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Optimization of Biodegradation of Long Chain n-Alkanes by *Rhodococcus* sp. Moj-3449 Using Response Surface Methodology

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Oil spills and subsequent pollution of marine ecosystems are associated with oil exploitation. The ultimate fate of unrecoverable portion of spilled oil is biodegradation by microorganisms. *Rhodococcus* sp. Moj-3449 has shown an ability to be grown on aliphatic hydrocarbons. In this study, response surface methodology was employed to optimize the biodegradability of Moj-3449 by changing salinity, pH, temperature and n-C_{16} concentration. The duplicate experiments conducted were based on a 5-level rotatable and orthogonal central composite design (CCD) performed in 2 separate blocks. The effect of each factor and their interactions on the biodegradation of n-C_{16} revealed that the optimal conditions for the biodegradation are 0% salinity at pH = 6.97, T = 28 °C and 52.98% wt/V n-C_{16} concentration. Under these conditions, the maximum biodegraded amount was predicted to be 45.52% wt/V out of 52.98% wt/V. The experimentally biodegradation amount obtained at the optimum condition was 44.27 ± 0.07% wt/V. The ability of the strain to effectively break down long chain n-alkanes at concentration up to 55% wt/V shows that this strain is an outstanding candidate for industrial bioremediation of crude oil spills in marine environment.

Keywords: Biodegradation, Bioremediation, Long chain n-alkanes, Crude oil degradation, Response surface methodology

INTRODUCTION

Crude oil will remain as a major energy source for the coming decades. The United States Energy Information Administration has anticipated that by 2025 the global oil demand will increase up to 123 million barrels per day [1]. Oil exploitation is inevitably accompanied by accidental spills and subsequent pollution of marine ecosystems. It is estimated that 140 large oil spills which has thus far occurred resulted in the release of more than 7 million tons of oil into the environment [2]. For example, Deepwater Horizon disaster in Gulf of Mexico released more than 700,000 tons of crude oil [3]. Oil spills cause extensive environmental impact resulting in a long-lasting threat to the ecosystem. Initial remediation strategies include mechanical containment and recovery of spilled oil, addition of dispersants, and physical cleanup of the shorelines [4]. Although above-mentioned physicochemical technologies are routinely used for oil spill cleanup, the spilled oil must ultimately be degraded by microorganisms. As a result, microbial degradation of long chain n-alkanes have remained as the ultimate process for the remediation of the oil contaminated areas [5-7].

Biodegradation of marine spilled oil occurs when a microorganism 1) produces biosurfactants that increase bioavailability of crude oil [8] and 2) consumes

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hydrocarbons in crude oil as a carbon source [4]. Numerous microorganisms are isolated that can grow on different fractions of crude oil and degrade various classes of hydrocarbons such as aliphatics [9-12], monoaromatics [13,14], polyaromatics [15-17], and resins [18,19]. It is also self-evident that the isolation of the right microbial candidate is an important initial step for a successful microbial process.

Strain *Rhodococcus* sp. Moj-3449 has shown a unique ability to degrade high concentration of the long chain n-alkanes, as well as crude oil [20]. The strain reaches its maximum growth (14.3 g cdw/L) within 2 days in a culture supplemented with 12% wt/V of n-hexadecane. It prefers C_{14} and C_{16} rather than C_{12} and C_{18} when a pure substrate is supplied as carbon source. Biodegradation of crude oil by the strain Moj-3449 resulted in degradation of 36% of the initial crude oil (15% wt/V). The strain degrades n-alkanes ranging from C_{10} to C_{35} while it grows on crude oil; however, it prefers C_{14} to C_{19}. Strain *Rhodococcus* sp. Moj-3449 only follows sub-terminal degradation pathway. The unique ability of strain Moj-3449 is anticipated to be due to its biosurfactant production as well as its sub-terminal oxidation pathway.

Selection of the right microbial candidate for bioremediation requires detailed knowledge of the optimum environmental condition for strain biodegradability [21]. To optimize the biodegradability of strain by manipulating the environmental condition (pH, salinity, temperature, and carbon source concentration). In this study, response surface methodology (RSM) is employed to obtain the optimum environmental condition for biodegradation by strain Moj-3449. The basic fundamental and theoretical aspects of RSM is explained elsewhere [22]. RSM clearly defines the effect of individual parameters or their interaction effect which is critical in biochemical processes due to synergism, and antagonism. It reduces the number of experiments required to evaluate the effect of multiple parameters on the response of interest. The mathematical model generated by RSM can be used to develop, improve, or optimize the process [23].

In this study, the most preferred substrate of *Rhodococcus* strain Moj-3449, n-C_{16} [20], was chosen as the carbon source. pH, salinity, temperature and concentration of n-C_{16} were selected as the independent variables to find the optimal condition for the biodegradation of the long chain hydrocarbon, such as hexadecane, by *Rhodococcus* sp. Moj-3449. The first 3 variables are the important factors affecting biodegradation of crude oil [24] and the last factor is added into the RSM model to investigate the maximum concentration of long chain hydrocarbon at which the bacteria is able to break down efficiently.

MATERIALS AND METHODS

Chemicals

n-Undecane (n-C_{11}), and n-hexadecane (n-C_{16}) were purchased from Sigma Aldrich or Fluka, all in analytical grade.

