



Effects of Time on pH, Total Bacteria Counts, and Total Hydrocarbon Contents in the Bioremediation of Crude Oil Contaminated Soil Using Indigenous Bio-stimulants

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Abstract

This study was conducted to investigate the effect of time on pH, total bacteria counts, and total hydrocarbon contents in the bioremediation of crude oil contaminated soil using pig waste and sawdust as bio-stimulants. Dried soil samples (500 g each) were weighed each into seven different plastic bowls, and each bowl was contaminated with 10 g of crude oil. Six of the contaminated soil samples in six of the plastic bowls were amended with a combination of pig waste and sawdust as bio-stimulants in the ratio of 1:1 in amounts of 150 g, 125 g, 100 g, 75 g, 50 g, and 25 g, while the seventh bowl served as control. The bio-stimulants were not added to the control sample. The effect of time on pH, total hydrocarbon contents (THC), and total bacteria counts (TBC) were monitored in all seven samples (test samples and control) for seven weeks. Results showed that changes in the pH values for the amended samples with masses 150 g, 125 g, 100 g, 75 g, 50 g, and 25 g were within the range of 6.00–7.00 (slightly acidic to neutral) which is suitable for most plants to thrive. There were relative increase and decrease in total bacteria counts with time reflecting an unsteady trend. There was also a steady decrease in THC with an increase in detention time in all samples which indicated steady decomposition of the crude oil contaminants during storage. Also, a significant level of bioremediation was achieved using the locally sourced biomaterials as amendments.

Keywords: Bioremediation; Biodegradation; Time; Contaminated soils; Bio-stimulant

Introduction

Bioremediation is a contamination control technology that employs the mechanism of biodegradation to clean up contaminated environment by utilizing metabolic activities of micro-organisms to convert various toxic chemicals into harmless products. Complete mineralization of these contaminants produces carbon dioxide, water, and cell biomass. This technology involves different useful strategies in both aquatic and terrestrial environments for

cleaning up crude oil successfully. The strategies could be in-situ or ex-situ remediation strategies which involve the use of indigenous or non-indigenous microbial populations to achieve or enhance degradation. Several investigations have shown that biodegradation using indigenous microbes has better effects over bio-augmentation (addition of non-indigenous microbial population) (Lee and Levy 1991, Venosa et al. 1992). Some

researchers argue that bio-augmentation is only effective in the laboratory and not in the field. However, studies have shown that 80–90% remediation has been achieved through bio-augmentation (Boopathy 2001, Brodkorb and Legge 1992, Venosa et al. 1992).

In-situ methods refer to the direct applications of microorganisms at the site of contaminated soil or groundwater. It is a very useful remediation option for treatment or cleanup of crude oil contaminated soil. Examples of in-situ methods include bioventing, biosparging, phytoremediation, mycoremediation, and enhanced bioremediation. In ex-situ methods, the contaminated material could be collected from polluted sites and bioremediation is carried out using the required microorganisms. Examples of ex-situ methods are biopiles, windrows, composting, slurry phase biological treatment, and farming. The ex-situ methods are more rapid for decontaminating polluted environments and studies have shown that they are very effective (Bragg et al. 1994, Satyanarayana 2005, Vidali 2001).

Bioremediation processes could be aerobic, anaerobic, anoxic, or a combination of these three based on the microorganisms used. Although in-situ or ex-situ methods are useful, they also have their limitations on the physical environment which may make conditions less suitable for life forms or communities present in the ecosystem. Generally, the limitation of microbial degradation of contaminants is that it is a slow process. Therefore, mutually stimulating actions of microorganisms would be necessary for the degradation process. Also, the presence of some substances like halogens in the oil pollutants can inhibit microbial degradation; while other substances like xenobiotics can get absorbed in the soil, and therefore become unavailable for microbial degradation.

Therefore, it is necessary to develop cost-effective indigenous technologies for the remediation of crude oil-contaminated soils. Reports have shown that the use of animal manure for remediating crude oil contaminated

soil has helped to correct nutrient loss due to hydrocarbon pollutants. This is highly effective because it ensures an adequate supply of nutrients such as nitrogen, phosphorous, and potassium to soils. This improves soil quality and enhances soil performance (Okolo et al. 2005, Ibekwe et al. 2006, Odjegba 2007, Ikpe and Powel 2002). However, nutrient contents in animals vary with the species of animal, age, nutrition, digestibility of the feed, microbes, and residues from the digestive system. The nitrogen contents of wastes, depending on the animal, are increased due to feed digestibility especially in ruminants (Van Horn et al. 1994, Wilkerson et al. 1997).

