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Effect of Initial Oil Concentration and Dispersant on Crude Oil Biodegradation in Contaminated Seawater

Mohammad Ali Zahed · Hamidi Abdul Aziz · Mohamed Hasnain Isa · Leila Mohajeri

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Abstract The effects of initial oil concentration and the Corexit 9500 dispersant on the rate of bioremediation of petroleum hydrocarbons were investigated with a series of ex-situ seawater samples. With initial oil concentrations of 100, 500, 1,000 and 2,000 mg/L, removal of total petroleum hydrocarbons (TPHs) with dispersant were 67.3%, 62.5%, 56.5% and 44.7%, respectively, and were 64.2%, 55.7%, 48.8% and 37.6% without dispersant. The results clearly indicate that the presence of dispersant enhanced crude oil biodegradation. Lower concentrations of crude oil demonstrated more efficient hydrocarbon removal. Based on these findings, bioremediation is not recommended for crude oil concentrations of 2,000 mg/L or higher.

Keywords Petroleum · Oil spill · Bioremediation · Bioaugmentation · Dispersant

Hydrocarbon contamination has been addressed as the main critical challenge in aquatic ecosystems. In 2007, the IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) estimated that oil enters marine environments from ships and other sea-based activities at a rate of 2,640,000 tons/year.

Bioremediation can be successful only if hydrocarbon-degrading microorganisms are present in the contaminated environment and they have the degradative capacity to deal with a high proportion of the hydrocarbons present. Pioneering studies by Atlas and Bartha (1973) demonstrated that the available concentrations of nitrogen and phosphorus in seawater are limiting factors for the growth of hydrocarbon-degrading microorganisms. Thus, the addition of nitrogen and phosphorus fertilizers stimulates the biodegradation of petroleum. Treatment of oil spills with dispersants in temperate marine environments has been common practice for many years. A major reason for using these chemicals is to prevent spilled oil from reaching the shore. However, it is thought that dispersed oil presents an increased toxicity to marine life compared to untreated oil, as a result of the detrimental effects of surfactants and elevated hydrocarbon dissolution (Epstein et al. 2000). Therefore, marine biota may be affected by both dispersant and oil as well (Anderson et al. 2009; Belore et al. 2009; Singer et al. 2000; Wolfe et al. 1999). Lindstrom and Braddock (2002), however, reported that nutrient addition may be important to consider in environmental application of dispersant Corexit 9500 since the dispersant appears to be a good microbial substrate.

The goal of this research was to evaluate biodegradation rates of crude oil (CO) and dispersed crude oil (DCO) at different initial oil concentrations with and without the addition of the Corexit 9500 dispersant in a simulated marine environment. The results compared with related natural attenuation and abiotic control.

Materials and Methods

Four locations were selected on and near Penang Island in northwest Malaysia for source material used in the experiments: (1) Batu Ferringhi Beach on the northern side of

M. A. Zahed · H. A. Aziz (✉) · L. Mohajeri
School of Civil Engineering, Universiti Sains Malaysia, 14300 Nibong Tebal, Penang, Malaysia
e-mail: cehamidi@eng.usm.my

M. H. Isa
Civil Engineering Department, Universiti Teknologi PETRONAS, 31750 Tronoh, Perak, Malaysia

Table 1 Conditions at stations

Property	Station 1	Station 2	Station 3	Station 4
Latitude	5°28′2.52″N	5°20′31.22″N	5°23′41.15″N	5°22′52.67″N
Longitude	100°14′23.65″E	100°18′43.22″E	100°21′49.59″E	100°22′15.57″E
Seawater pH	7.4 ± 0.1	7.5 ± 0.2	7.8 ± 0.2	8.1 ± 0.1
Temperature (°C)	26.0 ± 2.5	26.5 ± 3.5	27.0 ± 3.0	27.5 ± 1.5
DO (mg/L)	7.2 ± 0.1	5.2 ± 0.2	3.7 ± .02	4.1 ± 0.6
COD (mg/L)	530 ± 40	710 ± 30	920 ± 80	760 ± 120
TPHs (mg/L)	ND	2.7 ± 0.4	6.2 ± 3.6	3.4 ± 1.2
Total nitrogen (mg/L)	0.9 ± 0.2	1.1 ± 0.2	1.5 ± 0.3	2.0 ± 0.4
Total phosphorus (mg/L)	0.03 ± 0.01	0.03 ± 0.02	0.05 ± 0.03	0.04 ± 0.02

Penang Island, (2) Penang Bridge on the south western side near Jerejak Island, (3) in Perai area near the ferry station in Butterworth, and (4) in Perai at the Butterworth river estuary. A selection of conditions measured at these four stations is summarized in Table 1 Station 4 exhibited the best microorganismic acclimatization, presumably because it is located near an industrial area supporting high biodiversity.

