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RESEARCH ARTICLE

MODELLING THE BTX CONTAMINANTS DEGRADATION IN SOIL ENVIRONMENT USING BIOREMEDIATION TECHNIQUES

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ABSTRACT

A number of studies on the bioremediation techniques on the degradation of benzene, toluene and xylene (BTX) contaminants by microorganisms were reported in the 1980s and 1990s. Petroleum exploration, exploitation and production in Niger Delta Area of Nigeria has resulted to high environmental degradation. However, challenges have existed for the implementation of degradable environment due to BTX contaminants as well as instability of soil characteristics due to high production effect. The research was carried out to determine the usefulness of bioremediation techniques in the degradation of BTX contaminants in soil environment. This paper thus reviews the effect of soil characteristics and its overall performance of microbes as functional parameters of BTX contaminants degradation as well as discusses the evalution of rate constant of Benzene, Toluene, Xylene and maximum specific rate constant. The result obtained from the research showed that there was significant decrease in the concentration of toluene from 46.07 mg/kg to 40.05 mg/kg, Benzene (39.06 – 36.42) mg/kg and Xylene (53.09 -52 .72) mg /kg meaning that the microorganisms are friendly with Toluene compared to other contaminants as investigated. It was recommended that bioremediation should be used in monitoring and predicting the degradation rate of BTX contaminants in soil environments upon the influences soil characteristics and composition.

INTRODUCTION

The bioremediation techniques of benzene, toluene and xylene known as (BTX) contaminants in soil environment was investigated by using samples obtained at Onuigwe Aluu in Ikwerre Local Government Area of Rivers State. The research was conducted to evaluate and to see how microorganisms in the soil were used to reduce or break down the hazardous nature of contaminants in the soil environment. Bioremediation is referred to the treatment processes that use microorganism such as bacteria, yeast, or fungi to break down hazardous substances into less toxic or non-toxic substances. Bioremediation application can be used to clean-up contaminated soil or ground water. In-situ bioremediation treats contaminated or ground water in the location in which it is found whereas ex-situ bioremediation process, contaminated soil is excavated or groundwater is pumped to the surface before they can be treated. (Ruiz-Aquifer *et al*., 2002; O' Reilly, Alvarez, 2002. 'Anderson and Lovly, 2000; Lovanh and Alvarez, 2003. Homez, Becker *et al.,* 1995; Atlas, 1991; Fetter, 2001 and Dooher *et al*., 1995; Atlas, 1991; Fetter, 2001 and Powers *et al.,* 2001).

Benzene, Toluene and xylene are pollutants of high priority concerns because of their toxicity and possible accumulation in the environment. Benzene, toluene and xylene are introduced into soil environment from spillage and discharge of industrial effluents (Ukpaka *et al.,* 2009) Bioremediation technology is currently being used extensively for the removal of organic and inorganic micro-pollutant from soil environment. There are many microorganisms in use. Bacteria is the most widely used for the removal of a variety of pollutants from soils, but the advantage associated with it is the high environmental friendly substance produced (Beck, Kaus and Orberts; 2001 Jiang *et al*., 2006, Muyssen and Janssen, 2005; Gao, Zhu and Ling, 2005 Li *et al*., 2005 and Hua , 2007) Soil contamination by recalcitration toxic organic substances poses environmental concerns because of the consequent long-term effects on food chain and groundwater qualities. Investigation conducted by various researchers revealed that the remediation of persistent organic pollutants in soil with plants (Phytoremediation) is a potentially economic technology because of its low operational costs, landscape preservation, self-sustained process, and reduced human exposure (Newman *et al*., 1997 and Pilon-smits, 2005). The microbial community has a direct influence on the chemical degradation/mineralization process (Cavigelli and Robertson, 2000). The soil nutrients may enhance the

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microbial growth rate as well as benzene, toluene and xylene degradation by creating specific micro environments for pollutant degrading. For organic compounds, existing studies mostly focus on the degrading bacteria in soil (Johnsen *et al*; 2002 and Wick *et al.,* 2003) and the biostimulation and bioaugmentation of land farming (Straube *et al*., 2003). It is necessary to gain more knowledge about the effect of benzene, toluene and xylene contaminants on soil, microbial activity and whether the effects are due to changes in dominant species and/or in physiochemical activities of the microbial community/soil characteristics as well as the composition can be assessed by the phospholipids fatty acid (PLFA) analysis, since different PLFA patterns (Frostegard, Thnlid and Baath, 1991).

Benzene toluene and xylene hydrocarbon (BTXH) are common soil contaminants that are derived from natural and anthropogenic sources (Freeman and Lattel, 1990), depending on their physicochemical properties, these compounds exhibit toxic mutagenic and carcinogenic effect or anti-estrogenic activities (Hirose *et al.* 2001). Owing to their wide occurrence, BTXH are among the most important contaminants at present especially in some coastal and industrial areas of Niger Delta of Nigeria. Some work (Paul, 1975) has been done regarding the benzene, toluene and xylene (BTX) contaminant degradation in soil environment. These studies were limited only to benzene toluene and xylene contaminants effect on plant uptake, microbial growth and biokinetic model. No realistic investigation was conducted to study the rate of degradation of benzene, toluene and xylene contaminants in Niger Delta soil environment. It is the purpose of this study therefore to formulate mathematical equations by correlating functional parameters to simulate degradation rate of benzene, toluene, and xylene in soil environment as well as establishing the degradation rate constant parameters.

