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Application of statistical experimental methodology to optimize bioremediation of n-alkanes in aquatic environment

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ABSTRACT

Response surface methodology (RSM) was employed to optimize nitrogen and phosphorus concentrations for removal of n-alkanes from crude oil contaminated seawater samples in batch reactors. Erlenmeyer flasks were used as bioreactors; each containing 250 mL dispersed crude oil contaminated seawater, indigenous acclimatized microorganism and different amounts of nitrogen and phosphorus based on central composite design (CCD). Samples were extracted and analyzed according to US-EPA protocols using a gas chromatograph. During 28 days of bioremediation, a maximum of 95% total aliphatic hydrocarbons removal was observed. The obtained Model *F*-value of 267.73 and probability $F < 0.0001$ implied the model was significant. Numerical condition optimization via a quadratic model, predicted 98% n-alkanes removal for a 20-day laboratory bioremediation trial using nitrogen and phosphorus concentrations of 13.62 and 1.39 mg/L, respectively. In actual experiments, 95% removal was observed under these conditions.

1. Introduction

In 1946, Zobell from Scripps Institution of Oceanography discovered that many microorganisms are capable of utilizing hydrocarbons as the sole source of energy for metabolism [1]. Historically, the most valuable data about hydrocarbon bioremediation were collected during bioremediation of the Exxon Valdez oil spill in 1989 [2].

Petroleum hydrocarbons can typically be divided into four classes: saturates, aromatics, asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and resins (pyridines, quinolines, carbazoles, sulfoxides, and amides). Biodegradation rates have been shown to be highest for the saturates, followed by the light aromatics, with high-molecular-weight aromatics and polar compounds exhibiting extremely low rates of degradation [3–5]. In contrast, higher naphthalene biodegradation than hexadecane from a freshwater lake were reported [6]. Rapid biodegradation of aromatics were also reported in a petroleum bioremediation process [7], and dissimilar degradation rates have been documented in literature [2,4,8,9]. Rontani et al. [10] investigated the trends of n-alkanes removal and showed that the disappearance of low molecular-weight alkanes is stimulated by fertilizer addition. It is generally thought that n-alkanes of shorter chain length are more easily used as an energy source than the longer chain ones [11].

Biostimulation involves the addition of nutrients (mainly nitrogen and phosphorus) to accelerate the biodegradation process [12]. In most marine ecosystems heavily contaminated with hydrocarbons, nitrogen and phosphorus are limiting factors in oil biodegradation. Bioremediation of oil spills has therefore focused on countering this limitation by adding fertilizers to petroleum-contaminated marine environments [13]. Several laboratory experiments have shown that the addition of nutrients might be effective for increasing the biodegradation of organic compounds because it stimulates bacterial growth [14,15]. The predominant mechanism of n-alkanes degradation involves terminal oxidation of the corresponding alcohol, aldehyde, or fatty acid functional groups. Branched alkanes are less readily degraded in comparison to n-alkanes [2].

Since several variables are involved in the biological degradation of hydrocarbons, investigation of bioremediation can be very time consuming if the parameters are optimized following the classical approach of changing one parameter at a time [16]. Additionally, synergistic and antagonistic effects of process parameters may not be reflected and may lead to biased results. Hence, the statistical tool of response surface methodology (RSM) was employed in this study. This method is based on polynomial approximation and its design is in accordance with statistical requirements [17].

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Table 1
Experimental matrix and results for n-alkanes removal (%).

