Biodegradation Ofspilledoil Sludge Using Mixed Aerobicculiture From Sewage Treatment Plant

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Abstract :

The present study investigates the utilization of mixed aerobic culture from sewage treatment plant to biodegrade the oil spill samples collected from Chennai coast. The long chain paraffinic and aromatic hydrocarbons present in the spilled oil sludge pose serious problem in air, water and soil environment. Microbial degradation of oily sludge is an alternative way to address the various environmental issues effectively and economically. Literature reports the need of pure strains of bacterial cultures like (Pseudomonas, Bacillus)or enriched consortiums the current study explores the possibility of utilizing the waste sludge from sewage treatment plant for the bioremediation of petroleum sludge. Experiments have been conducted to observe the efficiency of aerobic microorganisms to biodegrade the fuel oil sludge at concentrations of 100-500 mg/L in presence of mineral salt medium. The solubilization rate and degradability of TPH was determined by gas chromatography mass spectrometry (GC-MS). The solubilization of various hydrocarbons present in the spilled oil such as Decane to Nonacosane (C10-C29) was increased considerably after the residence time of 5 d and reached the maximum for 100 mg/Lon 21st d in the aqueous phase. GC-MS results confirms that the microbes present in the aerobic culture are capable of utilizing spilled oil sludge as the sole source of carbon and energy. Biodegradation altered the relative abundance of n-alkanes distribution present in the spilled oil sludge at lower sludge concentration. The ultimate biodegradability was studied using Fourier transform infrared spectroscopy (FTIR) analysis. The peak in the spectra at 1700 cm^{-1} stands for the C=O group and confirms the production of carboxylic acid post-degradation which confirms the degradation of TPH present in the spilled oil sludgeat lower sludge concentration.

Keywords : Chennai oil spill, bio degradation, aerobic culture, alkanes

1. Introduction

Several constituents of oily sludge (oil and sludge solids) are toxic, mutagenic and carcinogenic (Liu et al., 2010). The constituents in oil include: n-alkanes, branched alkanes, cycloalkanes, polynuclear aromatic hydrocarbons, nitrogen, sulphur and oxygen containing heterocyclics, asphaltenes, and resins (Mrayyan and Battikhi, 2005). Various n-alkanes, branched alkanes and cycloalkanes are known to cause respiratory, renal andcentral nervous system disorders.The objective of this study was to assess thebiodegradability of oily sludge collected from Chennai Oil spill under various bioaugmentation scenarios after conducting a thorough characterization of oily sludge.

2 Materials and methods

2.1 Sample collection

Heavy fuel oil stored in the ship was collected for the reference. A number of ocean- weathered, first arrival oil spill samples were collected from various beaches in Chennai. Floating mousse sample recovered near Ernavoorshown in Fig. 1on January 31, 2017, as the reference ocean-weathered oil spill sample. During each sampling

event, representative field samples were collected. Total weight of samples recovered from various locations ranged from 0.5 kg to several kilograms.These homogenized field samples were prepared separately and stored in different containers to avoid cross contamination.





Figure1 - Oil spill floating on the beachand deposition on sea shore rocks

2.2 Bio degradation studies on oil spill sludge

Oily sludge biodegradation studies were set-up in 250 ml flasks containing different concentration (100, 250, 500 mg/L) of oily sludge in 100 ml mineral medium provided as sole substrate. One un-inoculated control was used in the study with oily sludge. Un-inoculated controls indicated possible microbial activity due to presence of indigenous culture. Experimental flasks were bioaugmented with 10 ml of the aerobic sludge adapted using cultures grown up to end of log phase and the initial count of inoculum was kept constant. The flasks were incubated in a rotary shaker operated at 120 rpm and 30 C. The contents were filtered, extracted using hexane extraction and the residual total petroleum hydro carbon was quantified using GC-MS. Residual TPH was measured after 60 days of bio degradation.

