Recovery of Aliphatic Hydrocarbons from Oil Field Sludge using Bacillus sp

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ABSTRACT

Bioremediation of aliphatic HC (Hydrocarbons) in the oily sludge of Kunnar oil and gas field, Pakistan was attempted by means of previously isolated and developed Bacillus sp. Both autoclaved and non-autoclaved sludge samples were analyzed for a reaction time of 30 days with pH 7 and temperature of 38^0C in 50 ml define MSM growth media for the sludge concentration of 5, 10 and 50% with 2, 4 and 6 ml of Bacillus sp. relatively, in air atmosphere. Stabilization of the samples by microbial activity resulted in the decrease in TPH (Total Petroleum Hydrocarbon) concentration by 60, 69 and 87% in autoclaved samples in contrast to the decrease of 70, 84 and 94% observed in non-autoclaved samples, relatively. Hydrocarbon degradation in oily sludge was investigated via GC (Gas Chromatograph) which transpired that 97 and 99% concentration of aliphatic hydrocarbons in autoclaved and non-autoclaved samples was removed at 5% of TPH concentration, relatively. However, with 10% TPH concentration aliphatic hydrocarbons reduction was 68% in autoclaved samples to that of 87% in non-autoclaved samples. Further increase in the hydrocarbons concentration by 50% yielded in the removal of aliphatic hydrocarbons by 65% in autoclaved samples as compared to 98% decrease in non-autoclaved samples.

Key Words: **Oily Sludge, Aliphatic Hydrocarbons, Bioremediation, Bacillus sp., Optical Density.**

1. INTRODUCTION

Chemical industries in Pakistan mostly use petroleum products as their primary energy source [1-2]. As a result, these industries during the course of production process essentially generate bulk quantities of a waste material known as oily sludge, which is mechanically separated within the plant to be stored into nearby ditches. Due to the harmful sludge characteristics, collection and management of oily sludge is a critical matter for the industries. However, sludge disposal into muddy ditches for its impounding inevitably results in soil pollution via leaching of sludge contaminants into the soil [3]. To overcome this problem, newly constructed ditches are plastic-coated to avoid the leaching of

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pollutants into soil. On other hand, construction of such specially built oily sludge pits are costly, difficult to sustain and require more area to store the oily sludge in the open pits [4]. Thus, sludge waste handling is one of the main issues being countered by the oil industry in order to protect the environmental resources including surface and groundwater as well as aquatic life. Oily materials are composed of hydrocarbons such as aromatic, aliphatic, asphaltenes, etc. Aromatic components are less biodegradable than aliphatic, while asphaltenes tend to be non-biodegradable that can pose severe environmental problems when such spills occur [5]. Therefore, safe throwing away of oily sludge is a foremost priority for the refineries in terms of environmental protection. Oily sludge is also recognized to contain harmful ingredients with potential health concern [6]. Different treatment methods have been applied to remove hydrocarbons from polluted soils that include soil vapors extraction, soil incineration and chemical treatment [7]. Traditional treatment methods such as filtration and evaporation to remove contaminants from oil sludge such as TPH don't eliminate them from the source rather convert them from primary to secondary contaminants. However, bioremediation is increasingly getting prominence for being simple and effective technique in removing pollutants from the environment without turning them into secondary pollutants, which are neither toxic nor harmful. Bioremediation with specific bacterial community is an effective method in which bacteria play a significant role in the elimination of hydrocarbons thereby removing the primary pollutants from the given pollution media.In addition, bioremediation is a low-cost, simple and energy-efficient method as against traditional mechanical methods involving costly machinery, which are complex in operation as well as energy and cost-intensive [8]. Oil and gas field activities generate lot of in-situ oily sludge, which is highly reactive and toxic in nature. Thus, the handling of this waste sludge in terms of its viable treatment is a major issue for such companies. Dumping of oily sludge leads to extensive pollution of soil mainly due to the presence of higher concentration of TPH, which can be a potential source of environmental pollution.

This research study was, therefore, aimed at keeping this issue in view and hence the objective of this work was to attempt removal of aliphatic hydrocarbons from the sludge material using bioremediation technique that involved the application of indigenously isolated bacteria in order to reduce the presence of pollution-causing substances present in the sludge such as aliphatic hydrocarbons.

