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# Box-Behnken design application to study enhanced bioremediation of soil artificially contaminated with spent engine oil using biostimulation strategy

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## Abstract

This work studies the biodegradation of spent engine oil in soil using Box-Behnken design under response surface methodology. NPK fertilizer (inorganic nutrient), Tween 80 (nonionic surfactant), and pig manure (organic nutrient) concentrations were used as independent biostimulant variables, while total petroleum hydrocarbon (TPH) and hexavalent chromium (Cr (VI)) reductions as dependent variables (response) in a 42-day remediation period. A statistically significant second-order quadratic regression model for TPH and Cr (VI) removal was obtained. The coefficient of determination ( $R^2 = 0.9995$  for TPH and  $0.9988$  for Cr (VI)) and probability value ( $P < 0.0001$ ) demonstrated significance for the regression model. Numerical optimization technique based on desirability function was carried out for initial spent engine oil concentration of 10% w/w to optimize the biodegradation process. The optimum values for biostimulation agents to achieve a predicted maximum TPH and Cr (VI) removal of 67.20% and 53.20%, respectively, were found to be as follows: NPK fertilizer, 4.22 g; Tween 80, 10.69 mg/l; and pig manure, 47.76 g. At this optimum point, the observed TPH and Cr (VI) reductions were found to be 66.47% and 52.33%, respectively. The statistical analyses and the closeness of the experimental results and model predictions show the reliability of the regression model, and thus, biostimulation of indigenous microbial density and activity can reduce remediation period of petroleum hydrocarbon and heavy metal-contaminated environment and subsequently the cost of remediation.

**Keywords:** Biodegradation, Biostimulation, Nutrients, Second-order quadratic regression model, Spent engine oil

## Background

Spent engine oil (SEO) which is also known as used motor oil is produced when new engine oil (or motor oil) is subjected to high temperature and high mechanical strain [1]. It is a brown-to-black liquid mixture of heavy metal contaminants such as zinc, lead, and chromium that come from engine parts as they wear down, including low to high molecular weight ( $C_{15}$  to  $C_{18}$ ) aliphatic and aromatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, lubricative additives, and decomposition products [1,2]. There are improper disposals of SEO during manual oil changing operation

which is not recycled but spilled and dumped by automobile and generator mechanics into runoff, gutters, water drains and open vacant plots, and farmlands used as automobile workshops [3-5], thereby polluting both soil and water. In addition, the oil is also released into the environment from the exhaust system during engine use and due to engine leaks [6]. In Nigeria and some developing countries, about 20 million gallons of SEO are generated annually from mechanic workshops and discharged carelessly into the environment [7].

Chromium which is a heavy metal component of SEO has been designated as a priority pollutant by US EPA [8] and found to be toxic, carcinogenic, and teratogenic when present at high concentration [9]. Chromium (Cr) does not occur naturally in elemental form, only in compounds. Chromium exists in nine valence states ranging

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from 2 to +6; nevertheless, trivalent chromium (Cr (III)) and hexavalent chromium (Cr (VI)) are of major environmental significance because of their stability in the natural environment [10]. Hexavalent chromium (chromate) compounds are highly water soluble and, therefore, can overcome the cellular permeability barrier, entering via sulfate transport pathways since it bears structural similarity with  $\text{SO}_4$ . It has a high tendency to bind with oxygen, and unless it is rapidly reduced, it can oxidatively damage the DNA via the production of free radicals [9]. It has been reported that hexavalent chromium causes lung cancer [11], chromate ulcer, perforation of nasal septum, and kidney damage in humans, and it is also toxic to other organisms as well. Trivalent chromium is an essential micronutrient for many microorganisms, relatively insoluble in water, and 100 times less toxic than the hexavalent form. Thus, reduction of Cr (VI) to Cr (III) represents a potentially useful detoxification process. Therefore, prolonged exposure to high SEO concentration may cause the development of liver or kidney disease, possible damage to the bone marrow, and an increased risk of cancer [12,13].

Soil is a key component of natural ecosystems because environmental sustainability depends largely on a sustainable soil ecosystem [14]. When soil is polluted, the ecosystem is altered, and agricultural activities are affected. Contamination of the soil by SEO creates an unsatisfactory condition for life in the soil, which is due to the poor aeration it causes in the soil, immobilization of soil nutrients, loss of water-holding capacity, lowering of soil pH, and reduction in soil catalase enzyme activity [15-17], as well as inhibitory effect on the nitrate reductase activities of plants [18]. The soil remediation processes leading to the eventual removal of petroleum hydrocarbon and heavy metal pollutants from the environment involve the trio of physical, chemical, and biological alternatives [19]. However, due to limitations of the physicochemical technologies which are expensive to implement at full scale, not environmentally friendly, their technologies are complex, can lead to destruction of soil texture and characteristics, and do not always result in complete neutralization of pollutants [20,21], bioremediation technology through the mechanism of biodegradation has been recognized to be a valuable alternative for the detoxification and disposal of toxic substance. This is because of their cost-effectiveness, environmental friendliness, simplicity in technology, and conservation of soil texture and characteristics [21-25].

Bioremediation is an ecologically acceptable technology that employs the use of microorganisms to efficiently degrade pollutants. Nevertheless, bioremediation is an emerging technology and still has its limitations or disadvantages which are as follows: partial degradation of pollutants to metabolites that can potentially be more

toxic and highly mobile than the parent compound [26,27], it is not always possible to obtain complete pollutant removal because there might be a threshold concentration below which rates of biodegradation are slow or negligible [28], bioavailability of pollutants may decrease as biodegradation proceeds, and thus, recalcitrant compounds may persist [29], retardation or inhibition of the bioremediation process may occur when there are high variations in environmental conditions and concentration of the pollutant, microorganisms may be susceptible to toxins or natural predators in the environment [30], and it requires longer treatment time. The rate of hydrocarbon biodegradation can be limited by many factors which are physicochemical, biological, and environmental such as microorganism type, nutrients, pH, temperature, moisture, oxygen, soil type and properties, and concentration, chemical structure, and type of contaminant [31-33]. The basic principle of bioremediation is superficially to optimize the environmental conditions so that microbial degradation can occur rapidly and as completely as possible [34]. A number of bioremediation strategies have been developed to treat waste and contaminated sites [35]. However, selecting the most appropriate strategy to treat a specific site can be guided by considering three fundamental principles [29]: the amenability of the pollutant to be biologically transformed to less toxic products (biochemistry), the accessibility of the pollutant to microorganism (bioavailability), and the opportunity for optimization of biological activity (bioactivity).

Biostimulation is an effective bioremediation strategy that can improve or accelerate pollutant degradation [36-38] by optimizing conditions such as bioavailability, addition of nutrients, aeration, pH, and temperature control [39]. Biostimulation can be considered as an appropriate remediation technology for SEO removal in soil and requires the evaluation of the environmental parameters involved in the kinetics of the *in situ* process. Three of these parameters are bioavailability, nutrients, and aeration which can be improved in bioremediation systems by the use of surfactants and inorganic/organic nutrients. Biostimulation in ecological systems where different physical and chemical factors were controlled has been well reported in the literature [40,41]; however, only very few works have been reported in the literature on the bioremediation of soil contaminated with used motor oil through the use of biostimulation [42,43]. Abdulsalam et al. [43] focused on the use of NPK fertilizer (biostimulation) and mixture of *Pseudomonas aeruginosa* and *Bacillus subtilis* (bioaugmentation), while Abioye et al. [42] studied the enhanced bioremediation of used motor oil using organic wastes that comprised banana skin, brewery spent grain, and spent mushroom compost. Nevertheless, in

developing countries, fertilizers are not sufficient for agriculture, let alone for cleaning oil spills. It therefore necessitates the search for cheaper and environmentally friendly options of enhancing petroleum hydrocarbon degradation. One of such option is the use of pig manure which acts as both bulking agents and bacterial biomass suppliers. The addition of organic waste materials such as pig manure to the soil facilitates aeration through small pores and increases the water-holding capacity of the soil, thus enhancing bioremediation [44,45]. The potential of this manure in enhancing the cleanup of soil contaminated with petroleum hydrocarbons has been investigated by very few workers [46,47]; however, there is a lack of information of its use in accelerating the degradation of petroleum hydrocarbon and reduction in heavy metal components of SEO. The information on *ex situ* uptake of heavy metal component of SEO (for example chromium) contaminated soil is rarely reported [5]. Hence, further works are still needed to fully establish the potential of pig manure in the enhancement of soil bioremediation. Knaebel et al. [48] had observed that microbial mineralization of organic chemicals added to soil is controlled by the interactions between the chemicals present in the soil. While Okolo and Amadi [49] had also observed that there are conflicting results obtained from the addition of biostimulating or amendment agents in soil treatment and that this may probably be due to the heterogeneity of soil and oil sample as well as the variability and possible interactions between the soil amendments and the natural soil constituents. As a result of this, there is a dearth of information in the literature on the interaction effects of animal manure (organic nutrient), inorganic fertilizer (inorganic nutrient), and surfactant in enhancing bioremediation of soil contaminated with mixtures of petroleum hydrocarbons and heavy metals such as SEO. Response surface methodology is a statistical experimental design that provides information about direct effects, pair-wise interaction effects, and curvilinear variable effects. This statistical technique has been successfully applied in many fields [50-53].

