BTX Degradation: The concept of microbial integration

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Abstract

The concept of microbial integration was carried out to examine bacterial and fungal activity on benzene, toluene and xylene (BTX) degradation in a batch reactor. The investigation was conducted for thirty five day of exposure of contact of members and substrate which yielded enzyme substrate complex as well disintegrated to produce products and free enzyme. Bacterial and fungal concentration was monitored per week and the results obtained recorded. The gas chromatography results of Ngara soil sample investigated reveals the concentration of M, P, and O-Xylene for different days of exposure. Increase in both bacterial and fungal was experienced with decrease in BTX concentration, whereas increase in bacterial is more than fungi, indicating the high activity of bacterial in the reactor than that of fungi. Although, both of them were well integrated in bioremediation programme to enhance effective remediation of BTX contaminants in Ngara soil, Omuigwe Alun Community, Niger Delta Area of Nigeria.

INTRODUCTION

The bioremediation technique of benzene, toluene and xylene known as (BTX) in the soil of Omuigwe Aluu in Ikwerre Local Government Area of Rivers State was evaluated to see how microorganisms in the soil were used to reduce or breakdown the hazardous nature of contaminants in the soil. The analysis of the Ngara soil collected at different levels, were carried out to know the best level to be used for the bioremediation process. The analysis was carried out to determine soil pH, electrical conductivity, available phosphorus, total nitrogen, organic carbon, organic matter, moisture content, particle density, porosity, sand, silt and clay and textural class was sandy clay. Because of the fact that sand, silt and clay and textural class was sandy clay. Because of the fact that level 0 – 1 has significant available phosphorous of 8.52(mg/kg), organic carbon (%) of 0.90 higher than other levels it was chosen as the best for the remediation process (Zurcher and Thuer, 1978; USEPA, 1986; Lovley, 2000; Domenico and Schwartz, 1990; Fetter, 2001 Abowei and Susu, 1989 and Cole, 1994). Microbial population or growth test was carried out to determine the total heterotropic bacteria (THB) and total heterotrophic fungi (THF) of the contaminants with time. It was recommended that bioremediation should be used in monitoring pollution emanating from oil spillage on environments, and the producers and users of these contaminants should be careful to avoid the spill or release of these contaminants on the environment to prevent pollution.
Bioremediation is referred to the treatment processes that use microorganism such as bacteria, yeast, or fungi breakdown hazardous substances into less toxic or nontoxic substances. Bioremediation can be used to clean-up contaminated or ground water in the location in which it found for ex-situ bioremediation process, contaminated soil is excavated or groundwater is pumped to the surface before they can be treated (Wami and Ogoni, 1997; Thomas and Ward, 1989, 1989; Dupont et al; 1991; Felske et al; 1998; Premizic and Lin 1991; Lovarh and Alvarez, 2003; Reinhard et al; 1997; Lovley et al 1989 and Ruiz-Aquifer and et al 2002).

Methanogenic through aquifer columns were used to investigate the potential of bio-argumentation to enhance anaerobic benzene-toluene-ethylbenzene-xylene (BTEX) degradation in ground water contaminated with ethanol – blended gasoline. Toluene was the only hydrocarbon degraded within 3 years in columns that were not bio-augmented, although anaerobic toluene degradation was observed after only 2 years of acclimation Mata-Alvarez and Cecchi (1989; Onwioduokit, 1993; johnson et al; 1998; Bragg, 1992; Borden et al; 1995; Atlas et al; 1989; Griffin et al; 2009; Amadi et al; 1993).

