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ORIGINAL PAPER

Role of *Serratia marcescens* ACE2 on diesel degradation and its influence on corrosion

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Abstract A facultative anaerobic species *Serratia marcescens* ACE2 isolated from the corrosion products of diesel transporting pipeline in North West, India was identified by 16S rDNA sequence analysis. The role of *Serratia marcesens* ACE2 on biodegradation of diesel and its influence on the corrosion of API 5LX steel has been elucidated. The degrading strain ACE2 is involved in the process of corrosion of steel API 5LX and also utilizes the diesel as an organic source. The quantitative biodegradation efficiency (BE) of diesel was 58%, calculated by gas-chromatography–mass spectrum analysis. On the basis of gas-chromatography–mass spectrum (GC–MS), Fourier Transform infrared spectroscopy (FTIR) and X-ray diffractometer (XRD), the involvement of *Serratia marcescens* on degradation and corrosion has been investigated. This basic study will be useful for the development of new approaches for detection, monitoring and control of microbial corrosion.

Keywords *Serratia marcescens* · Diesel pipeline · Biodegradation · Biocorrosion

Introduction

Corrosion is a major cause of pipe failure and is a significant component affecting the operation and maintenance costs of gas industry pipelines [1, 3, 8, 13, 17, 27, 34, 41]. The estimated pipeline corrosion loss to the gas industry in 1996 was about $840 million/year [8] while the estimated annual cost of all forms of corrosion to the oil and gas industries in 2001 was $13.4 billion, of which microbially influenced corrosion accounted for about $2 billion [27]. It was estimated that 40% of all internal pipeline corrosion in the gas industry can be attributed to microbial corrosion [17]. Many studies have shown that microbial contamination of stored hydrocarbon fuels can lead to blocking of pipelines, filters and reduced fuel quality and corrosion of storage tanks [14, 38]. The problems in India are particularly acute for diesel fuel, which frequently presents a substantial biomass at the fuel/water interface. Generally, the major bacteria involved in the corrosion of petroleum production systems are the anaerobic sulfate-reducing bacteria (SRB) [22, 46, 47]. However, aerobic bacteria and fungi may also participate in the corrosion process [6, 38, 40, 44]. These microorganisms influence the corrosion by altering the chemistry at the interface between the metal and the bulk fluid [29, 25, 7]. Therefore, most of the research on biocorrosion has focused on SRB [45, 9, 18]. Recent studies suggest that other types of bacteria such as iron-oxidizing bacteria, manganese-oxidizing bacteria, acid-producing bacteria and methanogens could also be involved [22, 28, 33, 37, 39, 51, 52]. Many genera of microorganisms have the ability to degrade recalcitrant compounds and use them as a source of carbon or energy. Such phenomena are not commonly encountered in enteric bacteria [25]. The enteric bacteria in the family enterobacteriaceae are mainly regarded as inhabitants of animal guts [12], but *Leclercia adecarboxylata* was noticed in oily-sludge contaminated soil. In the present study an enteric bacterium, *Serratia marcescens*, in a cross-country pipeline in North India is reported. Microbial corrosion studies involving the use of natural individual species

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obtained from industrial systems are scarce. However, such studies would better address the actual problem and increase the understanding of the microbial species involved in the degradation of diesel as well as their role in microbial corrosion and their interactions with the metal surfaces.

Background information of the work

Samples (diesel and muck) were collected from a cross-country pipeline (of 1,400 km length) in North India. This pipeline has intermittent petroleum product delivery with pressure booting stations at different locations. The muck (corrosion product) is pushed out of the pipeline by pigs (cylindrical devices that move with the flow of oil and clean the inner portion of the pipelines), which are introduced into the pipeline in a preceding station and received at a succeeding station. Carboxylic acid- and ester-based inhibitor is added in the petroleum product pipeline in order to control corrosion and bacterial proliferation. Despite the presence of the inhibitor, about 200–400 kg of muck within 2 months containing about 70% iron particles were accumulated over 200 km of the pipeline during pigging. An examination of the pipeline revealed that microbial corrosion was the cause of the problem. The bacterial count in the corrosion product was too numerous to count (above 10^{12} CFU/mL) while in the petroleum product it was too low to count [32].