Therefore, the purpose of this work is to study through the use of response surface methodology (RSM) the bioremediation of a tropical agricultural soil artificially contaminated with SEO using NPK fertilizer (inorganic nutrient), Tween 80 (surfactant), and pig manure (organic nutrient) as biostimulating agents. Also, in this study,  $2^3$  full-factorial Box-Behnken designs of experiment were implemented in order to evaluate the interaction effects of the biostimulating agents (variables) on the biodegradation rate of petroleum hydrocarbons and reduction (or uptake) of hexavalent chromium components of SEO as well as to optimize the variables for maximum SEO removal.

## Methods

### Soil sample and characterization

An un-impacted soil samples from Ladoke Akintola University of Technology (LAUTECH) Agricultural Farms, Ogbomosho were collected from the surface layer of the vadose zone 15 to 30 cm below the land surface. The soil samples were air-dried, homogenized, passed through a 2-mm (pore size) sieve, and stored in a polyethylene bag at room temperature. Soil samples were characterized for their physicochemical and microbial parameters according to standard methods. Total organic carbon and total nitrogen of soil were determined using Walkley-Black and Macro-Kjeldahl methods, respectively [54,55]. Soil pH was determined using pH meter fitted with a combined glass pH and reference electrode [55]. Soil moisture content was determined by evaporation on Whatman filter paper No. 1 at 103°C to 105°C in an electrical oven. Available phosphorus was determined using Bray No. 1 method [54,55].

The total hydrocarbon-degrading bacteria (THDB) population was determined by the pour plate method [56]. In this method, 10 g of soil was transferred into 250-ml Erlenmeyer flasks containing 100 ml of mineral salt medium (MSM). The MSM [57] consists of 2.13 g  $\text{Na}_2\text{HPO}_4$ , 0.5 g  $\text{NH}_4\text{Cl}$ , 0.2 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.3 g  $\text{KH}_2\text{PO}_4$ , 0.01 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 g  $\text{NaCl}$ , 20 g agar, and 1% spent engine oil in 1-L de-ionized water, pH 7.4. The flasks were shaken for 3 days at 180 rpm and  $28 \pm 2^\circ\text{C}$ . After the third day, 10 ml of the supernatant was transferred to 125 ml of fresh medium. The incubation transfer to the fresh medium was repeated three times. Thereafter, serially diluted samples (0.1 ml) were plated on nutrient agar medium (Oxoid) supplemented with 50  $\mu\text{g}/\text{ml}$  nystatin to suppress the growth of fungi. The oil agar plates were incubated at 30°C for 5 days, and the colonies were counted and randomly picked. Pure isolates were obtained by repeated sub-culturing on nutrient agar (Oxoid). The bacterial isolates were characterized using microscopic techniques and biochemical tests. The identities of the isolates were determined by comparing their characteristics with those of known taxonomy as described in Bergey's manual of determinative bacteriology [58].

The determined soil parameter values are as follows: moisture content  $5.95 \pm 0.05\%$ , total nitrogen  $0.25 \pm 0.04\%$ , available phosphorus  $0.12 \pm 0.02\%$ , potassium  $0.31 \pm 0.05\%$ , total organic carbon  $1.21 \pm 0.03\%$ , pH  $5.9 \pm 0.2$ , and THDB  $3.7 \times 10^5 \text{ cfu g}^{-1}$ . The hydrocarbon-degrading bacterial types isolated from the soil samples were mainly *Acinetobacter*, *Bacillus*, and *Pseudomonas* species. The soil characterization showed that the soil did not fulfill the nutrient (NPK) requirements for an efficient biodegradation process. Therefore, these elements were added in the form of NPK inorganic fertilizer (20:10:10) to provide the proper nutrients required for the biodegradation process.

### Chemicals

The spent engine oil was obtained from an automobile mechanic workshop located in Ogbomoso, Nigeria. This study employed a biodegradable nonionic surfactant Tween 80 manufactured by Sigma-Aldrich, St. Louis, MO, USA, which has an average molecular weight of 1,310 and a critical micelle concentration value of 15 mg/l. NPK fertilizer (20:10:10) was purchased from an agro-chemical store in Ogbomoso, Nigeria. Hexane solvent (BDH Chemicals Ltd., Poole, England) used for extraction of oil from soil was bought from a chemical store in Lagos, Nigeria.

### Microcosm preparation and bioremediation experimentation

To optimize the range of experimentation for 2<sup>3</sup> full-factorial Box-Behnken design, the following experiments were performed in earthen pots (used as bioreactors) maintained at room temperature. Soil samples (200 g) were placed in earthen pots (microcosm) and were artificially contaminated with SEO to a level of 10% w/w. The SEO-contaminated soil in each microcosm was amended with different amounts of NPK fertilizer (2 to 10 g), Tween 80 (5 to 25 mg/L), and pig manure (20 to 100 g), respectively. Soil used as control was not amended with any nutrient or surfactant. In total, 16 microcosms were settled and incubated for 42 days. All microcosms were mixed manually once per week to enhance oxygenation and kept moist during the 42-day experimental period. Samples were withdrawn at intervals of 1 week for residual total petroleum hydrocarbon (TPH) and chromium analysis.

### Experimental design and data analysis

The Box-Behnken factorial experimental design employed had three independent variables *viz.*, NPK (20:10:10) fertilizer, Tween 80 (surfactant), and pig manure (Table 1). Each of the independent amendment variables was studied at three levels (1, 0, and +1), with 17 experimental runs (Table 2). The levels were selected based on the above preliminary study results. The variables optimized were NPK fertilizer in the range of 2 to 6 g; Tween 80, 5 to 15 mg/l; and pig manure, 20 to 60 g, respectively. Change in TPH (i.e., percentage of TPH reduction) and change in chromium (Cr (VI)) were considered as experimental responses. Efficiency

**Table 1 Experimental range and the levels of the variables**

Dependent variable	Low level	Medium level	High level
	(-1)	(0)	(+1)
NPK fertilizer (A), g	2.0	4.0	6.0
Tween 80 (B), mg/l	5.0	10	15
Pig manure (C), g	20	40	60

**Table 2 Coded and uncoded Box-Behnken design for the three independent variables**

Run	NPK fertilizer (A)		Tween 80 (B)		Pig manure (C)	
	Code	Value (g)	Code	Value (mg/l)	Code	Value (g)
1	-1	2	-1	5	0	40
2	+1	6	-1	5	0	40
3	-1	2	+1	15	0	40
4	+1	6	+1	15	0	40
5	-1	2	0	10	-1	20
6	+1	6	0	10	-1	20
7	-1	2	0	10	+1	60
8	+1	6	0	10	+1	60
9	0	4	-1	5	-1	20
10	0	4	+1	15	-1	20
11	0	4	-1	5	+1	60
12	0	4	+1	15	+1	60
13	0	4	0	10	0	40
14	0	4	0	10	0	40
15	0	4	0	10	0	40
16	0	4	0	10	0	40
17	0	4	0	10	0	40
18 (control)	-	-	-	-	-	-

of SEO removal was assessed after 42 days. Table 3 shows the coded and actual values of factors and levels used in the experimental design. SEO-contaminated soil without biostimulation was also assayed as a control. The statistical software Design Expert6.0.8, (Stat-Ease Inc., Minneapolis, MN, 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.

### Estimation of total petroleum hydrocarbon

The TPH in the treatments was determined spectrophotometrically following the methods of Mishra et al. [13] and Vu-Duc et al. [59] for 30 min. Thereafter, the mixtures were allowed to settle, and extracts were decanted into a volumetric flask and plug. The above procedure was repeated two times using 20 ml of hexane each time. The total extracts were combined and was then filtered using a Whatman filter paper, and the filtrate was dried at room temperature by evaporation of the hexane solvent under a gentle nitrogen stream in a fume hood. After evaporation of the solvent, the extracted TPH in the filtrate (liquid phase extract) was detected spectrophotometrically at an absorbance of 400 nm using visible range spectrophotometer (model 6100, PYE UNICAM Ltd., England, UK). The TPH concentration was estimated from the standard curve of

**Table 3 Experimental design and results for bioremediation of spent engine oil**

Run	Percentage of TPH reduction		Percentage of Cr (VI) reduction	
	Observed value	Predicted value	Observed value	Predicted value
1	43.92	44.10	31.88	32.01
2	45.25	45.40	32.65	33.11
3	44.65	44.50	32.22	31.76
4	47.84	47.66	35.44	35.31
5	45.38	45.49	34.25	34.41
6	43.68	43.82	32.93	32.75
7	48.94	48.80	36.12	36.30
8	55.04	54.93	42.76	42.60
9	50.80	50.51	40.55	40.27
10	49.29	49.33	39.62	39.92
11	55.25	55.21	45.12	44.82
12	58.78	59.07	46.83	47.11
13	66.89	66.65	52.65	52.46
14	66.51	66.65	52.40	52.46
15	66.92	66.65	52.25	52.46
16	66.15	66.65	52.11	52.46
17	66.78	66.65	52.88	52.46
18 (control)	28.80	-	10.4	-

absorbance against concentration derived from fresh SEO that has been diluted with hexane. Values of TPH concentration obtained were multiplied by a dilution factor to give the actual concentration.

**Extraction and analysis of hexavalent chromium (Cr (VI)) and total chromium**

For the extraction of Cr (VI) and total chromium from soil, an alkaline digestion method and nitric acid/sulfuric acid digestion method as per standard methods [55] were used, respectively. The hexavalent chromium was measured colorimetrically at 540 nm by reaction with diphenylcarbazide in acidic conditions [55]. In the case of Cr (III), potassium permanganate was used to oxidize Cr (III) to Cr (VI). Cr (III) concentration was determined by taking the difference between total chromium concentration and Cr (VI) concentration.