The Model Degradation Rate Model

The model for the comparison of the experimental results was developed in this paper. The mathematical formulations were established as well developed to predict the degradation rate of petroleum hydrocarbon using a first order biotransformation model for the test of concentration of benzene, toluene and xylene was established in this investigation by considering the material barbance equation as shown below

$$\begin{pmatrix}\text{Rate of}\\ \text{Inflow of}\\ \text{material into}\\ \text{the control volue}\end{pmatrix} - \begin{pmatrix}\text{Rate of}\\ \text{Outflow}\\ \text{of material}\\ \text{from the}\\ \text{control volume}\end{pmatrix} + \begin{pmatrix}\text{Rate of}\\ \text{Foromation by}\\ \text{biochemical reaction}\\ \text{within the control volume}\end{pmatrix} = \begin{pmatrix}\text{Rate of}\\ \text{Accumulation}\\ \text{with in the control}\\ \text{volume}\end{pmatrix} \quad \ldots(1)$$

(Inflow of material) = FS_0……(2)

(Outflow of material) FS…….(3)

(Formation by biochemical reaction) R_sV……...(4)

(Accumulation) = Vds/dt……..(5)

Substituting equations (2), (3), (4) and (5) into (1)FS_0 - FS + R_sV =Vds/dt ……....(6)

Vds/dt = FS_0 - FS + R_sV

Vds/dt = F (S_0 –S) + R_sV ……..…..(7)

Dividing all through of equation (7) by V

$$\frac{ds}{dt} = S_0 - S + R_s \quad \ldots\ldots(8)$$

R_s = K_sSs…….……...(9)

Substituting equation (12) into equation (8) we shall have

$$\frac{ds}{dt} = S_0 - S + {}_sS \quad \ldots\ldots(10)$$

At t = 0, S = S_0 Using the above condition in equation (10)

$$\frac{ds}{dt} = K_sS \quad \ldots\ldots(11)$$

$$\frac{ds}{dt} = -K_sS \quad \ldots\ldots(12)$$

Equation (12) is the mathematical model that will describe the dynamic behavior of concentration of the substrate with respect to time. This can be solved to obtain the effect of microorganism on the BTX contaminants in the soil profile. Equation (12) is a first order differential equation, which can be solved using the separation of variable method. Step 1. Multiply both sides of the equation (12) by dt/s.

$$\frac{ds}{dt}\frac{dt}{S} = -K_S\frac{dt}{S}$$

$$\frac{ds}{S} = -K_s dt \quad \ldots\ldots(13)$$

Integrating both sides of equation (13)

$$\int_{s0}^{s}\frac{ds}{S} = K_s\int_0^1 dt$$

$$\text{i}\left[Ins\right]_{S0}^{S}\ 2 - K_S\left[t\right]_0^1 + C\ \text{Ins}$$

$$Ins - Ins_0 = -K_S\left[t - 0\right] + C$$

If the constant parameter C does not influence the system thus equation (13a) can be writhen as

$$\text{In}\left(\frac{S}{S_0}\right) = -K_s t \quad \ldots\ldots(14)$$

Dividing both sides of equation (14) by -1

$$\text{In}\left(\frac{S}{S_0}\right) = -K_s t$$

$$\text{In}\left(\frac{S}{S_0}\right) = -K_s t \quad \ldots\ldots(15)$$

$$\beta = \frac{S}{S_0}$$

K_s can be obtained by plotting in $\frac{S_0}{S}$ against substituting equation (16) into equation (15)

In

$$\frac{1}{\beta} = K_s^t \quad \text{.....(17)}$$

Taking exponential on both sides of equation (17)

$$\ell^{In}\ \frac{1}{\beta} = \ell^{Kst}$$

$$\frac{1}{\beta} = \ell^{Kst} \quad \text{.....(18)}$$

Dilutionrate $\frac{F}{V} = D\ where D = dilution rate\ from equation$ (7)

$$-\frac{V\frac{ds}{dt}}{V} = \frac{F}{V}(S_0 - S) + K_{S^S}$$

V = Volumetric flow rate.

$$-\frac{ds}{dt} = \frac{F}{V}(S_0 - S) + K_{S^S}$$

V = Volumetric flow rate.

$$-\frac{ds}{dt} = \frac{F}{V}(S_0 - S) + K_{S^S}$$

$$\frac{ds}{dt} = \frac{F}{V}(S - S_0) + K_{S^S}$$

At steady state $\frac{ds}{dt} = 0$ equation (19) reduces to

$$\frac{F}{V}(S - S_0) - K_S^S = 0 \quad \text{.....(20)}$$

$$\frac{F}{V} = D \quad \text{.....(21)}$$

Substituting equation (10) into equation (21) we have

$$D(S - S_0) - K_{S^S} = 0$$

$$D_1 = \frac{K_S S_1}{S_1 - S_{01}} \quad \text{.....(22)}$$

Using regression equation of the form S = at + b, where a and b are the slopes and intercepts on the S –axis respectively. S is the concentration and t is time. It can be shown that if S = at + b is the least – square line.