The widespread contamination of surface and groundwater resources by the oxygenate methyl tert – butyl ether (MTBX) is leading to its phaseout. Ethanol, a likely candidate to substitute Mbox, is increasingly being used as a gasoline additive to meet renewable and Clean Air Act requirements Power, Rice, Al-Raber et al; (1989). These conditions could contribute to longer BTEX plumes, increasing the probability that a potential down gradient receptor will potential down gradient receptor will be exposed (Puiz-Aquifer, Reilly and Alvar (2002). Enhanced anaerobic BTEX biodegradation has been reports following the addition of nitrate and sulfate. Nevertheless, anaerobic bio-stimulation may not be sufficient to ensure BTEX degradation if the aquifer material does not contain specific degraders in sufficient numbers to exert measurable degradation rates. In such cases, the addition of anaerobic microorganisms with the desired catabolic capacity directly into the contaminated zone should be evaluated for its ability to enhance the natural attenuation of BTEX and ethanol mixtures. (Anderson and Lovely, 2000). To date, the ubiquity of methanogenic consortia capable of degrading benzene has not been established (Lovely, 2000). The effect of ethanol on BTEX biodegradation activity using a bio-reporter strain in continuous culture (Lovanh and Alvarez, 2013; Ghoice and Wilson, 1988; Freeze and Cherry, 1979; Anderson and Lovley, 2000; and Atlas, et al; 1989; Brauner and Killingsta, 1996; Coleman et al; 1984).

The research student in the study of bioremediation of (BTX) in the soil profile of Ngara Omuigwe Aluu soil. Most application of bio-remediation processes have been area of crude oil, hence the study is geared towards investigating the suitability of the use of microorganisms already available in the Ngara Omuigwe Aluu soil to remediate the affected soil by monoaromatic hydrocarbon.

The objectives of the study are as follows:—conceptual development of the dissolution or dilution rate of monoaromatic hydrocarbon (BTX) in soil, determination of the concentration of substrate with respect to time, determination of bacterial population with respect to time of the various contaminants, determination of the fungi population with respect to time of the various contaminants, determination of the dissociation kinetic constant Ks for the various components, determination of the maximum specific rate of reaction for single and multiple catalysed reactions with time and the test of the developed model using regression equation with respect to time.

The scope of this research work is to carry out experiment which microorganisms in the soil will be used to remediate the soil to determine the concentration and microbial population of the various mono aromatic hydrocarbons (BTX). The model for literature will be used to determine the first order rate constant, monod constant, maximum specific rate for both single and multiple enzymes catalysed reactions. The developed model on first order bio-transformation will be used to determine the dilution rate of the contaminants. The model developed using regression equation will be tested with time. The main parameters monitored for the duration of the experiment are microbial population and the concentration. Microsoft Excel and MATLAB were used to determine the concentration of the contaminants daily for 35 days cure of Ngara soil sample collected at Omuigwe Aluu Contaminants daily for 35 days cure Ngara soil sample collected at Omuigwe Aluu.

Various problems have been identified to be associated with the application of microorganisms in remediating contaminated environment (water or soil phase). These problems are responsible for the failure of bioremediation programmes. However, these problems are largely due to wrong application because of the failure in accomplishing bioremediation process. The overall affect caused by these problems will affect the environment.

This research work shall provide the necessary tool, which can be used to achieve and correct the failure of bioremediation programme and increase good planning by eliminating the associated problems.

The application of the models to be developed is geared towards increasing environmental cleanup in a solid phase contaminated areas. Models which will make it possible for bioremediation process to be accomplished are developed by considering the effect of microorganisms in BTX contaminants in aquifer. The limitation of this research work is carry out experiments and used some already existing models from literature and model developed by the
researcher to achieve my objectives. The parameters monitored are microbial growth and concentration. The model will be developed using concentration and be tested using the time in days. Gas chromatography was used. MATLAB and excel programme were used.

**MATERIAL AND METHODS**

**Particle size analysis**

Apparatus: Multimix machine with baffled "milkshake cups, 1 litre capacity glass cylinder, Special hydrometer for measuring density of soil suspension with bouyouos scale in g/litre, Thermometer (centigrade), 2mm sieve.

