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Sludge Pollution Control from Crude Oil Tank Cleaning

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Abstract

This study investigated the use of microbial analysis as a bioremediation option for remediating petroleum sludge, which is part of the waste stream generated in the petroleum industry. The aim is to reduce environmental burden caused by the discharge of untreated sludge. Sludge sample was cultured in other to isolate microorganisms for the sludge treatment. The selected strain of the organisms after screening were *Aspergillus flavus*, *Aspergillus niger*, *Verticillus* sp, *Penicillum* sp, and *Microsporium audouinii*. Bioreactors (labeled A, B, C, D and O) were designed for the treatment of petroleum sludge. These reactors contain 2.0×10^{-2} m^3 of the diluted sludge samples and the isolated organisms for the treatment process. On a weekly basis, the control reactors received 1.5×10^{-3} m^3 of fresh and saline water respectively. After 12 weeks of treatment, sludge physicochemical characteristics showed distinct variations. From the result, reactor D was the best in terms of remediating the sludge as compared to other reactors. Friedman non-parametric test was performed to check if the weeks of treatment affected the reduction of the total hydrocarbon content (THC) in the five reactors and also checked for significant differences in the THC after treatments. The drop in the THC of the treated sludge ranged from 56.0% to 67.3%. These results showed the possibility of enhanced biodegradation of petroleum sludge by hydrocarbon utilizing microorganisms (fungi).

Keywords

Petroleum Sludge, Sludge Pollution Control, Crude Oil Tank Cleaning, Microbial Analysis, Bioremediation, Bioreactor

1. Introduction

During the process of oil and gas production and exploration, "the extracted

crude from oil well contains crude oil, condensates and gas fractions". Wastes are generated which include petroleum wastewater, drilling fluid, completion fluid, petroleum effluent treatment plant sludge and crude storage tank residue [1]. [2] Reported that 0.3% to 0.5% of sludge is generated from one ton of crude oil refining from a refinery with 105,000 barrel/year capacity. Petroleum sludge is generated during crude oil transportation, storage, refining and production. In crude oil storage tanks (cargo tank), the sludge formed at the base of the tank contains toxic substances such as asphaltenes, water, hydrocarbons, inorganic solids (e.g., sand, iron sulfides, and iron oxides). It is made up of complex chemical mixtures with varied petrochemical attributes [3] [4]. Petroleum sludge is a recurrent problem leading to corrosive effects and a reduction in oil storing capacity. Hydrocarbon is the principal component of petroleum sludge which is formed when crude oil's properties are changed as a result of changes in external conditions. The formation of petroleum sludge is commonly caused by cooling below the cloud point, evaporation of light ends, mixing with incompatible materials, and the introduction of water to form emulsions [5]. Disposing of sludge without adequate treatment poses a threat to the environment. Petroleum industry waste vary in concentrations of hydrocarbons (40% - 60%), water (30% - 90%), and mineral particles (5% - 40%). They are either hazardous or non-hazardous waste. Any waste that is reactive, inflammable, explosive, combustible, corrosive, or poisonous is classified as hazardous. The hazardous waste can be classified into 1) solid hazardous waste (batteries, catalysts, fluorescent, tubes, sampling bottles, pipes, filters, oil contaminated rags, oily contaminated soils), 2) liquid hazardous waste (used lubricants, expired chemicals, spent acids), 3) soil/sludge (oily soil/sludge from oil spills and crude oil storage tanks) [6] [7]. Non-hazardous waste is any form of industrial waste that does not pose a direct threat to human health or the environment, but still cannot be dumped of in a dumpster or sewage system due to rules. In the petroleum industry, the non-hazardous wastes could arise from general waste (mix garbage), tires (vehicle tires), food waste (from offices and cafeterias), concrete (construction and demolition waste), paper, wood (wooden pellets), glass (soft drinks, lab reagent bottles), plastic and metals (drums, steel pipes, metal scrap, redundant flow lines, aluminum soft drink tins). Specifically, the major petroleum industry waste is petroleum sludge (PS).

Accumulation of sludge reduces the storage capacity of the tank and this together with the possibility of corrosion makes it necessary to periodically remove these deposits. Conventional petroleum industry waste treatment strategies present various shortcomings, including high costs and need for specialized equipment and skilled personnel.

Figure 1 shows the amount of petroleum sludge generated from 2018 to 2020 in seven regions across the globe. Several methods for treating this petroleum sludge include chemical treatments, various distillation processes, cracking, hydro-treating, solvent treatment and bioremediation. Some of the conventional

