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## Development of model for bioremediation of crude oil using moringa extract

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### ARTICLE INFO

#### Article type:

Research article

#### Article history:

Received June 2015

Accepted August 2015

January 2016 Issue

#### Keywords:

Model

Moringa extract

Bioremediation

Crude oil

Development

Soil

### ABSTRACT

Crude oil spillage on land is a major undeniable challenge we face in the Niger Delta, this is as a result of the oil exploration and exploitation activity done by the big oil multinationals and also those done by indigenous private firms, the petroleum could find its way to the soil via occurrences including pipeline leakages and explosions, corrosion of underground pipes transporting crude oil and petroleum product and also it could come purely in form of untreated industrial waste. As a result of the foregoing research work was conducted using Moringa leave extract, and the component of interest included phosphates, potassium and nitrogen which are the major stimulators of bioremediation, were found to be abundant in the Moringa Oleifera leave extract. The application of Moringa leave extract was found to be useful in the enhancing of crude oil polluted lands, and by so doing it facilitates the rehabilitation of the contaminated soil as well as reinstating the soil constituents for agricultural purposes. This is a new research investigation which show high efficiency in bioremediation program the maximum specific rate and the rate constants as well as they overall order of the bioremediation reaction were determined, using the principle of the rate laws as well as they Monod's equation from which the line waver burke plot was obtained. Therefore the experiment as well as the theoretical model developed can be used to monitor, predict and simulate the rate of degradation of hydrocarbons present in a polluted soil undergoing bioremediation under the influence of Moringa Oleifera leave extract.

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**Capsule Summary:** Moringa oleifera leave extract was applied the remediation of land contaminated with of crude oil, and by so doing it facilitates the rehabilitation of the contaminated soil as well as reinstating the soil constituents for agricultural purposes. The models developed can used to monitor, predict and simulate the rate of degradation of hydrocarbons undergoing bioremediation.

**Cite This Article As:** C. P. Ukpaka. Development of model for bioremediation of crude oil using moringa extract. Chemistry International 2(1) (2016) 19-28

### INTRODUCTION

Moringa is the sole genus in the family of plants moringaceae Moringa oleifera. is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands, it ranges in size from tiny herbs to massive trees. It iss the most widely cultivated species in the family moringaceae Because M. oleifera has been naturalized

in many tropic and sub-tropic regions worldwide, the plant is referred to by a number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree, and "Mother's Best Friend". The Moringa tree was introduced to Africa from India at the turn ofthe twentieth century where it was to be used as a health supplement. The Moringa plant has been consumed by humans throughout the century in diverse culinary ways (Amadi et al., 1993). Almost all parts of the plant are used culturally for its nutritional value, purported medicinal properties and for taste

and flavor as a vegetable and seed (Amadi et al., 1991). The leaves of *M. oleifera* can be eaten fresh, cooked, or stored as a dried powder for many months reportedly without any major loss of its nutritional value. Epidemiological studies have indicated that *Moringa oleifera* leaves are a good source of nutrition and exhibit anti-tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic and anti-convulsant activities (Bertrand et al., 1993). The investigation of the different parts of the plant is multidisciplinary, including but not limited to nutrition, ethnobotany, medicine, analytical chemistry, chemical engineering and anthropology. *Moringa oleifera* leaves and seed powder has also been used in various chemical engineering processes such as water purification and decoagulation. This study is aimed at investigating the activity of moringa leaf extract as an activator of the moringa leave bagasse in cleaning up crude oil polluted soils (Siron et al., 1993; Ukpaka, 2006).

*Moringa* is a tree ranging in height from 5-12 m with an open umbrella-shaped crown, straight trunk (10-30 cm thick) and a corky, whitish bark. The plant (depending on climate) has leaflets 1-2 cm in diameter and 1.5-2.5 cm in length. The tree produces a tuberous tap root which explains its tolerance to drought conditions. Originally considered a tree of hot semi-arid regions (annual rainfall 250-1500 mm), *Moringa* is adaptable to a wide range of environmental conditions from hot and dry to hot, humid, wet conditions. The tree is tolerant to light frosts, but does not survive as a perennial under freezing conditions. *Moringa* grows more rapidly, reaching higher heights, when found in well-drained soils with ample water, but tolerates bot sandy soils, heavier clay soils and water limited conditions. The tree can be established in slightly alkaline soils up to pH 9 as well as acidic soils as low as pH 4.5 and is well suited for a wide range of adverse environments that would not be suitable for other fruit, nut and tree crops. *Moringa* can be found in the wild or cultivated and sold as a supplement on the health market In India and different parts of Africa, it is cultivated on a large scale in nurseries or orchards. Cultivation entails collection of seeds from the tree, development of plantlets in the greenhouse for 2 to 3 months and transplantation of mature stems (1-1.5 m long) to the main fields. The leaves, seeds, flowers, pods (fruit), bark and roots are all seen as a vegetable and each part is uniquely harvested and utilized. For example, fresh leaves are picked, shade dried, ground to a powder, and then stored for later as a food flavoring or additive. Dried or fresh leaves are also used in foods such as soups and porridges, curry gravy and in noodles, rice or wheat. Farmers have added the leaves to animal feed to maintain a healthy livestock while utilizing the manure and vegetable compost for crop growth<sup>10 - 20</sup>. Newer applications include the use of *Moringa* powder as a fish food in aquacultural systems and the *Moringa* leaves as a protein supplement for animals, such as cows. With the leaves being rich in nutrients, pregnant women and lactating mothers use the powdered leaves to enhance their child's or children's nourishment, especially in developing countries suffering from malnutrition. The seeds contain much of the plant's edible oil which is used as cooking oil for frying and as a salad oil for dressing (Ukpaka, 2006). Some important uses of *moringa oleifera* leave seed and its extracts can be classified as:

Most of the parts of the plant possess antimicrobial activity. They are well known for their pharmacological actions too and are used for the traditional treatment of diabetes mellitus hepatotoxicity, rheumatism, venomous bites and also for cardiac stimulation, Grinded moringa seeds have been used for water clarification in order to remove suspended matter and other physically combined impurities in water, researches have been done on its food preservation ability, generally *moringa oleifera* has a good antibacterial activity thus they can serve as good food preservatives under certain conditions, moringa leaf tea has been found to cure stress related illnesses such as hypertension, high blood pressure e.t.c., moringa leaf is a good source/ reservoir of nutrients generally for both plants animals and certain microbes, *moringa oleifera* leaf and seed can be eaten in the raw form as a source of food, moringa can also used for tired blood (anemia) anhrithis and other joint pains, diarrhea, epilepsy, and stomach pain thyroid disorders etc, it can also be used as an aphrodisiac, it can also be used as an additive in haircare products and moringa is also used to reduce swellings in the body.