2. MATERIALS AND METHOD

2.1 Sample Collection

The oily sludge samples were collected in sterilized plastic bags via a sterilized spoon under standard conditions of temperature and pressure from oil and gas field discharge pit. The samples were then passed from a 0.5cm screen to eliminate bulky and other unwanted material.

2.2 Estimation of Total Petroleum Hydrocarbons

Petroleum hydrocarbons from oily sludge were separated via soxhlet-extraction method (EPA technique 354^{o}C). Before extraction was applied, anhydrous sodium sulfate was added into oily sludge sample followed by its transfer to extraction thimble. The extraction was carried out with 100 ml each of n-hexane, dichloromethane and chloroform. All the three extracts were collected, evaporated in a rotary vacuum evaporator before being cooled at room temperature and weighed.

2.3 Microorganisms and Bacterial Inoculums Preparation

Bacteria utilized in this study were already isolated from the collected sample of polluted field (Aftab, et. al, [8]). To make the required microbial culture, recommended components were added with their specified ratios (Table 1). Microbial colony, as observed, was picked on agar plate and cultivated in minimal salt medium (OD600 nm) at 38^{0}C, 160 rpm for 24 hours.

2.4 Biodegradation of Oily Sludge

Hydrocarbons remediation prospective of decontaminated inoculums was examined in 250mL sample in corresponding restraining 50 mL minimal salt media by 5, 10, 50% (v/v) autoclaved and non-

TABLE 1. COMPOSITION OF INOCULUMS

Component	Quantity (g)
Beef Extract	0.15
Peptone	0.25
Sodium Chloride	0.25

autoclaved oily sludge as only carbon source. A permeable yarn cap and enclosed with aluminum foil was worn for proper aeration. Bacterial inoculums volumes of 2, 4, and 6ml from sample were aseptically sub-cultured into another 250 sample every restraining autoclaved and non- autoclaved oily sludge as a cause of carbon and liveliness for the microorganisms development. The isolates purified cultures were cultured before within minimal salt media for one day added component have OD 0.9 equal toward of 10^8 CFU per mL were applied. All samples were kept warm on rotating vibrator at 38^0C in favor of thirty days. Reduction of hydrocarbons was quantified based on the increase in concentration of the isolates the whole time the incubation period of 0, 3, 6, 10, 20 and 30 days. The purity of the oily sludge was confirmed using dispense method on agar plate.

2.5 Bacterial Growth Assessment

OD of the entire medium broth was calculated at 600nm adjacent to distilled water. The last consequence was get with multiplication of the analysis using suitable dilution factor, which must be smaller than 0.5 [9]. One mL of culture was dispensed in dust liberated sampler through autoclaved situations were maintained in a laminar flow cabinet and established turbidity evaluation was calculated.

2.6 Analysis of Oily Sludge

The oily sludge consisting of residual petroleum hydrocarbons in the liquid cultural medium was collected using sample dropper at the end of thirty day treatment time via liquid–liquid extraction. Primarily, the culture medium was acidified to pH 2.0 for stopping the microbial activity further. The samples were shaken vigorously to suspend the solid materials in order to obtain homogenous sample. The oily sludge was extracted with mixture of equivalent amount of n-hexane: acetone: twice. The organic phase containing hydrocarbons was estranged as of solvent segment during short speed centrifuge for 15 min in glass tube to split the oil in water mixture as shown in. The higher film was removed and lesser coating oil containing phase was filtered into pointed sample. The separated oil was dehydrated via mixing sodium sulfate to eliminate the moisture and concentrated by evaporating the hexane and acetone in a rotary evaporator 35^0C-40^0C [10-11]. The amount of oily sludge recovered was quantified via gravimetric technique [12-13].

