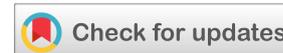




(RESEARCH ARTICLE)



Toxicity of raw and bio slurry treated oily sludge on *Nitrobacter* species and *Tilapia guineensis*

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Abstract

Toxicity of raw and bio-slurry treated oily sludge (OS) on *Nitrobacter* species was determined using standard analytical procedures. The total petroleum content of the OS was 116.44 ± 3.57 g/kg. The TPH was rich with Saturate (40.46 ± 0.73 %). This was followed by Aromatics (27.94 ± 0.50 %) and Asphaltenes (26.58 ± 0.90 %), while NSO fraction had the least proportion (5.75 ± 0.47 %). Contamination of coastal soil with the OS increased the residual TPH concentration in the soil from 5.0561 ppm to 24.2305 ppm. The OS-laden soil were subjected to biodegradation using 1.5-litter borosilicate glass bioreactors bioaugmented with single population of known OS utilizer (*Pseudomonas aeruginosa*) and consortium of known OS utilizer and biosurfactant producing bacterial species (*Bacillus subtilis*). Findings revealed that the bioslurry augmented with the bacterial consortium reduced the concentration of the residual TPH in the soil to 6.313 ppm (73.95% reduction) while 11.5751 ppm (52.23%) was recorded for the single bacterial population. Findings also revealed that the consortium were able to remarkable reduce the toxicity of the raw OS (LC₅₀ = 20.94 ppt) on *Nitrobacter* species. The LC₅₀ for the consortium treated OS was 104.64 ppt while that of single bacterial population treatment was 36.25 ppt. The reduction in the toxicity of the OS treated with the bacterial consortium indicates the potentials of the synergistic action between the biosurfactant producer and OS utilizer to reduce OS toxicity. The findings of this study can be explored as a cost-effective method for oily sludge waste management in the Oil and Gas industry.

Keywords: Bioslurry; Biosurfactant; *Nitrobacter*; Oily sludge; Toxicity

1. Introduction

Crude oil is a major source of energy globally, however the processes involved in processing crude oil results in the generation of considerable amount of waste. Of the various types of waste generated in the refineries, oily sludge is of major environmental concern as most of its constituents have been reported to be mutagenic or carcinogenic [1,2,3]. The poor and improper disposal of this waste has resulted in serious degradation of environmental and aesthetic qualities of the mangroves ecosystems and soil especially in the Niger Delta region of Nigeria. Several authors have reported the efficiency and effectiveness of employing bioremediation technique in treating petroleum contaminated sites [3,4,5,6]. The efficiency of several bioremediation techniques such as bio-augmentation and composting [7], biostimulation [8] and landfilling [9,10] in treating oily sludge contaminated sites have also been well documented. Of the biological treatment processes, bioreactors are considered the most efficient as it can be easily controlled. Toxicity test is used to determine the effect of contamination on the survival, growth, reproduction, behaviour and/or other attributes of an organism. The test also helps to determine whether the contaminant concentrations in a site/or medium is high enough to cause adverse effects in organisms. Several organisms such as fish, shrimps, microorganisms,

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earthworms etc have been used to determine the toxicity of the petroleum products [11]. This study is designed to investigate the efficacy bioslurry treatment in reducing the toxicity of oily sludge on *Nitrobacter* sp.

2. Methodology

2.1. Sample collection

The oily sludge (OS) for this study was collected from Universal Energy Operation Site in Mbo Local Government Area of Akwa Ibom State. The site is situated in the Niger Delta oil rich region of Nigeria where most of the countries crude oil installations and export terminals are located. The area experiences heightened navigational activities and as such spills of petroleum hydrocarbons from both crude oil and refined products occur regularly. The area is characterized by a mean annual rainfall of 2369 mm and a mean maximum daily-temperature of 28 °C [12]. The source of the oily sludge sample was crude tank bottom sludge, product tank bottom sludge and American Petroleum Institute (API) separator unit.

