Remediation of Simulated Oil Contaminated Sites using Shells of Clams and Oyster - *Total Petroleum Hydrocarbons of Simulated Oil Contaminated Sites, before and after Remediation*

Chikwe*, T. N. and Ogbole, S. N.

[1]*Department of Pure and Industrial Chemistry, University of Port Harcourt, P.M.B 5323, Choba, Port Harcourt, Nigeria.*

*(*Corresponding author: Tel:* (phone +234-8035478512; e-mail: templechikwe@yahoo.co.uk)

ABSTRACT

Aquatic contaminated sites due to oil spillage were simulated using five crude oil samples (Usan crude, Ogbele condensate, Ogbele crude, Abo crude and Kokori crude oil) in a water body at constant volume and temperature. The Total Petroleum Hydrocarbon (TPH) of the five contaminated sites were determined before and after remediation using Gas Chromatographic analyses. The TPH of the five contaminated samples before remediation were: 977, 817, 1029, 1131, 1209 mg/L respectively. Powdery oyster and clam shells of different particle sizes (60 µm, 30 µm and 10 µm) were used as adsorbents to remediate each of these contaminated samples through bioremediation. The same percentage reduction of TPH were obtained for four samples(contaminated with Usan crude, Ogbele condensate, Ogbele crude, Abo crude)after remediation with 10 µm powdery oyster shell (POS) and 10 µm powdery clam shell (PCS) respectively; The values obtained were: 99.9 %, 99.88 %, 99.90 % and 99.90 % respectively, thereby leaving a TPH of 1 mg/L for each of the four samples after remediation. Adsorbents with the smallest particle size of 10µm gave the best result with the highest percentage reduction of Total Petroleum Hydrocarbon (TPH) for the five contaminated samples. Samples contaminated with Kokori oil were more resistant to adsorption due to suspected higher molecular weight hydrocarbons that are non-biodegradable, hence the percentage reduction of Total Petroleum Hydrocarbon were 57.82% and 68.32% after remediation with 10 µm powdery oyster and clam shell respectively, thereby making clam shell a better adsorbent. The effectiveness of the adsorbents is directly proportional to their surface area which is a function of the particle size. The smaller the particle size of the adsorbents the larger the surface area. Total Petroleum hydrocarbons in aquatic environment can lead to the death of aquatic lives in addition to making the water unfit for domestic and industrial uses.

Keywords: Biodegradation, adsorption, adsorbents, contamination, hydrocarbons, spillage, simulation, bioremediation.

INTRODUCTION

Crude oil is a complex combination of several hydrocarbon components in different ratio, it also contain metals, nonmetals and some other component in varying proportion which has been formed and accumulated together over several millions of years due to some chemical and physical forces[1]. Crude oil can be drilled, treated and separated into various component fractions using distillation process and its constituents differ from each other in terms of compositions, properties, amount and quantity[2]. Crude oil spillage is the release of petroleum hydrocarbons into the environment due to human activities, it is predominant within the marine ecosystem however spills may also occur on lands. Total petroleum hydrocarbon (TPH) refers to hydrocarbon mixture found in aquatic oil. It is an aggregation of extractable petroleum hydrocarbons and volatile petroleum hydrocarbons[3].Crude oil spillage has caused serious environmental consequences all over the world ranging from degradation, pollution and contamination of water bodies (seas and oceans), landsand depletion of the environment which often leads to destruction of environmental assets[4]. Oil spillage within the marine ecosystem can lead to the death of marine lives by way of drastically depleting dissolved oxygen in water which is essential for the survival of aquatic organisms, in addition to making the water unfit for domestic and industrial use[5]. Oil spillage on land can drastically deplete soil nutrient and destroy farm land. Farm crops are also being destroyed when crude oil spillage occurs and soil fertility is negatively affected because of the presence of hydrocarbon which hinders seed germination, mitigate plant growth, decrease soil fertility and affect soil nutrients[6].