A modified medium, containing 1 g/L NH_4NO_3, 1 g/L KH_2PO_4, 1 g/L K_2HPO_4, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.05 g/L $FeCl_3$, and 0.02 g/L $CaCl_2$, was used to culture bacteria. The mixture was stirred, aerated and maintained at room temperature and pH 7.0–7.8 under natural light conditions as described by Mohajeri et al. (2010). Light crude oil was obtained from Shell Refining Company (F.O.M.) Berhad (Port Dickson, Malaysia). The components of the crude oil samples used for experiments and their characteristics are presented in Table 2.

Laboratory scale bioremediation trials were carried out on seawater samples collected from station 4. The oil concentrations at experiment begin were 100, 500, 1,000 and 2,000 mg/L. Erlenmeyer flasks were used as bioreactors: 250 mL oil-contaminated seawater was transferred to each flask. Four types of bio reactors were prepared as presented in Table 3.

Table 2 Crude oil component and their characteristics

	Unit	Tapis	Bintulu	Miri light	Sutu den
Density at 15°C	kg/L	0.798	0.842	0.864	0.849
API	API degree	45.8	36.5	32.3	35.1
Barrel factor	BBL/T	7.898	7.483	7.296	7.423
Sulphur content	Wt%	0.03	0.06	0.08	0.05
Pour point	°C	12	−6	−9	36
Viscosity at 20°C	Pa.s	3.51	4.08	4.69	73.6
Viscosity at 40°C	Pa.s	2.14	2.51	3.00	23.8
Reid vapor pressure	kpa	28	20	29	7.4
Crude oil components	%	54	17	5	24

Table 3 Experiment conditions

No	Experiment	Nutrient	Microorganism	Dispersant	Biocide
1	CO bioremediation	+	+	−	−
2	DCO bioremediation	+	+	+	−
3	Natural attenuation	−	−	−	−
4	Abiotic control	−	−	−	+

In CO experiments, bioreactors were supplemented with acclimatized microorganism and nutrients with a C:N:P ratio of 100:10:1 for each reactor. KNO_3 and K_2HPO_4 were used as nitrogen and phosphorus sources. 1.00 mL microorganism inoculums were added to each bioreactor containing 1.2×10^7 cell/mL.

In DCO experiments bioreactors were supplemented the dispersant Corexit 9500 at a ratio of 20:1 (w/w) crude oil to dispersant, as suggested by Lindstrom and Braddock (2002).

In natural attenuation controls for each oil concentration, bioreactors were prepared without nutrient or microorganism supplementation.

In an abiotic control, a bioreactor was treated with the biocide $HgCl_2$ to show the effect of evaporation, photo oxidation and other physical reactions in the absence of microbial activity. Reactors were shaken continuously on an orbital shaker and samples were removed for assay at 7, 15, 30, and 45 days.

Nitrogen and phosphorus were determined according to APHA 2005, and total petroleum hydrocarbons (TPH) were measured by the US-EPA Gravimetric Method-1664 (US-EPA 1999). Calibration verification and analysis of blanks were performed daily, and percent recovery (*S*) was calculated using Eq. (1)

$$S = \sqrt{\frac{\sum x^2 - \frac{\left(\sum x\right)^2}{n}}{n-1}} \quad (1)$$

where n refer to number of samples and x is percent recovery in each sample.

In addition, matrix spike (MS) was tested to ensure the accuracy of analysis: the relative percent difference (RPD) between the matrix spike and matrix spike duplicated (MSD) were calculated with Eq. (2):

$$\text{RPD} = \frac{|D_1 - D_2|}{(D_1 - D_2)/2} \quad (2)$$

where D_1 is the concentration of hexane extractable material in the sample and D_2 is the concentration of hexane extractable material in the duplicate sample. Quality assurance and quality control were performed following US-EPA 1999. Method detection limit (MDL) was 2 mg/L, average recovery was 88.67% and precision (relative standard deviation) was 11.17%.

