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Characterization of oily sludge from a refinery and biodegradability assessment using various hydrocarbon degrading strains and reconstituted consortia

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ABSTRACT

Oily sludge obtained from a refinery in India contained 10–11% oil associated with fine particulates. Along with Fe, Ca and Mg various toxic elements were associated with the sludge solids (Pb, Mn, Cu, Zn, As, Bi, Cd, Cr, Co, Ni and V). The oil contained 41–56% asphaltenes and the maltenes comprised of 49 ± 4%, 42 ± 2% and 4 ± 2%, aliphatic, aromatic and polar fractions, respectively. Biodegradation studies with the maltene fraction of oil provided as sole substrate revealed higher degradation by various 3-5 membered reconstituted consortia compared to pure bacterial strains and up to 42 ± 8% degradation could be achieved over 30 days. In contrast, over the same period up to 71.5 ± 2% oil degradation could be achieved using dried oily sludge (15% w/v) as sole substrate. Significant biodegradation observed in the un-inoculated controls indicated the presence of indigenous microorganisms in oily sludge. However, large variability in oil degradation was observed in the un-inoculated controls. Greater biodegradation of the maltene fraction led to significant enrichment of asphaltenes in residual oil associated with the sludge.

1. Introduction

Petroleum refineries generate significant quantity of oily sludge in oil storage tanks and oil-water separation systems. More than 28,220 tonnes of oily sludge is generated annually by refineries in India (Bhattacharyya and Shekdar, 2003). Improper handling and disposal of this hazardous sludge pose a risk of soil/groundwater contamination and air pollution through release of volatile organic compounds (VOCs). 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 and central nervous system disorders. The low molecular weight PAHs are commonly associated with acute toxicity while the high molecular weight polynuclear aromatic hydrocarbons (PAHs) are reported to be mutagenic and carcinogenic. The concentration of toxic heavy metals (such as, Ni, Cr, Zn, Pb, Mn, Cd, and Cu) in oily sludge is also relatively higher than in soil (Marin et al., 2006; Mishra et al., 2001; Venkateswar Reddy et al., 2011). The total petroleum hydrocarbon (TPH) content may vary from 5% to 86% depending on the source and batch to batch variability is also reported (Ramaswamy et al., 2007; Tahhan et al., 2011; Wang et al., 2010).

Owing to the high oil content and diverse physical properties, oily sludge is often treated through physicochemical treatment technologies such as, froth flotation, pyrolysis, oxidative thermal treatment, centrifugation using cyclotrons, and electrode-emulsification prior to landfarming or landfilling (Al-Futaisi et al., 2007; Elektorowicz and Habibi, 2005; Ramaswamy et al., 2007; Wang et al., 2010). However, physicochemical treatment technologies are cost intensive. Cost effective and environment friendly alternatives include, recycling of oil and recycling of valuable materials followed by bioremediation. Biological treatment reduces the volume of the waste and also decreases toxicity (Gallego et al., 2007). Various researchers have successfully demonstrated the potential of bioremediation for treatment of contaminated soil and sludge (Cameotra and Singh, 2008; Gallego et al., 2007; Gojgic-Cvijovic et al., 2012; Verma et al., 2006). Bioremediation of oily sludge can be influenced by a number of factors, such as, composition and concentration of TPH in the oily sludge, treatment duration and availability of suitable microorganisms. Various

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studies have reported the ability of microorganisms to use petroleum hydrocarbons as the sole source of carbon and energy (Biswal et al., 2009; Mohanty and Mukherji, 2008; Mukherji et al., 2004). Bioaugmentation with microbial consortia is practiced more commonly, since pure microbial strains can metabolize only limited substrate types. Complex scenarios typically benefit from the wider enzymatic activities and metabolic networks available in a microbial consortium (Gallego et al., 2007; Ghazali et al., 2004; Mrayyan and Battikhi, 2005; Rahman et al., 2003, 2002). Moreover, mixed microbial cultures can facilitate biodegradation of recalcitrant compounds through cometabolism and commensalism. Petroleum hydrocarbon degradation typically follows the trend: alkanes > isoalkanes > low molecular weight aromatics > cyclic alkanes (naphthenes) > polyaromatics > polar compounds > asphaltenes (Gojgic-Cvijovic et al., 2012). In addition to recalcitrance and structural complexity of the components, biodegradation may also be hindered by aging or weathering processes that reduce bioavailability of oil associated with the sludge (Peters et al., 2005; Van Hamme et al., 2003). Therefore, before applying biological treatment, characterization of oily sludge and studies on treatability assessment are recommended.

