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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HIGHLIGHTS

- Hydrocarbon degrading bacteria were isolated from deep crude oil reservoir sediment (2000 m).
- Biosurfactant plays a key role for the utilization of crude oil.
- *Streptomyces parvus* B7 was identified as a potent crude oil degrader and its involvement in corrosion of carbon steel API 5LX was deciphered.
- Biofilm play key role in acceleration of the MIC.
- Understanding of the diversity of bacterial species involved in corrosion will be useful for the development of a new approach to control MIC.
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$_2$O$_3$), iron oxide (Fe$_3$O$_4$), manganese oxide (Mn$_3$O$_4$), 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 ± 0.2 × 10$^{-3}$ A/cm$^2$, compared to abiotic control 1.2 ± 0.15 × 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.

Keywords: Biocorrosion; Carbon steel; Biofilm; Biodegradation; Electrochemical impedance spectroscopy
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 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., 2007). 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 (Javaherdashti et al., 2006; 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 to 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\(_2\)PO\(_4\), 1.0 g L\(^{-1}\) K\(_2\)HPO\(_4\), 1.0 g L\(^{-1}\) (NH\(_4\))\(_2\)(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 x 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. All the flasks were kept at 37 °C for 20 days at 200 rpm. All the testing were
carried out in triplicate. A set of flasks were retrieved at 2 days interval, and utilized for the
bacterial count in standard plate-count agar (Hi-Media, Mumbai, India) by the plate counting
technique. At the end of the incubation period, biodegradation of crude oil hydrocarbons was
examined using GC-MS and FT-IR as described in Parthipan et al. (2017a).

2.5. Bio-corrosion studies

MIC of carbon steel was investigated as previously described by Rajasekar et al.
(2010), with minor modifications by using crude oil instead of diesel. Carbon steel API 5LX
for weight loss studies and electrochemical studies was prepared as described in Parthipan et
al. (2017c). The control system consisted of coupons placed in a 1 L Erlenmeyer flask with
500 mL crude oil including 20% (v/v) sterile produced water. The experimental system was
similar to the control, except that the flask was inoculated with 2 mL of mixed bacterial
consortia including B. pumilus B1, B. subtilis B5, B. megaterium B6 and S. parvus B7 (each
10^6 CFU mL⁻¹). Triplicates were performed for each system. Metal coupons were retrieved
with two days of interval until the 20th day of incubation and total viable count was observed
using formed biofilm to monitor the bacterial growth through the plate count method, using
standard plate-count agar (Hi-Media, Mumbai, India). In addition the biofilm samples was
also utilized for identifying living/dead cells at two days interval using dual staining of
fluorescin in isothiocyanate and propidium iodide as described in Dhandapani et al. (2012).
Electrochemical impedance spectroscopy (EIS) coupons recovered from both systems were used for EIS studies. The corrosive medium as collected from both systems was used as the electrolyte solution for EIS studies as described in Parthipan et al. (2017c).

2.6. Surface analysis

After the weight loss experiment, the coupons were recovered, and the rust materials were carefully detached for subsequent surface analysis. All the coupons were cleaned using Clark solution as prescribed in Rajasekar et al. (2011) and subjected to the further analysis. For surface analysis, metal coupons were prepared as described in Rajasekar et al. (2017), further scanning electron microscopy (JEOL JSM-5600LV) with 15 kV beam of electrical energy was used to visualize the biofilm morphology. Final weight of the coupons were used to measure the corrosion rates as suggested by the American Society for Testing and Materials, using this formula: 

$$\text{CR} = \frac{K \times W}{A \times T \times D}$$

where, $K =$ a constant $(8.76 \times 10^4)$, $W =$ mass loss in grams, $A =$ area in cm$^2$, $T =$ exposure time in hours and $D =$ density in g/cm$^3$ (Rajasekar et al., 2017). In addition to SEM, surface pits were also studied using atomic force microscopy (AFM) (Rajasekar et al., 2008). The standard deviations for all systems were also calculated. Corrosion products collected from both bio-corrosion systems was analyzed using X-ray diffractometer (XRD) as described in Parthipan et al. (2017c). FT-IR was used to find out the character of oxides/functional material obtained from both biotic/abiotic systems (Rajasekar et al., 2007a).

2.7. Nucleotide sequence accession number
The sequence used in current study was allocated the accession numbers KP895567-KP895570 by the National Centre for Biotechnology Information (NCBI). *Streptomyces parvus* B7 strain has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen depository (DSMZ-Germany) with the code DSM 101525, and in the National Collection of Industrial Microorganisms, CSIR - National Chemical Laboratory (NCIM-NCL) – Pune, India, under the number of NCIM- 5587.

3. Results

3.1. Molecular identification of the isolates

The physiochemical properties of produced water are presented in Table 1. The produced water included with considerably high amount of chloride, 4-5% carbonate, sulphate, as well as trace amounts of other elements. Preliminary biochemical identification revealed the identity of crude oil degrading strains (CDSs) as belonging to the Gram positive genera only (Table 2). The phylogenetic relationship (*Firmicutes* and *Actinobacteria*) was verified by analyzing each relevant species predicted by the categorization and taxonomic hierarchy, and completed with the NCBI and Ribosomal Database Project-II Release 10. Phylogenetic tree was assembled using neighbor-joining method for the isolates (Fig. 1) to evaluate the relations amongst the bacteria with interrelated species from the GenBank database. 16S rRNA sequence alignment analysis revealed more than 99% similarity between *Bacillus pumilus* B1, *B. subtilis* B5, *B. megaterium* B6 and *Streptomyces parvus* B7.