*Moringa oleifera* as pointed out earlier has been subject to a number of researches, many research work has been done on its medicinal, nutritional, water purification and even its ability to extract heavy metals from solutions in which they are dissolved, but little to no attention has been given to investigating its bioremediation activity in crude oil soiled environment thus the aim of this research work is to investigate the activities of *moringa oleifera* in remediation of crude oil soiled environment (Wami, 1993).

In the Niger Delta region of Nigeria the activities of oil exploration and exploitation companies has led to a high level of pollution of the soil that constitute valuable farmlands with crude oil and its process effluents this has led to loss of arable lands and heavy economic losses it has also damaged the flora and the fauna of the region, also the constant pipeline explosions and leakages in waterways and creeks has rendered the marine and aquatic life of the region at a high risk thus there is a need to proffer solutions to all these industrial inefficiencies and challenges if we hope to sustain an industrialised society and good economic returns in the Niger Delta region of Nigeria (Ukpaka, 2006ab; 2009 ; 2011ab).

Aims and objectives of the study includes: To study the activity of moringa leave extract as a bio-stimulator in the bioremediation of crude oil soiled environment, to relate the kinetics of the bioremediation work to the Monod's equation, to come up with models to predict the rate of bioremediation with *Moringa* leaf extract as a bio-stimulator, to study the effect of P.H and nutrients in the inhibition of bioremediation reactions and to proffer viable solution to the problems of oil spillage on the lands of the Niger Delta. This study would investigate the constituents of the species *moringa oleifera* leave extract also this paper would also study they action of moringa leaf as an activator bioremediation of crude oil polluted soil environment we would also investigate the active components of the moringa leave extract in the biodegradation process and relate the process to the Monod's equation in order to help us ascertain the kinetics of the bioremediation process. This exploration of the micro-organism present in the soil sample used is beyond the scope of this work this work is specifically concerned with the

bioremediation process and not the micro-organism responsible for the bioremediation.

### Material and Methods

Development of the model to relate reduction in concentration and time



Can be simplified into the following



We can also represent the rate equation of the bioremediation having an nth order and a rate constant k as

$$\int_{C_0}^C \frac{dC}{C^n} = -KC^n \quad (3)$$

Equation (3) can be rearranged to obtain

$$\int_{C_0}^C \frac{dC}{C^n} = \int_0^t -k dt \quad (4)$$

Integrating equation (4) and taking its limits

$$\frac{C^{1-n} - C_0^{1-n}}{1-n} = -kt \quad (5)$$

$$\frac{C^{1-n} - C_0^{1-n}}{1-n} = -kt \quad (6)$$

Equation (6) can then be written as

$$C^{1-n} = C_0^{1-n} - (1-n)kt \quad (7)$$

The above equation can be used to predict the concentration of crude oil present in the soil at any time  $t$  as the remediation proceeds

In order to obtain the  $K$  and  $n$  of the reaction we make use of the fractional conversion method. As stated in chemical reaction engineering by octave and levienspiel

$$T_F = \frac{F^{1-n}-1}{K(n-1)} C_0^{1-n} \quad (8)$$

Where,  $T_f$  = time needed for fractional conversion,  $F$  = fractional conversion

$K$  = rate constant of reaction,  $n$  = order of the reaction

Next we take the logarithm of both sides of the equation to obtain

$$\log T_F = \log \left( \frac{F^{1-n}-1}{K(n-1)} \right) + (n-1) \log C_0 \quad (8i)$$

In order to obtain the value of  $n$  we plot a graph of  $\log T_f$  versus  $\log C_0$  in which

$$\text{Slope} = (n-1) \quad (9)$$

From which the value of  $n$  can be obtained.

To obtain the value of  $k$  we substitute the value of  $n$  and the other known values we are left with only  $k$  as the unknown from which we can solve to obtain the value of  $k$

We can further develop a model that relates specific rate of the bioremediation reaction, the initial concentration of the Moringa oleifera extract and the rate constant by means of the monod's equation

$$V = \frac{V_{\max}[S]}{K+[S]} \quad (10)$$

Where  $S$  = initial concentration of Moringa leaf extract  $[M]$ ,  $K$  = specific rate constant of the bioremediation reaction stimulated by Moringa Oleifera extract

$V_{\max}$  = maximum attainable rate of crude disappearance

Equation (10) can be re-written as :

$$V = \frac{V_{\max}[M]}{K+[M]} \quad (11)$$

Model of the pH as an inhibitor or activator

The monod's equation for the mechanism of inhibition is stated as

$$V = \frac{V_{\max}[M]}{K+[M]} \times I \quad (12)$$

In a situation where the pH is an activator that is the increment in pH favours the bioremediation reaction the inhibitor is represented as

$$I = P^H \quad (13)$$

Therefore,

Equation (12) becomes

$$V = \frac{V_{\max}[M]}{K+[M]} \times P^H \quad (14)$$

This only holds if an increase in the pH favours the bioremediation reaction.

In a situation where an increase in pH acts as an inhibitor to the bioremediation reaction the inhibition is represented as

$$I = \frac{1}{P^H} \quad (15)$$

Equation (12) theoretically becomes

$$V = \frac{V_{\max}[M]}{K+[M]} \times \frac{1}{P^H} \quad (16)$$

Equation (16) is only valid in a bioremediation reaction in which the increment in the pH inhibits .

Equation (7) can also be related to equation (11) this can help us establish the concentration of the crude oil in the soil undergoing bioremediation at any time  $T$  knowing the initial concentration of Moringa introduced into the soil.

