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Maximizing diesel removal from contaminated sand using *Scirpus mucronatus* and assessment of rhizobacteria addition effect

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A R T I C L E I N F O

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TPH

A B S T R A C T

Phytoremediation is one of the green technologies that is friendly to nature, utilizes fewer chemicals, and exhibits good performance. In this study, phytoremediation was used to treat diesel-contaminated sand using a local aquatic plant species, Scirpus mucronatus, by analyzing the amount of total petroleum hydrocarbons (TPHs). Optimization of diesel removal was performed according to Response Surface Methodology (RSM) using Box-Behnken Design (BBD) under pilot-scale conditions. The quadratic model showed the best fit to describe the obtained data. Actual vs. predicted values from BBD showed a total of 9.1 % error for the concentration of TPH in sand and 0 % error for the concentration of TPH in plants. Maximum TPH removal of 42.3 ± 2.1 % was obtained under optimized conditions at a diesel initial concentration of 50 mg/kg, an aeration rate of 0.48 L/min, and a retention time of 72 days. The addition of two species of rhizobacteria (Bacillus subtilis and Bacillus licheniformis) at optimum conditions increased the TPH removal to 51.9 ± 2.6 %. The obtained model and optimum condition can be adopted to treat diesel-contaminated sand within the same TPH range (50–3000 mg/kg) in sand.

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1. Introduction

Soil pollution is the mixing of compounds and the accumulation of unwanted particles in the soil matrix [1]. Several compounds may contaminate the soil, including organic [2] and inorganic pollutants [3]. Soil pollution may come from daily domestic activities [4] and from industrial activities [5]. Hydrocarbon pollution in soil is currently increasing due to increased fuel utilization and industrialization in many areas [6–9]. Diesel is one of the compounds commonly found to contaminate soil [10,11]. Diesel in soil was reported to reach 18,231 mg/kg in highly contaminated areas [12] and ranging within 50–500 mg/kg in non-severely contaminated areas [13].

Treatment of diesel-contaminated soil has been done using various techniques, including land excavation [14], burning [4], and soil washing [15]. The mentioned technologies are considered costly to be applied in such a large, contaminated area. The application of these techniques also requires skilled personnel to handle the operation and maintenance procedures [16]. The attention is currently shifted to the utilization of biological treatment or bioremediation to remove diesel from contaminated soil, which offers a considerably lower operation and maintenance cost [10,17–19]. One of the biological methods that have reliable performance to treat diesel-contaminated soil is phytoremediation [5,20].

Phytoremediation utilizes plants' natural mechanisms to resist, adapt, and utilize organic compounds in soil to grow [5,21]. Various types of plants have been widely used in hydrocarbon removal, such as *Glycine max* [22], *Festuca arundinacea* [23], *Scirpus grossus* [24], and *Medicago sativa* [25]. Grass-type species have often been reserved as impressive plants for treating polluted soil hydrocarbons due to their fibrous root systems [26,27]. The rhizosphere area played an important role due to the interaction between plants and the rhizo-microbial community to degrade and remove organic pollutants from soil [28]. Bacteria is one of the most commonly mentioned rhizo-microbial species involved in phytoremediation processes [29]. The addition of certain bacteria species is also reported to enhance the hydrocarbon degradation process in soil [30].

Most of the reported studies utilize terrestrial plants as hydrocarbon treatment agents in phytoremediation [6,31,32]. The utilization of aquatic plants, especially native local plants, to treat diesel-contaminated soil is still limited. In addition, Response Surface Methodology (RSM) is currently gaining attention in terms of modeling an optimization for research; however, phytoremediation of diesel-contaminated soil optimization studies using native local plant species is still rare. This research was aimed to (i) optimize, via RSM, the condition of phytoremediation using native aquatic plant species of *Scirpus mucronatus* for maximum removal of diesel represented by Total Petroleum Hydrocarbons (TPHs) and (ii) analyze the effect of rhizobacteria addition to plant growth and diesel removal from sand. The presented result is expected to give a clear understanding of the optimum conditions for diesel removal from sand and also to give a direct view of the effect of rhizobacteria addition during the phytoremediation process.

2. Materials and methods

2.1. Optimization via response surface methodology (RSM)

Optimization of phytoremediation conditions for maximum TPH removal in sand was conducted through Response Surface Methodology (RSM) using Design of Expert (Version 6, Stat-Ease, USA). The interaction between the relationship of key factors, namely diesel concentration in sand, retention time, and aeration, and TPH removal in contaminated sand and plants was analyzed using Box Behnken Design. The results obtained were then analyzed to develop an appropriate model for these factors. The optimum conditions obtained through RSM were then confirmed by comparing them with the experimental results.

The response value used was TPH concentration data on sand and plants. While the independent factors included were diesel

Table 1
Run order based on Box Behnken Design.

