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Corrosion behavior of SRB *Desulfobulbus propionicus* isolated from an Indian petroleum refinery on mild steel

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In this study, *Desulfobulbus propionicus* (*D. propionicus*), a sulfate reducing bacterium (SRB) was isolated and identified in cooling towers by molecular biologic techniques. This bacterial species has been reported for the first time in the cooling towers of an Indian petroleum refinery. Corrosion behaviors were analyzed by electrochemical and weight loss methods. The high corrosion rate and the enhancement of anodic current in increased chloride environment was noticed in the presence of *D. propionicus* indicating that this SRB species enhances the pitting corrosion of mild steel. Propionate metabolism in H_2S production is discussed as a new pathway of corrosion enhancement.

1 Introduction

Many industries experience corrosion problems in steel structures, such as pipeline cooling systems, storage tanks, etc., that use natural water or aqueous solution. Part of the corrosion cases of industrial equipment have been ascribed to microorganisms [1, 2] and this process is described as microbiologically influenced corrosion (MIC) [3, 4]. The participation of sulfate reducing bacteria (SRB) in corrosion processes was identified in oilfields, and heat supply systems [5, 6]. They are considered to be a serious problem because they produce corrosive and toxic H_2S, and they had been the main focus of concern in oilfield microbiology [7]. Oil, gas, and shipping industries are seriously affected by the sulfides generated by SRB [8]. SRB are a group of diverse anaerobes, which carry out dissimilatory reduction of sulfur compounds such as sulfate, sulfite, and thiosulfate to sulfide [9, 10]. The temperature, pH, salinity, in situ pressure, and degraded hydrocarbon products encourage the growth of thermophilic and mesophilic SRB in hydrocarbon-rich environments. Many sulfate-reducing bacteria can use molecular hydrogen as electron donor (Gram-negative mesophilic sulfate-reducing bacteria) and can thus be considered as facultative chemolithotrophs [11].

Studies on corrosion of mild steel have been started with *Desulfovibrio* sp. since 1960s [12]. Extensive research has been done on biofilm formation and MIC caused by the species of genus *Desulfovibrio* [13–18]. A previous study on *Desulfobulbus propionicus* claimed that the bacteria have not induced corrosion when compared to *Desulfovibrio* sp. [19].

The aim of this study is to analyze the corrosion behavior of mesophilic SRB other than *Desulfovibrio* sp. especially about *D. propionicus* present in cooling towers of process industries. In the present study, isolation, identification, and characterization of mesophilic SRB has been carried out by sensitive and specific molecular techniques. This technique helped in the identification of SRB with their functional gene sequences. Then electrochemical techniques and weight loss studies have been adopted for analyzing the corrosion behavior of the isolate. Attempts were also made to find out the mechanism of corrosion by surface characterization studies.

2 Experimental procedures

2.1 Sample collection

Corrosion product sediment in pipeline was collected aseptically from a cooling tower of a petroleum refinery, Chennai, South India, kept in an ice bucket and transported to Sourashtra College Laboratory, Madurai. The physical and chemical characteristics of the cooling water were analyzed by titrimetry and spectrophotometry as recommended [20].

2.2 Isolation and enumeration of SRB isolate

The sample was enriched for 7 days anaerobically in sterile modified Baar's liquid medium (1 L of medium contained 2.0 g of $MgSO_4$, 5.0 g of sodium citrate, 1.0 g of $CaSO_4$, 1.0 g of NH_4Cl, 0.5 g of KH_2PO_4, and 1.0 g of yeast extract; 3.5 g of carbon source such as sodium lactate, sodium acetate, and sodium propionate were added separately in each 1 L of medium).

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Fivefold serial dilutions ranging from 10^{-1} to 10^{-3} of the well-mixed sample were made in the dilution medium with three different carbon sources; lactate, acetate, and propionate. One milliliter of each dilution was inoculated in a set of five tubes containing 9 mL liquid medium. All the inoculated tubes along with uninoculated control were incubated at 37 °C in anaerobic condition. At the end of incubation period, the tubes were observed for formation of black precipitate. Most probable number (MPN) of SRB isolate was computed on the basis of tubes showing positive results. The enriched culture observed in propionate medium was inoculated in sterile modified Baar's medium with propionate. The samples inoculated were kept in anaerobic jar and incubated at 37 °C for 3 days.

2.3 Morphologic and biochemical characterization of SRB isolate

The strains isolated were examined for their morphology and biochemical characters by Gram's staining, carbon source oxidation, motility test, spore staining, and Desulfoviridin test [21] for the presence of desulfoviridin. In Desulfoviridin test, 10 mL culture was centrifuged in a glass tube at 7000 rpm for 10 min. The pellet was resuspended in 1 mL of the medium. The tube was placed under UV light (365 nm) and one drop of 2 M NaOH solution was added. The presence of desulfoviridin can be observed by the production of red fluorescence.

