

Modeling and Optimizing the Use of Aerobic Bioreactors for Sustained Removal of PAHs from Crude Oil Sludge

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Abstract - The presence of polycyclic aromatic hydrocarbons (PAHs) in air, soil sediments and water resources can be critical concern to human health and risk of fatal diseases. PAHs are organic chemicals usually with two or more benzene rings, so have potential toxic effects on humans due to their carcinogenic, teratogenic, and mutagenic risks, hence their removal from contaminated environments is crucial. This study explores the use of bioreactors from crude oil sludge for the optimal removal of PAHs. The Box-Wilson experimental design was adopted to ascertain the associated variables such as, dosage of oxygen, PAHs concentration and pH. To determine the effect of experimental factors on the bioreactor process, the method was also applied at different oxygen dosage, PAH concentration, and pH of the sludge suspension in the treatment process. A second-order polynomial model was incorporated to fit the experimental data and enable optimize the treatment discretely on a laboratory scale (implying working volume less than 1 liter) mechanically stirred-tank bioreactor. The results of the analyses show that the most functional requirement and so advantageous in the treatment process was 0.4g, 20mg/L and 6 for oxygen dosage, PAHs concentration and pH respectively. This implies that the mathematical model efficiently simulated the treatment process hence, enhanced the performance of aerobic bioreactor plants in removing PAHs from crude oil sludge.

Keywords: PAHs, crude oil sludge, aerobic bioreactors, oxygen dosage, bioremediation.

I. INTRODUCTION

Human activities have increasingly undermined the contribution of mineral hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and heavy metals in contaminating the ecosystem that essentially supports the basic needs of human health, including feeding and clothing. In the crude oil rich Niger Delta of Nigeria, the most common contaminant of the air, surface/groundwater and terrestrial plane can easily be attributed to crude oil exploration and production activities. These oil industries generate large quantities of viscous oily

residues formed during processes leading to refining and final consumption (Salameh and Kabrick, 2012). The viscous residues or oily sludge constitute oil, water and solids (Vidonish *et al*, 2016). The resulting multiphase structure becomes highly recalcitrant and so difficult to reutilize. Among the products of these anthropogenic activities are petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and complex compounds, like the asphaltenes, with very high molecular weight (Euiso and Euisin, 2018). PAHs (polychlorinated biphenyls) enter the environment via natural and human activities.

We have focused on PAHs in this study due to their widespread presence in contaminated sites as characteristic pollutants. Sixteen (16) different compounds of PAHs have been classified as priority pollutants by the United States Environmental Protection Agency (US EPA) and European Union due to their toxic nature and common occurrence at hazardous waste sites such as crude oil sludge, with a view to limiting their releases into the environment (ATSDR, 1995; Manoli and Samara, 2008). They are lipophilic carbon-based compounds, with fused aromatic rings, seven (7) of which are thought to be recalcitrant humancarcinogens (Blanchard *et al*, 1999; Sweetman and Jones, 2000; Kanaly and Harayama, 2000). Aromatics having at least five rings are considered to be resistant to absorption with the tendency to persist in serene environments (Vidonish *et al*, 2016). PAHs can also pose acute health problems to life in the marine ecosystem (Jing *et al*, 2014). They are ubiquitous compounds formed during the combustion of organic materials (Abdel-Shafy and Mansour, 2016). PAHs are resistant to degradation and toxic, which makes their removal from contaminated soils and sediments difficult with other biological processes hence the search for sustainable technological options for quick and effective treatment (Crawford and Crawford, 2005).

Crude oil sludge is a byproduct generated during the extraction, transportation, storage and refining stages of crude oil (Xu *et al.*, 2018; 2019), and it is classified as hazardous waste needing immediate and effective disposal measures (Xu *et al.*, 2018; 2019). At the Port Harcourt Refinery, Nigeria, the

estimated total oil processed is approximated to be 1% and this is discharged as oily sludge, after being accumulated in storage tanks for several years (SPDC, 2011). This sludge cannot be recommended to be incinerated because of the high cost of energy, air pollution risks and the persistence of PAHs. This research is therefore motivated by a quest for alternatives due to the inadequate disposal sites for such toxic residue in landfills.