Materials and methods

Collection of samples and characterization of bacteria

The bacteria were isolated from corrosion product as described by Maruthamuthu et al. [32] and Rajasekar et al. [38]. The isolated bacteria were grouped into various genera as per Bergey's Manual of Determinative Bacteriology [19]. These cultures were characterised based on their morphology, Gram staining, spore staining, motility, oxidase, catalase, oxidation fermentation, gas production, ammonia formation, nitrate and nitrite reduction, indole production test, methyl-red and Voges–Proskauer test, citrate and mannitol utilisation test, hydrolysis of casein, gelatin, starch, urea and lipid. The following genera were present in the corrosion product: *Bacillus* sp. *Micrococcus* sp. *Vibrio* sp. *Pseudomonas* sp. *Thiobacillus* sp., *Ochrobium* sp., *Xanthobacter* sp., *Gallionella* sp., *Legionella* sp. and *Acinetobacter* sp. Five bacterial species were selected as effective diesel hydrocarbon degraders for further study, which were identified and confirmed at the Institute of Microbial Technology, Chandigarh, India by employing biochemical analysis as *Bacillus licheniformis* (MTTC6535), unidentified strain (MTCC6536), *Bacillus megaterium* (MTTC6537) *Enterobacter aerogenes*, (MTTC6538), *Bacillus* sp. (MTTC6539). For further confirmation two isolates, *Bacillus* sp. (MTTC6539) and unidentified strain (MTCC6536) were identified by 16S rDNA analysis. The latter strain (MTCC6536) was identified as *Serratia marcescens* ACE2, and was selected for further biodegradation and corrosion studies.

Amplification, cloning and sequencing of 16S rRNA gene

Genomic DNA was extracted according to Ausubel et al. [5]. Amplification of genes encoding small subunit ribosomal RNA was carried out using Eubacterial 16S rDNA primers (forward primer 5′ AGAGTTTGATCCTGGCT CAG 3′ (*E. coli* positions 8–27) and reverse primer 5′ ACGGCTACCTTGTTACGACTT 3′ (*E. coli* positions 1494–1513) [49]. Polymerase chain reaction (PCR) was performed with a 50-μL reaction mixture containing 2 μL (10 ng) of DNA as the template, each primer at a concentration of 0.5 μM, 1.5 mM $MgCl_2$ and each deoxynucleoside triphosphate at a concentration of 50 μM as well as 1 μL of *Taq* polymerase and buffer as recommended by the manufacturer (MBI Fermentas). PCR was carried out with a Mastercycler Personal (Eppendorf) with the following program: initial denaturation at 95 °C for 1 min; 40 cycles of denaturation (3 min at 95 °C), annealing (1 min at 55 °C), and extension (2 min at 72 °C); followed by a final extension (at 72 °C for 5 min). The amplified product was purified using GFX™ PCR DNA and Gel Band Purification kit (Amersham Biosciences) and cloned in pTZ57R/T vector according to the manufacturer's instruction (InsT/Aclone™ PCR Product Cloning Kit, MBI Fermentas) and transformants were selected on LB medium containing ampicillin (100 μg/mL) and X-gal (80 μg/mL). Sequencing reaction was carried out using ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems). For sequencing reaction Big Dye Ready Reaction Dye-Deoxy Terminator Cycle Sequencing kit (Perkin–Elmer) was employed. A search of the GenBank nucleotide library for sequences similar to those determined was made by using BLAST [1], through the National Center for Biotechnology Information (NCBI) internet site (http://www.ncbi.nlm.nih.gov/BLAST).

Biodegradation test

Bacterial strain *Serratia marcescens* ACE2 was grown in batch cultures in 500 mL Erlenmeyer flasks containing 300 mL of Bushnell Haas broth (consisting of magnesium sulphate 0.20 g/L, calcium chloride 0.02 g/L, monopotassium phosphate 1 g/L, dipotassium phosphate 1 g/L, ammonium nitrate 1 g/L, ferric chloride 0.05 g/L, pH 7) supplemented with 1 g of diesel fuel as a sole carbon

source. Diesel samples were sterilized by filtering through a Millipore 0.45-μm pore size membrane filter. The flasks were inoculated with 2 mL of inoculum of 10^8 CFU/mL and were incubated aerobically at 27 °C on a rotary shaker operated at 200 rpm for 30 days. An uninoculated control flask was incubated in parallel to monitor abiotic losses of the diesel substrate. The purity of the culture was also checked during degradation study by the pour plate method. All the experiments which included uninoculated controls were performed in duplicate. At the end of the experiment, the residual diesel fuel in the entire flask was extracted first with an equal volume of dichloromethane. Evaporation of solvents was carried out in a water bath at 40 °C. The residual diesel was dissolved in dichloromethane and 1 μL of the resultant solution was analyzed by Thermo Finnigan gas chromatography/mass spectrometry (Trace MS equipped with a RTX-5 capillary column (30 m long × 0.25 mm internal diameter) and high purity nitrogen as carrier gas. The oven was programmed between 80 and 250 °C at a heating temperature of 10 °C/min. The degradation of diesel as a whole was expressed as the percentage of diesel degraded in relation to the amount of the remaining fractions in the appropriate abiotic control samples. The biodegradation efficiency (BE), based on the decrease in the total concentration of hydrocarbons, was evaluated by using the following expression as described by Luigi Michaud et al. [30]: BE (%) = $100 - \left(\frac{A_S \times 100}{A_{ac}}\right)$ where A_s = total area of peaks in each sample, A_{ac} = total area of peaks in the appropriate abiotic control, BE (%) = efficiency of biodegradation.