Run Order	Factor 1	Factor 2	Factor 3
	A: Diesel Concentration (mg/kg)	B: Retention Time (day)	C: Aeration (L/min)
1	50	39.5	2
2	50	7	1
3	3000	7	1
4	1525	39.5	1
5	50	39.5	0
6	1525	39.5	1
7	50	72	1
8	1525	72	2
9	3000	39.5	0
10	3000	39.5	2
11	1525	39.5	1
12	1525	7	0
13	1525	39.5	1
14	3000	72	1
15	1525	72	0
16	1525	39.5	1
17	1525	7	2

concentrations (50 and 3000 mg/kg), retention time (7 and 72 days), and aeration (0 and 2 L/min), The minimum concentration value (50 mg/kg) was determined based on soil quality standards for industrial areas containing benzene. While the maximum concentration (3000 mg/kg) was determined based on the ratio of mass to plant, the plant is still alive at the diesel concentration based on our earlier findings. The retention time in the plant site pilot plant used is from the 7th day until the 72nd day. The 7th day is determined because on that day the plants still look healthy and have not experienced whitening. The 72nd day is determined because on that day, some plants have suffered from lethargy and changed color from green to yellow or brown. Aeration was set at 0 and 2 L/min to analyze the effect of supplying aeration on the TPH removal performance, following the protocols by Al-Baldawi et al. [33] and Tangahu et al. [34].

Based on the variable factors used, the sampling that must be completed is 17 times with diesel concentrations of 50, 1,525, and 3000 mg/kg and aeration of 0, 1, and 2 L/min. The concentration of 1525 mg/kg was the median value of the diesel concentration. Similarly, 1 L/min of aeration is the middle value of the ventilation factor. In determining the optimal condition of the pilot plant, sampling was conducted as shown in Table 1. In Table 1, the first column is the number of runs, and the next three columns are the experimental conditions compiled by Box-Behnken Design. An ANOVA test was performed on all three factors and the obtained response. Subsequently, the results obtained were used in the operation of the *Scirpus mucronatus* plant site pilot plant to treat diesel-containing sand.

The optimum condition obtained from RSM was then verified by performing a validation run similar to the condition suggested by the model. The results obtained from the validation run and prediction were then compared to calculate the percentage error.

2.2. Reactor set-up and operational condition

A pilot plant study was conducted using 12 pilot tanks, as depicted in Fig. 1, each with dimensions of 1.18 $m \times 0.9$ m x 0.9 m. Each tank was filled with coarse gravel media (⌀ 2 cm) with a thickness of 0.1 m, fine gravel (⌀ 0.5 cm) with a thickness of 0.1 m, and sand with a thickness of 0.3 m. In this optimization study, the mass of gravel and sand applied was 491 kg, and a total of 60 one-month-old healthy plants (*Scirpus mucronatus*) were used in each tank. The plants were obtained from a shallow lake in Bangi, Selangor, Malaysia, and propagated in a greenhouse until the first generation was produced. Sand and gravel were used in this study instead of soil to ensure plants only depend on pollutants for growth since no additional nutrients are available in sand or gravel. Referring to Fig. 1, the whole set-up consisted of three rows; each row was set at one aeration rate (0, 1, and 2 L/min). Each row comprised one control tank without contaminants and another three tanks representing vegetated reactors with different diesel concentrations (50, 1525, and 3000 mg/kg). All the pilot reactors were operated batchwise for 72 days according to the experimental design by RSM, as listed in Table 1.

2.3. Effect of rhizobacteria addition

The optimum condition obtained from the DOE model was then validated in two conditions, namely without and with the addition of rhizobacteria. The rhizobacteria added were the combination of the two bacteria that provided the highest percentage of TPH removal for which this study had previously been carried out [35]. The bacterial species added were *Bacillus subtilis* and *Bacillus licheniformis*. The amount of bacterial mixture added to the reactor was 10 % (v/v) of the saturated sand [36,37]. The sand retention capacity is 26 mL in every 100 g of sand [38,39]. When the mass of sand in the reactor is 491 kg, the combined content of water in the reactor was 127.7 L. Thus, the combined content of bacteria added to the reactor was 12.7 L. The bacteria used were two species, so the combined content of each bacterial species was 6.5 L. In providing the added bacteria, the first step to be carried out was the culture of *Bacillus subtilis* and *Bacillus licheniformis* in TSA agar medium (R&M Chemicals, U.K.) for 24 h [40]. The next step is the culture of

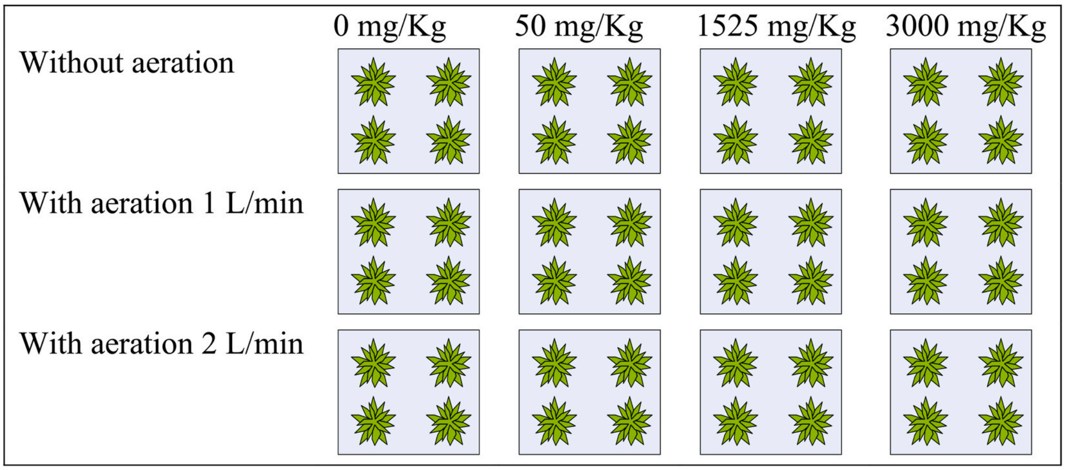


Fig. 1. Schematic diagram of reactor set-up for phytoremediation optimization study. All experiments were conducted in triplicate.

bacteria in TSB liquid medium (R&M Chemicals, U.K.) for 24 h [41]. The amount of mixture for the two bacteria was 13 L.