Reagent: Sodium hexamata-phosphate dispersing agent, 50% (calgin).

Procedure: The following procedures were used such as:

1. Air-dried soil (102g for coarse textural soil or 51g for fine-textural soil) and placed in a 500ml-dispersing cup.
2. Cup was filled up to 5 cm of the top with distilled water.
3. 20 mL of dispersing solution was added and soaked for about 15min.
4. Baffle was inserted into the cup and lowered the stirrer blade into the suspension and stirred the contents for 10 min.
5. The suspension in the cylinder was filled up to 125 mL mark (if 102g of soil was used) or 100 mL mark (if 51g soil was used) and hydrometer was immersed on the suspension samples.
6. The hydrometer was removed; top was covered of cylinder with the hand and inverted several times and placed the cylinder on flat surface for reading. After about 1/2 min, placed the hydrometer slowly and carefully in the suspension and reading was noted after 40 sec.
7. Hydrometer was removed and recorded the temperature of the suspension (Placed the thermometer in and out of the suspension very carefully).
8. After 2 h, the hydrometer inside the suspension was replaced and took the reading. Temperature of the suspension was noted.

9. The hydrometer reading was corrected by adding 0.3 for every degree centigrade that the temperature is about the calibration temperature of the instrument (marked on the stem) or by subtracting 0.3 for every degree that the temperature is below the calibration temperature. Also subtract 2.0 from every hydrometer reading to compensate for the added dispersing agent.

Calculations: The silt + clay, clay and sand were calculated as shown in Eqs. 1-3. The $H_1$ and $H_2$ are the hydrometer reading and $T_1$ and $T_2$ are the temperature ($^\circ F$) at 40 sec and 2h, respectively. Let $T^\circ F$ be the calibration temperature of the hydrometer, 20 $^\circ C$.

\[ \text{Silt + clay} = \frac{H_1 + 0.2(T_1 - T) - 2.0}{50} \times 100 \]  

(50 in the denominator for 51 g sample –100 was used for 102 g sample).

\[ \text{Clay} = \frac{H_2 + 0.2(T_2 - T) - 2.0}{50} \times 100 \]  

(50 in the denominator for 51 sample –100 was used for 102 g sample).

\[ \text{Sand} = 100 - \% (\text{silt + clay}) \]  

(51g sample)

**Soil pH**

Apparatus: A pH meter with a glass electrode and a reference electrode (calomel electrode) was used. Sometimes these two electrode into one pair called an excitation electrode.

Reagents: Distilled water, 0.01M cacl2: Dissolve 11.1gm cacl2 in 1 litre of distilled water, 1Mkcl: Dissolve 74.6gmKcl in 1 litre of distilled water and Buffer solutions of pH 4.0, 7.0 and 9.0. These buffers are prepared by dissolving standard buffer tablets or by diluting buffer concentrations as instructed by the supplier. Distilled water free of CO2 must be used. If commercial buffer solutions or tablets are not available, prepare standards as follows:

- pH 4.0: Dissolved 5.106 g of reagent grade potassium hydrogen phthalate (KHCH$_4$0$_4$) and made the 500 mL with CO2 free distilled water.
- pH 4.0: Dissolved 5.106 g of reagent grade potassium hydrogen phthalate (KHCH$_4$0$_4$) and made the 500 mL with CO2 free distilled water.

Procedure: pH in H$_2$O (1:2.5 soil water ratio) i.e., 10g soil and 25 mL distilled H$_2$O. To 20 g of air-dried soil (passed through 2mm sieve) in a 50ml beaker, and 20 mL of distilled water and allow to stand 30 minutes with occasional stirring with a glass rod. Insert the electrodes into the buffer solutions having pH values close to that expected of the soil and adjust the meter needle to read the buffer pH. Great care should be taken in inserting the electrodes into the solution as the electrodes are quite fragile and easily broken. They should extend at least 2 cm into the solution.

