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A statistical experiment design approach for optimizing biodegradation of weathered crude oil in coastal sediments

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ABSTRACT

This work studied the bioremediation of weathered crude oil (WCO) in coastal sediment samples using central composite face centered design (CCFD) under response surface methodology (RSM). Initial oil concentration, biomass, nitrogen and phosphorus concentrations were used as independent variables (factors) and oil removal as dependent variable (response) in a 60 days trial. A statistically significant model for WCO removal was obtained. The coefficient of determination ($R^2 = 0.9732$) and probability value ($P < 0.0001$) demonstrated significance for the regression model. Numerical optimization based on desirability function were carried out for initial oil concentration of 2, 16 and 30 g per kg sediment and 83.13, 78.06 and 69.92 per cent removal were observed respectively, compare to 77.13, 74.17 and 69.87 per cent removal for un-optimized results.

1. Introduction

Hydrocarbons present in the marine environment can originate from natural oil seepage and human activities including extraction, transportation, refining, storage, and utilization of petroleum (crude oil and natural gas) (Ebuehi et al., 2005; Kennish, 2001). Crude oil causes a variety of risks when released into the environment. It is physically, chemically and biologically harmful to soil because of the presence of many toxic compounds, such as polycyclic aromatic hydrocarbons, benzene and its substituted and cycloalkane rings, in relatively high concentrations. The fate and effects of spilled crude oil and its products in soils have already been the subject of several studies (Murakami et al., 2008; Ebuehi et al., 2005; Kulakow and Erickson, 2000; Leahy and Colwell, 1990).

Biodegradation of hydrocarbon compounds is one of the most important processes involved in the weathering and eventual removal of oil from the environment, particularly for its non-volatile components. Thus, potentially biodegradation can be used for recovery of sensitive areas such as contaminated shorelines, marshes, and wetlands. Bioremediation technologies have been developed for soils and coastal areas using the addition of nutrients and microbes (Lin and Mendelssohn, 2009; Lei et al., 2008; Joo et al., 2008; Greenwood et al., 2008).

When a major oil spill occurs in freshwater and marine environments, amount of carbon increase and the availability of nitrogen and phosphorus usually becomes the limiting factor for oil degradation (Leahy and Colwell, 1990). Huang et al. (2008), Borresen and Rike (2007), and Boopathy (2000) determined optimum nutrient supplement levels at laboratory incubation experiments.

Ongoing research and development seeking to improve methods by minimizing the number of experiments provide information about the direct additive effects of the study variables and interaction effects using design of experiment methods. Recently, this statistical technique has been successfully applied in many fields (Huang et al., 2008; Rigas et al., 2007; Pala et al., 2006; Ahmadi et al., 2005). The use of the technique enables selection of the best materials, equipment and process conditions for focusing on the correct variables and ranges for further study.

The statistical experiment designs most widely used in optimization experiments are termed response surface designs (Mohan et al., 2009; Da Silva et al., 2009; Huang et al., 2008; Rigas et al., 2007; Pala et al., 2006; Ahmadi et al., 2005). These designs provide information about direct effects, pairwise interaction effects and curvilinear variable effects. In this experiment coastal sediments artificially polluted with weathered crude oil (WCO) were studied. The application of central composite face centered design (CCFD) under response surface methodology (RSM) assisted in both modeling and optimization of the weathered crude oil biodegradation.

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2. Methods

2.1. Sampling

Coastal sediments were collected from the upper 20 cm surface of Butterworth Beach, Penang, Malaysia. Sediment was transported to the laboratory in a cool box and used for experiments. Physical properties of soil samples used in the laboratory experiments are listed in Table 1. The moisture content of the sample was in the range of 60 ± 3 per cent. Sediment cores were sectioned into 2 cm intervals to remove large roots, macro fauna and stones. The sediments were dried in an oven at 105 °C and passed through a 2 mm sieve (Hamdi et al., 2007). Seawater was periodically collected and stored at 4 °C prior to use. The seawater had a pH of 7.5, total *N* concentration of 2 mg per L and total *P* concentration of 0.04 mg per L.

2.2. Bacteria acclimatization

One liter seawater, 10 g soil sample and 1 mL weathered oil (as the carbon source) were inoculated into a 2 L conical flask containing growth medium under static condition. The acclimatized sample was stirred, aerated and maintained at room temperature under natural light conditions and pH 7.0. The modified medium containing 1 g per L NH_4NO_3, 1 g per L KH_2PO_4, 1 g per L K_2HPO_4, 0.2 g per L $MgSO_4{\cdot}7H_2O$, 0.05 g per L $FeCl_3$, and 0.02 g per L $CaCl_2$ was used to culture bacteria (Ghazali et al., 2004; Dutta and Arayama, 2000).

