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ORIGINAL PAPER

Biodegradation and corrosion behaviour of *Serratia marcescens* ACE2 isolated from an Indian diesel-transporting pipeline

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Abstract A facultative anaerobic species *Serratia marcescens* ACE2 isolated from the corrosion products of a diesel-transporting pipeline in North West India was identified by 16S rDNA sequence analysis. The role of *Serratia marcesens* ACE2 on biodegradation of commercial corrosion inhibitor (CCI) and its influence on the corrosion of API 5LX steel has been enlightened. The degrading strain ACE2 is involved in the process of corrosion of steel API 5LX and also utilizes the inhibitor as organic source. The quantitative biodegradation efficiency of corrosion inhibitor was 58%, which was calculated by gas chromatography mass spectrum analysis. The effect of CCI on the growth of bacteria and its corrosion inhibition efficiency were investigated. Additionally, the role of this bacterium in corrosion of steel has been investigated by powder X-ray diffractometer (XRD) and scanning electron microscope studies. The presence of high-intensity ferric oxides and manganese oxides noticed from the XRD indicates that ACE2 enhances the corrosion process in presence of inhibitor as a carbon source. This basic study will be useful for the development of new approaches for the detection, monitoring and control of microbial corrosion in petroleum product pipelines.

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Introduction

Many studies have indicated the importance of microbial contamination of stored hydrocarbon fuels, leading to the blocking of pipelines and filters, reducing fuel quality and resulting in corrosion of the pipes (Gaylarde et al. 1999). Corrosion, the major hallmark of pipeline failure, is the main component affecting the operation and maintenance costs of petroleum transporting pipelines (Buck et al. 1996; Pope and Pope 1998; Koch et al. 2001; Muthukumar et al. 2003; Groysman 2005; Rajasekar et al. 2005) and 40% of all internal pipeline corrosion in the gas industry can be attributed to microbial corrosion (Graves and Sullivan 1996). It has been estimated that microbially influenced corrosion (MIC) causes hundreds of millions of dollars damage to the production, transport and storage of oil every year in the United States oil industry alone (Koch et al. 2001). In oil pipelines, water can also stratify at the bottom of the line, if the velocity is less than that required to entrain water and sweep it through the pipeline system. Organic film-forming corrosion inhibitors used in the oil and gas industry are generally of the cationic/anionic type: amines, oxyalkylated amines, fatty acids, dimer, trimer acids, naphtheneic acid, phosphate esters and dodecyl benzene sulphonic acids. Oil-soluble, water-dispersible, film-forming amine-type organic corrosion inhibitors can disperse sufficiently into stratified water layers to prevent corrosion beneath the water. These organic corrosion inhibitors adsorb on the metal surface

and form a complex, which inhibits corrosion. Microorganisms enter the fuel from the contaminated water at the pipeline, from the biofilm present on the tank walls, if the latter has not been sufficiently well cleaned (Rajasekar et al. 2005; Maruthamuthu et al. 2005). Apart from the hydrocarbons, organisms may gain nutrients from the fuel organic additives and amine-based corrosion inhibitors (Videla et al. 2000; Freiter 1992; Videla and Characklis 1992; Prasad 1998; Rajasekar et al. 2006). It is expected that since the corrosion inhibitor mixture is the sole carbon source for bacteria, it loses its efficiency of corrosion inhibition (Muthukumar et al. 2006). Dominguez et al. (1998) reported the loss in efficiency of organic corrosion inhibitors in the presence of *Pseudomonas fluorescens* isolated from injection water system used in off-shore oil production. Maruthamuthu et al. (2005) noticed the degradation of inhibitor and its effect on the corrosion process in a petroleum product-transporting pipeline in Northwest India. Moreover, ten bacterial species were identified in the same pipeline (Maruthamuthu et al. 2005), of which five were also identified as effective diesel degraders. Among the isolates, *Serratia marcescens* was identified by 16S rDNA analysis. In the present study, the role of *S. marcescens* ACE2 on the degradation of corrosion inhibitor has been investigated and its involvement in the corrosion process has been studied.

Materials and methods

Collection of samples and characterization of bacteria

The bacteria were isolated from corrosion product as described by Maruthamuthu et al. (2005) and Rajasekar et al. (2006). The isolated bacteria were grouped into various genera as per *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994). These cultures were characterized depending on their morphology, gram staining, spore staining, motility, oxidase, catalase, oxidative fermentation, gas production, ammonia formation, nitrate and nitrite reduction, indole production test, methyl-red and Voges–Proskauer test, citrate and mannitol utilization test, hydrolysis of casein, gelatin, starch, urea and lipid (Aranson 1970).

