

A PRELIMINARY INVESTIGATION OF THE INVOLVEMENT OF NATURAL ATTENUATION PROCESSES IN THE FATE AND TRANSPORT MECHANISM OF PHENOL IN A NIGER DELTA REFINERY EFFLUENT MICROCOSM

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ABSTRACT

The natural attenuation processes involved in the fate transport mechanism of phenol in the Port Harcourt refinery effluent wastewater were established. In the study using microcosms with 4.91mg/l of phenol for 42 days, photooxidation, evaporation and volatilization collectively accounted for 2.74mg/l (55.8%) of phenol removed. Adsorption was responsible for removal of 0.29mg/kg (5.91%) of phenol. Biodegradation accounted for the removal of 1.88mg/l (38.3%) of phenol in the study. The statistical analyses showed no significant differences in the means of the pH and temperature values in the different treatments and in the log₁₀ total viable count for treatments where no biocide was added. Significant differences were observed in the weekly pH and temperature values as well as the phenol values recorded weekly and in the different treatments. Using a mineral salts base, a total of 25 phenol utilizing bacteria were isolated. Characterization and identification tests of the phenol utilizing bacteria revealed that they belong to the genera *Pseudomonas*, *Bacillus*, *Micrococcus*, *Corynebacterium*, *Staphylococcus*, *Chromobacter* and *Serratia*. The study has shown that there is a rich consortium of phenol utilizing bacteria present in the refinery effluent and that natural attenuation processes including biodegradation play a significant role in the fate transport of phenol released from the Port Harcourt refinery effluent wastewater. There is great need, however, in effecting a more efficient treatment for the removal of phenol in the refinery effluent wastewater. In this way, the receiving Okrika River would not be turned into a "phenol river".

KEYWORDS: Natural attenuation, phenol, fate and transport mechanism, refinery effluent, Niger Delta, microcosm

INTRODUCTION

Aqueous effluents from petro-chemical and related industries contain organic pollutants such as phenol (a hydroxyl derivative of benzene), which is toxic and causes considerable damage and heat to the ecosystem and human health. At very high concentrations of phenol, conventional biological oxidation processes are inapplicable and this causes a need for the development of efficient and inexpensive treatment process (Christoskova and Stoyanova, 2001).

Phenol is considered to be refractory with regard to the number of oxidative methods required for its removal from waste streams. It is probably the most important class of toxic organics both in petroleum refining and coal conversion processes (Bandy *et al*, 1985, Obiukwu and Abu, 2003). Thus an ideal phenol treatment method should provide for a complete mineralization of the toxic compound without any hazardous residues remaining.

Wastewaters can be treated by physico-chemical processes though this is considered to be generally less environmentally friendly, generating large volumes of chemical sludge and often requiring a pre-dilution of the wastewater to be treated. According to Mantzavinos *et al*, (2001), an attractive potential alternative to complete treatment by means of chemical oxidation would be the use of an integrated chemical and biological treatment process comprising a chemical pretreatment step to convert initially bio-resistant compounds to more readily biodegradable intermediates, followed by biological oxidation of these compounds to achieve sewer or river course discharge limits. These wastewaters can undergo natural attenuation, which is a passive remedial approach often referred to as a no-action option that depends upon natural processes to degrade and dissipate contaminants in soil and ground water (Kao *et al*, 2001). Natural attenuation for the bulk removal of phenol is considered a preferred option (Lop and Tar, 2000). There are classically seven physico-chemical and biological natural attenuation processes, which

include adsorption, evaporation, volatilization, photooxidation, biodegradation, dilution and dissolution.

Natural attenuation has the following advantages: less intrusive as few surface structures are required; may be applied to all or part of a given site depending on site conditions and clean-up objectives; may be used in conjunction with or as a follow-up to other (active) remedial measures; overall cost will likely be lower than active remediation; above all, the microorganisms for the biodegradation component of natural attenuation are found almost everywhere and biodegradation can be very safe and effective. When natural attenuation is chosen as a cleanup method, a monitoring programme is still required to assess the performance of natural attenuation (Venosa *et al*, 2001).

Hence this work is aimed at defining and establishing through microcosm studies, the fate of phenol contained in the effluent waste water of the Port Harcourt refinery, and thus determine the natural attenuation processes involved in the removal of phenol in this Niger delta setting. Such studies are currently lacking and thus the fate of phenol as a pollutant in the effluent wastewater of the refinery and the receiving environment is poorly understood.

