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Application of biosurfactant for enhancement of bioremediation process of crude oil contaminated soil

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ABSTRACT

In the present study, a biosurfactant produced by *Pseudomonas aeruginosa* SR17 was utilized to evaluate its efficiency in enhancement of bioremediation of oil contaminated soil. The degradation of total petroleum hydrocarbon (TPH) on application of rhamnolipid biosurfactant at 1.5 g L^{-1} was found to be 86.1% and 80.5% in two soil samples containing 6800 ppm and 8500 ppm TPH respectively. The efficiency of biosurfactant was also compared with a frequently used synthetic surfactant, sodium dodecyl sulphate (SDS) that resulted in 70.8% and 68.1% degradation of TPH from the same contaminated soil samples. GC-MS based analysis showed the presence of poly aromatic hydrocarbons (PAHs) namely indene, chamazulene, naphthalene, phenanthrene, anthracene, fluorene, floranthene, benz(b)fluorene and benz(d)anthracene in the soil samples. Rhamnolipid treatment eliminated 3 PAHs namely floranthene, benz(b)fluorene, and benz(d)anthracene completely within six months and the remaining PAHs were depleted up to 60–80%, within the same period. The efficient degradation of PAHs and other components of TPH on application of the biosurfactant were attributed to enhanced heterotrophic bacterial population. It was also found that the degradation of oil contaminants led to alteration of certain vital physico-chemical properties of the soil.

1. Introduction

Petroleum oil is the main source of energy for most of the industrialized nations of the world. Various oil exploration activities, transportation, and accidental seepage or leakage of oil during different processes of oil recovery, leads to the release of tremendous amount of hydrocarbon wastes into the environment which causes major pollution (Peng et al., 2008). Hydrocarbon contaminants are highly hydrophobic, recalcitrant and persistent in nature as they are very difficult to remediate from the environment. In soil, contamination of hydrocarbon often leads to possibilities of uptake of the contaminants by the plants that are grown in the contaminated sites (Fismes et al., 2002). Moreover, from those plants, the hydrocarbon contaminants can further be introduced to animal and human populations through food chain (Alagic et al., 2015). Polyaromatic hydrocarbons (PAHs) are the components of crude oil that are given prime attention due to their possible mutagenic and carcinogenic property (Ghosh et al., 2014). PAHs have been placed in the 9th position on the ATSDR (Agency for Toxic Substances and Disease Registry) list and studies also reported that exposure to PAHs can lead to various forms of cancer (Lee and Shim, 2007; Yoon et al., 2007). Thus considering the harmful effects of the hydrocarbon oil contaminants, it is utmost necessary to take measures for mitigating their level in the environment. Various techniques such as land filling, incineration, and chemical treatments are widely used for cleaning up oil polluted sites, but those techniques are highly expensive and can also pose additional risks to the environment due to their toxic and non-biodegradable nature. One of the promising methods to rehabilitate the oil polluted sites is the use of bioremediation technology, which is an eco-friendly, cost-effective and sustainable approach (Guntupalli et al., 2016). Many researchers have conducted studies involving microorganisms to remediate oil contaminated environment (Roy et al., 2014; Zhang et al., 2014). A large number of indigenous microorganisms inhabiting in the oil polluted sites, posses the ability to degrade hydrocarbon contaminants, although the degradation process of such compounds is hindered by their high recalcitrant and low bioavailability nature (Calvo et al., 2008). A suitable method that can be adopted to fasten the biodegradation process of such organic compounds is the involvement of biosurfactant in the biodegradation process.

Various strains of microorganisms such as *Serretia mercencis*,

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Pseudomonas spp., *Mycobacterium* spp., *Candida* spp., *Rhodococcus* spp., *Arthrobacter* spp., etc have the potential to produce biosurfactant naturally or during stress response (Shekhar et al., 2015). Biosurfactants are amphiphilic biomolecules which and are of heterogeneous composition such as glycolipid, fatty-acids, lipidic, polysaccharide, as well as complex polymer, etc. (Mukherjee and Das, 2010). Biosurfactants are nontoxic, biodegradable and highly stable over broad ranges of environmental conditions when compared with their synthetic counterparts (Anandaraj and Thivakaran, 2010). In the bioremediation process, the intended role of biosurfactant is to initiate the distribution of hydrophobic oil contaminants in the aqueous phase, thereby increasing their bioavailability which may be the critical limiting factor controlling biodegradation rate for most of the hydrophobic compounds (Kang et al., 2010). Biosurfactants are also expected to enhance the efficiency of the bioremediators which includes alive microorganisms by increasing their cell membrane hydrophobicity and changing the membrane permeability that enhances their ability to uptake the hydrocarbon compounds and utilize them, thereby leading to their biodegradation (Johnsen and Karlson, 2004). Thus, the involvement of biosurfactant in bioremediation processes can facilitate and accelerate the desired purpose. Over years, numerous studies were carried out to evaluate the efficiency of biosurfactant producing microbial strains for biodegradation of hydrocarbon compounds (Rahman et al., 2002). Chang et al. studied the effect of biosurfactant producing strain *P. aeruginosa* ATCC 9027, on the degradation of phenanthrene amended soil (Chang et al., 2015). The degradation of PAHs present in motor oil contaminates soil was investigated by Chebbi et al., who employed a *Pseudomonas* sp. that have the potential to produce biosurfactant (Chebbi et al., 2017). The effect of involvement of a biosurfactant producing strain in a consortium consisting of PAHs degrading bacteria was also studied (Nievas et al., 2008). Obayori et al. reported a degradation of 92.34 and 95.29% of crude oil and diesel oil respectively, by utilizing a biosurfactant producing strain *Pseudomonas* sp. LP1 (Obayori et al., 2009). Naphthalene and crude oil degradation was also performed by Ferradji et al., who employed biosurfactant producing bacteria *Streptomyces* spp. (Ferradji et al., 2013). Although the utilization of biosurfactant producing microbial strains for bringing about the degradation of hydrocarbon pollutants have been studied thoroughly, there are no existing reports on the use of biosurfactant directly without any other additional substrates in hydrocarbon contaminated soil in order to enhance the degradation efficiency of the indigenous soil microflora. Thus, the present study aims to evaluate the efficiency of biosurfactant produced by *P. aeruginosa* strain SR17 to enhance biodegradation of hydrocarbon compounds, including PAHs present in crude oil contaminated soil by the indigenous soil bacteria. The change in bacterial population and alteration in physico-chemical properties of soil during the degradation experiment were also evaluated.