Hydrocarbon determination was also comfored by Gas chromatographic analysis using US-EPA SW 846 procedures (US-EPA 1991). Samples were extracted by dichloromethane (DCM), analysis were carried out using a GC 2000 gas chromatograph equipped with a FID (Fisons Instruments, Milan, Italy). A DB-5 capillary column (J&W Scientific, Folsom, CA, USA; 60 m × 0.25 mm I.D., film thickness 0.25 μm) employed. Injector and detector temperatures seted at 300°C. Carrier and make-up gas was He and N_2, respectively. The oven temperature program was 2 min at 70°C, increasing by 5°C/min up to 180°C and by 10°C/min up to 270°C, and finally 3 min at 270°C. GC software was Chrom-Card version 2.0 (Thermo Electron, Rodano, Italy).

Statistical analysis of the results was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Analysis of variances (ANOVA) tests at the level of $p < 0.05$ were carried out for identification of correlations between results.

Results and Discussion

Under abiotic control conditions, the oil removal observed was 19.9%, presumable resulting from evaporation and photo-oxidation. This value is higher than average reported by Wang et al. (1998), due to the nature of light crude oil used in this study.

The natural attenuation control exhibited a crude oil removal of 31.8%, 29.2%, 26.4% and 25.2% for initial oil concentrations of 100, 500, 1,000 and 2,000 mg/L, respectively. These results indicate that removal through natural attenuation decreases with increasing oil concentration. Most of the oil removal both for abiotic control and natural attenuation occurred in the first week of the experiment.

Figure 1a illustrates percentage of residual oil for different days at initial oil concentrations of 100 mg/L. The best removal was obtained from this concentration. Most of the oil removal occurred within the first 30 days for all experiments, indicating that DCO bioremediation treatment may be effectively for 1 month.

For initial oil concentration of 500 mg/L, presented in Fig. 1b, removal for both CO and DCO bioremediation was significantly lower (55.7% and 62.5%, respectively) compare to results observed with 100 mg/L initial oil concentration. Hence high initial oil concentration results less removal during the same time period.

Figure 1c presents removal with initial oil concentrations of 1,000 mg/L. In DCO experiments, the rate of removal was faster in the first 4 weeks than subsequently. In CO experiments, the rate was the greatest in the final 2 weeks, presumably because bioavailability of biodegradable hydrocarbon was not efficient in the first 30 days. The lowest rates of removal were obtained for bioreactors with initial oil concentration of 2,000 mg/L, presented in Fig. 1d. No significant difference was observed between CO and DCO bioaugmentation in this test for the first 2 weeks, and the degradation rate was relatively slow throughout the experiment. A significant inverse correlation between initial oil concentration and amount of oil removal was observed. Based on these results, bioremediation is not recommended for initial oil concentrations of greater than 1,000 mg/L. The observed efficacy is greater than reported elsewhere: Gentili et al. (2006) observed 60% removal of hydrocarbons from crude-oil-polluted seawater and Da Silva et al. (2009) reported up to 50% of hydrocarbon removal in a 30 day trial. The effectiveness of Corexit 9500 on heavy fuel oils was investigated by Srinivasan et al. (2007). Venosa and Holder (2007) also reported that Corexit 9500 rates were slightly higher than the corresponding rates for the non-dispersed oil. Dissolved fractions of hydrocarbon in the aqueous phase are more available for microbiological degradation and, therefore, application of dispersant significantly enhanced crude oil biodegradation in all conducted experiments.

The following formula was used to calculate the dispersant efficiency (DE) percentage:

$$\text{DE} = \frac{R_{\text{DCO}} - R_{\text{CO}}}{R_{\text{DCO}}} \times 100 \quad (3)$$

where R_{CO} is the removal of crude oil (%) and R_{DCO} is the removal of dispersed crude oil (%) at different runs. The data were fitted to the Eq. (4) with R^2 of 0.9866.