MATERIALS AND METHODS

Experimental station

An experimental station was constructed. The station measuring 3.05m length, 2.45m width and 2.10m height was erected with wood. The roof was made of plastic translucent roofing sheets to allow sunlight pass through. The sides (up to above half the height) were covered with zinc roofing sheets to prevent unwarranted visitors. The station was constructed in an open field, hence subject to all natural attenuation processes applicable to a simulated condition. Different natural attenuation processes were simulated using impacted material contained in glass tanks.

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Glass tanks

Four glass tanks designated A, B, C and D each measuring 45cm length, 35cm width and 25cm height were constructed and used for the experimental set-up to simulate different natural attenuation processes in the station. The set-ups were as follows:

Tank A Ten litres of the refinery effluent + 20kg of sediment from Okrika River. This river receives the final effluent wastewater from the refinery. The sediments were collected upstream of the point of discharge of the effluent. This tank was used to monitor attenuation of phenol by biodegradation, adsorption, volatilization, evaporation, photooxidation and all other natural attenuation processes.

Tank B Ten litres of the refinery effluent + 20kg of sediment from Okrika River. A biocide (Sodium Azide) was added weekly and homogenized. This tank was used to monitor disappearance of phenol through natural attenuation processes than biodegradation.

Tank C Ten litres of the refinery effluent only. Sediment and biocide were not added. This tank was used to monitor phenol disappearance through biodegradation and other natural attenuation processes except adsorption.

Tank D Ten litres of the refinery effluent only. No sediment was added here but biocide was added and homogenized. The tank was completely covered with a black polythene sheet. This tank served as a control, where supposedly none of the natural attenuation processes would take place.

Biocide Treatment

Sodium azide (BDH Chemicals England) was used as the biocide to eliminate microbial growth. Some 15 grams of the biocide was added to each of the two tanks (B and D) every 7 days. Effectiveness of the treatment was tested by determining viable bacterial counts on mineral salts agar plates from treated materials.

Collection of effluent sample and testing for effect of dilution on the effluent

The effluent wastewater sample used was collected from the concrete drainage that sends the effluent wastewater from the refinery into the Okrika River. It was collected just at the point before it gets into the river. The sample was collected with four sterile 10 litre Jerry cans each for each of the glass tanks.

To test for the effect of dilution, effluent samples were collected first from the concrete drainage at the point of discharge into the river, then after every 10 meters till the third point along the river. This was done thrice in three weeks.

Isolation, enumeration and identification of phenol utilizing bacteria

The mineral salts medium of Mills *et al.* (1978) was used as the base. It comprised: $MgSO_4 \cdot 7H_2O$; 0.40g, KCl; 0.28g, KH_2PO_4 ; 0.80g, Na_2HPO_4 ; 1.20g, NH_4NO_3 ; 0.40g, NaCl; 15g, Agar No.2; 20g, and 1 litre of de-ionized water. The medium was enriched with 0.02% (0.002M) phenol concentration as the sole carbon source and inoculated with 0.1ml of diluted samples from the tanks, and incubated for 5-7 days at 37°C. The colonies that developed on the plates were randomly picked and re-inoculated onto Mineral salts agar with phenol concentration of 0.02%. After this the colonies that developed, were purified by sub culturing onto fresh Nutrient agar plates using the streak plate technique. Isolated colonies were then transferred onto Nutrient agar slants and stored for further tests. Identification and characterization tests were carried out using standard procedures (Bergeys Manual of Determinative Bacteriology, 1994).

Determination of phenol concentration and physico-chemical parameters of the experimental set-ups

Phenol concentration was determined as described by Allen *et al.* (1984). The method depends on the formation of indophenols from *p*-aminodimethylaniline (ADA) and phenol in the presence of potassium ferricyanide. Phenol utilization was tested by measuring disappearance of phenol (through phenol concentration assay) and by estimating increase in total viable bacterial count.

The phenol in sediment was tested on the last day of the experimental monitoring to assay for phenol adsorption to the sediment. This was established through the phenol concentration assay of Allen *et al.* (1984).

The temperature readings of the samples in the glass tanks were determined using a mercury-in-glass thermometer, while the pH was measured using a pH meter (Jenway 3015 model).

Statistical analyses.

