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BTX Degradation: The concept of microbial integration

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A B S T R A C T

The concept of microbial integration was carried out to examine bacterial and fungal activity on bezene, toluence 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 were well integrated in bioremediation program to enhance the effective remediation of BTX contaminants in Ngara soil, Omuigwe Alun Community, Niger Delta Area of Nigeria.

Capsule Summary: The bacteria and fungi BTX degradation efficiency were investigated and bacterial was found active than fungi and were integrated well in bioremediation to enhance effective remediation of BTX contaminants.

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INTRODUCTION

The bioremediation technique of bezene, toluence 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 phosphorous, 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

(Abowei and Wami, 1988; Power et al., 2001; Urlings et al., 1991; Lovley, 1997. 1993; Rooney-Varga et al., 1999; Weber and Corseuil, 1994; Eganhouse et al., 1996; Borja et al., 1995; Wilson Bouwer, 1997 and Nielsen, 1991). Also government should bring policy that will hold the companies producing or using these contaminants to avoid pollution. Programming was used to stimulate the K_s values of the contaminants to know the concentration on daily basis.

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 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 MTBX, is increasingly being used as a gasoline additive to meet renewal fuel 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; Ghcose and Wilson, 1988; Freeze and Cherry, 1979; Andercon 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 cur of Ngara soil sample collected at Omuigwe Aluu Contaminants daily for 35 days cure Ngara soil sample collected at Omuiqwe 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).
9. After 2 h, the hydrometer inside the suspension was replaced and took the reading. Temperature of the suspension was noted.
10. 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 (^{0}F) at 40 sec and 2h, respectively. Let T^{0}F be the calibration temperature of the hydrometer, 20 ^{0}C.

$$Silt + clay\ (\%) = [H_1 + 0.2(T_1 - T) - 2.0] * \frac{100}{50} \quad (1)$$

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

$$Silt + clay\ (\%) = [H_2 + 0.2(T_1 - T) - 2.0] * \frac{100}{50}$$

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

$$Clay\ (\%) = [H_2 + 0.2(T_2 - T) - 2.0] * \frac{100}{50} \quad (2)$$

Sand (%) = 100 - % (silt + clay) for 51g sample (3)

Soil pH

Apparatus: A pH meter with a glass electrode and a reference electrode (calomel electrode) was used. Sometimes these two electroded into one pair called an excitation electrode. Reagents: Distilled water, 0.01M $cacl_2$: Dissolve 1.11gm $cacl_2$ 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 CO_2 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 ($KHCH_4O_4$) and made the 500 mL with Co_2 free distilled water.

Procedure: pH in H_20 (1:2.5 soil water ratio) i.e., 10gx add 25mL distilled H_20. To 20 g of air-dried soil (passed through 2mm sieve) in a 50ml beaker, and 20ml 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 2cm 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_2CrO_7. Dissolve 49.04g of reagent grade $K_2Cr_2O_7$ (previously dried at 105^{0}C) in distilled water, and dilute the solution to 1 litre. Concentrated H_2SO_4. 0.5nFeSO_4. Dissolve 139gm of $FeSO_4$. $7H_2O$ in water. Add 15ml of conc. H_2SO_4 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 $(NH_4)_2$ $(SO_4)_2$ $6H_2O$ in about 800ml distilled water and 20ml conc. H_2SO_4 and diluting to 1 litre. 0.5NKMnO_4. Heat 16gms of K$mn0_4$ 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 the 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 $K_2Cr_2O_7$ into the flask and swirl gently to disperse the soil. Rapidly add 20ml conc.H_2SO_4 (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 30minutes.
3. Add about 300ml of distilled water and accurately 25ml of 0.5 Fe SO_4.
4. Titrate the suspension with standard $KMnO_4$ 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 $K_2Cr_2O_7$ and $FeSO_4$ added respectively. Let T and B be the volume of $KmnO_4$ used up in the titration of soil sample and blank respectively and N be the normality of $KmnO_4$.