3.2. Analysis of biosurfactant production
The four bacterial isolates *B. pumilus* B1, *B. megaterium* B6 and *S. parvus* B7 showed a positive zone of clearance in the hemolytic test, while *S. parvus* B7 and *B. pumilus* B1 displayed higher emulsification activity compared to *B. subtilis* B5 and *B. megaterium* B6 (Table 3). The all four strains were conferring positive for both drop collapse activity and oil spreading assay. These observations established the biosurfactant presences in the culture broth. The oil displacement activity was directly relative to the occurrence of the biosurfactant level in the solution. The emulsion index (E24) of the isolates with hexadecane ranged from 23 to 46%. This emulsification activity established unambiguously the production of biosurfactants by the isolates. Biosurfactant produced by strain *S. parvus* B7 reduces surface tension about 22.6±0.2 mN m⁻¹ from 72.42±0.2 mN m⁻¹.

Gas chromatography analysis revealed that major components present in the extracts were fatty acids only. *S. parvus* B7 (Fig. 2a) biosurfactant contained following fatty acids: n-hexadecanoic acid (C_{16}H_{32}O_{2}) (32.49%) (Fig. 2b), oleic acid or octadecanoic acid (C_{18}H_{34}O_{2}) (Davila et al., 1992) (40.33%) (Fig. 2c) and octadecanoic acid, methyl ester (C_{19}H_{38}O_{2}) (Figure 2d) accounting for 17% of the whole peaks present in the GC spectra. Hexanedioic acid, bis (2-ethylhexyl) ester (C_{22}H_{42}O_{4}) (Hien et al., 2013) was present in the remaining strains such as *B. pumilus* B1 (Fig. S1), *B. subtilis* B5 (Fig. S2) and *B. megaterium* B6 (Fig. S3). In addition palmitic acid (C_{16}H_{32}O_{2}) (Davila et al., 1992) also presents in *B. pumilus* B1 and palmitic acid, methyl ester (C_{17}H_{34}O_{2}) was present in *B. megaterium* B6. FT-IR analysis of the biosurfactant produced by *S. parvus* B7 (Fig. 3) confirmed it was a fatty acid in nature. FT-IR spectra revealed a peak at 599 cm⁻¹ arising from C–I (Carbon–Iodine) bond. The peak at 638 cm⁻¹ confirms the presence of C-Br. The peak at 3116 cm⁻¹ represents the cumulated system R₂C=N=N in the sample. An absorption band at 976 cm⁻¹ was found to be stretching of RCH=CH₂ which indicating the presence of alkenes. The wave numbers 3560, 2308 and 2390 cm⁻¹ reveals the stretching of N–H group. The transmittance at 1405 cm⁻¹ was caused by
the aliphatic chain of the C–H group. Intense stretching peaks at 1171 and 1645 cm\(^{-1}\) indicates the presence of R-NO\(_2\) groups. The presence of these chemical groups determinedly revealed that biosurfactant was fatty acid in nature (Sarafin et al., 2014).

3.3. Crude oil degradation analysis

Fig. 4 shows the growth curve of the isolates in being there of crude oil as sole energy source. Crude oil utilization capability of the bacterial isolates were constantly observed and noted that after the inoculation of isolates, clear BH medium turns into turbid within the 2\(^{nd}\) day of incubation. The turbidity of the growth medium was increased constantly with increasing incubation period. The maximum growth rate was recorded between 10-14\(^{th}\) day of incubation and further days the growth rate was slowly decreased. The GC–MS chromatogram of crude oil biodegradation is exposed in Fig. 5 and Table 4 shows the biodegradation efficiency of crude oil. The degradation of crude oil by \(B.\) pumilus B1, \(B.\) subtilis B5, \(B.\) megaterium B6, and \(S.\) parvus B7 strains showed biodegradation efficiency (BE) of about 66 %, 55 %, 52 %, and 82 % respectively. Mixed bacterial consortia (\(B.\) pumilus B1, \(B.\) subtilis B5, \(B.\) megaterium B6 and \(S.\) parvus B7) showed a maximum BE of 90% after 20 days of incubation. More precisely, \(S.\) parvus B7 showed a 95% BE in regards to C\(_{10}\)–C\(_{20}\), while strains \(B.\) pumilus B1, \(B.\) subtilis B5, and \(B.\) megaterium B6 had a 100% BE for C\(_{10}\)–C\(_{11}\). At the same time, degradation of other n-alkanes (C\(_{12}\)–C\(_{20}\)) was weak (about 40-65%), even after 20 days of incubation. \(S.\) parvus B7 showed a maximum BE of 82% and reached a population size of 2.92 x 10\(^5\) CFU mL\(^{-1}\). This observation suggests that \(S.\) parvus B7 has a high aptitude to utilize all molecular weight crude oil hydrocarbons. Besides \(S.\) parvus B7, mixed bacterial consortia also have high prospective to remove the broad range of hydrocarbons present in the crude oil.
The FT-IR spectra of crude oil, in the abiotic control system, showed characteristic bands of C–H aliphatic stretch, C=C stretch in aromatic nuclei, C-H bend alkanes, C–N stretch aliphatic amines and N–H wag of 1°, 2° amines (Fig. 6a). The FT-IR spectra of crude in the presence of CDSs *B. pumilus* B1, *B. subtilis* B5, *B. megaterium* B6, *S. parvus* B7 and mixed consortia, shows decreased bands intensity (Fig. 6b-f). Absence of aliphatic and amine peaks at 1092 cm\(^{-1}\) and 902 cm\(^{-1}\) was due to the degradation of respective hydrocarbons.