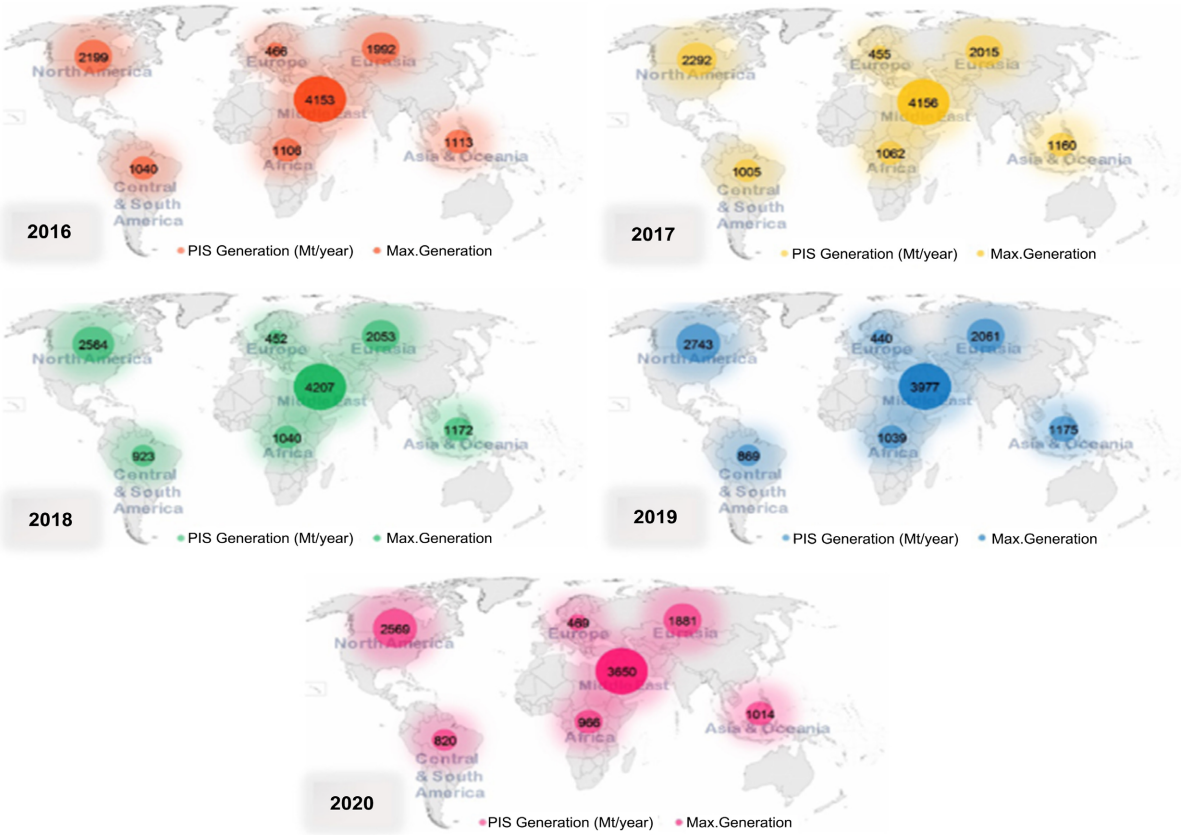

Figure 1. Amount of petroleum sludge generated from 2016 to 2020 in seven regions across the globe. Source: [10].

methods of sludge treatment are manual cleaning and incineration, solvent extraction, microwave heating, centrifugation, etc. The Department of Petroleum Resources (DPR) officially recommends incineration for the treatment of sludge. However, incineration is prohibitively expensive [8] and also exposes personnel to the resulting fugitive dust [9]. Concern about these drawbacks of the incineration method and the need to detoxify and reclaim this sludge prompts a need to adopt a treatment method that is cheap, simple and environmentally friendly. In this study, the method of bioremediation was applied in treating petroleum sludge before disposal to the environment. The objectives of this study were to characterize sludge deposit in crude oil storage tank, determine the composition of the toxic substances in the sludge and treat the solid and water parts of the sludge before disposal.

The average sludge generated in metric ton per year for the five year period listed is as follows: North America 2473.4; Central & South America 935.4; Europe 456.4; Africa 1035.4; Middle East 4028.6; Asia & Oceania 1126.8 and Eurasia 2000.4. Middle East accounts for the highest generation.

2. Materials and Methods

2.1. Sample Collection

The sludge samples used for this research were obtained from crude oil cargo

tank at XY FPSO located 220 miles south-east of Lagos and 70 miles offshore Nigeria. The samples were collected from four (4) different cargo tanks in the same FPSO. From the uppermost, 5 cm layer of the composite sludge samples were collected through random sampling in an air tight plastic container and then transported to the laboratory via ice pack for microbial analyses at a temperature of 4˚C until analyses were performed.

2.2. Characterization of Petroleum Sludge

Sludge sample collected from the crude oil storage tank was analyzed for physio-chemical parameters. The electronic centrifugal machine was used to separate the different components of the sludge such as water, free oil and basic sediments. The machine was set at 4000 RCF and ran for 1 hour continuously in order to recover centrifuged material. The total solid content was determined by evaporating the sludge sample to a constant weight at a temperature of 105˚C and the result expressed as a percentage of weight by weight. Volatile solid content was measured by weight loss on ignition of dried sludge test total solid residue (TSR) at 600˚C, and the result expressed as a percentage of the mass of organic material in the sludge of a certain mass to the mass of dry sludge solids.

The pH of the sludge extract (sludge:water = 1:2.5) was determined using a WTW Multi-340 pH-meter according to [11] method D4972. Total organic carbon (TOC) was determined according to BS 1377 method. Total nitrogen (TN) was determined according to [12] method 4500-NO3B. Available phosphorous was determined according to [12] method 4500-PO43.

The digestion method is used because phosphorus may occur in combination with organic matter, this method to determine total phosphorus is able to oxidize organic matter effectively to release phosphorus as orthophosphate. The nitric acid—sulfuric acid method was adopted.

Chlorine was analyzed by silver nitrate titration according to [11] method D512-04. Sodium was analyzed using a UNICAM-969 Atomic Absorption Spectrophotometer according to [12] method 3111C. Total hydrocarbon content (THC) was determined using a SHIMADZU Infrared Spectrophotometer according to [11] method D3921 by measuring light absorbance at the wavelength range of 3333 to 3704 nm. Bonny light crude was used to calibrate the equipment before use. The total heterotrophic bacteria (THB) was determined according to [11] method D5485.

2.3. Preparation of Agar Media for Organism Growth

Commercially prepared non-synthetic media were used for organism growth. Sabouraud Dextrose Agar (SDA) media for fungi growth was prepared by dissolving 65g of the powdered medium in a litre of distilled water. Chlorophenocle antibiotic was added to the SDA to inhibit growth of bacteria. It was then sterilized using autoclave at a temperature of 121˚C and a pressure of 1.5 N/m^2 for 15 minutes. The medium was left to cool after sterilization before usage. The

second medium was prepared with Nutrient Agar (NA) for bacteria growth. The medium was prepared by softening 28 g of the powdered medium (nutrient agar) in a litre of distilled water. It was then sterilized using autoclave at a temperature of 121˚C and a pressure of 1.5 N/m^2 for 15 minutes. The medium was left to cool after sterilization before usage.

2.4. Serial Dilution

Before serial dilution process, 9.0 ml of distilled water was dispensed in test tubes and then sterilized using autoclave at a temperature of 121˚C and a pressure of 1.5 N/m^2. The test tubes after sterilizing were left to cool to room temperature of about 25˚C to 27˚C. Total number of test tubes used were twenty-four (24), divided into four (4) according to the number of samples used. Therefore, six (6) test tubes containing 9 ml sterilized distilled water were allocated to each sample. Finally, 1.0 g of soil sample was added to the first set of the test tubes from each of the soil sample which already contains 9 ml of sterilized distilled water for serial dilution process.