Remove the electrodes, rinse them distilled water, insert them into soil suspensions (1), (2) and (3) (with the calomel electrode into the clear supernatant solution and the glass electrode into the sediment if the electrodes are supplied separately and record the pH meter readings to the nearest 0.05 unit (electrodes should be rinsed between each reading). At the end of experiment clean the electrodes with distilled water and then lower them into a beaker of distilled water.

**Organic carbon in soil measurement**

Apparatus: Analytical balance and Magnetic stirrer and a bulb-lamp. Reagents: INK$_2$CrO$_7$. Dissolve 49.04g of reagent grade K$_2$Cr$_2$O$_7$ (previously dried at 105°C) in distilled water, and dilute the solution to 1 litre.
Concentrated H₂SO₄ 0.5nFeSO₄. Dissolve 139gm of FeSO₄ 7H₂O in water. Add 15ml of conc. H₂SO₄ and dilute to 1 litre. Instead a 0.5N solution of ferrous ammonium sulphate can also be used. This is prepared by dissolving 196g of Fe (NH₄)₂ (SO₄)₂. 6H₂O in about 800ml distilled water and 20ml conc. H₂SO₄ and diluting to 1 litre.

0.5NKMnO₄. Heat 16gms of KmnO₄ in about 500ml distilled water, filter through a funnel containing a plug of glass wool and make up to 1 litre. Standardize the solution with sodium oxalate. Store the solution in a glass stoppered amber bottle.

Glassware: 500ml Erlenmeyer flasks, 50ml burette, 50ml measuring cylinder, 500ml measuring cylinder, 10ml pipette and 25ml pipette. Procedure adopted was as;

1. Weigh accurately about 1.00gm of soil into a 500ml Erlenmeyer flask (use 2.00gm of soil of organic carbon content is less than 1% and reduce the weight of soil if the organic carbon is too high).

2. Pepette 10ml of in K₂Cr₂O₇ into the flask and swirl gently to disperse the soil. Rapidly add 20ml conc. H₂SO₄ (measure out this volume by means of a measuring cylinder) into the flask and cover the flask immediately swirl the flask gently until soil and reagents are mixed, then more vigorously for one minute. Allow the flask to stand on a sheet of asbestos for about 30 minutes.

3. Add about 300ml of distilled water and accurately 25ml of 0.5 Fe SO₄.

4. Titrate the suspension with standard KMnO₄ from a burette using illumination from a bulb lamp. At the end point of the titration, colour changes from deep-grey to purple red.

5. Make a blank determination in the same manner, but without soil.

Let X be the Meq. of carbon in the soil sample, and Y and Z be the Meq. Of K₂Cr₂O₇ and FeSO₄ added respectively. Let T and B be the volume of KmnO₄ used up in the titration of soil sample and blank respectively and N be the normality of KmnO₄. 

\[ X = (T-B) \] \[ Y = Z - T \] \[ Z = Y - X \]

\[ W \] g soil contain \[ \frac{N(T-B)\times3}{W} \times \frac{100}{1000} \] g carbon \[ \text{Organic carbon in soil (\%) } \]

\[ \text{OC in soil (\%) } = \frac{N(T-B)\times3}{W} \times \frac{1}{10} \]

\[ \text{True % of organic carbon in soil } \]

\[ \text{Tru OC in soil (\%) } = \frac{N(T-B)\times3}{W} \times 100 \times \frac{1}{77} \]

Organic matter (\%) is calculated by multiplying % organic carbon by 1.724.