Also, the concentrations of nutrients depend on the amounts of dried matters present. It has been reported that fresh manure contains 70–85% moisture content, but may have less concentration of nutrients; while air-dried manure contains less moisture content (9–15%), but has more concentrations of nutrients on a weight and volume basis. Pig manure has very high nutritional quality (Samuel et al. 2018), and can therefore serve as an excellent fertilizer to improve soil structure. The chemical composition depends on the concentrations of amino acids containing sulfur (such as cysteine, cystene, or methionine) which is also responsible for the odor. Bioremediation technology has proven to be an alternative, environmentally friendly, and cost-effective measure for remediation of soil texture and other soil characteristics (Hoff 1991, Okoh 2006).

In this study, the efficiency of pig waste and sawdust as bio-stimulant for amending crude oil contaminated soil was investigated. The aim was to evaluate the overall efficiency of these amendments and to also encourage indigenous bioremediation technology. Therefore, the objectives of the study were to determine some remediation parameters such as pH, total bacteria counts, and total hydrocarbon contents of hydrocarbon contaminated soil, and to study the effects of time on the parameters using a seven-week detention time.

Justification of the study

Contaminated soil resulting from oil spills poses potential health hazards to man and animals in the environment. It affects the lands, making them highly unsuitable for agricultural and general activities. Organic manure (e.g. pig waste) which is nutrient-rich enhances plant growth. A combination of such manure with sawdust which is typically rich in carbon will provide microorganisms with rich sources of energy for degradation and soil clean-up. Therefore, pig waste and sawdust will be useful as an indigenous remedy for soils contaminated with crude oil.

Materials and Methods

Sample collection and preparation

The soil samples were obtained from an agricultural farm in Ugbowo, Ovia North East Local Government Area of Benin City, Edo State, Nigeria. The main occupations of the inhabitants are farming and trading. No history of crude oil contamination is known in the area. Crude oil was obtained from the Warri Refinery Delta State in Nigeria. The sawdust was obtained from a sawmill in Uselu, Benin City, while pig waste was obtained from Cami farms in Upper Mission Extension, Ikpoba-Okha, Benin City. The soil sample was air-dried and mechanically homogenized by removing materials such as pebbles, plastics, and metals and then screened using a 2.8 mm mesh size sieve. Preliminary analyses were carried out to determine moisture content, bulk density, and pH.

A soil sample (500 g) was measured into 6 different cells and contaminated with 10 g of crude oil each and mixed thoroughly using a hand trowel. Varying masses of the amendments used (pig waste and sawdust) in a 1:1 ratio were weighed and used as bio-stimulants in masses of 150 g, 125 g, 100 g, 75 g, 50 g, and 25 g, respectively. These masses were added to six of the cells which served as the test samples. One cell was labeled the control sample and contained only crude oil and soil. Total hydrocarbon contents in the control were determined as described by

Valcarcel et al. (2000). The six cells (i.e. the test samples and the control sample) were allowed to stand for a period of one week to allow for the growth and adaptation of indigenous microbes. Finally, bioremediation indication parameters such as pH, residual hydrocarbon contents (RHC), and total bacteria counts (TBC) were monitored for a period of seven weeks.

Sample analysis

Determination of pH

The pH was determined using a pH meter (model PHS-25, Techmel and Techmel, USA). The meter was calibrated using a buffer solution of pH 6.89. Ten grams (10 g) of the soil sample were weighed and transferred into a 50 mL beaker containing 25 mL of distilled water. The suspension was stirred for 30 minutes. Thereafter, the pH electrode was rinsed with the soil suspension, and the pH of the soil suspension was measured and recorded.

Determination of total bacteria count (TBC)

Total bacteria count was determined according to Samuel et al. (2018) with slight modifications using the spread plate technique. One gram of the sample was added to 9 mL distilled water and serially diluted up to 10^4 . Then one milliliter of the serially diluted sample (10^4) was plated out on a sterile nutrient agar plate containing 0.05 mg/mL of Ketoconazole to inhibit fungal growth. Duplicate plates were prepared and incubation was carried out in an inverted position at 30 °C for 48 hours. Colonies that developed after incubation were counted and the total bacteria expressed as colony-forming units per gram of sample.