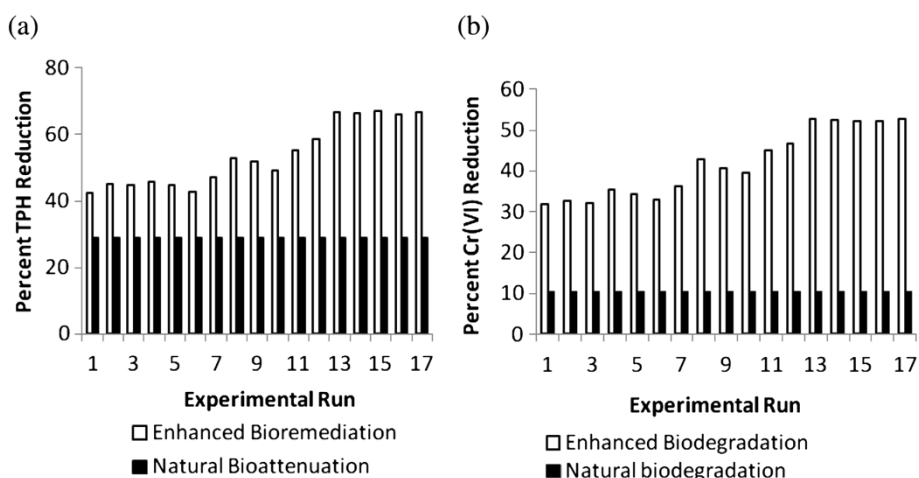
**Results and discussion**

**Natural bioattenuation and enhanced bioremediation**

After performing 17 runs of the Box-Behnken design (BBD) and one control, the results of the statistical experiment were analyzed with regard to the coded design matrix. The regression equation shows that the SEO degradation rate was an experimental function of test

variables in coded units. Table 3 shows that on day 42 (6th week), SEO content had decreased in all the soil microcosms. In control, natural biodegradation (natural bioattenuation) removed 28.8% of petroleum hydrocarbons and 10.4% of Cr (VI), respectively. The removal of 10.4% hexavalent chromium from the SEO-contaminated soil indicated that the intrinsic *Acinetobacter*, *Bacillus*, and *Pseudomonas* species in the soil have great potentials in accumulating or removing chromium heavy metal associated with SEO. Bader et al. [60] studied the potential of aerobic reduction of Cr (VI) by an indigenous soil microbial community and found that Cr (VI) in the soil was reduced by 33% within 21 days and suggested that Cr (VI)-reducing microbial populations is widespread in soil. Moreover, the respective reduction in petroleum hydrocarbon (TPH) and Cr (VI) content of soil microcosms containing amendments was much higher (Table 3) in the same period. This result indicated that the addition of biostimulant increased the rate of TPH biodegradation and hexavalent chromium uptake, respectively. This is in agreement with the report of Odokuma and Akponah [61] who observed an increase in crude oil biodegradation and heavy metal uptake from fresh and brackish waters impacted with crude oil when NPK fertilizer and poultry litters were used as nutrient supplements. A considerable decrease in SEO reduction was observed in runs 13 to 17, with a relatively high amount of NPK fertilizer, surfactant (Tween 80), and pig manure; the residual TPH and Cr (VI) reached 33.08% to 33.85% and 47.12% to 47.89%, respectively of the initial SEO concentration. Agarry et al. [62] in their study of enhanced bioremediation of soil artificially contaminated with kerosene have shown that natural attenuation removed 38.5% of kerosene oil after 42-day incubation, and when the soil was amended with NPK fertilizer, hydrogen peroxide, and Tween 80, 77.89% of the kerosene oil was removed. In comparing this study with the results of Agarry et al. [62], it could be seen that the percentage of hydrocarbon removal was lower in both the natural attenuation and biostimulation treatments. This may be due to the presence of heavy metals in the SEO which tends to inhibit the metabolic activities of the autochthonous hydrocarbon-degrading bacteria in the soil [63,64]. The comparison of SEO-enhanced biodegradation and natural bioattenuation for each run is shown in Figure 1.

At 10% (w/w) SEO concentrations, runs number 1 and 3, and 11 and 12 had the same remediation condition with different concentrations of Tween 80 (surfactant); the results show that the addition of surfactant can enhance SEO degradation. Similar observations have been reported for the use of nonionic surfactant as well as biosurfactant for the remediation of environment contaminated with petroleum hydrocarbons [62,65-69]. The



**Figure 1** Comparison of enhanced and natural biodegradations of SEO for (a) TPH and (b) Cr (VI) reductions.

observed enhancement as a result of the added surfactant may be due to the fact that surfactant might have played a role in emulsifying the SEO which made it readily available for degradation by the intrinsic hydrocarbon-degrading bacterial populations. However, one of the major factors that limit hydrocarbon biodegradation is their low availability to microbial cells [70].

Effect of different concentrations of pig manure were investigated at the same condition of NPK and Tween 80 (run numbers 6 and 8, and 10 and 12), and the findings demonstrated that the addition of pig manure can enhance the biodegradation process of SEO contaminant in soil. Yakubu [46], Agarry et al. [47], and Agarry and Owabor [71] in their respective work have demonstrated the positive effect of pig manure on enhanced biodegradation of petroleum hydrocarbons. Abioye et al. [42] have also reported the positive effect of organic waste (brewery spent grain, spent mushroom compost, and banana skin) on enhanced biodegradation of used motor oil. Relatively, run numbers 3 and 4 and run numbers 7 and 8 had the same condition with different concentrations of NPK; the results show that extra addition of NPK fertilizer (nutrient) improved SEO biodegradation. A similar observation has been reported for SEO biodegradation [43] as well as for kerosene degradation [62].

In addition, efficiency of bioremediation is a function of the microbial viability in the natural environment [72]. Factors, such as nitrogen, phosphorus, and microorganism presence, have been reported to affect bioremediation, for example, limiting nitrogen is reported to enhance bioremediation [73,74]. Abdulsalam et al. [43] and Abioye et al. [42] showed that natural attenuation removed 50% of oil and grease and 68% of TPH in

SEO after 70- and 84-day incubations, respectively. When the soil was supplemented with nutrients (nitrogen and phosphorus), 66% and 92% to 95% of the SEO was respectively removed. The results suggest that high dose of nutrient amendment can accelerate the initial SEO degradation rate and may shorten the period to clean up contaminated environments. The accelerating effect of amendment is stronger when nutrient availability is a limiting factor in the biodegradation of oil [51].

#### Second-order polynomial regression model and statistical analysis

The experimental data were fitted to a second-order polynomial regression model (Equation 1) containing three linear, three quadratic, and three interaction terms [75] using the same experimental design software to derive the equation for SEO removal from contaminated soil.

$$Y = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC, \quad (1)$$

where  $\beta_0$  is the value of the fixed response at the center point of the design;  $\beta_1, \beta_2,$  and  $\beta_3,$  the linear coefficients;  $\beta_{11}, \beta_{22},$  and  $\beta_{33},$  the quadratic coefficients;  $\beta_{12}, \beta_{13},$  and  $\beta_{23},$  the interaction effect coefficient regression terms, respectively; and  $A, B,$  and  $C,$  the levels of independent amendment variables. The significance of each coefficient in the equation was determined by  $F$  test and  $P$  values.  $F$  test indicated that all the factors and interactions considered in the experimental design are statistically significant ( $P < 0.05$ ) at the 95% confidence level. The regression equation obtained after analysis of variance gives the level of total petroleum hydrocarbon reduction and Cr (VI) reduction (SEO removal) as a function of the different

amendment variables: NPK, Tween 80, and pig manure. All terms regardless of their significance are included in Equations 2 and 3:

$$Y_{TPH} = 66.65 + 1.11A + 0.67B + 3.61C - 13.25A^2 - 7.98B^2 - 5.14C^2 + 0.47AB + 1.95AC + 1.26BC \quad (2)$$

$$Y_{Cr(VI)} = 52.46 + 1.16A + 0.49B + 2.94C - 12.96A^2 - 6.45B^2 - 2.98C^2 + 0.61AB + 1.99AC + 0.66BC, \quad (3)$$

where *A* is NPK concentration; *B*, Tween concentration; and *C*, pig manure concentration.

To test the fit of the model, the regression equation and determination coefficient ( $R^2$ ) were evaluated (Table 4). The model *F* value of 1,431.99 and 656.30 implies that the model is significant for TPH and Cr (VI), respectively. There is only a 0.01% chance that a model *F* value could occur due to noise alone for TPH and Cr (VI), respectively. The low probability value (<0.0001) for both TPH and Cr (VI) indicates that the model is significant. The value of the determination coefficient ( $R^2 = 0.9995$  for TPH and 0.9988 for Cr (VI)) being a measure of goodness of fit to the model indicates a high degree of correlation between the observed value and predicted values. The determination coefficient ( $R = 0.9997$  for TPH and 0.9994 for Cr (VI)) suggests that more than 99.97% and 99.94% of the variance are attributable to the variables and indicated a high significance of the model for TPH and Cr (VI), respectively.

Thus, 0.03% and 0.06% of the total variance cannot be explained by the model for TPH and Cr (VI), respectively. The fitted model is considered adequate if the *F* test is significant ( $P < 0.05$ ). The analysis of variance (ANOVA) quadratic regression model demonstrated that the model was highly significant for both TPH and Cr (VI) as was evident from the very low probability ( $P < 0.0001$ ) of the *F* test and insignificant result from the lack-of-fit model (Table 4). The lack-of-fit test is performed by comparing the variability of the current residual model to the variability between observations at replicate settings of the factors.