$\therefore$ NT = Z – (Y – X)

And NB = Z – Y

$\therefore$ X = N (T – B)

$\therefore$ Wgm soil contain N(T – B) x 3mg carbon

$$100\ g\ soil\ contain = \frac{N(T-B)*3}{W} * \frac{100}{1000}\ g\ carbon \qquad (4)$$

Organic carbon in the soil (%)

$$OC\ \text{in the soil}\ (\%) = \frac{N(T-B)*3}{W} * \frac{1}{10} \qquad (5)$$

True % of organic carbon in the soil

$$\text{Tru OC in the soil}\ (\%) = \frac{N(T-B)*3}{W} x \frac{100}{77}$$

$$= \frac{N(T-B)*3}{W} * 0.390 \qquad (6)$$

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 H_2SO_4, K_2SO_4–plus–catalyst mixture: - mixture contain 100gm K_2SO_4, 10gm $CuSO_4$. $5H_2O$ and 1gm Sodium hydroxide (NaOH), approximately 10N. Weigh 2.11kg of NaOH pellets in a heavy-walled 5 litre pryrex 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 CO_2 and allow it to stand for several days to permitary Na_2CO_3 present to settle. Siphon the clear supernatant solutions in a large Pyrex bottle which contains about 1 litre of CO_2 free water and mark to indicate a volume of 5 litres and make the solution to 5 litres by adding CO_2 - 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 CO_2.

Mixed boric acid - indicator solution: Dissolve 20gm of boric acid (H_3BO_3) 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 l00ml of ethanol. Add 0.lN 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 H_2S0_4, 0.0 1 N. Glassware: Kjeldahl flask (500ml), Burette (50m1), 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 500m1 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 K_2SO_4 – plus - catalyst mixture and 30ml cone. H_2SO_4 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 H_2SO_4 condenses about half way up the neck of the flask.
4. Allow the flask to cool and slowly add about l00ml 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_3BO_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_3PO_3.

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

PARAMETERS	SOIL DEPTHS (M)					
	0 – 1	1 – 2	2 – 3	3 – 4	4 – 5	REMARKS
Soil pHw (1:25)	5.10	4.80	5.20	4.80	5.00	
Elect. Cond. (µs/Cm)	93	141	60	90	59	
Available ρ (mg/kg)	8.52	5.46	3.18	3.42	1.68	
Total N. (%)	0.04	0.05	0.04	0.04	0.03	
Organic C. (%)	0.52	0.26	0.06	0.24	0.11	
Organic M. (%)	0.90	0.45		0.41	0.19	
Moisture Content (%)	13.88	13.82	14.92	15.12	16.49	
Particle Density (g/cm^{-3})	2.60	2.56	2.60	2.64	2.56	
Bulk Density (g/cm^3)	1.68	1.69	1.71	1.78	1.69	
Porosity (%)	35	34	34	33	34	
Sand (%)	57	55	57	57	55	
Silt (%)	1	3	1	1	2	
Clay (%)	42	42	42	42	43	
Textural Class	SC	SC	SC	SC	SC	Sandy Clay

Table 2: Microbial population of contaminated soil with respect to time

TIME (in Days)	Bacterial Population (cfu/g)			Fungi population (cfu/g)		
	$(THB)_B$	$(THB)_T$	$(THB)_X$	$(THF)_B$	$(THF)_T$	$(THF)_X$
0.00	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3
7.00	1.0×10^3	2.0×10^3	5.0×10^4	1.0×10^3	1.8×10^3	2.8×10^3
14.00	6.0×10^3	1.8×10^4	8.0×10^4	2.6×10^3	3.2×10^3	3.0×10^3
21.00	1.0×10^4	4.0×10^4	9.0×10^4	3.1×10^3	4.7×10^3	3.8×10^3
28.00	4.1×10^5	8.6×10^5	3.4×10^5	5.3×10^3	3.2×10^3	3.6×10^3
35.00	4.0×10^5	8.0×10^5	9.0×10^4	5.0×10^3	1.1×10^3	3.5×10^3

6. Clean the Kjeldahl flask and transfer the contents of the Erlenmeyer flask to the Kjeldahl flask, pour about l50ml 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_2SO_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_2SO_4.

Corrected Volume of H_2S0_4 = (T - B)ml

Amount of H_2S0_4 = N(T – B)mq

Amount of NH_3 in distillate = N(T – B)mq

Amount of N indistillate = N(T – B)mq

Corrected Volume of H_2S0_4 = (T - B)ml

Amount of H_2S0_4 = N(T – B)mq

Amount of NH_3 in distillate = N(T – B)mq

Amount of N indistillate = N(T – B)mq

= N(T – B) x 14mg

$$= \frac{N(T-B)*14\ mg}{1000}$$

$$W\ g\ soil\ contain = \frac{N(T-B)*14\ of\ N}{1000}$$

$$100\ g\ soil\ contain = \frac{N(T-B)*14*100}{1000*W}$$

$$Total\ N\ in\ soil = \frac{N(T-B)*14*100}{1000*W} \quad (7)$$

Available phosphorus in soil y bray and kurtz (Eq. 8)

Apparatus and reagents: Mechanical bottle shaker and B & L spectronic – 20 spectro–phometer

