RESPONSE SURFACE METHODOLOGY (RSM) APPLICATION FOR OPTIMIZATION OF PHYTOREMEDIATION PROCESS USING Paspalum srobiculatum L.Hack.

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ABSTRACT: Massive amounts of soil and water have been contaminated with total petroleum hydrocarbon (TPHs), including diesel and petrochemical products, due to the economic and industrial activities. Petroleum products contain chemicals that are carcinogenic and potentially mutagenic. Phytoremediation, the use of plant to treat the petroleum hydrocarbon contamination had been used in this study. Diesel was chosen to represent the petroleum hydrocarbon contamination and analysed as the total petroleum hydrocarbon (TPHs). The plant used as a phytoremediator was *Paspalum scrobiculatum* L.Hack. Response Surface Methodology (RSM) was employed, using a Box-Behnken design to optimize the three variables, which are diesel concentration, sampling day and aeration in order to get the maximum TPH degradation of diesel. From the analysis of variance (ANOVA), the value (0.8530) of the coefficient of determination (R^2) was obtained. The optimal condition was established at diesel concentration of 3.00 g/kg, 70 days of sampling day and 0.27 L/min of aeration. Under optimal value of parameters, the maximum TPH degradation of diesel was found to be 75%.

Keywords: Optimization, phytoremediation, total petroleum hydrocarbon (TPHs), diesel

I. INTRODUCTION

Petroleum products, including diesel oil, are common soil contaminants and are known to be carcinogenic and may contain potentially toxic compounds (Euliss et al. 2008). Diesel oil obtained by the distillation of crude oil has a carbon range between C_8 and C_{26} with high content of polyaromatic hydrocarbon (Jagtap et al. 2014). Since diesel oil is potentially phytotoxic and may interfere with normal plant development (Adam and Duncan, 1999), it was chosen as the model contaminant to represent petroleum hydrocarbons in this phytoremediation study. Plants have been shown to encourage organic contaminant degradation principally by providing an optimal environment for microbial proliferation in the rhizhosphere (Adam and Duncan, 2002).

Many soil remediation technologies, including soil washing and thermal treatment are applied to petroleum contaminated soil. However, these physical and chemical technologies destroy soil ecology and are very expensive. Phytoremediation has been proposed as a cost effective, non-intrusive and environmentally friendly technology for the restoration of soils contaminated with TPH (Jagtap et al. 2014). Phytoremediation, the use of plants to degrade or remove contaminants, has been shown to be effective for removing environmental petroleum. It has been assessed in a number of field and greenhouse studies (Euliss et al. 2008). While scientific interest in phytoremediation has recently arisen, in fact the use of plant-based systems to cleanse waters contaminated with organic and inorganic pollutants dates back hundreds of years.

In this study, *Paspalum scrobiculatum* L. Hack was used as phytoremediator. This plant is in grass type. This grass type of plant was chosen because of their fibrous root system, which can give large rhizoplane surface area, which is an advantage for establishing active microbial populations (Ogbo et. al, 2009). It also allows a close interaction between the rhizosphere microbial community and the contaminant (Kaimi et. al, 2007).

Response Surface Methodology (RSM) is one of the modelling tools used for optimization study (El-Ghenymy et al. 2012). RSM is a recommended method for modelling of pilot scale studies, especially for the phytoremediation process in order to save the time of doing experiment and cost (Al-Badawi et al. 2014).

The objective of this optimization study is to obtain the optimum conditions of operation of the pilot plant in treating sand containing diesel pollutants. Optimization was performed using *Design-Expert* software version 6.0.10 (Stat-Ease, USA, 2003), also called the *Design of Experiment* (DOE), and followed by the Response Surface Method (RSM) and Box-Behnken Design (BBD). Since the optimization of phytoremediation pilot scale study is rare, researchers have adopted RSM using BBD to optimize the performance of the phytoremediation of sand contaminated with diesel.

II. MATERIAL AND METHOD

a. Plant and Pilot Scale Setup

In this optimization study, the tank used has a dimensions of L1.0 x W0.9 x H1.0 m (Figure II.1). The tank was filled with coarse gravel (ø 20 mm), fine gravel (ø 2 - 3 mm) and sand (ø 0.15 – 1.18 mm) with depth of 10, 10 and 30 cm, respectively. There were 36 plants of *Paspalum Scrobiculatum* L. Hack being planted in the tank throughout this study. The experiment was run with three diesel concentrations and aeration rates for 70 days.