Analysis of variance (ANOVA) and the *t* - test were used to analyze the data generated in the microcosm set-ups.

RESULTS

Effect of Dilution

The phenol concentration assay on the samples collected from the river showed that the concentration of phenol decreased along the river course from the point of discharge. There was a steady decrease along the river course for the three weeks the sampling was done. The highest value recorded was 4.91 mg/l at the point of discharge while the least recorded was 0.63 mg/l after about 30m from the point of discharge along the river course. The average phenol concentrations recorded as the dilution test for the three weeks are as shown in Figure 1.

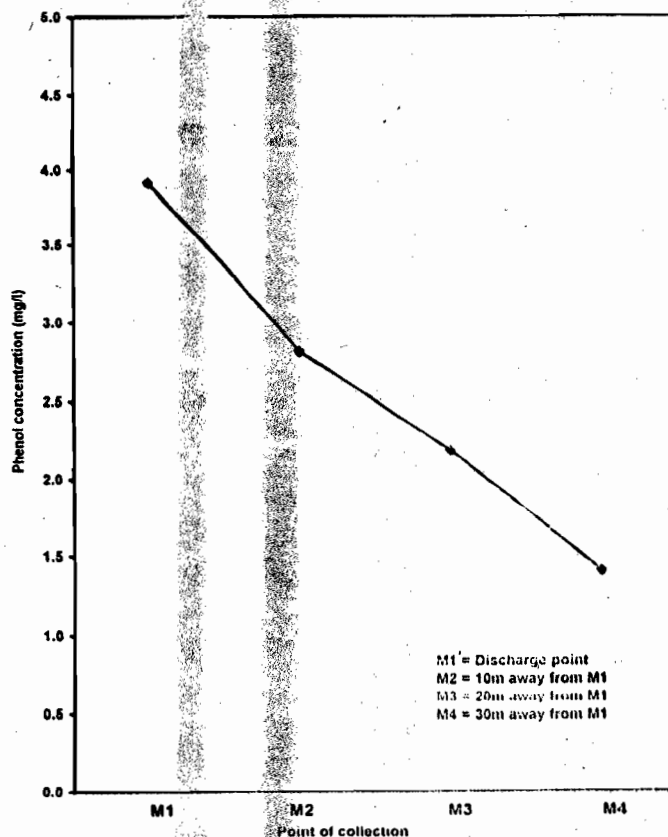


Fig. 1: Mean phenol concentrations at different points along the river from the point of discharge for Weeks 1 - 3.

Effect of other Natural Attenuation processes

The experimental set-ups in Tanks A - D were used to monitor other natural attenuation processes (except dilution) of phenol.

The weekly phenol concentration assay performed for six weeks for Tank A showed a gradual reduction in phenol concentration. This tank was used to monitor biodegradation and other natural attenuation processes such as evaporation, volatilization, photooxidation and adsorption. The phenol

concentration value dropped from the initial concentration of 4.91mg/l to 0.63mg/l after 6 weeks (Fig. 2). Thus 4.28mg/l (87.2%) of phenol was removed within the period. The phenol utilizing bacteria increased in number from the initial 1.89×10^5 cfu/ml to 3.0×10^8 cfu/ml in the third week. Then there was a decline to 5.6×10^5 cfu/ml in the sixth week (Table 1). There was a gradual increase in pH from the initial 7.95 to 9.62 in the fourth week then a decline to 8.98 in the sixth week. The temperature readings fluctuated between 24.5°C and 32.0°C.

