

Development of an Experimental Rig for Bioremediation Studies

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Abstract

In recent times cost of importing laboratory equipment for experimental studies is increasing at alarming rate and far beyond the reach of most educational and research institutions because of conflicting demands of these institutions. As a result, a study was carried out on the sizing, construction and testing of a robust and low cost experimental rig for bioremediation studies using indigenous technology. The experimental rig consists of air pretreatment unit, a fixed bed bioreactor, volatile organic compound trap or filter, air-flow meter, carbon dioxide (CO₂) trap and a stand. The fabricated rig was tested for biodegradation of Soil Contaminated with Spent Motor Oil (SCSMO) at control air-flow rate of 10 L/h. Results obtained revealed that the equipment was effective in the degradation of SCSMO; 75% removal of the initial oil and grease content was achieved in 10 weeks. Therefore, the developed rig can be used for bioremediation studies and in the same vein; it can be scaled up for commercial treatment of hydrocarbon contaminated soils.

Keywords: indigenous technology, spent motor oil, biostimulation, fixed bed bioreactor, low cost, oil and grease content.

1.0 Introduction

In the world today, most activities of oil industry, transportation and usages of petroleum and petroleum derived products have resulted in the contamination of many sites (soil and water). The conventional methods of cleanup have been successful in meeting up with the regulatory standards. However, these conventional techniques have their limitations, as they transfer pollutants from one place to the other, they are expensive, their technologies are complex, they do not always result in the complete neutralization of pollutant and above all, they are not environmentally friendly (Yerushalmi *et al.*, 2003). These limitations in the conventional cleaning technologies have spurred investigations into a simpler, environmentally safe, cost effective technology known as Bioremediation.

Bioremediation is a cleaning technology used from time immemorial to detoxify or reduce pollutant levels of harmful chemicals such as hydrocarbons, heavy metals, etc. to acceptable level by the regulatory authorities by the action of micro-organisms. Its use as a treatment option was unpopular until recently when attention has shifted to the area of biotechnology. Economic advantage, environmental friendliness and ease of application were in no doubt factors that make this remediation technology popular.

Bioremediation is not a pinnacle but meant to complement existing remediation options such as the thermal treatment, dig and dump method, chemical method, separation techniques and stabilization/solidification technology, which can be broadly classified as the physicochemical technologies. More so, the physicochemical technologies do not always result in complete neutralization of pollutants (Yerushalmi *et al.*, 2003). As a transformation mechanism, bioremediation achieves permanent treatment of contaminants.

Soil bioremediation may be broadly divided into "In Situ" and "Ex Situ" strategies. The former method refers to the treatment that does not involve the excavation of contaminated soil and whilst the latter does.

In this study emphases were laid on the "Ex Situ" strategy because of the conveniences of adoption in the laboratory and due to the fact that soil contaminated with minor oil spillages outweighs that of major oil spillages.

Ex-situ bioremediation is a biological process in which excavated soil is placed in a lined above-ground treatment area and aerated following processing to enhance the degradation of organic contaminants by the indigenous microbial population. Under aerobic conditions, specific micro-organisms can utilize organic contaminants such as petroleum hydrocarbon mixtures, polycyclic aromatic hydrocarbons (PAH), phenols, cresols and some pesticides as a source of carbon and energy and degrade them ultimately to carbon dioxide and water.

It is unusual to require the addition of microbial populations but usual to assess the nutrient requirement and amend the basic nutrients and organic substrate of the soil if any of these elements are deficient or absent. Oxygen (via the introduction of air) is essential to allow the microbial population to develop cultures capable of sustaining degradation.

Ex-situ bioremediation can remediate a wide range of hydrocarbon contaminants including but not limited to: general hydrocarbons, kerosene, phenols, cresols, polycyclic aromatic hydrocarbons, semi-volatile organic compounds, diesel range hydrocarbons, lubricating, oils, and straight chain aliphatics.

Non-chlorinated hydrocarbons within the carbon chain lengths C6 to C14 are readily treatable, non-chlorinated hydrocarbons with carbon chain lengths C15-C32 are treatable but require longer time periods to degrade. Chlorinated hydrocarbons and other more complex chains can be degraded but require detailed assessment and analysis to determine suitability.