2.4 DNA isolation, PCR amplification, cloning, and sequencing of 16S rRNA genes and dsrAB genes

Tris–EDTA (10 mM Tris–HCl, 1 mM EDTA; pH 8) buffer and lysozyme (10 mg/mL) were added in the pelleted cells and incubated for 30 min at room temperature [22]. SDS and proteinase K (10 U/μL) were added and incubated at 55 °C for 2 h. DNA was extracted with phenol, chloroform, and iso-amyl alcohol and DNA was precipitated with ethanol and dissolved in TE buffer. Polymerase chain reaction (PCR) was performed with a final volume of 50 μL in 0.2 mL thin walled tubes. The primers [23] used for PCR amplification of 16S rRNA gene are 8F 5′-AGA GTT TGA TCC TGG CTC AG-3′ and 1492R 5′-GGT TAC CTT GTT ACG ACT T-3′ (Sigma genosys). Each reaction mixture contained 2 μL of template DNA (100 ng), 0.5 μM of two primers, and 25 μL of enzyme master mix (Bioron). The PCR program consisted of an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of DNA denaturation at 92 °C for 30 s, primer annealing at 50 °C for 1 min, and primer extension at 72 °C for 2 min carried out in thermal cycler (Thermo Hybaid). After the last cycle, a final extension at 72 °C for 20 min was added. The *dsr*AB genes were amplified with primers [24] DSR1F – 5′-ACG CAC TGG AAG CAC G-3′ and DSR4R 5′-GTG TAG CAG TTA CCG CA-3′ as done for 16S rRNA gene amplification except the change in annealing temperature at 54 °C. The PCR products were purified by QIAquick PCR purification kit as described by the manufacturer and cloned using QIAGEN PCR cloning plus kit as described by the manufacturer. Clones were selected and isolated plasmids with insert were sequenced with M13 sequencing primers using ABI biosystems automated sequencer. The sequences obtained were matched with previously published sequences available in NCBI using BLAST [25].

2.5 Weight loss method

Mild steel coupons (1 cm × 5 cm) were sequentially ground with a series of grit silicon carbide papers (grades 180, 500, 800, 1200, and 1500) to a smooth surface and were finally polished to a mirror finish surface using 0.3 μm alumina powder and then degreased using trichloro ethylene. The polished coupons were rinsed with deionized water, and surface sterilized by immersion in a 70% ethanol solution for 1 min and finally dried. The prepared coupons were used for weight loss studies and surface analysis. Two hundred and fifty microliters of modified Baar's medium has been taken as the system and polished coupons were immersed in the medium with the inoculum of the SRB isolate incubated at 37 °C. After 7 days, the coupons were removed and pickled in pickling solutions, washed in water and dried with air drier. The average of final weights of triplicate systems of coupons in each system was taken. The standard deviation for each system is also presented. Final weights of the three coupons in each system were taken and the average corrosion rates were calculated and the weight loss has been normalized to the surface area.

2.6 Polarization study

Mild steel coupons were embedded in araldite with an exposed area of 1.0 cm^2 as a working electrode. The specimens were immersed for 7 days at 37 °C in sterile as well as SRB isolate inoculated in modified Baar's medium with sodium chloride to the final 2000 ppm (mg/L) of chloride ions (Cl). Conventional three-electrode cell assembly was used for polarization measurements. Polarization experiments were conducted with and without bacteria using a computer controlled potentiostat (PGP 201, potentiostat with voltammeter-1 software) in a 100 mL polarization cell. A three-electrode setup was used consisting of test coupon as the working electrode, saturated calomel electrode (SCE) as the reference and a platinum foil as the auxiliary. The test coupon was first immersed in the corrosion cell for 10 min to allow equilibrium with the electrolyte. The Tafel polarization curves were obtained by scanning from open circuit potential (OCP) to 300 mV *versus* SCE anodically and cathodically with the scan rate of 10 mV/min. Current and resistance (IR) drop compensation was not needed since this was a high conductivity electrolyte.

