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Research article

Enhanced biodegradation of hydrocarbons in petroleum tank bottom oil sludge and characterization of biocatalysts and biosurfactants

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ABSTRACT

Petroleum hydrocarbon removal from tank bottom oil sludge is a major issue due to its properties. Conventional physicochemical treatment techniques are less effective. Though the bioremediation is considered for the hydrocarbon removal from tank bottom oil sludge, the efficiency is low and time taking due to the low yield of biocatalysts and biosurfactants. The focal theme of the present investigation is to modify the process by introducing the intermittent inoculation for the enhanced biodegradation of hydrocarbons in the tank bottom oil sludge by maintaining a constant level of biocatalysts such as oxidoreductase, catalase, and lipase as well as biosurfactants. In addition, the heavy metal removal was also addressed. The microbial consortia comprising *Shewanalla chilikensis, Bacillus firmus,* and *Halomonas hamiltonii* was used for the biodegradation of oil sludge. One variable at a time approach was used for the optimum of culture conditions. The bacterial consortia degraded the oil sludge by producing biocatalysts such as lipase (80 U/ml), catalase (46 U/ml), oxidoreductase (68 U/ml) along with the production of lipoprotein biosurfactant (152 mg/g of oil sludge) constantly and achieved 96% reduction of total petroleum hydrocarbon. The crude enzymes were characterized by FT-IR and the biosurfactant was characterized by surface tension reduction, emulsification index, FT-IR, TLC, and SDS-PAGE. GC-MS and NMR also revealed that the hydrocarbons present in the oil sludge were effectively degraded by the microbial consortia. The ICP-OES result indicated that the microbial consortium is also effective in removing the heavy metals. Hence, bioremediation using the hydrocarbonoclastic microbial consortium can be considered as an environmentally friendly process for disposal of tank bottom oil sludge from petroleum oil refining industry.

1. Introduction

Petroleum industries generate huge amount of tank bottom oil sludge (roughly 28, 220 tons/year) and oily effluent soaked soil waste, which consists of both aliphatic and aromatic hydrocarbons, Total Petroleum Hydrocarbon (TPH) and heavy metals like nickel, chromium, zinc, manganese, cadmium, copper, and lead (An and Huang, 2012; Bhattacharyya and Shekdar, 2003). United States Environmental Protection Agency (US EPA) listed many of these components as priority pollutants due to their toxic, mutagenic and carcinogenic properties (Janbandhu and Fulekar, 2011). Improper disposal leads to soil contamination and groundwater pollution that in turn causes environmental imbalance.

Existing conventional methods like physical and chemical methods that include ultrasonic treatment, pyrolysis, chemical treatment, photocatalysis, incineration, solidification/stabilization and solvent extraction are used in the treatment and disposal of oil sludge (Hu et al., 2013). These methods suffer disadvantages like process ineffectiveness, uneconomical, generate secondary pollution, non-eco-friendly, a requirement of pre- and post treatment and sophisticated instrumentation along with skilled labors. The biological method like biodegradation such as land farming, biopile, bioslurry, and landfill is also used but, these methods require a large area and take a longer duration of time for degradation. The main problems with existing conventional methods are the generation of secondary pollution which contributes to the soil and groundwater contamination and causes secondary adverse effects such as GHG emissions, acidification, eutrophication, ozone depletion, ecological toxicity, etc. (Hou and Al-Tabbaa, 2014). Hence, there is a need for an efficient bioremediation process that could able to completely degrade the hydrocarbon in the oil sludge. And also the process must be eco-friendly and cost-effective.

Bioremediation utilizing the indigenous or exogenous microorganism for the degradation of petroleum hydrocarbon is an emerging viable green alternative method for treating the contaminants (Frutos et al., 2012). The usage of mixed microbial consortia in bioremediation process is considered more advantageous in removing the hydrocarbons

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in oil sludge due to its additive effects either by hydrocarbon uptake (soluble or emulsified forms) or development of hydrophobic cell surfaces (Ghosh et al., 2014). There is a possibility of one species removing toxic metabolite so that the others can survive and degrade the further metabolites effectively (Mukred et al., 2008). For an effective degradation, the survivability and the potent degrading capacity of the microorganism are essential. Optimizing the environmental parameters and growth conditions like temperature, pH, agitation and aeration for biosurfactant production along with the cellular growth of the organisms can significantly affect microbial biodegradation rate of hydrocarbons.

Biosurfactants are amphipathic, surface active compounds synthesized by the microorganism reduces the surface and interfacial tension between two liquids (Liu, 2005). Biosurfactants are emulsifiers which forms stable emulsions with water. These emulsifiers increase the surface area of hydrophobic moieties and increase their bioavailability, thereby improving the growth of bacteria and the rate of bioremediation. They also play an important role in the degradation of hydrocarbons and removal of heavy metals (Ferradji et al., 2014; IWL deLima et al., 2015; Guangming et al., 2005). Heavy metals like arsenic, chromium, nickel, cadmium, and lead are present in the petroleum refinery oil sludge (Nkeng et al., 2012). The challenges with heavy metals are accumulation in soil due to non-degradable nature, causing serious health effects at higher concentration and distributed over large areas (O'Connor et al., 2018). Heavy metal remediation is another challenge for the total bioremediation of petroleum oil sludge.