2.4. Plant growth measurement

During the validation run to compare the estimated results with the experimental data and also during the exposure to rhizobacteria, plant growth through dry mass measurement was also recorded. The plant's weight was determined using the gravimetrical method [20,30]. One plant was taken from each reactor during a sampling period. The plant was rinsed using tap water and dried on filter paper (Whatman, U.K.). The dried plant was then weighed as a wet weight. The plant was then dried once again in a 70 °C oven (Memert+, Germany) for 72 h until a constant weight was achieved. The dry weight of the plant was weighed after 72 h in the oven.

2.5. Analysis of TPH in sand and plant

Throughout the optimization study, sampling was conducted in accordance with the results obtained on the selected optimization runs from the Design of Expert Application (detailed in Section 2.3). Samples of sand, water, and plants were taken from each pilot plant. Sand samples were taken compositely from three different points at a depth of about 5–10 cm. Sand samples were taken simultaneously at the same time. The sand sample was then placed in a 120-mL glass bottle. Water samples were also taken compositely from three different points. Plant samples were taken at the same time, and one plant was randomly taken per sampling point. Plants are uprooted from the sand, cleaned with tap water, and dried with a tissue before further analysis.

Three samples of sand and plant samples were taken for TPH extraction in each sampling period. Approximately 10 g of sand or plant sample was placed in 100 mL glass bottles; sodium sulfate (Merck, Germany) and 50 mL dichloromethane (DCM) (R&M Chemicals, U.K.) were then added to the bottles. The sample mixture was stirred well. The sample mixture was extracted using an ultrasonic cleaner (Termo-10D, U.S.A.) for 30 min at 50 °C. The supernatant is filtered through a glass wool (Supelco, U.S.A.) into a 5 mL glass bottle. The extract was left in the fume chamber for seven days to allow the solvent to evaporate. When the solvent had evaporated, 2 mL of DCM was added, and the sample was stored in a GC vial. Extracts were analyzed using GC FID (Agilent, Model 7890 A, U.S.A.) with HP-5 5 % column phenyl methyl siloxane (30 $m \times 0.32$ mm i. d. X 0.25 μm) with helium as carrier gas. The column temperature is programmed at 50 °C for 1 min, and then ramps at 15 °C per minute to 320 °C for 10 min.

2.6. Statistical data analysis

In this optimization study, the parameters that need to be analyzed using statistics to obtain the significance of the data are changes in plant physics to diesel concentration and TPH concentration in sand and plants. Statistical analysis using two-way ANOVA Statistics SPSS 16.0 (IBM, U.S.A.) at a 95 % confidence level or $p \leq 0.05$ [42,43].

3. Results and discussion

3.1. Optimization using response surface methodology

The result of TPH concentrations in sand and plants based on the run order provided by RSM as well as the predicted value by BBD

Table 2
Result of TPH concentration in sand and in plants (actual vs. predicted concentration via Box-Benken Design).

Run Order	Initial Diesel Concentration (mg/kg)	Retention Time (days)	Aeration (L/min)	TPH concentration in sand (mg/kg)			TPH concentration in plants (mg/kg)		
				Actual	Predicted	Error (%)	Actual	Predicted	Error (%)
1	50	39.5	2	12.41	12.41	0.00	139.97	139.97	0.00
2	50	7	1	29.83	29.83	0.00	342.50	342.50	0.00
3	3000	7	1	317.00	317.00	0.00	272.09	272.09	0.00
4	1525	39.5	1	66.38	64.50	2.83	288.04	288.04	0.00
5	50	39.5	0	5.81	5.81	0.00	162.87	162.87	0.00
6	1525	39.5	1	60.18	64.50	−7.18	288.04	288.04	0.00
7	50	72	1	29.43	29.43	0.00	416.60	416.60	0.00
8	1525	72	2	34.92	34.92	0.00	233.38	233.38	0.00
9	3000	39.5	0	120.58	120.58	0.00	161.05	161.05	0.00
10	3000	39.5	2	199.64	199.64	0.00	290.75	290.75	0.00
11	1525	39.5	1	76.28	64.50	15.43	288.04	288.04	0.00
12	1525	7	0	86.32	86.32	0.00	259.56	259.56	0.00
13	1525	39.5	1	64.17	64.50	−0.52	288.04	288.04	0.00
14	3000	72	1	104.08	149.28	−43.43	408.43	408.43	0.00
15	1525	72	0	39.02	75.88	−94.49	140.79	140.79	0.00
16	1525	39.5	1	55.50	22.84	58.85	288.04	288.04	0.00
17	1525	7	2	41.99	0.72	98.29	156.77	156.77	0.00

