have faster biodegradability than heavier ones. On the other hand, different crude oil components such as aliphatic, aromatic and polycyclic compounds have dissimilar degradation rates. Thus, prediction of petroleum biodegradation kinetics is complicated and difficult in most cases. Furthermore due to differences in experimental techniques or data analysis, variations in the biokinetic constants have been reported for the same conditions [18].

Although kinetic is essential to determine the equilibrium constant, the speed of reaction and control of the process in hydrocarbon bioremediation studies, there is still a lack of knowledge on the subject of hydrocarbon bioremediation kinetics. The aim of this study is to evaluate, model and analyse degradation kinetics for natural attenuation, crude oil (CO) bioremediation and dispersed crude oil (DCO) bioremediation. Effectiveness of the dispersant Corexit 9500 was monitored for the 45 day study as well.

2. Material and methods

2.1. Sampling

Samples were collected from Perai area, Butterworth, northwest Malaysia (latitude: 5°22′52.67″N, longitude: 100°22′15.57″E). Water characteristics at the sampling station are presented in Table 2. Microorganism acclimatization was carried out as described elsewhere [19]. The isolates were characterized and identified according to Cowan and Steel's Manual [20] and Bergey's Manual [21]. Light crude oil was obtained from Shell (Port Dickson, Malaysia). The crude oil was a mixture of Tapis, Bintulu, Miri Light and Sutu den with percentages of 54, 17, 5 and 24%, respectively.

Table 2
Water characteristics at sampling station.

Property	Amount
Seawater pH	8.1 ± 0.1
Temperature (°C)	27.5 ± 1.5
DO (mg/L)	4.1 ± 0.6
COD (mg/L)	760 ± 120
TPHs (mg/L)	3.4 ± 1.2
Total nitrogen (mg/L)	2.0 ± 0.4
Total phosphorus (mg/L)	0.04 ± 0.02

2.2. Bioremediation experimentation

Erlenmeyer flasks were used as bioreactors and 250 mL oil-contaminated seawater was transferred to each flask at oil concentration of 100, 500, 1000 and 2000 mg/L.

A reactor was treated with the biocide $HgCl_2$ as an abiotic control to show the effect of evaporation and other physical reactions in the absence of microbial activity. A natural attenuation test was carried out for each oil concentration; the bioreactors were prepared without nutrient or microorganism supplementation. For CO bioremediation experiments, bioreactors were supplemented with acclimatized microorganisms and nutrients with a C:N:P ratio of 100:10:1 for each reactor. One milliliter bacterial inoculums containing 1.2×10^7 cells/mL were added to each bioreactor. KNO_3 and K_2HPO_4 were used as nitrogen and phosphorus sources.

To evaluate the effectiveness of the dispersant Corexit 9500 (Exxon, NJ) the DCO experiments were performed by supplementation of Corexit 9500 at a ratio of 20:1 (w/w) crude oil to dispersant [13]. Reactors were shaken continuously on an orbital shaker and samples were collected after 7, 15, 30, and 45 days for analysis.

2.3. Chemical analysis and quality control

Seawater characteristics and nutrients were measured using standard methods for examination of water and wastewater [22]. Total petroleum hydrocarbons analyses were carried out in accordance with US-EPA procedures [23]. Quality assurance and quality control (QA/QC) were performed to ensure quality of analysis. Verification of calibration was carried out with each set of analysis, blanks were analyzed regularly as a check for possible contamination and interferences. Blanks did not contain any interference. Method detection limit was 2 mg/L, average recovery was 88.67% and precision (relative standard deviation) was 11.17%. The results were verified by gas chromatography analysis [10] using US-EPA test methods [24]. Iranian light crude oil was used as certified reference materials (CRM) and analyzed periodically [24]. An average error of 3.7 ± 1.2% indicated the test reliability.

Percent degradation (D) was calculated using the following formula:

$$D = \frac{C_0 - C_r}{C_0} \times 100 \quad (1)$$

where C_0 and C_r are the initial and residual oil concentrations, respectively.

2.4. Data analysis

One way analysis of variance (ANOVA) tests at the level of $p < 0.05$ were performed using statistical package for social sciences, version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. TPH removal

TPH removal by natural attenuation is presented in Fig. 1(a). Natural attenuation is a variety of processes that naturally act to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in the environment, and includes biodegradation, dispersion, sorption, dilution, volatilization, and chemical or biological stabilization, transformation, or destruction of contaminants [25]. The highest removal by natural attenuation is observed for 100 mg/L oil concentration. Fig. 1(b) illustrates degradation of crude oil without dispersant addition. Bioremediation was fast in the early stage for all concentrations. Low concentrations show significantly higher percentage removal.