Characterization was also done by FTIR (Perkin Elmer, Paragm 500) and the FTIR spectra were recorded in the mid-IR region of 400–4,000 cm^{-1} with a 16-scan speed. The samples were mixed with spectroscopically pure KBr in 1:100 ratio. Infrared peaks localized at 2,960 and 2,925 cm^{-1} were used to calculate the CH_2/CH_3 ratio (absorbance) and functional groups in diesel.

Hydrogen peroxide estimation and pH estimation

Hydrogen peroxide was estimated with peroxide test strips (Merckoquent, Merck), and pH at the interface between diesel and water was measured by a pH meter (Eutech, Merck).

Corrosion studies

Weight loss method

Steel [API 5LX grade (C 0.29 max, S 0.05 max, P 0.04 max, Mn 1.25 max.)] coupons of 2.5 cm × 2.5 cm were mechanically polished to mirror finish and then degreased using trichloroethylene. In this study, 500 mL of diesel with 2% water containing 120 ppm chloride was used as the control system, while 500 ml diesel with 2% of water containing 120 ppm chloride and inoculated with 2 mL of inoculum with 1% of BH broth of about 10^6 CFU/mL was used as the experimental system. All the weight loss studies were performed in duplicate. After 7 days, the coupons were removed and pickled, washed in water and dried with an air drier. The final weights of the six coupons in each system in duplicate were taken and the average corrosion rates were calculated as recommended by NACE, Houston.

Electrochemical analysis

For electrochemical studies, steel specimens (exposed area: 1.0 cm^2) embedded in Araldite were used as the working electrode. A standard calomel electrode (SCE) and a platinum wire were used as the reference and counter electrodes, respectively. In the present study, a mixture of 75 mL of 1% BH broth (containing 120 ppm chloride) and 150 mL of diesel was used as the control system, while a mixture of 75 mL of 1% BH broth (containing 120 ppm chloride) and 150 mL of diesel inoculated with 2 mL of inoculum of about 10^6 CFU/mL was used as the experimental system. The diesel–water mixtures were stirred vigorously for periods of 4 and 10 days to stimulate flow condition. After the fourth and tenth day, the water (electrolyte) was collected from the systems [11] and electrochemical studies viz polarization and impedance studies have been studied. A Pentium 166 computer interfaced with an AutoLab/PGSTAT 30 system was used for polarization and impedance studies. The measurements were made between 10 mHz and 100 kHz with a 5 mV perturbation signal at the corrosion potential. A coupon of 1 cm^2 API 5LX as working electrode, a standard calomel electrode (SCE) and platinum wire as counter electrode were employed for polarization study. The tafel polarization curves were obtained by scanning from the open circuit potential towards 200 mV anodically and cathodically. The scan rate was 120 mV/min.

Surface analysis

After the weight loss study, the coupons were taken out and the corrosion products were scratched carefully, dried and crushed into a fine powder and used for powder X-ray diffractometer analysis (X' per PRO PANalytic fitted with a nickel-filtered copper $K\alpha$ radiation source. The scattering angle (2θ) ranged from 5° to 85°.

Results and discussion

In the present study, the mechanism of diesel degradation by *Serratia marcescens* ACE2 was reported in a petroleum

transporting pipeline in Northwest India. Its possible role on corrosion also has been investigated.

Characterization of bacterium ACE2

The phenotypic profile of strain ACE2 is shown in Table 1. Amplification of the gene encoding for small subunit ribosomal RNA of ACE 2 was done using Eubacterial 16S rDNA primers. The 16S rDNA amplicons derived from ACE2 was cloned in pTZ57R/T vectors. The recombinant plasmid (pACE2, harboring 16S rDNA insert) was partially sequenced. The sequence obtained was matched with the previously published sequences available in NCBI using BLAST. Sequence alignment and comparison revealed more than 99% similarity with *Serratia marcescens*. The nucleotide sequence of 16S rDNA of ACE2 has been deposited in GenBank database under accession number DQ092416.

Table 1 Phenotypic profile of strain ACE2

Test	Results
Gram staining	–
Indole production	–
Methyl red production	–
Voges–Proskauer	+
Citrate liquefaction	+
Catalase	+
Cytochrome oxidase	+
Starch hydrolysis	–
Gelatinase	–
Casein hydrolysis	–
Utilization of D-glucose, Fructose	+
Maltose	+
Salicin	+
Trehalose	+
Xylose	–
Sucrose	–
Galactose	–
Adonitol	–
Arabinose	–
Cellobiose	–
Insulin	–
Sorbitol	–
Mannose	–
Lactose	–
Melibiose	–
Affinose	–
Rhamnose	–
Mannitol	–
Inositol	–

Biodegradation study

From GC–MS analysis, it was observed that the control system consisted of *n*-alkanes (C_{10}–C_{20}), branched alkanes, naphthalene derivatives, substituted naphthalenes and isopreniod alkanes (pristane, phytane) (Fig. 1a). The system inoculated with *Serratia marcescens* ACE2 degraded almost all the *n*-alkanes (C_{10}–C_{20}) and many of the branched alkanes (Fig. 1b). The GC–MS analysis suggested that ACE2 degraded almost all the hydrocarbon present in diesel (C_{10} and C_{22}). Moderately degraded branched alkanes and naphthalene derivatives could be found in the degraded products, which is a common feature for many other alkane-degrading microorganisms [43, 50]. The biodegradation efficiency of diesel hydrocarbon by strain ACE2 are presented in the Table 2. Bacterium ACE2 showed 69% degradation efficiency.