In addition, in recent times, the cost of importing laboratory equipment for experimental studies is increasing at an alarming rate and far beyond the reach of most educational and research institutions. The numbers of experiments undertaken by most of our graduates were so few due to lack of enough and adequate laboratory equipment which in turn lowers the quality of education. In an effort to solve this problem, a study was carried out on the development of experimental rig for bioremediation studies using indigenous technology. This study is also justified from the fact that previous works done on bioremediation were basic proof of concepts and practical oriented.

The aim of this study was to design and fabricate an experimental rig for bioremediation of soil contaminated with hydrocarbon pollutants and the objectives were to carry out bioremediation experiments for the degradation of SCSMO using the rig developed. The scope of this study includes sizing, construction and testing of the experimental rig using SCSMO.

2.0 Experimental

2.1 Development of the Experimental Rig/Apparatus

The experimental apparatus developed in this study consists of air compressor, pressure gauge, air pretreatment unit, humidifying unit, air flow meter, bioreactors, VOC filters or adsorbers, CO₂ absorbers and a stand. Of these components, the air compressor, pressure gauge, air flow meter were procured while the humidifying and air pretreatment units, and CO₂ traps were improvised using 20 L plastic bottle, 10 L Pyrex bottle and 1 L plastic containers respectively. Only the fixed bed bioreactors, VOC traps and the stand were sized or designed. Details of these three components were explained in detailed as follow:

2.1.1 Sizing of fixed bed bioreactor

The amount of contaminated soil treated in each bioreactor was 1.5 kg. Therefore, our basis for sizing the bioreactor was 1.5 kg of Soil Contaminated with Spent Motor Oil.

- Basis: 1.5 kg of SCSMO
- The bulk density of SCSMO was determined to be approximately 1 500 kg/m³ (See Table 3) using Eqn. (1):

$$\text{Bulk density} = \frac{\text{mass of contaminated soil}}{\text{volume of contaminated soil}} \quad \dots \quad (1)$$

$$1500 = \frac{1.5}{\text{volume}} \Rightarrow \text{volume of SCSMO} = 1 \times 10^{-3} \text{ m}^3$$

Therefore, the volume of contaminated soil equivalent to 1.5 kg is $1.0 \times 10^{-3} \text{ m}^3$.

Bioreactor geometry was chosen to be cylindrical with effective height of 22.86 cm (0.2286 m). Therefore, the effective volume of the bioreactor was calculated using the formula for the volume of a cylinder as follows;

$$V_{\text{bioreactor}}^{\text{effective}} = \frac{\pi D^2 H}{4} \quad \dots \quad (2)$$

where: D and H are diameter and effective height of the bioreactor respectively.

$$1.0 \times 10^{-3} = \frac{\pi D^2 \times 0.2286}{4}$$

$$D = 0.07464 \text{ m (2.94")} \approx 0.0762 \text{ m (3")}$$

From the above, the height to diameter ratio (H/D) was obtained to be 3.0. This ratio is in line with typical value of 2-3 (Soderberg, 1997). Figs. 1 and 2 show the detailed diagram of a bioreactor system with two sampling points; these sampling points were improvised to allow easy access to the internal part of the bioreactor and these sampling points were covered using rubber seals to prevent air and moisture from escaping from the bioreactor.

The fixed base designated Section A-A (Fig. 1) was lined with geotextile material in order to prevent losses of soil particles through the perforated holes. Summary of sizing parameters for the bioreactor is presented in Table 1;

Table 1: Summary of Sizing Parameters for the Fixed Bed Bioreactor

Parameter	Characteristic/Value
Configuration of bioreactor:	Cylindrical
Diameter of bioreactor (D), m:	0.076 2
Effective height of bioreactor (H), m:	0.228 6
Overall height of bioreactor, m:	0.335 2
Effective height to diameter ratio (H/D):	3
Effective bioreactor volume (V), m ³ :	1.042 6*10 ⁻³
Vessel thickness (m)	0.003
Perforated hole diameter (m)	0.001
Material of construction:	316 Stainless Steel

2.1.2 Sizing of volatile organic compounds (VOC) adsorber

The adsorber consists of granulated activated carbon used to capture VOC. This component was sized and constructed using indigenous technology. As in the case of the bioreactor, the perforated sections of the adsorber were to allow passages of air through the geotextile material and to prevent losses of activated carbon. The configuration and dimension of adsorber used are presented in Fig. 3:

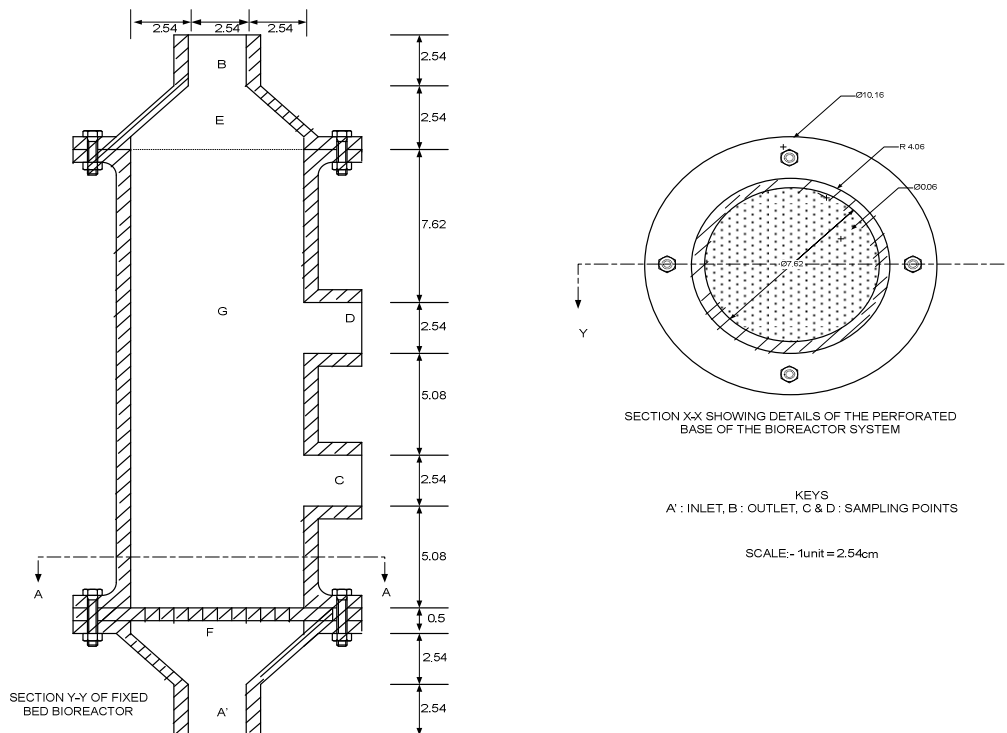


Figure 1: Sectional Views of a Fixed Bed Bioreactor with Two Sampling Points **Material of construction**

- Part G: 3-inches stainless steel pipe
- Parts A, B, C & D: 1-inch stainless steel pipe
- Parts E & F: Stainless steel sheet