Determination of total hydrocarbon content (THC)

For the hydrocarbon extraction, n-hexane (20 mL) was added to 5 g of the contaminated sample and then filtered into a cuvette in a UV spectrophotometer and the absorbance was determined at a wavelength of 480 nm. The total hydrocarbon content concentration was

extrapolated with a reference from a standard curve obtained from the graph of produced crude oil at varying concentrations (Valcarcel et al. 2000).

Determination of bulk density

The mass of the empty measuring cylinder was measured and recorded. The soil sample (2 g) was added to the empty cylinder and measured and the new mass was also recorded. The volume occupied by the soil in the cylinder was measured and bulk density was calculated (Akpauimam and Markakis 1981) as:

$$\text{Bulk density} = \frac{(\text{Mass of cylinder} + \text{soil}) - \text{Mass of empty cylinder}}{\text{volume of soil}}$$

Determination of moisture content

An aluminium dish was dried in an oven to constant weight for 12 hours (M_1). 5 g of air-dried soil were weighed into the aluminium dish, covered, and weighed again (M_2). The weighed dish and contents were dried in an oven to constant weight for 6 hours at 105 °C. After drying, it was removed from the oven, cooled in a desiccator for 30 mins and re-weighed (M_3). Moisture content was calculated (AOAC 2005, Gul and Safdar 2009) using the formula:

$$\text{Moisture content} = \frac{M_3 - M_1}{M_2 - M_1} \times 100$$

Where; M_1 = Weight of the dish,

M_2 = Weight of dish + weight of sample before drying,

M_3 = Weight of dish + weight of sample after drying.

Results and Discussion

Results obtained from the analysis are as shown in Tables 1 to 4 and Figures 1 to 3. Table 1 depicts the preliminary analysis carried out on the soil, pig waste, and saw-dust. The pH of the soil (6.42) and sawdust (6.00) were slightly acidic, while that of the pig waste (7.80) was slightly basic. The bulk densities of the soil, pig waste, and sawdust were 1.395, 0.14, and 0.7 g/mL, respectively while their

percentage moisture contents were 3.50%, 71.02%, and 28.2%, respectively.

Table 1: Preliminary analysis of soil, pig waste, and sawdust

Parameter	Soil	Pig waste	Sawdust
pH	6.42	7.80	6.00
Bulk density (g/mL)	1.395	0.14	0.70
Moisture content (%)	3.50	71.02	28.20

Table 2 depicts the results obtained when the pH values of samples were monitored for a period of seven weeks using various sample masses. Generally, it was observed that the pH of all the samples was neutral \pm 0.3. The pH values for the samples amended with masses 150 g, 125 g, 100 g, 75 g, 50 g, and 25 g were in the following range of values: 6.74–7.19, 6.90–7.19, 7.10–7.24, 7.30–7.29, 7.02–7.23 and 7.08–7.29, respectively and were within a close range. The ranges fall within the general pH range of 6.00–7.40 (slightly acidic to neutral), and this is suitable for most plants to thrive. When the pH of soil is high (alkaline) it leads to reduced uptake of some nutrients such as iron, nitrogen, calcium, magnesium by plants. Therefore, such nutrients remain bound in the soil which also can cause low phytochemical contents in the plants.

However, there were fluctuations in the pH values during the seven weeks. For the control sample, it was observed that the pH was almost the same in the seven weeks. The range of values was 6.67–6.90. The difference between the initial value (value at week 1) and final value (value at week 7) of pH for the control was +0.23. This means the final change in week 7 compared to week 1 is an increase of 0.23 (3.45%).

The pH changes for each mass of amendment with respect to time are also shown in Table 2. For samples with masses between 150 g and 75 g, the final differences between the initial and final pH values were in the range of \pm 0.04. But for samples with amendment masses of 50 g and 25 g, the differences

between the initial (week 1) and final (week 7) pH were -0.07 and +0.11, respectively. Therefore, the change in pH of the control sample was at least twice the change in pH of the other amended samples. This means that for samples with amendments, the change in pH was less compared with the control (sample without compost). The change (decrease) in the pH of the amended soil samples reflects a slightly acidic condition. This is in agreement with the report of Ebere et al. (2011) on enhanced remediation of hydrocarbon polluted soil. Slightly acidic conditions encourage the growth of indigenous microbes, hence promoting the degradation of crude oil. This

observation also agrees with the report given by Ayotamuno et al. (2006) who studied the bioremediation of petroleum-hydrocarbon polluted agricultural soil at different levels of water applications in Port Harcourt, Niger Delta part of Nigeria. Most soils in the Niger Delta region have been reported to exhibit weak soil acidity (Ayotamuno et al. 2006, Edwin-Wosu 2011). Yakubu (2007) in his study on biodegradation of Lagoma oil using pig dung from Minna, in the middle belt part of Nigeria, reported slightly acidic to neutral pH. A decrease in soil pH during the remediation process shows that bioremediation is in progress.