The lack of fit is designed to determine whether the selected model is adequate to describe the observed data or whether a more complicated model should be used. The predicted  $R^2$  values of 0.9957 and 0.9861 for TPH and Cr (VI), respectively, are correspondently in reasonable agreement with the adjusted  $R^2$  values of 0.9988 and 0.9973. Adequate precision measures the signal-to-noise ratio. A ratio >4 is desirable. The ratio of 96.000 and 62.746 obtained for TPH and Cr (VI), respectively, in this research indicates an adequate signal. This model can be used to navigate the design space. The coefficient of variation (CV) as the ratio of the standard error of estimate to the mean value of the observed response is a measure of reproducibility of the model; generally, a model can be considered reasonably reproducible if its CV is not greater than 10%. Hence, the low variation coefficient value (CV = 0.61% for TPH and 1.03% for Cr (VI)) obtained indicates a high precision and reliability of the experiments.

**Table 4 ANOVA for the quadratic response surface model fitting to the bioremediation data of spent engine oil**

Source	SS	DF	MS	F value	Probability > F(P value)
TPH					
Residual model	1,366.679	9	151.85	1,417.10	<0.0001
Lack of fit	0.33	3	0.11	1.07	0.4573
Pure error	0.42	4	0.10		
Total correlation	1,367.42	16			$R^2 = 0.9995$ Adjusted $R^2 = 0.9987$ Predicted $R^2 = 0.9956$ Adequate precision = 90.943
Cr (VI)					
Residual model	1,092.66	9	121.41	656.30	<0.0001
Lack of fit	0.91	3	0.30	3.18	0.1466
Pure error	0.38	4	0.096		
Total correlation	1,093.95	16			$R^2 = 0.9988$ Adjusted $R^2 = 0.9973$ Predicted $R^2 = 0.9861$ Adequate precision = 62.746

SS sum of square, DF degrees of freedom, MS mean square.

The coefficient of the model (parameter estimation) and the corresponding *P* values are presented in Table 5. The significance of regression coefficients was considered at a significance level of 95%. The *P* values of the regression coefficients suggest that among the independent test variables, linear, quadratic, and interaction effects of NPK fertilizer (inorganic nutrient), Tween 80 (surfactant), and pig manure (organic nutrient) are highly significant. In this study, *A*, *B*, *C*,  $A^2$ ,  $B^2$ ,  $C^2$ , *AB*, *AC*, and *BC* are significant model terms for TPH and Cr (VI), respectively. Thus, statistical analysis of all the experimental data showed that NPK fertilizer, Tween 80, and pig manure had a significant effect on TPH and Cr (VI) reductions in this study.

Moreover, it is observed that pig manure and NPK fertilizer concentrations exerted more pronounced linear effect (higher coefficient values) on TPH and Cr (VI) reductions, respectively, that is, TPH and Cr (VI) reductions were mostly and positively influenced by the addition of pig manure followed by NPK fertilizer and Tween 80, respectively. A higher positive linear effect of NPK fertilizer over Tween 80 in the biostimulation treatment of soil contaminated with kerosene had been reported in the previous work of Agarry et al. [62]. Furthermore, the pig manure exerted the highest positive linear effect (due to

higher coefficient) than the interaction effect between the amendment variables. The strong influence of animal-derived organic manure on petroleum hydrocarbon degradation has been shown in the previous works of Agarry et al. [47] and Agarry and Owabor [71]. The quadratic effect of the independent variables on the rate of TPH and Cr (VI) reductions was negative.

#### Influence of variable interaction on spent engine oil removal

Table 5 showed that SEO removal (i.e., TPH and Cr (VI) reductions) was influenced positively by the interaction of NPK fertilizer (*A*) and Tween 80 (*B*); NPK fertilizer (*A*) and pig manure (*C*); and Tween 80 (*B*) and pig manure (*C*), respectively. The interaction effect of NPK fertilizer (inorganic nutrient) and pig manure (organic nutrient) exerted more pronounced positive influence (due to higher coefficient) on SEO removal (i.e., TPH and Cr (VI) reductions) than the linear effect of NPK fertilizer. The interaction effect of NPK fertilizer and Tween 80 exerted less positive influence (due to lower coefficient) on SEO removal than the linear effect of NPK fertilizer and Tween 80, respectively. A similar observation has been reported [62]. On the other hand, Thavasi et al. [69] have reported that the biodegradation

**Table 5 Coefficient of the model for bioremediation of spent engine oil**

Factor	Coefficient estimate	Standard error	F value	P value	Remarks
TPH					
$\beta_0$	66.65	0.15	1,417.10	<0.0001	Significant
$\beta_1$	1.11	0.12	92.82	<0.0001	Significant
$\beta_2$	0.67	0.12	33.26	0.0007	Significant
$\beta_3$	3.61	0.12	971.59	<0.0001	Significant
$\beta_{11}$	-13.25	0.16	6,900.98	<0.0001	Significant
$\beta_{22}$	-7.98	0.16	2,503.76	<0.0001	Significant
$\beta_{33}$	-5.14	0.16	1,037.10	<0.0001	Significant
$\beta_{12}$	0.47	0.16	8.07	0.0250	Significant
$\beta_{13}$	1.95	0.16	141.94	<0.0001	Significant
$\beta_{23}$	1.26	0.16	59.26	0.0001	Significant
Cr (VI)					
$\beta_0$	52.46	0.19	656.30	<0.0001	Significant
$\beta_1$	1.16	0.15	58.57	0.0001	Significant
$\beta_2$	0.49	0.15	10.33	0.0148	Significant
$\beta_3$	2.94	0.15	372.53	<0.0001	Significant
$\beta_{11}$	-12.96	0.21	3,824.64	<0.0001	Significant
$\beta_{22}$	-6.45	0.21	946.27	<0.0001	Significant
$\beta_{33}$	-2.98	0.21	202.16	<0.0001	Significant
$\beta_{12}$	0.61	0.22	8.11	0.0248	Significant
$\beta_{13}$	1.99	0.22	85.63	<0.0001	Significant
$\beta_{23}$	0.66	0.22	9.42	0.0181	Significant