### 3.4. Bio-corrosion studies

#### 3.4.1. Weight loss studies

The carbon steel corrosion rate in different bio-corrosion systems is presented in Table 5. The abiotic control system displayed a weight loss of 40 ± 3 mg, whereas the presence of mixed consortia increased the weight loss up to 201 ± 3 mg (Table 5). The corresponding corrosion rates (0.297 ± 0.020 mm/y and 1.493 ± 0.015 mm/y) were considered high or severe respectively (Powell, 2015). Fig. 7 showed the growth pattern of the mixed consortia in the occurrence of crude oil as sole carbon source in the corrosive medium. Maximum growth (10^6) was reached at 5th day of the incubation and cell numbers was decreased slowly from 7th day of the incubation. Growth pattern confirmed that the active growth of the CDSs in the bio-corrosion system and no countable cells was found in the abiotic system. Fig. 8 showed the epi-fluorescence microscopic observations of the bacterial cells collected from biofilm. From this figure, the presence of green fluorescence specified the existence of viable bacterial cells (Fig. 8a-c). In later stages at 8th and 10th day of incubation some of the dead cells were observed and it was specified by the presence of the
red fluorescent spots in the Fig. 8d&e. This observation confirms that mixed consortia were active throughout the biocorrosion study periods.

3.4.2. Electrochemical impedance spectroscopy

Fig. 9a shows the potentiodynamic polarization curves for carbon steel API 5LX in abiotic control and mixed consortia inoculated systems. The polarization values such as corrosion potential (E$_{\text{corr}}$), the corrosion current density (I$_{\text{corr}}$), and the anodic tafel slope ($\beta_a$) and cathodic tafel slope ($\beta_c$) Tafel values were stated in Table 6. From the polarization information it can be observed that the I$_{\text{corr}}$ was increased in the existence 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$^2$. Similarly both $\beta_c$ and $\beta_a$ of the mixed consortia systems were increased in comparison with the abiotic system.

Fig. 9b demonstrates the electrochemical impedance data for the carbon steel API 5LX in different corrosion systems. The electron transfer function is thus represented by an equivalent circuit (Fig. 9b inside), which was used for the stimulation of impedance values for both corrosion systems. The impedance parameters such as charge transfer values (R$_{\text{ct}}$), solution resistance (R$_s$) and biofilm resistance (R$_b$) values of the both systems were shown in Table 6. The higher values of R$_{\text{ct}}$ was recorded in the abiotic system ($21.3 \pm 1$ $\Omega$·cm$^2$), compared to mixed consortia ($7.7 \pm 0.8$ $\Omega$·cm$^2$). This could possibly be attributed to the thin biofilm-iron oxide deposit on the carbon steel surface, in the control system, which enhances the corrosion.

3.4.3. Surface analyses
The micrographs of bacterial biofilm (Fig. 10a & Fig. 10b) revealed that these CDSs have the ability to form dense micro colonies with accumulated metabolites (EPS). Corrosion caused by these CDSs was evaluated by examining the pits on the surface of carbon steel, following the exclusion of the biofilm and corrosion products from the coupons. Examination of the metals under SEM revealed smooth surface in the abiotic control system (Fig. 11a), whereas pitting type corrosion was observed on the surface of carbon steel in the mixed consortia system (Fig. 11b). Further the pits were confirmed by AFM analysis, 2D and 3D images of the abiotic control coupon and mixed consortia coupons along with cross-sectional analysis of the coupons are shown in Fig. 12a & b. Bacterial strains accelerated the pitting corrosion on carbon steel API 5LX surface. The micro-pitting encouraged by bacterial strains looks greater in comparison with that uninoculated control system, as revealed by the standard AFM software on the pitted areas. Based on this analysis, depth of pits accelerated by bacterial strains as range between -500 to -1000 nm compared to control coupons (below -3nm). The depth of pits proliferates with time and lead to deeper pits on carbon steel surface. In aerobic corrosion processes, oxidation takes place at the cathodic positions to formation of hydroxides. Aerobic corrosion takes place while oxygen is retained from the surface of metal through microorganisms. Consequently pit formation or corrosion reactions occur rapidly beneath the biofilm by aerobic corrosive bacterial strains (Parthipan et al., 2017d).