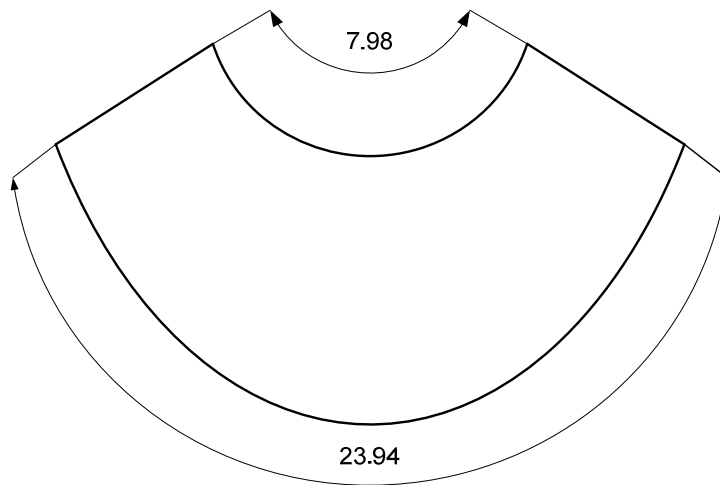


Figure 2: Working Drawing of E and F of Fig. 1

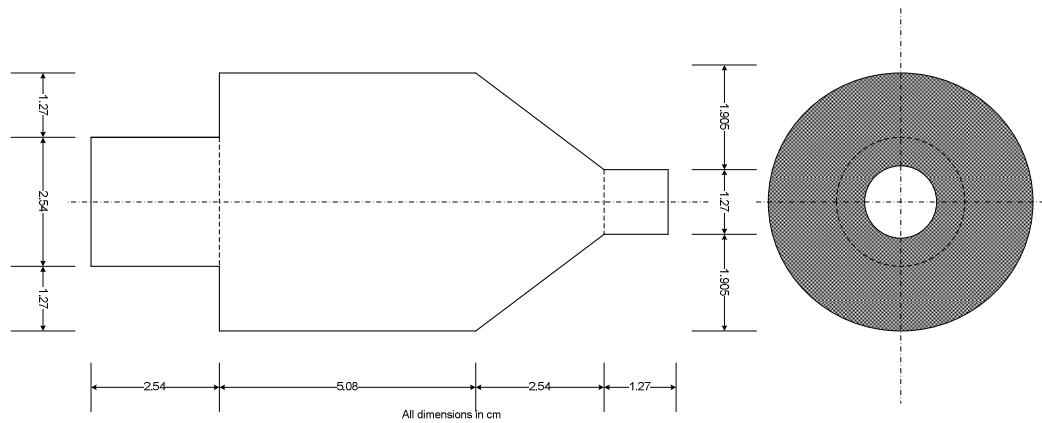


Fig. 3: Detail diagram of VOC Absorber The active volume is the region bounded by the two vertical short dashes (Fig. 3) consisting of a cylinder and a frustum. Therefore, the volume of the active space is given by;

$$V_{active} = V_{cylinder} + V_{frustum} \quad (3a)$$

Mathematically;

$$V_{active} = \frac{\pi D_1^2}{4} H_1 + \frac{\pi}{12} (D_1^2 H_{1*} - D_2^2 H_2) \quad (3b)$$

$$V_{active} = \frac{\pi \times 5.08^3}{4} + \frac{\pi}{12} (5.08^2 \times 2.54 - 1.27^2 \times 0.635) \cong 120 \text{ cm}^3 \text{ (or } 1.2 \times 10^{-4} \text{ m}^3)$$

Hence, the volume occupied by the granulated activated carbon is $1.2 \times 10^{-4} \text{ m}^3$

Summary of sizing parameters for the VOC trap is presented in Table 2:

Table 2: Summary of Adsorber Sizing

Parameter	Characteristic/Value
Configuration of Adsorber:	Cylindrical + Frustum
Maximum diameter of adsorber (D_1), m:	0.050 8
Minimum diameter of adsorber (D_2), m:	0.012 7
Effective height of adsorber (H), m:	0.076 2
Overall height of adsorber, m:	0.114 3
Effective adsorber volume (V), m^3 :	1.2×10^{-4}
Vessel thickness, m	0.003 0
Perforated hole diameter, m	0.001 0
Material of construction:	Galvanized steel

2.1.3 Design of the stand

The stand was designed and constructed using $\frac{3}{4}$ -inch iron pipe; it was trapezoidal in shape and partitioned at the middle on which bioreactors, VOC adsorbers, CO_2 absorbers and the piping systems were fastened. The bottom of the stand was covered with $\frac{1}{2}$ -inch plywood decorated with white Formica and the top (roof) was left

uncovered (see Figs. 8 and 10). The first angle orthographic projection of the stand is presented in Fig. 4. The bioreactors were fixed to the stand using iron clips and the connections between the bioreactors, adsorbers and absorbers were done using rubber hoses (See Figs. 8 - 10).

2.1.4 Construction and installations of components

All the components improvised, sized and/or designed were constructed using indigenous technology and supervised locally. The flow diagram of the experimental rig is shown in Fig. 5 and pictorial representations of the constructed components assembled together are depicted in Figs. 6 -10. In addition, piping of the experimental rig was carried out under strict supervision.