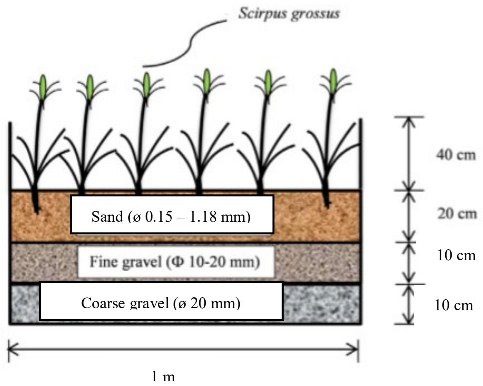

Figure II.1: Pilot Scale Setup

b. Total Petroleum Hydrocarbon (TPH) Analysis

The diesel concentration in this study was analyzed through total petroleum hydrocarbon (TPH). The sand extraction procedure was adapted from (Euless at al. (2008) and (Sun et al. (2004). The sand was first dried at room temperature to remove any water from the samples. After that, 10g of dried sand was placed into a 100 mL capped Teflon bottle with 50 mL dichloromethane (DCM) as a solvent. The sample was then extracted using Ultrasonic Bath (Thermo Line Ultra Sonic Cleaner, Fisher Scientific) for 30 minutes. The extract sample was then filtered and transferred into the 20 mL glass scintillation vial. These samples were left in fume hood overnight to allow the DCM to evaporate. After the DCM had been fully evaporated, another 2 mL of DCM was added and transferred into a GC vial for analysis of total hydrocarbon carbon (TPH) degradation. The degradation of TPH were analyzed using a GC-FID (Agilent 7890 A), equipped with HP-5 5% phenyl methyl siloxane column that was 30 m x 0.32 mm i.d x 0.25 micron and helium gas was used as the carrier gas. The column temperature was programmed to stay at 50°C for 1 minute, ramp 15°C per minute to 320°C (Al-Badawi at al. 2013).

c. Optimization Conditions with Response Surface Methodology (RSM)

In this optimization study, the TPH concentration in sand was set by using RSM and followed by Box-Behnken Design (BBD). The interaction between the main factor of diesel concentration, sampling day and aeration rate to the response of TPH degradation was investigated. The results were then analysed to develop an appropriate model for these factors. The variability factors included in the design were diesel concentration (0.05, 1525 and 3.00 g diesel/kg sand), sampling day (14, 43 and 72 days) and aeration rate (0, 1 and 2 L/min). The range of selected factors for variability was determined by preliminary experiments. The variables and their code level are shown in Table II.1.

Variable	Unit	Code level		
		-1	0	+1
Diesel concentration	g diesel/kg sand	0.05	1.525	3.00
Sampling day	Day	14	43	72
Aeration rate	L/min	0	1	2

Table II.1 : Variables and their level in experimental design

The aim for this study was to maximise the TPH degradation in sand as well as to find the optimize condition in order to achieve the desired result. The dependant variable was TPH degradation. Box-Behnken was used for the statistical design of experiments and data analysis.

III. RESULTS AND DISCUSSION

a. Optimization of Operational Condition

Optimization experiment was conducted by using *Design Expert* version 6.0.10 followed by RSM and BBD. The factors that affect the diesel TPH degradation in sand were diesel concentration, sampling day and aeration rate. Table III.1 shows the three factors (diesel concentration, sampling day and aeration rate) as the parameter in this study and the diesel TPH degradation as the response. Based on the table, there are six runs with the same conditions (run 15 to 20), with a concentration of 1.525 g diesel/kg sand, 43 days of sampling day and aeration rate of 1 L/min. Although the operating conditions were the same, but the response was obtained (percentage of TPH degradation) is different.

The results obtained for the degradation of diesel TPH in sand was analysed by using *Design-Expert* 6.0.10.Based on the Box-Behnken design, it was found that the quadratic model is appropriate (Agarry et al. 2012; Agarry & Ogunleye 2012& Zain 2012) for the response (degradation percentage of TPH-diesel).The quadratic model obtained as shown in Equation (III.1).This equation will be used in determining the degradation percentage of diesel TPH in the sand for the operation of the pilot plant in the optimal condition.

ANOVA was performed to test the significance and adequacy of the model. The ANOVA results of the suggested model for diesel TPH degradation was presented in Table III.2. Based on the table, the *F*-value for the model is 6.45. The *P*-value for the model is also less than 0.05, which indicates that the model was significant (Abu Hasan 2012) and quadratic equation formed can be applied for the degradation of diesel oil in the sand. The *F*-value of *lack of fit* obtained from Equation (III.1) is 4.45, which also shows the *F*-value is not significant. *F*-value of *lack of fit* is not good, so the value needed from the model must be so little.