Crude oil spillage can be treated by adsorption process through the use of a suitable adsorbent. Adsorption entails chemical or physical separation process, requiring the activity and presence of an adsorbent (substances with highsurface area possessing the tendency of attracting any substance/component to its surface, hence forming attachment with it through intermolecular forces of attraction) and an adsorbate (gaseous or liquid components or substanceseasily attracted to porous surface of adsorbent) in a given fluid phase or system[7]. Adsorption denotesphysicochemical processes during which adsorbents (such as zeolite, bentonite, alumina, activated carbon, animal shells and activated charcoal) removes adsorbates from any fluid systemthrough physical attraction onto its surface usingintermolecular forces. A material is said to be an adsorbent if it has the ability to contain a specific amount of liquid in small chambers similar to a sponge[8]. Considering the fact that sponges are members of the phylum *porifera* within the *Animalia* kingdom, it becomes imperative to try the adsorption capacities of several other animal sources, for instance shells of aquatic organisms like Clams and Oyster could serve as adsorbentswhen prepared in a powdery form. Clam is a common name for different types of bivalve Mollusca, they are sometimes called Thales and they fall within the Animalia kingdom. Clams have two shells of equal sizes connected by two adductor muscles and they have a strong burrowing foot. There are different classes of Clams ranging from hard shell Clams, soft shell Clams to manila Clams[9,10]. Oysters just like Clams are within the family of bivalve Mollusca within the Animalia kingdom, some classes of Oysters are also referred to as Clams however in the culinary sense and Clams do not live attached to a substrate but Oysters do[11]. Some pictures of Oyster and Clams are shown in Figures 1 & 2.

Environmental remediation refers to clean-up, abatement and subsiding to minimize, curtail and remove harmful and toxic compounds from human environment. Environmental remediation denotes contaminants and pollutants removal from human environment (consisting the soil, water bodies, ground water, atmosphere and surface water). biological, physical, chemical remediation depending on the nature of the adsorbents. Figure 3 shows a typical contaminated site in the Niger Delta region of Nigeria.

Figure 1: Oyster Shells

Figure 2: Clam Shells

Figure 3: Crude oil contaminated sites (River and farm land) in Niger delta region, Nigeria.

It denotes actions performed for safer and cleaner immediate surroundings, without violating the recommended international safety standards and criteria[12]. There are different types of remediation ranging from

Oil contaminations in the environment has caused several challenges in humans and the environment and this has prompted several researches in recent years. Several nations and scientist have expanded their resources just to develop a better way of remediating crude oil contamination and researches are still underway for a more effective way of remediation. The aim of this study is to remediate or treat crude oil contaminated water using shells of Oyster and Clams through bioremediation. This study will provide a cost effective and alternative route for the remediation of crude oil contaminated water with little or no environmental side effect; it will also provide a means of boosting farmers' income through the use of agricultural waste.

MATERIALS AND METHODS

Sample Collection and Preparation.

Clams and Oyster shells were purchased from local fishermen at Borokiri market in Rivers State Nigeria. These shells were properly washed using distilled water and oven dried at 35 ^{o}C at a well monitored and controlled laboratory temperature for 72 hours (3 days). The dried shells were thereafter crushed using electrical grinder machine, subjected to shaking using magnetic shaker into fine particles of 60 µm, 30 µm and 10 µm respectively.Five crude oil samples were obtained from five (5) different locations. These samples are: Usan Crude Oil, Ogbele condensate, Ogbele crude oil, ABO crude oil and Kokori Crude oil.

Sample Simulation

Five (5) thermoplastic containers of10 liter capacity were successively labeled A, B, C, D and E representing samples contaminated with Usan Crude Oil, Ogbele condensate, Ogbele crude oil, ABO crude oil and Kokori Crude oil respectively for proper identification. Eight (8) liters of distilled water was gently poured in each container. 100 ml of each of the water contaminants (crude oils and condensates) were simultaneously added to the thermoplastic containers (containing water) with the corresponding identification (A – E). The water – oil mixtures and the condensate – water mixture were allowed to partially equilibrate for three (3) hours without any agitation or disturbance.