Amplification, cloning and sequencing of 16S rRNA gene

Genomic DNA was extracted according to Ausubel et al. (1988) Amplification of the gene encoding for small subunit ribosomal RNA was done using eubacterial 16S rDNA primers (forwards primer 5′AGAGTTTGATCCTGGCTCAG3′ (*E. coli* positions 8–27) and reverse primer 5′ACGGCTACCTTGTTACGACTT3′ (*E. coli* positions 1,494–1,513) (Weisburg et al. 1991). Polymerase chain reaction (PCR) was performed with a 50-μl reaction mixture containing 2 μl (10 ng) of DNA as the template and 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, Newcastle, UK). PCR was carried out with a Mastercycler Personal (Eppendorf, Hamburg, Germany) with the following programme: 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, Piscataway, NJ, USA) and cloned in pTZ57R/T vector according to the manufacturer's instructions (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 was carried out using ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, Foster City, USA). For sequencing reaction Big Dye Ready Reaction Dideoxy Terminator Cycle Sequencing kit (Perkin-Elmer) was employed. The obtained partial 16S rDNA sequence was then submitted to a BLAST (Altschul et al. 1990) search to obtain the best homology sequences.

Biodegradation test

Bushnell–Hass broth (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, Hi-Media, Mumbai, India) was used for biodegradation studies. All the experiments were done in triplicate. Bacterium ACE2 was grown in BH medium containing 400 ppm of corrosion inhibitor (amine-based carboxylic acid) as a sole carbon source. An uninoculated control flask was incubated in parallel to monitor abiotic losses of the corrosion inhibitor substrate. The flasks were incubated at room temperature (27°C) for 30 days in an orbital shaker (150 rev/min). At 2-day intervals, a set of flasks was used for the enumeration of microbial population by using the pour plate technique on plate count agar (Hi-Media).

Analytical methods

Biodegradation of corrosion inhibitor was monitored quantitatively by gas-chromatography-mass spectrum analysis as described by Michaud et al. (2004). At the end of the 30-day incubation period, the residual corrosion inhibitor for each system of the entire flask was extracted with an equal volume of dichloromethane. Evaporation of solvent was carried out in a hot water bath at 40°C. The 1 μl of the resultant solution (corrosion inhibitor) was analyzed by GC-MS by means of Thermo Finnigan gas chromatography/mass spectrometry (Trace MS equipped with a RTX-5 capillary column 30 m long × 0.25 mm internal diameter and flame ionization detector (FID) 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 GC retention data of the corrosion inhibitor correspond to structural assignations was done after library (NIST) search with a database and by mass spectra interpretation. The degradation of corrosion inhibitor as a whole was expressed as the percentage of corrosion inhibitor 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 corrosion inhibitor hydrocarbons, was evaluated by using the following expression:

$$\mathrm{BE}(\%) = 100 - (A_s \times 100/A_{ac}),$$

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. Changes in functional group characterization of corrosion inhibitor during biodegradation were analyzed by Fourier transform infrared spectroscopy (FT-IR) and H^1 Nuclear magnetic resonance spectroscopy (NMR). FT-IR spectrum (Nicolet Nexus 470, Madison, WI, USA), which was taken in the mid i.r. region of 400–4,000 cm^{-1} with 16-scan speed. The samples were mixed with spectroscopically pure KBr in the ratio of 1:100 and the pellets were fixed in the sample holder, and the analysis was carried out. H^1 NMR (Bruker, Ettlingen, Germany, 300 m Hz) analysis was used to detect the protons of the nuclei in the diesel compound. The sample of corrosion inhibitor was dissolved using deuterated chloroform solvent and tetramethylsilane (TMS) was used as a reference standard.