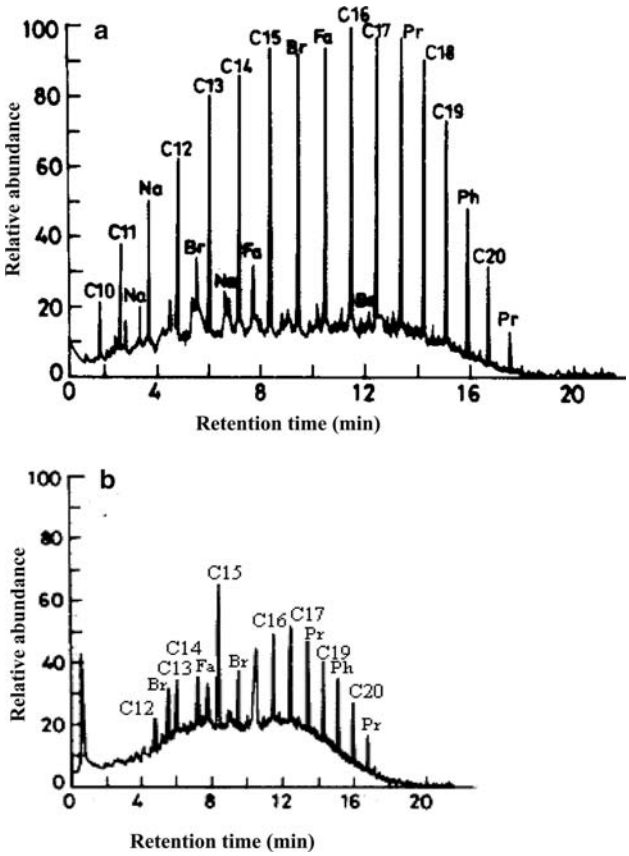


Fig. 1 GC–MS profiles of diesel oil extracted from the aqueous phase of MSM medium after 30 days of incubation period with and without inoculation with ACE2. **a** GC–MS profiles of abiotic control (uninoculated). **b** Inoculated with ACE2. C10 to C21, *n*-alkanes (*numbers* designate the number of C atoms); *Br* branched alkanes; *Na* substituted naphthalenes; *Pr* pristane; *Ph* phytane. The alkane, naphthalene, phytane, and pristane peaks were identified by comparison of their retention times and mass spectra with authentic standards

Table 2 Percentage of biodegradation efficiency of diesel degradation by ACE2

RT (Time)	Compounds	Control Relative abundance (RA)	Strain ACE2 Relative abundance (RA)	Biodegradation efficiency, BE (%)
2	C 10	21	0	100
3	C 11	38	0	100
5.1	C 12	61	20	67.21
6	C 13	80	36	55
7.1	C 14	83	38	54.22
8.2	C 15	97	64	34.03
11.8	C 16	100	45	55
12.6	C 17	99	46	53.54
14.5	C 18	95	0	100
15.1	C 19	78	38	51.29
16.5	C 20	38	24	26.85
3.7	Napthalene derivatives compound-I	18	0	100
4	Napthalene derivatives compound-II	50	48	4
5.8	Branched alkanes compound-I	21	0	100
9.5	Branched alkanes compound-I	100	39	61
16	Phytane compound-I	45	0	100
13.1	Pristane compound-I	98	43	56.13
18.1	Pristane compound-II	14	0	100
11.5	Farnithane compound-I	98	38	61.23
7.6	Farnithane compound-I	30	0	100
BE (Total percentage)				69

In Fig. 2a, the FTIR spectrum of the control system (pure diesel) shows bands at 2,904, 2,923 and 2,853 cm^{-1} (C–H aliphatic stretch); 1,607 cm^{-1} (C=C stretch in aromatic nuclei); 1,458 and 1,375 cm^{-1} (CH def for methyl group), 808 and 726 cm^{-1} (di-substituted benzene). The FTIR spectrum for diesel in presence of *Serratia marcescens* (Fig. 2b) shows characteristic bands at 3,331 cm^{-1} (OH peak); 2,905 cm^{-1} and 2,853 cm^{-1} (C–H aliphatic stretch); 1,647 cm^{-1} (C=C stretch); 1,458 and 1,375 cm^{-1} (CH aliphatic stretch for methyl groups). A new peak at 1,647 cm^{-1} indicates carbonyl group (C=C) stretch and OH peak at 3,331 cm^{-1}. Peaks due to aromatic nuclei at 847, 809, 872, 722, and 743 cm^{-1} (mono- and di-substituted benzene) disappeared because of degradation.