Gas Chromatographic (GC) Analysis

Gas chromatograph (model no. GC – FID - 1) was utilized for total petroleum hydrocarbon (TPH) determination in the samples from the five different locations. The gas chromatograph was carefully equipped with column of fused silica capillary (0.25 mm x 30 m x 0.25 mm), Helium gas (carrier gas) with a flow rate of 1.9 mL/min was used. Column temperature was formally set to periodically increase from 64 ^{o}C to 191 ^{o}C at 4.0 ^{o}C/min and also to 282 ^{o}C at 6.0 ^{o}C/min. It was held for 20 minutes at 274 ^{o}C. The samples (1 μL each) were properly injected in split less modes. The quadrupole, ion exchanges and interface temperatures were held constant at 162 ^{o}C, 153 ^{o}C and 276 ^{o}C respectively. Ionization was carried out in electrons impact mode at 67 eV and data were thereafter obtained by selected ion monitoring mode. TPHs identifications were based on selected ions, retention time comparison between sample and standard solutions. TPHs quantification was carried out using relative response factor of the target or specific TPH to internal standard. This was achieved using quantification ions and confirmation ions[13].

Remediation Processes

The remediation process was practically carried using solid – phase technique of extraction (SPTE). The EPA remediation method (EPA – 33535) was adopted for contaminated sample remediation. Measured amount (1Litre) of each contaminated samples was set up in a laboratory cartridge (cleaved on retort stand) containing ten gram of the different mesh sizes (60, 30 and 10µm) of the powdered Oyster shell (POS)respectively. The set up was uninterruptedly allowed to stand for duration of 10 days as the remediation time. Thereafter, the liquid was allowed to elute from the laboratory cartridge and the eluted liquid was subjected to gas chromatographic analysis. Same procedure was adopted for the different mesh sizes (60, 30 and 10µm) of the powdered Clam shell (PCS)respectivelyto clean up 1 Litre of each contaminated sample (A – E). The total petroleum hydrocarbons (TPHs) in the contaminated samples before and after the remediation process (using animal sources, POS and PCS) were analyzed and determined using gas chromatography[14].

RESULTS AND DISCUSSION

Table 1 shows the TPH of the samples contaminated with five crude oil samples ofUsan Crude Oil, Ogbele condensate, Ogbele crude oil, ABO crude oil and Kokori Crude oil represented as A, B, C, D and E respectively (before remediation). The Table also shows the TPH of the respective samples after remediation with powdered Oyster and Clam shell of 60 **µm** mesh size. Table 2 shows the TPH of the respective samples before and after remediation with powdered Oyster and Clam shell of 30 **µm** mesh size.

Table 1: Total Petroleum Hydrocarbon (TPH) of Contaminated Samples before & afterRemediation with 60 µm PCS/POS

SAMPLE	C_0 (mg/L)	C_e (mg/L) with POS	C_e (mg/L) with PCS
A	977.00	420.00	390.00
B	817.00	405.00	372.00
C	1029.00	421.00	381.00
D	1131.00	423.00	386.00
E	1209.00	1070.00	917.00

C_0 = Initial Concentration, C_e = Equilibrium Concentration,
POS = Powdered Oyster shell, PCS = Powdered Clam shell

Table 2: Total Petroleum Hydrocarbon (TPH) of Contaminated Samples before & afterRemediation with 30 µm PCS/POS

SAMPLE	C_0 (mg/L)	C_e (mg/L) with POS	C_e (mg/L) with PCS
A	977.00	206.00	172.00
B	817.00	180.00	162.00
C	1029.00	210.00	184.00
D	1131.00	215.00	178.00
E	1209.00	987.00	687.00

C_0 = Initial Concentration, C_e = Equilibrium Concentration,
POS = Powdered Oyster shell, PCS = Powdered Clam shell

Table 3 shows the TPH of the respective samples before and after remediation with powdered Oyster and Clam shell of 10 **µm** mesh size. Table 4 shows the percentage reduction of TPH for the respective samples after remediation with powdered Oyster and Clam shell of 10 **µm** mesh size.