Run	Factor 1: A: Diesel concentration (g/kg)	Factor 2 : B: Sampling day (Day)	Factor 3: C:Aeration rate (L/min)	Response: TPH degradation of Diesel (%)
1	0.05	14	0	12
2	3.00	14	0	36
3	0.05	72	0	36
4	3.00	72	0	52
5	0.05	14	2	38
6	3.00	14	2	8
7	0.05	72	2	64
8	3.00	72	2	59
9	0.05	43	1	54
10	3.00	43	1	85
11	1.52	14	1	19
12	1.52	72	1	87
13	1.52	43	0	33
14	1.52	43	2	36
15	1.52	43	1	63
16	1.52	43	1	69
17	1.52	43	1	60
18	1.52	43	1	77
19	1.52	43	1	76
20	1.52	43	1	76

Table III.1: Box-Behnken design matrix for three test factors along with the observed and predicted response for TPH degradation of diesel

$$\hat{y} = -7.98295 + (-3.41295A) + (1.35699B) + (62.06396C) + (3.30275A^2) + (-0.011123B^2) + (-27.81446C^2) + (0.049679AB) + (-6.35593AC) + (0.15948BC) \quad \text{(III.1)}$$

With, $\hat{y}$=estimation of the percentage degradation of diesel TPH in sand (%), A = diesel concentration (g/kg), B = sampling day (day) and C = aeration rate (L/min).

In the quadratic equation as well, given adequate precision for the degradation of diesel TPH in the sand was 7.801.This level of adequate precision is to measure the signal-noise ratio, where the ratio exceeds 4.000 is needed (Sharma et al. 2009). This shows that the model is adequate for use in the pilot plant design. Value for R^2 obtained for the design of RSM was 0.853. According to Chauhan and Gupta (2004), the RSM model with $R^2 > 0.75$ is acceptable. Thus, the model obtained in this study can also be accepted and used for degradation of diesel TPH in sand.

Optimization is performed to determine the optimum conditions for each factor that is used to produce the maximum of diesel TPH degradation in the sand. Response for percentage of diesel TPH degradation was set to be maximized to enable it to achieve the highest performance at the optimum condition. With optimization process, desirability and responsiveness of each factor were combined and then the maximum model (equation (III.1)) will be done.

Parameter	Sum of squares	Degree freedom	Min square	*F*-value	*P*-value	
Model	9090.27	9	1010.03	6.45	0.0037	Significant
Lack of fit	1278.46	5	255.69	4.45	0.0636	Not significant
Pure error	287.5	5	57.5	-	-	
Cor total	10656.23	19		-	-	
$R^2 = 0.8530$; $R^2_{adj} = 0.7208$; Adeq precision = 7.801						

Table III.2 ANOVA analysis of the quadratic model to the diesel TPH degradation in sand

b. Desirability and Relationship Between the Response and Variables Under the Optimum Condition

Optimization results are shown in Table III.3. Based on the table, there are four optimal conditions given. The first and second conditions have the same desirability, with the value of 0.859 but different in aeration rate. Aeration rate for the second condition is greater than the first condition. The third condition has the desirability and diesel TPH degradation with a small value compared to the first and second condition. For the fourth condition, the result shows that the desirability is very low with high value of aeration rate.

No.	Diesel concentration (g/kg)	Sampling day (day)	Aeration rate (L/min)	Diesel TPH degradation (%)	Desirability
1	3.00	70	0.27	75	0.859
2	3.00	70	0.28	75	0.859
3	3.00	69	0.25	74	0.858
4	0.05	65	0.57	65	0.722

Table III.3 Diesel TPH degradation and desirability for different optimal condition

Therefore, the optimum conditions proposed by the BBD is in the first condition, which the desirability is 0.859, diesel concentration is 3.00 g/kg, for 70 days of sampling day and aeration rate of 0.27 L/min. The highest desirability with optimum operating condition was shown in Figure III.1.