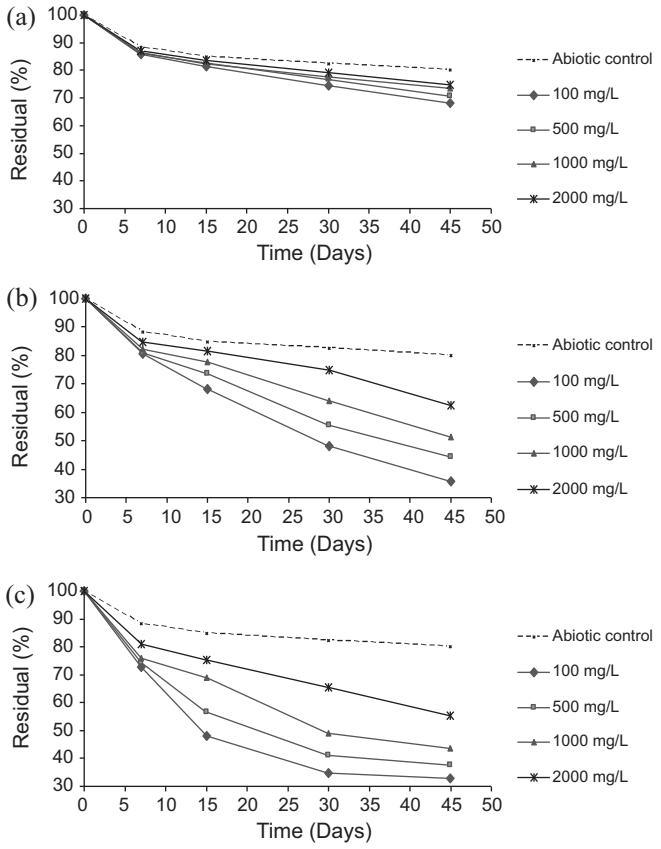

Fig. 1. Biodegradation of different concentrations of crude oil (a) natural attenuation, (b) crude oil without dispersant and (c) crude oil and Corexit 9500 dispersant.

With regard to the dispersed crude oil bioremediation data (Fig. 1(c)) it is notable that most of the hydrocarbon degradation was achieved in 30 days. Hence a one month bioremediation period is recommended to reduce the expenses of clean up for this type of crude oil. According to this figure, medium and low concentrations of hydrocarbons (1000 mg/L and below) exhibited good TPH elimination. Thus bioremediation is more practical for this range of petroleum pollution. High concentrations of hydrocarbons can cause inhibition of biodegradation due to toxic effects, although the inhibitory concentration varies with oil composition. Hence, there is an optimum oil concentration range for bioremediation applications [26]. Zahed et al. [11] stated that the efficiency of crude oil bioremediation in marine ecosystems is directly related to the oil concentration and application of dispersant.

3.2. Kinetic evaluation

Kinetic analysis is a key factor for biodegradation process understanding, bioremediation speed measurement and development of efficient clean up for a crude oil contaminated environment. Bacteria growth rate were described based on Monod expression [27]. Since biodegradability of crude oil is usually explained by first order kinetics [12,16,28–31], Eq. (2) was employed in this analysis.

$$C = C_0 e^{-kt} \quad (2)$$

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

Plotting the logarithm of hydrocarbon concentration versus time presents appropriate information about the biodegradation rate. Kinetic evaluation of different crude oil concentrations is illustrated in Fig. 2(a)–(d). The addition of dispersant improved TPH removal. Kinetic equation for different remediation strategies are tabulated in Table 3. In natural attenuation studies the process was fast in the first week and continued slowly during the study thus the lowest k was detected.