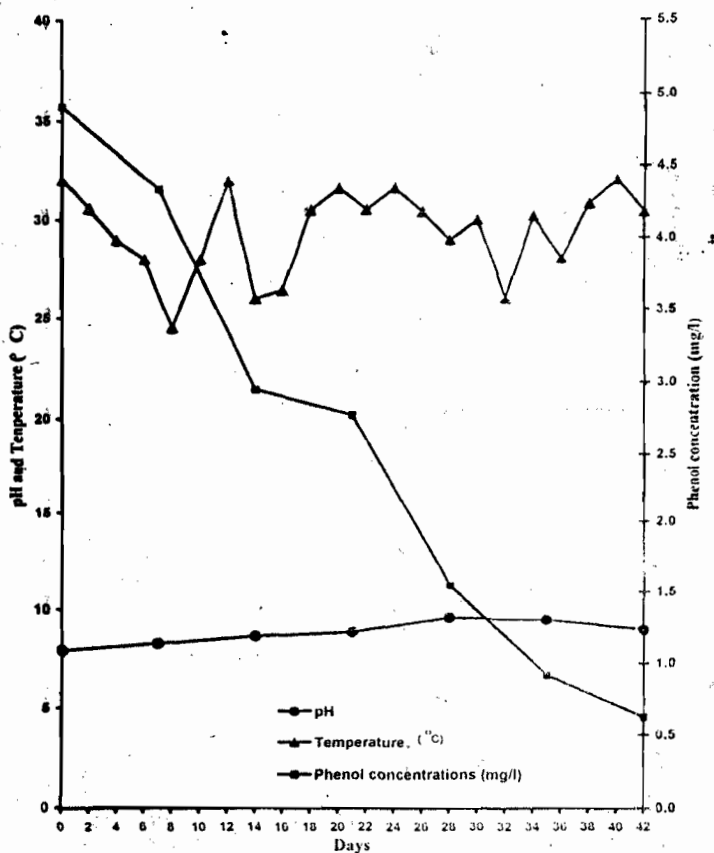


Fig. 2. Phenol concentration, pH and temperature values recorded in Tank A in the course of the study.

The phenol concentration assay for Tank B was used to monitor phenol removal by adsorption, photooxidation, volatilization and evaporation. The phenol concentration values here dropped from the initial concentration of 4.91mg/l to 2.51mg/l after 6 weeks (Fig. 3). Thus 2.40mg/l (48.9%) of phenol was removed within the period of 42 days. The phenol utilizing bacteria were completely inhibited following the weekly addition of the sodium azide biocide (15g per week), hence the total viable count for the phenol utilizing bacteria gave "too few to count" colonies or none at all. The pH values increased slightly as in Tank A from 7.95 to 8.98 in the fourth week and dropped to 8.00 in the sixth week (Fig. 3) while the temperature values fluctuated between 24.5°C and 34.6°C.

The phenol concentration analysis for Tank C used to monitor attenuation by evaporation, volatilization, photooxidation and biodegradation showed a reduction from the initial concentration of 4.91mg/l to 2.81mg/l in the third week and to 0.00mg/l in the sixth week (Fig. 4). Thus 4.91 mg/l (100% removal) of phenol was achieved in six weeks. Adsorption was not monitored here since no sediment was added. The phenol utilizing bacteria increased in number from 1.9×10^5 to 2.6×10^8 in the second week, and then decreased to 5.2×10^4 in the fourth week (Table 1). The pH increased

from 7.95 to 10.22 in the second week then decreased to 8.32 in the sixth week. The temperature values recorded were in the range of 25°C and 36.5°C (Fig. 4).

For Tank D, which served as the control, the phenol concentration decreased from the 4.91mg/l to 3.95mg/l in the sixth week showing 19.6% removal (Fig. 5). The total viable count of the phenol utilizing bacteria gave similar pattern of results as those of Tank B. The pH values increased from 7.95 to 8.60 in the sixth week then dropped to 8.07 in the sixth week while the temperature readings were in the range of 25°C and 31.0°C (Fig. 5). The graphical representation of the phenol concentrations, pH and temperature readings recorded over 6 weeks for these tanks are shown in figures 2-5.

Phenol in microcosm sediment

The phenol concentrations of the sediments in Tanks A and B due to adsorption are 0.36mg/kg and 0.21mg/kg respectively.

Total viable bacterial counts

The total viable bacterial counts for the tanks, presented as mean of two replicates, are as shown in Table 1.

Days	Tank A	Tank B	Tank C	Tank D
0	$1.9 \pm 0.2 \times 10^5$	$9 \pm 0.2 \times 10^5$	$1.9 \pm 0.2 \times 10^5$	$1.9 \pm 0.2 \times 10^5$
7	$3.8 \pm 0.5 \times 10^6$	TFTC	TFTC	
14	$1.5 \pm 0.8 \times 10^7$	TFTC	$4.5 \pm 1.0 \times 10^5$	
21	$3.0 \pm 1.4 \times 10^8$	TFTC	$2.6 \pm 0.6 \times 10^6$	
28	$5.1 \pm 0.9 \times 10^6$	TFTC	$3.4 \pm 2.5 \times 10^6$	TFTC
35	$4.8 \pm 1.7 \times 10^6$	TFTC	$5.2 \pm 1.6 \times 10^4$	TFTC
42	$5.6 \pm 2.2 \times 10^5$	TFTC	$4.7 \pm 0.4 \times 10^2$	TFTC