A large data bank exists on the use of biological processes to treat waste or waste contaminated materials (Atlas and Cerniglia, 2015). Soil-slurry bioreactors as a treatment process has been studied severally at the laboratory scale (implying at working volume less than 1 liter), with a view to identifying and quantifying the variables responsible in the removal process (Atlas and Cerniglia, 2015). The variables under consideration are bioaugmentation (employment of external microorganisms), constituents of soil, biostimulation (supplementation of nutrients), with operational parameters of temperature, air flow, pH, mixing rate and regime (Lewis, 1993; Li *et al.*, 2019; Collina *et al.*, 2004; Lee *et al.* 2001). The effectiveness of the remedial process is dependent on hydraulic retention time (HRT), solid retention time (SRT) or substrate loading rate (SLR), where the operational parameters are adjusted and optimized to achieve the target result.

The effectiveness in the removal process for high molecular weight PAHs contaminated soils or sediments are of less significance than the lighter PAHs due to bioavailability (Haritash *et al.*, 2009). This implies that high molecular weight PAHs are more hydrophobic and less soluble, and so are less bioavailable for microorganisms (Semple *et al.*, 2003; Fava *et al.*, 2004; Giordano *et al.*, 2004). PAHs found in wastewater can also be in sewage sludge as a result of their physical and chemical properties since they can be easily adsorbed by solids (Dat *et al.*, 2017).

The objective of this research is focused therefore on assessing the effects of the various operational parameters, including pH, PAH and oxygen concentration on the rate of the bioremediation of oily sludge using design of experiments (DOE) and to optimize the treatment processes in a laboratory scale mechanically stirred-tank bioreactors. The statistical models were validated by an additional set of experiments at the optimum conditions in line with the DOE results.

II. METHODOLOGY

2.1 Materials

A maximum volume 2L-M bioreactor consisting disk magnetic coupling system with sliding bearings was incorporated in an automatic control system (ACS) at

laboratory scale. This provides the opportunity of managing two bioreactors such that the ACS process is classified into components: in block dosimeters - for passing base, acid and foam slacked; and in block transformers - for basic values measured. The components of the system are then assembled discretely under control of a computer to allow for regulation of parameters of the treatment process, such as the flow rate, rotation speed, hydraulic retention time, temperature, phosphate concentration and oxygen concentration.

2.2 Data Collection

Crude oil sludge is a byproduct of crude oil storage and processing generated during the refining stages hence needs to be managed efficiently to curtail environmental impact and ensure regulatory compliance. The process of collecting crude oil waste/sludge samples at the Port Harcourt refinery involved multiple phases and considerations. The sludge sample was collected and stored in plastic containers to prevent spills and leaks.

2.3 Chemicals and Culture Development

The objective is to achieve pH and alkalinity settings, and neutralize the supernatant with NaOH and NaHCO₃. The laboratory scale experiments in this study followed the modified Bushnell-Hass (BH) culture medium, constituted by MgSO₄ (0.2 g/l), CaCl₂ (0.02 g/l), KH₂PO₄ (1 g/l), K₂HPO₄ (1 g/l), FeCl₃ (0.025 g/l) and NaCl (0.2 g/l). A substrate ratio of chemical oxygen demand (COD):N:P of 300:5:1 was maintained in the experiments, Nitrogen source was provided by adding NH₄Cl and K₂HPO₄ solutions. Subsequent growth conditions are corrected using micro-nutrient deficiency (Field, 2011). Constituents from the sludge, composed of oil, grease, asphaltenes, waxes, water, and other solid deposits were established before the experiment. The organic phase of the composition was extracted by n-hexane in a separation funnel and passed through a layer of Na₂SO₄ to remove the moisture. Accordingly, Gas Chromatography using a flame ionization detector, and total polyaromatics by UV absorption in the wavelength range of 220 - 450 nm (Fitzgerald *et al.*, 2010).

Following Aiba *et al.*, 2013, the Taguchi & Humphrey method was used to assess oxygen uptake rate (OUR). This was to restrict the aeration process and monitor the drop in oxygen concentration in the bioreactor. The Posten and Cooney, 2016 mode of 1012 cells/g bacteria and 109 cells/g yeast was assumed to express the specific oxygen uptake rate (SOUR) as a function of microbial biomass as measured by plate counts.

A variable speed peristaltic pump was used to feed the sludge to the bioreactor, and monitored through various hydraulic retention times. The flow rate of the influent sludge (Q_{inf}) was varied here, as the loading rate. The bioreactor was constantly aerated relative to the loading rate to achieve steady-state conditions where the gas production rate in bioreactor approaches constant. Subsequently, samples of the gas released are collated for analysis of influent and effluent COD, oil and grease, paraffins and total polyaromatics parameters. The experiments were carried out as functions of oxygen dosage, PAH concentration, and pH of the sludge suspension, at different stages to determine the effects on the bioreactor process.