Table 3: Total Petroleum Hydrocarbon (TPH) of Contaminated Samples before & afterRemediation with 10 µm PCS/POS

SAMPLE	C_0 (mg/L)	C_e (mg/L) with POS	C_e (mg/L) with PCS
A	977.00	1.00	1.00
B	817.00	1.00	1.00
C	1029.00	1.00	1.00
D	1131.00	1.00	1.00
E	1209.00	510.00	383.00

C_0 = Initial Concentration, C_e = Equilibrium Concentration,
POS = Powdered Oyster shell, PCS = Powdered Clam shell

Table 4:Percentage Reduction (%) of (TPH) for Contaminated Samples after Remediation with 10 µm PCS/POS

SAMPLE	C_0 (mg/L)	(%) with POS	(%) with PCS
A	977.00	99.90	99.90
B	817.00	99.88	99.88
C	1029.00	99.90	99.90
D	1131.00	99.90	99.90
E	1209.00	57.82	68.32

C_0 = Initial Concentration, % = Percentage Reduction of TPH,
POS = Powdered Oyster shell, PCS = Powdered Clam shell

Percentage reduction of TPH is obtained using Equation 1,

$$\% \, Reduction = \frac{C_0 - C_e}{C_0} \times 10 \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

where:

C_0 = TPH before remediation (Initial Concentration)

C_e = TPH after remediation (Equilibrium Concentration)

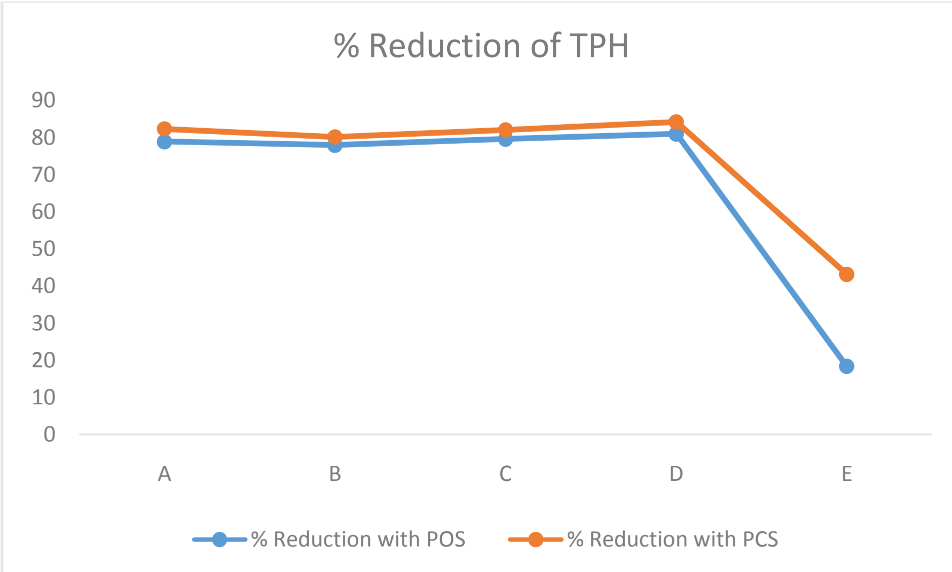

Figure 4: Percentage Reduction (%) of (TPH) for Contaminated Samples after Remediation with 30 µm PCS &POS