Defining equation (11) in terms of michealis menten terms it becomes

$$C = \frac{C_{\max}[M]}{K+[M]} \quad (17)$$

But from equation (7)

$$C^{1-n} = C_0^{1-n} - (1-n)kt$$

Therefore equation (17) becomes

$$C_0^{1-n} - (1-n)kt = \frac{[C_0^{1-n} - (1-n)kt]_{\max} \times [M]}{K+[M]} \quad (19)$$

The above equation can be written in terms of the line waver burk plot just like

The monod's equation is expressed as

$$\frac{1}{V} = \frac{K}{V_{\max}[S]} + \frac{1}{V_{\max}} \quad (20)$$

Thus we see that we can express equation (19) in the line waver burke plot

$$\frac{1}{C_0^{1-n} - Kt(1-n)} = \frac{K}{[C_0^{1-n} - Kt(1-n)]_{\max}[S]} + \frac{1}{C_0^{1-n} - Kt(1-n)_{\max}} \quad (21)$$

We can also obtain the specific bioremediation rate constant and the maximum rate of bioremediation by plotting the line weaver burke plot of

$$\frac{1}{V} = \frac{K}{V_{\max}[Co]} + \frac{1}{V_{\max}} ; -V = kC^n \quad (22)$$

Where,  $-V$  = rate of reaction,  $K$  = specific rate of bioremediation,  $C$  = concentration of crude present in the soil,  $K$  = rate constant of reaction

To obtain the values of  $k$  and  $-V_{\max}$  we plot a graph of  $\frac{1}{V}$  vs  $\frac{1}{Co}$  having an intercept of  $\frac{1}{V_{\max}}$  also,  $K$  can be obtained from slope

$$\text{given as } \frac{K}{V_{\max}[Co]}$$

### EXPERIMENTAL PROCEDURES

The work is concerned with the investigation of the Moringa leave extract as a stimulator for the bioremediation process. Before we can attempt to study the action of this leaf extract on crude oil polluted soil environment it is necessary to find out the characteristic constituents of the Moringa leaf extract and the

**Table 1:** Showing reactor and how they were set up

| Reactor           | Amount of Moringa (ml) | Conditions |
|-------------------|------------------------|------------|
| A <sub>w</sub> C  | 0                      | Moist      |
| C <sub>w</sub> 6  | 6                      | Moist      |
| B <sub>w</sub> 9  | 9                      | Moist      |
| H <sub>w</sub> 12 | 12                     | Moist      |
| G <sub>d</sub> 6  | 6                      | Dry        |
| U <sub>d</sub> 9  | 9                      | Dry        |
| F <sub>d</sub> 12 | 12                     | Dry        |
| D <sub>d</sub> C  | 0                      | Dry        |

**Table 2:** Illustrates the amount of extract, moist and dry conditions for the system

| Amount of extract | Moist conditions  | Dry conditions    |
|-------------------|-------------------|-------------------|
| 12                | H <sub>w</sub> 12 | F <sub>d</sub> 12 |
| 9                 | B <sub>w</sub> 9  | U <sub>d</sub> 9  |
| 6                 | C <sub>w</sub> 6  | G <sub>d</sub> 6  |
| 0                 | A <sub>w</sub> C  | D <sub>d</sub> C  |

composition of certain important elements/compounds that aid biodegradation that are present in the Moringa oleifera leaf extract and also find out if and microorganism that can thrive in the media of the Moringa oleifera leaf extract.

There are certain microorganisms that are actually responsible for the bioremediation process and these microbes secrete enzymes that help them to break down this crude oil in the soil into consumable substances. The response of these microorganisms to Moringa oleifera leaf extract is of utmost importance to this work. If the Moringa oleifera aids the growth of the microbes this in turn will help influence the bioremediation process

### ***The Moringa Leaf Sample Collection Extract Preparation***

The moringa oleifera leave was gotten from agudama epic community in yenagoa local government area of bayelsa state nigeria.

A juice extractor was used to extract the Moringa juice from the Moringa leaf immediately they were harvested and then the Moringa juice/ extract and the left over chaff were refrigerated immediately.

Two different kinds of analysis were carried out on the Moringa oleifera juice extract namely

1. microbial analysis; to find out if the extract can support micro-organisms.
2. Chemical analysis; to find out the presence and composition of certain chemical species in the extract.

### ***Microbial Analysis***

Materials : Conical flask, One 5millitre syringe, One 1millitre syringe, Distilled water, Nutrient agar, Moringa leaf extract, 5 Petri dishes, Masking tape, Cotton wool, Aluminium foil, Test tubes, Nutrient broth, Autoclave, Weighing balance

Procedures : First the conical flask, test tubes, and the petri dishes were washed with distilled water

Preparation of nutrient agar culture : The nutrient agar dilution culture is prepared in a standard culture of 28grams of agar dissolved in 1litre of distilled water next we, Using a spatula Slowly and carefully place the powdered nutrient agar on the foil, dissolve all the weighed out 3 grams of the nutrient agar in 100 ml of distilled water in a conical flask, and cover the conical flask with cotton wool and wrap up the cotton wool in an aluminium foil covering

Preparation of nutrient broth culture : Place a cut small piece of aluminium foil and on the digital weigh balance and zero the digital scale, using a spatula Slowly and carefully place the pellets of the nutrient broth on the foil until the weighing balance reads 5grams, dissolve all the weighed out 5 grams of the nutrient broth in 500ml of distilled water in a conical flask, using the syringe collect 20millitre of nutrient broth water and place in the 6 test tubes, and cover (cork) each test tube with cotton wool and wrap aluminium foil around it In order to prevent contamination after which the samples are sterilized by autoclaving.