2.4 The Design Model for Optimization

The three-factor model with three parametric levels of higher, central and lower was applied aimed at maximizing the removal of PAHs in combination with the characterization technique, response surface methodology (RSM). The independent factors used in the Taguchi design of experimental methodology (DOE) were pH (X_1), initial PAH concentration (X_2) and oxygen dosage (X_3) with the PAH removal (Y) as the response variable. Table 1 shows that each factor was coded at three levels, from -1 to $+1$. The Marandi *et al* (2011) procedure was applied to establish and decide the critical ranges of the factors.

$$Y = \beta_o + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=2}^n \beta_{ij} X_i X_j + c \quad (1)$$

The quadratic model shown in Equation (1) was used to formulate the model responses with a view to estimating the coefficients of the equation using least-squares regression (Montgomery, 2016). Such that β_o , β_i , β_{ii} , and β_{ij} represent constant, linear, quadratic, and cross-factor interaction coefficients, respectively; X_i and X_j are the coded independent variables with the subscript i or j as the number of independent variables; Y_i is the response forecasted; while c and n equal the residual term and the number of factors respectively.

Table 1: Coded levels of Factors for 3-Cube Central Composite Design (CCD)

Factors	Factor Levels		
	Lower Level (-1)	Central Level (0)	Higher Level (+1)
pH (X_1)	4	7	10
Concentration (mg/L) (X_2)	50	100	150
Adsorbent dosage (g/L) (X_3)	0.5	1	1.5

The coefficients of the response functions were estimated using the *Design-Expert* statistical software for graphical and regression analysis. Assessment of the significance of the independent variables, factor interactions, and model equations was explored with the analysis of variance (ANOVA) program at 95% confidence intervals (CI). This innovative process resulted into three-dimensional (3D) surfaces and two-dimensional (2D) contour plots while keeping another factor constant in the quadratic models. The experiments were further repeated with a view to validating the statistical models enabling maximum removal of the PAHs. In addition, optimal operating conditions were estimated using the numerical optimization method built in the software. Finally, further experimental runs were carried out to confirm the predicted optimal conditions for the response function and removal of PAH. To combine the desirable ranges for the response, the desirability multiple response method was used to obtain a simultaneous function that represents the geometric mean of all transformed responses (Myers *et al.* (2014)) in Equation (2).

$$D = \sqrt[n]{(d_1 \times d_2 \times \dots \times d_n)} = \sqrt[n]{\prod_{i=1}^n d_i} \quad (2)$$

Where D is the desirability objective function, d_i is response range, and n equals the number of responses. If the analyzed response is found to be outside of the desirability range, the overall desirability function becomes zero. Therefore, for a simultaneous optimization, response is required to be assigned low value for optimization. In this case, the percent removal of PAH (d_1) was maximized.

III. RESULTS ANALYSIS

The three-factor, three-level Central Composite Design (CCD) with observed and predicted values for the removal of PAH from the developed quadratic models are shown in Table 2. Hence, to enable prediction of the response function for the removal of PAH, a second-order polynomial equation was introduced as follows:

$$Q = 78.72 + 11.33X_1 + 17.15X_2 - 13.78X_3 + 7.05X_1X_2 - 10.26X_1X_3 + 9.96X_2X_3 - 3.27X_1^2 - 8.86X_2^2 - 1.69X_3^2 \quad (3)$$

Equation (3) implies that the initial PAH concentration influenced the amount of PAH removed much more significantly in all the factors considered during the course of the experiment. The model however showed that pH (X_1) must be increased to enable sufficient removal of the PAH. In addition, the model showed the significance of the first-order interaction between pH, the oxygen dosage (X_1X_3) and the dual interaction of initial PAH concentration (X_2^2) in the experiment. It was further observed that the dual interaction of pH (X_1^2) and oxygen dosage (X_3^2) produced quite low values of constants. The significance model coefficients test that was incorporated fails to provide any influence on the final outcome. This implies that oxygen dosage (X_3) has negative effect on the amount of PAH removed (Y).

However, the values of the constants resulting from the dual interaction of pH (X_1^2) and oxygen dosage (X_3^2) were very low such that the use of a significance model coefficients test showed these terms did not influence the final outcome. This implies that oxygen dosage (X_3) has negative effect on the amount of PAH removed (Y).