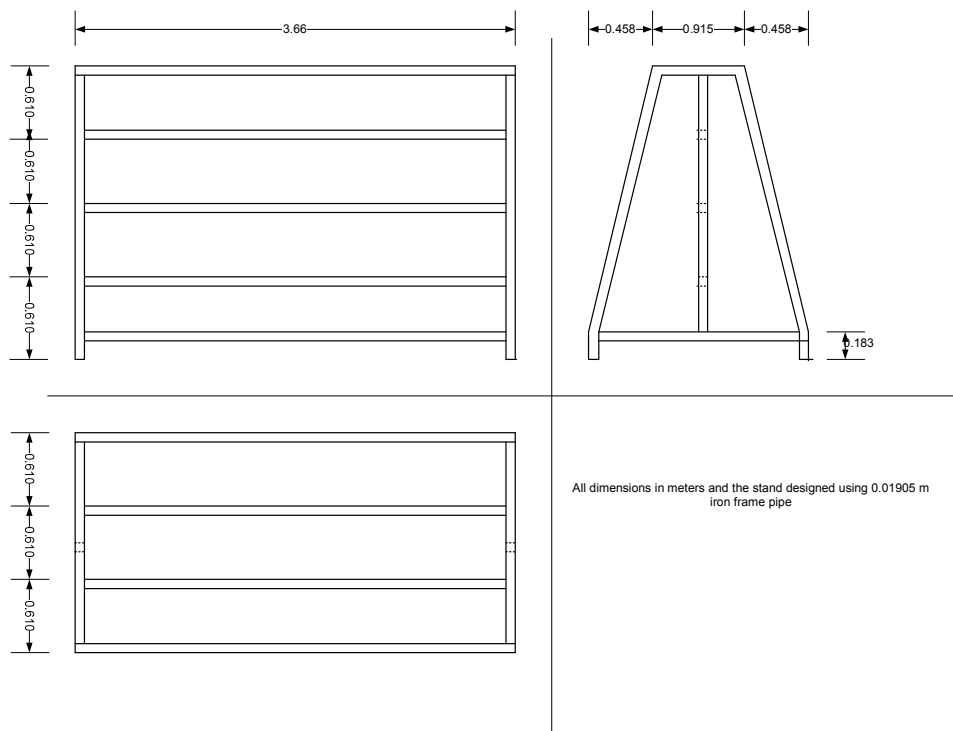


Fig. 4: Detailed Diagram of the Experimental Rig Stand

2.1.5 Pre-commissioning runs

Various process unit (bioreactors, adsorbers and absorbers) sized and fabricated were checked for possible leakages by pressurizing and ensuring that these units were airtight. After installation and piping works of the experimental rig, a pre-commissioning runs were conducted. This was done by passing compressed air through the rig and testing for leakages using detergent solution, where leakages were identified, they were rectified appropriately.

2.1.6 Commissioning and test running of experimental rig

The developed experimental rig was tested using a typical bioremediation experiment: "bioremediation of soil contaminated with spent motor oil". The experimental design and process description for this bioremediation experiment were described in section 2.2.

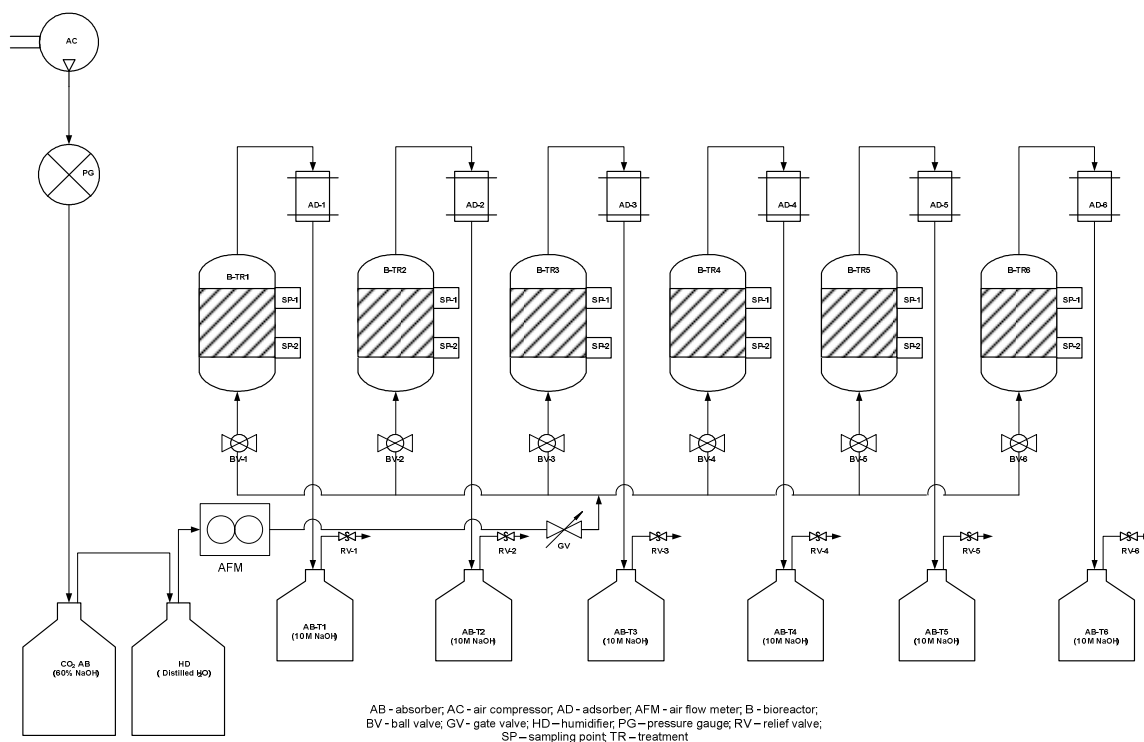


Fig. 5: Flow Diagram of Experimental Rig for Bioremediation of Hydrocarbon Contaminated Soil