S = at + b .....(23)

$$\alpha = \frac{\sum(t - \bar{t})}{\sum(t - \bar{t})^2} \quad \text{.....(24)}$$

$$b = \bar{s} - a\bar{t} \quad \text{.....(25)}$$

$$\sum(t - \bar{t})(s - \bar{s}) = \sum(st - t\bar{s} - t\bar{s} - s\bar{t} + t\bar{s})$$

$$= \sum st - \sum t\bar{s} - \sum \bar{t}s + \sum \bar{t}s \quad \text{.....(26)}$$

But, $\bar{t} = \frac{\sum t}{n}$ .....(27)

$$\bar{s} = \frac{\sum s}{n} \quad \text{.....(28)}$$

$$\overline{st} = \frac{\sum ts}{n} \quad \text{.....(29)}$$

Making $\sum t,\ \sum s\ and \sum st$ the subject of the formula in equation (27),

(28) and (29) we shall have;

$$\sum t = n\bar{t},\ \sum s = n\bar{s}\ and \sum st = ns\bar{t} \quad \text{.....(30)}$$

Substituting equation (30) into equation (26) we have

$$\sum st - \bar{s}nt - \bar{t}ns + ns\bar{t} = \sum st - 2ns\bar{t} + ns\bar{t} = \sum st - ns\bar{t} \quad \text{.....(31)}$$

Substituting equation (27) and (28) into equation (31) we have

$$\sum st - n\left(\frac{\sum s}{n}\right)\left(\frac{\sum t}{n}\right) = \sum st - \frac{n\sum s\sum t}{n^2} = \sum st - \frac{(\sum s)(\sum t)}{n}$$

$$\therefore \sum(t - \bar{t})(s - \bar{s}) = \frac{n\sum st - (\sum s)(\sum t)}{n}$$

$$\sum(t - \bar{t})(s - \bar{s}) = \frac{n\sum st - (\sum s)(\sum t)}{n} \quad \text{.....(32)}$$

$$\sum(t - \bar{t})^2 = \sum\left(t^2 - 2t\bar{t} + \bar{t}^2\right) \quad \text{.....(33)}$$

$$\sum t^2 - \sum 2t\bar{t} + \sum \bar{t}^2 \quad \text{.....(34)}$$

$$\sum t = n\bar{t} \quad \text{.....(35)}$$

$$\sum s = n\bar{s}$$

Substituting equation (34) into equation (33) we have

$$\sum t^2 - 2\bar{t}\ x\ n\bar{t} + n\bar{t}^2 = \sum t^2 - 2n\bar{t}^2 + n\bar{t}^2$$

$$\sum t^2 - n\bar{t}^2 = \sum t^2 - n\left(\frac{\sum t}{n}\right)^2 = \sum t^2 - \frac{(\sum t)^2}{n} = \frac{n\sum t^2 - (\sum t)^2}{n}$$

$$\sum (t-\bar{t})^2 = \frac{n\sum t^2 - (\sum t)^2}{n}$$

Substituting equations (32) and (37) into equation (24) we have

$$a = \frac{n\sum t^2 - \sum (t-\bar{t})(s-\bar{s})}{\sum (t-\bar{t})^2}$$

$$a = \frac{n\sum st - (\sum s)(\sum t)}{n} \Big/ \frac{n\sum t^2 - (\sum t^2)}{n}$$

$$a = \frac{n\sum st - (\sum s)(\sum t)}{n} \; x \; \frac{n}{n\sum t^2 - (\sum t^2)}$$

$$a = \frac{n\sum st - (\sum s)(\sum t)}{n\sum t^2 - (\sum t)^2}\,\bar{t} \quad \text{.....(39)}$$

Substituting equation (38) into equation (25) we have

$$B = \bar{s} - \frac{n\sum st - (\sum s)(\sum t)}{n(\sum t^2) - (\sum t)^2} \; \frac{\sum t}{n}$$

Substituting equation (38) and (40) into equation (23) we have

$$S = \left(\frac{n\sum st - (\sum s)(\sum t)}{n\sum t^2 - (\sum t)^2} \right) t + \frac{\sum s}{n} - \frac{n\sum st -}{n(\sum t^2) - (\sum t)^2} \; \frac{\sum t}{n} \quad \text{.....(41)}$$

equation (41) represent the developed. The equation representing the mathematical model is (41). The above equation can be tested using individual concentration of benzene, toluene and xylene. Equation (41) can be written in terms of BTX. Contaminants, thus,

For Benzene

$$S_B = \left(\frac{n\sum st - (\sum s)(\sum t)}{n\sum t^2 - (\sum t)^2} \right)_B t + \left(\frac{\sum s}{n} - \frac{n\sum st - (\sum s)(\sum t)}{n(\sum t^2) - (\sum t)^2} \; \frac{\sum t}{n} \right)_B \quad \text{.....(42)}$$

For Toluene

$$S_B = \left(\frac{n\sum st - (\sum s)(\sum t)}{n\sum t^2 - (\sum t)^2} \right)_T t + \left(\frac{\sum s}{n} - \frac{n\sum st - (\sum s)(\sum t)}{n(\sum t^2) - (\sum t)^2} \; \frac{\sum t}{n} \right)_T \quad \text{.....(43)}$$

For Xylene

$$S_B = \left(\frac{n\sum st - (\sum s)(\sum t)}{n\sum t^2 - (\sum t)^2} \right)_x t + \left(\frac{\sum s}{n} - \frac{n\sum st - (\sum s)(\sum t)}{n(\sum t^2) - (\sum t)^2} \; \frac{\sum t}{n} \right)_x \quad \text{.....(44)}$$

Equations (42), (43) and (44) can be simplified as

$$S_b = a_b t + b_B \quad \text{.....(45)}$$
$$S_t = a_t t + b_T \quad \text{.....(46)}$$
$$S_x = a_x t + b_x \quad \text{.....(47)}$$

For Benzene, Toluene, and Xylene

$n = 6,\ \sum t = 105,\ \sum S_b = 198.15,\ \sum t^2 = 2695,\ \sum S_b t = 3185.35 \sum S_t =$ 200.41,

$$\sum S_t t = 2941.26,\ \sum S_x = 313.03,\ \sum S_x t = 5433.19$$

$S_b = - 0.3292t + 38.786$

$S_t = - 0.66t + 44.95$

$S_x = - 0.052 + 53.08$

MATERIALS AND METHODS

Sample Collection

All the samples used in carrying out this research work was obtained in Niger Delta area of Nigeria.