Corrosion inhibition efficiency studies

Corrosion inhibition efficiency (IE) was studied by the rotating cage (RC) test described by Papavinasam (2000). API 5LX grade steel (C-0.29 max, S-0.05 max, P-0.04 max, Mn-1.25 max.) coupons of size 2.5 × 2.5 cm^2 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 as system-I; 500 ml of diesel with 2% water containing 120 ppm chloride and 10^8 c.f.u./ml of bacterium ACE2 as system-II; 500 ml of diesel with 2% water containing 120 ppm chloride and 10 ppm of corrosion inhibitor as system-III and 500 ml of diesel with 2% water containing 120 ppm chloride and 10 ppm of corrosion inhibitor and inoculated with 10^5 c.f.u./ml of bacterium ACE2 were used as system IV. The 1% BH broth for bacterial growth was added to systems II and IV. After 10 days, the coupons were removed and washed in Clark's Solution for 1 min to remove the corrosion products and rinsed with sterile distilled water, dried. Final weights of the three coupons for duplicate in each system were taken and the average corrosion rates were also calculated and standard deviations are also presented. The IE was calculated as follows:

$$\text{Inhibition efficiency (IE \%)} = \frac{W - W_{inh}}{W} \times 100,$$

where W_{inh} and W are the values of the weight-loss of API 5LX steel after immersion in solutions with and without inhibitor, respectively.

Surface analysis

After the RC experiments, the coupons were taken out and the corrosion product was scratched carefully, dried and crushed into a fine powder and used for powder X-ray diffractometer (XRD) analysis for determining the nature of oxides present in the corrosion product in presence/absence of ACE2 and corrosion inhibitor. A computer controlled XRD system, JEOL Model JDX—8030 was used to scan the corrosion products between 10° and 85°—2θ with copper K α radiation (Ni filter) at a rating of 40 kV 20 mA.

Results and discussion

In oil pipelines, water can stratify at the bottom of the line if the velocity is less than that required to entrain water and sweep it through the pipeline. Liquids (hydrocarbon) stratify along the bottom of the pipe and storage tank, with water forming a separate layer beneath them, where hydrocarbon degradation by microbes occurs easily at the interface (Maruthamuthu

et al. 2005; Bento et al. 2004, 2005). Hence, the role of bacteria in degradation and corrosion is an important area in pipeline technology. Ten morphologically different isolates were obtained in the corrosion product sample. All these isolates were characterized at genus level by morphological and biochemical characteristics. 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 of these bacterial species were selected as effective diesel hydrocarbon degraders for further study, and they were identified and confirmed at the Institute of Microbial Technology, Chandigarh, India by biochemical techniques as *Bacillus licheniformis* (MTTC6535), unidentified strain (MTCC6536), *Bacillus megaterium* (MTTC6537) *Enterobacter aerogenes*, (MTTC6538), *Bacillus* sp. (MTTC6539). For further confirmation two strains, *Bacillus* sp. (MTTC6539) and unidentified strain (MTCC6536) have been identified by 16S rDNA analysis. The unidentified strain (MTCC6536) was identified as *S. marcescens* ACE2.

Characterization of bacterium ACE2

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 *S. 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

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

Biodegradation analyzes

The total viable counts of bacteria in the presence and in the absence of corrosion inhibitor during degradation are presented in Fig. 1. The count is in the range between 10^6 and 10^9. The excellent growth of bacteria in the presence of corrosion inhibitor when compared to growth in its absence was noticed. It could be explained that ACE2 utilized the inhibitor as organic source with inorganic nutrients from the BH medium for their proliferation. This indicates that ACE2 utilizes corrosion inhibitor as a carbon source for their growth. Viable count data indicated that corrosion inhibitor had a positive effect on microbial growth (10^9 cells ml^{-1}).

The GC retention data of the corrosion inhibitor correspond to structural assignations performed after NIST (library search) with a database and by mass spectra interpretation are presented in Table 2. From

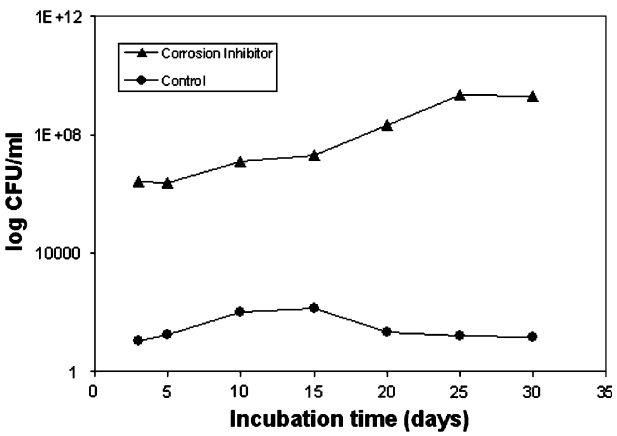

Fig. 1 Growth of *Serratia marcesens* in BH media in presence/absence of corrosion inhibitor