Table 2: The pH values of samples monitored for seven weeks

Sample	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Final ΔpH
Control	6.67	6.71	6.77	6.80	6.89	6.88	6.90	↑ 0.23
150 g	7.01	7.09	6.98	6.74	7.13	7.19	7.05	↑ 0.04
125 g	7.12	7.19	7.16	7.12	7.08	7.02	6.90	↓ 0.22
100 g	7.15	7.10	7.21	7.24	7.18	7.20	7.13	↓ 0.02
75 g	7.12	7.15	7.30	7.29	7.24	7.19	7.15	↑ 0.03
50 g	7.12	7.13	7.15	7.16	7.23	7.02	7.05	↓ 0.07
25 g	7.08	7.15	7.24	7.29	7.30	7.21	7.19	↑ 0.11

↑= Increase, ↓= Decrease, Δ= Change.

Figure 1 shows the change in pH with respect to time using various masses of amended samples. The minimum pH was 6.74 and was observed in soil amended with 150 g

sample, while maximum pH which was 7.30 was observed in soil amended with 75 g and 25 g samples, respectively.

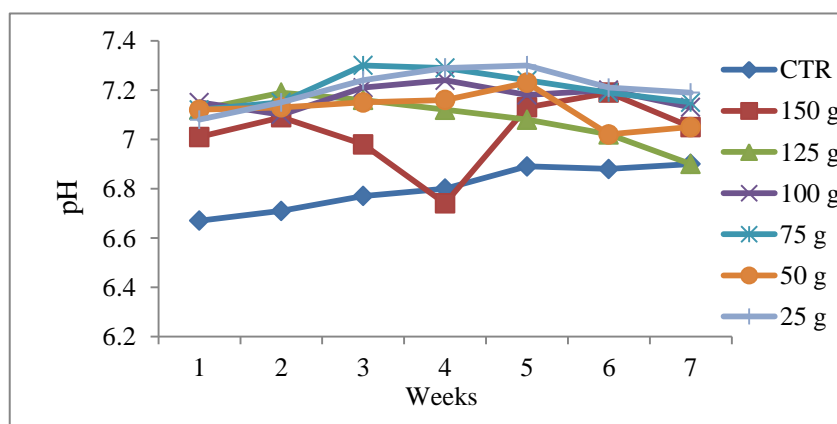


Figure 1: Effect of time on pH with time.

Table 3: Total bacteria counts (cfu/g) of samples monitored for seven weeks

Sample	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Final Δ TBC
150 g	20000	18000	16000	15000	13000	13000	20000	0
125 g	18000	16000	17000	15000	10900	12000	19000	↑ 1000
100 g	21000	13000	15000	20000	19000	11000	17000	↓ 4000
75 g	16000	12000	15000	16000	18000	14000	12000	↓ 4000
50 g	14000	11000	12000	15000	15000	13000	10700	↓ 3300
25 g	10900	19000	10000	12000	13000	10000	14000	↑ 3100

↑ = Increase, ↓ = Decrease

Generally, there was an unsteady trend in total bacteria counts with respect to time for all the masses of samples used. For the 150 g sample, it was observed that there was a steady decrease in bacteria counts until week 7 which increased by 7,000 (CFU/g). For weeks 1 and 2, a decrease in bacteria counts was observed for all the masses of compost except the 25 g compost which was higher in the second week. Samples with 125 g, 100 g, and 75 g compost showed unsteady trends. However, samples with 50 g experienced a steady reduction in total bacteria counts from week 3 with stable values in weeks 4 and 5 which later increased in week 7.