Total nitrogen estimation (Eq. 7)

Apparatus: Macro Kjeldahl digestion apparatus (in fume cupboard) and Macro Kjeldahl digestion apparatus

Reagents: Concentrated H₂SO₄ K₂SO₄–Plus–catalyst mixture: - mixture contain 100gm K₂SO₄ 10gm CuSO₄ 5H₂O and 1gm Sodium hydroxide (NaOH), approximately 10N. Weigh 2.11kg of NaOH pellets in a heavy-walled 5 litre pyrex bottle or flask. Add 2 litres of distilled water and swirl the flask until the alkali is dissolved. Cool the solution with a stopper in the neck of the flask to prevent absorption of atmospheric CO₂ and allow it to stand for several days to permitary Na₂CO₃ present to settle. Siphon the clear supernatant solutions in a large Pyrex bottle which contains about 1 litre of CO₂ free water and mark to indicate a volume of 5 litres and make the solution to 5 litres by adding CO₂-free water. Swirl the bottle vigorously to mix the content and fit the nest with some arrangement, which permits the alkali to be stored and dispensed with protection from atmospheric CO₂.

Mixed boric acid–indicator solution: Dissolve 20gm of boric acid (H₃BO₃) in about 800ml distilled water in a 1 litre Erlenmeyer flask (having the litre mark) by heating on a hot plate at low heat, Cool the solution and add 20ml of mixed indicator solution prepared by dissolving 0.099gm of bromocresol green and 0.066gm of methyl red in 100ml of ethanol. Add 0.1N NaOH through a burette until the solution becomes reddish purple in colour (pH 5.0). Dilute the solution with distilled water to 1 litre. Mix the solution thoroughly before use 5. Standard Hcl or H₂SO₄ 0.0 1 N.

Glassware: Kjeldahl flask (500ml), Burette (50ml), Erlenmeyer flask (1 litre), Erlenmeyer flask (500ml), Measuring cylinder (50ml), Measuring cylinder (250ml). Procedure adopted was as;

1. Weigh accurately about 1g of soil sample containing about 10mg N (air-dried; ground to pass 0.5mm-sieve) in a dry 500ml kjeldahl flask. Add 20ml of distilled water and after swirling the flask for a few minutes allow it to stand for 30 minutes.

2. Add 11gm of K₂SO₄ – plus - catalyst mixture and 30ml cone. H₂SO₄ through automatic pipette (or measuring cylinder under a fume cupboard).

3. Heat the flask cautiously at low heat on the digestion stand. When the water has been removed and frothing has ceased, increase the heat until the digest clears. Then boil the mixture for about 5 hours, rotating the flask at intervals. The heating should be regulated in such a way that H₂SO₄ condenses about half way up the neck of the flask.

4. Allow the flask to cool and slowly add about 100ml of water to the flask carefully transfer the digest in to a 1 litre
Erlenmeyer flask. Retain all sand particles in the digestion flask because sand can cause severe bumping during distillation (Bumping can be further reduced by steam distillation instead of direct heating). Wash the sand residue with 50ml of distilled water four times and transfer the aliquots into the Erlenmeyer flask.

5. Add 50ml H_{3}BO_{3} - indicator solution into a 500ml Erlenmeyer flask and place it under the condenser of distillation apparatus so that the end of the condenser is below the surface of the H_{3}PO_{4}.

6. Clean the Kjeldahl flask and transfer the contents of the Erlenmeyer flask to the Kjeldahl flask, pour about 150ml of 10N NaOH down the neck of the Kjeldahl flask and quickly attach it to the distillation apparatus (check for good fit of the flask with the condenser before adding NaOH). Mix the contents thoroughly b swirling and commence distillation.

7. Keep condenser cool by allowing sufficient cold water to flow through and regulate heat to minimize frothing and prevent suck-back.

8. Collect about 150ml of distillate, remove the receiver flask and then stop distillation.

9. Titrate the NH_{4}^{+} in the distillate with standard HCl or H_{2}SO_{4}. The colour change at the end point is from green to pink.