Materials used in the serial dilution process : 1ml syringe, 6 sterilized test tubes with nutrient broth, moringa oleifera leaf extract and bunsen burner

Steps for tenfold serial dilution : Collect 1ml of Moringa oleifera leaf extract and place in one of the test tubes with the nutrient broth, shake the test tube vigorously to make sure of sufficient mixing, take 5ml from the same test tube and place in another test tube, shake vigorously and also take another 1ml from this test tube and add into another and we did this until I obtained about 6 test tubes that have been serially diluted each with powers ranging from  $10^{-1}$   $10^{-2}$   $10^{-3}$   $10^{-4}$   $10^{-5}$   $10^{-6}$

Preparation of the culture : Pour about 20ml of nutrient agar into each of the sterilized petri dishes, invert the petri dishes so that the nutrient agar hangs from the top of each of the dishes, wait until the nutrient agar sticks to the top of the petri dishes, after which 1ml each of these serially diluted broth culture  $10^{-1}$   $10^{-2}$   $10^{-3}$   $10^{-4}$   $10^{-5}$  is taken and placed in one Petri dish each, and the petri dishes are ten placed into an incubator like heater where the temperature was maintained at 37<sup>0</sup>C

Chemical Analysis

**Materials :** Muffle furnace, Crucibles, Evaporating dish, Kjehdahl digesting set, Kjehdahl flask 100ml (2), Conical flask (10), Soxhlet extraction set, Beakers 250ml (5), Walch glass (5), Hot-air drying oven.

**Chemicals and reagents :** Samples (Moringa oleifera leaf extract), Sulphuric acid, Copper sulphate, Sodium sulphate, 40M sodium hydroxide, 0.1M hydrochloric acid, Mixed indicator (methyl red and methylene blue, 1.25% HCl

Determination of nitrate

The method of determination of nitrate used was the production of diazonium ion by the reaction of N-alpha naphthyl amine ana sulphuric acid in the presence of nitrate ions. The

**Table 3:** Concentration of TPH of the samples as recorded on a 2 day interval for 16 days

| Reactor Time(days) | A <sub>w</sub> C (ppm) | D <sub>d</sub> C (ppm) | H <sub>w</sub> 12 (ppm) | F <sub>d</sub> 12 (ppm) | B <sub>w</sub> 9 (ppm) | U <sub>d</sub> 9 (ppm) | C <sub>w</sub> 6 (ppm) | G <sub>d</sub> 6 (ppm) |
|--------------------|------------------------|------------------------|-------------------------|-------------------------|------------------------|------------------------|------------------------|------------------------|
| 0                  | 0.062                  | 0.06                   | 0.059                   | 0.058                   | 0.057                  | 0.063                  | 0.059                  | 0.062                  |
| 2                  | 0.061                  | 0.06                   | 0.054                   | 0.057                   | 0.051                  | 0.057                  | 0.056                  | 0.06                   |
| 4                  | 0.060                  | 0.059                  | 0.047                   | 0.049                   | 0.049                  | 0.051                  | 0.05                   | 0.058                  |
| 6                  | 0.061                  | 0.06                   | 0.041                   | 0.045                   | 0.048                  | 0.046                  | 0.049                  | 0.054                  |
| 8                  | 0.059                  | 0.06                   | 0.039                   | 0.04                    | 0.047                  | 0.045                  | 0.049                  | 0.053                  |
| 10                 | 0.060                  | 0.059                  | 0.031                   | 0.039                   | 0.044                  | 0.044                  | 0.048                  | 0.053                  |
| 12                 | 0.058                  | 0.06                   | 0.028                   | 0.038                   | 0.043                  | 0.043                  | 0.048                  | 0.052                  |
| 14                 | 0.058                  | 0.059                  | 0.027                   | 0.037                   | 0.039                  | 0.041                  | 0.047                  | 0.051                  |
| 16                 | 0.057                  | 0.059                  | 0.025                   | 0.035                   | 0.038                  | 0.041                  | 0.045                  | 0.05                   |

**Table 4:** Showing logT<sub>f</sub> vs logC<sub>o</sub> for reactor C

| Co    | 0.8Co  | Time needed to decompose from Co to 0.8 Co | Log T <sub>f</sub> | logCo   |
|-------|--------|--|--------------------|---------|
| 0.059 | 0.0472 | 5.8 days                                   | 0.763              | -1.229  |
| 0.058 | 0.0464 | 12.4 days                                  | 1.073              | -1.3365 |
| 0.057 | 0.0456 | 14.5 days                                  | 1.161              | -1.4441 |

**Table 5:** Showing logT<sub>f</sub> vs logC<sub>o</sub> for reactor B

| Co    | 0.8Co  | Time needed to decompose from Co to 0.8Co | Log T <sub>f</sub> | logCo   |
|-------|--------|---|--------------------|---------|
| 0.057 | 0.0456 | 8.7 days                                  | 0.9395             | -1.2441 |
| 0.046 | 0.0346 | 10.7 days                                 | 1.0294             | -1.3372 |
| 0.037 | 0.0296 | 12.8 days                                 | 1.1072             | -1.4317 |

addition of nitric tablets (a combination of the above reagents) In the presence of NO<sub>3</sub><sup>-</sup> in the Moringa oleifera leaf extract sample produce varying diazonium colours which is read at 570nm in the UV/visible light spectrophotometer. The absorbance values are read on a direct concentration table that gives the result of the NO<sub>3</sub><sup>-</sup>.

#### Moisture content (A.O.A.C)

5g of Moringa oleifera leaf extract was weighed in an hot air drying oven an hot air drying oven and set at 105<sup>0</sup>C in a porcelain crucible this was kept in the oven for 24 hours after which it was weighed again in an analytical balance the weight loss was determine by

$$\% \text{ Moisture} = \frac{b - c}{b - a} \times \frac{100}{1}$$

#### Ash content (A.O.A.C)

2g of dried samples were placed in a preheated crucible and placed in a muffled furnace and the temperature set at 550<sup>0</sup>C and heated for 16 hours. At the end of heating the muffled furnace was allowed to cool and they as in it was weighed

$$\% \text{ Ash} = \frac{\text{Ash weight}}{\text{sample weight}} \times 100$$

#### Crude protein content (A.O.A.C)

0.5g of sample was weighed into a 100ml kjeldahl nitrogen flask containing 1.5g of NA<sub>2</sub>SO<sub>4</sub> and 1.5g of CUSO<sub>4</sub>. Some anti bumps

were also added . 5ml of cone sulphuric acid/ nitric acid. Digestion flask was eated and placed on the digester and heated for 1 hour slowly and vigorously until the contents form a clear solution after froting for up to 8 hours

