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Biological Treatment of Oil Sludge from Petroleum Product Storage Tank

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ABSTRACT

Oil sludge which contains water, sediment, hydrocarbons and other non-hydrocarbons needs to be treated to recover the useful content and appropriately dispose the waste. Biological treatment had been found to be most economical and more environmental friendly among the various methods for treating oil sludge. In this study, oil sludge samples collected from Fort Oil Plc in Nigeria were subjected to microbial degradation using positive gram bacteria (*Bacillus firmus*) to recover the useful oil therein. Different quantities of the oil sludge were inoculated with the microbes and allowed to undergo different incubation period ranging from 3 to 18 days. The *Bacillus firmus* used was isolated from culture of the oil sludge and identified using Analytical Profile Index (API) test kits. The hydrocarbon composition of the oil recovered was anaylsed using Gas Chromatography with flame Ionization Detector (GC-FID). Also, the pH and the density of the recovered oil were determined. The average density of the recovered oil was 0.781g/cm^3 and pH value of 6.92. The average amount of oil recoverable from the oil sludge was 20.7 g per 100 g of oil sludge. It was further observed that the oil sludge was greatly biodegraded by *Bacillus fermus* based on the oil recovered and left over residue after the biodegradation process up to about 70.2%, which is close to the values obtained by previous researchers. The result showed that decane constitutes the least % mole fraction of the recovered oil with value of 0.0049% while tetratricontane was the highest component with % mole fraction value of 40.1433%. *Bacillus fermus* demonstrated capability to biodegrade oil sludge from petroleum products tank and as well made room for recovery of some of the oil inherent in the sludge. Hence the use of this microorganism is proposed for treatment of oil sludge from petroleum products tanks.

INTRODUCTION

Oil sludge generation in the oil and gas industry is a great concern majorly because of the hazardous effects of some of its composition. It has been classified by the United States Environmental Protection Agency (US EPA) as a hazardous organic complex [1-2]. Ordinarily, oil sludge may be regarded as a thick, soft, wet, mud. It is a complex viscous mixture. As a result of its hazardous content there is need for adequate treatment of oil sludge before disposing the treated effluent into the environment [3]. It was observed that about 50 tons of oily sludge per year could be generated by a petroleum refinery with a production capability of 105,000 drums per day [4]. This will constitute a huge source of environmental hazard to man and other living organisms at the end of the year if not properly handled. The composition of oil sludge depends on its source. It may be from crude oil storage tanks, petroleum products storage tanks or refinery-wastewater treatment plants [5-7]. Oil sludge found in crude oil storage tanks, is mainly made up of Saturates, Aromatics, Resins And Asphalthenes (SARA) just as the typical composition of a crude oil is. Other non-hydrocarbon components of oil sludge are phenols, heavy metals, chlorinated hydrocarbons and inorganic solids such as sand, iron sulfides and iron oxides. In addition to these hydrocarbons and non-hydrocarbons, materials or chemical used in the refinery wastewater treatment plants may add to the list of oil sludge obtained from this source. Asia *et al.* [8] observed that elemental composition of petroleum sludge consists of Nitrogen, Phosphorous, Potassium, Iron, Copper, Calcium, Magnesium, Cadmium, Phosphate, Chromium, Zinc, Sodium, and Lead. [9] in their study noted that oil sludge from crude oil storage vessels could be typically made up of sulphides, phenols, heavy metals, aliphatic and Polycyclic Aromatic Hydrocarbons (PAHs) of 4, 5, 6 and more rings, in over 10-20 fold concentration [9]. However, United States Environmental Protection Agency observed that high content of aromatic hydrocarbons ranging from C_1 to C_{40} could be found in oil sludge [1]. Most common methods for treating petroleum and petroleum products oil sludge are chemical treatment [10 – 11] and biological treatment [7, 12-14]. Biological method seems to be more environmentally friendly and cost effective than the chemical method [12]. It was noted that indigenous microorganisms isolated from the oil sludge will do a great deal in treatment of the oil sludge biologically, as the microorganisms can degrade the components and have a higher tolerance to toxicity that may wipe off other introduced species [15]. Few reports are in the literature regarding the isolation of bacteria directly from oil sludge but far more common to find references on bacteria isolated from hydrocarbon-contaminated soils [16]. It has been found that several strains of the genera