Particle Size Analysis

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

Reagent: Sodium hexamata –phoshate dispersing agent, 50% (calgin)

Procedure: the following procedures were used such as: Weigh a quantity of air-dried soil (102g for coarse textural soil or 51g for fine –textural soil) and place in a 500ml –dispersing cup. Fill the cup to within 5cm of the top with distilled water. Add 20ml of dispersing solution and allow to soak for about 15min. Insert the baffle into the cup and lower the stirrer blade into the suspension and stir the contents for 10 minutes. Make the suspension in the cylinder up to 1250ml mark (if 102g of soil was used) or 1000ml mark (if 51g soil was used). Do this with the hydrometer in the suspension. Remove the hydrometer. Cover top of cylinder with the hand and invert several times. Place cylinder on flat surface and note time. after about ½ minutes place the hydrometer slowly and carefully in the suspension. At 40sec. Exactly take the hydrometer reading. Remove the hydrometer and record the temperature of the suspension. (Place the thermometer in and out of the suspension very carefully). Just before 2 hours have elapsed replace the hydrometer inside the suspension and take a reading. Note again the temperature of the suspension. Correct the hydrometer reading 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.

Calculation: Let H_1 and H_2 be the hydrometer reading and T_1 and T_2 be the temperature (^{0}F) at 40sec and 2hrs respectively. Let T^{0}F be the calibration temperature of the hydrometer. (20^0c) % (silt + clay) $= [H_1 + 0.2(T_1 - T) - 2.0] \times \frac{100}{50}$ (50 in the denominator for 51g sample – use 100 for 102g samples). % Clay $= [H_2 + 0.2(T_2 - T) - 2.0] \times \frac{100}{50}$

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

Soil pH

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

Reagents: Distilled water, 0.01M $CaCl_2$: Dissolve 1.11gm of KCl in 1 litre of distilled water. 1.0M of KC1: Dissolve 74.6gm of KCl in 1 litre of distilled water. Buffer solutions of

pH 4.0, 7.0 and 9.0. These buffers are prepared by dissolving standard buffer tablets or by diluting buffer are prepared by dissolving standard buffer tablets or by diluting buffer concentrations as instructed by the suppliers. 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:- Dissolve 5.106g of reagent grade potassium hydrogen phthalate ($KHCH_4O_4$) and make up to 500ml with CO_2 must be used. If commercial buffer solutions or tablets are not available, prepare standards as follows: pH 4.0:- Dissolve 5.106g of reagent grade potassium hydrogen phthalate ($KHCH_4O_4$) and make up to 500ml 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 20gm of air-dried soil (passed through 2mm sieve) in a 50ml beaker, and 20ml of distilled water and allow to stand 30minutes 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 with 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 Soils By The Method Of Walkley And Black (1934)

Apparatus: Analytical balance, magnetic stirrer and a bulb- lamp 500ml Erlenmeyer flasks 50ml burette, 50ml measuring cylinder, 500ml measuring cylinder, 10ml pipette , 25ml pipette.

Reagents: 1.0N of $K_2Cr_2O_7$. Dissolve 4 9. 04g of reagent grade $K_2Cr_2O_7$ (previously dried at 105°C) in distilled water, and dilute the solution to 1litre. Concentrated H_2SO_4. 0.5 N of $FeSO_4$. 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. Tin's is prepared by dissolving I96g of Fe $(NH_4)_2$ $(SO_4)_2$ $6H_2O$ in about 800ml distilled water and 20ml cone. H_2SO_4 and diluting to 1 litre. 0.5N of $KMnO_4$. Heat 16gms of $KmnO_4$ 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 oxaJate. Store the solution in a glass Stoppard amber bottle.

Procedure: Weigh accurately about l.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). Pepette 10ml of in $K_2Cr_2O_7$ into the flask and swirl gently to disperse the soil. Rapidly add 20ml conc.H2S04 (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. Add about 300ml of distilled water and accurately 25ml of 0.5N of $FeSO_4$. 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. Make a blank determination in the same manner, but without soil.

Calculations: 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 $N(T - B) \times 3$mg carbon

$\therefore$ 100gm soil contain $\frac{N(T-B)x3}{W} x \frac{100}{1000} g\ carbo$

$\therefore$ % organic carbon in the soil according to the organic carbon in the soil $\frac{N(T-B)\ x\ 3}{10W} x$

$\therefore$ True % of organic carbon in the soil

$= \frac{N(T-B)}{10W} x \frac{100}{77}$

$= \frac{N(T-B)}{W} x\ 0.390$ % organic matter is calculated by multiplying % organic carbon by 1.724.

Total Nitrogen In Soils By Kjeldahl Digestion And Distillation

Apparatus: Macro Kjeldahl digestion apparatus (in fume cupboard), Macro Kjeldahl digestion apparatus, Kjeldahl flask (500ml) , burette (50ml), Erlenmeyer flask (1 litre), Erlenmeyer flask (500ml), Measuring cylinder (50ml), Measuring cylinder (250ml).

Reagents: Concentrated H_2SO_4 K_2SO_4, - plus - catalyst mixture: - mixture contain l00gms $KiSO_4$, 10gm $CuSO_4$. $5H_2O$ and 1. 0gm se. Sodium hydroxide (NaOH), approximately I0 N. 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 O_2. Mixed boric acid - indicator solution: Dissolve 20gm of boric acid (H_3BO_3) in about 800ml distilled water in a i 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 H_2SO_4, 0.01N.