The test organisms (*Nitrobacter* sp.) used in the study were isolated from soil within University of Uyo, Uyo, Akwa Ibom State using DSMZ heterotrophic *Nitrobacter* medium. Grayish, mucoid, flat colonies, stained as Gram negative, with pear shape were selected according to Colwell and Zambrushki's scheme [13]. The isolates were sub-cultured on the same medium and stored at 4°C until when needed. *Pseudomonas aeruginosa* (the best oily sludge utilizer) and *Bacillus subtilis* (Best biosurfactant producer) used in this study were obtained from previous research [14].

2.2. Treatment of Oily Sludge Using Bio slurry

Three kilograms of coastal soil was contaminated with 18 kg oily sludge and mixed properly. This mixture ratio (i.e. soil: oily sludge = 1:6) agrees with that used by Ouyang *et al.*, [7]. One (1) kg of the contaminated soil was then measured into 3 sets of 1.5 liter borosilicate glass bioreactors labelled A, B and C for the bioslurry study. The bioslurry study was carried out in a bioreactor as described by Kuyukina *et al.*, [15] and Chikere *et al.*, [16]. To each of the bioreactors, 500 ml of estuarine surface water samples were added and mixed to form a slurry. A compressor was used to supply air to the bioreactors at a regulated flow just enough to mix the slurry while the temperature and pH of the slurry was maintained at between 28 – 30 °C and 7 respectively throughout the experimental period. Bioreactor A (Control) received no further treatment however Bioreactor B was bioaugmented with 10 ml of 24-hour broth culture of *P. aeruginosa* (best Oily sludge hydrocarbon utilizer from previous study) while Bioreactor C was bioaugmented with 10ml each of 24 hours culture broth culture of *P. aeruginosa* and *B. subtilis* (Best biosurfactant producer). Bioreactor B and C received the same treatment after every 3 day for 30 days degradation study.

2.3. Determination of Concentration of Residual (TPH) Total Petroleum Hydrocarbon

At the end of the 30-day degradation, the concentration of the residual TPH in the bioreactors were measured and compared with that of the raw sludge with any treatment. The concentration of the residual hydrocarbon fraction was determined gravimetrically using EPA method 3540C. The gravimetric method was carried out by modifying the method described by Joseph [17]. The sample was mixed with anhydrous sodium sulphate and consecutively soxhlet- extracted with n-hexane, dichloro methane and chloroform (100ml each) in an extraction thimble. All the extracts were pooled and evaporated in a rotary vacuum evaporator to about 2 ml. The distilling head was removed, and dried in vacuum, cooled, and weighed. The concentration of TPH in the original sample was calculated as.

$$TPH (mgkg^{-1} \text{ dry weight}) = \frac{\text{Gain weight of the flask (mg)}}{\text{weight of solid (g)}} \times 1000$$

2.4. Acute Toxicity Test of Raw and Treated Oily Sludge on *Nitrobacter* species

The Acute toxicity of the raw and treated oily sludge on *Nitrobacter* sp. was determined followed the method described by Ernest and Laurelta [11]. The isolate was inoculated into a Bacto Peptone medium and incubated at 30°C for few hours then used to prepare a stock culture by stock culture containing 180 ml peptone and 20 ml of the inoculum. A preliminary range finding test using three concentrations was carried out to determine the appropriate dilution to be used in the test. Five geometric concentrations (312.5 ppt, 625 ppt, 1250 ppt, 2500 ppt and 5000 ppt) based on the result of the range finding test was sterilized and used for the test. For the *Nitrobacter*, a loopful of the bacterium was inoculated into 20ml peptone water and allowed to stand for 4 hours at 30 °C the aliquot was used to prepare a stock culture. Different concentrations (312.5 ppt, 625 ppt, 1250 ppt, 2500 ppt and 5000 ppt) and into a 25 ml conical flask, sterilized and allowed to cool. Ten (10) ml of the stock solution were then inoculated into the different flask. A control was also set up by inoculating 10 ml of the stock solution into sterile water. The flask was mixed and incubated at 30 ±

2°C. The number of viable cells at 0, 8, 16 and 24 hours were then monitored by inoculation the aliquot on an already prepared DSMZ Nitrobacter agar incubated at 30°C for 24 hours. The EC₅₀ was determined using the Probit method of analysis [18].