Fig. 13a and 13b show the XRD spectra of the corrosion product collected during the bio-corrosion studies. Iron oxide hydroxide (FeO(OH)), ferrous hydroxide (Fe(OH)₂) manganese dioxide (MnO₂) and ferrous chloride (FeCl₂) were detected in the control system (Fig. 13a). More intense peaks of ferric oxide (Fe₂O₃), iron oxide (Fe₃O₄), manganese oxide (Mn₃O₄), and manganese dioxide (MnO₂) were instead found in the mixed consortia system (Fig. 13b) (Rajasekar et al. 2007c; Parthipan et al., 2017c&d).
The FT-IR analysis of the rust products collected from different corrosion systems are shown in Fig. 14. In both control and experimental systems, broad bands were found at 3427 and 3435 cm$^{-1}$ and were endorsed to the OH group. In the control system, peaks ranged from 2924 to 2850 cm$^{-1}$, and were consigned to $-$CH$-$stretching of aliphatic hydrocarbons present in the crude oil. The peak at 1628 cm$^{-1}$ is owing to COO$^-$ (carboxylate anion) and the one at 602 cm$^{-1}$ specifies the stretch of iron oxides (FeO). The peak at 1633 cm$^{-1}$ is owing to C=O (stretch (amide I) related to proteins) and is attributed to the formation of bacterial exopolymer secretion (EPS) (Badireddy et al., 2010). New peaks were noticed at 1365 cm$^{-1}$ representative to the existence of C–H alkanes on the metal surface. A peak at 1024 cm$^{-1}$ identifies the stretching intended for $-$C–O$-$ stretch for $-$C–O–C$-$ group. One peak at 877 cm$^{-1}$ specifies the existence of FeO whereas the peak at 568 cm$^{-1}$ was attributed to C–Cl bond (Rajasekar et al., 2007a).

4. Discussion

The produced water samples collected from an Indian crude oil reservoir contains considerable level of chloride, carbonate and sulphate. These chemicals, together with the crude oil as carbon source, support microorganisms in the oil reservoir. The ability of Gram positive bacteria (bacilli) to form endospores is a vital adaptation machinery among the microorganisms living in extremes and unstable environments, such as those with high temperature, pressure, marine sediments, semi-arid circumstances, and with hot summers (Shimura et al., 1999). The growth of microorganisms in crude oil is often linked to the production of biosurfactants (Rajasekar et al., 2008). Production of biosurfactant allows microorganisms to uptake the hydrocarbons, with a positive effect on their growth, which has significant implications in the oil reservoir (Maruthamuthu et al., 2005; Parthipan et al.)
The surface reducing nature of the strain B7 confirms that produced biosurfactant has the capabilities to reduce the surface tension of the medium in presence of the crude oil as substrate and it will enhance the solubility of the crude oil (Sakthipriya et al. 2015).

While the CDSs used throughout this study were isolated from a crude oil reservoir, they can also easily adapt to, and survive in the oil-contaminated aqueous medium. All the bacterial strains produced different biosurfactant compounds which are classified as fatty acid in nature.

The bacterial isolates showed luxuriant growth in crude oil by using it as carbon source; they also exhibited efficient crude oil degradation corresponding to an increase in cell population. The GC-MS spectra (Fig. 5) confirm that the bacterial strains have the capability to utilize crude oil hydrocarbons. During degradation, the cationic moieties of the biosurfactants have attraction towards negatively charged bacterial membrane in connection with crude oil. The hydrophobic part of the biosurfactant is believed to allow the peptides to sliver and permeate into the membrane (Mulligan and Gibbs, 2004).

From the utilization of low molecular weight hydrocarbons, bacteria produce biosurfactants, which assist in the crude oil solubilization and bacterial growth. Cell growth was then promoted by the ‘degraded’ oil products and additional emulsifying agents were then produced (Radhika et al., 2014). In the present work, synthesis of the biosurfactant by bacterial strains leads to highest biodegradation efficiency of hydrocarbon by increasing their solubility. Thavasi et al. (2011) described that degradation of crude oil by Corynebacterium kutscheri, B. megaterium, and Pseudomonas aeruginosa was enhanced by the production and action of biosurfactants. The GC spectra analysis of the degraded residual compounds confirmed that all the bacterial strains are capable of breaking down the complex hydrocarbons found in the crude oil. Rajasekar et al. (2007a) reported the ability of Serratia marcescens to degrade diesel/naphtha hydrocarbon.
EIS measurements were considered to elucidate the consequence of bacterial strains on biocorrosion of carbon steel API 5LX. EIS is a non-destructive method for distinguishing electrochemical process at metal/biofilm interfaces and observing development of corrosion products and biofilms during microbial corrosion. Potentiodynamic polarization observations confirmed that the corrosion current and anodic/cathodic tafel slope were enhanced in bacterial system. This finding further confirmed that these bacterial strains increased the corrosion rate (1.493 ± 0.015 mm/y) of carbon steel through inducing cathodic reactions. In a biofilm, electrons are accepted from metal surface, creating an alleyway of electron flow from carbon steel (anode) to the collective electron acceptor oxygen (cathode); and as a result accelerated bio-corrosion (Tsai and Chou, 2000).