Table 2 GC-MS data of corrosion inhibitor in presence/absence of ACE2

Retention time (min)	Compounds
0.90	1-Hexadecanol
1.00	3-Nonen-2-one
1.07	1-Octadecyne
1.10	1-Methyl-2-propylcyclohexane
1.24	2-Propenic acid octyl ether
1.35	1,2,3,5-Tetramethylbenzene
1.72	1-Chlorotetradecane
1.55	1,2-Diethylbenzene
11.22	*n*-Hexadecanoic acid
13.16	Oleic acid
13.35	2-Tridecylpyridine
14.30	2-Tridecylpyridine
15.14	2-Tridecylpyridine
15.94	2,6-Di(*t*-butyl)-4(cyclohexancylidene)aminophenol
Strain ACE2	
13.01	Oleic acid
15.12	2-Tridecylpyridine

the GC-MS analysis (Table 2), it was observed that the corrosion inhibitor consists of aliphatic hydrocarbons including cyclohexane, octadecyne, *n*-hexadecanoic acid, propenic acid octyl ether, tetradecane and aromatic carbons including 1,2-diethylbenzene, pyridine and phenol. The major component is oleic acid (Fig. 2). The enlarged view of the relative abundance 1.0–3.5 (RA) is shown in Fig. 2a. In presence of ACE2 (Table 2), the absence of compounds cyclohexane, octadecyne, *n*-hexadecanoic acid, tetradecane (Relative abundance 1.0–3.5) and aromatic carbons including 1,2-diethylbenzene, pyridine and phenol are noticed. All components in the corrosion inhibitor were highly reduced by ACE2. It indicates that ACE2 utilizes both aliphatic and aromatic compounds present in the inhibitor (Fig. 3). The BE of the inhibitor by

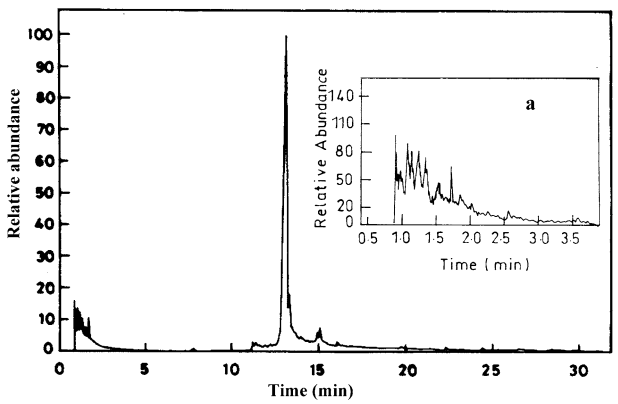

Fig. 2 GC-MS spectrum of pure corrosion inhibitor (uninoculated system)

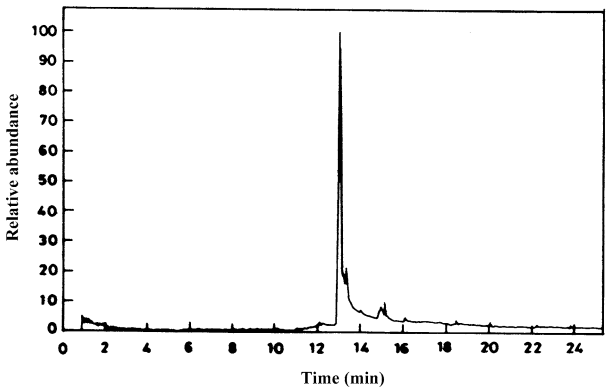

Fig. 3 GC-MS spectrum of corrosion inhibitor inoculated with *Serratia marcesens* ACE2

strain ACE2 is presented in Table 3. Bacterium ACE2 showed maximum 58% degradation efficiency and reached a population size of about 2.92×10^8 c.f.u./ml while using inhibitor as a carbon substrate. The compounds corresponding to the retention times of 1.0 1.24 1.72 1.55 11.22 13.35 are completely utilized by the ACE2, since the relative abundance for these compounds are zero in presence of ACE2.