The initial experiments showed the range within which the degradation occurs: salinity, 0-3% wt/V salinity; pH, 7-9; temperature, 28-40 °C; and concentration, 15-55% wt/V.

Bacterial Strains and Cultivation Condition

The strain was initially cultured on agar plates with Luria-Bertani (LB) medium at 37 °C for 3 days. Cells on the plates were inoculated into 5 ml of liquid LB medium in a 25 ml flask and shaken at 300 rpm at 37 °C for 1 day. 0.2 ml of the preculture was transferred into 20 ml E2 medium with 25% wt/V of n-C16 as sole carbon source in a 125 ml conical flask. The culture was shaken at 300 rpm in an orbital shaker (New Brunswick Scientific Edison, N.J.) at 37 °C for 72 h to achieve an optical density at 600 nm wavelength (OD_{600}) of 27.3 which corresponds to the end of the exponential phase [20]. Preculture was added to a series of 125 ml flasks containing 20 ml E2 medium at appropriate pH and salinity to achieve an OD_{600} of 0.3. The biodegradation process occurred for 3 days in an orbital shaker at 300 rpm.

E2 Medium

E2 medium was prepared by dissolving 7.0 g $NaNH_4HPO_4 \cdot 4H_2O$, 15.0 g $K_2HPO_4 \cdot 3H_2O$, and 7.4 g KH_2PO_4 in 2 l H_2O, sterilizing the solution in autoclave at 120 °C for 30 min, and adding 2 ml 1M $MgSO_4$ and 2 ml standard 1000MT solution (2.78 g $FeSO_4 \cdot 7H_2O$, 1.98 g $MnCl_2 \cdot 4H_2O$, 2.81 g $CoSO_4 \cdot 7H_2O$, 1.47 g $CaCl_2 \cdot 2H_2O$, 0.17 g $CuCl_2 \cdot 2H_2O$, and 0.29 g $ZnSO_4 \cdot 7H_2O$ in 1 l 1 N

HCl) through a sterile filter (0.22μm pore diameter).

Gas Chromatography (GC) Analysis

The total content of each shaking flask was mixed with 10 ml of n-hexane by shaking vigorously for 2 min on a vortex (Scientific Industries, Inc. G-560-E). The mixtures were centrifuged at 15000 rpm (21100 g) and 4 °C for 5 min using a high-speed refrigerated centrifuge (Hitachi Himac CR 22G). The hexane phase was collected and the process was repeated for 2 more times. The alkane phase after vortex was mixed in 1:9 ratio with hexane containing 0.002 M n-undecane, n-C_{11}, as the internal standard. The GC analysis was performed with an Agilent Technologies 6890N gas chromatograph equipped with an on-column injection, FID detector, and HP-5 capillary column (30 m×0.32 mm i.d, 0.25 μm thickness) (J & W Scientific). Helium was used as carrier gas and set at a constant flow rate of 2 m min^{-1}. Injector and detector temperature was 280 and 300 °C, respectively. For pure n-alkane analysis, oven temperature was set at 100 °C for 1 min, raised to 210 °C at a rate of 15 °C min^{-1}, and then kept at 210 °C for 1 min. At these conditions, the retention time of n-C_{16} and n-C_{11} are 7.308 and 3.298 min, respectively.

Experimental Design and Statistical Analysis

The most popular design of experiments to fit an empirical model of second-degree polynomial is the central composite design (CCD) which allows for a rotatable and orthogonal design. The former ensures the precision of the predicted response, y, while the latter enables experiments to be performed in blocks, meaning that the block effects do not affect the estimates of the first- and second-degree coefficients in the second-order model. For a rotatable, orthogonal experimental design, a four factor-five coded level CCD, 30 duplicate runs, were carried out in 2 blocks to fit into a second-order polynomial model of the general Eq. (1):

$$y = \beta_0 + \sum_{j=1}^{k} \beta_i X_j + \sum_{j=1}^{k} \beta_{jj} (X_j)^2 + \sum_{i}^{j-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \qquad (1)$$

where β_0, β_j, β_{jj} and β_{ij} are regression coefficients for the intercept, linear, quadratic and interaction coefficients, respectively, and X_i and X_j are coded independent variables.

The response measured in this study is denoted by y, which is the biodegradation amount of hexadecane in percentage weight per volume of medium (%wt/V) calculated according to Eq. (2):

$$y = \frac{R_{control} - R_{run}}{R_{control}} \times C_{C16} \qquad (2)$$

where $R_{control}$ is the ratio of area under chromatography graph for n-C_{16} peak over that of n-C_{11} peak for the control, R_{run} is the ratio of area under chromatography graph for n-C_{16} peak over that of n-C_{11} peak for the experimental run and Cc_{16} is the initial concentration of the hexadecane substrate for that run in %wt/V. The coding of the variables is essential as each factor in the experiment designed might have different ranges and units. Hence, for accuracy of the statistical analysis, the different levels for each factor were normalized. For the CCD employed, the range and levels of the independent parameters, in the actual and coded values, investigated in this study are shown in Table 1. Each of the coded variables is forced to range from -2 to 2, so that they are dimensionless. The following equation was used for coding:

$$X = \frac{X - \left| \frac{X_{\max} + X_{\min}}{2} \right|}{\left| \frac{X_{\max} - X_{\min}}{2} \right|} \qquad (3)$$

where x is the actual variable value, X is the coded variable, x_{max} and x_{min} are the maximum and minimum values of the actual variable, respectively. Tables 2 and 3 show the conditions for each duplicate run in the two blocks, in the coded levels, that were carried out. The 6 duplicate center points are split into 4 duplicates in the first block and 2 duplicates in the second block as suggested by the program Minitab 15 Statiscal Soft (Minitab Inc, USA), using the response surface design option. As each run was done twice and the experiments were done in 2 separate blocks, the software splits the constant term β_0 in the Eq. (1) into 4 different constant values to take care of the effect of each duplicate in each block. In this way, the terms block 3 and 4 have the same meaning of block 1 and 2 and the only difference is that it represents the second duplicate run.

Table 2. Levels of the Independent Variables in Actual and Coded Values

	Coded levels				
Factor-code	-2	-1	0	1	2
	Actual values				
X_1-NaCl Concentration (% wt/V)	0	0.75	1.5	2.25	3
X_2-pH	7	7.5	8	8.5	9
X_3-Temperature (°C)	28	31	34	37	40
X_4-*n*-C_{16} Concentration (% wt/V)	15	25	35	45	55

Table 2. Central Composite Design of Variables in Coded Levels, Block 1

Runs	X_1-NaCl	X_2-pH	X_3-Temperature	X_4-*n*-C16 Conc.
1	-1	-1	-1	-1
2	-1	-1	-1	1
3	-1	-1	1	-1
4	-1	-1	1	1
5	-1	1	-1	-1
6	-1	1	-1	1
7	-1	1	1	-1
8	-1	1	1	1
9	1	-1	-1	-1
10	1	-1	-1	1
11	1	-1	1	-1
12	1	-1	1	1
13	1	1	-1	-1
14	1	1	-1	1
15	1	1	1	1
16	1	1	1	1
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0

Table 3. Central Composite Design of Variables in Coded Levels, Block 2

Runs	X_1-NaCl	X_2-pH	X_3-Temperature	X_4-*n*-C16 Conc.
21	-2	0	0	0
22	2	0	0	0
23	0	-2	0	0
24	0	2	0	0
25	0	0	-2	0
26	0	0	2	0
27	0	0	0	-2
28	0	0	0	2
29	0	0	0	0

MATLAB 7.0 (MathWorks, Inc, USA) was then used to plot the three-dimensional response graphs, and Minitab 15 was used to calculate the lack-of-fit, *F*-value, and the multiple correlation coefficient, R^2. In general, the model was considered to be efficient and effective if the *P*-values for the independent variables used were significant, the lack-of-fit *F*-value was insignificant and R^2 was close to the value of 100%.

RESULTS AND DISCUSSION

Statistical Analysis

The second order polynomial model equation obtained for the biodegradation of hexadecane by *Rhodococcus* sp. Moj-3449 as a function of salinity, pH, temperature and concentration of n-C_{16} substrate, obtained by multiple regression analysis on the experimental data is:

$$y = 24.6479 + Blk_i - 4.2848X_1 - 1.243X_2 + 0.2252X_3 \quad (4)$$

All terms, regardless of their significance are included in the equation above. The experimental data used for the fitting of the model and the predicted value for the response calculated from the derived model are reported in Tables 4 and 5. The statistical analysis of the CCD experimental results, response surface modeling and optimization of the process variables were carried out using Minitab 15, which employs *t*-test and *F*-test [25]. The results of analysis of variance (ANOVA) are shown in Table 6 indicating that the fitted second-order model is statistically significant with *F*-test = 24.14 (p = 0.000). The *t*-test was used to determine the significance of the regression coefficients of the variables while *p*-value indicates the significance of the test [26]. In general, the bigger the value of *t* and the smaller the value of *p*, the more significant the corresponding term is. The regression coefficients, *t* and *p* values for all the linear, quadratic and interactive terms of the model are shown in Table 6. The determination coefficient R^2 for the predicted model was 90.32%, implying that 90.32% of the sample variation for the biodegradation of hexadecane is attributable to the independent variables included in the model. Only about 9.7% of the total variation cannot be explained by the model. Hence, Eq. (4) can be used to well predict the response at any combination of the 4 independent variables within the coded experimental range.