Impedance observations as well as confirmed bacterial attachment are corrosive nature that leads to the decreases of corrosion resistance. Lower impedance value in the presence of mixed consortia was due to the weakening of protective effects. The presence of biofilm and prevalence of bacterial metabolic activities can considerably involve in the decline of passivity while bacterial metabolites and chloride ions accumulate at metal surface. Consequently, the impedance parameters decreased over the period of exposure.

Bacterial biofilm play crucial role in the pit formation on carbon steel surface. Similar observations were observed recently by Machuca et al. (2016). There is no considerable pit was observed in carbon steel immersed in the abiotic control system, it could be due to the very less corrosiveness in the absence of bacterial consortia. These results were well supported by the SEM observations. Bacterial attachment and the subsequent biofilm development are the decisive steps in biological mediated metal deterioration (Parthipan et al., 2017d). From the epi-fluorescence microscopy analysis the biofilm formation was higher with active cells throughout the incubation period of the biocorrosion study (Fig.8a-c). In the current study, destructive ions, such as chloride, attached over the metal surface with the
CDSs and induced corrosion. Besides, the existence of bacteria on surface of carbon steel can encourage rigorous attack because of the alterations in the microchemistry of the metal surface modified by bacterial metabolism (Tsai and Chou, 2000).

The presence of Fe$_2$O$_3$ in the corrosion product confirms that the CDSs accelerated the corrosion of carbon steel API 5LX (Hamilton, 1985). These results revealed the presence of high intensity corrosion products including Fe$_2$O$_3$, Fe$_3$O$_4$, Mn$_3$O$_4$, and MnO$_2$, confirming the role of mixed bacterial consortia in iron/manganese oxidations, which accelerates the corrosion process (Parthipan et al., 2017c). Block/grey rust product was observed over the carbon steel in the mixed consortia system, it could be due to the occurrence of magnetite in the rust products as identified in XRD analysis.

Degraded hydrocarbons in crude oil promote the development of bacteria and augment the rust formation (Lenhart et al., 2014; Aktas et al., 2017). Also degraded hydrocarbons enhance the development of ferric oxide. Consequently, bacteria accelerate the corrosion reaction by forming Fe$_2$O$_3$. The occurrence of inorganic substances such as ferric, in rust product, indicate that mixed consortia accelerate the development of ferric/manganese complex products (Rajasekar et al., 2007b, 2010). Similar results were previously reported by Rajasekar et al. (2005), indicating that a number of crude oil consuming bacteria oxidize the Fe$^{2+}$ to Fe$^{3+}$ by addition of O$_2$ commencing from the biodegraded compounds, leading to the formation of organic complex. Because ferric has a higher attraction for O$_2$, it removes O$_2$ from the biodegraded product and boosts the development of Fe$_2$O$_3$ and enhances the corrosion process (Rajasekar et al., 2010)

4.1. Biocorrosion mechanism
The isolated CDSs identified here belong to the *Bacillaceae* and *Streptomycetaceae* families. These isolates consume hydrocarbon with a wide range of molecular weight. Among the identified species, *S. parvus* B7 displayed a maximum BE of 82% for hydrocarbons, including light and heavy hydrocarbons found in the crude oil (Fig. 5 and Fig. 6). Biosurfactant involved an exceptionally important function in enhancing the degradation of crude oil. In our study, the isolate *S. parvus* B7 acts as good crude oil degrader due to the production of biosurfactant and its higher emulsification abilities. These strains are facultative anaerobes, and biochemical tests confirmed that they express both cytochrome oxidase and catalase enzymes. All strains also express catalase, which neutralize the toxicity of H$_2$O$_2$ into H$_2$O and O$_2$. These strains then utilize oxygen and hydrogen in the respiration process. O$_2$ radicals, formed by bacterial metabolism, combined with the nearby iron atom present on surface of the metal, form a superoxide surface anion radical. Eventually, the metal surface anion reacts with H$_2$O, which directs the oxidation of Fe$^{2+}$ to Fe$_2$O$_3$ as rust compounds, besides the hydroxide anion (Fig. 13 and Fig. 14) (Rajasekar et al., 2011). This observation corroborates the work of Lenhart et al. (2014) who demonstrated that microorganisms utilise the hydrocarbon and ferrous ion as organic and inorganic sources respectively and thus accelerate the corrosion of carbon steel in crude oil reservoir (Ching et al., 2016; Aktas et al., 2017). In general, the results obtained in this study support the theory that the MIC of carbon steel takes place through the contribution of Fe$_2$O$_3$, which is a consequence of degradation of crude oil hydrocarbons.

Nowadays, addition of inhibitors/biocides is extensively used for managing corrosion in the oil industry. It is crucial to select appropriate and effective inhibitor/biocide, as many microorganisms present in oil and other petroleum products are capable of degrading these compounds and utilize them for their development and growth, hence unwittingly promoting corrosion as well (Maruthamuthu et al., 2005). It is therefore essential to have a basic
understanding of the physiology of bacterial communities present in crude oil reservoir, which will help selecting a suitable inhibitor/biocide for the control of MIC in crude oil reservoir.