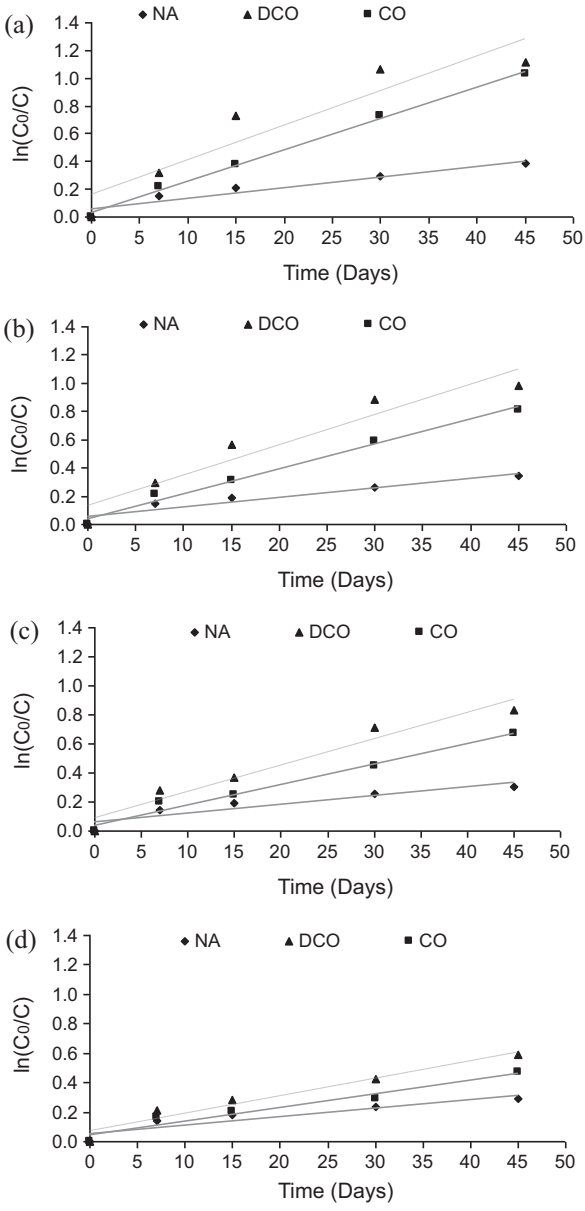

Fig. 2. Biodegradation kinetic evaluation of crude oil concentration of (a) 100 mg/L, (b) 500 mg/L, (c) 1000 mg/L and (d) 2000 mg/L.

The kinetic evaluation and biodegradability of crude oil in the presence of Corexit 9500 were described by Venosa and Holder [12], and they concluded type of oil, surfactant formulation in commercial dispersants, differences in uptake of the various hydrocarbon constituents of oil, effects of surfactants on bacterial attachment to oil droplets, effects of dilution, and their vast interrelationships are some of the factors that affect dispersant efficacy for enhancement of crude oil biodegradation.

3.3. Estimation of biodegradation half life times

The biological half-life is the time taken for a substance to lose half of its amount. Biodegradation half-lives are needed for many applications such as chemical screening [32] environmental

Table 3
Kinetic expression and half life times for different remediation strategies.

No.	Remediation strategy	Oil (mg/L)	Kinetic expression[a]	R^2	$t_{1/2}$ (days)
1	Natural attenuation	100	$y = 0.0077x + 0.0578$	0.9228	90
2	Natural attenuation	500	$y = 0.0068x + 0.0584$	0.9017	102
3	Natural attenuation	1000	$y = 0.0060x + 0.0646$	0.8508	116
4	Natural attenuation	2000	$y = 0.0056x + 0.0588$	0.8606	124
5	CO Bioremediation	100	$y = 0.0225x + 0.0336$	0.9961	31
6	CO Bioremediation	500	$y = 0.0175x + 0.0448$	0.9883	40
7	CO Bioremediation	1000	$y = 0.0139x + 0.0425$	0.9800	50
8	CO Bioremediation	2000	$y = 0.0092x + 0.0477$	0.9420	75
9	DCO Bioremediation	100	$y = 0.0248x + 0.1634$	0.8763	28
10	DCO Bioremediation	500	$y = 0.0215x + 0.1292$	0.9152	32
11	DCO Bioremediation	1000	$y = 0.0181x + 0.0877$	0.9490	38
12	DCO Bioremediation	2000	$y = 0.0120x + 0.0694$	0.9517	58

[a] $y = \ln C_0/C$, $x = t$ (days).

fate modeling [33] and describing the transformation of pollutants [34,35]. Biodegradation half life times ($t_{1/2}$) are calculated by Eq. (3) [33,35–37].

$$t_{1/2} = \frac{\ln(2)}{k} \quad (3)$$

where k denotes the rate constant. Rate constant is calculated according to Eq. (2) and presented in kinetic expression list in Table 3. The highest $t_{1/2}$ of 124 days observed for natural attenuation in oil concentration of 2000 mg/L. This was reduced to 75 days and 58 days for CO and DCO, respectively; thus, showing the positive effect of microorganisms and nutrient supplementation and dispersant addition.