Effects of Salinity, pH, Temperature and n-C_{16} Concentration

According to Table 6, the linear effect of parameters decreases in the order of n-C_{16} concentration, salinity, pH,

Table 4. Observed Experimental Values, Predicted Values and the Residuals-Part 1

Runs	Observed values	Predicted values	Residual
1- (1)	20.514	23.022	-2.508
1- (2)	21.248	22.873	-1.625
2- (1)	36.624	37.609	-0.985
2- (2)	35.125	37.46	-2.335
3- (1)	20.558	19.656	0.902
4- (1)	34.869	32.827	2.042
4- (2)	33.982	32.678	1.304
5- (1)	21.795	21.111	0.684
5- (2)	21.62	20.962	0.658
6- (1)	37.952	36.325	1.627
6- (2)	37.894	36.176	1.718
7- (1)	19.183	20.347	-1.164
7- (2)	19.529	20.199	-0.67
8- (1)	35.251	34.146	1.105
8- (2)	33.037	33.997	-0.96
9- (1)	15.235	15.579	-0.344
9- (2)	17.233	15.43	1.803
10- (1)	29.963	25.846	4.117
10- (2)	29.833	25.697	4.136
11- (1)	15.648	15.579	0.069
11- (2)	15.099	15.43	-0.331
12- (1)	25.402	25.846	-0.444
12- (2)	23.068	25.697	-2.63
13- (1)	16.16	14.971	1.19
13- (2)	17.027	14.822	2.205
14- (1)	24.772	24.449	0.323
14- (2)	26.373	24.3	2.073
15- (1)	15.449	14.971	0.478
15- (2)	15.612	14.882	0.79

Table 5. Observed Experimental Values, Predicted Values and the Residuals-Part 2

Runs	Observed values	Predicted values	Residual
16- (1)	26.617	24.449	2.168
16- (2)	27.215	24.3	2.915
17- (1)	23.77	27.602	-3.832
17- (2)	24.141	27.453	-3.312
18- (1)	23.149	27.602	-4.454
18- (2)	21.534	27.453	-5.919
19- (1)	29.087	27.602	1.484
19- (2)	28.784	27.453	1.331
20- (1)	25.144	27.602	-2.458
20- (2)	25.863	27.453	-1.59
21- (1)	33.416	32.451	0.965
21- (2)	33.209	31.947	1.262
22- (1)	12.67	15.312	-2.642
22- (2)	10.535	14.808	-4.273
23- (1)	21.025	21.064	-0.039
23- (2)	21.138	20.56	0.578
24- (1)	14.395	16.092	-1.697
24- (2)	12.058	15.588	-3.53
25- (1)	20.487	18.094	2.393
25- (2)	15.736	17.59	-1.854
26- (1)	15.722	18.995	-3.273
26- (2)	16.537	18.491	-1.953
27- (1)	0.466	0.016	0.449
27- (2)	0.118	-0.488	0.606
28- (1)	18.971	24.082	-5.111
28- (2)	22.946	23.578	-0.632
29- (1)	26.126	22.02	4.106
29- (2)	26.869	21.516	5.353
30- (1)	26.869	22.02	4.849
30- (2)	25.959	21.516	4.443

Table 6. Regression Coefficient and Corresponding T and P Values

Term	Coefficient	t	p
Constant	24.6479	28.070	0.000
Block 1	2.9544	4.599	0.000
Block 2	-2.6279	-3.291	0.002
X_1	-4.2848	-8.753	0.000
X_2	-1.2431	-2.077	0.044
X_3	0.2252	0.376	0.709
X_4	6.0164	13.892	0.000
X_1X_2	-1.0948	-1.343	0.187
X_1X_3	1.6114	1.976	0.055
X_1X_4	-1.0799	-2.036	0.048
X_2X_3	0.6506	0.950	0.347
X_2X_4	0.1568	0.256	0.799
X_3X_4	-0.3540	-0.578	0.566
X^2_1	0.4653	1.137	0.262
X^2_2	-0.8605	-2.103	0.041
X^2_3	-0.8689	-2.124	0.040
X^2_4	-2.4927	-6.093	0.000

and temperature. The quadratic effect of parameters decrease in the order of n-C_{16} concentration, temperature, pH, and salinity. The importance of binary interaction between parameters decreases in the order of salinity-temperature, salinity-pH, salinity-n-C_{16} concentration, pH-temperature, temperature-n-C_{16} concentration, and pH-n-C_{16} concentration. Based on the fitted coefficients in Table 6, an increase in the salinity (X_1) decreases biodegradation of n-C_{16} regardless of the pH, temperature, and n-C_{16} concentration. The interplay of other parameters (X_2, X_3 and X_4) does not follow such a monotonic trend.

To understand the effect of each factor on biodegradation, 3-dimensional plots are drawn as a function of 2 factors at a time, holding the other two factors at the center level (0). The 3D surface plots showing the effect of salinity and each of the other 3 factors are presented in Figs. 1-3. Figure 1 shows the effect of salinity and pH on biodegradation at 34 °C and 35% wt/V of n-C_{16}. High pH and high salinity have a severe adverse effect on biodegradation. At any salinity, increase in pH initially promotes biodegradation; however, at pHs higher than 8 the biodegradation further decreases. Moreover, at any given pH, an increase in the salinity decreases the biodegradation.

Figure 2 shows the effect of salinity and temperature at pH 8 and 35% wt/V of n-C_{16}. It can be seen that at any given temperature an increase in the salinity decreases the biodegradation, but this decrease is more pronounced at moderate temperatures.