5. Conclusions

To conclude, the isolate *S. parvus* B7 showed a BE of crude oil of up to 82%, aided by the high biosurfactant production. Mixed bacterial consortia converts Fe$^{2+}$ to Fe$_2$O$_3$ by adding oxygen during the degradation process, thus forming iron oxide complexes (rust) on carbon steel, the maximum corrosion rate was recorded in the mixed consortia system (1.493 ± 0.015 mm/y). Biofilm formation assisted pit formation on the carbon steel surface and it was evidenced from the SEM and AFM analysis. Corrosion current was increased in the presence of mixed consortia this observation confirmed that mixed bacterial consortia play key role in the corrosion of carbon steel. These observations enlarge the understanding of bacterial communities related to biocorrosion of carbon steel as well as distinguish the corrosive properties of bacteria belonging to the *Streptomyces* family.

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Conflicts of interest
The authors declare no competing financial interest.

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environments: The impact of different ultra low sulfur diesels and bioaugmentation.
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sludge microorganisms under glucose-controlled conditions. Water Res. 44, 4505-
4516.


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Figure Legends

**Fig. 1.** Neighbor-joining tree based on 16S rRNA gene sequences, showing phylogenetic relationships between sequences of the bacterial phylum *Firmicutes* (*Bacillus* related species) *Actinobacteria* (*Streptomyces* species). GenBank accession numbers are given in parentheses. The scale bar indicates sequence divergence.

**Fig. 2.** GC-MS analysis of biosurfactant from *S. parvus* B7 (a) GC spectrum of biosurfactant; (b) Mass spectra of n-hexadecanoic; (c) Mass spectra of octadecanoic acid and (d) Mass spectra of octadecanoic acid, methyl ester.

**Fig. 3.** FT-IR spectrum of partially purified biosurfactant isolated from *S. parvus* (B7).

**Fig. 4.** Bacterial growth curve of CDSs in BH medium with crude oil as a sole carbon source.

**Fig. 5.** Gas Chromatography mass spectrum (GC-MS) tracing of residual crude oil in the abiotic system control and experimental system (a) Abiotic system; (b) *B. pumilus* B1; (c) *B. subtilis* B5; (d) *B. megaterium* B6; (e) *S. parvus* B7 and (f) Mixed consortia.

**Fig. 6.** FT-IR spectrum of crude oil in abiotic control and experimental system inoculated with individual bacterial culture (a) Abiotic system; (b) *B. pumilus* B1; (c) *B. subtilis* B5; (d) *B. megaterium* B6; (e) *S. parvus* B7, and (f) Mixed consortia.

**Fig. 7.** Growth pattern of the mixed consortia in the bio-corrosion studies.

**Fig. 8.** Epi-fluorescence micrograph of bacterial biofilm (a) 2\textsuperscript{nd} day (b) 4\textsuperscript{th} day (c) 6\textsuperscript{th} day (d) 8\textsuperscript{th} day and (e) 10\textsuperscript{th} day.

**Fig. 9.** Electrochemical analysis of the carbon steel API 5LX coupon exposed in different bio-corrosion studies; (a) Polarization curves and (b) Impedance curves (equivalent circuit was presented inside of the impedance curves).

**Fig. 10.** SEM micrograph of biofilm formation on carbon steel API 5LX surface coupon exposed in bio-corrosion studies; (a) Over view of the biofilm on metal surface and (b) Magnified view of the biofilm and bacterial attachments.
Fig. 11. SEM micrograph of typical pits formed on surface of the carbon steel API 5LX immersed in bio-corrosion studies; (a) abiotic control (bare metal) and (b) Mixed consortia.

Fig. 12. Two (a1 and b1), three (a2 and b2) dimensional images of the AFM observation of carbon steel API 5LX coupon surface show that pit formation on surface of the experimental systems in presence of mixed consortia, cross-sectional (a3 and b3) analysis determining the depth of pit on the metal surface.

Fig. 13. Analysis of corrosion product on carbon steel exposed to mixed bacterial consortia by XRD analysis (a) Abiotic system, and (b) Experimental system.

Fig. 14. FT-IR spectrum of surface film on the metal surface in presence/absence of mixed bacterial consortia (a) Abiotic system, and (b) Mixed consortia.
Fig. 1.

Bacillus pumilus ISE_24 (KX035059)
Bacillus pumilus CSR_28 (KX035022)
Bacillus sp. NHPC-1 (KU644590)

- Bacillus pumilus B1 (KP895567)
- Bacillus pumilus CSR_14 (KX035021)

- Bacillus subtilis B5 (KP895568)
- Bacillus subtilis Xmb062 (KT986187)
- Bacillus subtilis Lmb031 (KT986096)
- Bacillus subtilis Lmb026 (KT986093)
- Bacillus subtilis LLP-4 (KU821697)

- Bacillus megaterium B6 (KP895569)
- Bacillus megaterium R12-40 (LT604384)
- Bacillus megaterium S2-91 (LT604345)
- Bacillus megaterium S2-86 (LT604344)
- Bacillus megaterium S1-115 (LT604244)

- Streptomyces parvus B7 (KP895570)
- Streptomyces sp. CAI121 (JN400113)
- Streptomyces parvus sj38 (JX0139651)
- Streptomyces parvus 13647j (EU741140)
- Streptomyces parvus 3151 (EF063462)
Fig. 2
Fig. 3.
Fig. 4.
Fig. 5.
Fig. 6.