FT-IR spectrum of the corrosion inhibitor shows (Fig. 4a), wave numbers 3,300–3,400 cm^{-1} indicate the presence of –OH or –N–H stretching bands. About 2,950 cm^{-1} indicate the –C–H stretching in aliphatic compounds. Near 3,000 cm indicate the presence of =C–H stretching in aromatic components. About 2,480–2,550 cm^{-1} indicate the presence of –N–H stretching for amino acids. The 1,670 indicate the –C=C stretch for aliphatic compounds and 1,585 cm^{-1} indicate –C=C stretch for aromatic compounds. About 1,306 and 1,280 cm^{-1} indicate the presence of –C–N stretch for primary aromatic amines and 1,050 cm^{-1} indicating that it should be a presence of –C–O stretch for primary alcohols. The wave number 1,715 cm^{-1} indicate the presence of aldehyde groups. The aromatic *para* disubstituted compounds observed at 800–850 cm^{-1} were identified. This indicates that the corrosion inhibitor consists of amine-based carboxylic acid compounds.

In the presence of ACE2 (Fig. 4b) the corrosion inhibitor showed significant changes in the functional groups during degradation at the wave number of 3,300–3,400 cm^{-1} when compared to control. The broad spectra of 3,300–3,400 cm^{-1} indicate the presence of intermolecular hydrogen bonding of –OH or –NH groups. This is due to the increasing substitution reaction producing the addition of hydroxyl or amine groups in the hydrocarbon by ACE2. This hydroxylation is due to a hydroxylase enzyme (Jørgensen et al. 2005) secreted by the bacterium ACE2. The wave numbers

Table 3 Percentage of biodegradation of corrosion inhibitor in presence of ACE2

R.T (time)	Compounds	Relative abundance (RA)	Strain ACE2	Biodegradation efficiency, BE (%)
0.90	1-Hexadecanol	6	5	16.66
1.00	3-Nonen-2-one	5	0	100
1.07	1-Octadecyne	5	4	20
1.10	1-Methyl-2-propylcyclohexane	5	3	40
1.24	2-Propenic acid octyl ether	4	0	100
1.35	1,2,3,5-Tetramethylbenzene	5	4	20
1.72	1-Chlorotetradecane	6	0	100
1.55	1,2-Diethylbenzene	6	0	100
11.22	*n*-Hexadecanoic acid	2	0	100
13.16	Oleic acid	100	100	0
13.35	2-Tridecylpyridine	11	0	100
15.14	2-Tridecylpyridine	6	4	33.33
15.94	2,6-Di(*t*-butyl)-4(cyclohexancylidene) aminophenol	8	6	25
BE (Total percentage)				58.08

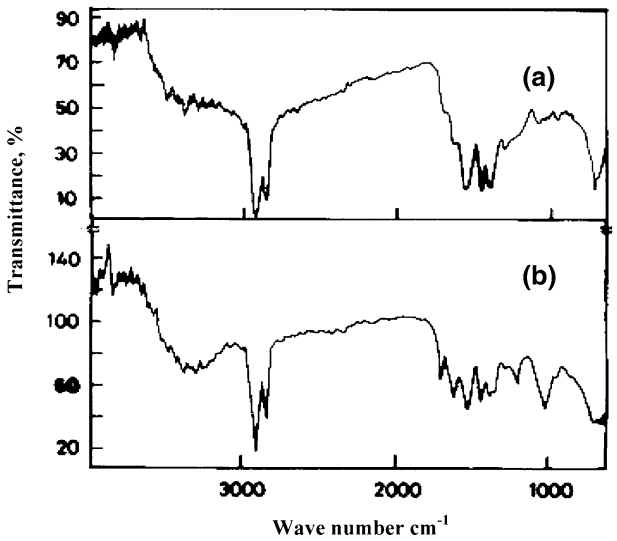

Fig. 4 Fourier-Transform Infrared Spectrum. (**a**) Corrosion inhibitor (uninoculated system—control). (**b**) Inoculated with *Serratia marcesens* ACE2