Fig. 6: Setup for the Bench-Scale Experimental Rig



Fig. 7: Air vessel, Air pretreatment and Humidifying Vessels for the Experimental Rig

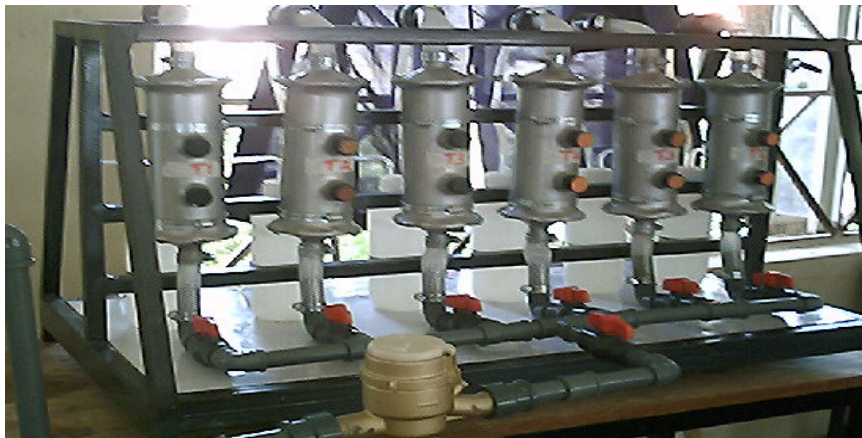


Fig. 8: Front View of the Bench-Scale Bioremediation Rig consisting of Six Bioreactors, Air flow meter and the Piping System



Fig. 9: Plan View of the Experimental Rig showing VOC Adsorbers



Figure 10: Back Elevation of the Experimental Rig Showing CO₂ Absorbers connected to VOC Adsorbers using Rubber Hoses

2.2 Experimental Design

The experimental designs for the six treatments studied in this research were presented as follow;

- Treatment 1 (TR1): Heat sterilization
- Treatment 2 (TR2): No treatment
- Treatment 3 (TR3): Heat sterilization, followed by addition of Bacteria
- Treatment 4 (TR4): Addition of Bacteria
- Treatment 5 (TR5): Application of NPK (20:10:10) and KH₂PO₄, followed by the addition of Bacteria

Treatment 6 (TR6): Application of NPK (20:10:10) and KH₂PO₄

2.3 Process Description and Methods

These biodegradation investigations were carried out in six aerobic fixed bed bioreactors (TR1, TR2, TR3, TR4, TR5 and TR6), with 1.5 kg of contaminated soil; this included, where appropriate, the various additives at room temperature. The bioreactors were completely closed in order to avoid CO₂ leakage to the environment before passing into the CO₂ traps. The CO₂ traps (AB) placed before the bioreactors contained solution of 60% (w/v) NaOH used to absorb CO₂ from the atmosphere and the humidifying unit (HD) to moisten the air before entering the bioreactors. The absorbers after the bioreactors contain 10 M solution NaOH each meant to absorb the CO₂ generated from the bioremediation processes.

The moisture content in all the six treatments was set at 20% of the total weight of the soil at the initiation of bioremediation. The air-flow rate was maintained in all cases at an average rate of 10 L/h using a flow meter for minimum of fourteen (14) hours daily for the ten weeks of investigation.

At the initiation of this experiment, the physicochemical and microbiological characteristics of the contaminated soil sample were analyzed in order to access its suitability for bioremediation. In addition, the progress of bioremediation process was assessed by the measurement of the oil and grease content (O&G) on weekly basis and carbon dioxide (CO₂) respiration rate monitored at 48 hourly basis.