The process of serial dilution was done by taking out 1 ml of the mixture of the soil and distilled water sample from the first test tube (dilution one) and injecting the 1 ml into the second test tube in series to achieve dilution two and shaking the test tube for proper mixture. Then another 1 ml of the solution was taken out from the second test tube (dilution two) and injecting into the third test tube. The process continued repeatedly until the sixth test tube was diluted with the soil content to achieve a series of dilution with dilution factor of 10^{-6}.

2.5. Inoculation

After the serial dilution process was completed, to know the number of viable cells in the different dilution numbers, inoculation process was then carried out. The inoculation process was carried out by dispensing 1 ml of the inoculant from the dilution factor of 6 into a sterile petri dish. Then a multimedia of SDA and NA (growth medium) prepared was also dispensed inside the Petri dish containing the inoculant and mixed together, the Petri dish containing a mixture of the inoculant and the growth medium was then left for 1 to 2 hrs to solidify.

2.6. Incubating the Plates

After inoculation, the Petri dishes containing the inoculant and the multimedia were then moved to an incubator at a suitable temperature for the growth of a particular organism after it was allowed for solidification. The bacteria plate which contains Nutrient Agar (NA) was incubated at 37˚C for 24 to 72 hrs (1 to 3 days) while the fungi plate which is SDA was incubated at an ambient temperature which range from 25˚C to 27˚C for 24 to 168 hrs (1 to 7 days). During the period of incubation, the bacteria and the fungi plates were often checked to

confirm proper culturing of the organisms and also checked to **know the actual time the organisms started growing.**

2.7. Counting of Microbial Cells

After the period of incubation, the colonies in the Petri dish were counted in order to determine the number of microorganisms present in the original sample. The counting of the number of the microbial cell grown on the plate was done by the method of plate counting. This was done by multiplying the total number of colonies counted in a plate by the reciprocal of the dilution factor and the volume of solution dispensed in the Petri dish during the period of inoculation. It is expressed mathematically as:

$$MC_n = C_t * R^{-1} \tag{1}$$

where MC_n is the number of microbial cell which is expressed in cfu/g, C_t is the total number of colonies in a plate and R is the dilution factor.

2.8. Isolation of the Organisms

Pure isolation was carried out by separating the different viable colonies to a single colony for the purpose of characterization. The organisms found in the Petri dishes were isolated into a new Petri dish which already contained growth medium (SDA or NA). The isolated organisms were then sub-cultured in the culturing box at suitable temperatures. Thereafter, the isolated organisms were preserved in a slant bottle containing a growth medium (SDA or NA) agar. This was done by picking a microorganism from the Petri dish as the inoculant and then inoculated in a slant bottle. The slant bottle containing the microorganism was incubated for 1 to 3 days for bacteria and 1 to 7 days for fungi at suitable temperatures for the organisms to grow and be preserved.

2.9. Identification of the Organisms

The fungi were named based on their morphological looks. A pure isolate was collected from the slate bottles where the organisms were kept. These portions of organisms collected were microscopically viewed through a binocular microscope connected to a computer for enlarge vision of the organism. The shape and nature of the organisms seen on the screen were compared to the fungi atlas. Identification of the organisms were carried out by Gram's staining which reviews the evolutionary trend of the microorganism and then followed by series of biochemical test such as catalase test, hydrogen sulphide production test, coagulase test, citrate utilization methyl red test, voges prausker test, haemolysis test, etc. and finally compared the characteristics with that of Taxas of Cowein and Steel 2010 to identify the bacteria.

2.10. Gram's staining

Smear was prepared using a clean grease free glass slide. Crystal violet was

flooded on the smear and allowed for 30 seconds, and was then diluted with gram's iodine which acted as mordant on the smear and left for another 30 seconds. Thereafter, the smear was drained and decolorized using 96% ethyl-alcohol for a minute. After the decolourisation, the smear was counterstained with safranine and left for 60 seconds. Finally, the smear was washed with distilled water and mopped with Whatman filter paper and viewed at ×100 (objective lens) using a binocular microscope. The gram's staining gave the evolutionary trend of bacteria through the gram's stain type which can be gram's positive or gram's negative. The gram's positive retains the color of dye crystal violet (dark purple), while the gram's negative retaining the color of the dye safranine (redish). The cell shape is either cylindrical (rod) or spherical (cocci); the arrangement of the cells is connected in pairs, scattered (clusters), or connected in long rod (chain) which was recorded to guide in bacteria identification.

2.11. Biochemical Test

2.11.1. Catalase Test

This test was carried out to determine the ability of the isolates to produce enzyme catalase which breakdown hydrogen peroxide (H_2O_2) when added. The test bacteria colour was picked and emulsified with 3% H_2O_2 on a clean glass slide. It was observed for bubble formation as the appearance of gas bubbles after a few seconds would show a positive catalase test and negative if no gas bubbles.

2.11.2. Oxidase Test

This test was carried out to detect the presence of the enzyme cytochrome oxidase, an enzyme of the bacterial electron transport chain. An oxidase strip containing oxidase reagent was used. A smear of the isolated test organism was made on the strip using a sterile wire loop. Within 10 seconds of this test, a violet or purple colour development signifies an oxidase positive organism.

2.11.3. Methyl Red Test

During mixed acid fermentation, a variety of end products would be elicited. These include ethanol, succinate, lactate, acetate, formate molecular hydrogen and carbon dioxide. The products vary depending on the bacterial species. In a situation where mixed acid fermentation is the primary fermentation pathway for bacteria species, acidic products will accumulate in sufficient amount. This will drastically reduce the pH of the spent broth. MR-VP medium measuring 15 g was dissolved in one litre of distilled water and 10 ml was dispensed into each tube and autoclaved. The isolates were inoculated into the cooled medium and incubated at 37˚C for 48 hours, after which three drops of 0.002% methyl red reagent was added to 5 ml of the culture and observed. A red colour reaction signifies positive result while a yellow colour indicates a negative result.