Pseudomonas, Stenotrophomonas and *Bacillus* isolated from hydrocarbons-contaminated soils are able to grow and degrade aliphatic and aromatic hydrocarbons [16 -19]. *Stenotrophomonas acidaminiphila* was isolated from anaerobic sludge in a lab-scale Upflow Anaerobic Sludge Blanket (UASB) reactor treating petrochemical effluents by Assih *et al.* [17], while *Pseudomonas aeruginosa* was isolated from hydrocarbon-contaminated soil and used as biosurfactant producers [18]. Also, Cerqueira *et al.* [16], isolated *Stenotrophomonas acidaminiphila, Bacillus megaterium and Bacillus cibi* from petrochemical oily sludge and *Pseudomonas aeruginosa* and *Bacillus cereus* from soil contaminated by petrochemical waste investigated their ability to degrade hydrocarbons. Furthermore, previous researchers have investigated the ability of A*cinectobacter sp., Rodococcus sp., mycobacterium sp., Pseudomonas sp., Arthrobacter sp., Staphylococcus sp., Bacillus megaterium* and *Bacillus cereus* strains to degrade hydrocarbons and produce biosurfactants [19 – 21]. However, reports regarding isolation from oil sludge, hydrocarbon degradation capacity and studies on biosurfactant production by *Bacillus fermus* were not found. It is pertinent to investigate this and also to study the possibilities of recovery of oil from oil sludge treated with *Bacillus fermus*. This study focus on treatment of the oil sludge with *Bacillus fermus* isolated from the sludge and investigate the possibilities of recovery some oil components of the sludge in the process.

MATERIALS AND METHODS

2.1 Sample collection

The oil sludge sample used in this study was collected from Forte Oil Plc. Hexane and other chemical reagents used in the study were analytical grade products from Qualikems Laboratory and BDH Limited Poole England.

2.2 Isolation of the microorganism

To isolate the microorganism used for this study, 5 g of crude oil sludge collected from Forte Oil Plc was weighed with foil paper. Three different 45 ml of sterile distilled water were prepared in conical flasks and the weighed 5 g crude oil sludge was transferred into first 45 ml of sterile distilled water. Serial dilution was made for the 3 conical flasks. Also, 100 ml of nutrient agar was prepared aseptically. This was done by weighing 2.8 g of nutrient agar powder into the flask and made it up to 100 ml with distilled water. It was autoclaved at 120 ℃ for 15 minutes in (SHENAN LDZX-50FB vertical heating pressure steam sterilizer). The detailed composition of the growth medium is shown in Table 1. Pour plate technique (a plate

prepared by mixing the inoculum with the cooled but still fluid medium before pouring the latter into the petric dish) was applied by plating out the inoculum into dilution one, two and three in the Petri dish. The plates were incubated at 37 ℃ for 18-24 hrs. The growth plates were harvested at 24 hrs. The API Kits (Biomerieux API kits) were used to confirm the specie of the bacteria as *Bacillus firmus*. The identification tests were carried out on the isolate at the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria.

Table 1. Composition of the growth medium for the *Bacillus firmus*

Composition of Growth Medium	**Amount per Liter of Solution**	**Functions**
Beef Extract/Yeast Extract	3.0g	The water content of these contribute vitamins, carbohydrates, nitrogen and salt
Peptone	5.0g	This provides the organic nitrogen
Agar (HIMEDIA M001-500G)	20.0g	This gives the mixture solidity
Distilled Water	1000ml	Water serves as a transport medium of the agar various substances
Sodium Chloride	8.0g	This gives the mixture proportions similar to those found in the cytoplasm of most organisms
Ph	7.0-7.2	Adjusted to normal