2.3 Enumeration of bacterial population and optical density determination

Total heterotrophic bacteria were enumerated by using a pour plate technique on plate count agar (Merck, UK) after 24h incubation at 30^{o}C, which also allowed growth of all members of the added bacterial consortium. Optical density of the inoculum was measured in UV-Vis spectro photo meter

2.4 Hydrocarbon analysis

Chemical finger printing of sludge was done through GC-MS (Agilent Technologies, USA). A HP-5 (30-m x 0.25-mm) silica based cross-linked column was used. The injector and detector temperatures were set at 300°C. The initial temperature was kept at 50°C for 1 min, ramped to 110°C at 10°C/min and held for 2 minutes, then ramped up to 250°C at 5°C/min and held for 2 minutes and to 300°C at 3C/min and held at that temperature for 15 min. An aliquot of 1µL was injected in the split less mode. Helium was used as a carrier gas at the rate of 1 mL/min. MS was scanned from 35 to 550 amuat 1.562 u/s by selecting full scan mode. MS source temperature was 230°C and MS quadrapole temperature was maintained at 150°C. Calibration curves were prepared through multiple dilution 20 element multi elemental total petroleum hydrocarbons (TPH) standard (Supelco, USA). The amount of TPH present in the oil spill sludge was quantified by employing Agilent Technologies Mass Hunter software. The standard solution contains decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane, eicosane, heneicosane, docosane, tricosane, tertracosane, pentacosane, hexacosane, heptacosane, octacosane and nonacosane. This twenty compound standard was used to build a GC/MS calibration curve with five calibration points at concentration levels of 5, 10, 30, 50 and 100 mg/L.

3.0 Results and Discussion

Oil samples were collected from 12 different locations, namely Tiruvanmiyur, Marina beach, Eliots beach, Kasikoilkuppam, Tiruchinnakuppam, Cooum river, Adyar river, Periyakuppam, Chennai port, Fishing harbor, Ennore port and Tiruvottiyur beach. These areas were monitored carefully and the samples were collected at regular intervals (1st, 3rd, 6th and 10th day) after the oil spill issue. The analyzed compounds present in the oil samples were observed to be ranging from $C_{10}H_{22}$ to $C_{29}H_{60}$. These hydrocarbons are highly paraffinic and hydrophobic in nature. More than 75% of the paraffins present in the oil sample analyzed using GCMS are solid (long chain) in structure. Total Petroleum hydro carbon present in day 1 sample was observed to be 40.34 mg/kg and it has been reduced to 16.68 mg/kg in 3 days. It is observed that the concentration of TPH has reduced in first 3 days and later it has increased to 81.81 mg/kg in 6 days and stabilized thereafter. TPH concentration present in the oil spill sludge is shown in Fig.2. GC MS chromatogram of oil spill sludge with 10 mg/L of saturated alkane standard is shown in Fig.3.

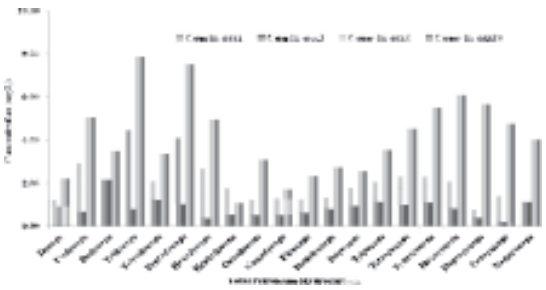


Figure 2 - Concentration of TPH present in oil spill sludge (1,3,6,10 days)

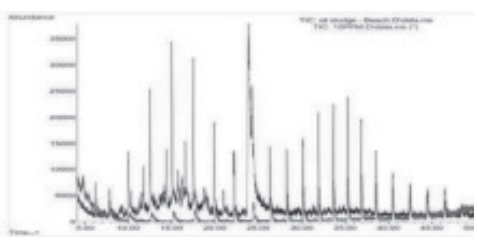


Figure 3 - GCMS overlay chromatogram of sample collected after 10 days of \ oil spill (black)and 10 ppm saturated alkane standard (blue).

3.1 Growth characteristics of microbial population

The maximum concentration of microbes on the oil spill sludge hasbeenobservedto be 19, 22 and 10 for 10^6 CFU/mLfor 100, 250 and 500 mg/L of oil spill sludge respectively. The concentrationof microbes hasincreasedupto21days,and decreased thereafter. Theoptical density of the consortia was measured in UV Vis spectrophoto meter at 610 nm to measure the MLSS concentration present in the mixture. For an increase in number of days, there is an increase in optical density measurement values shows that the MLSS concentration present in the mixture is increased.