Although numerous studies on bioremediation of hydrocarbon contaminated soil have been conducted by utilizing indigenous microbial species or consortia, their widespread applicability is restrained as it require additional adaptation due to diverse ecological conditions in different region of the globe. Again, chemicals and solvents are also utilized for enhancing the degradation of hydrocarbon by the indigenous microflora, but such chemicals are hazardous and are non biodegradable in nature. Thus, since biosurfactant are non toxic and highly stable over broad range of environmental conditions, they can be suitably applied over different geographical locations to expedite the natural bioremediation process performed by the native soil microflora. Further optimization and scaling up of the study can develop a novel technology which can be widely adopted to resolve the pollution problem caused by hydrocarbon contamination.

Table 1
Surface tension reduction (STR) with different carbon sources.

Carbon sources	Surface tension of control (mN m^{-1})	Surface tension of sample (mN m^{-1})	Surface tension reduction (%)
Glucose	69.4 ± 0.3	25.6 ± 0.1	63.12
Glycerol	69.6 ± 0.1	28.0 ± 0.4	59.77
Mannitol	68.5 ± 0.14	32.3 ± 0.25	52.84
Mollasses	65.6 ± 0.22	32.8 ± 0.04	50.00
n-Hexadecane	52.0 ± 0.21	38.1 ± 0.5	26.73

Surface tension values represented mean ± SD of three independent experiments.

2. Materials and methods

2.1. Chemicals and bacteria

Various microbial culture media viz. nutrient broth, nutrient agar, yeast extract powder, were purchased from Himedia and all mineral salts and different solvents used, were obtained from Merck, Germany. PAHs were obtained from Sigma Aldrich (Germany). A previously isolated biosurfactant producing bacterial strain, named *Pseudomonas aeruginosa* SR17 (GenBank accession number: KR028434), was used in the study (Patowary et al., 2016a).

2.2. Selection of carbon source

Different carbon sources namely glycerol, glucose, mannitol, molasses and hexadecane were used in mineral salt medium (MSM) to evaluate the suitable carbon source for maximum production of biosurfactant by *P. aeruginosa* SR17. Each carbon source was used at 2% (w/v) concentrations. The culture flasks were incubated at 35 °C at 150 rpm for five days and the surface tension (ST) was recorded every day. For better comparison, flasks containing the same media composition but no bacterial culture (control) were also studied.

The biosurfactant produced by *P. aeruginosa* SR17 in MSM containing the most suitable carbon source was then extracted and purified for further experiments.

2.3. Extraction, purification and characterization of the biosurfactant

The biosurfactant was extracted by solvent extraction method using ethyl acetate (Patowary et al., 2016b). Purification of the biosurfactant was done by performing column chromatography, using the method reported by George et al. with minor modifications (George and Jayachandran, 2012). 1 g of crude biosurfactant was dissolved in 5 mL of chloroform and poured in a silica gel (60–120 mesh size) chromatography column (26 × 3.3 cm^3). The loaded column was washed with chloroform and then chloroform-methanol mobile phases were applied in sequence; 50:3 v/v (300 mL), 50:5 v/v (200 mL) and 50:50 v/v (100 mL) at a flow rate of 1 mL min^{-1}. Finally a 50:50 v/v chloroform-methanol was applied to remove any remaining biosurfactant from the column. Biosurfactant containing fractions were combined and dried in a rotary evaporator at 40 °C and then used for analysis.

Primary characterization of the biosurfactant was done by performing certain biochemical analysis: molisch's test, emulsion test and rhamnose test, following the standard methodology (Goswami et al., 2012; Patowary et al., 2015).

The functional groups and existing bond types, present in the column purified biosurfactant were identified by Fourier Transform Infrared Spectrometer (FTIR), using a NICOLT 6700 FTIR Spectrophotometer (USA). Analysis was carried out in ATR (Attenuated Total Reflectance) mode in a range of 4000–400 cm^{-1}.

Various structural congeners of the biosurfactant were identified using LC-MS (Thermo-Scientific Exactive plus LC-MS mass spectrometer by following the procedure mentioned in Patowary et al. (2016b)).