TFTC = Too few to count colonies
No colonies

Table 1. A time series display of the total viable bacterial counts for Tanks A - D during the course of the study

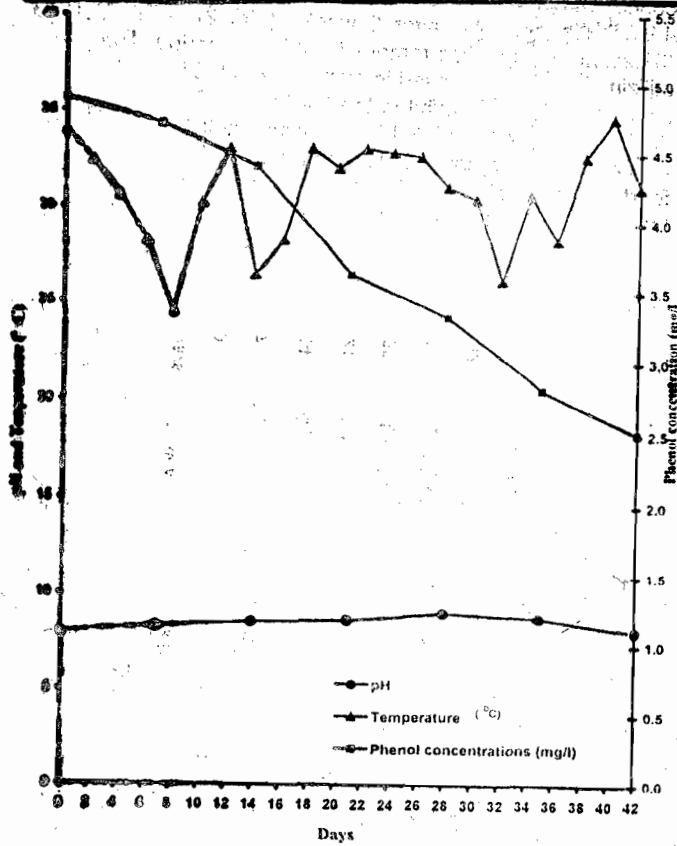


Fig. 3 Phenol concentration, pH and temperature values recorded in Tank B in the course of the study.

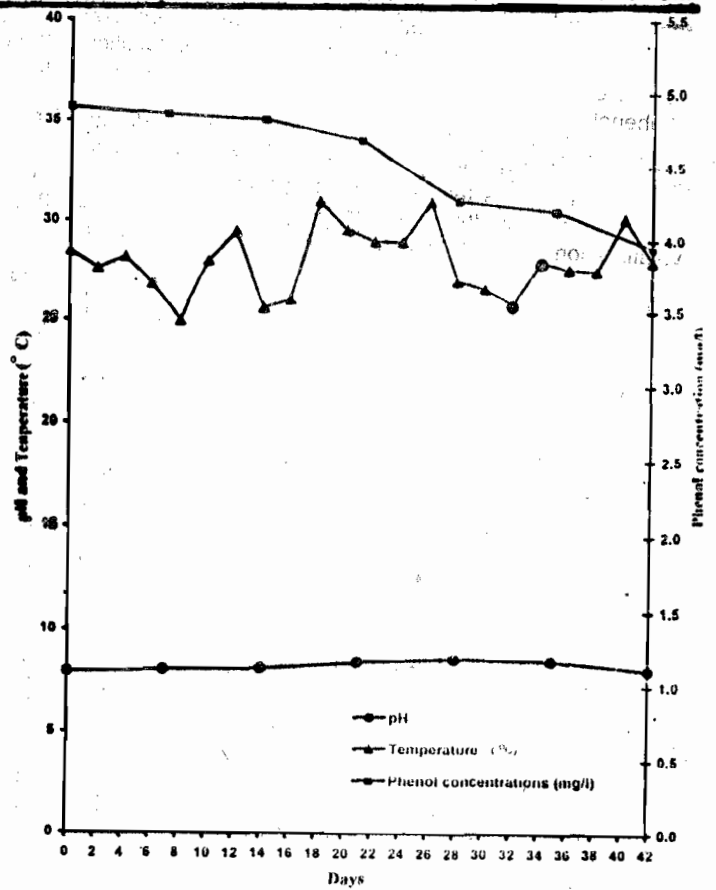


Fig. 5 Phenol concentration, pH and temperature values recorded in Tank D in the course of the study.