The coefficients of the model components X_3 , X_1X_3 , and X_2^2 were also negative, hence detrimental to the PAH removal process. However, the positive coefficients of X_1 , X_2 , X_1X_2 , and X_2X_3 favored the PAH removal process. It follows that when there is an increase in the concentration (X_2) of PAH and pH (X_1), the dependent term (Y) representing amount removed increases.

Another factor considered is the effect on the amount adsorbed (Y) and the PAH concentration (X_2) under high concentration conditions. The model here showed that the positive increase on the amount of PAH removed (Y) due to the interface of PAH concentration and oxygen dosage (X_2X_3) (Figure 3) suggests that effect of oxygen dosage (X_3) was much less (Figure 1).

Accordingly, the model satisfied that the contribution of pH on the amount of PAH removed (Y) was much more positive than the oxygen dosage X_3 (see Figure 2) in relation to the effect of negative interface between the pH and the oxygen dosage (X_1X_3).

It was also observed that the interactive effects of PAH concentration (X_2^2), pH (X_1^2) and oxygen dosage (X_3^2) shows negative in relation to the amount removed. It shows that decrease in these factors also decrease the removal process in the amount of PAHs.

3.1 Application of Analysis of Variance (ANOVA)

Analysis of Variance (ANOVA) was applied to the coded quadratic model for response (Table 3) and for the coded factors, the model (Equation (4)) was used, neglecting the insignificant terms:

$$Q = 78.72 + 11.33X_1 + 17.15X_2 + 13.78X_3 + 7.05X_1X_2 - 10.26X_1X_3 + 9.96X_2X_3 - 8.86X_2^2 \quad (4)$$

Table 2: Results from 3-Cube Factorial CCD with Observed and Predicted PAH removal (Y)

Run	Coded Values			Experimental Values			Response Y (mg/g)
	X_1	X_2	X_3	X_1	X_2	X_3	
1	-1	0	1	4	100	1.5	54.95
2	0	0	0	7	100	1	80.24
3	1	0	1	10	100	1.5	64.3
4	-1	1	0	4	150	1	72.47
5	0	1	1	7	150	1.5	78.29
6	1	0	-1	10	100	0.5	113.07
7	0	-1	1	7	50	1.5	31.15
8	0	0	0	7	100	1	81.33
9	0	0	0	7	100	1	77.11
10	0	0	0	7	100	1	75.75
11	1	-1	0	10	50	1	46.59
12	-1	-1	0	4	50	1	45.23
13	0	0	0	7	100	1	79.15
14	1	1	0	10	150	1	102.04
15	-1	0	-1	4	100	0.5	62.67

16	0	-1	-1	7	50	0.5	77.93
17	0	1	-1	7	150	0.5	85.28

A 95% confidence level by ANOVA using the Fisher’s (F) exact test was used to confirm the implication of the model and further compared to probability (p) values greater than F . The resulted model was observed to be significant with the factor “ $\text{Prob} > F$ ” showing less than 0.05. The computed model agreed favourably with literature (Tukey, 2017; Kutner *et al.*, 2014; Box and Cox, 2014) considering that the larger F -value with the associated P value show less than 0.05 confidence intervals, hence the experimental systems can be modelled effectively with less error. This translate the implication that the modeled F -values for PAH removal was significant as summarized ANOVA results (Table 3).The results showing p -values of 0.0417, 0.0085, 0.0099 and 0.0149 from the ensued interactions involving the pH of the oily sludge and the PAH concentration (X_1X_2), pH and the oxygen dosage (X_1X_3), PAH concentration and the oxygen dosage (X_2X_3) and the dual interaction of the PAH concentration (X_2^2) respectively, were statistically satisfactory in the AO7 dye removal. In comparison however, the p -values of 0.5590 and 0.2745 from the dual interaction results of pH (X_1^2) and dual interaction of the oxygen dosage (X_3^2) respectively, were not statistically significant in the removal of PAH.