10. Carry out similar distillation with blank (without soil).

Let Wg be the weight of soil used, Tml, burette reading for the sample, Bml burette reading for the blank; N, then normality of H_{2}SO_{4}.

\[
\text{Corrected Volume of } H_{2}SO_{4} = (T - B)\text{ml} \\
\text{Amount of } H_{2}SO_{4} = N(T - B)\text{mq} \\
\text{Amount of NH}_{3}\text{ in distillate} = N(T - B)\text{mq} \\
\text{Amount of } N\text{ in distillate} = N(T - B)\text{mq} \\
\text{Corrected Volume of } H_{2}SO_{4} = (T - B)\text{ml} \\
\text{Amount of } H_{2}SO_{4} = N(T - B)\text{mq} \\
\text{Amount of NH}_{3}\text{ in distillate} = N(T - B)\text{mq} \\
\text{Amount of } N\text{ in distillate} = N(T - B)\text{mq} \\
\text{Amount of } N\text{ in distillate} = N(T - B)\text{mq} \\
\text{Amount of } N\text{ in distillate} = N(T - B)\text{mq} \\
= N(T - B) \times 14\text{mg} \\
= \frac{N(T - B) \times 14 \text{mg}}{1000} \\
W \text{ g soil contain} = \frac{N(T - B) \times 14 \text{mg}}{1000} \\
100 \text{ g soil contain} = \frac{N(T - B) \times 14 \times 100}{1000 \times W} \\
\text{Total } N \text{ in soil} = \frac{N(T - B) \times 14 \times 100}{1000 \times W} \quad (7)
\]

Available phosphorus in soil y bray and kurz (Eq. 8)
6. Measure absorbance of the sample containing the soil extract and determine the p concentration from the standard curve.

Let the concentration of p in the diluted soil extract by ppm

\[
P_{\text{conc. in undiluted extracts}} = \frac{50}{10} \text{ ppm}
\]

\[
P_{\text{in 200 mL diluted extracts}} = \frac{50}{10} \times y \times 200 N_g
\]

This is present in 2.85gm soil

\[
1 \text{ g soil contain} = \frac{50}{10} \times y \times 20 \text{ ppm}
\]

Available P in soil \[
= \frac{50}{10} y \times 20 \text{ ppm} \quad (8)
\]

**Experimental set-up for bioremediation of BTX**

Material equipment and apparatus: Weight balance, Glass rod, 250ml plastic containers, Pipette 0.5ml and 1 ML capacity, Measuring cylinder (150ml), Screw cap bottle, Distilled water, 0.5M of individual contaminants and Gas chromatography

Experimental procedure: The Ngara soil samples were collected at Omuigwe Aluu in Ikwerre Local Government of Rivers State, at different levels. The soil samples were analyzed to determine the textural composition of the soil and other parameters that will make the research work effective.

Empty cylindrical plastic containers were weighed to there are various weights and recorded. The total numbers of cylindrical containers used were fifteen (15).

A 200 g of the soil sample of level 0 - 1 was weighed because of its high content of organic matter and organic carbon, and added into the containers. The first five containers were labeled A1, A2, A3, A4, and A5, for benzene. The five containers were labeled B1, B2, B3, B4, and B5 for toluene, while the three first five containers were labeled C1, C2, C3, C4, and C5, for xylene. Pipette was used to collect 10ml of benzene and added to A-series, 10ml of toluene to B-series and 10ml if xylene to C-series. The samples were kept at room temperature, and collected for 7 days interval for 35 days cure.

**Enumeration of bacteria and fungi in the bioremediation samples**

From each of the bioremediation samples, 1g of the samples was dispersed into 9.0ml of normal saline (diluent) in test tubes to give 10⁻¹ dilutions. Further serial dilutions up to 10⁻³ were done. Two rops (0.1 ml) aliquots of 10⁻² and 10⁻³ dilutions were inoculated into the surface of sterile nutrient agar plates (for enumeration of bacteria) and onto (for enumeration of fungi). The inocula (0.1ml aliquots) were evenly spread on the surface of the agar using a sterile bent glass rod; after which the inoculated at 37°C for 24 - 48hours. After incubation, the plates were examined and, colonies that developed were counted and recorded; and taken as the population of bacteria and fungi in colony forming unit (CFU) per gram sample.