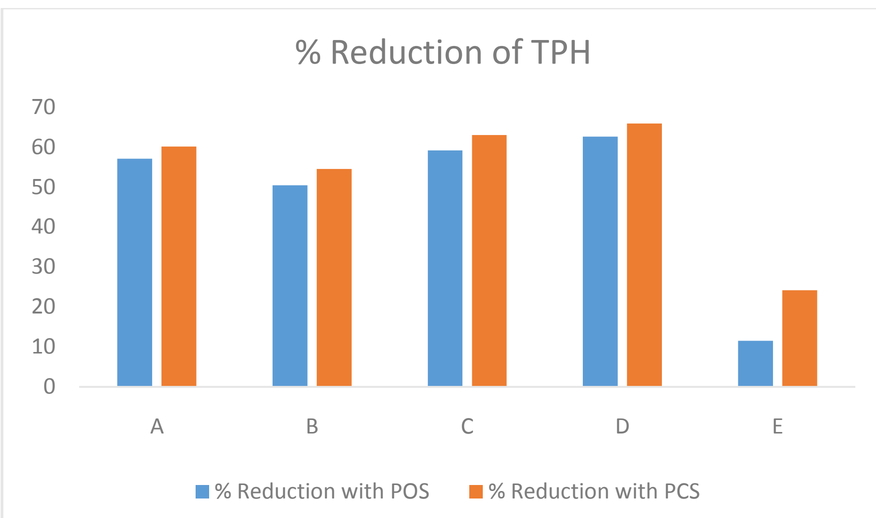

Figure 5: Percentage Reduction (%) of (TPH) for Contaminated Samples after Remediation with 60µm PCS &POS

Total Petroleum Hydrocarbon (TPH) refers to a mixture of hydrocarbons found in crude oil. There are several of these compounds hence it is not practicable to measure one separately however it is of great importance to measure the total amount (TPH) at a site. It is also worthy to note that not all the petroleum hydrocarbons occur in one sample, the TPH of a sample depends on the origin of the sample. TPH isthe sum of the volatile petroleum hydrocarbons, including poly aromatic hydrocarbons (PAH) and extractable petroleum hydrocarbon present in the sample[15].Table 1 shows the TPH of the five contaminated samples before remediation (indicated by the initial concentration C_0) and after remediation with 60µm powdered oyster shell (POS) and powdered clam shell (PCS) (indicated by the equilibrium concentration C_e). Comparing results obtained from Tables 1,2 and 3 it can be deduced that the reactivity of the adsorbents which is indicated by the adsorption of TPH in the contaminated sites increases with decrease in the particle size of the adsorbents. The smaller the particle size of the adsorbents, the smaller the TPH after remediation hence the higher the percentage reduction of TPH as shown in Table 4 and Figures 4 and 5. The surface area of adsorbents increases with decrease in particle size thereby increasing the rate of adsorption. From Table 3 it can be deduced that 10 µm powdery clam and oyster shells used separately reduced the TPH of samples A, B, C, D to 1 mg/L respectively which is far more environmentally and human friendly compared to the TPH of the four samples before remediation. Results obtained shows that sample E has the highest TPH before remediation as well as the least percentage reduction of TPH after remediation with 10µm powdery oyster shell (POS) and powdery clam shell (PCS). Comparing the percentage reduction of TPH after remediation of sample E with 10µm POS and PCS, results obtained from Table 4 shows that PCS is a better adsorbent than POS having adsorbed 68.32% of TPH in sample E against the 57.82% adsorbed by POS.The adsorption of TPH using POS and PCS was made possible through bioremediation. Bioremediation is the use of microorganisms to detoxify or remove pollutants. The detoxification of pollutants through the use of microorganisms is made possible through the different metabolic abilities of these organisms. The adsorption characteristics of these shells (POS and PCS) is mainly due to the functional groups in the shells which is a function of the microorganisms inherent in the shells[16]Biodegradation of petroleum hydrocarbons is the basis for bioremediation and it is a complex process that depends on the nature and amount of hydrocarbons present. Petroleum hydrocarbons can be divided into saturates, aromatics, asphaltenes and resins. The tendency of hydrocarbons to undergo microbial degradation is generally ranked as: linear alkanes > branched alkanes > small aromatics > cyclic alkanes. High molecular weight polycyclic aromatic hydrocarbons (PAH) may not be degraded at all[17].Though results show that sample E has the highest TPH it is important to note that the percentage reduction of TPH in samples A, B, C, and D using the same adsorbents are not commensurate to the percentage reduction in sample E and the simple reason is due to the nature of petroleum hydrocarbons in Sample E considering the fact that the remediation of the five contaminated samples were carried out using the same adsorbents and under the same conditions. Other factors that can increase the biodegradation of hydrocarbons are increase in temperature, presence of nutrients such as nitrogen, phosphorus and in some cases iron as well as presence of aerobic conditions[16].Reduction of the particle size of POS and PCS (using this study as a reference) can also increase the percentage reduction of TPH after remediation for Sample E.