2.11.4. Voges Proskauer Test

This test was carried out to determine the ability of the isolates to ferment carbohydrate and produced acetyl methyl carbinol or reduced product 2, 3 buty-

lenes glycol (CH_3 CHOH·CH_3). A 24-hour grown broth culture of bacteria was mixed with 2.0 ml of potassium hydroxide (40% KOH) and 1.0 ml of 5% alcoholic alpha naphthol. After 5 minutes, the colour was observed to change from amber to pink or red which indicates a positive reaction, whereas any other colour will indicate negative to the test.

2.11.5. Indole Test

This test was done to demonstrate the ability of the bacteria to decompose amino acid tryptophan to indole which accumulates in the medium, and peptone water was adopted as the medium. The culture was grown in 3 ml of peptone water and incubated at 37˚C for 48 hours. After incubation, 0.5 ml of Kovac's reagent was added to the culture broth and the tube was rocked gently. The appearance of a dark pink/red band coloration in the alcohol within 1 - 2 minutes would indicate a positive indole test.

2.11.6. Citrate Test

This test was based on the ability of the organism to use citrate as its sole source of carbon and energy, and ammonium salt as the sole source of nitrogen. The medium used was Simmon's citrate agar. The medium was boiled for 15 minutes at 100˚C, and dispensed into test tubes which were then autoclaved for 15 minutes at 15 psi. After sterilization, the medium was allowed to set in 121˚ slanting position before inoculating the organism. The culture was incubated at 37˚C for 48 hours. A colour change from green to blue indicates a positive result while no colour change signifies a negative result.

2.11.7. Starch Hydrolysis

Some bacteria produce exoenzymes such as amylases that act on starch that are transported outside of the cell. The exoenzyme activity can be demonstrated by a change in the substrate. When starch has been hydrolyzed, it would no longer produce a blue colour when gram's iodine is added, the opaque colour of the organism becomes clear. Accordingly, nutrient (starch) agar was prepared with addition of 0.3% soluble starch (that is 0.3 g of soluble starch plus 100 ml of NA) and then mixed and sterilized. The bacteria were inoculated into the starch agar in the plate and incubated at 37˚C for 24 hours and then flooded with iodine solution for 5 minutes, after which the iodine solution was poured away. If the colonies form a blue/black colour it means that the test is negative, otherwise the test is positive meaning that starch has been hydrolyzed.

2.11.8. Hydrogen Sulphide Production Test

This test was carried out to determine if the organism is acidic or alkaline, and can produce gas or hydrogen sulfide. The medium was autoclaved at 121˚C for 15 minutes at 15 psi and allowed to cool in a slant position. The organism was inoculated into the tube by streaking the slant and stabbing the butt. The tubes were incubated at 37˚C for 24 to 48 hours. Slant and butt show yellow when acidic or red when basic. Dark coloration in tube indicates hydrogen sulfide

production and a crack indicates gas production.

2.12. Screening for Heterotrophic Organisms from Isolated Microorganisms

Preliminary screening of the bacterial and fungal isolates was carried out depending on the zones of clearance formed by the organisms on the solid multi-media in the petri dish. All the isolated and identified organisms were cultured on petri dishes containing Nutrient agar and Saubourand dextrose agar (SDA) media supplemented with 3 ml of olive oil for fungal isolates and 0.2 g of starch for bacterial isolates. The Nutrient agar plates were inverted and incubated in the incubator for 24 - 48 hours at 37˚C, while the Saubourand dextrose agar petri dishes were incubated at ambient temperature of 25˚C to 28˚C inside the incubation box for 72 hours and then screened for the formation of zones of clearance around the colonies of microbial isolates. The zones of clearance were then measured and calculated in millimeter (mm).

2.13. Experimental Design

A biological reactor was designed to support microbial treatment of petroleum sludge (Figure 2). The bio-reactor was a vessel where biological process was carried out, involving microbial organisms or biochemical active substances which were derived from the organism. The environmental condition inside the bio-reactor, including nutrient concentration, dissolved gasses, pH and temperature, affected the productivity of the organisms. The reactor has four (4) major components, namely agitator for mixing of contents inside the reactor to achieve a homogenous mixture, baffle for breaking vortex formation inside the reactor, sparger for supplying adequate oxygen, and jacket for maintaining constant temperature. Before commencing the treatment of the petroleum sludge, the sludge was firstly diluted with sandy-loam soil so as to reduce the concentration of total organic content from a higher mg/kg to a lower mg/kg. This process was carried out to enhance aeration and nutrients infiltration because the petroleum sludge was highly compacted.

Figure 2. Biological reactor for microbial treatment of petroleum sludge.

The diluted sludge sample was put in an incubator and left for 3 days (72 hours) before transporting the sludge into five (5) separate bio-reactors (O, A, B, C and D) containing various isolated microorganisms and were immediately homogenized with 160 g of 20:10:10 NPK fertilizer in turn. The fertilizer was applied in liquid form twice a week until the end of the treatment period. This was done to provide Nitrogen, Phosphorus and Potassium, which are major limiting nutrients in the growth of sludge microorganisms. According to [13], the quantity of fertilizer corresponded to a fertilizer application rate of 8.5 kg/m^3 of diluted sludge sample and provided each reactor with approximately 4.3 kg of nitrogen, 2.1 kg of phosphorus and 2.1 kg of potassium per application. In addition, an equal level of oxygen exposure was maintained in the five reactors. This was achieved through tilling, and was done five times a week throughout the remediation period. Control reactors A and B received 5.0×10^{-4} and 1.5×10^{-3} m^3 of fresh water respectively while "treatment" reactors C and D received 5.0×10^{-4} and 1.5×10^{-3} m^3 of saline water (containing 4.54 g/l of NaCl) respectively on a weekly basis. Reactor O, which served as the counterfactual, was only rain-fed. At six weeks intervals, triplicate composite sludge samples were randomly collected from each reactor for laboratory analyses.

2.14. Statistical Analysis

Comparative analysis was carried out to establish any significant difference in the value of THC of sludge between periods of treatment. This was carried out using Friedman test. Multiple pairwise analysis and regression modeling on sludge parameters (physiochemical characteristics of treated sludge) were carried out.