After digestion, the digest was cooled and quantitatively transferred into a 50ml standard flask and made up to the mark. 10ml of the solution was transferred into a micro kjedah Flask containing 10ml of 40% NaOH solution and then heated

The evolving is ammonia gas which is distilled into an Erlen Meyer flask containing 10ml Of 5% boric acid into which two drops of mixed indicator ( methyl red + methylene blue) the distillate was titrated wit 0.1M HCl until the green colour was turned to a pink end product was achieved.

$$\% \text{ Nitrogen} = \text{ml HCL}(s) - \text{ml HCL}(b) \frac{N \times 14 \times 50 \times 100}{10(3) \times 10 \times 0.5}$$

Where S and b are representing sample and blank,

% protein = % N \*6.25

14 = atomic weight of nitrogen

50= from the procedure

10= from the procedure

100= percentage

0.5= weight of sample

6.25= gravimetric factor of protein

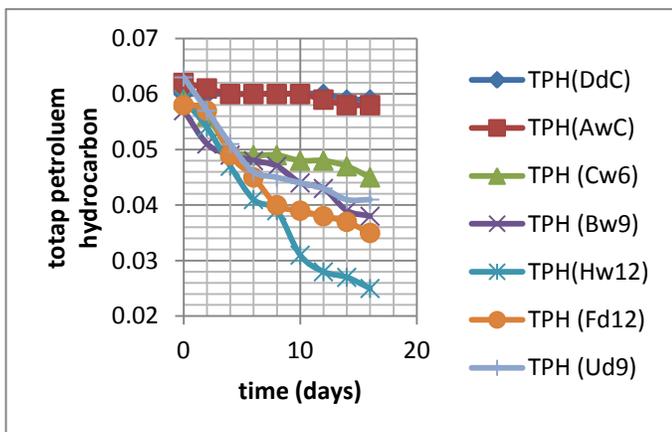
10<sup>3</sup> is constant

#### Crude lipids or ether fibre extract (A.O.C.A)

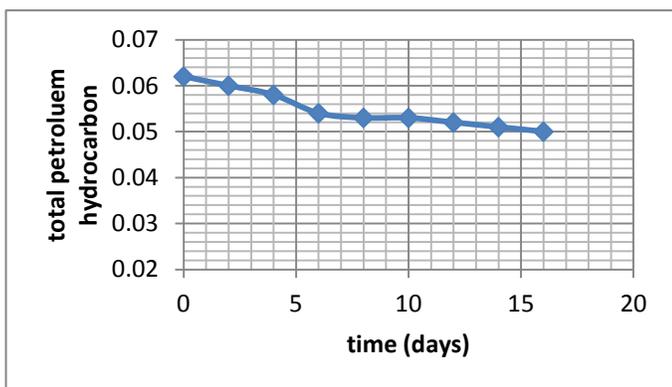
5g of sample were weighed into a weighed extraction thimble and placed in 100ml soxhlet extractor. 200ml of petroleum ether was poured into the flask and heated in a heating mantle. After several siphoning process with the anti-bumps in the flask. The flask was cooled and connected to the rotary evaporator to recover the solvent. The flask was further dried and weighed. The difference in weight of the empty flask and the flask with the extract was obtained

$$\% \text{ lipids} = \frac{\text{Extract weight}}{\text{Sample weight}} \times 100$$

#### Crude fibre (A.O.C.A)



**Fig. 1:** Graph of ppm of TPH versus time showing the reduction rate of the TPH for reactors (U,F,H,B,C,A,D)



**Fig. 2:** Graph of ppm of TPH versus time showing the reduction rate of the TPH for reactor G

2g of the fat free sample from above was weighed and quantitatively transferred into a 250ml beaker which was marked at the 150ml mark 50ml of 1.25%  $H_2SO_4$  was added and boiled after the addition of distilled water up to the 150ml mark. The content was boiled for 30 minutes. This was filtered through a buchner funnel with the aid of a pump. The residue was washed with hot water until it was acid free the residue was quantitatively transferred to yet another 250ml beaker and digested with 50ml of 1.25% NaOH and made up to the 150ml mark. The mixture was then boiled for 30 minute with constant stirring.

It was then filtered and washed with hot distilled water until it was base free the residue was further washed with 95% methanol/ this was then transferred into a pre-weighed porcelain crucible and dried In an oven at  $105^{\circ}C$  the crude fibre was ten weighed

$$\% \text{ lipids} = \frac{\text{Fibre weight}}{\text{Sample weight}} \times 100$$

#### Dry matter

5g of sample place in a porcelain crucible and dried in an hot air drying oven at  $105^{\circ}C$  until a constant weight is achieved the dried matter of the sample is therefore given as

$$\% \text{ dry matter} = 100\% - \% \text{ moisture}$$

#### Determination of phosphate ( $PO_4$ )

##### Reagents

1. Concentrated hydrochloric acid
2. Ammonium molybdate
3. Ammonium vanadate

##### Apparatus

1. Uv-vis spectrophotometer

##### Procedure vanadate-molybdate reagents

Solution A: 25g of ammonium molybdate was dissolved in 200ml of distilled water.

Solution B: 1.25g of ammonium tri-oxo-vanadate was dissolved in a boiling 300ml of distilled water to which 330ml concentrated hydrochloric acid was cool to room temperature. Pour solution A into solution B in 1L flask. Make up to 1L with distilled water

25ml of digested sample was placed into a 50ml volumetric flask. 10ml of vanado-molybdate reagent was then added and made up to the mark with the distilled water. The sample was allowed to stand for 10minutes in order for it to develop the colour. The spectrophotometer was set at 470nm. The absorbance values were then extrapolated from the standard curve.