(calculated by the model) are tabulated in Table 2. Based on the Box-Behnken design method, it is found that the quadratic model is suitable for both factors. The quadratic models obtained are as shown in Equations (1) and (2). The model was used in determining the concentration of TPH on sand and plants during plant site pilot plant operation at optimal conditions.

$$y_1 = 11.31384 + (0.014121 * A) + (6.73179E - 003 * B) + (42.19012 * C) + (2.73896E - 005 * A^2) - (4.44663E - 003 * B^2) - (24.70798 * C^2) - (1.52430E - 004 * A * B) - 0.034716 * A * C) + (0.30949 * B * C) - (5.62031E - 007 * A^2 * B) + (1.54092E - 005 * A^2 * C) + (9.59880E - 006 * A * B^2) \quad \text{Equation (1)}$$

$$y_2 = 319.31290 + (0.034103 * A) - (7.66664 * B) + (191.56701 * C) - (3.42649E - 005 * A^2) + (0.093943 * B^2) - (130.82931 * C^2) + (4.84993E - 004 * A * B) - (0.015137 * A * C) + (1.50288 * B * C) + (8.93128E - 007 * A^2 * B) + (1.34430E - 005 * A^2 * C) - (3.65124E - 005 * A * B^2) \quad \text{Equation (2)}$$

with, y_1 = TPH concentration in sand (mg/kg), y_2 = TPH concentration in plant (mg/kg), A = TPH initial concentration (mg/kg), B = retention time (day) and C = aeration (L/min).

The results of the ANOVA analysis of the two quadratic models are shown in Table 3. Based on Table 3, the F-values for both models (TPH in sand and in plants) were 129.87 and 63, 660, 000, respectively. This value indicates that the quadratic model obtained was significant ($p < 0.05$). An adequate level of precision is needed to measure the noise-to-noise ratio, for which a ratio exceeding 4 is required [44]. From the quadratic model obtained, the noise-to-noise ratio value is 45.76 for the concentration of TPH in sand. As for the concentration of TPH in plants, there is no value for the signal-noise ratio. This is due to the values of $R^2 = 1$ and $R^2\text{adj} = 1$, indicating that each model was sufficient for use and in accordance with the experimental design of a plant site pilot plant. Moreover, the p value of the second model was less than 0.05, which indicates that the model was significant, and the quadratic equations formed can be applied to determine the concentration of TPH in sand and plants [11]. The F-value of the lack of fit obtained from Equation (1) was 32.3, while Equation (2) does not have a lack of fit value due to the total congruency.

The results obtained through experiments and estimated by the model are shown in Table 2. In Table 2, run 5 gives the lowest TPH concentration in the sand (5.8 mg/kg), while the TPH concentration in the plant is 162.9 mg/kg under the condition of 50 mg/kg diesel

Table 3
Result of ANOVA analysis for a quadratic model for TPH in sand and plants.

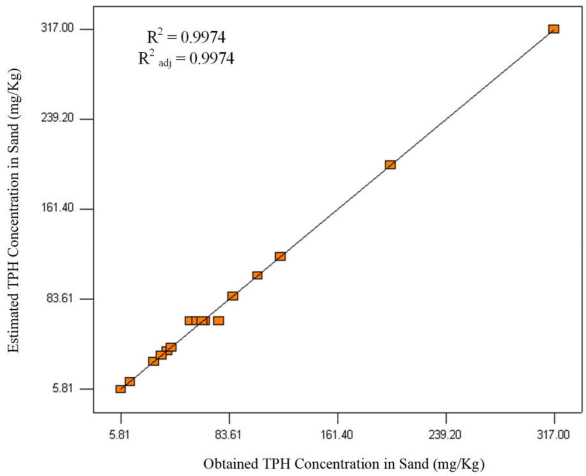
TPH in sand						
Source	Sum of square	DF	Mean Square	F-value	Prob > F	Remark
Model	94237.64	12	7853.14	129.87	0.0001	Significant
A	22800.94	1	22800.94	377.06	<0.0001	
B	738.84	1	738.84	12.22	0.0250	
C	586.28	1	586.28	9.70	0.0357	
A^2	8456.28	1	8456.28	139.84	0.0003	
B^2	487.92	1	487.92	8.07	0.0468	
C^2	2570.46	1	2570.46	42.51	0.0029	
AB	11291.21	1	11291.21	186.72	0.0002	
AC	1312.82	1	1312.82	21.71	0.0096	
BC	404.68	1	404.68	6.69	0.0609	
A^2B	3158.53	1	3158.53	52.23	0.0019	
A^2C	2247.81	1	2247.81	37.17	0.0037	
AB^2	447.28	1	447.28	7.40	0.0530	
Pure Error	241.88	4	60.47			
Cor Total	94479.52	16				
$R^2 = 0.9974$	R^2 adj = 0.9898	Lack of fit = 45.762				
TPH in plants						
Source	**Sum of square**	**DF**	**Mean Square**	**F-value**	**Prob > F**	**Remark**
Model	117589.68	12	9799.14	63,660,000	<0.0001	Significant
A	5547.27	1	5547.27	63,660,000	<0.0001	
B	444.57	1	444.57	63,660,000	<0.0001	
C	25.97	1	25.97	63,660,000	<0.0001	
A^2	4165.22	1	4165.22	63,660,000	<0.0001	
B^2	6877.01	1	6877.01	63,660,000	<0.0001	
C^2	72068.66	1	72068.66	63,660,000	<0.0001	
AB	968.21	1	968.21	63,660,000	<0.0001	
AC	5821.69	1	5821.69	63,660,000	<0.0001	
BC	9542.82	1	9542.82	63,660,000	<0.0001	
A^2B	7976.12	1	7976.12	63,660,000	<0.0001	
A^2C	1710.77	1	1710.77	63,660,000	<0.0001	
AB^2	6471.86	1	6471.86	63,660,000	<0.0001	
Pure Error	0.00	4	0.00			
Cor Total	117589.68	16				
$R^2 = 1.000$	R^2 adj = 1.0000	Lack of fit = –				