Figure 2 depicts the variation of TBC with time using various masses of pig waste compost. The relative increase and decrease in TBC values as shown in Table 2 and Figure 2 could be due to many factors. Generally, the rate of bacterial growth can be influenced by environmental factors such as acidity (pH), temperature (warmth), moisture, nutrient contents, oxygen levels, and toxins. Variations in TBC may have been due to temperature fluctuations during storage or other environmental conditions which may have caused interferences during storage and analysis.

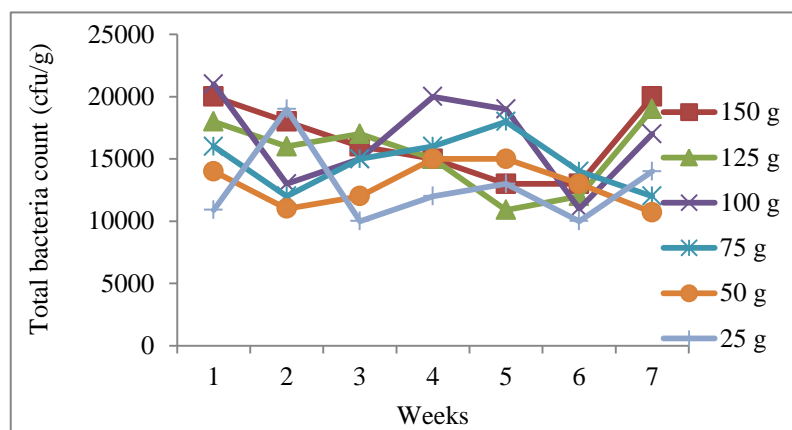
**Figure 2:** Effect of time on total bacteria counts.

Table 4 shows the values of THC obtained for samples of different masses monitored for 7 weeks. Generally, there was a steady decrease in THC in all the amended soil samples from

weeks 1 to 7 which shows that there was enhanced hydrocarbon degradation by some bacteria present in the samples during storage. The decrease in THC observed for the control

sample shows that degradation can also occur by natural processes, but at a much slower rate. For samples with 150 g amendment, the THC were reduced from 3.645 in week 1 to 0.703 and percentage degradation was 82.9%, whereas, for samples with 25 g amendment, reduction in THC was from 1.200 in week 1 to 0.112 in week 7, with percentage degradation of 97.28%. Other amendments with masses of 125 g, 100 g, 75 g, and 50 g had their percentage degradations as 86.25%, 91.36%, 93.81%, and 95.44%, respectively. However, the highest percentage degradation achieved with the 25 g amendment suggests that increasing the mass of either pig waste or sawdust amendment will not be necessary for the soil correction. The sawdust which is organically rich in carbonaceous materials and low in nitrogen would utilize nitrogen from the nutrient-rich pig waste for microbial decomposition. This might have affected nitrogen availability in the contaminated soil, and therefore degradation. Also, the increased microbial load which may result from increasing the amendments can regulate the functional diversity of the microorganisms, and thus influence the remediation process. Kelechi et al. (2019) in their study on bioremediation of crude oil polluted soil using fowl droppings also reported a 69.3% decrease in total

hydrocarbon contents after a period of six weeks. Degradation of hydrocarbon contents resulting from the application of animal manure enhances bacterial utilization of the hydrocarbon contents of the soil and degrades the soil in a less toxic condition. Therefore, degradation accelerated by microorganisms leads to nutrient release, and thus an increase in the nutrient contents of the soil (Sutherland et al. 1995, Mohammadi-Sichani et al. 2017). In a previous work carried out by Okafor and Nwankwegu (2016) on the effect of wood chips on bioremediation of crude oil contaminated soil, 50% wood chips as bio-stimulant achieved 75% contaminant removal. Samuel et al. (2018) on the effect of pig manure on the microbial remediation of crude oil-polluted soil reported that amendment with only pig manure (40%) achieved degradation of 84.65% in 14 weeks. The present study shows that a combination of pig waste and sawdust amendments strategically enhanced remediation of the crude oil contaminated soil in seven weeks. With the steady decrease in THC observed, there may be a need to increase the storage time to allow more time to complete the remediation process particularly in cases where there are recalcitrant organic compounds in the sample.