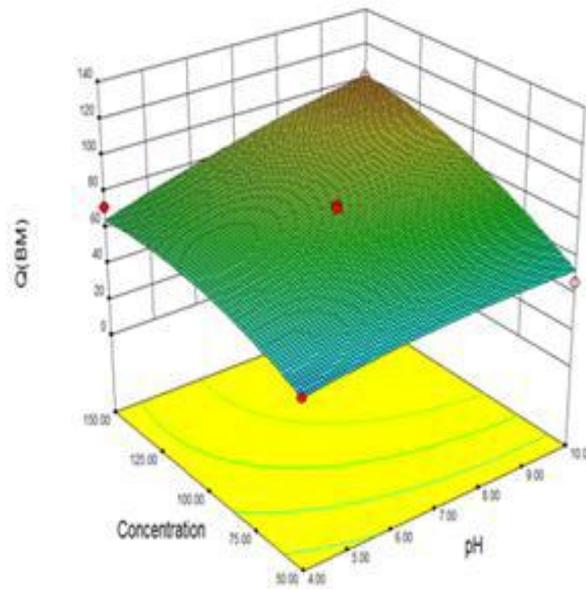


Figure 1: Surface Plots of Cross Factor Interaction effect of concentration of PAH and the pH (X_1X_2)

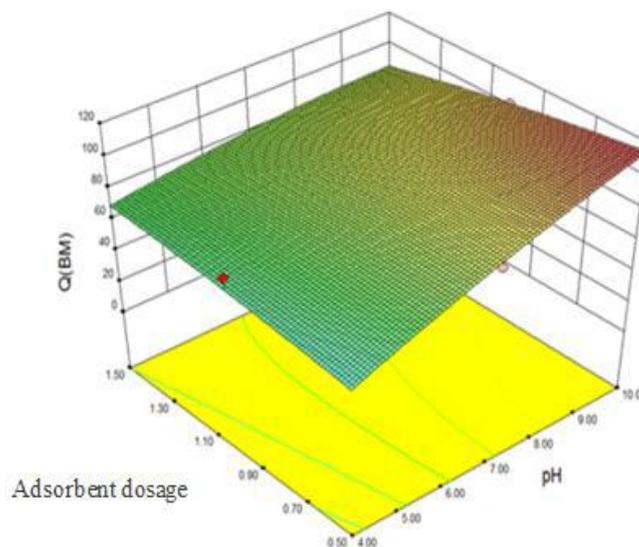


Figure 2: Surface Plot of Cross Factor Interaction effect of pH and the oxygen dosage (X_1X_3)

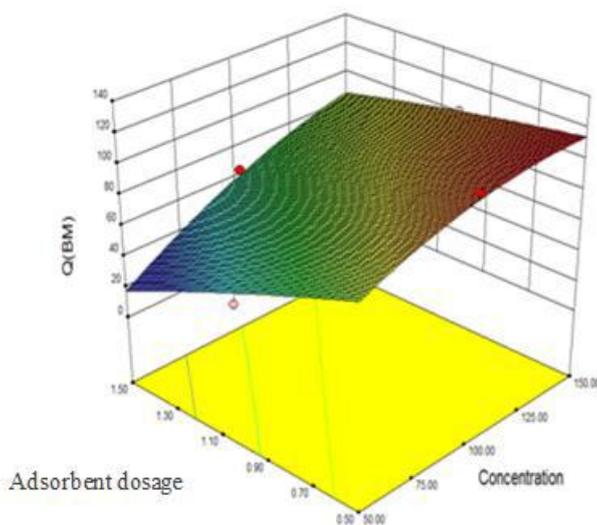


Figure 3: Surface Plot of Cross Factor Interaction effect of PAH concentration and the oxygen dosage (X_2X_3)

Table 3: ANOVA for Predicted Results for PAH removal (Y)

Source	Sum of squares	Df	Mean square	F value	p-value Prob > F
Model	6329.148	9	703.239	21.891	0.0003
X_1 -pH	1027.858	1	1027.858	31.996	0.0008
X_2 -Concentration	2352.294	1	2352.294	73.223	<0.0001
X_3 -adsorbent dosage	1519.658	1	1519.658	47.305	0.0002
X_1X_2	198.951	1	198.951	6.193	0.0417
X_1X_3	421.276	1	421.276	12.117	0.0085
X_2X_3	395.811	1	395.811	12.321	0.0099
X_1^2	45.140	1	45.140	1.405	0.2745
X_2^2	330.469	1	330.469	10.288	0.0149
X_3^2	12.086	1	12.086	0.376	0.5590
Residual	224.874	7	32.125		

3.2 Model Analysis

The quadratic model described the observed data well as validated by the R-squared and adjusted R-squared values of 99%, ensuring acceptable variation relative to the experimental values, for the removal of PAH. Figure 4 shows a correlation of the observed and predicted values for the removal of PAH while Figure 5 depicts the residual values. The occurrence of some negligible inconsistencies can be seen on the straight line trend showing that the observed and predicted values fit well. This implies the predicted outcome for the removal of PAHs was significantly acceptable, which is in agreement with the correlation from the mathematical model and the experimental data. In essence, the residue does not exceed the amount adsorbed and so is evenly distributed in space with due handling.