concentration and a retention time of 39.5 days without aeration. The highest TPH concentrations in plants (416.6 mg/kg) were achieved on run 7, at 50 mg/kg diesel concentration, 72 days of retention time, and 1 L/min of aeration.

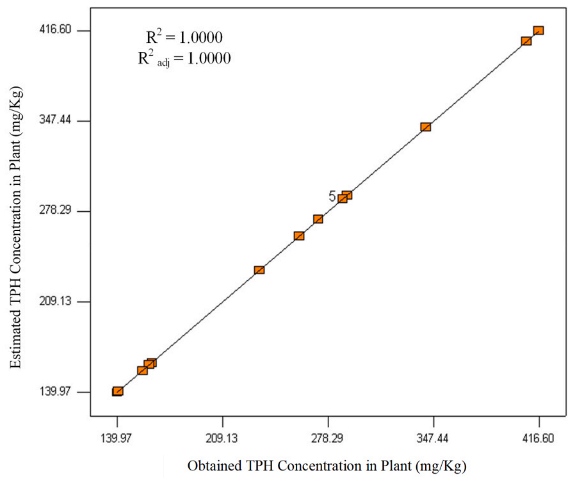
Fig. 2 demonstrates a comparative analysis between the concentration of TPH in sand and plants for each run obtained through experiments and that estimated by Box-Behnken. Based on the diagram, the concentration of TPH on sand estimated by Box-Behnken was obtained according to the removal pattern obtained from the experiments. However, there was a slight pattern difference in runs 14 to 17 (Fig. 2(a)). TPH concentrations in plants do not indicate a pattern of difference between actual and predicted data. This was because the value of $R^2 = 1$. A greater R^2 indicated that the obtained data were more closely matching the prediction; in this case, it means that the experiment was indeed equal to the prediction [45,46]. An illustration of the actual value of the response obtained through the experiments arranged by Box-Behnken and the estimated value obtained through Equations (1) and (2) are also shown in Fig. 2. The values of coefficients R^2 and R^2adj for the estimated concentration of TPH in sand are 0.9974 and 0.9898. While the estimated concentration of TPH in plants has a value coefficient of 1 for both R^2 and R^2 adj. The R^2 and R^2adj were also used as indicators to show satisfactory development between simulated data; values higher than 0.95 indicated a good fit [47]. In addition, Sidek et al. [48] mentioned that a significant *p*-value (<0.05), an R^2 above 0.9, and adequate precision >4 indicated the good soundness of the model with a surface contour plot.

3.2. Model desirability for the optimization study

Determining the optimal factor conditions carried out was to minimize the concentration of TPH in sand and maximize the



(a)



(b)

Fig. 2. Comparison of TPH concentrations obtained through experiments with those estimated by Box-Behnken in (a) sand and (b) plants.

concentration of TPH in plants. The required goal for operating conditions is that the initial concentration of diesel in the sand was set in the range of 50–3000 mg/kg, the retention time was set in the range of 7–72 days, and the aeration supply was set to a minimum to reduce electricity consumption. The response of TPH concentrations in sand was minimized (i.e., targeting maximum removal of diesel from sand), and TPH concentrations in plants were maximized. Through the optimization program, the likelihood of each factor and response was combined, and then the search for the maximum model (Equation (1) and (2)) was performed. The optimization results are shown in Fig. 3, where the optimum conditions at the predetermined goal are selected at a desirability of 0.82 with a diesel concentration of 50.0 mg/kg, a retention time of 72.0 days, and an aeration rate of 0.59 L/min. A desire value higher than 0.8 is considered a good scenario to be implemented for a specific objective function [49], with a higher value (closer to 1) indicating a better solution for optimization [49,50].

The desirability of the optimum operating conditions is shown in Fig. 4(a). Experiments were conducted to confirm the optimum conditions obtained from the model estimation to eliminate the concentration of TPH in sand and increase the concentration of TPH in plants [51,52]. Fig. 4(b) and (c) show that the optimal diesel concentration in the operation of this plant site is 50.0 mg/kg, with a sand retention time of 72 days at the plant site and an aeration supply of 0.6 (approximated from 0.59) L/min. Based on the optimal conditions obtained, sampling of sand, water, and plants was done from day 0 to day 72 to obtain the concentration of TPH on sand and plants. At this optimum condition, the estimated concentration of TPH in sand using Equation (1) was 17.11 mg/L (Fig. 4(b)), while the estimated concentration of TPH in plants using Equation (2) is 325.62 mg/kg (Fig. 4(c)).