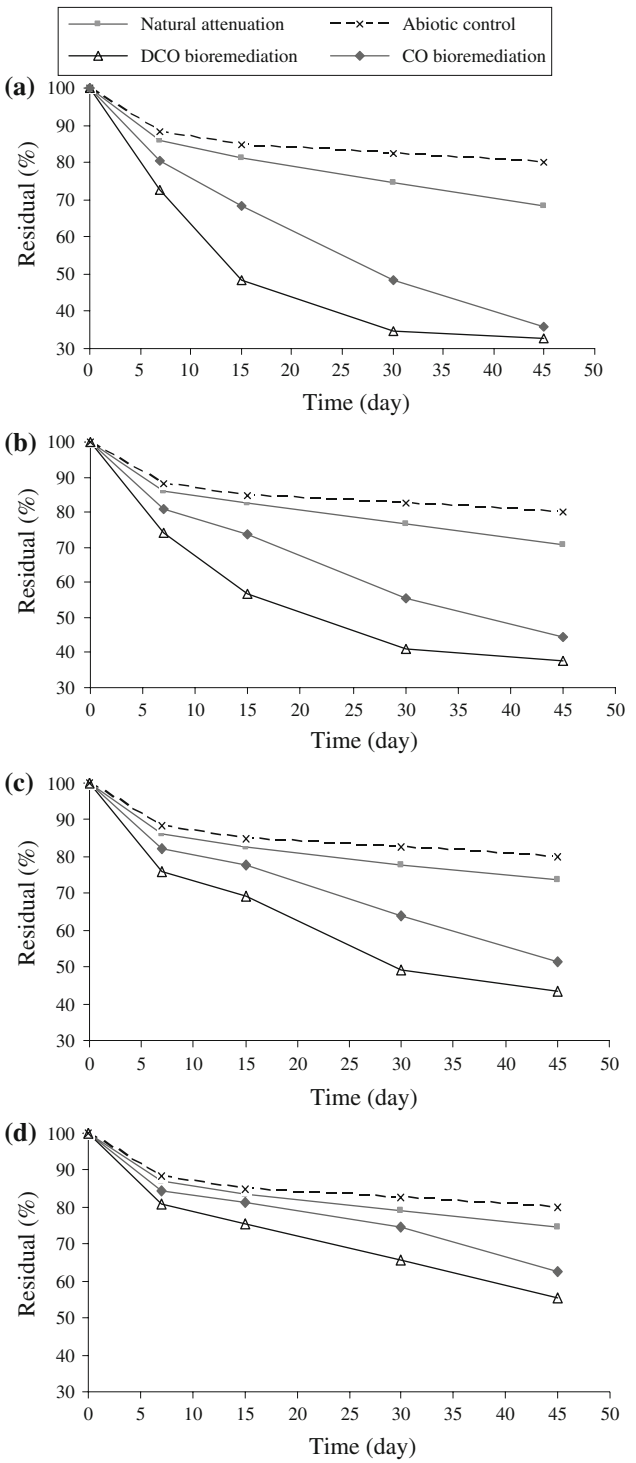

Fig. 1 Reduction of oil concentration in bioreactors containing an initial oil concentration of **a** 100 mg/L, **b** 500 mg/L, **c** 1,000 mg/L and **d** 2,000 mg/L

$$y = -5E - 06x^2 + 0.0182x + 3.4749 \quad (4)$$

As can be seen in Fig. 2, DE was increased by rising oil concentrations and dispersant, enhancing biodegradation. Therefore, the use of dispersant is recommended for

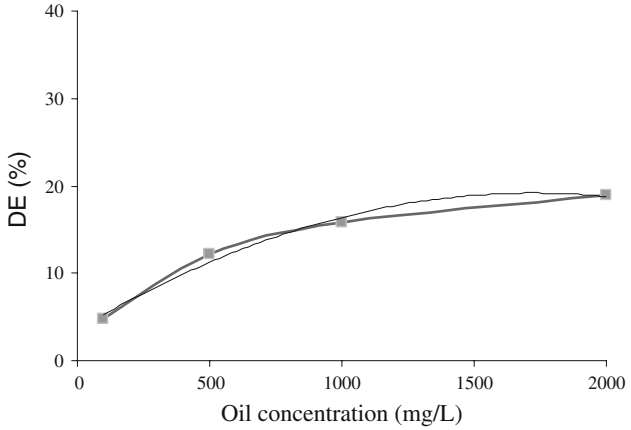

Fig. 2 Dispersant effectiveness percentage for different oil concentration (mg/L)

bioremediation of high oil concentrations. It is thought that chemical dispersant alters oil droplets to make them smaller and less hydrophobic and, hence, more easily broken up and dispersed through physical agitation.