The NMR spectrum (Fig. 3a) of the control system shows some major peaks at 0–3 chemical shifts (δ), suggesting the presence of aliphatic protons. The other peaks at 6–7 chemical shifts indicate the presence of benzene ring. In the presence of *Serratia marcescens* ACE2 (Fig. 3b), the new peaks obtained at 4–5 δ indicate the presence of (–O–CH_2–) after the degradation by bacterial strains. At the end of the degradation processes, the amount of hydrogen peroxide was in the range 5–10 ppm while the pH was 5.2 at the diesel–water interface [13, 29]. It appears that the inclusion of oxygen in degraded products may be due to dissociation of hydrogen peroxide. The reduction of pH was due to the acidity formation by peroxide or by metabolic activity of the bacterium at the diesel–water interface. This study provides new insights into the influence of degradation of diesel fuel on corrosion by enteric bacteria. However, only a handful of reports have addressed utilization of aromatic compounds by *Enterobacteria*, particularly those of the genera *Klebsiella*, *Escherichia*, and *Hafnia* [13, 16, 20, 39]. Sarma [36] discovered a new enteric bacterium *Leclercia adecarboxylata* PS 4040 in oil sludge-contaminated soil and reported the degradation of PAH at the Digboi Oil Refinery in Northeastern India. Recently Wongosa et al. [48] identified the strain *S. marcescens* which has the ability to degrade a broad spectrum of hydrocarbons. Muthukumar et al. [33] reported that *Brucella* sp. and *Gallionella* sp. could degrade diesel in a transporting pipeline in Northwest India while Rajasekar et al. [37] detected the bacterial genera *Pseudomonas* sp., *Bacillus* sp., *Gallionella* sp., *Siderocapsa* sp., *Thiobacillus* sp., *Thiospira* sp., *Sulfolobus* sp. and *Vibrio* sp. in a naphtha pipeline, and reported on interactions between heterotrophs and chemolithotrophs in naphtha-transporting pipelines in Southwest India. Hence, the role of bacteria on degradation and corrosion is an important area in petroleum product pipelines.

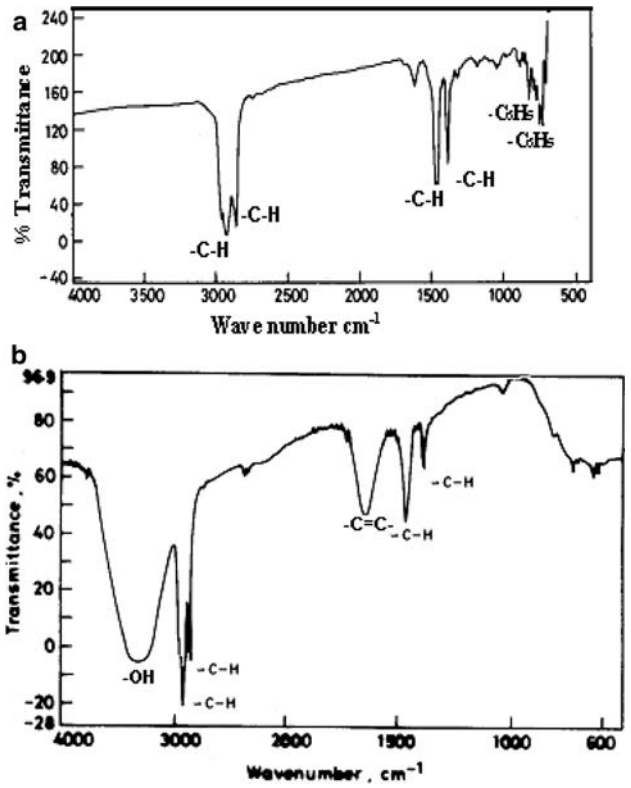


Fig. 2 Fourier-transform infrared spectrum **a** Pure diesel, **b** inoculated with *Serratia marcescens* ACE2