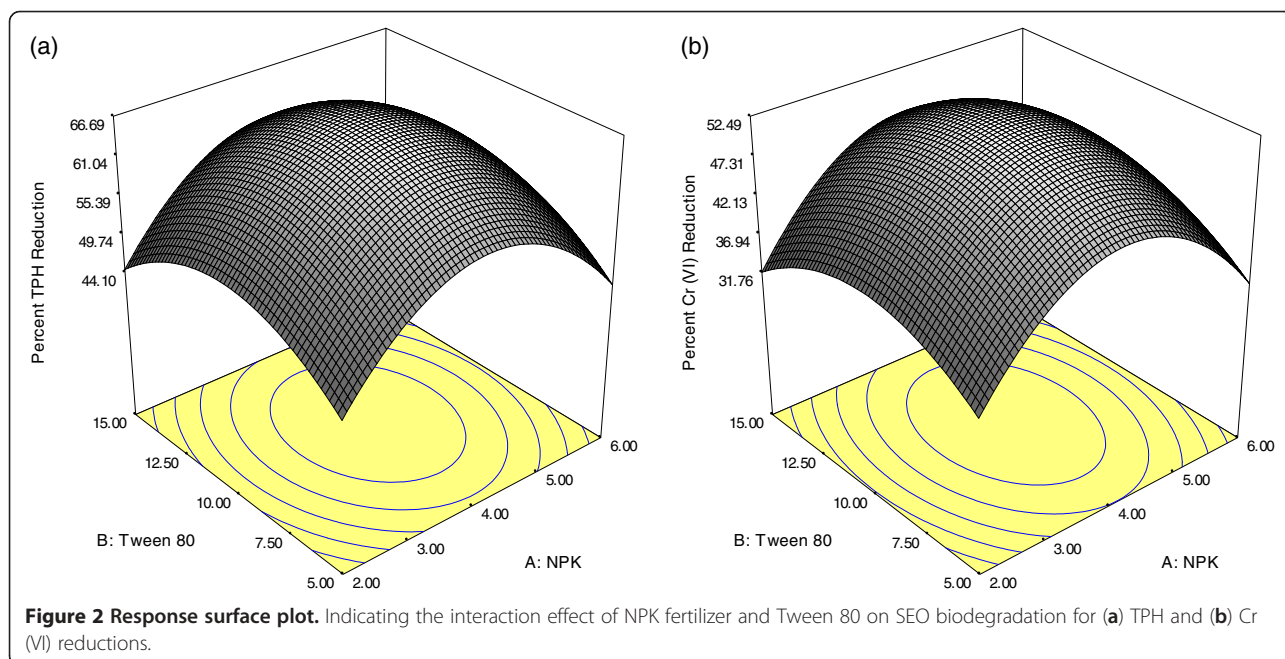


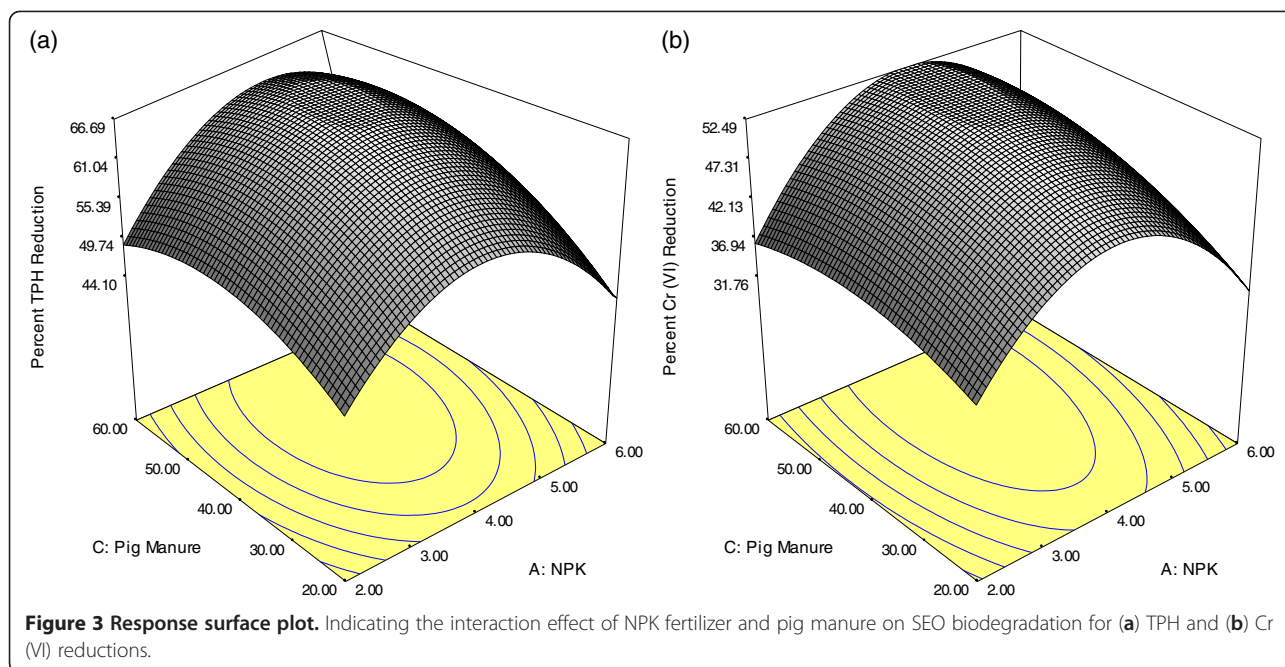
of crude oil in soil was more enhanced by the addition of biosurfactant + fertilizer than the addition of biosurfactant alone, but the difference between them was minimal. Thus, they concluded that biosurfactants alone are capable of promoting biodegradation process without using fertilizers. Furthermore, the interaction effect of Tween 80 and pig manure exerted higher positive influence on SEO removal than the linear effect of Tween 80. Generally, the interaction effects of NPK fertilizer and pig manure as well as Tween 80 and pig manure exerted more pronounced positive effects on SEO removal than the individual linear effects of NPK fertilizer and Tween 80, respectively. Okolo and Amadi [49] have reported the positive influence of the mixture of cow dung + poultry manure + Corexit (surfactant) on the biodegradation of crude oil.

The graphical representation of the response shown in Figures 2,3,4 helped to visualize the effect of NPK fertilizer (A), Tween 80 (B), and pig manure (C) on percentage of TPH and Cr (VI) reductions (SEO removal). The interaction effect of NPK fertilizer and Tween 80 on SEO biodegradation is illustrated in Figure 2. The plot shows that higher rate of TPH reduction was attained with increase in NPK fertilizer (nutrient) and Tween 80 (surfactant) concentrations (Figure 2a). The maximum TPH degradation yield of 66.68% was obtained with 10 mg/l of Tween 80 surfactant and 4 g/l (or 0.02 µg/kg) of NPK fertilizer at a fixed pig manure concentration of 40 g/l (0.2 µg/kg). A similar effect was observed for Cr (VI) percentage reduction (Figure 2b), where it can be seen that increasing NPK fertilizer and Tween 80 concentrations in the soil also caused an increase in Cr (VI)

reduction. This may be due to better bioavailability of substrate for the intrinsic microorganisms. However, availability of hydrocarbon and heavy metal-utilizing microorganism is a key issue in SEO biodegradation [76]. The positive influence of NPK fertilizer and Tween 80 interaction on petroleum hydrocarbon degradation in SEO is higher (due to high coefficient value of 0.47) though with lower maximum TPH yield (66.85%) as compared to the same interaction effect of NPK fertilizer and Tween 80 on petroleum hydrocarbon in kerosene (coefficient value of 0.27) with maximum degradation yield of 74.95% at a fixed concentration of hydrogen peroxide as presented in the previous work of Agarry et al. [62] where they used Box-Behnken design of experiment to study the effect of NPK fertilizer, Tween 80, and hydrogen peroxide as biostimulating agents on the degradation of kerosene in soil. The lower maximum TPH degradation value obtained in this study may probably be due to the presence of heavy metals in SEO which tends to inhibit the metabolic activities of the autochthonous hydrocarbon-degrading bacteria in the soil [63,64] as well as due to the heterogeneity of soil and SEO sample [49]. Metals may interfere with the biodegradation of pollutants through either interaction with enzymes directly involved in biodegradation or interaction with enzymes involved in general metabolism. In either case, inhibition is mediated by the ionic form of the metal [64,77].

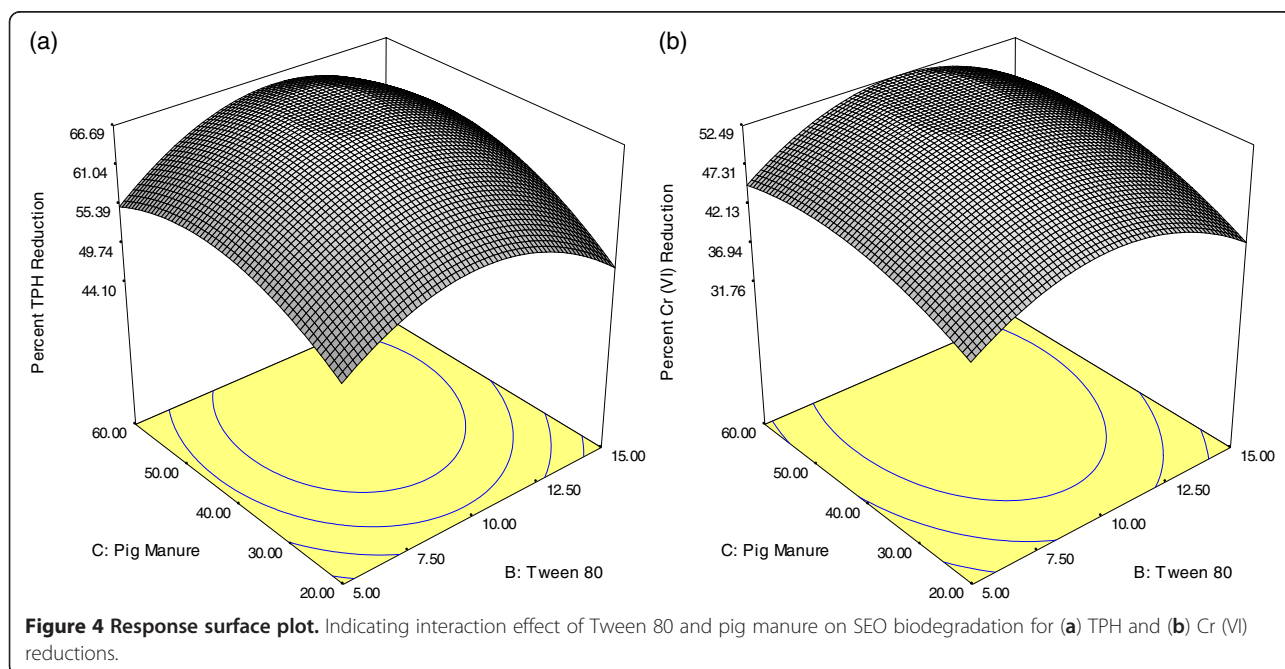
Figure 3 shows the 3D response surface plot of the interaction effect between NPK fertilizer and pig manure concentrations. This three dimensional plot explains that both NPK fertilizer and pig manure have individual





impact on SEO removal as the individual coefficient of both NPK fertilizer and pig manure is positive, and their interaction effect is positive. However, the impact of pig manure is more than the NPK fertilizer concentration as the individual coefficient value is higher for pig manure than for NPK fertilizer. It is seen that a higher percentage of TPH reduction was obtained at a higher pig manure concentration and relatively high amount of NPK fertilizer (Figure 3a). A similar effect was observed for Cr (VI) reduction in the contaminated soil (Figure 3b).

The maximum removal yield of 67.34% TPH and 53.27% Cr (VI) reductions were achieved, respectively, with around 40 g (or 200,000 mg/kg) of pig manure and 4 g (or 20,000 mg/kg) of NPK fertilizer. Further increase in the amount of NPK fertilizer (>4.0 g), a significant decrease in bioremediation yields occurred. This suggests that at a fixed concentration of Tween 80, the amount of NPK fertilizer can be decreased and that of the pig manure concentration has to be increased for higher reduction in TPH and Cr (VI) yields. Nevertheless,



eutrophication and harmful algal blooms in the aquatic environment may occur due to excessive nutrient concentration [55,78,79]. The positive influence of NPK fertilizer and pig manure interaction on petroleum hydrocarbon degradation in SEO is higher (due to high coefficient value of 1.96) though with lower maximum TPH yield (67.34%) as compared to the interaction effect of NPK fertilizer and hydrogen peroxide on the biodegradation of petroleum hydrocarbon in kerosene (coefficient value of 1.02) with a maximum degradation yield of 75.07% at a fixed concentration of Tween 80 as presented in the previous work of Agarry et al. [62]. Though the maximum degradation yield may be low due to the earlier suggested reasons, however, the comparisons imply that pig manure (animal organic waste material) can perform the purpose of improving aeration process for the soil microorganisms just as the hydrogen peroxide (an oxygen-releasing compound) serves to do.