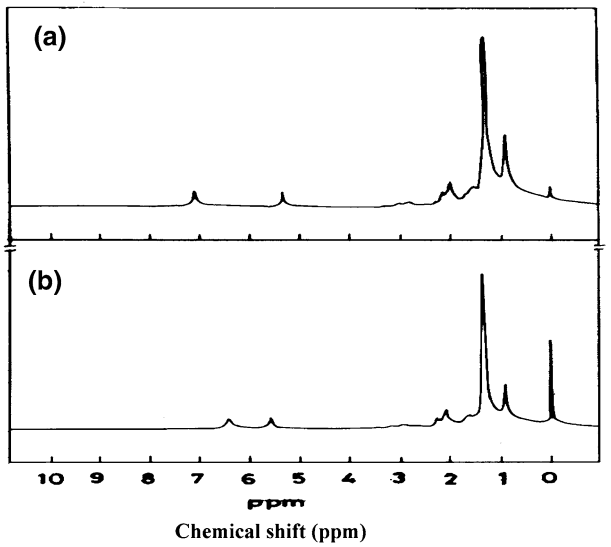

Fig. 5 H^1 NMR Spectrum (**a**) Corrosion inhibitor (uninoculated system—control) (**b**) Inoculated with *Serratia marcesens* ACE2

1,750 and 1,720 cm^{-1} indicating the –C=O stretching for aldehyde or ketone or acid or ester groups, is due to the oxidation of reactive groups by the action of an oxygenase enzyme. In the control system the corrosion inhibitor 1,715 cm^{-1} indicates the presence of aldehyde group, but ACE2 oxidizes the aldehyde group into acid or keto group (1,750 and 1,720 cm^{-1}).

The NMR spectrum of the corrosion inhibitor is presented in Fig. 5a. The chemical shifts at 0.98–2.3 ppm indicate the presence of aliphatic protons. The olefinic proton is observed at 5.3 ppm and the aromatic proton peak is observed at 7.8 ppm as a singlet peak. There is no significant change in the aliphatic proton peak (0.98–2.3 ppm) in the presence of ACE2 system (Fig. 5b). But the aromatic proton chemical shift at 7.8 shifted into lower chemical shift value at 6.5 ppm, which is due to the substitution reaction of electron-donating tendency groups like hydroxyl or amine groups. This observation is confirmed by FT-IR spectrum at 3,300–3,400 cm^{-1}. It reveals that ACE2 is able to utilize the hydrocarbon present in the corrosion inhibitor as a sole carbon source and reduces their IE. Videla et al. (2000) also noticed microbial degradation of film-forming inhibitor and observed that *Pseudomonas* sp. isolated from injection water, degraded several aromatic compounds and generated energy for its metabolic activity. They also suggested that dimethylamine, imidazoline, morpholine, cyclohexylamine

and quaternary ammonium compounds are biodegradable.

The present finding observed that strain ACE2 converts aldehyde groups into acid by hydroxylation during degradation of the hydrocarbon. There are limited reports describing the involvement of *Serratia* in biodegradation of hydrocarbons and its influence on corrosion in refined petroleum product-transporting pipeline in tropical countries and they are mostly thought of as degraders of aromatic compounds. (Dela Fuente et al. 1991; Rojas-Avelizapa et al. 2002; Wongsa et al. 2005; Verma et al. 2006)

Corrosion inhibition efficiency

The IE of corrosion inhibitor is presented in Table 4. The corrosion rate of API 5LX in control system (without inoculum) is about 0.1041 mm/year in 240 h. In the presence of ACE2, the corrosion rate is 0.0466 mm/year. This indicates that the adsorption of bacterium on API 5 LX steel in low-chloride system inhibits corrosion but it may be a temporary inhibition. Moreover, SEM studies have been carried out to check the surface morphology of the coupons. Fig. 6a–d shows the SEM observation of API 5LX. Pitting corrosion was noticed in presence of ACE2 (system II) and system IV (Fig. 6b, d). Uniform corrosion was observed in system I (Fig. 6a). Corrosion inhibitor alone gives an IE of 48% (0.0538 mm/year) in the absence of bacterium ACE2 (Fig. 6c) while adding inhibitor with bacterium the corrosion rate (Fig. 6d) increased (0.2082). It indicates that the efficiency of corrosion inhibitor has been decreased by bacterial degradation (Rajasekar et al. 2006). Generally, bacteria utilize energy from the environment by consumption of carbon and phosphorus in the ratio 40:1; if these consume excess, P is released as glucose-6-phosphate and fructose-6-phosphate (Malony et al. 1990). In the present study 1% of BH broth was added in the corrosion inhibition and degradation study, respectively, because inorganic nutrients are also needed for bacterial physiological activity. Since organic and inorganic nutrient sources are needed for bacterial physiology, BH broth was added with bacteria for the corrosion evaluation study. Although 1% BH broth consists of 10-ppm phosphate and 10-ppm nitrate, the effect of nutrient on the metal surface should also be considered as an important factor. Cohlen (1976) suggested that phosphates can influence the anodic reaction in aerated solution, whereas the phosphate film was formed by reaction with dissolved oxygen. Because the ferrous phosphate has some solubility in water, the corrosion reaction is not totally eliminated but is rather reduced to manageable degree (Pryor and Cohen 1953). Franklin et al. (1990, 2000) investigated the effect of biofilm on mild steel surface in the presence of phosphate and other anions (chloride and sulfate). They found that bacterial biofilm enhanced the propagation of pits, probably because of the uptake of phosphate by the microorganisms.