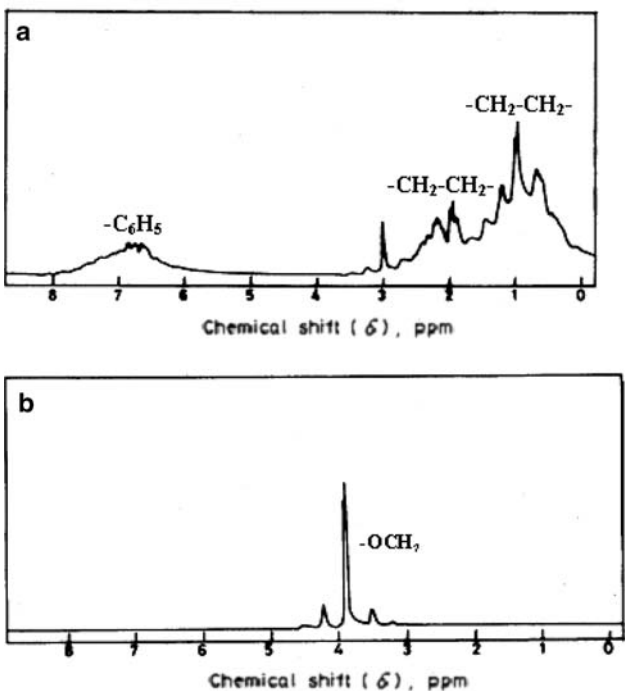


Fig. 3 NMR spectrum, **a** Pure diesel, **b** Inoculated with ACE2

Corrosion studies

Weight loss method

The corrosion rates of API 5LX steel in the presence and absence of bacteria are presented in Table 3. The corrosion rate in the absence of ACE2 was 0.0026 mm/year. However, in the presence of ACE2, the corrosion rate was higher, 0.0687 mm/year.

Electrochemical analysis

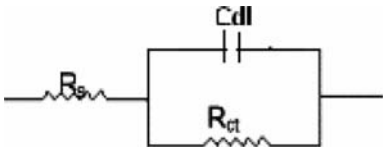
Figure 4 shows the polarization curve for API 5LX steel in diesel–water system with and without the bacteria on the fourth and tenth days. It can be seen that on the fourth day, the corrosion current for the control system was 7.26×10^{-6} A/cm^2 while in presence of the bacteria it was 1.08×10^{-5} A/cm^2 (Table 4). The corrosion potential for the control system was –607 mV while it was –708 mV in the presence of the bacteria at fourth day. It indicates that the potential of carbon steel is shifted by ACE2 towards the cathodic side. On fourth day that the anodic current was higher at –550 mV whereas the cathodic current was lower as compared to the control at –750 mV the increasing current was due to the anodic dissolution. E_{corr} for control system at tenth day was –651 mV and in presence of bacteria by the E_{corr} potential was –602 mV. On the tenth day, the corrosion current for the control and experimental systems were 1.36×10^{-6} and 2.94×10^{-6} A/cm^2, respectively. The nature of the curves indicates that the anodic current was lower as compared to that of the control. Besides, an insignificant difference in the cathodic currents between bacteria-inoculated and uninoculated systems can be noticed in all the potentials. On the fourth day, the increase in the anodic current may be due to utility of electrons from ferric ion, which will be converted as ferric oxide by ACE2. The reduction in the cathodic current may be due to consumption of oxygen by ACE2 which reduces the oxygen reduction process. On the tenth day, the products of degraded components along with nutrients (1% BH) may adsorb on the metal surface and reduce the anodic current. At the same time, the converted ferric ions may be reduced to ferrous and supply electrons to bacterium and encourage the cathodic reduction process [2]. Hence, it can be concluded that the formation of organic metallic complex may adsorb on the metal surface and reduce the anodic current. Busalman et al. [10] suggested the extracellular appearance of bacterial metabolites with a catalytic effect on the oxygen reduction in the presence of bacteria. Based on the present study, it can be explained that the bacterial production of catalase (catalase positive) indicates the production of hydrogen peroxide, which encourages an

Table 3 Corrosion rates of carbon steel API 5LX

System	Weight loss (mg)	Corrosion rate (mm/year)
Control		
500 mL oil + 2% water (120 ppm chloride)	45 ± 2	0.0026
Test		
500 mL oil + 2% water (1% BH, 120 ppm chloride) + ACE2 culture	118 ± 1	0.0687

autocatalytic mechanism by which the oxygen reduction current increases.

Data obtained from the impedance measurements were analyzed using the Zview program, in order to find an equivalent circuit and to give physical meaning to the corrosion process observed during the experimental work. The equivalent circuit shown below was used to adjust the impedance curve obtained for the steel exposed in the presence and in the absence of bacterium ACE2. The circuit includes the following elements. A resistor (R_s), which represents the solution resistance. A parallel combination of a resistor (R_{ct}) and a capacitance (Cdl) as following diagram.



Equivalent circuit used to adjust the impedance diagram obtained for API 5LX steel exposed to ACE2

Values of R_s and R_{ct} were derived from impedance measurements and are presented in Table 5 and Fig. 5. The R_s value for the all the systems was in the range 138–265 Ω cm^2. The R_{ct} value for the control system on the fourth day was 9.3 kΩ cm^2, which was higher as compared to that of the bacteria-inoculated system (1.57 kΩ cm^2). On the tenth day, the R_{ct} value for the control and bacteria-inoculated systems were 0.46 and 0.60 kΩ cm^2, respectively. The nature of the curves on the fourth day suggest an activation control reaction both in the presence and in the absence of the bacterial system. In both the systems, two capacitive loops can be noticed, the first one being small. The first capacitive loop appears well defined on tenth day with a higher frequency range. The high-frequency part represents the formation of the adsorbed film [4] whereas the low-frequency data points are associated with the Faradaic processes occurring on the bare metal through defects and pores in the adsorbed diesel components on the metal surface.