Procedure: Weigh accurately about Ig 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. Add 11gm of K_2SO_4 -plus- catalyst mixture and 30ml concentration H_2SO_4 through automatic pipette (or measuring cylinder under a fume cupboard). 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 $H2S0_4$ condenses about halfway up the neck of the flask. 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. 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. 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, swirling and commence distillation. Keep condenser cool by allowing sufficient cold water to flow through and regulate heat to minimize frothing and prevent suck-back. Collect about 150ml of distillate, remove the receiver flask and then stop distillation. Titrate the NH^+ in the distillate with standard Hcl or H_2SO_4. the colour change at the end point is from green to pink. Carry out similar distillation with blank (without soil).

Calculation: Let Wg be the weight of soil used, Tml, burette reading for the sample, Bml burette reading for the blank; N, men normality of H_2SO_4Corrected Volume of H_2SO_4 = (T - B)ml
Amount of H_2SO_4 = N(T - B)mq
Amount of NH_3 in distillate = N (T - B)mq
Amount of Nindistillate = N(T - B)mq

$$= N(T \sim B) \times 14mg$$

$$= \frac{N(T-B)x14mg}{1000}$$

$$\text{Wg of soil contain} = \frac{N(T-B)x14of\ N}{1000}$$

$$\therefore \text{100g soil contain} = \frac{N(T-B)x\ 14\ x100}{1000\ x\ W}$$

$$\therefore \text{Percentage total N in the soil} = \frac{N(T-B)x\ 14\ x100}{1000\ x\ W}$$

Available Phosphorus in Soil By Bray and Kurtz

Apparatus: Mechanical bottle shaker B & L spectronic -20 Spectro- photometer Test tubes with stoppers, Funnels, 1 ml pipette, 4ml pipette, 50ml graduated pipette, 10ml graduated pipette, 10ml of graduated cylinder.

Reagents: Ammonium fluoride (NH_4F), IN: Dissolve 3.7g of NH_4F in distilled water and dilute the solution to 100ml. and put this solution in a polyethylene bottle. Hydrochloric acid (Hcl), 0.5N: Dilute 20.2ml conc. Hcl to a volume of 500ml under a fume hood. Extracting solution (0.03N NH_4F and 0.025NHcl): Add 15ml of 1.0N of NH_4F and 25ml of 0.5NHcl to 460ml distilled water.

Reagent A: (a) Dissolve 12g of ammonium molybdate $(NH_4)_6$. $MO_7O_{24}4H_2O$ in 250ml distilled water (b) Dissolve 0.2908g of potassium antimony tartarate ($KsbOC_4H_4O_6$) in 100ml of distilled water. (c) Prepare $5NH_2SO_4$ by diluting approximately 148ml conc. H_2SO_4 in about 100ml of distilled water. (d) Mix solutions (a), (b) and (c) Together in a 2 litre volumetric flask and make up to mark with distilled water. 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. 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 100ppm p. pipette 5ml of 100ppm p solution into a 100ml 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.

Procedure: Weigh 2.85g soil into a tube and add 20ml of the extracting solution. Shake the tube for I minute, and filter the content through whatmann No. 42 paper. If the filtrate is not clear quickly pour the solution back through the filter. Pipette 10ml alique of the soil extract into a 50ml volumetric flask and add 10ml of distilled water. Add 4ml of reagent B and make up to volume with distilled water. Allow, the colour to develop for 15 minutes. 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 5 ppm p stock to 50ml.) Each of the standards should contain 10ml of the extracting solution and 4ml of reagent B. Allow the colour to develop for 15minutes and measure absorbance of the standards on a spectrophotometer at 6,60N. Draw a standard curve by plotting absorbance Vs concentration in a graph paper. Measure absorbance of the sample containing the soil extract and determine the p concentration from the standard curve.

Calculations: Let the concentration of p in the diluted soil extract by yppm

$$\therefore \text{P concentration in the undiluted extract} = \frac{50}{10} yppm$$

$$\therefore \text{Amount of P in the 20ml undiluted extract} = \frac{50}{10} y\ x20Ng$$

This is present in 2.85gm soil

$$\therefore \text{1 gm soil contain } \frac{50}{10} y\ x\ \frac{20}{2.85}\ Ng$$

$$\therefore \text{Available } \rho \text{ in the soil} = \frac{50}{10} y\ x\ \frac{20}{2.85}\ ppm$$

Experimental Set-Up for Bioremediation of BTX

Material Equipment And Apparatus: Weight balance, Glass rod, 250ml plastic containers, Pipette 0.5ml and IML capacity, Measuring cylinder (150ml), Screw cap bottle, Distilled water, 0.5m of individual contaminants, 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 weighted to determine their various weights and recorded. The total number of cylindrical containers used were fifteen (15) 200g of the soil sample of level 0 -1 was weighted 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, 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} was done. Two rops (0.1ml) aliquots) of 10^{-2} and 10^{-3} dilutions were inoculated into 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 bacterial 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: BTX standard mix, Methanol (chromatographic grade) Distilled water, Agilent 6890N Gas chromatography, Glass grew cap vials, Micro – syringes, Analytical balance, Pipettes.

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

RESULT AND DISCUSSION

The results of laboratory analysis on the soil sample obtained from Ngrar soil of Omuigwe Aluu in Rivers State of Nigeria is shown in Table 1. 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 microorganisms in the soil were used for the remediation process.

Table 1. Results of laboratory analysis of five (5) Ngara Soil Samples obtained at Omuigwe Aluu

Parameters	Soil Depths (m)				
	0-1	1-2	2-3	3-4	4-5
Soil pH (1:25)	5.10	4.80	5.20	4.80	5.00
Electrical conductivity (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	0.10	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

Contaminants Concentration

The results of the concentration of the contaminants with respect to time are presented in Table 2.