The most important interactive effect observed is salinity and n-C_{16} concentration, as shown in Fig. 3. The

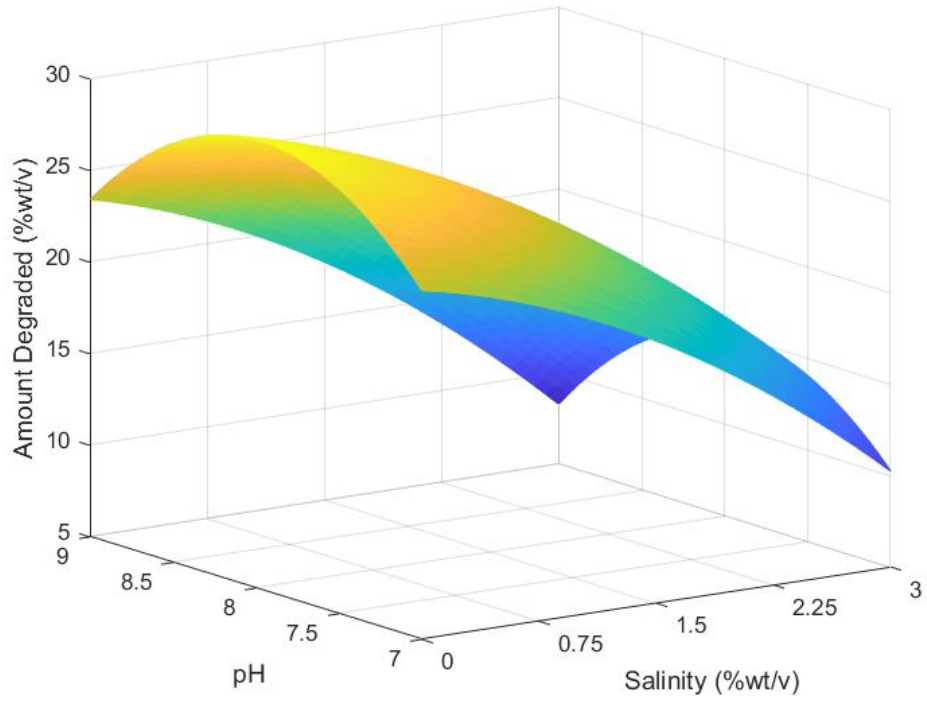

Fig. 2. Response surface plot of amount of biodegradation as a function of salinity and pH at 34 ºC and n-C16 concentration of 35% wt/V.

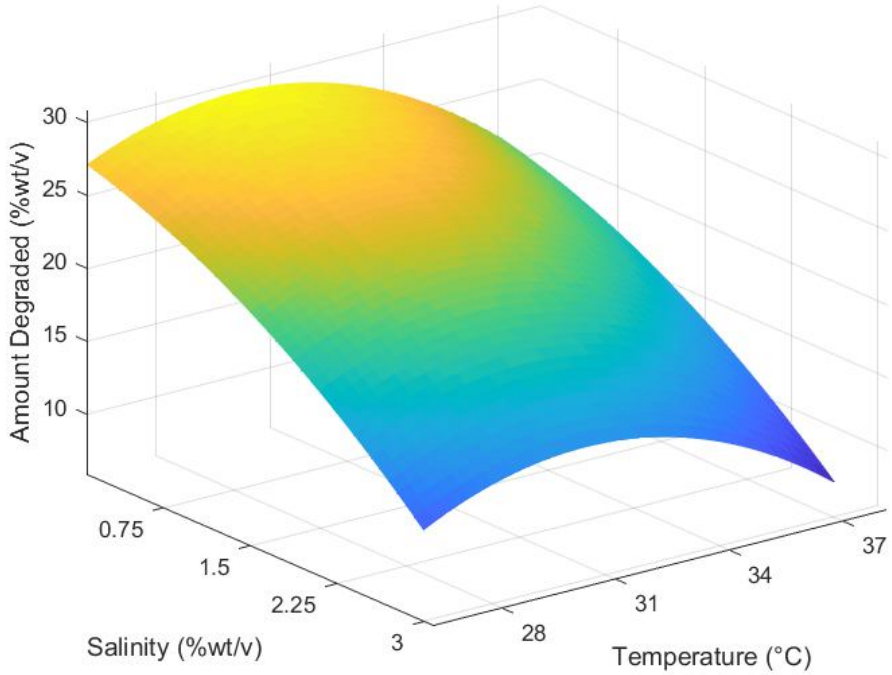

Fig. 2. Plot of biodegradation amount as a function of salinity and temperature at pH 8 and n-C_{16} concentration of 35% wt/V.