The total number of culturable bacteria was determined by plating serially diluted samples on nutrient agar plates (0.5 per cent peptone, 0.3 per cent yeast extract, 1.5 per cent agar and pH adjusted to neutral at 25 °C) (Chen et al., 2005; Ruberto et al., 2003). The bacterial count of the final culture was 1.2×10^6 cells per mL.

2.3. Bioremediation experimentation

Experiments were performed in cylindrical plexiglass reactors (30 cm × 15 cm ID) maintained at room temperature. One kilogram of dried sediment was placed in each reactor. Fresh seawater was passed through the reactors 2–3 times to allow equilibration of physical and chemical parameters.

Light crude oil was obtained from the Shell Refining Company, Port Dickson-Malaysia. It was artificially weathered in a 2 L tank by air stripping the volatile fraction until the oil volume reduced by 25 per cent (Mills et al., 2004; Page et al., 2002). The sediment in the reactors was thoroughly mixed with pre-decided amounts of weathered crude oil and inorganic nutrients were added 24 h after oil addition. Ammonium chloride was used as nitrogen source and dipotassium hydrogen phosphate as phosphorus source. All reactors were mixed manually once per week to enhance oxygenation, and kept moist during the 60 day experiment period.

2.4. Chemical analyses

Soil dry weight was determined after heat treatment (24 h at 105 °C). The pH of soil and aqueous soil leachate in the reactors were determined with a glass electrode (Standard Test Methods for pH of Water and Soil; ASTM D1293-99-2005). The inorganic nutrient contents were determined with a HACH DR2000 direct reading spectrophotometer using HACH proprietary reagents (Hach Method-10071 and 8190, 1995a,b).

Duplicate samples (5 g) were taken from all reactors after 60 days. For solid liquid extraction, 5 g wet sediment was weighed and mixed with 2 times its weight of sodium sulphate (Na_2SO_4) to bind water. This mixture was extracted with dichloromethane (CH_2Cl_2) by sonication according to protocol 3550 of the US-EPA (US-EPA, 1991). Extracts were concentrated to small volumes with a gentle stream of dry nitrogen gas (99.99 per cent) (Mishra et al., 2001). When reduced to a few ml, the extracts were filtered through a column chromatograph (30 cm L, 2 cm ID) containing glass wool at the bottom, 10 cm silica gel and Na_2SO_4 on top of glass vials and using *n*-hexane as a solvent. Total petroleum hydrocarbons (TPHs) were measured with the Gravimetric Method (US-EPA, 1999) and Gas Chromatography (US-EPA, 1991). A GC 2000 Series equipped with a flame ionization detector (Fisons Instruments, Milan, Italy) was employed. A DB-5 capillary column (J&W Scientific, Folsom, CA, USA) (60 m × 0.25 mm ID, film thickness 0.25 μm) was used. The operating conditions were as follows: injector temperature 300 °C; detector temperature 300 °C; carrier gas helium 99.999 per cent; make-up gas nitrogen at 30 ml per s; oven temperature program was 1 min at 60 °C, then increasing by 10 °C per min up to 160 °C then 10 min in this temperature followed by 4 °C per min up to 300 °C, and finally 10 min at 300 °C. Splitless mode injections were carried out with the splitless time at 0.8 min. The chromatographic data were analyzed using Chrom-Card data system version 2.1 software (Thermo Electron, Rodano, Italy).

Quality assurance and quality control were performed according to same procedures.

Response surface methodology was used to determine the optimum conditions for bioremediation of WCO in sediment.

2.5. Experimental design and data analysis

The central composite face centered design employed had four independent variables viz., concentration of weathered oil (*A*), biomass (*B*), concentration of nitrogen (*C*), concentration of phosphorus (*D*). Each of the independent variables was studied at three levels (−1, 0, +1), with 30 experiments and three controls with three different WCO concentrations. The soil organic carbon content was chosen as the control variable. Three control reactors were prepared with sediment and seawater that had been sterilized three times at 120 °C for 2 h. Efficiency of oil removal was assessed after 60 days. Coded and actual values of variables used in the study are presented in Table 2 and experimental matrix for central composite design for general optimization is presented in Table 3.

The statistical software Design Expert® 6.0.7, (Stat-Ease Inc., Minneapolis, USA) was used to evaluate the analysis of variance ($P < 0.05$) to determine the significance of each term in the fitted equations and to estimate the goodness of fit in each case. The levels were selected based on preliminary study results and literature

Table 1
Physical properties of soil samples used in laboratory experiments.

Sieve size (μm)	Passing (%)	Texture	Component (%)
>2000	100	Gravel	0
50–2000	84.5	Sand	19.4
2–50	48	Silt	57.7
<2	20	Clay	22.9

Table 2
Coded and actual values of variables used in the response surface study.

Factor	Symbol	Coded levels of variables		
		Low level (−1)	Center (0)	High level (+1)
Oil (g)	*A*	2	16	30
Biomass	*B*	0	1	2
Nitrogen (g)	*C*	0.2	1.6	3
Phosphorus (g)	*D*	0.02	0.16	0.3