2.7 GC Study of TPH

The biodegraded oil was further fractionated into aliphatic hydrocarbons.1µl aliquot was separated from diluted concentration appropriate for GC for aliphatic fractions. Individual compounds present in the aliphatic fractions were detected via harmonizing the retention times by authentic standards obtained from Sigma-Aldrich, Pakistan. Extracted TPH fraction was analyzed by GC plus (Shimadzu- 14B series, Japan) fixed with flame ionization detector. Residual TPH analysis for aliphatic and aromatic fraction was carried out via 30m length DB5 column (0.3µm inner dia, 0.25m film thickness). 1 µL sample was injected onto the column via micro-syringe through rubber septum. Throughout the investigation, the insertion and the finding arrangement temperature for GC was kept at 280^0C. The oven temperature was set between 80 and 240^0C for 5^0C rise per min and detained at 240^0C for 30 min, while nitrogen as a carrier gas was supplied at 40 ml/min with make-up gas at 30 ml/min.

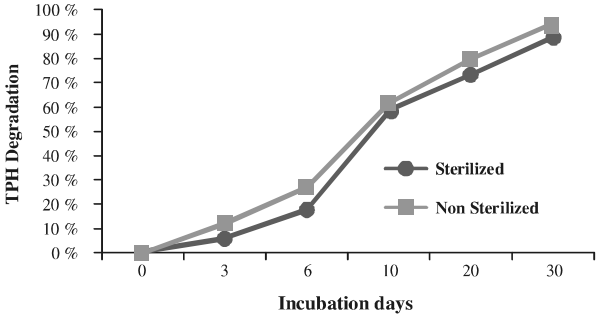
3. RESULTS AND DISCUSSION

3.1 Oil Sludge Biodegradation

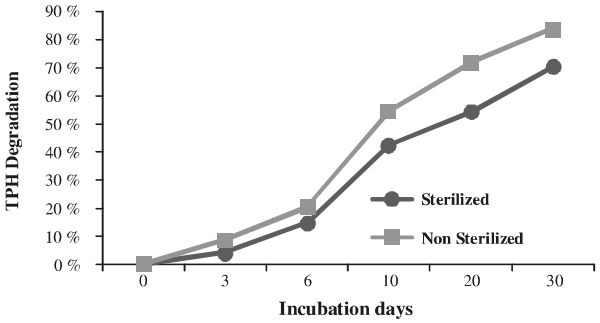
Throughout remediation of oily sludge in autoclaved oily sludge the hydrocarbons concentration reduced via bacteria were 87.3, 69.7 and 60.1% and in non-autoclaved oily sludge 93.9, 84.1 and 69.9% TPH concentration was reduced at 50, 10 and 5% of oily sludge correspondingly. TPH removal was observed from day six until the end on 30^{th} day indicating that hydrocarbon concentration was showing a consistent decline during this time as highlighted in Fig. 1(a-c). The differing results obtained for both autoclaved and non-autoclaved samples meant that resident microorganisms in the non-autoclaved sample had to with higher reduction in the amount of hydrocarbons as compared to that observed for autoclaved sample. This observation led to the inoculation of all ochthonous or external source of bacteria in bacillus sp. for efficient removal of the hydrocarbons from the samples. The consistent decrease in TPH concentration by the specified bacteria implied that the sludge composition was conducive to the optimum growth of the bacteria, which led to the efficient degradation of the hydrocarbons from the sample.

3.2 Optical Density Analysis

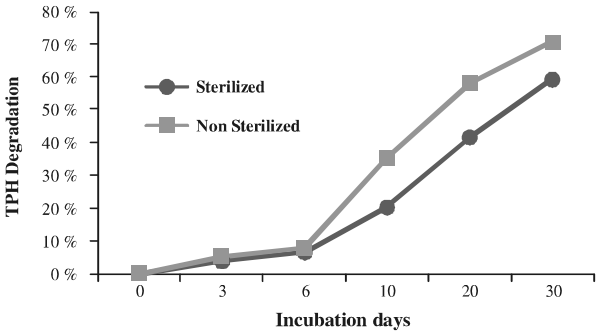
OD (Optical Density) test was carried out on the samples at 600nm absorbance to examine the development and growth of the bacteria in the sample medium. The growth of bacteria in the medium was an indirect indication of the uptake of hydrocarbons by the bacteria from the sample contents causing reduction in the hydrocarbons concentration of the sample. In addition, the inoculated bacteria were proved to be labile as optimum growth of the bacteria indicated that they were quite capable of consuming the hydrocarbons as a source of carbon and energy for them. Fig. 2(a-c) showed consistent rise in OD value with the passing of incubation time. It was observed during initial days of incubation that there was no release of color by the bacteria in the sample medium. However, OD value was increased up to 3.0 by the end of the process, once bacteria started to consume hydrocarbons from the sample. Higher value of OD implied towards the emergence of bacterial colonies in the medium as a result of the uptake of nutrients from the medium for their growth [6]. During the course of oil biodegradation, the characteristic change in the medium color was noticed from 3rd day until 10th day, which indicated towards improved metabolic behavior by the bacteria. This also meant that the given sample of oily sludge was feasible to be treated in terms of hydrocarbons removal via the use of specified bacteria.



