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Characterization of hydrocarbon degrading bacteria isolated from Indian crude oil reservoir and their influence on biocorrosion of carbon steel API 5LX

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ABSTRACT

The role of biosurfactants producing hydrocarbon-degrading bacteria (HDB) on biodegradation and bio-corrosion was evaluated. Biodegradation efficiency (BE) of *Streptomyces parvus* B7 was found to be 82% when compared to other bacteria. Increased production of biosurfactants directly influences the rate of crude oil BE. Corrosion of carbon steel was found to be more severe in mixed bacterial consortia (1.493 ± 0.015 mm/y). X-ray diffraction confirmed the presence of high intensity of ferric oxide (Fe_2O_3), iron oxide (Fe_3O_4), manganese oxide (Mn_3O_4), and manganese dioxide (MnO_2) in corrosion product of mixed bacterial system. Biofilm formation was assist to pit formation on the carbon steel surface and it was evidenced from the atomic force microscopy (AFM) and scanning electron microscopy (SEM) analysis. Corrosion current was increased in the presence of mixed consortia $1.6 \pm 0.2 \times 10^{-3}$ A/cm^{-2}, compared to abiotic control $1.2 \pm 0.15 \times 10^{-4}$ A/cm^{-2}, this values were well supported with charge transfer values and these observations confirmed that mixed bacterial consortia play key role in the corrosion of carbon steel. This is the first report to show degradation of crude oil by *Streptomyces parvus* B7 and its effects on the corrosion of carbon steel in oil reservoir.

1. Introduction

Biodegradation is a naturally occurring process in polluted environment where microorganisms take part as a pivotal portion. Consequently, it is very essential to comprehend the activities of microorganisms which are responsible for the biodegradation of compounds, including crude oil hydrocarbon (Hassanshahian, 2014; Parthipan et al., 2017a,b). In general, crude oil biodegradation affects the physiochemical nature of petroleum, follow-on in a drop off of hydrocarbon level and an increase in viscosity, acidity, sulphur content and oil density, which in turns lead to negative financial outcomes for the oil production industry and the refining process (Roling, 2003; Tsesmetzis et al., 2016; Parthipan et al., 2017a,b). Water flooding is commonly used to increase the reservoir pressure for improving oil recovery. This process also introduces microorganisms as well as chemicals which act as micronutrients, encouraging microbial proliferation, and which can lead to reservoir souring (Youssef et al., 2009). The prevention of entry of microorganism in fuel and crude oils both in oilfields after drilling, and in storage tanks is challenging. Both aerobic/anaerobic microorganisms form microbial colonies in the oil pipelines as well as in oil and fuel storage equipments. Complex microbial groups, including hydrocarbon utilizing microbes and anaerobic microorganisms, use metabolites synthesized by other microorganisms for their growth.

High/low molecular weight hydrocarbons present in crude oil, depend upon the physiochemical properties of the oil field (Uzoigwe et al., 2015; Pi et al., 2016; Parthipan et al., 2017b). The ability of microorganisms to use hydrocarbons as carbon source has drawn considerable attention presently (Laczi et al., 2015; Chen et al., 2017). Crude oil is naturally hydrophobic compounds that usually need to be softened earlier to their utilization by microorganisms (Radhika et al., 2014; Liu et al., 2014; Parthipan et al., 2017a). While growing on hydrocarbons, many microorganisms produce emulsifiers with the purpose of increasing hydrocarbons bioavailability and consequent

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degradation by the microbial consortium (Radhika et al., 2014; Uzoigwe et al., 2015). Emulsification is an important process that can influence the density of crude oil. Emulsifier contains hydrophilic head along with hydrophobic tail in nature (Bharali et al., 2011). In general, it is recognized that microbes grow on hydrocarbons and other substrate and leads to production of biosurfactants, which emulsify substrates and enable their transport into cells. Biosurfactants are surface-active agents and are complex biomolecules (which include fatty acids, peptides and polysaccharides) which have the aptitude to reduce surface tension (Youssef et al., 2009; Das and Ma, 2013; Parthipan et al., 2017b). This is achieved by solubilising fatty acids that coexist in the crude oil, consequently directs to efficient utilization of hydrocarbon by microorganisms. Biosurfactants have several physiological roles and provide environmental advantages to their synthesizers. These are originating in diverse environment, while more in location that are highly contaminated with pollutants, such as oil sludge, petroleum waste, than in un-contaminated environments (Hassanshahian, 2014). They play a critical role in bioremediation by boosting their bioavailability through the circulation of pollutants into the aqueous phase. Moreover, they may also manipulate the competence of the microorganisms applied for bioremediation (Kavitha et al., 2014).

Microbiologically induced corrosion (MIC) is an biological process, where microorganisms instigate, assist, or step up the corrosion mechanism over the surface of metal and leading to metal deterioration (Jan-Roblero et al., 2004; Rajasekar et al., 2007a; Machuca et al., 2014; Parthipan et al., 2017c; Wade et al., 2017). Leakage of crude oil due to the internal corrosion on transporting pipelines has been well reported globally. For instance important pipeline crashes (Prudhoe Bay, AK) (Brouwer et al., 2006; Lenhart et al., 2014) suggest that microbial corrosion may be a causative factor. Microbiological activity in oil reservoir leads to fuel contamination, unacceptable level of turbidity, metal corrosion in pipelines, storage tanks and souring of oil products (Hamilton, 1985; Rajasekar et al., 2010). Besides, water can as well stratify at the substructure of oil pipeline if the oil rapidity is not adequate to entrain water and brush it through the transporting pipeline (Rajasekar et al., 2007b). The occurrence of microbes is the important thing liable to the corrosion concern in oil industries (Lenhart et al., 2014; Machuca et al., 2014).