3. Results and Discussion

3.1. Isolated and Identified Organisms from Cultured Sludge

Results obtained during the process of culturing petroleum sludge sample both for bacteria and fungi as well as their identification are presented in **Tables 1-4**. The sludge samples were collected from four (4) different positions in the same field. The samples were coded as S1, S2, S3 and S4. The samples were then cultured in two (2) different media, namely Nutrient Agar (NA) media for bacteria and in Saubourand Dextrose Agar (SDA) media for fungal. For bacteria, sample S1 contained more microbial cells than samples S2, S3 and S4, while for fungal, sample S2 had higher microbial cells compared to samples S1, S3 and S4. Nine (9) different bacteria were identified from the cultured NA media while six (6) fungi were identified from the cultured SDA media.

3.2. Screening of the Isolated Organisms for Sludge Treatment

The result of screening the isolated organisms for possible petroleum sludge treatment is presented in **Table 5** to **Table 6**. The bacteria isolates were screened for their capacity to express sludge treatment. The degrading activities of bacteria

were assessed in terms of the zone of clearance diameter. None of the bacteria strain showed a better possibility of degrading the oily sludge (Table 5). On the contrary, a total of 5 out of 6 fungal strains were selected for sludge treatment

Table 1. Colonies of samples in nutrient agar media.

Sample code	Dilution factor	Colony count	No. of microbial cells
S1	10^{-6}	89	89×10^6 Cfu/g
S2	10^{-6}	15	15×10^6 Cfu/g
S3	10^{-6}	18	18×10^6 Cfu/g
S4	10^{-6}	31	31×10^6 Cfu/g

Table 2. Colonies of samples in saubourand dextrose agar media.

Sample code	Dilution factor	Colony count	No. of microbial cells
S1	10^{-6}	27	27×10^6 Cfu/g
S2	10^{-6}	42	42×10^6 Cfu/g
S3	10^{-6}	32	32×10^6 Cfu/g
S4	10^{-6}	11	11×10^6 Cfu/g

Table 3. Biochemical characteristics of bacteria isolated from sludge.

Isolate code	Gram Reaction	Shape	Catalase	Coagulase	Starch Hydrolysis	Citrate	Indole test	Methyl-red test	VP	Urease	Probable organism
S1A	+	Rods	+	−	+	−	−	−	+	−	*Bacillus badius*
S1B	+	Rods	+	−	+	−	−	+	−	−	*Bacillus megaterium*
S1C	+	Cocci	+	+	+	+	−	+	−	−	*Staphylococcus aureus*
S2A	+	Rods	+	−	+	−	−	−	+	−	*Bacillus badius*
S2B	+	Rods	+	−	+	+	−	−	+	−	*Bacillus subtilis*
S2C	−	Rods	−	−	−	+	+	+	−	−	*Escherichia coli*
S2D	+	Rods	+	−	+	−	−	+	−	−	*Bacillus anthracis*
S2E	+	Rods	+	−	+	+	−	−	+	−	*Bacillus subtilis*
S3A	+	Rods	+	−	+	+	−	−	+	−	*Bacillus subtilis*
S3B	+	Rods	+	−	+	−	−	+	−	−	*Bacillus megaterium*
S3C	+	Cocci	+	−	+	+	−	+	−	−	*Staphylococcus epidermidis*
S3D	+	Rods	+	−	+	+	−	−	+	−	*Bacillus cereus*
S4A	−	Rods	+	−	−	+	−	+	−	+	*Klebsiella* species
S4B	−	Rod	+	−	−	+	+	+	−	−	*Escherichia coli*
S4C	+	Rods	+	−	+	+	−	−	+	−	*Bacillus cereus*
S4D	+	Rods	+	−	+	−	−	+	−	−	*Bacillus anthracis*

+positive, −negative.

Table 4. Morphological and microscopic characteristics of fungi isolated from sludge.

Isolate code	Cultural morphology	Microscopy	Probable organism
S1	Whitish cotton wool like face	Canoe-shaped conidia, clustered conidiophores.	*Saccharomyces cerevisiae**
S2	Green powdery colonies at both front and reverse view of the plate	Single branch and a separate hyphae with conidia lined at the tips of each hyphae.	*Penicillium* sp*
2A1	Dark grayish surface, dark on reverse side view	Separate hyphae with branched conidiophores bearing vesicles that produce chorines of conidia.	*Aspergillus* sp*
3A1	Mycelium extensive and cotton wool like, white in color	Canoe-shaped, has a distinct foot cell in chains divided by several cross walls.	*Microsporium* sp*
3A2	Greenish dark grey surface	Separate hyphae, unbranched conidiophores bearing vesicles that produce chains of conidia.	*Aspergillus* sp*
2A2	Green powdery colonies at both front and reverse view of the plate	Single branch and a separate hyphae with conidia lined at the tips of each hyphae.	*Verticillus* sp*

*Selected.

Table 5. Zone of clearance produced around bacterial colonies.

S/N	Name of organism	Zone of clearance (mm)
1	*Bacillus subtilis*	1.00
2	*Bacillus megaterium*	1.13
3	*Bacillus anthracis*	1.11
4	*Staph aureus*	1.00
5	*Bacillus cerus*	0.00
6	*Klebsilla* species	2.33
7	*Escherichia coli*	1.33

Table 6. Zone of clearance produced around fungal colonies.

S/N	Name of organism	Zone of clearance (mm)
1	*Saccharomyces cerevisiae*	1.00
2	*Aspergillus flavus*	7.67*
3	*Aspergillus niger*	6.88*
4	*Verticillus* sp	13.45*
5	*Penicillium* sp	11.23*
6	*Microsporum audouini*	4.70*

*Selected for sludge treatment.

based on their large diameter for the zone of clearance (Table 6). The selected organisms were used for the treatment of the sludge in the bio-reactors. The five organisms selected were distributed to five reactors, viz reactor A, B, C, D and O containing *Verticillus* sp, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp and *Microsporum audouini*, respectively.