Surface analysis

Figure 6a and b present the details of XRD data corresponding to the phases present in the corrosion product sample collected from control and experimental systems. α-Iron oxide hydroxide (FeO(OH)), ferrous hydroxide ($Fe(OH)_2$)and ferrous chloride ($FeCl_2$) were observed in the control system (Fig. 6a). More intensity peaks of α-Ferric oxide (Fe_2O_3), Iron oxide (FeO) and manganese dioxide (Mn_2O_3) were noticed in the experimental system (Fig. 6b). XRD results reveal the presence of ferric oxides (Fe_2O_3) and manganese oxides (Mn_2O_3) indicating the role of ACE2 on manganese/iron deposition during the formation of corrosion product and accelerate the microbial corrosion.

In the same pipeline in Northwest India, *Brucella* sp. and *Gallionella* sp. were reported by Muthukumar et al.

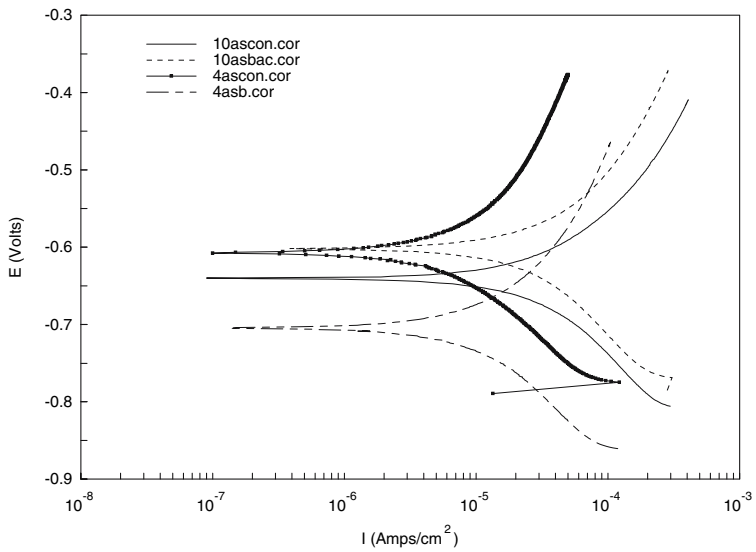


Fig. 4 Polarization curves for API 5LX in diesel water system in presence/absence of ACE2 at different periods. *4ascon* - Control system (4th day); *4asb* - ACE2 inoculated system (4th day); *10ascon* - Control system (10th day); *10asbac* - ACE2 inoculated system (10th day)

Table 4 Polarization studies on API 5LX in the presence/absence of ACE2

Systems	Immersion periods (days)	E_{corr} (mV)	b_a (mV/decade)	b_c (mV/decade)	I_{corr} A/cm^2
Control system					
75 mL of 1% BH broth (containing 120 ppm chloride) and 150 mL of diesel	4	–607	0.210	0.148	7.26×10^{-6}
	10	–651	0.113	0.073	1.36×10^{-6}
Experimental system					
75 mL of 1% BH broth (containing 120 ppm chloride) and 150 mL of diesel inoculated with 2 mL of inoculum about 10^6 CFU/ml	4	–708	0.189	0.155	1.08×10^{-5}
	10	–602	0.188	0.168	2.94×10^{-6}

[33]. *Brucella* sp. was reported as a major aromatic degrader where *Gallionella* sp. was reported as an aliphatic degrader. Besides, *Gallionella* sp. produced more (–O–CH_2–) group due to very rapid addition of oxygen. The observation noticed in the presence of *Serratia marcescens* supports the findings of Muthukumar et al. [33]. In the present study, *Serratia marcescens* breaks both the aromatic and aliphatic hydrocarbons as energy source. The addition of oxygen can also be noticed because of the presence of catalase enzyme. Hence, it can be concluded that *Serratia marcescens* encourages the formation of ferric and manganese oxides. Zhu et al. [52] reported the presence of *Escherichia coli* (AF511430) in a gas pipeline and associated with microbiologically influenced corrosion. Jobson et al. [24] also reported that intermediate hydrocarbon degradation products make energy sources available for the physiological activities of the corrosion bacterium *Desulfovibrio* sp. This supply of utilizable hydrocarbon degradation products explains why corrosion is intense in the Pembiana oil pipeline. Toluene and ethyl benzene were used as sources of carbon and energy by microbes, whereas ethyl benzene was degraded by monooxygenase enzyme [35]. Furthermore, *Rhodococcus rhodochrons* S-2 produces extracellular polysaccharides that help to live in aromatic fraction [21]. From the present study it can be concluded that ACE2 degrades both aliphatic and aromatic hydrocarbons and encourages oxygen addition in aliphatic compounds. It is possible that the converted compounds accelerate the formation of ferric oxide. Subsequently, the same microbe encourages the corrosion process by forming Fe_2O_3.