Surface analysis

Figure 7 presents the details of XRD data corresponding to the phases present in the corrosion product samples collected from various systems. Iron oxyhydroxide (FeO(OH) and ferrous hydroxide ($Fe(OH)_2$) were observed in the control system (Fig. 7a). Ferric oxide (Fe_2O_3), iron oxide (FeO) and manganese dioxide (MnO_2) were noticed in the ACE 2-inoculated system (Fig. 7b). But in the corrosion inhibitor system there is no significant ferric oxides peak is observed (Fig. 7c). This indicates that the corrosion inhibitor adsorbs on the metal surface and suppresses the corrosion reaction. In the corrosion inhibitor inoculated with ACE2, greater intensity of ferric and manganese oxide peaks were observed when compared to all the systems (Fig. 7d). The presence of ferric oxide (Fe_2O_3) and manganese oxides (MnO_2) peaks indicates the role of ACE2 in manganese/iron deposition during the formation of the corrosion product and thereby accelerating the microbial corrosion directly on the pipeline (Jones 1986). It reveals that ACE2 consumes hydrocarbon as a sole carbon source present in the corrosion inhibitor and reduces the corrosion IE.

Table 4 Weight loss of API 5LX steel exposed to strain ACE2 in presence/absence of corrosion inhibitor

S. No	System	Weight loss (mg)	Corrosion rate (mm/year)	Inhibition efficiency (%)
1	500 ml diesel + 2% water	29 ± 1	0.1041	–
2	500 ml diesel + 2% water + bacterium ACE2	13 ± 2	0.0466	–
3	500 ml diesel + 2% water + corrosion inhibitor (10 ppm)	15 ± 1	0.0538	48
4	500 ml diesel + 2% water + corrosion inhibitor (10 ppm) + bacterium ACE2	58 ± 2	0.2082	–

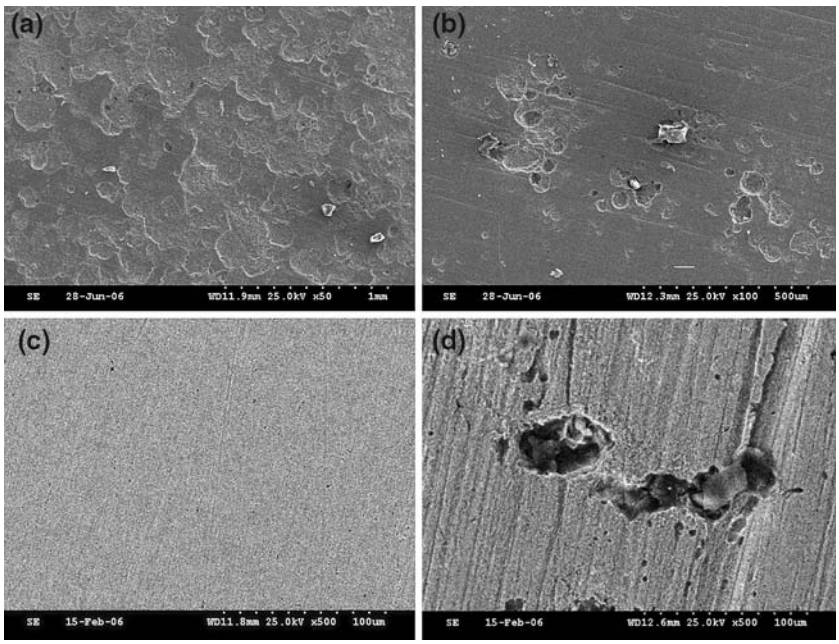

Fig. 6 Scanning electron microscopy (*SEM*) indicating that the bacterial strain ACE4 is capable of influencing corrosion. (**a**) SEM of API 5LX (control system) (**b**) SEM micrograph of steel API 5LX surface, showing localized attack, following exposure to ACE2. (**c**) SEM of API 5LX in presence of corrosion inhibitor (**d**) SEM of API 5LX in presence of corrosion inhibitor inoculated with ACE2