**Enumeration of BTX using gas chromatography**

Test method: Gas chromatography - EPA 8240. Direction injection method was applied in place of purge and trap. GC/FID and capillary column techniques was applied in place of GC/MS and packed column. Equipment and material: BTEX standard mix, methanol (chromatographic grade), distilled water, Agilent 6890N Gas chromatography, glass grew cap vials, micro–syringes, Analytical balance and Pipettes

Testing procedure: Sample Extraction: Weigh 10 - 20g of fresh sample into 50ml sample bottle. Add 10ml methanol. Replace cap of sampling bottle and shake through for 30min. Allow organic layer to separate. Collect organic layer into vial.

Preparation of BTEX standard mixture: Add 50, 100, 150, 200 and 250 η of 0.2 mg/ml BTEX stock standard solution into separate 1ml vials. Make up the final volume to 1ml with methanol. The concentration of the BTEX standard is 10, 20, 30, 40 and 50 mg/l, respectively.

**RESULTS AND DISCUSSION**

The results of sample analysis are presented in Tables 1-2 and Figures 1-2.

The percentage organic carbon and organic matter were greater in the first level compare to other levels. From Table 1 level (6-1) has 0.52% organic carbon, 0.90%. Organic matter, level (1-2) has 0.26% organic carbon, 0.45% organic carbon, level (2-3) has 0.06% organic carbon, 0.10% organic carbon, level (3-4) has 0.24% organic carbon, 0.41% organic matter, level (4-5) has 0.11% organic carbon, and 0.19% organic matter. This implies that first level is preferred to others because of the fact that the micro-organisms in the soil were used for the remediation process.

**Microbial population**

The results of microbial population and differentiating the microbes present are presented in Table 2. FromTable 2. In the first 28 days Toluene sample has the highest population of 8.6 x 10⁵cfu/g, followed by benzene sample of 4.1 x 10⁵cfu/g before Xylene sample 3.4 x 10⁵cfu/g. The microbial population of bacteria and fungi decreases as days decrease in the concentration of the contaminants and the microbial organisms involved may not have enough to feed an and some will die. This implies that the microorganisms are remediated the affected aquifer.
Bacterial population

The variation of bacterial population with time is shown in Figure 1. The bacterial population of benzene sample has its optimum value of $4.1 \times 10^5$ cfu/g at 28 days and minimum value of $1 \times 10^3$ cfu/g at 0 and 7 days. Toluene sample has optimum value of $8.6 \times 10^5$ cfu/g at 28 days and minimum value of $1 \times 10^3$ cfu/g at 0 day. While Xylene has its optimum value of $3.4 \times 10^5$ cfu/g at 28 days and minimum value of $1 \times 10^3$ cfu/g at 0 day.

Fungi population

The variation of fungi population with time is shown in Figure 2. The fungi population of benzene sample has its optimum value of $5.3 \times 10^5$ cfu/g at 28 days and minimum value of $1.0 \times 10^3$ cfu/g at 0 day 7 days. Toluene sample has its optimum value of $4.7 \times 10^5$ cfu/g at 28 days and minimum value of $1.0 \times 10^3$ cfu/g at 0 day. Xylene sample has its optimum value of $3.6 \times 10^5$ cfu/g at 28 days and minimum value of $1.0 \times 10^3$ cfu/g at 0 day. These findings are in line with previous studies, which support the (Bossier et al., 2016; Hassan et al., 2016; Jia et al., 2016; Li et al., 2016; Parelho et al., 2016; Sadhukhan et al., 2016; Varanasi et al., 2016; Wang et al., 2016).