3. Results and Discussion

The OS sample was black in coloration with a nearly solid consistency and sticky in nature with a total petroleum content of 116.44 ± 3.57 g/kg. The petroleum hydrocarbon content of the OS was fractionated into four fractions. Among the four fractions of petroleum hydrocarbons (Saturate, Aromatics, NSO and Asphaltenes), Saturate had the highest proportion (40.46 ± 0.73 %). This was followed by Aromatics (27.94 ± 0.50 %) and Asphaltenes (26.58 ± 0.90 %), while NSO fraction had the least proportion (5.75 ± 0.47 %). The ash content of the asphaltene fraction was 15.71 ± 0.97 %. A summary of this result is as presented on Table 1.

Table 1 Total Petroleum Hydrocarbon (TPH) of the Oily Sludge

TPH	Composition % (wt/wt)			
	Sample 1	Sample 2	Sample 3	Mean
Saturate (Hexane extract)	40.21	41.28	39.89	40.46 ± 0.73
Aromatic (benzene extract)	27.65	28.52	27.66	27.94 ± 0.50
NSO	5.27	5.78	6.21	5.75 ± 0.47
Asphaltenes	26.40	25.78	27.55	26.58 ± 0.90
n-pentane insoluble ash content (%) of asphaltenes	16.71	15.66	14.77	15.71 ± 0.97
Total				116.44 ± 3.57