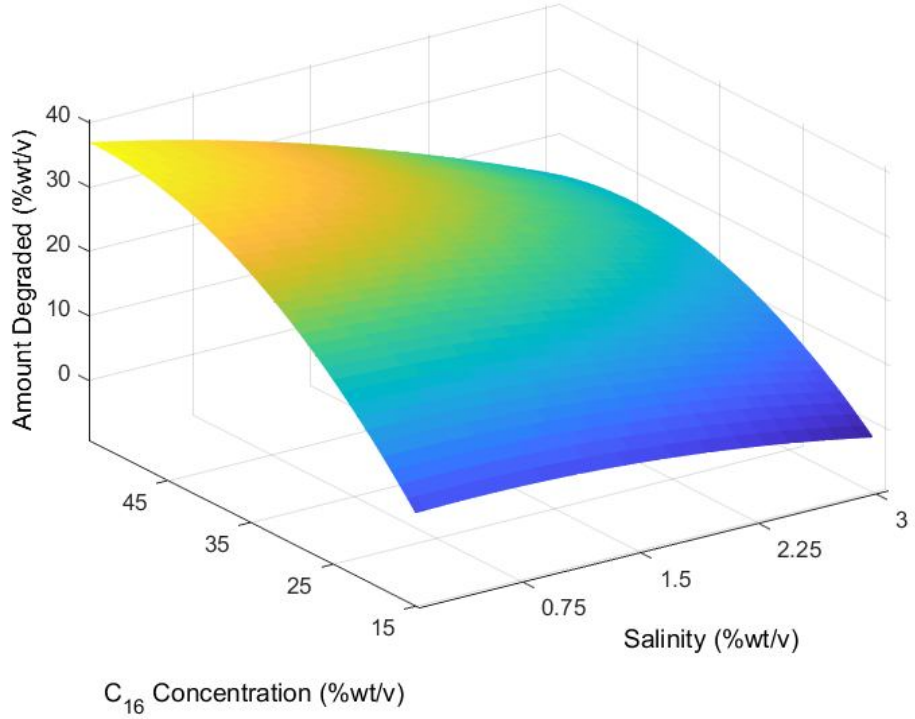

Fig. 4. Plot of biodegradation amount as a function of salinity and n-C16 concentration at pH 8 and 34 ºC.

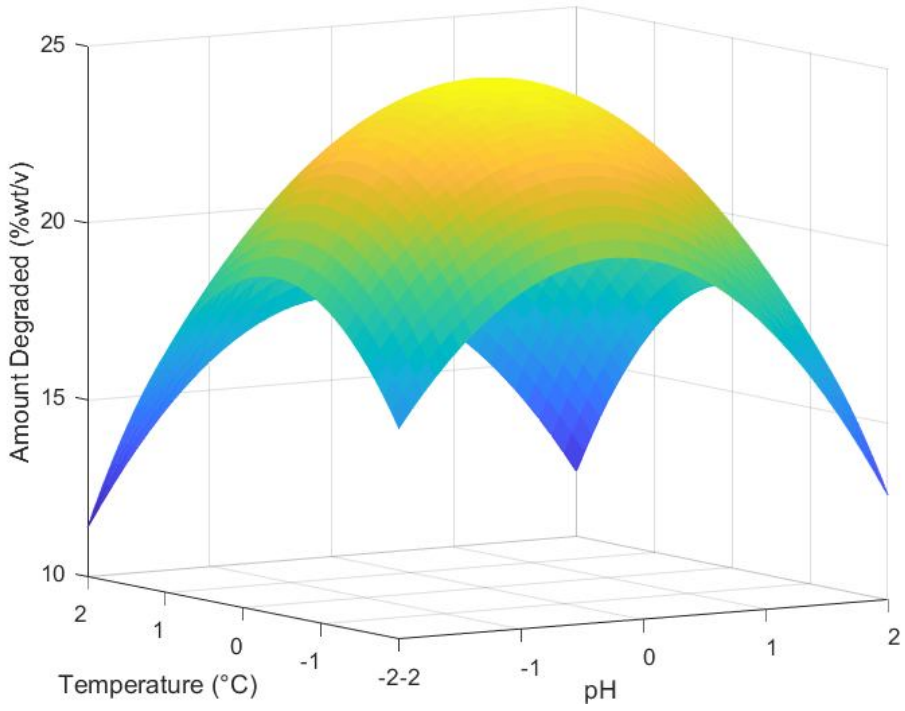

Fig. 4. Plot of biodegradation amount as a function of pH and temperature at salinity of 1.5% wt/V and n-C_{16} concentration of 35% wt/V.

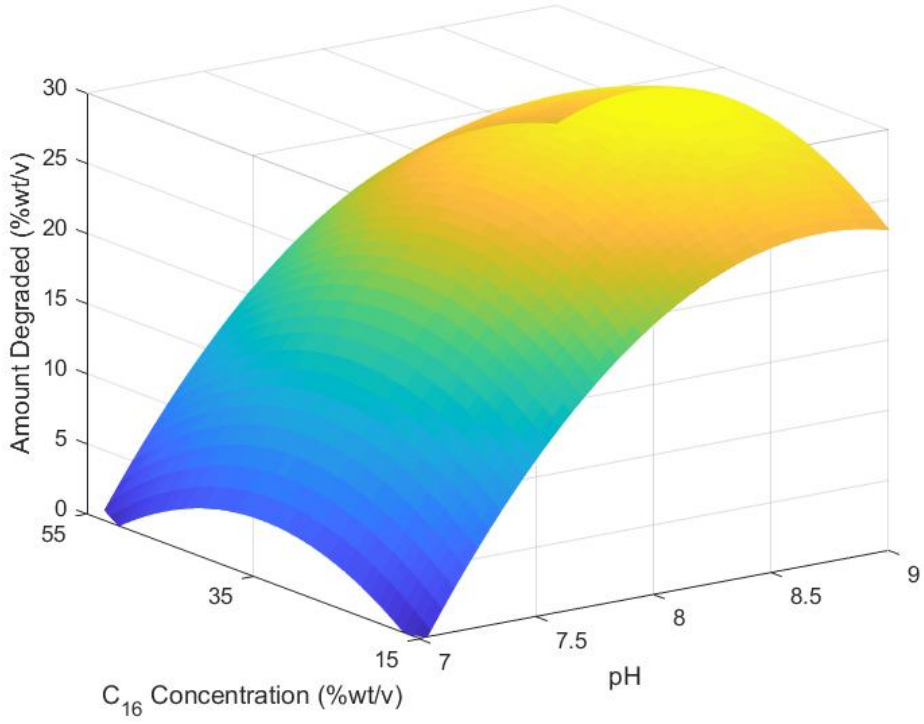

Fig. 6. Plot of biodegradation amount as a function of pH and n-C16 concentration at 34 ℃ and salinity of 1.5% wt/V.