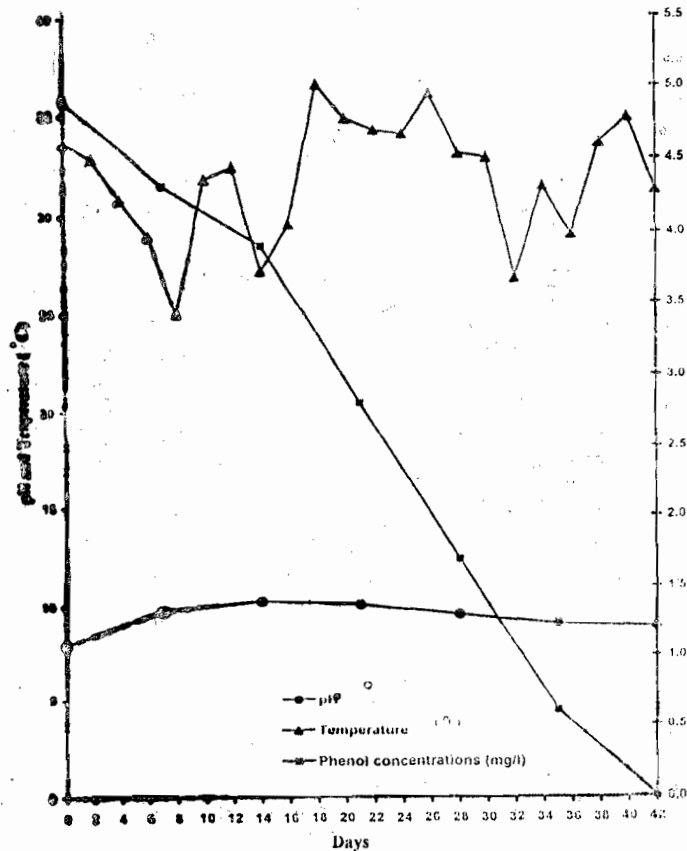


Fig. 4 Phenol concentration, pH and temperature values recorded in Tank C in the course of the study.

Bacterial isolates and characteristics

A total of 25 bacterial isolates were characterized as phenol utilizers. The colonial morphology and the biochemical characteristics (Bergey's Manual of Determinative Bacteriology, 1994) of the isolates revealed that they belong to the genera *Pseudomonas*, *Bacillus*, *Micrococcus*, *Corynebacterium*, *Staphylococcus*, *Chromobacter* and *Serratia*.

DISCUSSION AND CONCLUSION

The fate transport mechanism of phenol in the Port Harcourt refinery effluent wastewater was determined through the study of natural attenuation processes. The research station and glass tanks used as microcosms modeled the wider environment.

The result of the dilution test showed a steady decline in the phenol concentration from the point of discharge till 30m along the river course. For the three weeks the sampling was done, the average phenol concentration values at the point of discharge and after 30m along the river were 3.92mg/l and 1.41mg/l, respectively. Thus 2.51mg/l (51.1%) of phenol diluted off or dissolved within 30m along the river from the point of discharge for the three weeks the sampling was done. As contaminants move from the source area, they may become diluted by uncontaminated recharge water (Kap *et al.*, 2001). In general, phenol is quite soluble in water due to the OH group on the benzene ring. At lower concentrations, microorganisms can readily metabolize it. Part of the attenuation of the phenol in the body of water could be attributable to microbial oxidation and degradation.