3.3. Characterization of Petroleum Sludge Sample

Table 7 shows the initial and after dilution physicochemical characteristics of the petroleum sludge from crude oil cargo tank, while Figures 2-5 show the percentage reduction of total hydrocarbon content of the sludge after treatment. Sludge samples collected for analyses after 6 and 12 weeks of remediation showed an increase in the pH values in all the reactors, which suggested that there was a release of by-products during the degradation of hydrocarbons. As the duration of the treatment increased, the pH of the sludge changed from acidic to alkaline. Reactor D had the highest increase in pH value while reactor O had the least increase. The pH value of reactor D increased by 30% while that of reactor O increased by only 16%. All pH values were well within the pH range of 7.0 to 8.0, reported to be the optimal range for degrading microflora [14]. This indicates that the bio-treatment of the oily sludge was amenable to saline water.

Table 7. Physiochemical characteristics of the petroleum sludge.

Parameters	Initial sludge sample				Sludge sample 3 days after dilution with sandy-loam soil				DPR limit
	S1	S2	S3	S4	S1	S2	S3	S4	
MC (%)	20.6	20.5	23.2	23.88	25.6	25.5	26.1	26.12	-
pH	5.48	5.44	5.75	5.68	5.52	5.57	5.92	5.82	-
THC (mg/kg)	98,000	98,400	93,500	95,000	64,200	64,700	59,250	62,350	50,000
P (mg/kg)	14.05	14.8	12.8	10.28	28.00	31.5	25.33	21.33	-
TOC (%)	0.88	0.95	0.88	0.89	0.68	0.72	0.69	0.70	-
TN (%)	0.19	0.20	0.18	0.20	0.24	0.25	0.25	0.25	-
C/N ratio	4.63	4.75	4.89	4.45	2.83	2.88	2.60	2.69	-

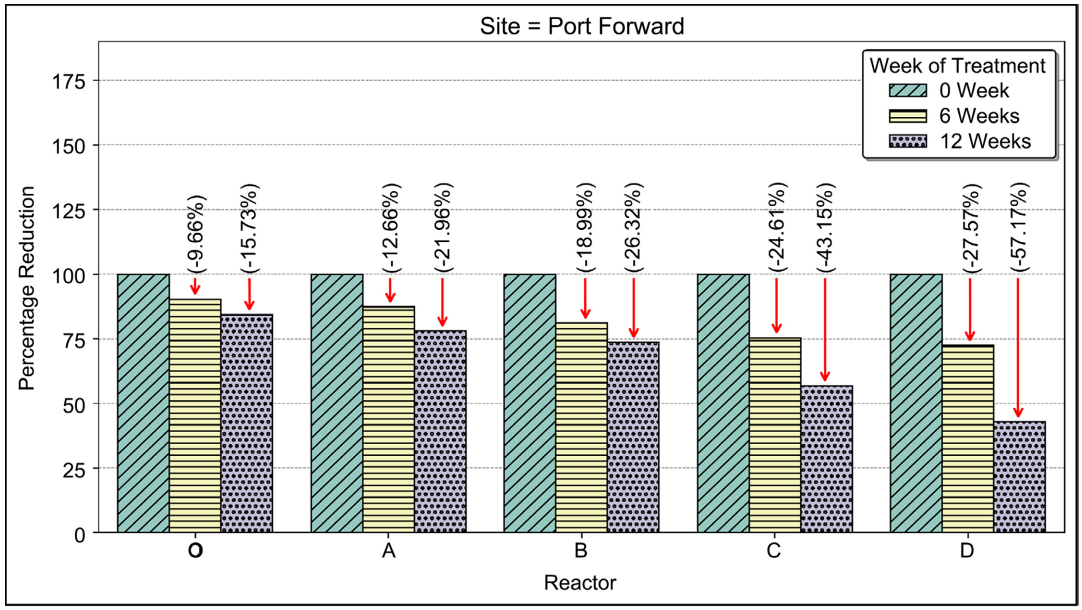

Figure 3. Percentage THC reduction of port forward site sludge after treatment.

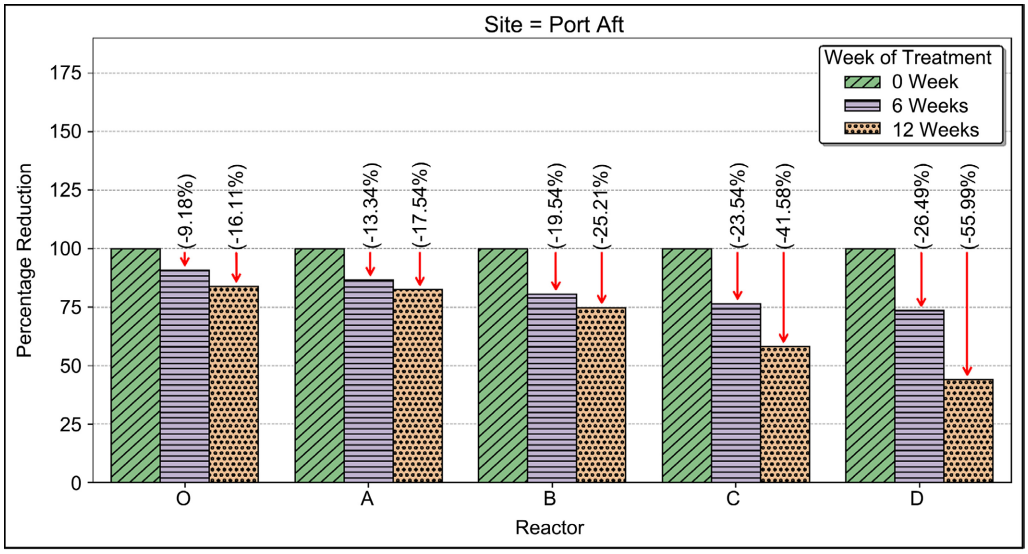

Figure 4. Percentage THC reduction of port aft site sludge after treatment.