Run	Point type	Factors			n-Alkanes removal (%)		
		A:N (mg/L)	B:P (mg/L)	C:time (day)	Observed	Predicted	Residual
1	Fact	0.0	0.0	7	10.89	11.38	−0.49
2	Fact	20.0	0.0	7	21.92	20.13	1.79
3	Fact	0.0	2.0	7	18.12	17.34	0.78
4	Fact	20.0	2.0	7	36.66	38.02	−1.36
5	Fact	0.0	0.0	28	33.45	32.07	1.38
6	Fact	20.0	0.0	28	59.22	59.98	−0.76
7	Fact	0.0	2.0	28	52.73	54.50	−1.77
8	Fact	20.0	2.0	28	94.86	94.35	0.51
9	Axial	5.0	1.0	18	79.43	81.60	−2.17
10	Axial	15.0	1.0	18	91.03	93.75	−2.72
11	Axial	10.0	0.5	18	74.49	78.38	−3.89
12	Axial	10.0	1.5	18	92.07	88.46	3.61
13	Axial	10.0	1.0	12	69.13	70.63	−1.50
14	Axial	10.0	1.0	23	91.10	89.88	1.22
15	Center	10.0	1.0	18	88.58	87.67	0.91
16	Center	10.0	1.0	18	85.69	87.67	−1.98
17	Center	10.0	1.0	18	90.68	87.67	3.01
18	Center	10.0	1.0	18	89.04	87.67	1.37
19	Center	10.0	1.0	18	90.35	87.67	2.68
20	Center	10.0	1.0	18	87.07	87.67	−0.60
21	–	0.0	0.0	18	26.05	–	–

The main goal of the present study is to model and optimize bioremediation of n-alkanes in dispersed crude oil by performing a series of controlled laboratory experiments designed and analyzed by central composite design (CCD) and response surface methodology (RSM). The effects of both time and nutrients on biodegradation are investigated.

2. Materials and methods

2.1. Sampling and microorganism acclimitization

Seawater samples were collected from Butterworth Beach, Penang, Malaysia. A media 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 [18,19]. The sample mixture of seawater, nutrients, and hydrocarbon was stirred, aerated and maintained at room temperature under natural light conditions and pH 7.0–7.8. Details of bacterial consortium acclimatization have been reported earlier [20].

2.2. Bioremediation experiments

Seawater samples were mixed with light crude oil (Shell Refining Company Berhad, Port Dickson, Malaysia) and the dispersant Corexit 9500 in a ratio of 20:1 (w/w). Erlenmeyer flasks were used as bioreactors, each containing 250 mL oil-contaminated seawater with initial oil concentration of 100 mg/L and different amounts of nitrogen and phosphorus as listed in Table 1. Furthermore, one extra test (Run 21) was carried out for determination of removal on day 18 due to natural attenuation. Ammonium nitrate and dipotassium hydrogen phosphate were used as nitrogen and phosphorus sources, respectively. Each bioremediation test reactor received 1 mL bacterial inoculum containing 1.2×10^7 cells/mL. The reactors were shaken and samples were collected at 7, 12, 18, 23 and 28 days for analysis.

2.3. Chemical analysis

Analytical grade chemicals were used and all analyses were done according to Standard Methods for the Examination of Water and Wastewater [21]. Samples were extracted three times by dichloromethane (DCM) following US-EPA test methods [22] and hydrocarbons quantification was performed using a GC 2000 series gas chromatograph equipped with a FID flame ionization detector (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) was employed. Splitless mode injections were carried out with the purge valve opened at 1 min; injector and detector temperatures were set at 300 °C; helium (He) was used as carrier gas; make-up gas, N_2, flow rate was 30 mL/s; the oven tempera-

Table 2
Analysis of variance for response surface quadratic model terms.

Source	Sum of squares	DF[a]	Mean square	*F*-value	Prob > *F*	Remarks
Model	15375.10	8	1921.89	267.73	<0.0001	Significant
A	1254.67	1	1254.67	174.78	<0.0001	Significant
B	863.65	1	863.65	120.31	<0.0001	Significant
C	3150.94	1	3150.94	438.95	<0.0001	Significant
B^2	71.42	1	71.42	9.95	0.0092	Significant
C^2	217.18	1	217.18	30.25	0.0002	Significant
AB	71.22	1	71.22	9.92	0.0092	Significant
AC	183.65	1	183.65	25.58	0.0004	Significant
BC	135.71	1	135.71	18.91	0.0012	Significant
Residual	78.96	11	7.18			
Lack of fit	60.58	6	10.10	2.75	0.1437	Not significant
Pure error	18.39	5	3.68			
Cor total	15454.06	19				

[a] DF = degree of freedom.