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**Table 1:** Result of sample analysis of five (5) Ngara soil samples Omuiqwe Aluu

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>SOIL DEPTHS (M)</th>
<th>REMARKS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0 – 1</td>
<td>1 – 2</td>
</tr>
<tr>
<td>Soil pHw (1:25)</td>
<td>5.10</td>
<td>4.80</td>
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<tr>
<td>Elect. Cond. (µs/Cm)</td>
<td>93</td>
<td>141</td>
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<tr>
<td>Available ρ (mg/kg)</td>
<td>8.52</td>
<td>5.46</td>
</tr>
<tr>
<td>Total N. (%)</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Organic C. (%)</td>
<td>0.52</td>
<td>0.26</td>
</tr>
<tr>
<td>Organic M. (%)</td>
<td>0.90</td>
<td>0.45</td>
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<tr>
<td>Moisture Content (%)</td>
<td>13.88</td>
<td>13.82</td>
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<tr>
<td>Particle Density (g/cm³)</td>
<td>2.60</td>
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<tr>
<td>Bulk Density (g/cm³)</td>
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<td>1.69</td>
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<tr>
<td>Porosity (%)</td>
<td>35</td>
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<tr>
<td>Sand (%)</td>
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<tr>
<td>Silt (%)</td>
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<td>Textural Class</td>
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**Table 2:** Microbial population of contaminated soil with respect to time

<table>
<thead>
<tr>
<th>TIME (in Days)</th>
<th>Bacterial Population (cfu/g)</th>
<th>Fungi population (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(THB)₆</td>
<td>(THB)₇</td>
</tr>
<tr>
<td>0.00</td>
<td>$1.0 \times 10^3$</td>
<td>$1.0 \times 10^3$</td>
</tr>
<tr>
<td>7.00</td>
<td>$1.0 \times 10^3$</td>
<td>$2.0 \times 10^3$</td>
</tr>
<tr>
<td>14.00</td>
<td>$6.0 \times 10^3$</td>
<td>$1.8 \times 10^4$</td>
</tr>
<tr>
<td>21.00</td>
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<tr>
<td>28.00</td>
<td>$4.1 \times 10^5$</td>
<td>$8.6 \times 10^5$</td>
</tr>
<tr>
<td>35.00</td>
<td>$4.0 \times 10^5$</td>
<td>$8.0 \times 10^5$</td>
</tr>
</tbody>
</table>
CONCLUSION

The following conclusion can be drawn from the investigation such as:
1. The fungi concentration of Xylene is higher than the others under the same condition.
2. The best microorganism that can be used to remediate affected area with the contaminants is bacteria.
3. Toluene degrades faster than the other two other contaminants under the same condition.
4. Integration of the microbes isolated and identified from the substances under considered will fascinate the rate of biodegradation, when they are well cultured and empower to achieve energy level at ATP.
5. The results of the tests carried out on microbial population of fungi and bacteria concentration shows that microorganisms can be used to remedy an affected area with contaminants.

RECOMMENDATIONS

The bioremediation is most effective in xylene for rate of reaction, concentration, bacterial population and fungi
population. For dilution rate benzene is effective. From these results, the following recommendations are hereby made:

1. For economical reason, the first layer of soil is recommended for bioremediation process using an aquifer.
2. The bioremediation of BTX should be incorporated in the design or bio-treatment plant.
3. The use of gas chromatograph for the test of concentration of the contaminants (substrates) is recommended.
4. The bioremediation should be used in monitoring pollution emanating from oil spillage on land and aquatic environments.
5. The values obtained should be used in determining the residence time for the design of bio-treatment reaction.
6. The values obtained should be used in estimating the period of biodegradation of mono aromatic hydrocarbon base industrial effluent.
7. The values of the rate constants obtained in this research should be used for design of bio-treatment reaction.
8. The producers/users of the contaminants should be careful to the spill or release of the contaminants to prevent pollution.

REFERENCES


bioassays and microbial biosensors. Environment international 92-93, 106-118.