3.3. *Validation run at optimal conditions*

In this section, the results obtained during the operation of the optimum-condition pilot plant will be compared with the results from the DOE model. This validation run was performed to confirm the validation of the results obtained from the DOE optimization [51]. Validation runs can be carried out using various methods, including t-tests and further runs using suggested conditions [53,54]. This research validation was conducted using further runs on the suggested condition, with the parameters analyzed being TPH concentrations in sand and plants. Table 4 summarizes the results of the confirmation run on the optimal condition of the plant site pilot plant compared to the results from DOE. The results given by the DOE model for TPH concentrations in sand and plants were 17.1 (↓32.9 %) and 325.6 mg/kg, respectively. Meanwhile, through the confirmation run conducted, the results for TPH concentrations in sand and plants were 18.7 ± 0.94 (↓31.3 %) and 325.2 ± 16.28 mg/kg, respectively. With this, the percentage of errors given is 9.1 % and 0.1 % for TPH on sand and plants, respectively. This RSM model can also be used for large-scale plant modeling, as long as the hydrocarbon concentrations used are still in the range of 50–3000 mg/kg. Previous research mentioned that *Scirpus grossus* was able to remove up to 81.5 % TPH, with the maximum extraction of diesel reaching 223.5 mg/kg [55]. Other plant species, *Festuca arundinacea* and *Lathyrus sativus,* achieved 54 % and 46 % of diesel removal from contaminated soil, respectively [56]. Diesel removal performance in phytoremediation is highly related to plant species and initial concentrations, which affect the degradation mechanisms and plant metabolism [16,57,58].

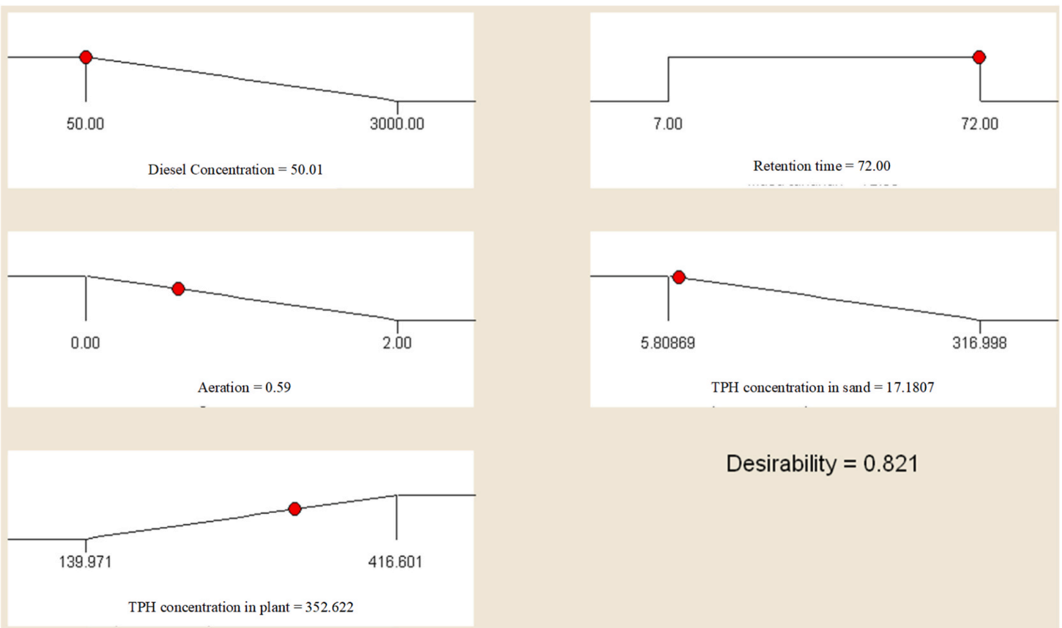
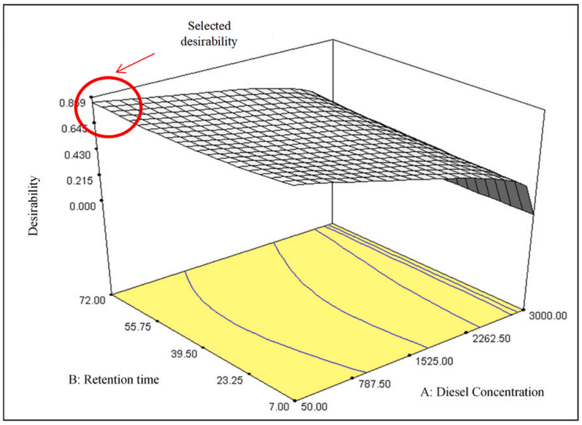
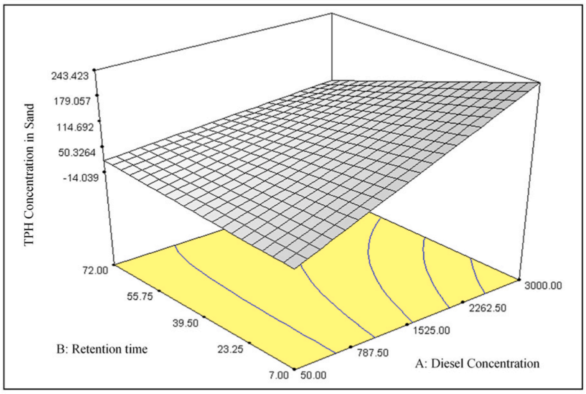