(a) 5% of TPH Concentration

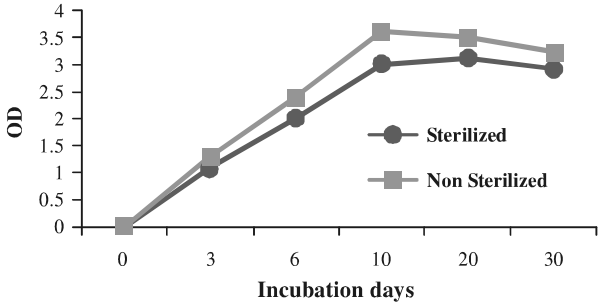


(b) 10% of TPH Concentration

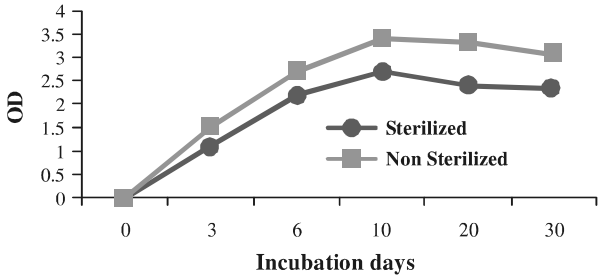


(c) 50% of TPH Concentration

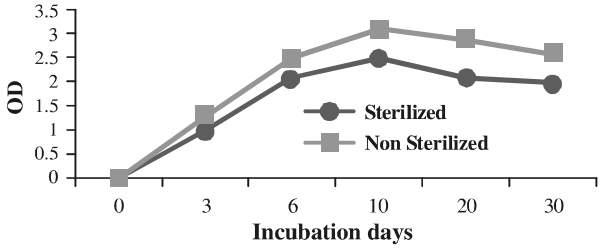
FIG. 1. DEGRADATION OF TOTAL PETROLEUM HYDROCARBONS



(a) 5% TPH, 2ml Inoculums



(b) 10% of TPH, 4ml Inoculums



(c) 50% of TPH, 6ml Inoculums

FIG. 2. BACTERIAL GROWTH CURVE IN OILY SLUDGE DEGRADATION

3.3 Aliphatic Hydrocarbons Analysis

TPH analysis of the samples was carried out via GC to assess the level of pollution contained by the oily sludge. Before introduction of the sample into GC, all the hydrocarbons present in the sample were extracted from the sample via the use of mixed solvents solution of acetone and hexane. Fig. 3 shows the evolution of hydrocarbons present in the injected sample at their given processing time. Each peak as shown in the chromatogram stood for its characteristic hydrocarbon compound along with the area of the peak, which was taken as the basis for variation in the concentration of characteristic component of the peak. It was observed that both sample chromatograms and the standard GC had the identical compounds in qualitative terms. Every carbon number was identified by running in parallel the standard chromatogram except one of the component which was recognized as an unknown compound. The GC of oily sludge samples showed high concentrations of aliphatic hydrocarbons inside discard ditch. Oily sludge contains hydrocarbons in the range of C6-C46, hence, it was attempted to obtain lesser molecular weight hydrocarbons (C6-C8) as well. However, lesser molecular weight hydrocarbons (<n-C8) were not found to be present, probably due to the effect of sample evaporation via either sample dispensation or otherwise. Whereas, the oily sludge analysis via GC showed the presence of aliphatic hydrocarbons from C9-C43.