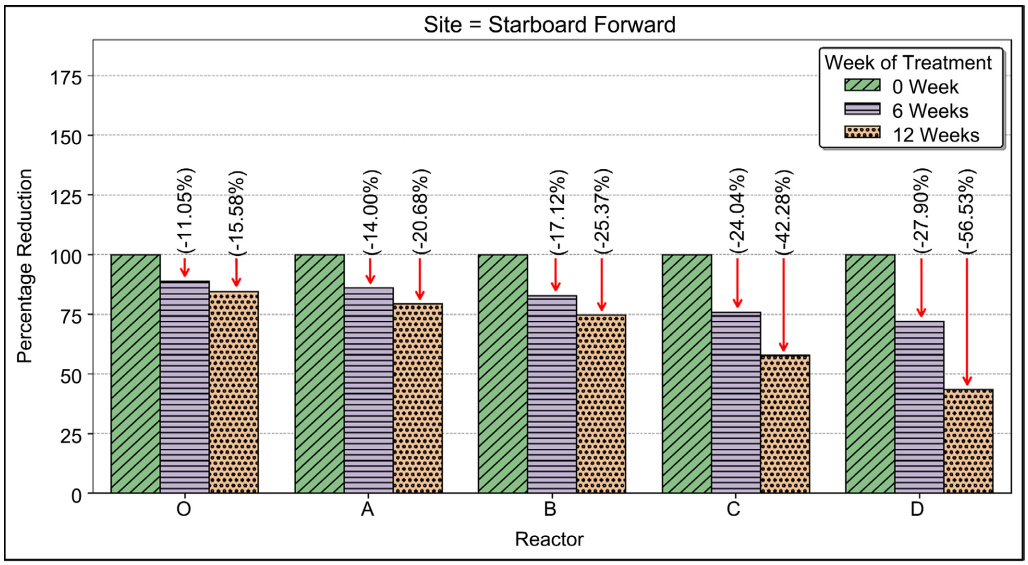

Figure 5. Percentage THC reduction of starboard forward site sludge after treatment.

The total hydrocarbon content (THC) of the oily sludge that range from 98,400 to 93,500 mg/kg for the unbulked sludge and 64,700 to 59,250 mg/kg for the bulked sludge sample were way too far from the 50,000 mg/kg discharge limit prescribed by the Government Department of Petroleum Resources (DPR). This implies that the oily sludge is not safe to be discharged on land without prior treatment. Reactor D had the best reduction ability out of the 5 reactors. After 6 weeks of treatment of the oil sludge, just two reactors (C and D) reduced the THC content to the acceptable DPR limit of 50,000 mg/kg. After 12 weeks of treatment, reactor B was able to reduce the THC content to acceptable DPR limit but reactor O and A failed to reduce the THC initial value to acceptable limit. There were 57.2%, 56.0%, 56.5% and 67.3% reduction in the THC contents after treatment of the oil sludge in reactor D (**Figures 3-6**).

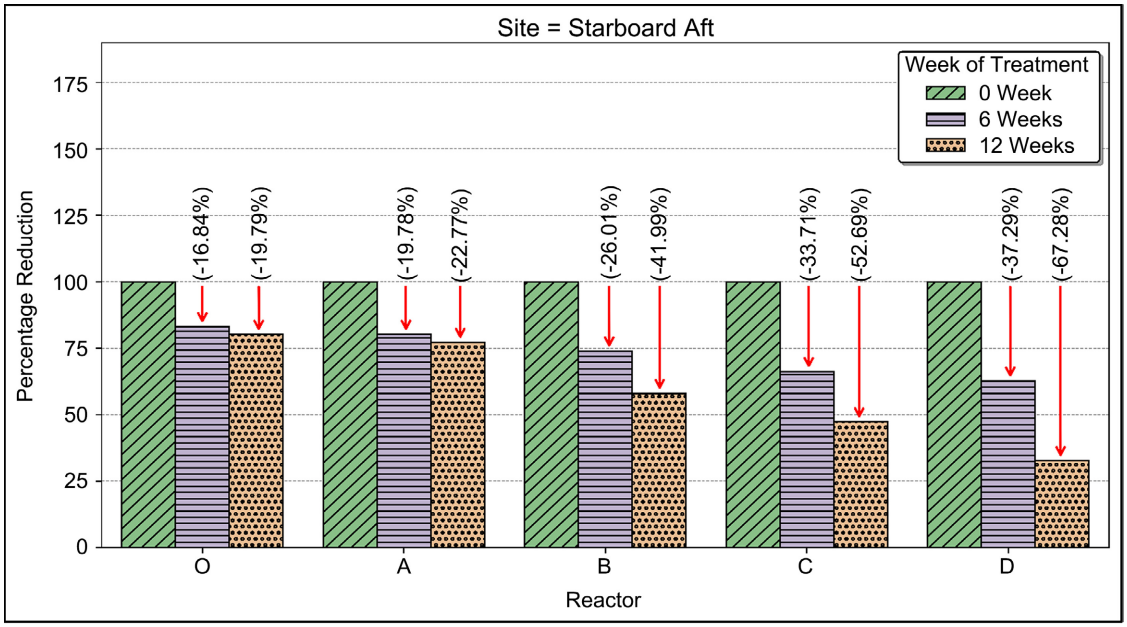

Figure 6. Percentage THC reduction of starboard aft site sludge after treatment.

Generally, there was a drastic reduction in the phosphorus (P) content of the sludge. Reactor C gave the highest reduction (98%) after the 12 weeks treatment period. Reactor O had the least reduction (97%). There was an increase in total organic carbon (TOC) after 6 weeks of treatment in all reactors but dropped drastically after 12 weeks of treatment in all reactors. Reactor D had the highest reduction of TOC after 12 weeks of treatment while reactor O had the least. The reduction was 85.2% in reactor D while it was 79% in reactor O. The total Nitrogen (TN) of the various reactors decreased with increasing period of treatment. This was not expected considering that nitrogenous fertilizer was periodically applied to the degrading sludge and apparently would have increased the total nitrogen of the sludge. This huge loss of nitrogen might largely be due to the biochemical activities of denitrifying fungi isolated from the degrading sludge. This observation is corroborated by a previous study on crude oil-polluted agricultural soil [15] [16].

Carbon-nitrogen (C/N) ratio increased until the 6th week and thereafter dropped in the 12th week in all the reactors except in the control where this drop was minimal. Apparently, the change in C/N ratio within the treatment period seemed very much dictated by the changes in the total organic carbon and nitrogen. The initial increase in the C/N ratio might be linked to trapped leachate and the accumulation of organic carbon (OC) due to evaporation in the reactors and the partial degradation of hydrocarbons by microbes while the drop in the C/N ratio towards the end of the treatment period might be attributed to the use of the carbons as sources of energy by the environmental bacterial population.