Oil spill on land affect physicochemical properties of different soil (pH, nutrient status, structure and temperature). Oil film formation on soil surfaces after oil spillage scuttles soil aeration through barrier formation between soil and air, hence leading to soil dispersion and soil texture breakdown as well as destruction of soil biomass[6].The water bodies (aquatic ecosystems) are more susceptible to crude oil contamination, when water bodies are contaminated, both aquatic animals and plants are drastically affected,photosynthetic rate, growth and stem height are reduced due to oil contamination. The dissolved oxygen in the water is depleted due to the transformation of organic compounds into inorganic component during the decay process of dead organism (aquatic animals and plants) emanating from oil contamination. Eutrophication and loss of biodiversity are also consequences of crude oil contamination which ultimately affects the food chain. Aquatic animals are also likely to suffer the following diseases; hemorrhagic septicemia, epidermal hyperplasia and lymphocytosis because of polyaromatic hydrocarbons (PAHs) which are also the key constituents of crude oil[18, 19].The PAHs contents of crude oil are responsible for growth retardation, low reproduction, inability to survive and developmental problems in various aquatic organisms[6]. Human exposure to the PAHs contents can lead to infectious diseases, thereby increasing the mortality rate of human and causing problems to the reproductive systems of humans[20].

The link between the human health (which is an old science) and the environmental health (which is a modern science) has demonstrated that humans are prone to various infectious and deadly diseases, when exposed to PAHs from crude oil contamination in the environment through contaminated food, water and vapour emission[21]. The toxic constituent present in the crude oil is capable of inhibiting protein synthesis and disrupting the transport system of the membrane. Mutagenesis, carcinogenesis, hemorrhagic tendencies and reproductive system impairment are also possible consequences of crude oil contamination. The volatile constituents of aquatic oil can lead to rising aging of human lungs, bronchitis and asthma diseases. Liver and kidney diseases may also occur from human exposure to this contamination[22].

CONCLUSION

Proper remediation on crude oil affected regions and contaminated sites with the use of effective adsorbents is very essential to minimize the effect of aquatic oil contamination on human and other living organisms (fauna and flora). Powdered oyster shell (POS) and powdered clam shell (PCS) proved to be very effective in the remediation of water bodies contaminated with various crude oil samples(Usan Crude Oil, Ogbele condensate, Ogbele crude oil and ABO crude oil) by drastically reducing the total petroleum hydrocarbon (TPH) of the crude oil contaminated samples to unity after remediation. The effectiveness of the adsorbents is directly proportional to their surface area, hence smaller particle sized adsorbents gave a better result in terms of adsorption. Sample contaminated with Kokori crude contains more quantities of high molecular weight poly aromatic hydrocarbons as such will require another type of adsorbent with better bio degradative ability than 10 µm POS and PCS to reduce the TPH to unity or possibly an appreciable low level which will be both environmentally and human friendly.

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