3.4 Comparative GC Analysis of Oily Sludge Degradation for Both Autoclaved and Non Autoclaved Samples

GC investigation of TPH extract revealed that bacillus sp: degraded approximately 97.5% of aliphatic HCs following 30 days of incubation at 5% of autoclaved oily sludge. It was found that the various number of aliphatic fraction of hydrocarbons were entirely degraded i.e. C-10, C-11, C-13, C-15, C-16, C-18, C-23, C-30, C-31, C-32, C-34, C-35, C-37, C-39, and C-40 as shown in Fig. 4(a). Peak area in each part of the figure signifies hydrocarbons bioremediation. However, with 10% TPH concentration, biodegradation of aliphatic hydrocarbons stood at 68.3% after 30 days incubation, as shown in Fig.4(b). In addition, the results obtained with 10% TPH concentration suggested that some of the hydrocarbon fractions such as C-23, C-30, C33, and C-38 were completely despoiled during the course of biodegradation. Similar trend was observed with higher TPH concentration of 50% when 65.1 % of aliphatic hydrocarbons were decomposed with the same number of hydrocarbons being despoiled completely.

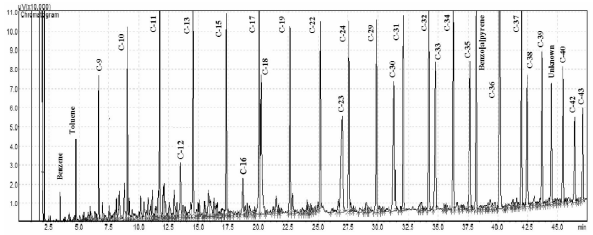
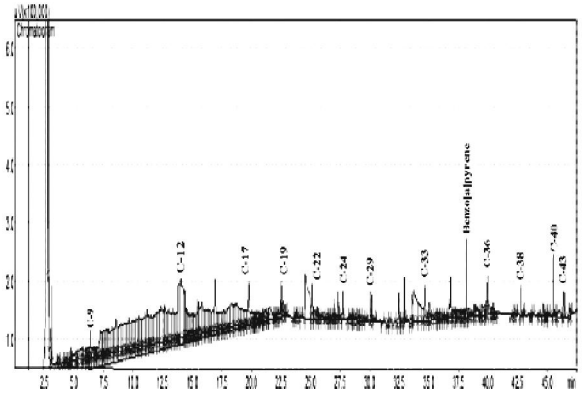


FIG. 3. CHROMATOGRAM SHOWING PRESENCE OF ALIPHATIC HYDROCARBONS WITH RESPECT TO TIME

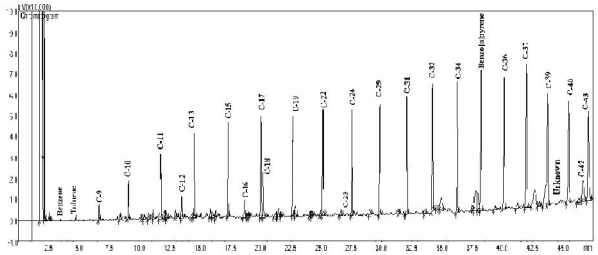
GC assessment of hydrocarbons, alienated from oily sludge by means of bacillus sp., indicated that 90% reduction had occurred during the course of this bioremediation in non-autoclaved oily sludge (Fig. 4(d)). These results suggested that aliphatic hydrocarbons were almost entirely bio-remediated with respect to their concentration in the sludge samples likely due to elevated movement of the bacteria in a given conducive environment of greasy mud. When concentration of hydrocarbons increased with the induction of 10% TPH, then aliphatic HC degradation was 87%in non-autoclaved samples, as shown in Fig. 4(e). However, further increase in the TPH concentration by 40% induced a decrease in the hydrocarbon reduction percentage, when 77% removal of aliphatic hydrocarbons was possible with 50% TPH concentration in non-autoclaved samples (Fig. 4(f)).