The decrease in phenol concentration and increase in total viable bacterial count in Tank A could be attributed to utilization of the phenol as sole source of carbon and energy being metabolized for growth. The reduction in the bacterial count after the third week could be as a result of the reduction in the phenol (growth substrate) concentration. There have been similar reports for phenol utilizing bacteria with phenol as sole source of carbon and energy (Shen and Wang, 1995). It can be reasoned that the increase in the bioload led to the decrease in phenol concentration. Phenol degradation at pH 6.8 - 9.0 has been reported (Paula and Young, 1998). Phenol is acidic but degradation products or intermediates could be neutral to basic. The average value for temperature readings taken for Tank A was 29.10°C with the highest value being 32.0°C and the lowest value being 24.5°C. In general, Port Harcourt has been shown to have a high/dense cloud cover (Kuye and Jagtap, 1992), but the radiant energy reaching the surface is still enough to produce temperatures in the mesophilic range for microbial metabolism/biodegradation; most aerobic metabolism occurs in the mesophilic temperature range i.e., 25°C to 37°C. This temperature range is also enough to effect such physico-chemical processes as evaporation, photooxidation and volatilization.

Biodegradation of phenol was not monitored in Tank B because of the biocide added weekly. Sodium azide inhibits the cytochrome enzymes of the electron transport chain of aerobic organisms. Unlike in Tank A where biocide was not added and algal growth bloomed, the sample in Tank B was a clear liquid. The alkaline pH recorded in this tank could also be as a result of the phenol degradation products and intermediates. The average value for temperature got in this tank was 30.22°C with the highest and the least values being 34.6°C and 24.5°C, respectively. This high peak appears to support degradation by photooxidation volatilization and evaporation based on the fact (Kuye and Jagtap, 1992) that Port Harcourt receives a reasonable amount of radiant energy despite the long periods of cloud cover.

A drastic reduction in phenol concentration was recorded in Tank C over the test period. By the sixth week, the sample in this tank almost dried up. This could be due to excessive evaporation since there was no sediment in the tank. The trend recorded in the total viable bacterial count and pH could also be as a result of the reasons adduced for Tank A. The average temperature recorded in this tank was 31.31°C with 36.5°C and 25.0°C being the highest and the least values, respectively. Aside the reasons given for Tank A as it relates to biodegradation, these temperature values recorded here will also support attenuation through photooxidation and evaporation, since Port Harcourt has been shown to receive radiant energy (Kuye and Jagtap, 1992) that would promote these natural attenuation processes. In addition, these temperature ranges can support build up of vapour pressure according to Raoult's law:

$$P_k = X_k P_k^\circ \text{ where}$$

(P_k) is the vapour pressure of a particular contaminant in the gas phase; X_k is the mole fraction of the component k in the non aqueous phase liquid (NAPL) and P_k° is the vapour pressure above the pure component (Palmer and Johnson, 1991); this can contribute to the reduction of the contaminant through volatilization.

Tank D, served as the control and was covered with a black, thin polyethylene sheet to prevent other natural attenuation processes from taking place. The biocide added weekly reduced the activity of the organisms supposedly there to the barest minimum. There was little or no reduction in the volume of effluent sample poured into this tank, unlike in the other tanks. The slight decrease in phenol concentration can be attributed to dissolution and partly to evaporation. The total

viable count (cfu/ml) of phenol utilizers taken weekly gave similar results with those of Tank B where biocide was also added. The pH values recorded were somehow different from what was obtained in the other tanks possibly because there was little degradation products or intermediates, thus the fluctuations in pH values were not as much as those in the other tanks. The average temperature reading was 27.76°C with the highest and lowest values being 31.0°C and 25.0°C, respectively. These temperature ranges can support evaporation of the sample and the phenol hence the slight decrease in phenol concentration.

The results of the Natural attenuation studies showed that 4.28mg/l of phenol was removed in Tank A which was used to monitor phenol attenuation by evaporation, volatilization, photooxidation, adsorption and biodegradation. About 2.40mg/l of phenol was removed in Tank B used in monitoring attenuation by evaporation, volatilization, photooxidation and adsorption. The difference (i.e. 4.28mg/l - 2.40mg/l = 1.88mg/l; 38.3%) could be attributed to biodegradation. This makes biodegradation a major process among the natural attenuation processes involved in the natural attenuation of phenol in the refinery effluent wastewater. The contribution of biodegradation is very significant because it is a process that leads to the total destruction of a pollutant to yield CO₂, H₂O and biomass. Microbial biodegradation of phenol is essentially aerobic involving such enzyme systems as the oxygenases (Gottschalk, 1986). The effectiveness of the sodium azide biocide in reducing biodegradation is on the basis that it acts as a poison to the cytochrome enzymes of the electron transport chain; thus it is only effective against those organisms with a functional electron transport system. The effect of dilution is also significant, but for very toxic and carcinogenic substances, dilution may offer only a temporary solution to the effect of pollution.