The objective of this study was to access biodegradability of oily sludge under various bioaugmentation scenarios after conducting a thorough characterization of oily sludge obtained from a refinery in India. A hypothesis was that reconstituted consortia formed by combining known aliphatic and aromatic hydrocarbon degraders would cause enhancement in oil biodegradation in comparison to biodegradation achieved by the individual strains. Another hypothesis was that the rate and extent of biodegradation of oily sludge by the reconstituted consortia would be lower than that of the maltenes due to presence of recalcitrant asphaltenes, toxic heavy metals and other solids in the sludge. The first hypothesis was tested by using the maltene fraction of oil extracted from sludge, since asphaltenes are highly recalcitrant. Oil and hydrocarbon degradation potential of several of the pure bacterial strains used in this study have been reported in previous studies (Biswal et al., 2009; Jeswani and Mukherji, 2013; Mohanty and Mukherji, 2008). The novelty of this work lies in testing the impact of various bioaugmentation scenarios using well characterized hydrocarbon degrading strains.

2. Materials & methods

2.1. Source of chemicals

High purity solvents, such as, dichloromethane (DCM), hexane, heptane, toluene and methanol, and various other chemicals were procured from Merck India Ltd (Mumbai, India). Nutrient broth, bacteriological agar and gram staining kit were obtained from Hi Media (Mumbai, India).

2.2. Source of bacterial cultures

The various oil degrading bacterial strains used in this study were *Exiguobacterium aurantiacum* (AS1), *Sphingomonas* sp. (MSY), *Bacillus* sp. (BST), *Ochrobacterium* sp. (BSW), *Burkholderia multivorans* (HN1), *Burkholderia multivorans* (NG1) and *Rhodococcus* sp. (WSO). These bacterial strains were originally isolated from oil contaminated sites in and around Mumbai (India) using various hydrocarbon substrates (Table S1 in supplementary material).

2.3. Source of oily sludge

Oily sludge was obtained from the weathering pit of a petroleum refinery in Mumbai, India in August, 2010 (details provided in supplementary material). The sludge was stored at 4 °C and was dried, homogenized and sieved manually before use in the characterization and biodegradability assessment studies (conducted within four months of collection).

2.4. Characterization of oily sludge

Physical characterization of the oily sludge and the associated solids involved determination of pH, moisture content, organic matter content and particle size distribution. The pH of the oily sludge samples were measured after mixing the samples with water (1:5 w/v). Moisture content, organic matter content and volatile suspended solids were determined using standard procedures (Biswal et al., 2009; Clesceri et al., 1998). Particle size of solids in dried oily sludge after oil extraction was determined using the dry sieving technique and the Laser diffraction particle size analyzer (LSI3 320, Beckman Coulter Counter, Miami, FL). Dried sludge solids were suspended in distilled water and kept in an ultrasonic bath for 10 min to obtain a uniform distribution of the particles before this analysis was conducted.

Chemical composition (heavy metals and mineralogical constituents) of the sludge solids was also analyzed. The basic elemental composition such as carbon (C), hydrogen (H), oxygen (O), sulphur (S) and nitrogen (N) in the sludge solids after oil extraction was determined using a CHNS analyzer (FLASH EA 1112 series, Thermo Finnigan, Italy). The metals and mineralogical constituents of the sludge solids were analyzed using Scanning electron microscopy – Energy dispersive X-ray spectroscopy (SEM-EDS, S-3400N, Hitachi, Japan). For sample preparation, dried samples of the extracted sludge solids were coated with gold, a conducting material that helps in neutralizing charges on the surfaces of the specimen. Multiple images of the dried sludge solids were taken and the various elements were identified semi-quantitatively using EDS.

Quantitative estimation of metals and other elements in the sludge solids were also performed. First, oil was extracted from the dried sludge after acidification. The oil free extracted solids (300 mg) were dissolved in aqua regia (9 ml conc. HNO_3 and 3 ml conc. HCl) and digested in a microwave digester (Milestone, MLS 1200, MEGA, Microwave Lab Systems, Monroe, CT, USA). The digested samples were diluted and filtered successively through 0.45 μm and 0.2 μm membrane filters (Ultipor Nylon 66 membranes, Pall Corporation, India). The sample was then analyzed using inductively coupled plasma – atomic emission spectroscopy (ICP-AES, Ultima, 2000, Horiba Jobin Yvon, France). Calibration curves were prepared through multiple dilutions of a 25 element multi-elemental standard (VHG Labs, USA) and elemental composition was determined using these calibration curves.

2.5. Quantification of oil in oily sludge

Quantification of oil in oily sludge is essential both before and during biodegradation. Oil contained in oily sludge (15 g) was extracted by soxhlet extraction using DCM (150 ml) as solvent over 6 h (Biswal et al., 2009). The oil extracted from oily sludge was separated into asphaltene and maltene fractions as per ASTM D2007-08 with minor modifications, i.e., using n-heptane as the standard precipitant instead of n-pentane. After separation of the asphaltenes, the maltene fraction was subjected to silica gel column chromatography to further separate the maltenes into aliphatic, aromatic and polar fractions by sequentially using solvents of increasing polarity (hexane, toluene and methanol, respectively; Biswal et al., 2009). The mass fractions were estimated gravimetrically after solvent removal. Subsequently, each fraction was diluted in 10 ml DCM and was analyzed by gas