Finally, Figure 4 shows the 3D response surface plot of the effect of interaction between Tween 80 (surfactant) and pig manure (organic nutrient) concentrations. This plot demonstrates that both Tween 80 and pig manure have positive mutual impact on the biodegradation process. At a fixed concentration of NPK fertilizer, it was observed that increase in Tween 80 and pig manure concentrations resulted in higher TPH and Cr (VI) reductions. The maximum reductions in TPH (67.32%) and Cr (VI) (53.21%) were obtained with 10 mg/l of Tween 80 and 4 g (i.e., 20,000 mg/kg) of NPK fertilizer.

#### Factor plot

The factor effect function plot (Figure 5) was used to assess the effect of each factor graphically. From the trace plot as shown in Figure 5, it can be seen that each of the three variables used in the present study has its individual effect on SEO (TPH and Cr (VI)) removal by the intrinsic microbial populations in the soil. Gradual increase in NPK fertilizer (A), Tween 80 (B), and pig manure (C) concentrations from low level (coded value -1) to a higher level (coded value +1) resulted in both increase and decrease of TPH (Figure 5a) and Cr (VI) (Figure 5b), respectively. Moreover, it is also to be noted from Figure 5a,b that over the range of -1 (2 g) to +1 (6 g) of NPK fertilizer, the TPH and Cr (VI) degradation changed in a wide range. This was also the case for Tween 80; however, for pig manure, it did not change over a wide range. This clearly indicates that keeping pig manure at the optimum level, a change in NPK fertilizer (nutrient) and Tween 80 (surfactant) will affect the biodegradation process more severely than done otherwise.

#### Optimization and validation

Numerical optimization technique based on desirability function was carried out to determine the workable

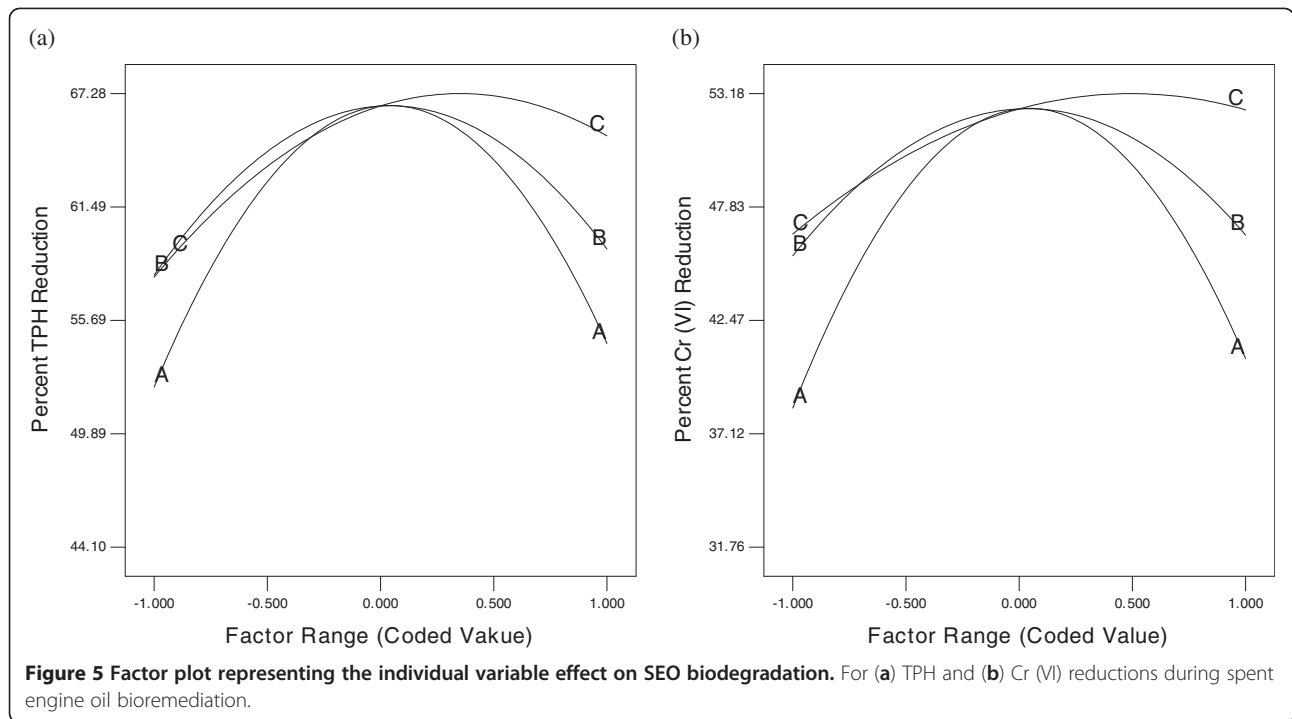
optimum conditions for the SEO bioremediation process. In order to provide an ideal case for biodegradation, the goal for NPK fertilizer, Tween 80, and pig manure was set in range based upon the requirements of the oil biodegradation, and TPH and Cr (VI) removal was set to maximum. The predicted optimum (uncoded) values of NPK fertilizer, Tween 80, and pig manure were correspondingly found to be 4.22 g (or 20,100 mg/kg), 10.69 mg/l, and 47.76 g (or 238,800 mg/kg) to achieve 67.20% and 53.20% maximum TPH and Cr (VI) reductions, respectively, while desirability was 1.000 for the experiment (Figure 6). Nevertheless, validation experiment was conducted to determine the optimum SEO removal when the amendment variables were set at the favorable optimum levels established above, through BBD and RSM. Standard deviation and percentage error were investigated for validation of the experiments. Errors between predicted and actual values were calculated according to Equation 4:

$$\text{Error} = \frac{\text{Actual value} - \text{Predicted value}}{\text{Actual value}} \times 100 \quad (4)$$

In the optimized condition for initial SEO of 10% (w/w) concentration, 66.47% and 52.40% TPH and Cr (VI) reductions were obtained, respectively. The percentage error between the predicted and actual values was found to be -1.10 and -1.53 for TPH and Cr (VI), respectively. The results clearly indicated that no significant difference was observed.

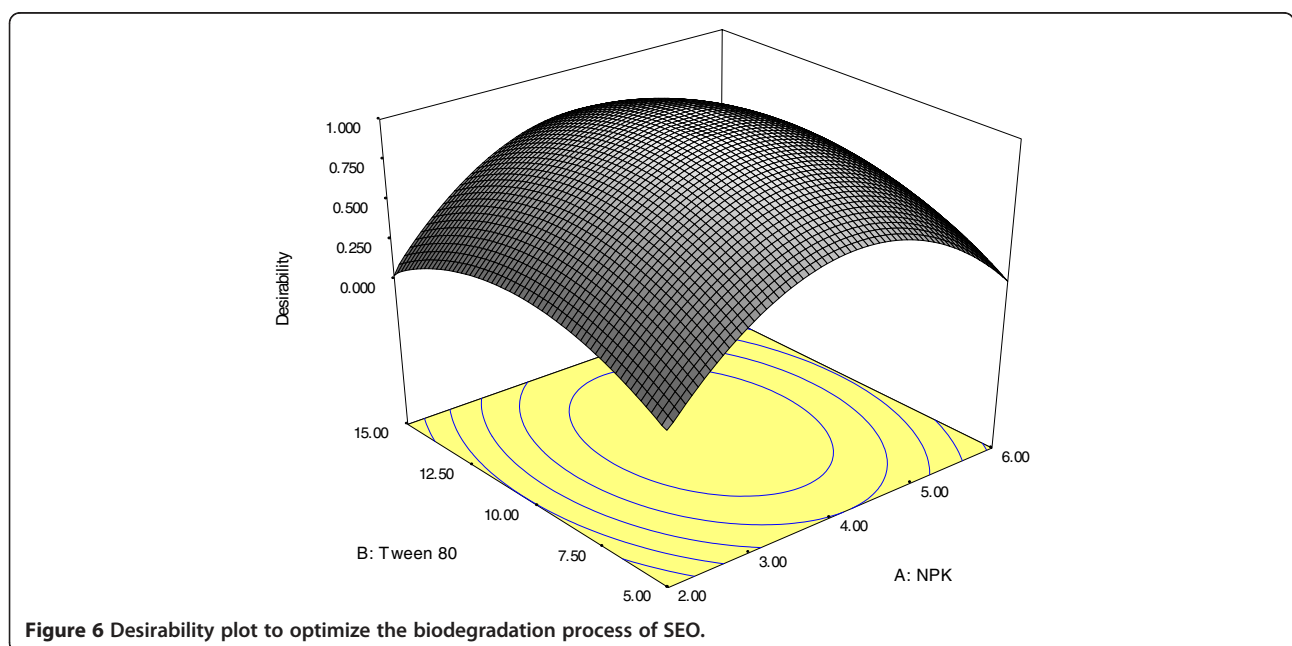
#### Conclusions

RSM is a reliable and powerful tool for modeling and optimization of spent engine oil bioremediation processes. The variations in total petroleum hydrocarbon and hexavalent chromium contents of spent engine oil bioremediation pattern with respect to NPK fertilizer, Tween 80, and pig manure contents were observed to be very significant. The results indicated that bioremediation of spent engine oil-contaminated soil with the use of NPK fertilizer, Tween 80, and pig manure as biostimulating agents resulted in the acceleration or improvement of petroleum hydrocarbon and hexavalent chromium reductions. However, the presence of heavy metals in spent engine oil tends to reduce the degradation rate (percentage degradation) of its petroleum hydrocarbon component. Pig manure (organic nutrient) alone or in combination with either NPK fertilizer (inorganic nutrient) or Tween 80 (surfactant) can greatly and significantly enhance the biodegradation of petroleum hydrocarbon and heavy metal uptake or reduction in soil than the addition of either NPK fertilizer alone or Tween 80. Thus, based on the coefficient values of the second-order polynomial regression model, the order of biostimulation effectiveness among the biostimulating