3.4. Effectiveness of Corexit 9500

The effectiveness of Corexit 9500 was tested. Through evaluation of CO and DCO bioremediation, dispersant efficiency (DE) was calculated at different times using Eq. (4) [11]:

$$\mathrm{DE} = \frac{R_{\mathrm{DCO}} - R_{\mathrm{CO}}}{R_{\mathrm{DCO}}} \times 100 \quad (4)$$

where R_{CO} is the removal of crude oil (%) and R_{DCO} is the removal of dispersed crude oil (%). The results of DE in different times are illustrated in Fig. 3. The best fit equation of the trendline is as follows:

$$y = -0.03x^2 + 1.1523x + 19.546 \quad (5)$$

where y is dispersant efficiency and x is time (days).

As presented in Fig. 3, the highest DE of 38% was observed on day 15, indicating that Corexit 9500 is more effective in early stage of bioremediation. The results of this study clearly indicated that application of Corexit 9500 dispersant can enhance biodegradation of crude oil via increased bioavailability of petroleum hydrocarbons to marine microorganisms. The dispersant is capable to break emulsions, disperse the oil and enhance the biodegradation of dispersed oil by providing a digestible substrate that stimulates the growth of organisms and makes more of them available to use hydrocarbons as source of energy [14].

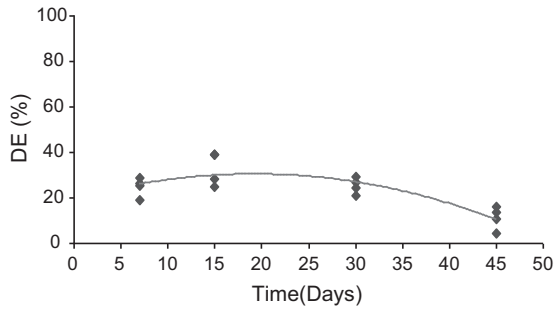

Fig. 3. Dispersant efficiency in different times.

Bioavailability may be the critical limiting factor controlling biodegradation rates for many organic compounds with low water solubility. This phenomenon was also reported by Lindstrom and co authors. They stated that the Corexit 9500 could increase the effectiveness of Corexit 9500 which enhances the bioavailability of crude oil in cool region [38].

3.5. Comparison with other reports

Results presented in this paper showed that addition of nutrients as well as microorganisms enhanced TPH removal. Bacterial consortiums were identified as *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Pseudomonas* and *Vibrio*. The performance of strain was detailed in Mohajeri and co authors [19]. A maximum of 64.2 and 67.3% TPH removal in seawater contaminated with 100 mg/L was observed for CO and DCO bioremediation, respectively in this study.

Gentili and co-authors [39] reported hydrocarbon removals of 40% by using bacterial strain immobilized on chitin and chitosan flakes. TPH removal has also been reported to be about 61% using *Spongia officinalis* [40] and 70% using Guano as fertilizer [41]. When biostimulation was employed, hydrocarbon metabolization was more efficient, also showing a higher velocity of microbial action due to the appropriate C/N/P ratio [41].

The use of dispersants has been thought to stimulate the natural process of biodegradation, because microbial attack is at the oil–water interface and the dispersion of the oil dramatically increases the area available for microorganisms [14]. The results of this study also confirmed the effectiveness of Corexit 9500 dispersant in improving bioavailability of hydrocarbons [13,42–44].

4. Conclusions

A comparison of bioremediation of crude oil and dispersed crude oil at different initial oil concentrations were carried out using indigenous microorganisms. The present experiments confirm that the use of dispersant improved the rate of biodegradation in bioreactors simulating marine environments contaminated with crude oil. Dispersant was more effective for higher oil concentration and a maximum dispersant efficiency (DE) of 38% was observed on day 15. A significant correlation between initial oil concentration and amount of TPH reduction was observed: lower initial oil concentrations exhibited higher removal efficiencies in all experiments. First order kinetics described the crude oil biodegradation with and without dispersant. Half life times of 31, 40, 50 and 75 days were observed for crude oil concentration of 100, 500, 1000 and 2000 mg/L, respectively. These were reduced, respectively, to 28, 32, 38 and 58 days with the usage of dispersant. The best hydrocar-

bon removal of 67% was obtained for initial crude oil concentrations of 100 mg/L. Furthermore, the most efficient removal for low initial concentrations of dispersed crude oil occurred within the first 30 days.

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