Knowledge of the oil biodegradation rate under different environmental conditions is important for assessing the potential fate of targeted compounds, evaluating the efficacy of bioremediation, and determining appropriate strategies to enhance oil biodegradation. Oil biodegradation rates are difficult to predict due to the complexity of the environment. The rates of biodegradation vary greatly among the various components of crude oils and petroleum products. The presence of other substrates may affect the degradation rates of the compounds of interest. Environmental factors such as temperature, nutrient concentrations, and oxygen tension also influence the kinetics of oil degradation. The heterogeneity of oil distribution on shorelines or wetland sediments makes kinetics studies even more difficult (Head and Swannell 1999).

The findings of this research clearly support the use of bioremediation for initial oil concentrations of 1 g/L and below. Bioremediation is effective for at least 45 days. The results also indicated that hydrocarbon degradation increases greatly as a result of bioavailability and adding dispersant significantly increase bioremediation rate of crude oil in the marine environment.

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References

Anderson BS, Arenella-Parkerson D, Phillips BM, Tjeerdema RS, Crane D (2009) Preliminary investigation of the effects of dispersed Prudhoe Bay crude oil on developing topsmelt embryos, *Atherinops affinis*. Environ Pollut 157:1058–1061

APHA (2005) Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association, Washington, DC

Atlas RM, Bartha R (1973) Stimulated biodegradation of oil slicks using oleophilic fertilizers. Environ Sci Technol 7:538–541

Belore RC, Trudel K, Mullin JV, Guarino A (2009) Large-scale cold water dispersant effectiveness experiments with Alaskan crude oils and Corexit 9500 and 9527 dispersants. Mar Pollut Bull 58:118–128

Da Silva AC, Oliveira FJ, Bernardes DS, Franca FP (2009) Bioremediation of marine sediments impacted by petroleum. Appl Biochem Biotechnol 153:58–66

Epstein N, Bak RPM, Rinkevich B (2000) Toxicity of third generation dispersants and dispersed Egyptian crude oil on red sea coral larvae. Mar Pollut Bull 40:497–503

Gentili AR, Cubitto MA, Ferrero M, Rodriguéz MS (2006) Bioremediation of crude oil polluted seawater by a hydrocarbon-degrading bacterial strain immobilized on chitin and chitosan flakes. Int Biodeterior Biodegrad 57:222–228

GESAMP (2007) Estimates of oil entering the marine environment from sea-based activities (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep Stud GESAMP No. 75, London

Head IM, Swannell RPJ (1999) Bioremediation of petroleum hydrocarbon contaminants in marine habitats. Curr Opin Biotechnol 10:234–239

Lindstrom JE, Braddock JF (2002) Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant Corexit 9500. Mar Pollut Bull 44:739–747

Mohajeri L, Aziz HA, Isa MH, Zahed MA (2010) A statistical experiment design approach for optimizing biodegradation of weathered crude oil in coastal sediments. Bioresource Technol 101:893–900

Singer MM, Aurand D, Bragin GE, Clark JR, Coelho GM, Sowby ML, Tjeerdema RS (2000) Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. Mar Pollut Bull 40:1007–1016

Srinivasan R, Lu Q, Sorial GA, Venosa AD, Mullin J (2007) Dispersant effectiveness of heavy fuel oils using baffled flask test. Environ Eng Sci 24:1307–1320

US-EPA (1991) Test methods for evaluating solid waste-SW 846, United States environmental protection agency. Cincinnati, Ohio

US-EPA (1999) Method 1664, revision A: N-hexane extractable material (HEM; oil and grease) and silica gel treated N-hexane extractable material (SGT-HEM; non-polar material) by extraction and gravimetry. United States Environmental Protection Agency, Cincinnati

Venosa AD, Holder EL (2007) Biodegradability of dispersed crude oil at two different temperatures. Mar Pollut Bull 54:545–553

Wang Z, Fingas M, Blenkinsopp S, Sergy G, Landriault M, Sigouin L, Foght J, Semple K, Westlake DWS (1998) Comparison of oil composition changes due to biodegradation and physical weathering in different oils. J Chromatogr A 809:89–107

Wolfe MF, Olsen HE, Gasuad KA, Tjeerdema RS, Sowby ML (1999) Induction of heat shock protein (hsp) 60 in *Isochrysis galbana* exposed to sublethal preparations of dispersant and Prudhoe Bay crude oil. Mar Environ Res 47:473–489