Table 5 Impedance parameters for API 5LX in the presence and in the absence of ACE2

Systems	Immersion periods (day)	Solution resistance R_{ct} (k Ω cm^2)	Charge transfer resistance R_s (Ω)
Control system			
75 mL of 1% BH broth (containing 120 ppm chloride) and 150 mL of diesel	4	138	9.3
	10	229	0.46
Experimental system			
75 mL of 1% BH broth (containing 120 ppm chloride) and 150 mL of diesel inoculated with 2 mL of inoculum about 10^6 CFU/mL	4	238	1.57
	10	265	0.60

Corrosion mechanism

S. marcescens ACE2 is a facultative anaerobe and biochemical tests indicate the presence of catalase and cytochrome oxidase. ACE2 has a peroxidase enzyme, which produces hydrogen peroxide for the degradation of

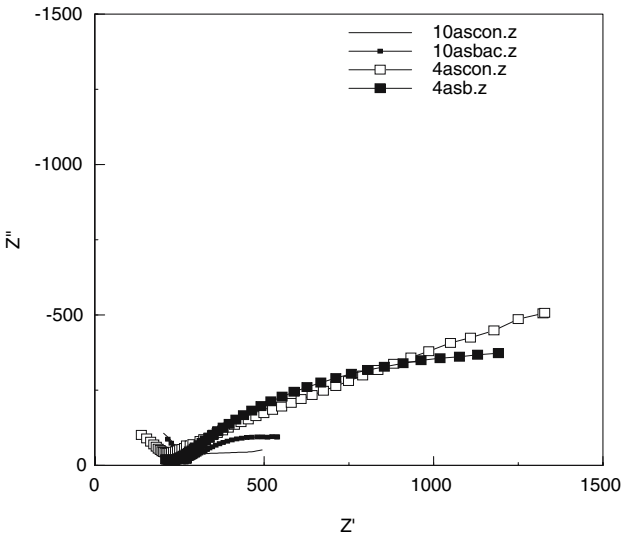


Fig. 5 Impedance curves for API 5LX in diesel water system in presence/absence of ACE2 at different periods. *4ascon* - Control system (4th day); *4asb* - ACE2 inoculated system (4th day); *10ascon* - Control system (10th day); *10asbac* - ACE2 inoculated system (10th day)

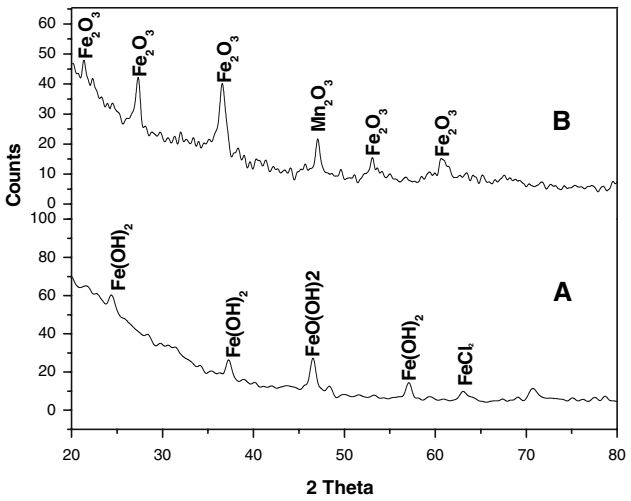


Fig. 6 XRD pattern of corrosion product. **a** Control system, **b** experimental system, inoculated with ACE2

petroleum products. Moreover, it produces catalase that overcomes the toxic nature of hydrogen peroxide, breaking peroxide into water and oxygen [10]. During the respiratory process, oxygen is consumed by ACE2 and converted into water, wherein H^+ is utilized from the degraded product and electrons are supplied by cytochrome oxidase enzyme.

$$\frac{1}{2}O_2 + 2H^+ + 2e \rightleftarrows H_2O.$$

It can also be assumed that ACE2 favors the Fenton reaction [42] by reducing ferric iron, leading to the production of hydroxyl radicals which can damage any biological macromolecules.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}.$$

It is inferred that the formation of Fe^{3+} combines with OH ions and degraded products and form as iron organic as end product. Since hydrogen and carbon are consumed by ACE2 from diesel, (–O–CH_2–) is formed. The oxygen from peroxide and H^+ molecules from the degraded products combine with Fe^{++} to produce Fe^{+++}. Since this bacteria live in low pH environments, [34] Fe^{+++} formation is encouraged by peroxide production and degradation according to the equation

$$Fe^{2+} + \frac{1}{4}O_2 + H^+ \rightarrow Fe^{3+} + \frac{1}{2}H_2O.$$

ACE2 converts ferric to ferric oxides by inclusion of oxygen from the degraded products and finally (–CH_2–CH_2–) is formed in the degradation pathway. Finally the ferric organic complex may be formed as a corrosion product on the metal surface. In the petroleum product pipeline *Serratia* is a rare species, which is involved in the corrosion reaction individually without any help from other bacteria.

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