All analyses carried out were conducted using established methods: Particle size distribution were determined based on the Unified soil classification (ASTM-D2487); Bulk and particle densities were determined using the gravimetric analysis and soil porosities calculated from bulk and particle densities values (Brady *et al.*, 1999); Soil pH were determined using the method of Bates (1954) and moisture contents by the ASTM-D2216; Organic carbon and available phosphorus in soil samples were determined using the spectrophotometer (Abdulsalam, 2011) and total nitrogen content by the Kjeldahl method; oil and grease content was determined using method well detailed in Abdulsalam (2011); the temperature was measured using a digital thermometer, total heterotrophic bacteria counts (THBC) were carried out by employing the standard plate counting technique, total hydrocarbon bacteria degrading counts (HDHC) were carried out by employing the Most Probable Number (MPN) analysis with 5 tubes using the Bushnell-Haas medium (Atlas, 1994), and the CO₂ respiration rates were determined using the titrimetric analysis.

3.0 Results and Discussion

3.1 Physicochemical Characteristics

The physicochemical and microbiological characteristics of the soil were investigated in order to determine the success or failure of bioremediation on the soil sample. Results obtained are presented in Table 3; the high level

of carbon content was due to the presence of spent motor oil in the soil, which is related to the oil and grease content. The value of the oil and grease content exceeds the safe limit of 500 mg/kg specified by the Nigeria Ministry of Environment and therefore, needs intervention for public safety and environmental health. The soil texture and pH value obtained from the test soil were adequate for bioremediation to thrive. In addition, the microbiological analyses revealed that the THBC was above the minimum value of 10^5 required for effective bioremediation (Forsyth *et al.*, 1995) and HDBC was reasonable in number for effective bioremediation to occur. Hence, the numbers of indigenous bacteria in the test soil were adequate for effective bioremediation to occur. Therefore, the soil sample investigated was amenable to biodegradation.

Table 3: Physicochemical and microbiological characteristics of SCSMO

Parameter	Characteristic/Value
Soil texture	Loamy Sand
Particle density (g/cm^3)	2.040
Bulk density (g/cm^3)	1.490
Soil porosity (%)	26.89
pH	7.430
O&G (mg/kg)* 10^5	1.410
TOC (%)	18.42
WC (%)	2.090
WAC (%)	34.13
OC (mg/kg)* 10^4	6.420
N (mg/kg)* 10^3	1.400
P (mg/kg)	17.96
THBC (CFU/g) * 10^8	2.600
HDHC (MPN/g)* 10^4	2.760

Data presented are averages of triplicate determinations

3.2 Oil and Grease Biodegradation

The percentage reductions in the oil and grease content at different periods during the course of this study are shown in Fig. 11. From this figure, it could be seen that the percentage O&G content removal increased with time, which is typical of any degradation process. The process was characterized by a period of fast increase in percentage O&G content removals i.e. during the first five weeks (40, 45, 51, 40, 59 and 63% for TR1, TR2, TR3, TR4, TR5 and TR6 respectively), followed by a period of slower activity (past Week 5). The degradation pattern followed shifting order (1-0) degradation (Okpokwasili and Nweke, 2005).

At the initiation of bioremediation (at time zero), the concentrations of O&G contents in bioreactors TR1, TR2, TR3, TR4, TR5, and TR6 were 29 010, 37 966, 35 519, 33 746, 32 027 and 38 592 mg/kg dry weight respectively. After 70 days, their concentrations reduced to 14 439, 14 088, 12 085, 14 438, 10 115 and 9 830 mg/kg dry weight, which were equivalent to 50, 63, 66, 57, 68 and 75% removals in O&G contents as indicated in Fig. 11.

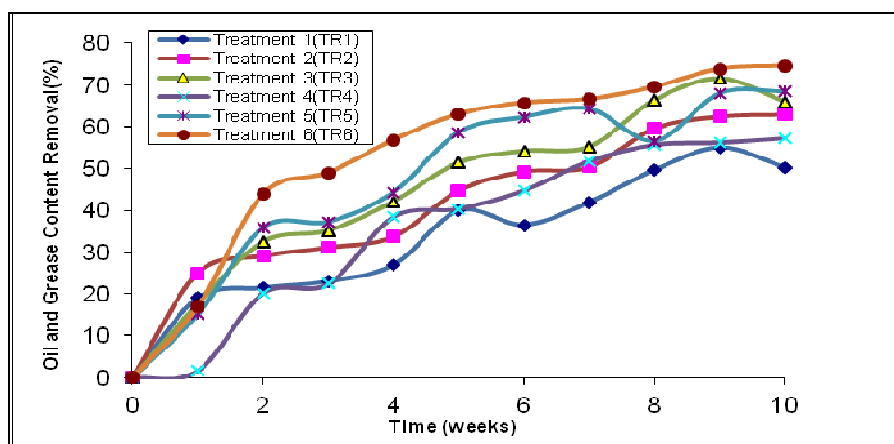


Fig. 11: Rate of Oil and Grease Content removals for Soil Contaminated with Used Motor Oil in aerobic fixed bed Bioreactors