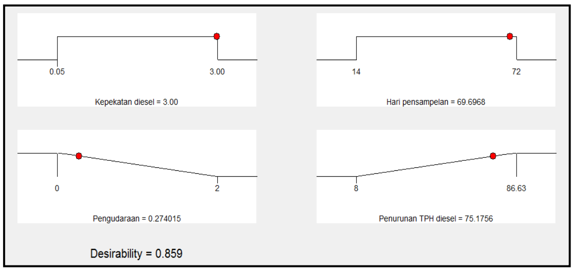

Figure III.1: Optimal condition and highest desirability of diesel TPH degradation in sand

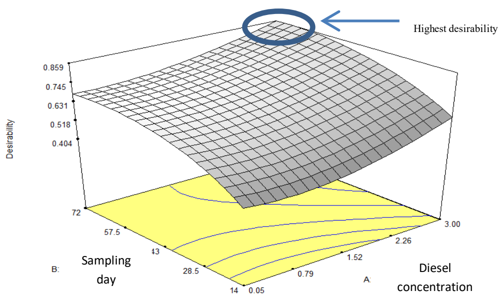

Figure III.2: The optimum condition for the operation of the pilot plant and the estimated percentage of diesel TPH degradation at desirability of 0.859 given by the BBD.

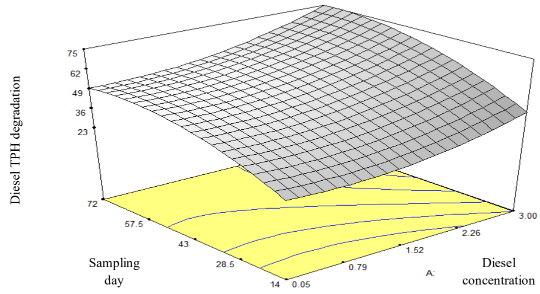

Figure III.3 Percentage degradation of diesel TPH in sand at the optimal condition (A: Diesel concentration, B: Sampling day)

Figure III.4 depict the effect of diesel concentration (A) and aeration rate (C) on diesel TPH degradation in sand. It was observed that diesel TPH degradation increased when aeration rate was decreased. This might due to the effect of aeration rate which was not significantly different on diesel TPH degradation.

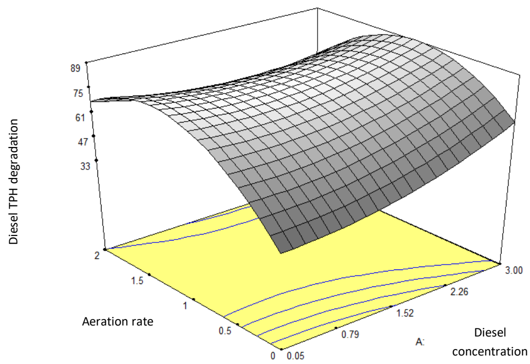

Figure III.4: Percentage degradation of diesel TPH in sand at the optimal condition (A: Diesel concentration, C: Aeration rate)

Figure III.2 shows the optimal conditions for the operation of pilot plant. As shown in the figure, the optimal concentration of diesel in the operation of pilot plant was 3.00 g/kg, with sampling days of 70 days and the aeration rate of 0.27 L/min.

In optimal conditions, the estimated percentage of diesel TPH degradation in the sand by Equation (III.1) was 75% (Figure III.3 and III.4). Figure III.3 and III.4 shows the 3D response surface plot on how diesel TPH degradation relates to the factors of diesel concentration (A), sampling day (B) and aeration rate (C). The effect of diesel concentration (A) and sampling day (B) on diesel TPH degradation was shown in Figure III.3. It can be seen that higher diesel concentration and sampling day led to higher diesel TPH degradation. For the sampling day, the increasing is generally believed that the long retention time of a phytoremediation operation leads to higher of diesel TPH degradation (Al-Badawi et al. 2014).

The optimization results in this study, which is 75% of TPH degradation was in line with other studies. Agarry et al. (2012) determined the degradation of kerosene oil and found that 75.06% of removal was predicted by BBD. The study conducted by Al-Badawi et al. (2014) which determined the TPH removal of diesel in water found that the optimized value was 76.3%. Different articles were reported up to 54.12% removal of hydrocarbon (Martinez Alvarez et al. 2015), 98% of COD removal in petroleum refinery wastewater (Pakravan et al. 2015) and 89.7% of COD removal in contaminated coastal sediment (Subha et al. 2015).

IV. CONCLUSION

In this diesel TPH degradation optimization studies, the modelling method used was *Design-Expert* Version 6.0.10 (Stat-Ease, USA, 2003), followed by a response surface method (RSM) and Box-Behnken design. The coefficient of determination (R^2) for the model equation was 0.853 demonstrating significance for the regression model to predict the response. The optimum conditions were as follows: the diesel concentration of 3.00 g/kg, 70 days of sampling day and aeration rate of 0.27 L / min. Estimated percentage degradation of diesel TPH suggested by BBD was 75%. From this estimated diesel TPH degradation value, it shows that this RSM and the Box-Behnken Design can be used in the optimization process of phytoremediation in diesel TPH degradation by using *Paspalum scrobiculatum*.

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