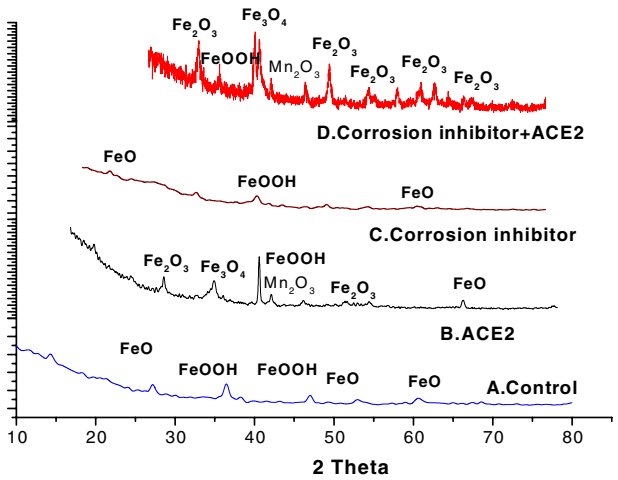

Fig. 7 XRD pattern of corrosion product collected in presence/absence of ACE2 and corrosion inhibitor

Hypothesis on corrosion inhibitor degradation and its impact on corrosion

Serratia marcescens ACE2 is a facultative anaerobe. Biochemical characteristics indicate the presence of catalase and cytochrome oxidase. This bacterium was isolated from a petroleum product pipeline where carboxylic acid was added as a corrosion inhibitor (Maruthamuthu et al. 2005). Since ACE2 has a peroxidase enzyme, which produces hydrogen peroxide for the degradation of hydrocarbon, it also produces catalase to overcome the toxic nature of hydrogen peroxide, breaking peroxide into water and oxygen (Busalmen et al. 2002). During the respiratory process, oxygen is consumed and hydrogen is utilized from the degraded corrosion inhibitor hydrocarbon product (Muthukumar et al. 2003, Maruthamuthu et al. 2005; Rajasekar et al. 2005). Since the ferric/manganese present in the metal API 5LX has a high affinity for oxygen, it takes oxygen from the product obtained from hydrogen peroxide and encourages the formation of ferric/manganese oxides and accelerates the corrosion. It can be explained that since electrons are needed continuously for bacterial metabolic activity, it converts the Fe^{++} to Fe^{+++}, Mn^{++} to Mn^{+++} and forms ferric oxides (Fe_2O_3) and manganese oxides (MnO_2) by continuous addition of oxygen (Fig. 6). It can also be assumed that ACE2 favours the Fenton reaction (Touati 2000) by reducing ferric iron, leading to production of hydroxyl radicals, which can damage any biological macromolecules.

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

It is inferred that the formation of Fe^{3+} combines with OH ions and degraded products and forms iron oxides as end product. Jones (1986) suggests that the insoluble product is a mixture of ferric oxide (Fe_2O_3) and ferric hydroxide ($Fe(OH)_3$), in which an approximate formula is $FeO(OH)_n$. The ferric ions are not precipitated completely, especially in the acidic crevice regions. Ferric ions in solution serve as highly oxidizing species and tend to accelerate corrosion. Moreover, in waters containing chloride ions, iron-oxidizing bacteria may be directly involved in the production of ferric chloride which is an extremely corrosive substance that

can concentrate under nodules (Tiller 1983; Borenstein 1988). In the present study, due to the presence of organic and inorganic nutrients, bacterium ACE2 degrades the corrosion inhibitor for energy purposes, which adsorbs on to the metal surface and determines the electrochemical behaviour of API 5 LX. Hence, the degradation should be avoided by employing non-degradable corrosion inhibitor to control corrosion.

Conclusions

The performance of an organic film-forming corrosion inhibitor was assessed towards its corrosion inhibitive efficiency in controlling the corrosion of API 5LX steel in petroleum product containing bacterial contaminant *S. marcescens* ACE2. In the present study, it can be concluded that *S. marcescens* utilizes the corrosion inhibitor as a sole carbon source and the converted compounds accelerate the formation of ferric oxides. The present study reveals that a non-degradable corrosion inhibitor is needed for petroleum product pipelines to avoid the microbial degradation of corrosion inhibitors.

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