Biocatalysts are the degradative enzymes like lipase, catalase, and oxidoreductases such as alkane hydroxylase, methane monooxygenase, and alcohol dehydrogenase play a major role in hydrocarbon degradation (Luan et al., 2006). Catalase decomposes hydrogen peroxide into water and oxygen and alleviates induced oxidative stress by heavy metals and hydrocarbons (Achuba and Peretiemo-Clarke, 2014). Lipase catalyzes the hydrolysis of oil compounds into simpler compounds and finally transforming it into compounds which is possible for the microorganism to take up as nutrient (Svendsen, 2000). Oxidoreductases are the largest class of enzymes responsible for redox reactions (da Fonseca et al., 2015). These enzymes oxidize and reduce toxic hydrocarbons and biotransform them into simpler compounds.

Even though many reports are available on the bioremediation of hydrocarbon (Janbandhu and Fulekar, 2011; Mnif et al., 2011; Saimmai and Kaewrueng, 2012), the petroleum industries are still struggling to dispose of the oil sludge in an efficient manner by using existing bioremediation processes. This could be due to the production of the lesser amount of biomolecules throughout the degradation process and takes long time duration (40–240 days) to degrade the hydrocarbons in the sludge. Very few reports are available on the bioremediation of hydrocarbons present in the oil sludge (Cerqueira et al., 2011; Yudono et al., 2010). Hence, there is a need for an alternate process to bioremediate the oil sludge in an efficient manner and in short duration. Therefore, the present study is dealt with the bioremediation process modification to ensure the constant levels of biomolecules throughout the degradation process. The biomolecules are produced by the microbial consortium comprising *Shewanalla chilikensis, Bacillus firmus* and *Halomonas hamiltonii* isolated from oil sludge contaminated soil were inoculated at regular intervals to maintain the production of enzymes and biosurfactants increasingly constant level in its attempt to enhance the bioremediation process.

2. Materials and methods

The petroleum oil sludge and oil sludge contaminated soil used in this study were collected from a petroleum oil refinery situated in Chennai, Tamil Nadu. Chemicals and media used were of analytical grade.

2.1. Characterization of oil sludge

The tank bottom oil sludge was collected from a petroleum oil refinery situated in Chennai, Tamil Nadu. The automated soxhlet extractor coupled with gas chromatography-mass spectrometry (GC-MS) and NMR was used to characterize the oil sludge. The ICP-OES was used to determine the heavy metals present in the oil sludge.

2.2. Screening, isolation and identification of oil sludge degrading microorganisms

In order to isolate efficient strains for the degradation of oil sludge, 1 g of hydrocarbon oil sludge contaminated soil sample was inoculated to mineral salt medium (MSM) containing (g/L) $MgSO_4$, 1; NaH_2PO_4, 0.2; K_2HPO_4, 0.8; $CaCl_2$, 0.5; $FeSO_4$, 0.05 and NaCl, 0.5 along with 0.1% of tank bottom oil sludge. The pH of the medium was adjusted to 7.0 and incubated for 7 days at 37 °C at 120 rpm. Cultures grown in MSM were periodically transferred successively to the MSM containing an increased concentration of oil sludge from 0.1 to 1.0% to obtain the enriched bacterial strains. After enrichment, the bacterial strains were selected based on their ability to utilize the oil sludge hydrocarbons as the sole source of carbon and energy thereby causing the degradation of oil sludge. The 3 strains S1, S2, and S3 were capable of producing biocatalysts and biosurfactants and also degraded hydrocarbons. But, the synergistic effect of the individual strain was found to be higher in degrading hydrocarbons and producing the biocatalysts as well as biosurfactants when compared to the individual strains. Hence, the 3 strains that survived and utilized the oil sludge as the sole carbon and energy source and produced biomolecules are formed as microbial consortia. The bacterial strains which showed better degradation and the biomolecules production were selected and subjected to identification by 16s rDNA sequencing. The phylogenetic tree was constructed using the neighbor-joining method.

2.3. Optimization of culture conditions for the maximal enzyme and biosurfactant production for the oil sludge degradation

The microbial consortia were prepared by growing isolated strains in mineral salt medium containing 1% oil sludge as the carbon source. In order to degrade the oil sludge, the organisms must produce degradative enzymes and biosurfactants. So the optimization of culture conditions for the effective production of degradative enzymes and biosurfactant was carried out by varying the temperature (30–50 °C), pH (4–8), oil sludge concentration (0.25–1.25% (w/v)) and biomass concentration (5–25%). The activity of enzymes like lipase, catalase and oxidoreductase, biosurfactant production yield and surface tension reduction was monitored. The presence of biosurfactant was determined by drop collapse method.

2.4. Enzyme assays

The activity of lipase was measured by titrimetric assay according to the protocol followed by Ramani et al. (Ramani and Sekaran, 2012). Briefly, olive oil 10% (v/v) was emulsified in distilled water containing 2% (w/v) of polyvinyl alcohol using an ultrasonic sonicator. To 5 ml of the olive oil emulsion, 2 ml of 0.03% Triton X-100, 2 ml of 0.075% $CaCl_2$, 1 ml of 3M NaCl and 4 ml of phosphate buffer were added and incubated for 5 min in a shaker. Then, 1 ml of enzyme solution was added and incubated for 15 min in a shaker. After incubation, 10 ml of acetone: ethanol (1:1) solution was added and titrated against 0.02N NaOH solution. One unit activity of lipase was defined as the amount of enzyme that released 1 mM of fatty acid per min under assay conditions (Suganthi and Ramani, 2016).

For catalase activity determination (Bergmeyer, 1974), 2.0 ml sample was taken in a cuvette (light path: 1 cm) and equilibrated at 25 °C for about 5 min and 1.0 ml of 30% H_2O_2 solution (prepared in

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