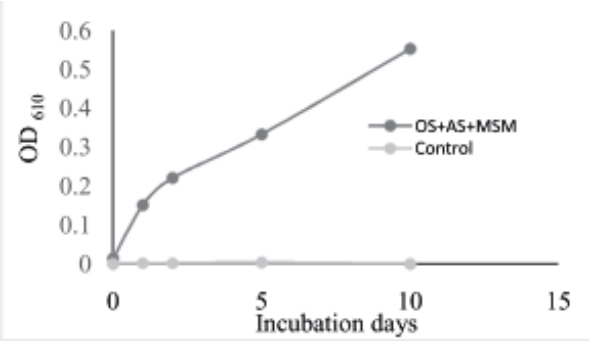


Figure 4 - Optical density measurement of oil spill sludge

3.2 Bio degradation of oil spill sludge using mixed aerobic culture

Bio degradation of oil spill sludge was studied at various concentration of 100, 250, 500 mg/L in presence of mineral salt medium. Gas chromatographic analyses revealed all hexane soluble n-alkanes in the range of nC8–nC40, which were relatively abundant in spilled fuel oil sludge. The degradation of the compounds range from decane toNonacosane (C_{10}- C_{29})concentration were measured for 60 days.The concentration of nC10–nC20, nC21–nC30. The solubilization and degradation rate ofdecane toNonacosane compounds at different concentration with respect to time were shown in Fig. 5,6 and 7.

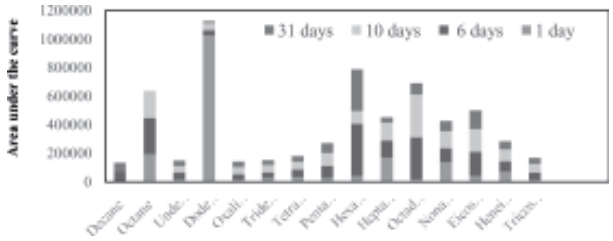


Figure 6 - Bio degradation of 250 mg/L oil spill sludge

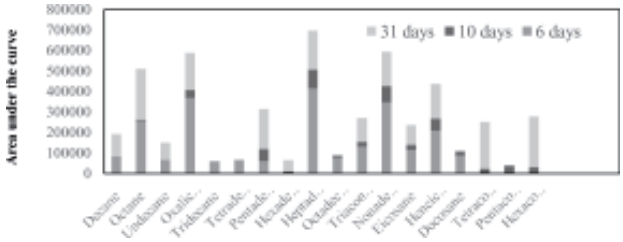


Figure 7 - Bio degradation of 500 mg/L oil spill sludge

For an increase in concentration of oil spill sludge in the presence of mixed inoculums with mineral salt medium increased the solubilization rate of TPH present in the sludge. For an increase in number of days there is an increase in solubilization rate of nC20 compounds.The solubilization of various hydrocarbons present in the spilled oil such as Decane to Nonacosane (C10-C29) was increased considerably after the residence time of 5 d and reached the maximum for 100 mg/L on 21st d in the aqueous phaseMedium size carbon chain molecules from nC10 - nC20 solubilization rate is high for lower concentration of oil spill sludge (i.e 100, 250 mg/L etc.,). Differences in their reactivities are primarily determined by the occurrence, type and arrangement of unsaturated bonds. The most possible degradation pathway for the substrate n-alkane is oxidized to the corresponding alcohol by substrate-specific terminal monooxygenases/ hydroxylases. The alcohol is then oxidized to the corresponding aldehyde, and finally converted into a fatty acid(Sakthipriya et al, 2015). The ultimate biodegradability was studied by analysing the functional group present in the sludge using Fourier transform infrared spectroscopy (FTIR) analysis. The FT-IR spectrum of spilled oil sludge and degraded oil sludge was shown in Fig. 8.

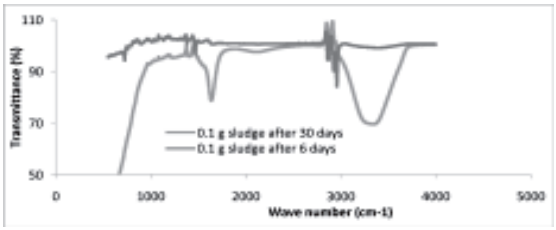


Figure 8 - FTIR spectrum of oil spill sludge after 6, 30 days of bio degradation

The peak in the spectra at 1700 cm^{-1} stands for the C=O group and confirms the production of carboxylic acid post-degradation which confirms the degradation of TPH present in the spilled oil sludge at lower sludge concentration.

4.0 Conclusion

The current work focused on the degradation of oil spill sludge using mixed aerobic culture from sewage treatment plant. For an increase in number of days there is an increase in MLSS concentration in the inoculum. For an increase in oil spill sludge concentration above 250 mg/L in the inoculated culture reduced the rate of solubilization and degradation. FT-IR analysis shows the presence of carboxylic acid after 30 days of bio degradation, which confirms the complete degradation of oil spill sludge at lower concentration.

5. References

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