2.3 Treatment of the oil sludge by the microorganism

Samples of the oily sludge were collected and subjected to microbial degradation aimed at recovering the oil from the oily sludge solution by introducing a gram positive bacterial called *Bacillus firmus* to it. The biodegrading abilities of *Bacillus firmus* on oily sludge solution was tested in 250 ml mineral salts medium containing 3-5% (v/v) oily sludge solution and 50 ml inoculums. The mixture was incubated at 28 °C in an Edmund Buhler Johanna Otto incubator shaker at 200 rpm for 3, 6, 9, 12, 15, 18 days. The microorganism grew in the medium by

feeding on the oily sludge (biodegradation) and the residual oil was thereafter separated from the solution using a centrifuge. The sample was transferred into the centrifuge where the oil was separated from the mixture. This was done repeatedly for varying masses of the oily sludge (30 g, 25 g, 20 g, 15 g, 10 g, and 5 g). The volume and mass of the oil extracted were determined and recorded using measuring cylinder and digital weighing balance (RADWAG WTB 2000) respectively. Also, the mass of the final residue from the treated oil sludge was determined and recorded. All the laboratory works were carried out in the Biochemical Engineering Laboratory of University of Lagos and all materials were acquired in Lagos.

2.4 Analysis of the recovered oil

2.4.1 pH determination

The pH of sample of the recovered oil was determined using JENWAY 3505 pH meter. The meter was adjusted to read zero by dipping the pointer into the buffer solution after which the pointer was dipped into the recovered oil and the reading was taken. This was done for all the oil extracted from the different masses of the oil sludge.

2.4.2 Hydrocarbon composition of the Extracted Oil

The hydrocarbon composition of each extracted oil was determined using SRI instruments Gas Chromatograph (Model: 8610C) with Flame Ionization Detection, GC-FID. The extracted oil sample was transferred into test tubes and put inside the machine and the results were taken on the system. Helium was used as the carrier gas for the Flame Ionization Detector (FID) while hydrogen was used as the fuel gas. Also, the make-up gas used in analysis was compressed air.

RESULTS

3.1 Microorganism (Isolate) Identification

From the API kits test of the isolated microorganism, it was confirmed that the microorganism was *Bacillus firmus* (Figure 1). The Analytical profile index tests carried out on the isolated microorganism indentified the microorganism to be *Bacillus firmus* using the API 20 E and API 50 CH (Figure 1).

Figure 1 The API tests result of the isolated microorganism

3.2 Analysis of Recovered oil

The average pH of the recovered oil from the oil sludge after the biodegradation was 6.92. It was observed from the results that required incubation period for the microorganism growth for recovery of oil from the sludge increases with decrease in quantity of the sludge to be treated as shown in Figure 2. The highest incubation period of 432 hours (18 days) was recorded for oil sludge of 5 g while the least incubation period was recorded for oil sludge of 72 hours (3 days) was recorded for oil sludge of 32 g. These could be as a result of the little substrate nutrients in the 5 g oil sludge that will enhance the growth of the microorganisms while the 30 g oil sludge contained large quantity of nutrients that speed up the biological growth of the microorganisms. The observed time lag in biological treatment, especially during treatment of low quantity of oily sludge, may be attributed to the stability of the compounds, their complex molecular structures and the ability of oil sludge components to adsorb onto sediments [22]. Conversely, the volume of recovered oil increases with increase in quantity of sludge treated (Figure 2). Highest oil recovery of 5.12 ml was recorded from 30 g oil sludge while the least oil recovery of 2.12 ml from 5 g oil sludge. The average amount of oil recoverable from the oil sludge was 20.7 g per 100 g of oil sludge. It was further observed that the oil sludge was greatly biodegraded by *Bacillus fermus* based on the oil recovered and left over residue after the biodegradation process up to about 70.2%, which is close to the values obtained previous researchers [16, 23-24]. Verna *et al* [23] obtained 59% from biodegradation of mineral liquid medium of oil sludge using isolate *Bacillus* sp. SV9 for 5 days. Zhang *et al* [24] verified an increase in the biodegradation rate of refinery oily sludge from 69.4% to 77.4% after optimizing the fermentation conditions of *Bacillus* HJ-1 growing in liquid medium for 7 days

while Cerqueira *et al* [16] obtained 83.1–87. 4% of oily sludge degradation related to the initial content for isolates and microbial consortium of *Stenotrophomonas acidaminiphila*, *Bacillus megaterium, Bacillus cibi, Pseudomonas aeruginosa* and *Bacillus cereus.*