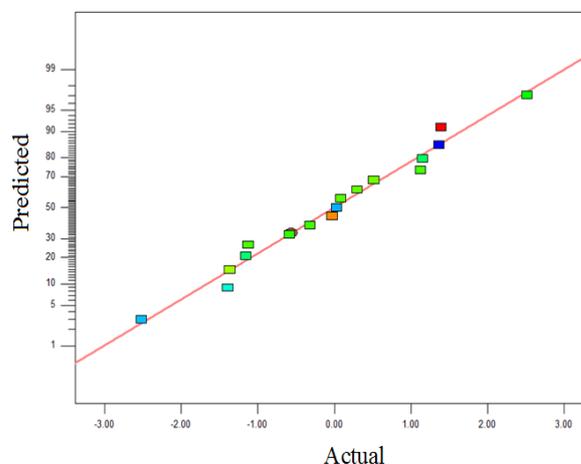


Figure 4: Tests of Model Assumptions from Experimental and predicted response for AO7 dye removal

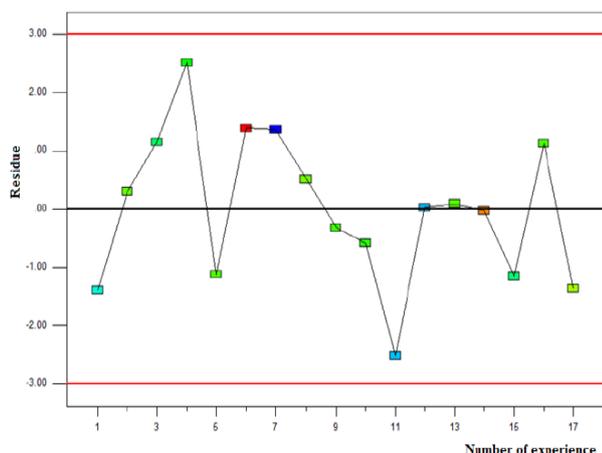


Figure 5: Tests of Model Assumptions from Analysis of Residual for the Response

3.2 Optimizing Operational Conditions from the Implementation Process

The optimization, by the choice of operating conditions and practical implementation process was evaluated using the RSM for the three independent variables to get maximum percent removal of PAHs. The model (Equation (4)) was used as function for the removal of PAHs where the range of independent factors is taken as model constraints. The optimum conditions for removal of PAHs reached maximum of 99% with dosage of oxygen (0.4g), initial PAH concentration (20mg/L) and pH (6). This enabled an additional run to validate the predicted values resulting in PAH removal of 97.8% experimentally. The R-squared confidence interval of 95% here confirmed the reliability of the model.

IV. CONCLUSION

The objective of this research focused on assessing the effects of the various operational parameters, including pH, PAH and oxygen concentration on the rate of the bioremediation of oily sludge using design of experiments (DOE). The aim was to optimize the treatment processes in a laboratory scale mechanically stirred-tank bioreactors. The statistical models applied were validated by an additional set of experiments at the optimum conditions in line with the DOE results. The mathematical model developed in this study provides a comprehensive exploration of the cross-factor interactive effects of the independent variables on the responses. Following this success future study can be made with a view to regenerating the exhausted biomass, recovering the removed PAHs and designing continuous oily sludge treatment systems.

The results of the analyses show that the most functional requirement and so advantageous in the treatment process was 0.4g, 20mg/L and 6 for oxygen dosage, PAHs concentration

and pH respectively. This implies that the mathematical model efficiently simulated the treatment process hence, enhanced the performance of aerobic bioreactor plants in removing PAHs from crude oil sludge. The study therefore has advantage to enable improving the performance of aerobic waste treatment plants.

ACKNOWLEDGEMENT

The authors wish to express their profound gratitude to the Tertiary Institution Trust Fund (TETFUND) for completely sponsoring this research through TETFUND Institution Based Research (IBR) project intervention of Fund Number: FUI/AP/TETF.24/IBR/ 001.

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Citation of this Article:

Okuroghoboye D. Itugha, Andy O. Ibeje, & Esther C. Udochukwu. (2025). Modeling and Optimizing the Use of Aerobic Bioreactors for Sustained Removal of Pahs from Crude Oil Sludge. *International Research Journal of Innovations in Engineering and Technology - IRJIET*, 9(10), 284-291. Article DOI <https://doi.org/10.47001/IRJIET/2025.910035>