Table 2. Concentration of the various contaminants with time

Time (Day)	Concentration of benzene (S_b)g/ml	Concentration of toluene (S_t)	Concentration of xylene (S_x)g/ml
0.00	39.06	46.07	53.09
7.00	36 .42	40.05	52.72
14.00	33.96	34.82	52.35
21.00	31.66	30.27	51.99
28.00	29.52	26.32	51.62
35.00	27.53	22.88	51.26

Benzene Concentration: The variation of concentration of benzene with time is shown in Table 2. The concentration of benzene in benzene samples has optimum value of 36.06g/ml at 0 day, then decrease in descending order as the days are increasing 36.42g/ml at 7 days, 33.96 at 14 days, 31.66g/ml at 21days, 29.52g/ml at 28 days and 27.53g/ml at 35 days.

Toluene Concentration: The variation of concentration of Toluene with time is depicted in Table 2. The concentration of Toluene in Toluene samples has optimum value of 46.07g/ml at 0 day, then decreases to 40.05g/ml at 7 days, 34.82g/ml at 14 days, 30.27g/ml at 21 days, 26.32g/ml at 28 days, and 22.88g/ml at 35 days.

Xylene Concentration: The variation of concentration of xylene with time is depicted in Table 2. The concentration of xylene samples has its optimum value of 53.09g/ml at 0 day, then decreases to 52.72g/ml at 7 days, 52.35g/ml at 14 days, 51.99g/ml at 21 days, 51.62g/ml at 28 days and 51.26g/ml at 35 days.

Concentration Inverse and Specific Rate Inverse

Table 3. depicts the concentration inverse and specific rate inverse of the contaminants.

Reaction Rate Constants for Contaminants

Result presented in table 4 depicts the determination of reaction rate constants of various contaminants with respect to time.

Table 3: Computation of specific rate of benzene, toluene and xylene ($\mu_B = K_B S_B$; $\mu_T = K_T S_T$ and $\mu_x = K_x S_x$)

μ_B	μ_T	μ_x	$^1/\mu_B$	$^1/\mu_T$	$^1/\mu_x$	$^1/S_B$	$^1/S_T$	$^1/S_x$
0.39	0.92	0.053	2.56	1.09	189	0.026	0.022	0.0188
0.36	0.80	0.053	2.78	1.25	189	0.027	0.025	0.0190
0.34	0.69	0.052	2.94	11.45	19.2	0.029	0.029	0.0191
0.32	0.61	0.052	3.13	1.64	192	0.032	0.033	0.0192
0.30	^0.53	0.052	3.33	1.89	192	0.034	0.038	0.0194
0.28	0.46	0.051	3.57	2.17	196	0.044	0.044	0.095

Table 4: Computation of substrate logarithm

Time (day)	S_B/S_{BO}	S_T/S_{TO}	S_x/S_{xo}	$\ln S_B/S_{BO}$	$\ln S_T/S_{TO}$	$\ln S_x/S_{xo}$
0.00	1.0000	1.0000	1.0000	0.000	0.000	0.000
7.00	0.9324	0.8693	0.9930	-0.069	-0.140	-0.007
14.00	0.8694	0.7558	0.9861	-0.140	-0.280	-0.014
21.00	0.8105	0.6570	0.9793	-0.280	-0.560	-0.021
28.00	0.7558	0.5713	0.9723	-0.280	-0.560	-0.028
35.00	0.7048	0.4966	0.9655	-0.3498	-0.700	-0.035

Determination of Rate Constant of Benzene, Toluene and Xylene

This is determine by plotting In (S_B/S_Bo) against time for benzene the slope of the line is the rate constant of benzene as shown ie. Rate constant of benzene, Toluene and Xylene are presented as shown K_{SB}

$$= \frac{\Delta(InS_B\, IS_{BO})}{\Delta t}, K_{rt} \quad \frac{\Delta(InS_t\, IS_{ttO})}{\Delta t} \; and \; K_{rx} = \frac{\Delta(InS_x\, IS_{xo})}{\Delta t}$$

Dissociation Constants, Monod Constant and Maximum Specific Rate Constant

Result presented in table 5 shows the dissociation constant of the various contaminants**.**

Table 5. Computation of various dissociation constant of Benzene, Toluene and Xylene

Parameter	Benzene	Toluene (K_{ST})	Xylene (K_{SX})
K_S (day^{-1})	0.01	0.02	0.001
K_m(g/mld)	143	333	90
u_{rms}(g/mld)	2	7	0.14

Determination of Percentage Rate Constant

Mathematical computation for determination of percentage rate constant was obtained by summing of the rate constants of the contaminants, and take percentage of each as shown below. $K_{STOT} = K_{SB} + K_{ST} + K_{SX}$, %Rate constant of benzene = $\frac{K_{SB}}{K_{STOT}}\, x\, 100$

for benzene, toluene and xylene

The above percentages of the contaminants obtained is as shown, that is % rate constant of benzene is 32.26%, toluene is 64.52%, and Xylene is 3.23%

Maximum Specific Rate of Reaction for single Enzyme Catalysed Reaction

Recalling the Monod's equation, thus:

$$\mu^S_{\max}(t) = -K_{si}\, In\beta_i + 1 - \beta_i$$

For Benzene

$$\mu^S_{\max}(t) = -K_{sB}\, In\beta_B + 1 - \beta_B$$

At time t = 0, $\mu^S_{\max}(0) = 0.01\, x\, 0 + 1 - 1 = 0$, the same procedure was repeated for the other time.

For Toluene: $\mu^S_{\max}(0) = 0.02\, x\, 0 + 1 - 1 = 0$ The same procedure was repeated for the other time.