Of the six treatments employed in this study, TR6 in which the indigenous microorganisms were stimulated with NPK (20: 10: 10) and KH_2PO_4 resulted in the maximum bioremediation response of 75% removal of the initial O&G content. This observation is in line with the literature that biostimulation thrive well in aged contaminated

sites (Kosteck and Calabrese, 1991). The percentage degradation of 75% achieved in 70 days in this investigation is superior to similar experiment carried out by Benyahia *et al.* (2005), they achieved 74% degradation in 120 days although bioaugmentation was used and aeration was on continuous basis. In addition, Gogoi *et al.* (2003) achieved 75% degradation of the initial petroleum hydrocarbon in the contaminated soil which was bioaugmented and biostimulated with nutrients. Their runs lasted for one year. More so, the percentage degradation achieved in this work (75%) was also higher than the 45% degradation of the initial petroleum hydrocarbon in contaminated soil for a 45 day run by Baptista *et al.* (2005).

3.3 CO₂ Generation in Bioreactors

The CO₂ evolution was also used as indicator of bacteria respiration (a product of bioremediation process). CO₂ generation in each of the bioreactors (TR1 to TR6) was monitored on 48-hourly basis. The 48-hourly measurements of CO₂ generation enabled the calculation of cumulative CO₂ generation over the treatment period (Fig. 12). All the treatment options (TR1 to TR6), appears to show a trend of adaptation period (1 to 10 days), maximum oil degrading period (25 to 55 days) and a decaying rate of oil degradation period (past 60 days). The profiles of cumulative CO₂ obtained were similar to those obtained in other studies (Renato *et al.*, 2009).

The cumulative CO₂ generation in each bioreactor increased with pollutant or oil degradation. The cumulative CO₂ generation for TR1, TR2, TR3, TR4, TR5 and TR6 were 4 276, 5 226, 5 493, 5 279, 5 667 and 6 249 mg/kg respectively. Treatment option 6 (TR6), gave the best CO₂ generation, which corresponds to the best O&G content removal (75% in 10 weeks). The control (TR1), showed the least CO₂ generation, which also corresponds to least O&G content removal (50% in 10 weeks).

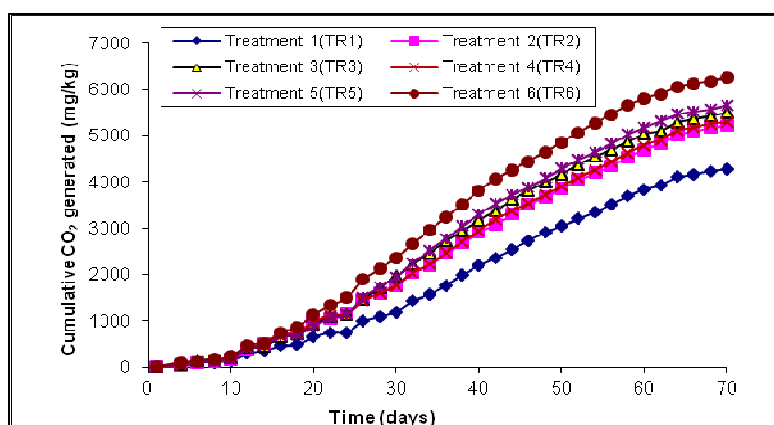


Fig. 12: Cumulative rate of generation of CO₂ in degradation of Soil Contaminated with Used Motor Oil in aerobic fixed bed Bioreactors

4.0 Conclusions

Based on the results obtained in this investigation, the following conclusions can be drawn: (i) biodegradation of soil contaminated with spent motor oil was successful to an appreciable extent in a closed bioreactor (solid-phase) system, (ii) the experimental rig developed was effective in the rehabilitation of soil contaminated with spent motor oil under the operating conditions used in this study (iii) petroleum removal efficiencies in term of oil and grease content removals can reach 75% over a period of 70 days in aerobic fixed bed bioreactor within the range of experimental conditions in this study and (iv) the biostimulation option gave the best result and could be used to develop a full scale treatment technology for SCSMO). More so, the experimental rig developed can be used for experimental studies at low cost.

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