Biocorrosion is one of vital characteristic of pipeline letdown, and also it is significant factor for the increases in the process and repairs cost in the oil and gas industries (Lee et al., 2010; Suflita et al., 2012). In general, nearly 40% of pipeline problems in the oil and gas industries originate from microbial activities (Rajasekar et al., 2007b). Biocorrosion has synergistic effect among the metal surface, corrosive medium and rust products created in biofilm over the surfaces of metal (Machuca et al., 2016; Eckert and Skovhus, 2016). Extracellular polymeric substances (EPS) contribute a key function in formation of biofilm on metallic/non-metallic surfaces (Little et al., 1991; Little and Lee, 2007; Reyes et al., 2008). Biofilm development begins with affections of microbes on firm exterior, and higher emission of EPS metabolites show the way to the expansion of a thicker biofilm and further spreading of individual cell which yet over again commence to form new biofilms on near metal surfaces (Rajasekar et al., 2007a; Forte Giacobone et al., 2011; AlAbbas et al., 2013).

The intention of the current investigation is to identify mesophilic crude oil hydrocarbon degrading bacteria isolated from crude oil reservoir, and to elucidate their effect on carbon steel corrosion. Bacterial isolates were screened for biosurfactant production to understand their role in crude oil degradation. Additionally, impact of the crude oil degrading bacteria on biocorrosion of carbon steel was examined.

2. Materials and methods

2.1. Sample collection

Crude oil and produced water samples were collected from the crude oil reservoir, Karaikal, India (latitude: 10.7694 and longitude: 79.6155) using sterilized sample containers. The temperature at the sampling point ranged from 30 to 70 °C and the depth of the reservoir was 1200–2000 m. The collected samples were transported immediately to the environmental molecular microbiology research laboratory, Thiruvalluvar University, Vellore, India. Samples were sustained at 4 °C until further studies.

2.2. Isolation and molecular identification of bacteria

Bushnell-Haas medium (BH) comprising: 0.2 g L^{-1} $MgSO_4$, 0.02 g L^{-1} $CaCl_2$, 1.0 g L^{-1} KH_2PO_4, 1.0 g L^{-1} K_2HPO_4, 1.0 g L^{-1} $(NH_4)(NO_3)$, 0.5 g L^{-1} $FeCl_3$, and 15.0 g L^{-1} agar (Hi-Media, Mumbai, India) was utilized to isolate hydrocarbon degrading bacteria. Enumeration procedure was followed as previously described in Rajasekar et al. (2010). Sterile crude oil (1% v/v) was added as the sole carbon source, for the enumeration and isolation of crude oil degrading bacteria. The samples (both produced water and crude oil) were successively diluted up to 10^{-6} dilution and 1 mL of every dilution was plated in triplicate by pour plate technique. The plates were kept at 37 °C for 24–48 h, following which the bacterial colonies were calculated and dissimilar (morphology and appearance) colonies were picked from each plate. The picked colonies were further purified using BH plates (with 1% crude oil as carbon source) by streak plate method and the pure isolates thus obtained were maintained in BH slants (with crude oil) for additional examination. Selected dissimilar isolates were further screened for the following biochemical characterizations: Gram staining, methyl red, motility, indole production, Voges-Proskauer, citrate, catalase, carbohydrate fermentation, oxidase, gelatine, starch and lipid hydrolysis test as described in Holt et al. (1994). Further strains were used for molecular identification up to species level by 16S rRNA gene sequencing. DNA of selected isolates was extracted as described by Ausubel et al. (1988). The 16S rRNA gene was amplified using primers (27F/1492R) and amplifications and sequencing were the same as described in Rajasekar et al. (2010).

2.3. Screening for biosurfactant production and characterization

Selected bacteria were screened for biosurfactant production as described in Parthipan et al. (2017a). Biosurfactants production was confirmed using a series of screening assays including drop collapse test (Jain et al., 1991), oil displacement method (with crude oil), emulsification activity (with hexadecane) (Hassanshahian, 2014; Padmavathi and Pandian, 2014) and hemolytic test (Hassanshahian, 2014). All the assays were performed in triplicate and sterile distilled water was used as control. Biosurfactant extracted from strain B7 was used for surface tension measurement as described by Sakthipriya et al. (2015). Further extracted biosurfactant was characterized using gas chromatography and mass spectrometry (GC-MS) as described in Parthipan et al. (2017a). Functional groups were confirmed using fourier transform infrared spectrometry (FTIR, model: Perkin–Elmer, Nicolet Nexus – 470). Briefly, obtained biosurfactant was mixed with the KBr in the ratio of 1:100 and the prepared pellet was preset in the sample holder, and analyzes was performed in the mid IR region 400–4000 cm^{-1} (Parthipan et al., 2017a).

2.4. Crude oil biodegradation

Before the biodegradation studies were performed, the identified isolates were pre-grown overnight at 37 °C with crude oil as substrate. Degradation of crude oil was evaluated following the protocol as mentioned by Rahman et al. (2002). Pre-grown individual bacterial culture and mixed consortia (2.1×10^4 CFU mL^{-1}) were transferred in a 250 mL Erlenmeyer flask, each included 100 mL of BH broth added with 1% (v/v) sterile crude oil as sole carbon source. An un-inoculated flask was also used to examine the abiotic loss of crude oil hydrocarbon.