For Xylene: $\mu^S_{\max}(t) = -K_{sx} In\beta_x + 1 - \beta_x$ At time t = 0

$\mu^S_{\max}(0) = -0.001\, x\, 0 + 1 - 1 = 0$

The same procedure was repeated for the other time

Table 6. Computation of maximum specific rate of reaction for single enzyme catalyzed reaction with time of the contaminants

Time (Day)	Maximum Specific Rate (g/mlday)		
	Benzene	Toluene	Xylene
0.00	0.0000	0.0000	0.0000
7.00	9.9346	46.7927	0.637
14.00	20.1506	93.5682	1.2739
21.00	40.2295	186.991	1.9107
28.00	40.2842	187.0767	2.5477
35.00	50.3166	233.8134	3.1845

Table 7. Comparative results between the experimental and model computation of concentration of benzene (S_b)

Time	Experimental	Model	Error
0.00	39.06	38.79	0.27
7.00	36.42	36.48	-0.06
14.00	33.96	34.18	-0.22
21.00	31.66	31.88	-0.22
28.00	29.52	29.68	-0.16
35.00	27.53	27.26	0.27

Table 8: Comparative results between the experimental and model Computation of Concentration of Concentration of Toluene (S_t)

Time	Experimental	Model	Error
0.00	46.07	44.95	1.12
7.00	04.05	40.33	-0.28
14.00	34.05	35.71	-1.66
21.00	30.27	31.09	-0.82
28.00	26.32	26.47	-0.15
35.00	22.88	21.85	1.03

Table 9: Comparative results between the experimental and model computation of concentration of concentration of Xylene (S_x)

Time	Experimental	Model	Error
0.00	53.09	53.08	0.01
7.00	52.72	52.72	0.00
14.00	52.35	52.35	0.00
21.00	51.99	51.99	0.00
28.00	51.62	51.62	0.00
35.00	51.26	51.26	0.00

Table 1: Shows the results of analysis for the five Ngara soil samples at different levels. The analysis of microbial population of bacteria and fungi for the various contaminants are shown in Table 2. The increase in time increases the microbial population in bacteria up to 28 days and declination occur at 35 days for both toluene and xylene expect benzene that maintained constant population at 28 days. While fungi shows increase in population up to 28 days and decline at 35 days for all the contaminants. The higher molecular mono aromatic hydrocarbons shows increase in there concentration. There were decreases in the concentration of the contaminants with time of the contaminants. Table 3 shows the concentration inverse and specific rate inverse of the contaminants with time. Table 4 shows the determination of reaction rate constants of the various contaminants with time. Table.5 shows the dissociation constant, Monod constant specific rate constant of the various contaminants. Table 6 shows the dilution rate for the various contaminants with time. Table 7 shows the percentage of dilution rate of the contaminants with time. The values of maximum specific rate of reaction for single enzyme catalysed reaction $\mu^{S}_{\max}$ were determined using the data in Table 4 and presented in Table 6. Comparative results between the experimental and model computation of concentration of benzene is shown in table 7. Comparative results between the experimental and model computation of concentration of toluene is shown in Table 8, and Comparative results between the experimental and model computation of concentration of xylene is shown in Table 9. The specific rate of reaction seen to be decreasing with time and concentration. This is in line with previous reports by Ukpaka, (2008), the results analysed also show that bioremediation rate increases with increase in molecular weight. This phenomenon confirms previous research findings which indicate that the lighter hydrocarbons are more toxic to micro-organism due to their extraction effect or as a result of enzyme specificity Ukpaka (2006).

CONCLUSION

The results of the tests carried out on microbial population of fungi and bacteria, and concentration shows that microorganisms can be used to remediate an affected area by the contaminants. The following conclusions are made based on the result obtained in the research. The best microorganism that can be used to remediate affected area with the contaminants is bacteria. Toluene degrade faster than the other two contaminants under the same condition. The dilution rate of benzene is higher than the other two contaminants under the same condition. The concentration of toluene with time decreases faster than the others. The bacteria population of xlyene is higher than the other under the same condition. The fungi population of xlyene is higher than the others under the same condition.

NOMENCLATURE

C = Contaminant concentration as a function of x , y, z, t

R = Retardation factor representing equilibrium sorption

V = Uniform pore-water velocity (m/s)

D_L = Longitudinal dispersion coefficient (in the direction of flow) = $\alpha_L V$

□L = Longitudinal dispersivity (ml)

□ = First order transformation rate coefficient (m/s) = In2/$t^{1/2}$

$t^{1/2}$ = Half - life of transformation rate (s).

$\mu^{S}_{\max}$ = Maximum specific rate of reaction for single enzyme catalysed reaction (cfu/ml/day).

t = Time (day)

μ^{m} = Specific rate of reaction for multiple enzyme catalysed reaction (cfu/ml/day).

$\mu^{m}_{\max}$ = Maximum specific rate of reaction for multiple enzyme catalysed reaction (cfu/ml/day).

K_S = First order constant $(mol\%)^{-1}$

K_m = Monod constant or dissociation contant $(cfu/ml)^{-1}$

$\mu_{\max}$ = Maximum specific rate (cfu/ml/day)

F = Flow rate (am^3/day)

V = Rate reactor volume (Am^3)

D = Dilution rate $(day)^{-1}$

Rs = Rate of substrate reaction (cfumol%/ml)

REFERENCES

Anderson, R.T and lovely, Di.R (2000). Anaerobic bioremediation of benzene under sulfate –reducing condition in a petroleum contaminated aquifer. *Environ. Sci. Technol*. 34, pp. 2261 -2266.