Tank C gave the fastest removal rate of phenol. This could be attributed to the fact that no sediment was added here, thus evaporation, volatilization and photooxidation were rapid coupled with biodegradation. This also could be seen from the rate at which the sample almost volatilized or evaporated completely within 6 weeks. Hence the phenol removed in Tank C (4.91mg/l) can be attributed to photooxidation, evaporation, volatilization and biodegradation. All of these natural attenuation processes are feasible in Port Harcourt and the Niger Delta. This is because despite a prolonged cloud cover (Kuye and Jagtap, 1992) the solar radiance reaching this region is enough to promote these natural attenuation processes.

Tanks A and B accounted for 1.88mg/l phenol removed through biodegradation. The values of 0.36mg/kg and 0.21mg/kg were the phenol concentrations recorded for the sediments in Tanks A and B respectively, indicating attenuation due to adsorption. Thus on the average, adsorption was responsible for removal of 0.29mg/kg (5.91%) concentration of phenol. Thus for Tank C, this means that photooxidation, evaporation, and volatilization accounted for 2.74mg/l phenol removed (i.e. 4.91mg/l - (1.88mg/l + 0.29mg/kg) = 2.74mg/l.) (55.8%).

The degradation of phenol in Tank D cannot be fully ascribed to evaporation, volatilization or photooxidation. So the value cannot be used for these parameters. Since the tank was covered, the effect of the sun must be felt but not as fully as it was in the other open tanks. The values for Tank D were used for setting background.

A total of 25 phenol-utilizing bacteria belonging to the genera, *Pseudomonas*, *Bacillus*, *Micrococcus*, *Corynebacterium*, *Staphylococcus*, *Chromobacter* and *Serratia* were isolated. *Pseudomonas* has been isolated in connection with phenol and hydrocarbon degradation (Atlas, 1984; Focht

and Westlake, 1987; Leahy and Colwell, 1990; Shen and Wang, 1995; Obiukwu and Abu, 2003). *Bacillus* also has been isolated in connection with phenol degradation (Gurujealashimi and Oriel, 1989; Obiukwu and Abu, 2003). *Micrococcus*, *Corynebacterium* and *Chromobacter* are known hydrocarbon degraders (Leahy and Colwell, 1990) but no literature specifically corroborate their involvement in phenol degradation. Degradation of phenol by *Staphylococcus* species, a known halophile has also been reported (Obiukwu and Abu, 2003). *Serratia* is an enterobacterium but has also been isolated from phenol wastes (Christoskova and Stoyanova, 2001). Thus, these aerobes with their oxygenases were able to withstand the toxic effects of phenol. The setting up of the experiment in an open area modeled what obtains in the real environment in the Niger Delta and enhanced the growth and activity of these mesophilic aerobes.

The statistical analyses (using analysis of variance, ANOVA and the t - test) of the data obtained showed that there was no significant difference ($P=0.05$) in the means of the \log_{10} cfu/ml for Tanks A and C. No significant difference was observed in the means for the different days for all the tanks with respect to pH and temperature values. But significant differences were observed for weekly analyses with respect to pH and temperature values. On the other hand, significant differences were observed between the days and the weekly analyses of the phenol concentrations for different tanks. The noticeable differences in pH values could be as a result of biodegradation, its intermediates and products. The differences were more pronounced in Tanks A and C. The significant differences observed in the phenol concentration values for the different tanks and on weekly basis showed the efficiency of the different natural attenuation processes monitored in these tanks in phenol removal.

In conclusion, the fate transport mechanism of phenol in the Port Harcourt refinery effluent wastewater was studied and the natural attenuation processes involved were established. Phenol could naturally attenuate through biodegradation, volatilization, evaporation, photooxidation and adsorption with a myriad of microorganisms involved in its biodegradation. There is need to properly treat or reduce the phenol concentration in the effluent before it is discharged into the river considering its toxicity to man, plants, animals and microorganisms alike, and considering also that the Okrika River where the effluent is finally discharged should not be turned into a 'phenol river' since it is a source of drinking water for people living nearby. More so, because the sediments of this river are prone to adsorbing phenol. Since biodegradation played a very significant role in the attenuation of phenol in the effluent wastewater, bioremediation measures can be applied to enhance or speed up the removal of phenol in the impacted media.

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