The hydrocarbon compositions of the oil recovered from treatment of the oil sludge ranges from octane to hexatricontane (Figure 3). The result showed that decane constitutes the least % mole fraction of the recovered oil with value of 0.0049% while tetratricontane was the highest component with % mole fraction value of 40.1433%. It was observed that octane, decane, eicosane, tricosane, hentricontane, and pentatricontane were present in traces of approximately 0 % in the recovered oil. Furthermore, octadecane, phytane, nonadecane, heneicosane, docosane, tetracosane, pentacosane, hexacosane, heptcosane, octacosane, dotricontane and hexatricontane were between approximately 1 % to 5% in the recovered oil. Between 5% and 10% approximately were tricontane and triatricontane hydrocarbons in the recovered oil. Only Nanocosane which was second to the highest component in the recovered oil was approximately between 10% and 20% (about 16%). Definitely, the components of the hydrocarbons in the recovered oil is a reflection of the source of the oil sludge and its initial compositions in addition to the other hydrocarbon that may have been generated as a result of the biodegradation of higher hydrocarbons in the original oil sludge. These are in agreement of the findings of previous researchers [16]. This large composition of tetratricontane ($C_{34}H_{70}$) in the recovered oil may an indication that the microorganism has little capability for biodegrading ($C_{34}H_{70}$). This is in agreement with findings of other researchers that the biodegradability of petroleum compounds follows a decreasing preferential order: n-alkanes > branched-chain alkanes > branched chain alkenes > monoaromatic > cycloalkanes > polyaromatic > asphaltenes [25]. Also, Cerqueira *et al.* [16] noted that there was efficient degradation of aliphatic compounds of n-$C_{11}H_{24}$ to n-$C_{28}H_{58}$ by isolates and microbial consortium of *Stenotrophomonas acidaminiphila*, *Bacillus megaterium, Bacillus cibi*, *Pseudomonas aeruginosa* and *Bacillus cereus.* Furthermore, the presence of phytane or 2, 6, 10, 14-tetramethylhexadecane ($C_{20}H_{42}$) in larger percentage (4.73 %) than eicosane ($C_{20}H_{42}$) percentage (0.34 %) is in agreement with the findings of other researchers that isoprenoid alkanes like phytane present in fuel are relatively resistant to biodegradation and are more slowly degraded when compared with linear alkanes due to their branch nature [26].