agents and their interactions are as follows: pig manure > NPK fertilizer + pig manure > Tween 80 + pig manure > NPK fertilizer > Tween 80 > NPK + Tween 80. Therefore, the application of these biostimulation strategies can reduce the length of remediation time in the bioremediation of hydrocarbon and heavy metal-contaminated ecological system and subsequently the cost of remediation. The

success and efficiency of these biostimulation techniques may vary considerably from one site to another; hence, there is no universal soil treatment regimen for the bioremediation of all petroleum hydrocarbon-contaminated soils. The effectiveness of any soil treatment applied for such purpose has to be evaluated on a case-specific basis. However, two important issues need to be addressed before



taking such technology to the field (1) evaluation of the metabolic potential of the intrinsic microbial species and (2) evaluation of the environment where such bioremediation process is needed for lack of essential nutrients.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

SEA participated in the design and coordination of the study, interpreted the data, and drafted and corrected the manuscript. OOO participated in the interpretation of the data and corrected the draft manuscript. All authors read and approved the final manuscript.

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#### References

1. ATSDR (Agency for Toxic Substances and Disease Registry) Toxicology: Profile for used mineral base crankcase oil. Department of Health and Human Services, Public Health Service Press, Atlanta (1997)
2. Wang, J, Jia, CR, Wong, CK, Wong, PK: Characterization of polycyclic aromatic hydrocarbon created in lubricating oils. *Water Air Soil Pollut.* **120**, 381–396 (2000)
3. Anoliefo, GO, Edegba, BO: Effect of spent engine oil as a soil contaminant on the growth of two eggplant species *Solanum melongena* (L) and *S. incanum* (L). *J. Agric. Forestry Fisheries* **1**, 21–25 (2000)
4. Odjegba, VJ, Sadiq, AO: Effect of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amaranthus hybridus* L. *Environmentalist* **22**, 23–28 (2002)
5. Ikhajagbe, B, Anoliefo, GO: Natural attenuation of a 14-month-old waste engine oil polluted soil. *J. Soil Sci. Environ. Manage.* **2**(7), 184–192 (2011)
6. Osubor, CC, Anoliefo, GO: Inhibitory effect of spent lubrication oil on the growth and respiratory function of *Arachis hypogea* L. *Benin Sci. Dig.* **1**, 73–79 (2003)
7. Faboya, OOP: Industrial pollution and waste management. In: Akinjide, O. (ed.) *Dimensions of environmental problems in Nigeria*, pp. 12–25. Friedrich Ebert Foundation, Washington D.C (1997)
8. Urvashi, T, Rasesh, P: Reduction of chromate by cell-free extract of *Brucella* sp. isolated from Cr VI contaminated sites. *Bioresour. Technol.* **98**, 1541–1547 (2007)
9. Poornima, K, Karthik, L, Swadhini, SP, Mythili, S, Sathivelu, A: Degradation of chromium by using a novel strains of *Pseudomonas* species. *J. Microbial Biochem. Technol.* **2**, 095–099 (2010)
10. Schroeder, DC, Lee, GF: Potential transformations of chromium in natural waters. *Water Air Soil Pollut.* **4**, 355–365 (1975)
11. De Flora, S: Threshold mechanisms and site specificity in chromium(VI) carcinogenesis. *J. Carcinog.* **21**, 533–541 (2000)
12. Propst, TL, Lochmiller, RL, Qualis, CW, McBee Jr., K: In situ (mesocosm) assessment of immune-toxicity risks to small mammals inhabiting petrochemical waste sites. *Chemosphere* **38**, 1049–1067 (1999)
13. Mishra, SJ, Jyot, RC, Kuhad, BL: Evaluation of inoculum addition to stimulate in situ Bioremediation of oily-sludge-contaminated soil. *Appl. Environ. Microbiol.* **67**(4), 1675–1681 (2001)
14. Adriano, DC, Chlopecka, A, Kaplan, KI: Role of soil chemistry in soil and ecosystem conservation, pp. 361–386. Soil Science Society of America Special Publication, Madison (1998)
15. Sathiya-Moorthi, P, Deecaraman, M, Kalachelvan, PT: Bioremediation of automobile oil effluent by *Pseudomonas* sp. *Advanced Biotech.* **31**, 34–37 (2008)
16. Atuanya, EJ: Effect of oil pollution on physical and chemical properties of soil: a case study of waste oil contaminated delta soil in Bendel State Nigeria. *J. Appl. Sci.* **55**, 155–176 (1987)
17. Achuba, FI, Peretiemo-Clarke, BO: Effect of spent engine oil on soil catalase and dehydrogenase activities. *Int. Agrophysics* **22**, 1–4 (2008)
18. Odjegba, VJ, Atebe, JO: The effect of used engine oil on carbohydrate, mineral content and nitrate reductase activity of leafy vegetable (*Amaranthus hybridus* L.). *J. Appl. Sci. Environ. Manage.* **11**(2), 191–196 (2007)
19. Okoh, AI: Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. *Biotechnol. Mol. Biol.* **1**, 38–50 (2006)
20. Zhang, H, Dang, Z, Zheng, LC, Yi, XY: Remediation of soil co-contaminated with pyrene and cadmium by growing maize (*Zea mays* L.). *Int. J. Environ. Sci. Tech.* **6**(1), 69–76 (2009)
21. Yerushalmi, L, Rocheleau, S, Cimpoia, R, Sarrazin, M, Sunahara, G, Peisajovich, A, Leclair, G, Guiot, RS: Enhanced bioremediation of petroleum hydrocarbons in contaminated soil. *Bioremed. J.* **7**(1), 37–51 (2003)
22. Vidali, M: Bioremediation: an overview. *J. Appl. Chem.* **73**(7), 1163–1172 (2001)
23. Mandri, T, Lin, J: Isolation and characterization of engine oil degrading indigenous microorganisms in Kwazulu-Natal, South Africa. *Afri. J. Biotechnol.* **6**(1), 23–27 (2007)
24. Adams, RH, Guzmán-Osorio, FJ: Evaluation of land farming and chemico-biological stabilization for treatment of heavily contaminated sediments in a tropical environment. *Int. J. Environ. Sci. Tech.* **5**(2), 169–178 (2008)
25. Fouébé, TA, Kengni, TL, GurnadhaRao, VVS, Ndam, NJR: Transfer of moisture through the unsaturated zone in the tropical forest using the neutron probe. *Int. J. Environ. Sci. Tech.* **6**(3), 379–388 (2009)
26. Sutherland, JB: Detoxification of polycyclic aromatic hydrocarbons by fungi. *J. Ind. Microbiol.* **9**, 53–62 (1992)
27. Bradley, PM: Microbial degradation of chloroethanes in groundwater systems. *Hydrogeology J.* **8**, 104–111 (2000)
28. Allard, AS, Neilson, AH: Bioremediation of organic waste sites: a critical review of microbiological aspects. *Int. Biodeter. Biodegrad.* **39**, 253–285 (1997)
29. Kim, S-J: Bioaugmentation for the remediation of pesticide contaminated soil with microorganisms directly enriched in soil or compost. The Ohio State University, (2003). Dissertation
30. Singleton, I: Microbial metabolism of Xenobiotics: fundamental and applied research. *J. Chem. Technol. Biotechnol.* **59**, 9–23 (1994)
31. Ting, YP, Hu, HL, Tan, HM: Bioremediation of petroleum hydrocarbons in soil microcosms. *Resour. Environ. Biotechnol.* **2**, 197–218 (1999)
32. Semple, KT, Reid, BJ, Fermor, TR: Impact of composting strategies on the treatment of soils contaminated with organic pollutants. *Environ. Poll.* **112**, 269–283 (2001)
33. Bundy, JG, Paton, GI, Campbell, CD: Microbial communities in different soil types do not converge after diesel contamination. *J. Appl. Microbiol.* **92**, 276–288 (2002)
34. Smith, J: *Biotechnology*, 3rd edn, p. 143. Press Syndicate Publishers, Great Britain (1996)
35. Agary, SE, Owabor, CN, Yusuf, RO: Studies on biodegradation of kerosene in soil under different bioremediation strategies. *Bioremediation J.* **14**(3), 135–141 (2010)
36. Hollender, J, Althoff, K, Mundt, M, Dott, W: Assessing the microbial activity of soil samples, its nutrient limitation and toxic effects on contaminants using a simple respiration test. *Chemosphere* **53**, 269–275 (2003)
37. Semple, KT, Dew, NM, Doick, KJ, Rhodes, AH: Can mineralization be used to estimate microbial availability of organic contaminants in soil? *Environ. Poll.* **140**, 164–172 (2006)
38. Walworth, J, Pond, A, Snape, I, Rayner, J, Ferguson, S, Harvey, P: Nitrogen requirements for maximizing petroleum bioremediation in a sub-Antarctic soil. *Cold Regions Sci. Technol.* **48**, 84–91 (2007)
39. Margesin, R, Zimmerbauer, A, Schinner, F: Monitoring of bioremediation by soil biological activities. *Chemosphere* **40**, 339–346 (2000)
40. Elektorowicz, M: Bioremediation of petroleum-contaminated clayey soil with pretreatment. *Environ. Technol.* **15**, 373–380 (1994)