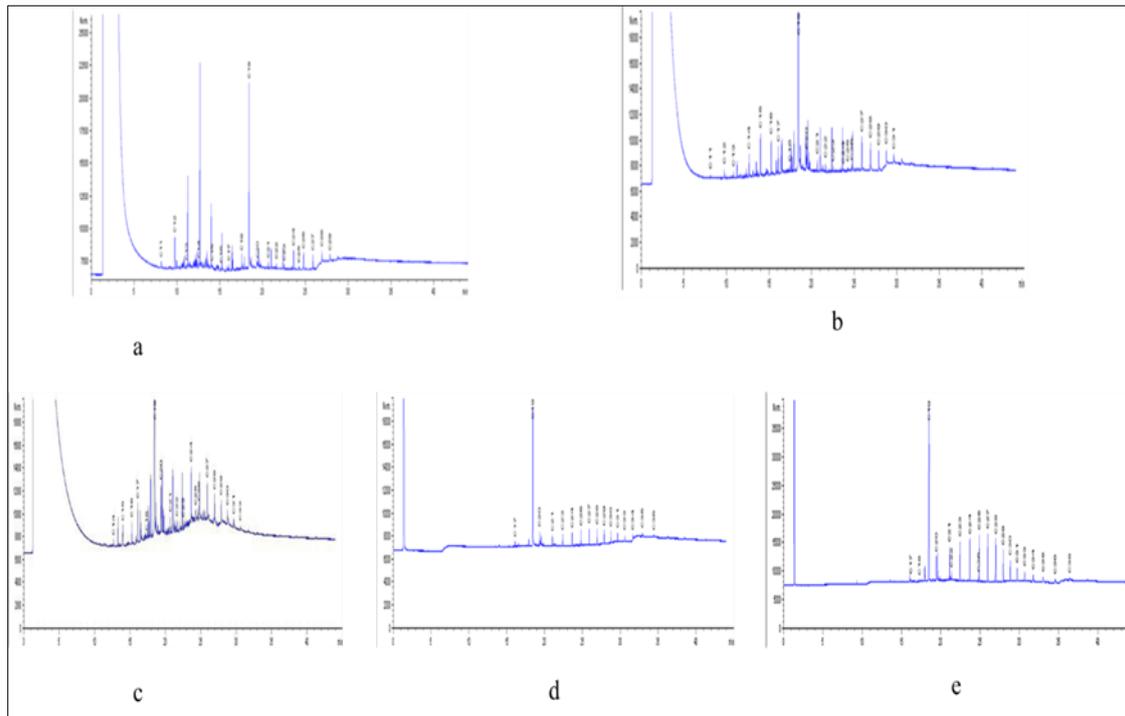


Figure 1 Chromatograms Residual TPH content in the (a) Uncontaminated soil, (b) Contaminated soil, (c) Bioslurry A, (d) Bioslurry B and (e) Bioslurry C

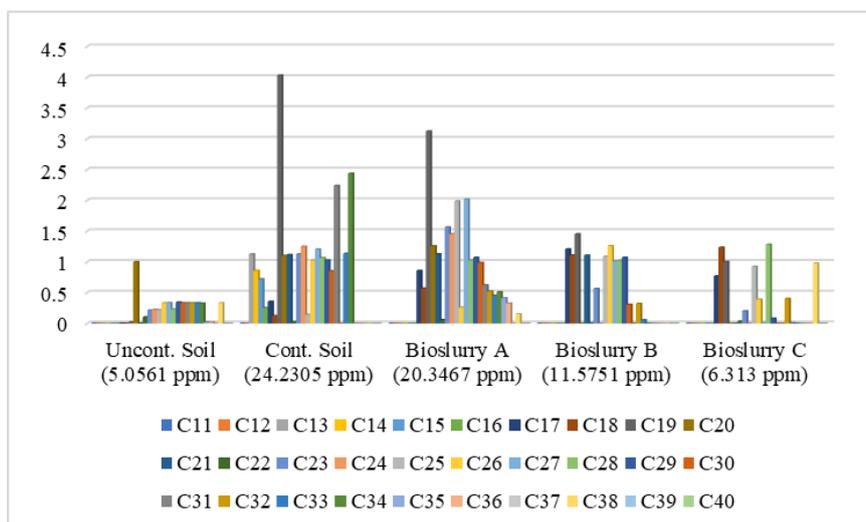


Figure 2 TPH fraction in the Uncontaminated, Contaminated and Treated Soil

Analysis of the concentration of residual TPH in the uncontaminated soil, contaminated soil before and after treatment with bioslurry technique revealed that most volatile fractions of hydrocarbon (C8 – C11) were not detected in the soil prior and after contamination, there were not also present in the treated soil (Figure 1). However, on comparison, contamination of the soil with oily sludge increased the concentration of the C13 to C34 fraction of hydrocarbon in the soil as well as increased the total concentration of Petroleum Hydrocarbon in the soil from 5.0561 mg/kg to 24.2305 mg/kg (79.1%). Treatment of the contaminated soil with bioslurry technique showed vary TPH reduction potentials. The highest reduction (73.95%) in residual TPH concentration was recorded for Bioslurry C which was augmented with cultures of both biosurfactant producer and OS utilizing bacteria while the single population treatment (Bioslurry B) reduced the TPH by 52.23% (Figure 2).

The toxicity analysis of the raw and the residual oily sludge in the bioslurry and biopile was carried according to DPR (2018) standard. The analysis revealed that the raw sludge had a high toxicity on *Nitrobacter* species. However, analysis of the toxicity of the remedied soil treated with consortium of best biosurfactant producer and best OS utilizer had the least toxicity on *Nitrobacter* sp. ($LC_{50} = 104.64$ ppt) followed by Bioslurry B ($LC_{50} = 36.25$ ppt) (Figure 3)