1. Ammonium fluoride (NH_4F), IN: Dissolve 3.7g of NH_4F in distilled water and dilute the solution to l00ml. And put this solution in a polyethylene bottle.
2. Hydrochloric acid (HCl), 0.5N: Dilute 20.2rnl cone. HCl to a volume of 500ml under a fume hood.
3. Extracting solution (0.03N NH_4F and 0.025NHCl): Add 15ml of 1.0NNH4F and 25ml of 0.5NHCI to 460ml distilled water.
4. Reagent A: (a) Dissolve 12g of ammonium molybdate $(NH_4)_6$. $MO_7O_24H_2O$ in 250ml distilled water (b) Dissolve 0.2908g of potassium antimony tartarate ($KSbOC_4H_40_6$) in 10ml of distilled water. (c) Prepare $5NH_2SO_4$ by diluting approximately 148ml cone. H_2SO_4 in about 100ml of distilled water. (d) Mix solutions (a), (b) and (c) Together in a 2 litres volumetric flask and make up to mark with distilled water.
5. Reagent B: Dissolve 1.056g of ascorbic acid in 200ml of reagent A and mix. This reagent cannot keep for more than 24hrs. Prepare it fresh every 24hrs.
6. Standard P stock solution: Dissolve 0.4393g of oven - dry KH_2PO_4 in distilled water and make up to 1 litre in a volumetric flask. The solution contains l00ppm p. pipette 5ml of 100ppm p solution into a l00ml volumetric flask and make up to volume with distilled water. The solution contains 5ppm p. store this solution in a brown bottle inside a refrigerator.

Glassware: Test tubes with stoppers, Funnels with filler papers (whatmann No. 42), 1 ml pipette, 4ml pipette, 50ml graduated pipette, l0ml graduated pipette and l0ml of graduated cylinder. Procedure adopted was as;

1. Weigh 2 85 g soil into a tube and add 20ml of the extracting solution.
2. Shake the tube for 1 minute, and filter the content through whatmann No. 42 paper, if the filtrate is not clear quickly pour the solution back through the filter.
3. Pipette l0ml alique of the soil extract into a 50ml volumetric flask and add 10ml of distilled water.
4. Add 4ml of reagent B and make up to volume with distilled water. Allow, the colour to develop for 15 minutes.
5. Prepare a set of standard p solutions of 50ml containing 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0ppm (dilute 1, 2, 3, 4, 6 and 8ml of 5ppm p stock to 50ml.) Each of the standards should contain l0ml of the extracting solution and 4ml of reagent B. Allow the colour to develop for 15 minutes and measure absorbance of the standards on a spectrophotometer at 660N, Draw a standard curve by plotting absorbance Vs concentration in a graph paper.
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 yppm

$$P\ conc.in\ undiluted\ extracts = \frac{50}{10}\ yppm$$

$$P\ in\ 200\ mL\ diluted\ extracts = \frac{50}{10} * y * 200\ Ng$$

This is present in 2.85gm soil

$$1\ g\ soil\ contain = \frac{50}{10}\ y * \frac{20}{2.85} Ng$$

$$Available\ P\ in\ soil\ = \frac{50}{10}\ y * \frac{20}{2.85}\ ppm \quad (8)$$

Experimental set-up for bioremediation of BTX

Material equipment and apparatus: Weight balance, Glass rod, 250 ml 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 A_1, A_2, A_3, A_4, and A_5, for benzene. The five containers were labeled B_1, B_2, B_3, B_4, and B_5 for toluene, whole the third five containers were labeled C_1, C_2, C_3, C_4, and C_5, for xylene. Pipette was used to collect 10ml of benzene and added to A-series, l0ml of toluene to B-series and l0ml 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