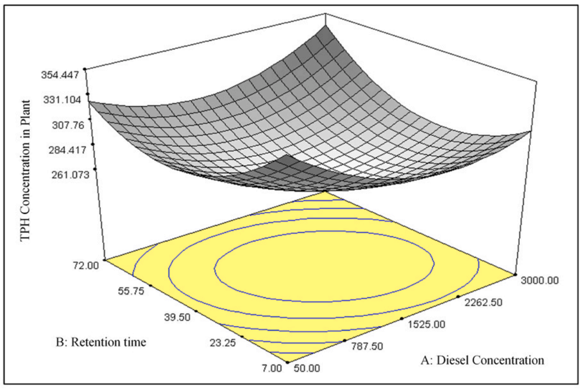
Fig. 3. Optimized Condition of TPH treatment in various operational parameters.



(a)



(b)



(c)

Fig. 4. (A) Desirability for optimized conditions during TPH removal; (b) TPH concentration in sand under optimized conditions; and (c) TPH concentration in plants under optimized conditions.

3.4. Effect of rhizobacterial addition

Growth changes expressed in wet and dry weight throughout the optimization study are depicted in Fig. 4. During the optimization study, the wet and dry weight of plants in the treatment with the addition of rhizobacteria was significantly higher than without the addition of rhizobacteria. This result indicated that the addition of rhizobacteria may support the growth of plants, as indicated by the

Table 4
Result of Confirmation run at optimum conditions.

Parameter	Predicted value by RSM model (mg/kg)	Experimental results from validation run (mg/kg)	Error (%)
TPH concentration in sand	17.1	18.7	9.1
TPH concentration in plants	325.6	325.2	0.1

significant increment in not only wet but also dry weight [5,24,28]. The increase in dry weight is clear evidence of cell growth, while the increase in wet weight might be biased due to the higher water uptake by plants [59–61]. Based on Fig. 4, at the end of the optimization period (Day 72), it was found that the wet and dry weight of plants given the addition of rhizobacteria was significantly higher than without the addition of rhizobacteria. Rhizobacteria may promote the growth of plants via several interactions, including phytostimulation, biofertilization, and biocontrol [62–64]. Phytostimulation is a phenomenon in which rhizobacteria release growth hormones, benefiting the host plant [65]. Biofertilization is a mechanism related to nitrogen fixation that converts nitrogen gas into ammonium, which is easier to uptake by plants [62]. In addition to that, rhizobacteria may also produce antibiotics and siderophores, which protect the host plant from phytopathogens and abiotic stress [62].

In general, the TPH concentration in the sand decreased with both treatments. In the treatment without the addition of rhizobacteria, the TPH concentration ranged between 18.7 ± 0.94 and 74.1 ± 3.7 mg/kg (Fig. 5). The TPH concentration in the sand at the beginning of the study was compared to that at the end of the study period, and the percentage of TPH removal in the sand was 42.3 ± 2.1 %. The TPH concentration range in sand on treatment with the addition of rhizobacteria was 16.2 ± 0.81 to 43.3 ± 2.2 mg/kg. The percentage of TPH removal achieved on day 72 under rhizobacteria addition was 51.9 ± 2.6 % with an increment of 9.6 % ($p > 0.05$) compared to the non-rhizobacteria addition. Based on statistical analysis, the addition of rhizobacteria made a not-significant difference in the TPH concentration in the sand. This result might have occurred since the measurement of compounds was TPH. The non-complete degradation of hydrocarbon compounds by rhizobacteria may still be counted during the TPH measurement in soil, thus resulting in no significant difference in the TPH concentration after treatment [8,11]. In this case, TPH removal can also indicate the complete degradation or bioconcentration of hydrocarbon compounds in plants [66] (see Fig. 6).

The concentration of TPH in plants treated without the addition of rhizobacteria increased from day 0 to day 14. After that, the TPH concentration decreased and increased again until the 42nd day and decreased so that it reached a concentration of 325.2 ± 16.3 mg/kg at the end of the study period (72 days). The TPH concentration in plants at the start of the study was 207.5 ± 10.4 mg/kg (Fig. 5), resulting in an increase of 56.8 % for the TPH concentration in plants. The TPH concentration in plants treated with the addition of rhizobacteria shows the same trend. The TPH concentration in plants increased from day 0 to day 14 and decreased on day 28. On day 42, the TPH concentration in plants increased and decreased at the end of the study period (day 72). The TPH concentration in plants on that day was 414 ± 20.7 mg/kg. If the TPH concentration in plants at the end of the study period was compared to the beginning of the study period, it shows that there is an increase in the concentration of 117.4 % with a significant difference of 60.6 % ($p < 0.05$). The concentration of TPH in plants for treatment with the addition of rhizobacteria is mostly located in the roots of plants, and the addition of rhizobacteria can increase TPH and polycyclic aromatic hydrocarbons (PAH) uptake by plants significantly. A similar result was obtained by Al-Baldawi et al. [24], which stated that the addition of three rhizobacterial strains (mostly Bacillus) during TPH degradation from soil increased the performance by up to 18 %. As previously mentioned, rhizobacteria assisted in the removal of TPH from soil by promoting the degradation of petroleum-related hydrocarbons into simpler compounds that can be absorbed more easily by plants. In addition, plants may provide a good environment for bacterial growth by excreting exudates, which promote rhizobacterial growth, resulting in the higher degradation of TPH in the rhizosphere area [9,59,67]. This is also indicated by the higher wet