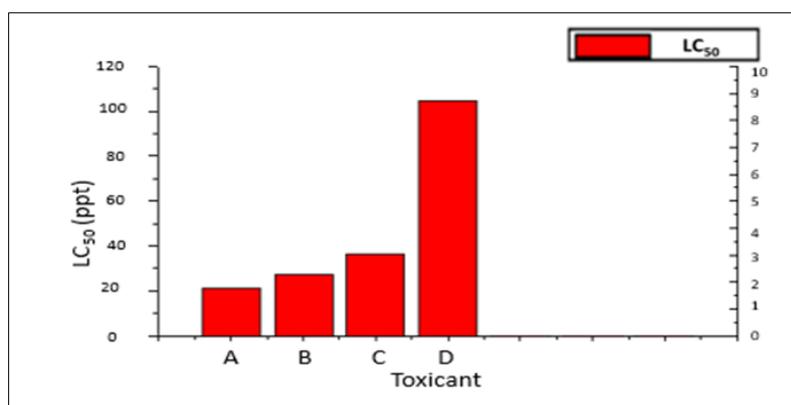


Figure 3 Toxicity of Raw and Treated OS on *Nitrobacter* species

The Oil and Gas is one of the major economic sectors globally, however activities associated with crude oil exploration, production and refining has led to the release of various contaminants onto the environment. One of such pollutants which have received increasing attention is oily sludge. Several factors such as the type and volume of sludge, concentration, presence of toxic or inhibitory substances, type of microorganisms available, etc have been outlined to affect the efficiency and effectiveness of remediation of oily sludge especially when microorganisms are used. The petroleum hydrocarbon content of oil sludge can be categorized into four (aliphatic, aromatics, nitrogen sulphur oxygen (NSO) containing compounds, and asphaltenes) fractions [19]. The oily phase of petroleum sludge contains 40 to 60% saturated hydrocarbons, 25 to 40% aromatic hydrocarbons, 10 to 15% resins and 10 to 15% asphaltenes [20,21]. The

findings of the study also revealed that the oily sludge had a total petroleum content of 824.14 ± 5.98 g/kg. The TPH was rich with Saturate (40.46 ± 0.73 %). This was followed by Aromatics (27.94 ± 0.50 %) and Asphaltenes (26.58 ± 0.90 %), while NSO fraction had the least proportion (5.75 ± 0.47 %). This composition agrees with Hu *et al.*, [22] to stated that the total hydrocarbon content in oily sludges ranges from 5 to 86.2% with an average range of 15 – 50 % while water and solids occupy about 85% and 5 – 46% respectively.

Treatment of the contaminated soil using bioslurry with single bacterial population (*Pseudomonas aeruginosa*) and consortium (*P. aeruginosa* and *Bacillus subtilis*) techniques reduced the residual concentration of the hydrocarbons at various degrees, with the highest percentage of degradation (73.95%) observed in the consortium (Bioslurry C). The high degradation observed in Bioslurry C may be attributed to the activity of biosurfactants [1]. The finding of this study agrees with several studies which have reported that biosurfactant enhances biodegradation by increasing the bioavailability of the pollutant [23, 24]. Biosurfactant producing bacterial species have also been reported to enhance the biodegradation of crude oil [25, 26]; PAHs [27] and oily sludge [28].

The high toxicity of the raw sludge reported in the study is a pointer to the stipulated regulation by the Department of Petroleum Resources (DPR), which recommends that petroleum refinery oily sludge should be treated before disposal in a method that does not pose any danger to human life and living organisms nor cause significant pollution to ground and surface water [29]. One of such treatment methods is the use of bioremediation (bioreactor, land farming and biopile). Treatment with bioslurry remarkably reduced the toxicity of the OS on *Nitrobacter* species. The toxicity of the oily sludge in the decreasing order is as follows Raw sludge > Bioslurry A > Bioslurry B > Bioslurry C.

4. Conclusion

The findings of this study have revealed that petroleum refinery oily sludge if not treated properly before disposal could pose serious risk to the receiving environment. The reduction in the toxicity of the OS treated with the bacterial consortium indicates the potentials of the synergistic action between the biosurfactant producer and OS utilizer to reduce OS toxicity. The findings of this study can be explored as a cost -effective methods for oily sludge waste management in the Oil and Gas industry.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have declared that there is no conflict of interest.

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