- Abiotic control
- *B. pumilus* B1
- *B. subtilis* B5
- *B. megaterium* B6
- *S. parvus* B7
- Mixed consortia

Wavenumber, cm$^{-1}$
Fig. 7.

Graph showing the change in CFU/cm² over the incubation period (days) for Abiotic control and Mixed consortia.
Fig. 9.
Fig. 10.
Fig. 11.
Fig. 12.
Fig. 13.

The X-ray diffraction patterns for (a) and (b) show the presence of various iron and manganese compounds. Peaks at specific 2 Theta values correspond to different compounds such as Fe₂O₃, FeO(OH), Fe₃O₄, MnO, and MnO₂. The intensity of these peaks varies between the two patterns, indicating differences in the crystal structures or compositions of the samples.
Fig. 14.
Table 1

Physiochemical characters of the produced water collected from Indian crude oil reservoir

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Present values (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Suspended Solids</td>
<td>194</td>
</tr>
<tr>
<td>2</td>
<td>Oil &amp; Grease</td>
<td>34.2</td>
</tr>
<tr>
<td>3</td>
<td>Total Dissolved Solids</td>
<td>59793</td>
</tr>
<tr>
<td>4</td>
<td>Salinity as NaCl</td>
<td>59303</td>
</tr>
<tr>
<td>5</td>
<td>Chloride as Cl⁻</td>
<td>35988</td>
</tr>
<tr>
<td>6</td>
<td>Hardness as CaCO³</td>
<td>6700</td>
</tr>
<tr>
<td>7</td>
<td>Calcium as Ca²⁺</td>
<td>1800</td>
</tr>
<tr>
<td>8</td>
<td>Magnesium as Mg²⁺</td>
<td>529</td>
</tr>
<tr>
<td>9</td>
<td>Sodium as Na⁺</td>
<td>20600</td>
</tr>
<tr>
<td>10</td>
<td>Iron as Fe³⁺</td>
<td>32.9</td>
</tr>
<tr>
<td>11</td>
<td>Bicarbonate as HCO₃⁻</td>
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</tr>
<tr>
<td>12</td>
<td>Sulphate as SO₄²⁻</td>
<td>354</td>
</tr>
<tr>
<td>13</td>
<td>pH</td>
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Table 2
Biochemical characterization of the CDSs isolated from Indian crude oil reservoir

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>B1</th>
<th>B5</th>
<th>B6</th>
<th>B7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram staining</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Motility test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Indole Production test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Methyl red test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Voges-Proskauer test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Citrate test</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Utilization of hydrocarbon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude oil</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Production of acid from</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Dextrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
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<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Catalase test</td>
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<td>Oxidase test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Starch hydrolysis test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

B1- *B. pumilus*, B5- *B. subtilis*, B6- *B. megaterium*, B7- *Streptomyces parvus*
Table 3

Screening for biosurfactant production: drop collapse assay, oil spreading assays and emulsification activity of the isolates

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of bacteria</th>
<th>Hemolytic activity</th>
<th>Drop collapse assay</th>
<th>Oil spreading Assay</th>
<th>Emulsification index (E24%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>B. pumilus</em> B1</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td><em>B. subtilis</em> B5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td><em>B. megaterium</em> B6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td><em>Streptomyces parvus</em> B7</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>46</td>
</tr>
</tbody>
</table>

Hemolytic activity: +, Positive response; -, Negative response

Drop collapse assay

‘+++’ - Drop collapse within 1 minute, ‘++’ - Drop collapse after 1 minute and ‘+’ - Drop collapse after 2 minutes of biosurfactant addition.

Oil spreading assay

‘+’ - Oil spreading with a clear zone of 0.5-1.0 cm, ‘++’ - Oil spreading with a clear zone of 1.5 to 2.0 cm, ‘+++’ - Oil spreading with a clear zone of 2.0 to 3.0 cm.