41. Piehler, MF, Swistak, JG, Pinckney, JL, Paerl, HW: Stimulation of diesel fuel biodegradation by indigenous nitrogen fixing bacterial consortia. *Microbial Ecol.* **38**, 69–78 (1999)
42. Abioye, PO, Abdul Aziz, A., Agamuthu, P: Enhanced biodegradation of used engine oil in soil amended with organic wastes. *Water Air Soil Pollut.* **209**, 173–179 (2009)
43. Abdulsalam, S, Bugaje, IM, Adefila, SS, Ibrahim, S: Comparison of biostimulation and bioaugmentation for remediation of soil contaminated with spent motor oil. *Int. J. Environ. Sci. Tech.* **8**(1), 187–194 (2011)
44. Jobson, AM, McLaughlin, M, Cook, FD, Westlake, DWS: Effects of amendments on microbial utilization of oil applied to soil. *Appl. Microbiol.* **27**, 166–171 (1974)
45. Amadi, A, Ue Bari, Y: Use of poultry manure for amendment of oil-polluted soils in relation to growth of maize (*Zea mays* L.). *Environ. Int.* **18**, 521–527 (1992)
46. Yakubu, MB: Biodegradation of Lagoma crude oil using pig dung. *Afr. J. Biotechnol.* **6**, 2821–2825 (2007)
47. Agarry, SE, Owabor, CN, Yusuf, RO: Bioremediation of soil artificially contaminated with petroleum hydrocarbon mixtures: evaluation of the use of animal manure and chemical fertilizer. *Bioremediation J.* **14**(4), 189–195 (2010)
48. Knaebel, DB, Federie, TW, McAvoy, DC, Vestal, JR: Effects of mineral and organic soil constituents on microbial mineralization of organic compounds in natural soil. *Appl. Environ. Microbiol.* **60**, 4500–4508 (1994)
49. Okolo, JC, Amadi, EN: Optimising crude oil biodegradation in a sandy loam soil using a mixture of cow dung and poultry manure. *Int. J. Agric. Rural. Dev.* **5**, 69–76 (2004)
50. Ahmadi, M, Vahabzadeh, F, Bonakdarpour, B, Mofarrah, E, Mehranian, M: Application of the central composite design and response surface methodology to the advanced treatment of olive oil processing wastewater using Fenton's peroxidation. *J. Hazard. Mater.* **123**, 187–195 (2005)
51. Pala, DM, de Carvalho, D, Pinto, J, Sant'Anna Jr, G: A suitable model to describe of a petroleum-contaminated soil. *Int. Biodeter. Biodegr.* **58**, 254–260 (2006)
52. Rigas, F, Papadopoulou, K, Dritsa, V, Doulia, D: Bioremediation of a soil contaminated by lindane utilizing the fungus *Ganoderma australe* via response surface methodology. *J. Hazard. Mater.* **140**, 325–332 (2007)
53. Huang, L, Mab, T, Li, D, Liang, F, Liu, R, Li, G: Optimization of nutrient component for diesel oil degradation by *Rhodococcus erythropolis*. *Mar. Pollut. Bull.* **56**, 1714–1718 (2008)
54. Black, CA: *Method of Soil Analysis II*, pp. 573–590. American Society of Agronomy, Madison (1965)
55. APHA: *Standard Methods for Examination of Water and Wastewater*. American Public Health Association, Washington D.C (1985)
56. Colores, GM, Macur, RE, Ward, DM, Inskeep, WP: Molecular analysis of surfactant-driven microbial population shifts in hydrocarbon-contaminated soil. *Appl. Environ. Microbiol.* **66**, 2059–2964 (2000)
57. Kästner, M, Breuer-Jammali, M, Mahro, B: Enumeration and characterization of the soil microflora from hydrocarbon contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons. *Appl. Microbiol. Biotechnol.* **41**, 267–273 (1994)
58. Krieg, NR, Holt, JG, Sneath, PHA, Stanley, JT, Williams, ST: *Bergey's Manual of Determinative Bacteriology*, 9th edn. Williams and Wilkins, Baltimore (1994)
59. Vu-Duc, T, Huynh, CK, Lafontaine, M, Bonnet, P, Binet, S: A spectrophotometric method for the determination of organic soluble matter in bitumen fumes. *Appl. Occupational Environ. Hygiene* **17**(7), 495–500 (2002)
60. Bader, JL, Gonzalez, G, Goodell, P, Ali, AM, Pillai, S: Aerobic reduction of hexavalent chromium in soil by indigenous microorganism. *Biorem J.* **3**, 201–212 (1999)
61. Odokuma, LO, Akponah, E: Effect of nutrient supplementation on biodegradation and metal uptake by three bacteria in crude oil impacted fresh and brackish waters of the Niger Delta. *J. Cell and Animal Biol.* **4**(1), 001–018 (2010)
62. Agarry, SE, Owabor, CN, Yusuf, RO: Enhanced bioremediation of soil artificially contaminated with kerosene: optimization of biostimulation agents through statistical experimental design. *J. Pet. Environ. Biotechnol.* **3**, 120 (2012)
63. Al-Saleh, ES, Obuekwe, C: Inhibition of hydrocarbon bioremediation by lead in a crude oil. *Int. Biodeter. Biodegr.* **56**(1), 1–7 (2005)
64. Al-Mahin, A, Chowdhury, AZ, Alam, MK, Aktar, Z, Fakhruddin, ANM: Phenol biodegradation by two strains of *Pseudomonas putida* and effect of lead and zinc on the degradation process. *Int. J. Environ.* **1**, 27–33 (2011)
65. Wang, S, Mulligan, CN: An evaluation of surfactant foam technology in remediation of contaminated soil. *Chemosphere* **57**, 1079–1089 (2004)
66. Tsai, TT, Kao, CM, Surampalli, RY, Chien, HY: Enhanced bioremediation of fuel-oil contaminated soils: laboratory feasibility study. *J. Environ. Eng.* **135**(9), 845–853 (2009)
67. Calvo, C, Manzanera, M, Silva-Castro, GA, Uad, I, González-López, J: Application of bioemulsifiers in soil oil bioremediation processes. *Future prospects. Sci. Total. Environ.* **407**, 3634–3640 (2009)
68. Rahman, KSM, Banat, IM, Thahira, J, Thayumanavan, T, Lakshmanaperumalsamy, P: Bioremediation of gasoline contaminated soil by bacterial consortium amended with poultry litter, coir pith and rhamnolipid biosurfactant. *Bioresour. Technol.* **81**, 25–32 (2002)
69. Thavasi, R, Jayalakshmi, S, Banat, IM: Effect of biosurfactant and fertilizer on biodegradation of crude oil by marine isolates of *Bacillus megaterium*, *Corynebacterium kutscheri* and *Pseudomonas aeruginosa*. *Bioresour. Technol.* **102**, 772–778 (2011)
70. Mohajeri, L, AbdulAziz, H, Isa, MH, Zahed, MA: A statistical experiment design approach for optimizing biodegradation of weathered crude oil in coastal sediments. *Bioresour. Technol.* **101**, 893–900 (2010)
71. Agarry, SE, Owabor, CN: Anaerobic bioremediation of marine sediment artificially contaminated with anthracene and naphthalene. *Environ. Technol.* **32**(12), 1375–1381 (2011)
72. Joo, H, Ndegwa, PM, Shoda, M, Phae, C: Bioremediation of oil-contaminated soil using *Candida catenulata* and food waste. *Environ. Pollut.* **156**, 891–896 (2008)
73. Odokuma, LO, Dickson, AA: Bioremediation of a crude oil polluted tropical mangrove environment. *J. Appl. Sci. Environ. Manage.* **7**(2), 23–29 (2003)
74. Mohan, SV, Reddy, BP, Sarma, PN: Ex situ slurry phase bioremediation of chrysene contaminated soil with the function of metabolic function: process evaluation by data enveloping analysis (DEA) and Taguchi design of experimental methodology (DOE). *Bioresour. Technol.* **100**, 164–172 (2009)
75. Montgomery, D.C.: *Design and Analysis of Experiments*, 7th edn. John Wiley, New York (2008)
76. Zhu, X, Venosa, AD, Suidan, MT, Lee, K: *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands*. US Environmental Protection Agency Office of Research and Development National Risk Management Research Laboratory Land Remediation and Pollution Control Division, Cincinnati (2001)
77. Angle, JS, Chaney, RL: Cadmium resistance screening in nitrilotriacetate-buffered minimal media. *Appl. Environ. Microbiol.* **55**, 2101–2104 (1989)
78. Atlas, RM: Petroleum biodegradation and oil spill bioremediation. *Mar. Pollut. Bull.* **31**, 178–182 (1995)
79. Tam, NFY, Wong, YS, Wong, MH: Novel technology in pollutant removal at source and bioremediation. *Ocean Coast. Manage.* **52**, 368–373 (2009)

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