#### Determination of pH

##### Materials

1. Soil sample
2. Distilled water

##### Apparatus

1. P.H meter

##### Procedure

Take 5 grams of soils, dissolve in 10ml of distilled water with P.H of approximately 7 insert the probe into the suspension of soil in distilled water and take the P.H reading when the device is ready

#### Determination of total petroleum hydrocarbon (TPH)

##### Reagents

1. Hydrochloric acid
2. Tetra-chloromethane

##### Apparatus

1. Buck Model HC-404 System

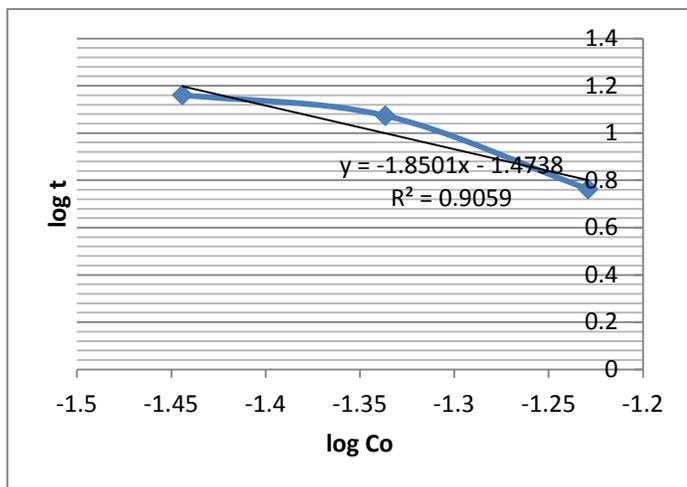
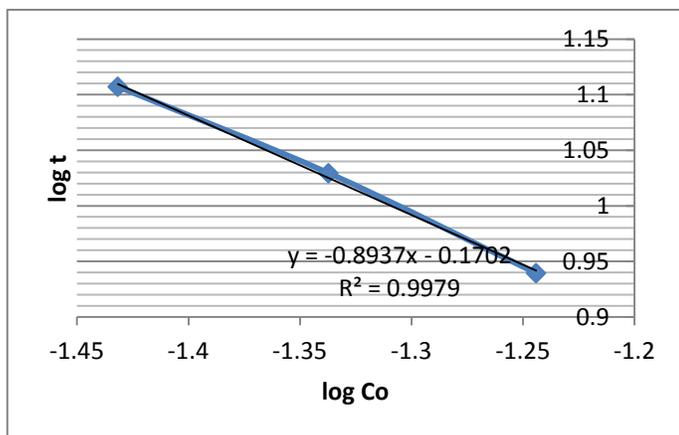
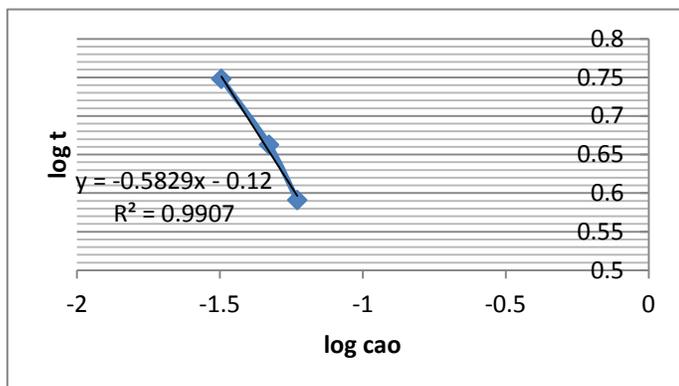
Weigh out 5g of oven dried soil, after which acidify soil with HCl to minimize contaminants and kill microbes. Pipette in 60ml of  $CCl_4$  and combine it with the soil to extract the TPH materials, after which filter solution containing the extract from the suspension. Transfer filtrate into cuvette and place into the Buck Model HC-404 with standard 10mm IR Quartz Cuvette instruments, cell holder the ppm of TPH present in sample will be displayed in the visual display unit of the instrument.

#### Bioremediation Experiment

Materials: 8 plastic bottles cut open used as reactors, Swampy soil, Crude oil (bonny Light) And moringa oleifera leaf extract

##### Procedures

60 g of crude oil was introduced into the soil to pollute the soil, the soil samples was then well mixed and stirred to obtain uniform concentration, Moringa oleifera extract was then added

Fig. 3: Graph of  $\log T_f$  vs  $\log C_o$  for reactor CFig. 4: Graph of  $\log T_f$  vs  $\log C_o$  for reactor BFig. 5: Graph of  $\log T_f$  vs  $\log C_o$  for reactor H

into the reactors in the following way as well as presented in Table 1 and 2.

Thus the experiment was setup in a way to compare the effect of moringa oleifera and the effect of moisture

bioremediation process also reactors A and reactor D were designed as control reactors.

In the reactors operated under moist conditions 5ml of water was added to them every 5 days to replenish their moisture content, whereas water was never added to the reactors operated under dry conditions. The pH and the TP was measured every two days starting from day zero when they experiment was set up up to day 16 when the bioremediation experiment was halted.

## RESULTS AND DISCUSSION

Based on the experiment conducted it was observed that the rate of disappearance of the TPH increased as the amount of the bio-stimulant *Moringa Oleifera* leave extract increased notice that reactor H its concentration reaches d lowest value by a lot of margin and in reactor C, its TPH reduced but it isn't as much as it is in reactor H, this is as a result of the fact that the *Moringa Oleifera* contains in it certain nutrients in the form of phosphates and nitrates that are very important in bioremediation process because the help to supply nutrients to the soil and in so doing the microbes responsible for the bioremediation feed on this nutrient and as they do that the reproduce and thus their population increases as the population increases more microbes become available to breakdown the crude oil in the soil thus the crude oil present in the soil experiences a continual decrease. Thus an increase in the quantity of *Moringa Oleifera* brings about an increment in the nutrients supplied to the soil this in turn stimulates the bioremediation (Octave and Levienspiel, 2004).

Furthermore notice that the crude oil in the control reactors A and C never experienced any appreciable decrease in their concentration over the 16 day period, even though some of the crude present in these reactors disappeared its quantity and its rate of disappearance becomes negligible when compared to that of the reactors that were stimulated this is simply because naturally bioremediation occurs in the environment but this natural bioremediation is slow and it may take hundreds of years before a soil polluted with crude oil and that was left to nature to remedy will return to a TPH free state. Thus the natural process of bioremediation was stimulated that is its speed was increased by the *Moringa oleifera* leave extract (Luizer, 1992).