Table 4: Total hydrocarbon contents ($\mu\text{g/g}$) of soil samples monitored for seven weeks

Sample	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Final ΔTHC	% Degradation
Control	4.129	4.016	3.932	3.772	3.561	3.502	3.426	3.342	\downarrow 0.787	19.06
150 g	4.129	3.645	2.541	2.482	2.187	1.293	0.711	0.703	\downarrow 3.426	82.98
125 g	4.129	2.811	2.650	1.841	1.571	1.116	0.568	0.568	\downarrow 3.561	86.25
100 g	4.129	2.144	1.731	1.622	1.411	0.989	0.391	0.357	\downarrow 3.772	91.36
75 g	4.129	2.060	1.799	1.343	0.812	0.534	0.323	0.256	\downarrow 3.873	93.81
50 g	4.129	1.327	1.090	0.821	0.551	0.298	0.247	0.188	\downarrow 3.941	95.44
25 g	4.129	1.200	0.711	0.441	0.323	0.154	0.129	0.112	\downarrow 4.016	97.28

\downarrow = Decrease

The effect of time on total hydrocarbon contents using various sample masses of the organic bio-stimulants is shown in Figure 3. The plot shows that the total hydrocarbon

contents of samples attained maximum value in the first week and subsequently began to decline steadily with time.

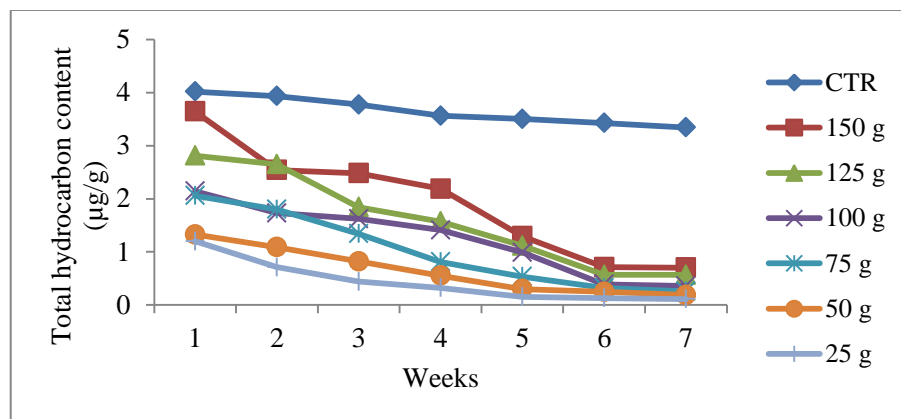


Figure 3: Effect of time on total hydrocarbon contents.

The change in percentage degradation observed with different sample masses of the amendments is as represented in Figure 4. This shows that degradation was reasonably

achieved with either high or reduced amounts of amendments added.

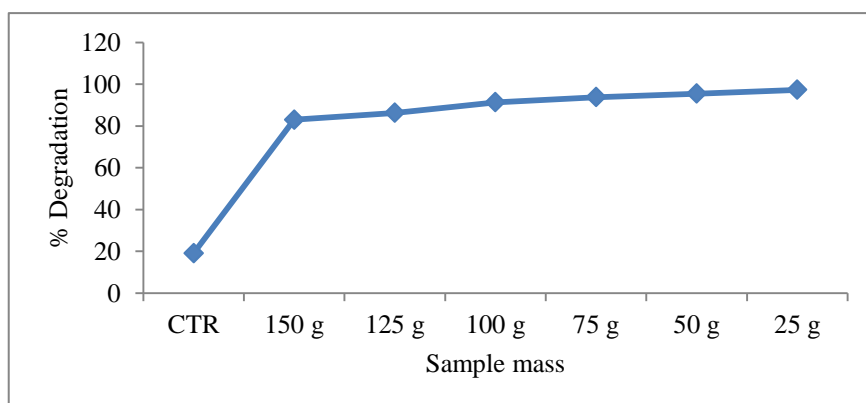


Figure 4: Variations in percentage degradation with sample masses.

Conclusion

This work studied the effect of time on pH, total bacteria counts, and total hydrocarbon contents in the bioremediation of crude oil contaminated soil. Results from this analysis showed that the bioremediation process involved materials (such as pig waste and sawdust) that were readily available and cost-effective. The organic origin and nutrient-rich nature of the materials render them useful for correcting nutrient loss in soil due to hydrocarbon contamination and enhancing soil

quality for improved agriculture. The slightly acidic-to-neutral pH observed is suitable for plant growth. The steady reduction in THC observed from the results showed that the pig waste compost and sawdust which are usually discarded and regarded as wastes in the environment are effective for bioremediation. Also, a combination of both pig waste and sawdust as bio-stimulants strategically enhanced remediation of the crude oil polluted soil.

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