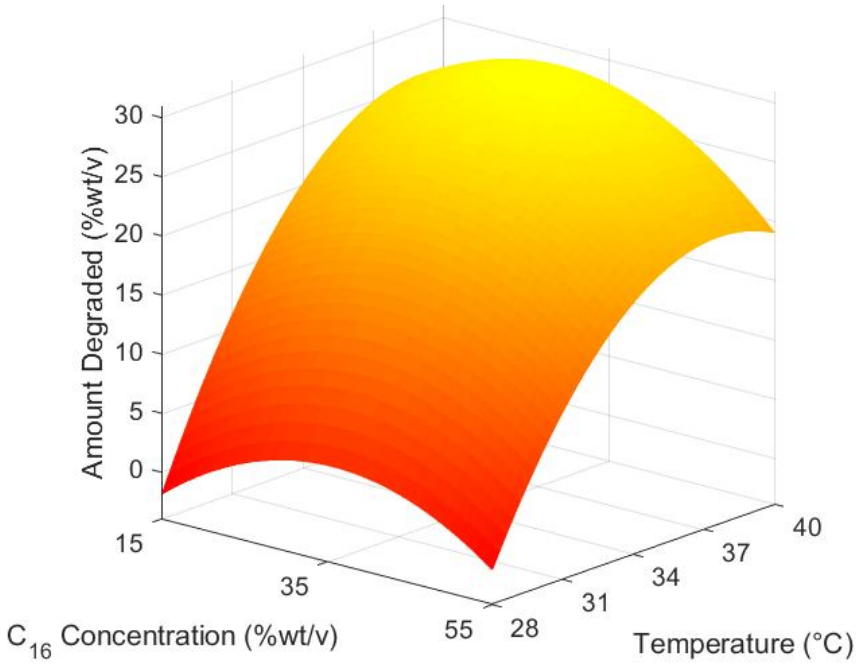

Fig. 6. Plot of biodegradation amount as a function of temperature and n-C_{16} concentration at pH 8 and salinity of 1.5% wt/V.

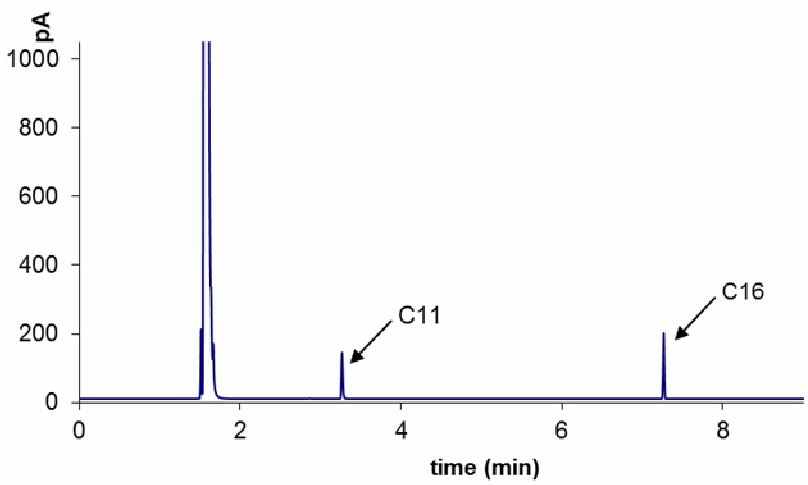

Fig. 8. The GC chromatogram of biodegradation of 52.98% wt/V n-C_{16} with *Rhodococcus* sp. Moj-3449 at salinity 0% wt/V, pH 7.97, and temperature 28.0 °C. The sample was taken from the bacterial culture.

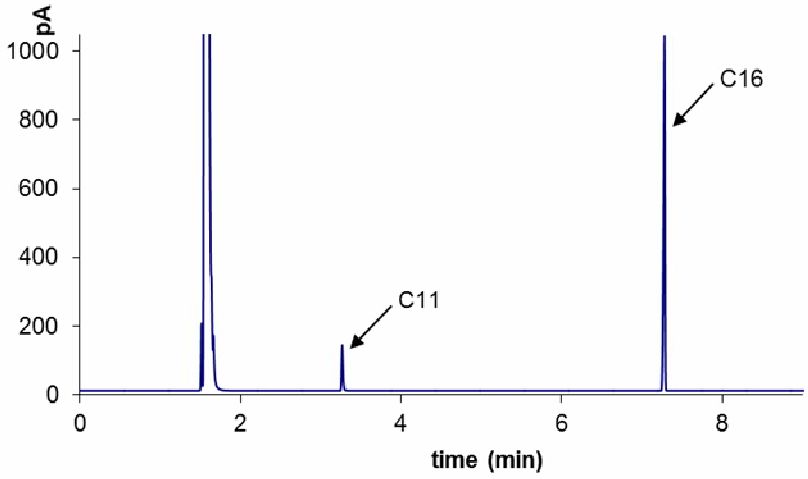

Fig. 8. The GC chromatogram of biodegradation of 52.98% wt/V n-C_{16} with *Rhodococcus* sp. Moj-3449 salinity 0% wt/V, pH 7.97, and temperature 28.0 °C. The sample was taken from control flask.