It was also observed that of the reactions, the reactors that were operated under wet condition experienced a much higher bioremediation rate than those under dry conditions, this is due to the fact that the water supplied to the micro-organism under wet conditions weren't supplied to those that were operated under dry conditions and water is of paramount importance to both micro and macro life because it is part of the metabolic pathway by which micro-organism feed, produce energy and grow thus as a result of that the moist reactors experienced a faster bioremediation rate than those done under dry conditions (Tabarabaci et al., 2010 ; Murphyt et al., 1995 ; Ogoni, 2001).

Thus we can conveniently say that they higher they *Moringa oleifera* leave extract they faster they bioremediation process this statement only olds to the extent of inhibition of bioremediation caused by excessive supply of nutrients to the soil to be bioremediated

Obtaining The Order(n) And The specific reaction rate constant (K) Of The Reaction.

**Table 8:** Showing  $\log T_f$  vs  $\log C_o$  for reactor U

| Co    | 0.8Co  | Time needed to decompose from Co to 0.8Co | Log $T_f$ | logCo   |
|-------|--------|---|-----------|---------|
| 0.063 | 0.0504 | 4.2 days                                  | 0.6232    | -1.2    |
| 0.054 | 0.0456 | 5.4 days                                  | 0.7323    | -1.2376 |
| 0.044 | 0.0424 | 6.5 days                                  | 0.8129    | -1.3565 |

**Table 9:** Showing  $\log T_f$  vs  $\log C_o$  for reactor G

| Co    | 0.8Co  | Time needed to decompose from Co to 0.8Co | Log $T_f$ | logCo   |
|-------|--------|---|-----------|---------|
| 0.062 | 0.0496 | 16.4 days                                 | 1.2148    | -1.2076 |
| 0.05  | 0.0400 | 35.6                                      | 1.3136    | -1.3010 |

**Table 10:** Table of n and k values

| Amount of Moringa | Reactor           | Order (n) | Rate constant(k) |
|-------------------|-------------------|-----------|------------------|
| 12ml              | H <sub>w</sub> 12 | 1.5829    | 0.3254           |
| 12ml              | F <sub>d</sub> 12 | 1.4177    | 0.1606           |
| 6ml               | C <sub>w</sub> 6  | 2.8501    | 3.8168           |
| 6ml               | G <sub>d</sub> 6  | 1.9453    | 0.2098           |
| 9ml               | B <sub>w</sub> 9  | 1.8937    | 0.3176           |
| 9ml               | U <sub>d</sub> 9  | 2.0832    | 1.1445           |

**Table 11:** Reactor and equations of reaction

| reactor           | Equation of reaction                    |
|-------------------|---|
| H <sub>w</sub> 12 | $C^{-0.5829} = Co^{-0.5829} + 0.1894t$  |
| F <sub>d</sub> 12 | $C^{-0.4177} = Co^{-0.4177} + 0.06708t$ |
| B <sub>w</sub> 9  | $C^{-0.8937} = Co^{-0.8937} + 0.2838t$  |
| U <sub>d</sub> 9  | $C^{-1.0832} = Co^{-1.0832} + 1.2383t$  |
| C <sub>w</sub> 6  | $C^{-1.8501} = Co^{-1.8501} + 7.06146t$ |
| G <sub>d</sub> 6  | $C^{-0.9453} = Co^{-0.9453} + 0.1983t$  |

**Table 12:** Showing  $\log T_f$  vs  $\log C_o$  for reactor F

| Co    | 0.8Co  | Time needed to decompose from Co to 0.8Co | Log $T_f$ | logCo   |
|-------|--------|---|-----------|---------|
| 0.057 | 0.0456 | 8.7 days                                  | 0.9395    | -1.2441 |
| 0.046 | 0.0346 | 10.7 days                                 | 1.0294    | -1.3372 |
| 0.037 | 0.0296 | 12.8 days                                 | 1.1072    | -1.4317 |

From the graphs given we can attempt to deduce the order and rate constant of the equation by using the formula presented in equation (8) and (8i) stated as

$$T_F = \frac{F^{1-n} - 1}{K(n-1)} Co^{1-n}$$

and

$$\log T_F = \log \left( \frac{F^{1-n} - 1}{K(n-1)} \right) + (n-1) \log Co$$

to obtain the n and k of all the batch reactors using  $F = 0.8$  where f stands for fractional conversion  
Reactor C<sub>6</sub>w

Reactor B<sub>w</sub>9

From slope n and k is obtained from equation (8) and (9)

$$K = 0.3176 \text{ and } n = 1.8937$$

Reactor H<sub>w</sub>12

From slope n and k is obtained from equation (8) and (9)

$$n = 1.5829 \text{ k} = 0.3254$$

Reactor F<sub>d</sub>12

From the graph n can be obtained as 1.4177 and k as 0.1606

Reactor U<sub>d</sub>9

From the graph  $n = 2.0832$  and the value of k is obtained from equation 3.8 to be 1.1445

Reactor G<sub>d</sub>6

From the graph  $n = 1.9453$  and  $k = 0.20948863$

Substitute the values of n and k into equation (7) so as to obtain equations of each reactor

$$C^{1-n} = Co^{1-n} - (1-n)kt$$

For reactor H<sub>w</sub>12

$$C^{1-1.5829} = Co^{1-1.5829} - (1-1.5829).3254t$$

Which can be further written as

$$C^{-0.5829} = Co^{-0.5829} + 0.1894t$$

For reactor F<sub>d</sub>12

$$C^{-0.4177} = Co^{-0.4177} + 0.06708t$$

For reactor C<sub>w</sub>6

$$C^{-1.8501} = Co^{-1.8501} + 7.06146t$$

For reactor G<sub>d</sub>6

$$C^{-0.9453} = Co^{-0.9453} + 0.1983t$$

For reactor B<sub>w</sub>9

$$C^{-0.8937} = Co^{-0.8937} + 0.2838t$$

For reactor U<sub>d</sub>9

$$C^{-1.0832} = Co^{-1.0832} + 1.2383t$$

The above sets of equations can be used to predict the concentration of the crude oil present in the soil at any time t for each of the reactors knowing the initial concentration of crude that was present in the soil at the time when they bioremediation reaction was initiated.