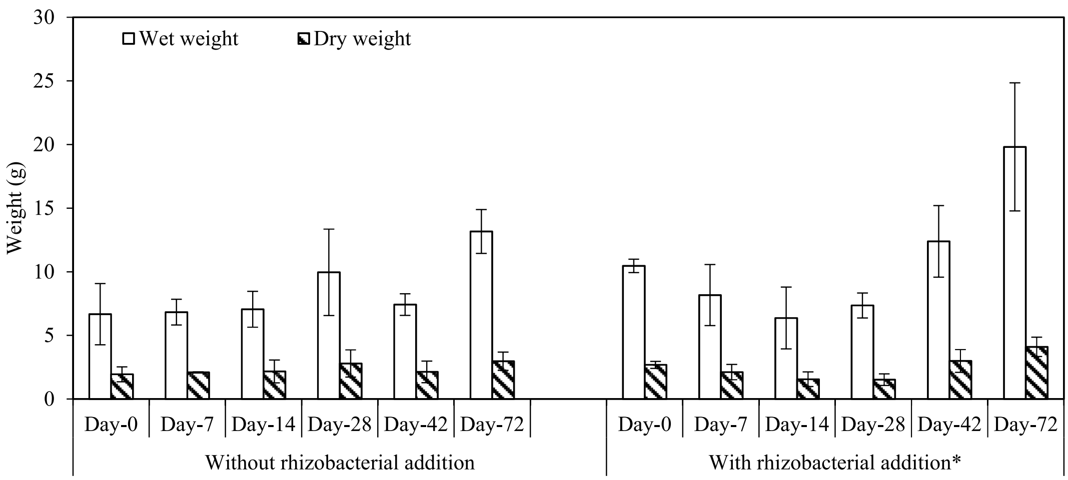


Fig. 5. Effect of rhizobacterial addition on plant growth. The asterisk (*) symbol indicates a significant difference (p < 0.05) in weight between without and with rhizobacterial addition.

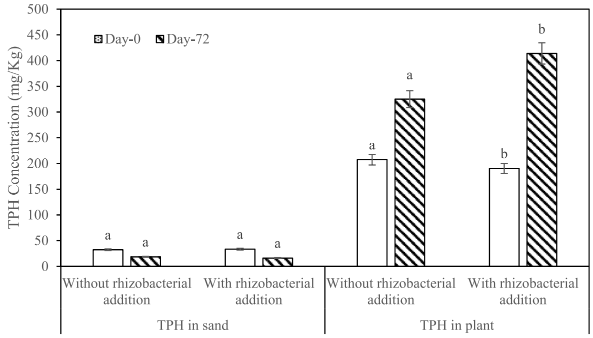


Fig. 6. Effect of rhizobacterial addition to the TPH concentration. The different letters above the bar (a–b) indicate a significant difference ($p < 0.05$) in TPH concentration between without and with rhizobacterial addition.

and dry weight of plants in the treatment with the addition of rhizobacteria versus without the addition of rhizobacteria [68–70]. However, based on statistical analysis, the addition of bacteria did not make a significant difference in the TPH concentration in plants.

4. Conclusions

Optimization of diesel-contaminated sand phytoremediation using Response Surface Methodology (RSM) showed that *Scirpus mucronatus* was feasible to use as a treatment agent. Under optimized conditions of an initial diesel concentration of 50 mg/kg, an aeration rate of 0.6 L/min, and a retention time of 72 days, a total of 42.3 ± 2.1 % total petroleum hydrocarbon (TPH) removal can be achieved. The model generated from the Box Behnken Design (BBD) showed a total 9.1 % error for TPH concentration in sand, while showing 0 % error for TPH concentration in plants when compared to the experimental data. The addition of rhizobacteria (*Bacillus subtilis* and *Bacillus licheniformis*) had no significant effect on the plant's growth parameter while giving a significant increment to the TPH removal from sand. A total of 9.6 % increment in TPH removal was obtained in the reactor with rhizobacteria addition. The obtained model and data were suggested to be adaptable to be scaled up with criteria of initial diesel concentration ranged between 50 and 3000 mg/kg.

CRediT authorship contribution statement

Ipung Fitri Purwanti: Conceptualization, Formal analysis, Investigation, Writing – original draft. **Siti Rozaimah Sheikh Abdullah:** Conceptualization, Data curation, Methodology, Supervision, Writing – original draft. **Ainon Hamzah:** Funding acquisition, Supervision. **Mushrifah Idris:** Funding acquisition, Supervision. **Hassan Basri:** Funding acquisition, Supervision. **Mohd Talib Latif:** Funding acquisition, Supervision. **Muhammad Mukhlisin:** Funding acquisition, Supervision. **Setyo Budi Kurniawan:** Data curation, Visualization, Writing – original draft, Writing – review & editing. **Muhammad Fauzul Imron:** Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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