3.4. Regression Modeling on Sludge Physiochemical Parameters

From the results, it is shown that reactor D has a better performance from other

reactors. Therefore, reactor D is used as the basis for developing the regression models for future prediction of sludge physiochemical parameters after treatment. From Figure 7 models were developed for the different physiochemical parameters of the petroleum sludge; namely, Linear model for pH and THC; Quadratic model for Phosphorus and Total Nitrogen; and Piece-wise model for TOC and C/N. The Piece-wise model represents a linear plot first with a positive slope to a maximum point and thereafter decreases with increase in time. A typical representation is as given in Equation (2):

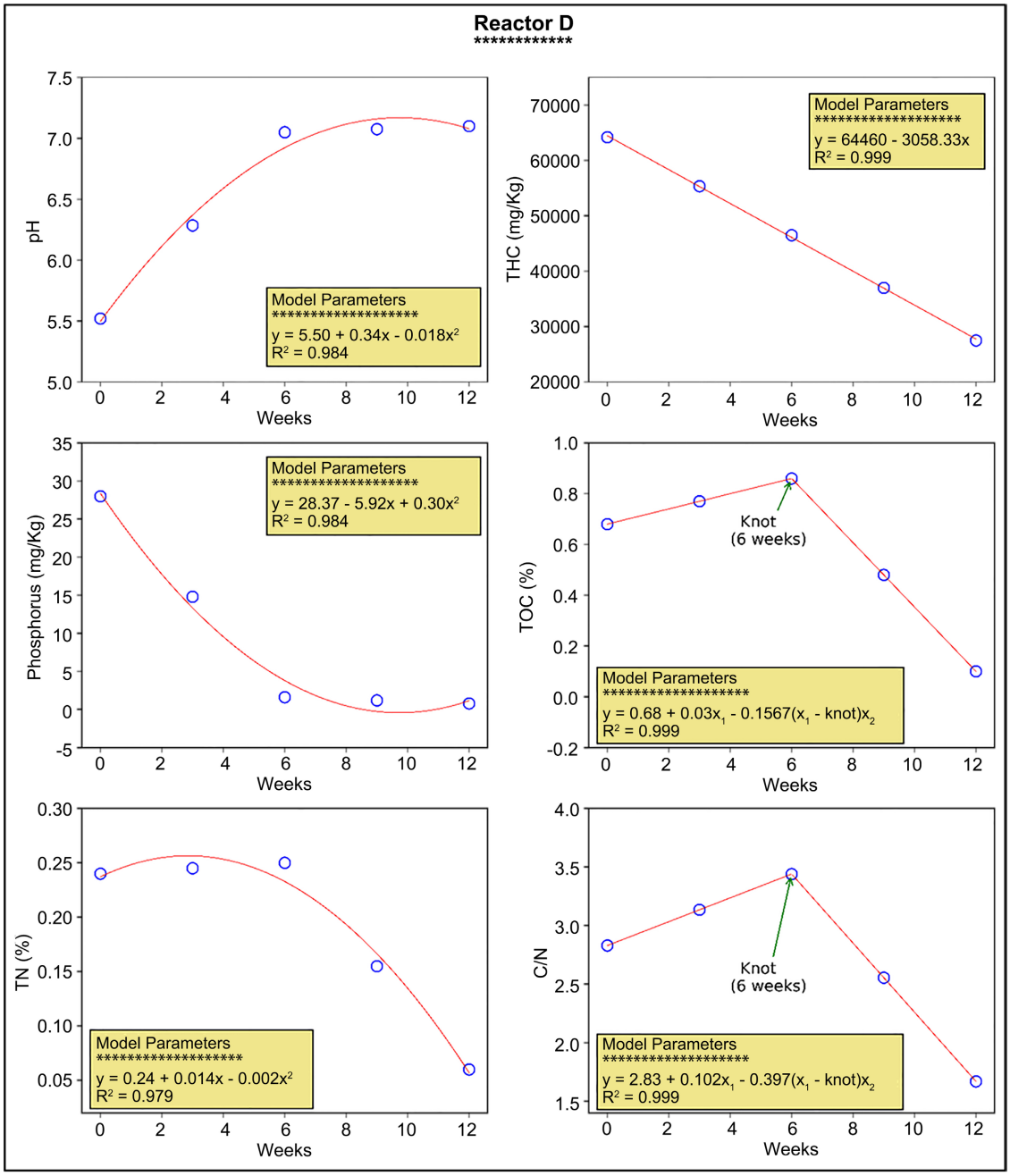

Figure 7. Modeling the effect of treatment of sludge physiochemical characteristic in reactor D after 12 weeks (Site = Port Forward).

$$y = 0.68 + 0.03x_1 - 0.1567\left(x_1 - \text{knot}\right)x_2; R^2 = 0.999 \quad (2)$$

3.5. Friedman Test

Friedman as non-parametric test was used to check if the weeks of treatment affected the reduction of the THC in the five reactors. The Friedman statistic (10.0) was greater than the critical value (5.9915), indicating that there is significant difference in the THC before and after treatment. Multiple pairwise comparison test was used to establish where the difference lied. The result from the multiple pairwise test showed that after 12 weeks the THC significantly reduced in concentration. But no noticeable significant reduction was observed after 6 weeks of treatment.

4. Conclusions

The optimum results showed that in sludge, the oil was 16.2%, water 62% and remaining 21.8% was sediment with emulsified oil. The maximum pH value in the sludge was found as 7.4 which is alkaline in nature. With high alkalinity, the sludge cannot be disposed into open pits.

It may cause hazards and may increase the chances of fire and emission of volatile matter. It may cause environmental pollution as well as damage to the physical properties of the soil at the point of disposal.

Isolated organisms showed the potential for remediating petroleum sludge through a zone of clearance during microbial screening. The petroleum sludge toxicity after treatment was reduced to or below DPR acceptable limit of 50,000 mg/kg THC. Reactor D shows the best performance which is as a result of the organism inside the reactor (*Penicillum* sp).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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