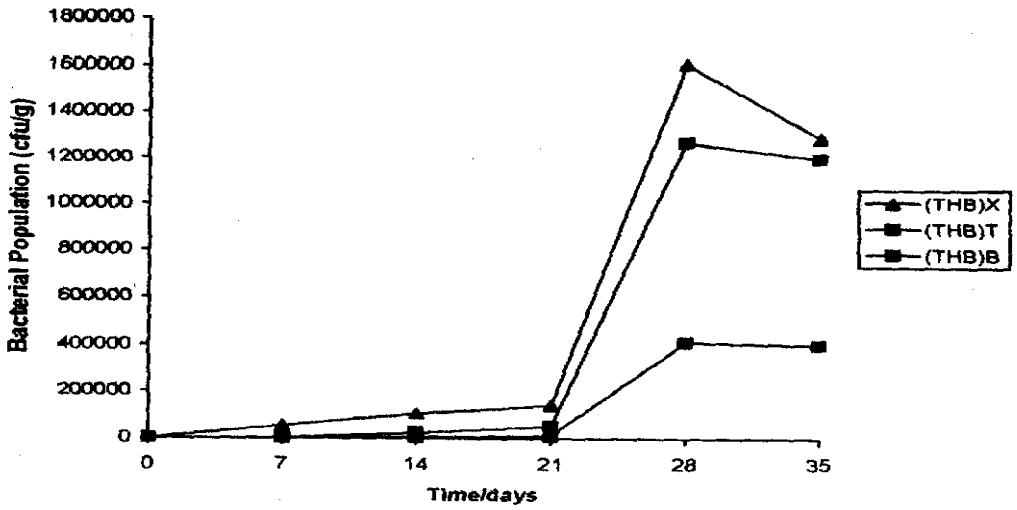

Fig. 1: Variation of bacterial population with time

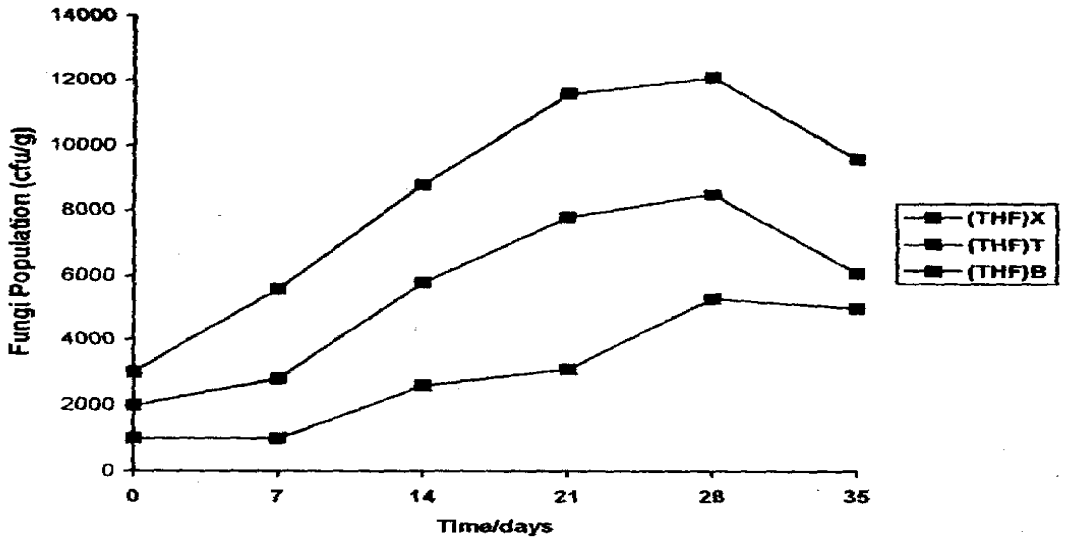

Fig. 2: Variation of fungi population with time

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 37^0C 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 l0ml 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. From Table 2, in the first 28 days Toluene sample has the highest population of 8.6 x 10^5cfu/g, followed by benzene sample of 4.1 x 10^5cfu/g before Xylene sample 3.4 x 10^5cfu/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 remediating the affected aquifer (Fernando et al., 2014; Huang and Angelidaki, 2008; Nauseef, 2007).

Bacterial population

The variation of bacterial population with time is shown in Figure 1. The bacterial population of benzene sample has its optimum value pf 4.1 x 10^5 cfu/g at 28 days and minimum value 1 x 10^3cfu/g at 0 and 7 days. Toluene sample has optimum value of 8.6 x 10^5cfu/g at 28 days and minimum value of 1 x 10^3cfu/g at 0 day. While Xylene has its optimum value of 3.4 x 10^5cfu/g at 28 days and minimum value of 1 x 10^3cfu/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 it's the fungi population of benzene sample has its optimum value of 5.3 x 10^3cfu/g at 28 days and minimum value at 1.0 x 10^3cfu/g at 0 day 7 days. Toluene sample has its optimum value of 4.7 x 10^3cfu/g at 28 days and minimum value of 1.0 x 10^3cfu/g at 0 day. Xylene sample has its optimum value of 3.6 x 10^3cfu/g at 28 days and minimum value of 1.0 x 10^3cfu/g at 0 day. These findings are in lines 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)

CONCLUSIONS

The following conclusion can be drawn from the investigation such as: The fungi concentration of Xylene is higher than the others under the same condition. The best microorganism that can be used to remediate affected area with the contaminants is bacteria. Toluene degrades faster than the other two other contaminants under the same condition. 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. 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.

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

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