The can be written in form of the line waver burke plot (Eqs. 23-28)

For reactor G

Table 3 illustrates the influence of moringa extract on the degradation of crude oil in defferent reactor set-up. The result presented in figure 1 illustrates the variation in the TPH upon the influence of time for various reactors and figure 2 shows the Total

Petroleum Hydrocarbon (TPH) degradation with increase in time for only reactor G.

The result presented in Table 4 shows the relationship between  $\log T_f$  and  $\log C_o$  for reactor C whereas figure 3 illustrates the graph of  $\log T_f$  anganist  $\log C_o$  for reactor C. The equation of the best fit was established as  $y = -1.850x - 1.473$  with the squire root value of  $R^2 = 0.905$ . The result presented in Table 5 shows the relationship between  $\log T_f$  and  $\log C_o$  for reactor B whereas figure 4 illustrates the graph of  $\log T_f$  anganist  $\log C_o$  for reactor B. The equation of the best fit was established as  $y = -0.893x - 1.78$  with the squire root value of  $R^2 = 0.997$ . The

$$\frac{1}{Co^{-0.5829}+0.1894t} = \frac{K}{[Co^{-0.5829}+0.1894t]_{max}[S]} + \frac{1}{[Co^{-0.5829}+0.1894t]_{max}} \quad (23)$$

for reactor H

$$\frac{1}{Co^{-0.4177}+0.06708t} = \frac{K}{[Co^{-0.4177}+0.06708t]_{max}[S]} + \frac{1}{[Co^{-0.5829}+0.06708t]_{max}} \quad (24)$$

For reactor F

$$\frac{1}{Co^{-0.8937}+0.2838t} = \frac{K}{[Co^{-0.8937}+0.2838t]_{max}[S]} + \frac{1}{[Co^{-0.8937}+0.2838t]_{max}} \quad (25)$$

For reactor B

$$\frac{1}{Co^{-1.0832}+1.2383t} = \frac{K}{[Co^{-1.0832}+1.2383t]_{max}[S]} + \frac{1}{[Co^{-1.0832}+1.2383t]_{max}} \quad (26)$$

For reactor U

$$\frac{1}{Co^{-1.8501}+7.0614t} = \frac{K}{[Co^{-1.8501}+7.0614t]_{max}[S]} + \frac{1}{[Co^{-1.8501}+7.0614t]_{max}} \quad (27)$$

For reactor C

$$\frac{1}{[Co^{-0.9453}+0.1983t]} = \frac{K}{[Co^{-0.9453}+0.1983t]_{max}[S]} + \frac{1}{[Co^{-0.9453}+0.1983t]_{max}} \quad (28)$$

result presented in Table 6 shows the relationship between  $\log T_f$  and  $\log C_o$  for reactor H whereas figure 5 illustrates the graph of  $\log T_f$  against  $\log C_o$  for reactor H. The equation of the best fit was established as  $y = -0.582x - 0.12$  with the square root value of  $R^2 = 0.990$ . The result presented in Table 7 shows the relationship between  $\log T_f$  and  $\log C_o$  for reactor F whereas figure 6 illustrates the graph of  $\log T_f$  against  $\log C_o$  for reactor F. The equation of the best fit was established as  $y = -1.083x - 0.647$  with the square root value of  $R^2 = 0.863$ . The result presented in Table 8 shows the relationship between  $\log T_f$  and  $\log C_o$  for reactor U whereas figure 7 illustrates the graph of  $\log T_f$  against  $\log C_o$  for reactor U. The equation of the best fit was established as  $y = -1.850x - 1.473$  with the square root value of  $R^2 = 0.853$ . The result presented in Table 9 shows the relationship between  $\log T_f$  and  $\log C_o$  for reactor G whereas figure 8 illustrates the graph of  $\log T_f$  against  $\log C_o$  for reactor G. The equation of the best fit was established as  $y = -0.945x - 0.099$  with the square root value of  $R^2 = 1$ .

Table 10 illustrates the relationship of  $n$  and  $k$  values for the various reactors investigated and Table 11 illustrate the various equation of reaction obtained from the research work. The variation in the various results obtained in each reactor can be attributed to the variation on the composition of the mixture as well as time and other environmental factors and present investigation is in line with previous studies (Prince, 1999; Ukpaka et al., 2009; Van Hamme et al., 2000)

## CONCLUSION

Based on the experiments conducted we can clearly observe that the *Moringa oleifera* stimulated the bioremediation of crude oil in the soil this is largely attributed to the nutrients supplied to the soil by the *Moringa oleifera* leave extract this further helps the micro-organisms present in the soil to grow as they grow the population of microbes is increased theoretically as a result of this more microbes are available in the soil to consume the crude oil and thus the bioremediation proceeds at a faster rate. Thus we can conclude that the bioremediation was induced by the extract. Furthermore as pointed out in the discussion biostimulants can in some ways act as inhibitors this happens when the biostimulants supply nutrients to the soil in an excess amount in so much that they become toxic to the micro-organism present in the soil and they do this by inhibiting the metabolism of the microbes this then to death and eventual population decrease of the microbes in the soil as a result of this the bioremediation process is truncated

Furthermore we observe pH increase favoured they bioremediation reaction this was as a result of the fact that the soil was acidic and increase in the P.H of the soil meant that the soil is tending towards neutrality this favoured the bioremediation because the micro-organisms responsible for bioremediation, example of which include *Pseudomonas*, *Aeromonas*, *Moraxella*, *Beijerinckia*, *flavobacteria*, *chrobacteria*, *Nocardia*, *Corynebacteria*, *Atinetobacter* all perform optimally in an alkaline environment as presented in the paper, but as the pH increases beyond 7 they environment becomes basic which could inhibit the activity of certain of this microbes, thus the bioremediation process activation by the *Moringa oleifera* extract is limited to the extent of the concentration of the nutrients present in biostimulants.

Finally the models developed can used to monitor, predict and simulate the rate of degradation of hydrocarbons present in a polluted soil undergoing bioremediation under the influence of *Moringa oleifera* leave extract, As well as to ascertain the amount of *Moringa oleifera* to be added to the soil to be remediated in order to prevent inhibition of the bioremediation process by excessive nutrient supplied.

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