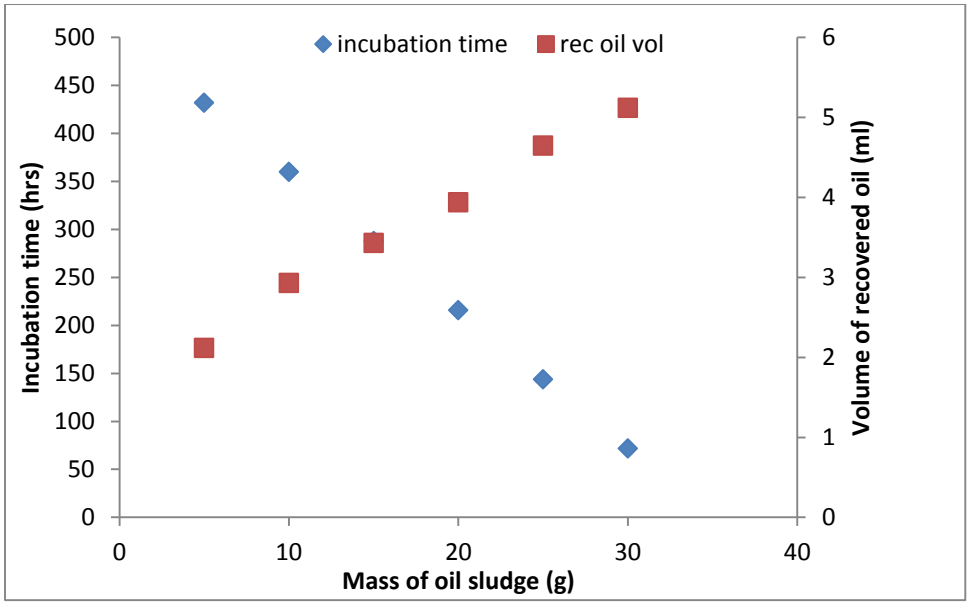

Figure 2 Graph of mass of sludge against volume of recovered oil and incubation time

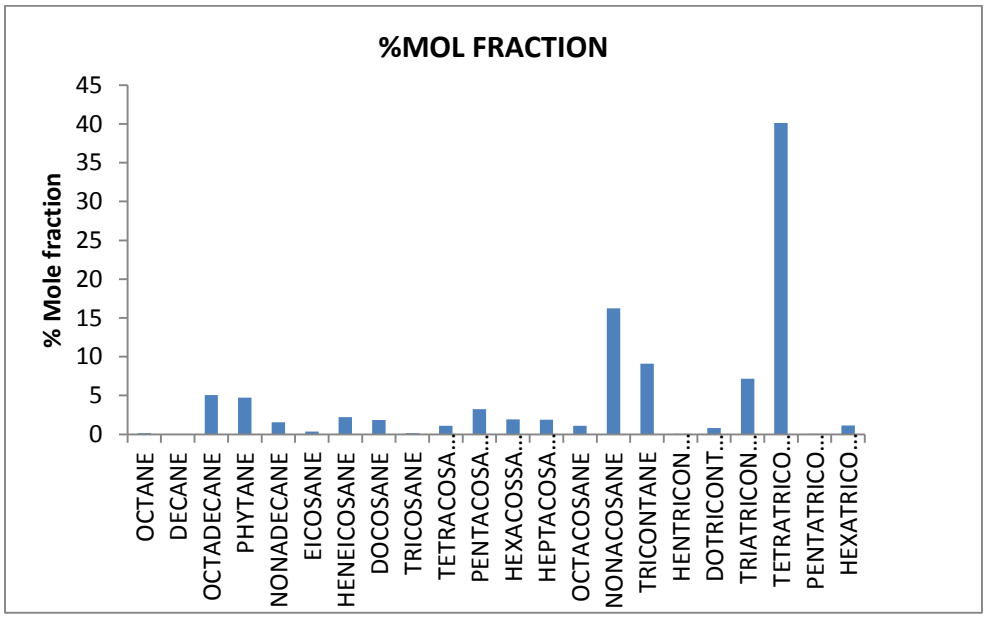

Figure 3 Hydrocarbon composition of the recovered oil from the oil sludge

CONCLUSION

Oil sludge samples collected from Fort Oil Plc in Nigeria were subjected to microbial degradation using positive gram bacteria (*Bacillus firmus*) to recover the useful oil therein. Different quantities of the oil sludge were inoculated with the isolated microbes from culture of the oil sludge and allowed to undergo different incubation period ranging from 3 to 18 days. The Analytical profile index tests carried out on the isolated microorganism indentified the

microorganism to be *Bacillus firmus* using the API 20 E and API 50 CH. The average density of the recovered oil was 0.781g/cm^3 and pH value of 6.92. The average amount of oil recoverable from the oil sludge was 20.7 g per 100 g of oil sludge. It was further observed that the oil sludge was greatly biodegraded by *Bacillus fermus* based on the oil recovered and left over residue after the biodegradation process up to about 70.2%, which is close to the values obtained previous researchers. The result showed that decane constitutes the least % mole fraction of the recovered oil with value of 0.0049% while tetratricontane was the highest component with % mole fraction value of 40.1433%. Bacillus fermus demonstrated capability to biodegrade oil sludge from petroleum products tank and as well make room for recovery of some of the oil inherent in the sludge. Hence the use of this microorganism is proposed for treatment of oil sludge from petroleum products tanks.

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