Beck, F; Kaus, R; & Orberts, M. (2001). Tran passive dissolution of iron to ferrate (vi) in concentrated alkali hydroxide solutions. *Electrochemical Acta.* 30 (2) pp.173-183

Borden, R.C. homez, C. A & Becker, M. T. (1995) Ground – water contamination, *Environ. Sci. Technol*.33, pp. 180 -189.

Cavigelli, M. A & Robertson, G. P (2000). The functional significance of denitrified community composition in a terrestrial ecosystem. *Ecology*, 81. pp.1402 – 1414.

Felter, C.M. (2001). Applied Hydrogeology. 4th edition, Macmillan Publishers, New York, pp. 105 – 357.

Freeman, D. J. & Cattel, F.C (1990). Wood-Burning as a source of atmospheric polyhydric aromatic hydrocarbons. *Environ. Sci. Technol*. 24, pp. 1581-1585

Frostegard, A; Tunlid, A & Baath, E. (1991). Microbial biomass measured as total lipid phosphate in soils of different organic content. *J. Microb. Meth*. 14 (5), P.163.

Gao, Y; Zhu, L; & Ling, W. (2005). Validation of the partition –limited model for plant uptake of organic chemical from soil and water. *Sci. Total Environ,* 336 (1-3) pp. 171 – 182. Hirose, T; Morito, K; Kizu, R; Toriba, A; Hayakawa, K; Ogawa, S; Inoue, S; Muramatsu, M; & Masamune, Y. (2001). Estrogenic /antestrogenic activities of benzo (a) pyrene monohydroxy derivatives. *J. Heath Sci*. 47, pp. 552 – 558.

Hua, Y; Yu, H.S; Yong, G.Z; Mei, M. C; Chen, B.D & Yun, X, L. (2007). Influences of polyhydric aromatic hydrocarbons (PAHs) on soil microbial community composition with or without vegetation, *J. Environ. Sci. and Health Part* A, 42, pp 65 -72.

Jiang, J. Q; Panagoulopoulos, A; Bauer, M. Pearce, P. (2006). The application of potassium ferrate for swage treatment. *J. Environ. Manag*. 79 (2), pp. 215 -220.

Johnsen, A. R; Winding, A; Karlson, U; Roslev, P. (2002) Linking of microorganisms to phenanthrene metabolism in soil by analysis of C -13 – Labeled cell lipids. *Appl. Environ. Microbiol.* 68 (12), pp. 6106 -6113.

Li, H; Sheng, G; Chiou, C.T; Xu, 0. (2005). Relation of organic contaminant equilibrium sorption and kinetic uptake in plants. *Environ. Sci. Techno*l. 39, pp. 4864 – 4870.

Lovanh Land Alvarez, p. J. J. (2003). Effect of ethanol in BTEX biodegradation activity using a bioreporter strain in continuous culture. 25th ACS National Meeting, 43 (1), pp. 669 -673, new Orleans, LA March 23 -27.

Lovely, D. R. (2000). Anaerobic Benzere degradation. Bioremediation II. *Journal of Industrial Microbiology and Biotechnology* 24 (22), pp. 107 -116.

Mugssen, B.T; & Janssen, C.R. (2005). Importance of acclimation to environmentally relevant zinc concentrations on the sensitivity of daphnia magna toward zinc. *Environm. Toxicol. Chem*. 24 (4), pp.895-901.

Newman, L.A; Strand, S.E; Choe, N; Duffy, J; & Ekuan, G (1997). Uptake and biotransformation of trichloroethylene by hybrid poplars. *Environ, Sci. Technol.* 31, pp. 1062 – 1067.

Paul, S. (1975). Pollution Engineering Hand Book. M.V – Graw- Hill Publish London, pp. 226 -407.

Pilon – Smits, E (2005). Phytoremediation. Annu. Rev. Plant Bio. 26, pp. 15 -39.

Powers, S.E.; Rice, D; Dooher, B. & Alvarez, P. J.J (2001). Will ethanol blended gasoline affect ground water quality using ethanol of MTBE as a gasoline oxygenate could be less harmful to the environment. *Environ. Sci. Technol*. 35, pp. 24A – 30A.

Ruiz - Aquifer, G.M.L, O' Reilly, K Alvarez, P.J.J (2002). A comparison of benzene and toluene plume lengths for sites contaminated with regular vs. ethanol –amended gasoline. *Ground Water Merit.* 23, pp. 48 – 53.

Straube, W.L., Nestler, C.C; Hansen, L.D.; Ringleberg, D; Pritchard, P.H; & Jones –Mechan, J. (2003). Remediation of Polyaromatic hydrocarbons (PAHs) through Land farming with biostimulation and bioaugmentation. *Acta biotechnol*. 23 (2-3), pp.179-196

Ukpaka C.P; Abowei, F.M and Okerie, U. (2009). Evaluation of Biostimulation rate of BTEX compounds of contaminated site. *Multidisciplinary Journal of Research Development*, 12 (2) pp.114 – 120.

Ukpaka, C.P (2006). Modeling Degradation Kinetics of Petroleum hydrocarbon mixture at specific concentration. *Journal of Research in Engineering* 3 (3), pp.47-45.

Ukpaka, C.P; (2009) Development of the Biokinetic model for the production of ammonia from urea in a continuous stirred tank reactor (CSTR), Knowledge Review: *A Multidisciplinary Journal,* 31 (1), pp. 76-80.

Wick, L.Y; Pasche, N; Bernasconi, S. M; Pelz, O; Harms, H. (2003). Characterization of multiple-substrate utilization by anthracene degrading mycobacterium frederiksbergense (B501). *Appl. Environ. Microbiol*. 69 (10), pp. 6133 – 6142.

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