Note: E24% checked using hexadecane.
Table 4

Percentage of biodegradation of crude oil in the presence of CDSs

<table>
<thead>
<tr>
<th>RT</th>
<th>Compounds</th>
<th>RA</th>
<th>B1</th>
<th>BE(%)</th>
<th>B5</th>
<th>BE(%)</th>
<th>B6</th>
<th>BE(%)</th>
<th>B7</th>
<th>BE(%)</th>
<th>Mix</th>
<th>BE(%)</th>
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<tbody>
<tr>
<td>3.0 &amp; 3.5</td>
<td>2-methylpentane</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>4.0</td>
<td>2,2-Dimethylpentane</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>11</td>
<td>89</td>
<td>1.4</td>
<td>99</td>
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<tr>
<td>5.0</td>
<td>2,4- Dimethylpentane</td>
<td>92</td>
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<td>1.4</td>
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<td>2-methylheptane</td>
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<td>10</td>
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<td>2.8</td>
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<td>89</td>
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<td>82</td>
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<td>83</td>
<td>5</td>
<td>94</td>
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<td>91</td>
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<td>29.6</td>
<td>Decane, 2,3,5,8-tetramethyl</td>
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<td>31</td>
<td>64</td>
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<td>71</td>
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<td>91</td>
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<td>51</td>
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<td>90</td>
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<td>4</td>
<td>93</td>
</tr>
<tr>
<td>56.8</td>
<td>Nonadecane</td>
<td>49</td>
<td>25</td>
<td>49</td>
<td>29</td>
<td>41</td>
<td>37</td>
<td>24</td>
<td>4</td>
<td>92</td>
<td>4</td>
<td>92</td>
</tr>
<tr>
<td>60.6</td>
<td>Eicosane</td>
<td>41</td>
<td>21</td>
<td>49</td>
<td>27</td>
<td>34</td>
<td>30</td>
<td>27</td>
<td>6</td>
<td>85</td>
<td>2.8</td>
<td>93</td>
</tr>
<tr>
<td>64.3,67.8,71,74.4,77.5 &amp; 80.5</td>
<td>Eicosane-10-methyl</td>
<td>21.6</td>
<td>11</td>
<td>50.3</td>
<td>15.5</td>
<td>30.3</td>
<td>17</td>
<td>23.5</td>
<td>7.6</td>
<td>61.3</td>
<td>1.9</td>
<td>90</td>
</tr>
<tr>
<td>83.2, 86.1, 88.9 &amp; 91.0</td>
<td>Heptadecane -9-octyl</td>
<td>6.5</td>
<td>3.6</td>
<td>43.7</td>
<td>4.8</td>
<td>21.7</td>
<td>4.8</td>
<td>25.2</td>
<td>2</td>
<td>69.2</td>
<td>1.2</td>
<td>81</td>
</tr>
<tr>
<td>92.4,93.5,94.8 &amp; Octadecane</td>
<td>6</td>
<td>3.2</td>
<td>42.6</td>
<td>3.3</td>
<td>44.3</td>
<td>4.1</td>
<td>30.6</td>
<td>1.6</td>
<td>71.6</td>
<td>1.4</td>
<td>75</td>
<td></td>
</tr>
</tbody>
</table>

Total biodegradation efficiency (%) 65.8 54.5 52.0 81.6 90.0

Note: RT= Retention time, RA= Relative abundance (%), B1= B. pumilus, B5= B. subtilis, B6 = B. megaterium, B7= Streptomyces parvus, Mix= Mixed consortia. Following compounds are given by mean values such as: 2-methylpentane, Hexadecane, Eicosane-10-methyl, Heptadecane -9-octyl and Octadecane.
Table 5

Corrosion rate of carbon steel in presence and absence of CDSs

<table>
<thead>
<tr>
<th>Systems</th>
<th>Weight loss (mg)</th>
<th>Corrosion rate (mm/y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control system: 500 mL crude oil with 20% of</td>
<td>40 ± 3</td>
<td>0.297 ± 0.020</td>
</tr>
<tr>
<td>produced water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental system: 500 mL crude oil + 20% of</td>
<td>201 ± 2</td>
<td>1.493 ± 0.015</td>
</tr>
<tr>
<td>produced water with mixed consortia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 6
Polarization and impedance parameters for carbon steel API 5LX in the presence/absence mixed bacterial consortia.

<table>
<thead>
<tr>
<th>Systems</th>
<th>Polarization data</th>
<th>Impedance data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\text{I}_{\text{corr}}$</td>
<td>$\text{E}_{\text{corr}}$</td>
</tr>
<tr>
<td></td>
<td>(A/cm$^2$)</td>
<td>(V vs. SCE)</td>
</tr>
<tr>
<td>Control system:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 mL crude oil with 20%</td>
<td>(1.2 ± 0.15) × 10$^{-4}$</td>
<td>-495 ± 6.4 ± 2.8</td>
</tr>
<tr>
<td>Experimental system: 500 mL</td>
<td>(1.6 ± 0.2) × 10$^{-3}$</td>
<td>-557 ± 9.3 ± 3.8</td>
</tr>
<tr>
<td>crude oil with 20% produced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and mixed consortia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$E_{\text{corr}}$ - Corrosion potential, $I_{\text{corr}}$-Corrosion current, $\beta_a$ - anodic tafel slope, $\beta_c$ – cathodic tafel slope, Rs- Solution resistance, Rct- Charge transfer resistance and $R_b$ – Biofilm resistance.
Supplementary Information

**Fig. S1.** GC-MS analysis of biosurfactant from *B. pumilus* B1 (a) GC spectrum of biosurfactant; (b) Mass spectra of hexanedioic acid, bis (2-ethylhexyl) ester and (c) Mass spectra of palmitic acid.

**Fig. S2.** GC-MS analysis of biosurfactant from *B. subtilis* B5 (a) GC spectrum of biosurfactant and (b) Mass spectra of hexanedioic acid, bis (2-ethylhexyl) ester.

**Fig. S3.** GC-MS analysis of biosurfactant from *B. megaterium* B6 (a) GC spectrum of biosurfactant; (b) Mass spectra of hexanedioic acid, bis (2-ethylhexyl) ester and (c) Mass spectra of palmitic acid, methyl ester.
Fig. S1.
Fig. S2.