4. CONCLUSION

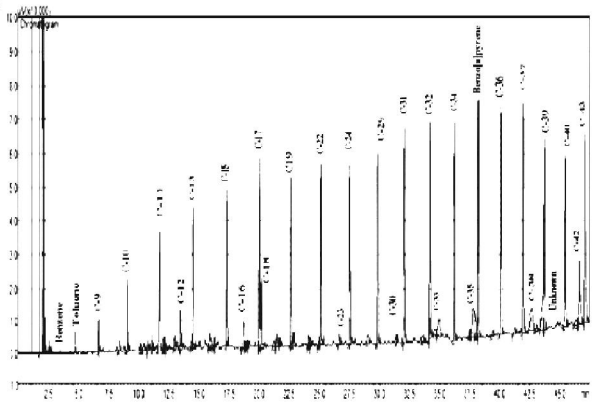
Aliphatic hydrocarbon recovery from oily sludge samples was successfully attempted by means of suitable bacteria i.e. Bacillus sp. It was found that hydrocarbons recovery was higher by 7% in non-autoclaved samples (94%) than in autoclaved samples likely due to sustained conducive growth conditions for the bacterial activity in non-autoclaved samples. The GC indicated that 99% removal of aliphatic hydrocarbons was possible during non-autoclaved oily sludge samples as compared to 92% removal from autoclaved oil sludge samples with 5% TPH concentration. It was also observed that increase in the TPH concentration by 5 and 45% resulted in lower removal of the hydrocarbons, which implied that higher the TPH concentration than the optimized concentration the lower would be the hydrocarbon recovery.



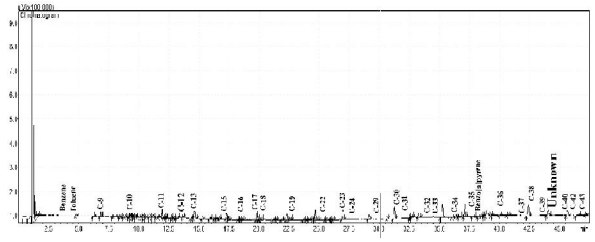
(a) 5%TPH CONCENTRATION (AUTOCLAVED SAMPLES)



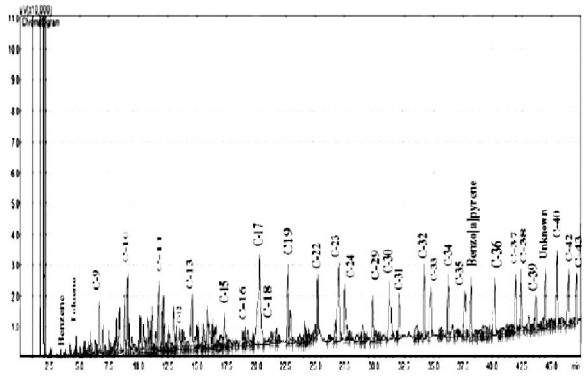
(b) 10% TPH CONCENTRATION (AUTOCLAVED SAMPLES)



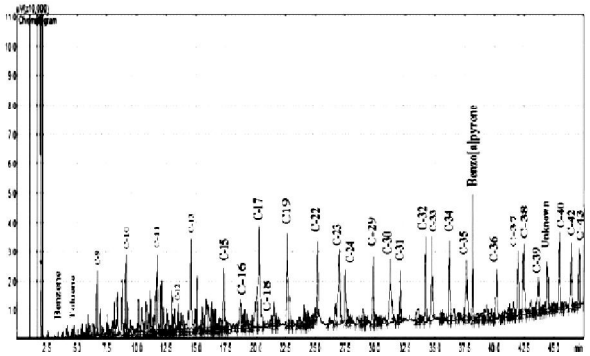
(c) 50% TPH CONCENTRATION (AUTOCLAVED SAMPLES)



(d) 5%TPH CONCENTRATION (NON-AUTOCLAVED SAMPLES)



(e) 10% TPH CONCENTRATION (NON-AUTOCLAVED SAMPLES)



(f) 50%TPH CONCENTRATION (NON-AUTOCLAVED SAMPLES)

FIG. 4. GAS CHROMATOGRAMS SHOWING DEGRADATION OF ALIPHATIC HYDROCARBON FRACTION AFTER 30 DAYS

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