negative sign of their interacting coefficient suggests that at low salinity (lower than 1.5% wt/V) and high hydrocarbon concentration (higher than 35% wt/V) the interaction will have a positive effect on degradation (high salinity and low n-C_{16} concentration is not physiologically favorable). It can be attributed to the fact that NaCl is a toxic chemical to the cells and it has an adverse effect on their biodegradability. On the other hand, higher n-C_{16} concentration increases the amount of n-C_{16} available to the cells; hence, it favors biodegradation. It is logical to expect that at the reverse condition (high salinity and low n-C_{16} concentration) degradation will be tremendously retarded.

The quadratic effect of pH and temperature at salinity of 1.5% wt/V and n-C_{16} concentration of 35% wt/V is shown in Fig. 4. The optimum biodegradation is at pH 7.2 and 31.3 °C at the experimental range of Fig. 4. It is apparent that extreme pH and/or temperature severely decreases the biodegradation.

Table 7. Microorganisms Capable of Growing on Oil at Concentrations Higher than 1% (W/V)

Microorganism	Highest oil concentration, %, (W/V)	Degraded oil, %, (W/V)	Incubation time (Days)	Ref.
Pseudomonas sp. BPS1-8	12	4.1	25	[27]
Pseudomonas aeruginosa DQ8	10	7.9	10	[28]
Acinetobacter Radioresistens	1	0.5	30	[29]
Candida catenulata KP324968	10.6	5.3	6	[30]
Acinetobacter sp. XM-02	0.5	0.4	10	[31]
P. aeruginosa TPHK-4	5	3.5	10	[32]
Rhodococcus sp. Moj-3449	52.98	44.26	3	This study

From Fig. 5, it can be seen that at 34 °C and salinity of 1.5% wt/V, higher pH improves biodegradation for all n-C_{16} concentrations. The fact that higher pH increases the biodegradation at salinity of 1.5% wt/V suggests that an increase in the pH alleviates the adverse effect of salinity.

The quadratic effect of n-C_{16} concentration and temperature at salinity of 1.5% wt/V and pH 8 is shown in Fig. 6. As seen in Fig. 6, for a given n-C_{16} concentration at salinity of 1.5% wt/V and pH 8 an increase in the temperature initially increases the biodegradation. The optimum temperature for the experimental range is between 36 and 38 ºC and further increase in the temperature slightly decreases the biodegradation. It should be noted that all of the biodegradation behavior depicted in Fig. 1 to Fig. 6 just show the interaction of 2 different parameters, while the biodegradation process is affected by the simultaneous interaction of all 4 parameters.

Optimization

The numerical optimization of the response model was performed using the Minitab 15 response optimizer tab. The optimal conditions for the biodegradation of hexadecane were: salinity of 0% wt/V, pH of 7.97, temperature of 28.0 °C, and n-C_{16} concentration of 52.98% wt/V. At these conditions, the predicted amount of biodegradation is 45.52% wt/V. In order to validate the optimization condition of the bacterial degradation process predicted by the RSM method, a set of duplicate experiments was performed at the model predicted optimal condition. The experimental results suggest degradation of 44.20 and 44.33% wt/V out of 52.98% wt/V for a duplicate run, which gives only 2.89 and 2.61% error from the model predicted value, respectively. Under optimum condition, 86% of the initial n-C_{16} was degraded. Figures 7 and 8 show the final n-C_{16} content in the bacterial culture and the control flasks, respectively.

A comparison between the biodegradation results of this study and other research findings is made in Table 7. It can be seen from Table 7 that the tolerance of bacterial strain *Rhodococcus* sp. Moj-3449 on high oil concentration is far higher than that reported for other microorganisms; moreover, its biodegradation rate and final amount of degraded oil make this strain a promising candidate for bioremediation of oil spills.

CONCLUSIONS

This RSM investigation has detailed the interplay of salinity, pH, temperature and n-C_{16} concentration on the biodegradation of n-C_{16}. Salinity and n-C_{16} concentration were found to be the most significant factors affecting the biodegradation of n-C_{16} by *Rhodococcus* sp. Moj-3449 in

the working range. The biodegradation response could be fitted into the second-order polynomial equation supported with good regression coefficient. The predicted value of the model and experimental values are in agreement, reflecting the applicability of RSM. The ability of the strain to effectively break down long chain *n*-alkanes at high concentrations up to 55% wt/V shows that this strain has a potential role in breaking down hydrocarbon and crude oil from spills in bioremediation industry. The result of this study can be used to design bioremediation processes using *Rhodococcus* sp. Moj-3449.

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