2.7 X-ray diffraction (XRD), Fourier transform infra red (FTIR) spectroscopy and scanning electron microscopy (SEM) analysis

A computer controlled X-ray diffraction (XRD) technique, JEOL Model JDX-8030 was used to scan the corrosion products between 10 and 85° – 2θ with Cu Kα radiation (Ni filter) at a rating of 40 kV, 20 mA. The dried corrosion products were collected and crushed into a fine powder and used for XRD analysis for determining the nature of oxides present in the corrosion product. To verify the adsorption of corrosion products on the metal surface in the control and inoculated system, the film

formed on the metal surface was carefully removed and dried, mixed thoroughly with potassium bromide (KBr) and made as pellets. These pellets were subjected to FT-IR spectra (Perkin-Elmer, Nicolet Nexus-470) to find out the nature of film formed on the surface of the metal coupons. For SEM preparation, the coupon exposed to SRB isolate taken out was cleaned with sterile water to remove all loose corrosion products. First it was fixed with 0.25% glutaraldehyde at 16 °C for overnight and then dehydrated with a series of ethanol–water combinations (20–100%) and then kept in vacuum desiccators. The surface morphologic characteristics of the treated coupon were observed under scanning electron microscope (SEM) (Hitachi model S-3000 H) at magnification ranging from 50× to 5000× operated at accelerating voltage of 30 kV.

2.8 Confocal laser scanning microscopy (CLSM) analysis

The mild steel specimen with biofilm was air-dried, stained using acridine orange, and observed under confocal laser scanning microscope (CLSM). A Leica (TCS-SP2-RS, Germany) CLSM equipped with argon ion laser light source with excitation wavelength of 488 nm was used to image the biofilm. The images were obtained in fluorescence mode and images were generated with an objective magnification of 20× and numerical aperture set at 0.5 (20×/0.5 NA), selecting a scan format of 512 × 512 pixels and scan speed of 4000 Hz and pinhole set at 0.63. The stage was moved vertically (z-direction) between the first and last detectable light reflex and a z series consisting of optical sections (~1.5 μm) as per numbers optimized by the software was generated.

3 Results

3.1 Isolation and enumeration

The physico-chemical parameters of the cooling water were presented in Table 1 as weight percentage, mg/L (ppm). Chloride (280 ppm) and sulfate (78 ppm) were analyzed among the other chemical characteristics of the cooling water. The growth of dominant SRB in different carbon sources and the MPN index of dominant SRB species was observed as 280 MPN index/100 mL in the medium containing propionate [26].

3.2 Morphological and biochemical characterization of SRB isolate

The dominant SRB strain isolated was Gram negative, non-spore forming, propionate oxidizing, and H_2S producing mesophilic bacilli (Table 2). No production of desulfoviridin was observed in the isolate. The Desulfoviridin test was performed to confirm whether the isolate belongs to genus *Desulfovibrio*.

3.3 Molecular identification

The sequences obtained were analyzed and showed the similarity with 16S rRNA genes of SRB species (Fig. 1a) along with some species of gamma proteobacteria. The isolate was confirmed as *D. propionicus* since *dsr*AB genes showed the 99% similarity with the same gene sequences of *D. propionicus* and other *Desulfobulbus* species in the database sequences (Fig. 1b).

3.4 Weight loss method

The corrosion rate of mild steel in presence and absence of bacteria is presented in Table 3. The corrosion rate was high (0.22 mm/year) in presence of bacteria when compared to the uninoculated system (0.14 mm/year).

3.5 Polarization study

Polarization studies for mild steel in presence and absence of bacteria are presented in Fig. 2. The E_{corr} value of mild steel in absence of bacteria was −720 mV *versus* SCE and shifted to −750 mV *versus* SCE in presence of *D. propionicus*. The i_{corr} value of mild steel in absence of bacteria was 3.5×10^{-6} A/cm^2 and in the presence of bacteria, the i_{corr} was 9×10^{-5} A/cm^2. It indicates that SRB accelerates corrosion on mild steel by enhancing the anodic current.

Table 1. Physical and chemical characteristics of the cooling water collected from the refinery; titration, and spectrophotometric analysis done for the sample collected

S. No.	Factors	Observations
1	Temperature	50 °C
2	pH	8.2
3	Total solids	285 ppm
4	Total dissolved solids	225 ppm
5	Total suspended solids	60 ppm
6	Dissolved oxygen	6.3 ppm
7	Chloride	280 ppm
8	Total hardness	150 ppm
9	Sulfate	78 ppm
10	Calcium	68 ppm
11	Magnesium	82 ppm

ppm – weight percentage, mg/L.

Table 2. Morphological and bochemical characteristics of SRB isolated from the cooling water system; especially desulfoviridin, an enzyme test under UV illumination to qualify the presence or absence of *Desulfovibrio* sp.

S. No.	Characters	Isolate
1	Shape	Rod
2	Gram reaction	Negative
3	Motility[(a)]	+
4	Spore formation	−
5	Optimal growth temperature	37 °C
6	Oxidation of organic substrates	Propionate
7	H_2S[(b)] production	+
8	Desulfoviridin test[(c)]	−

[(a)]+, Motile.
[(b)]+, H_2S produced.
[(c)]−, No desulfoviridin.

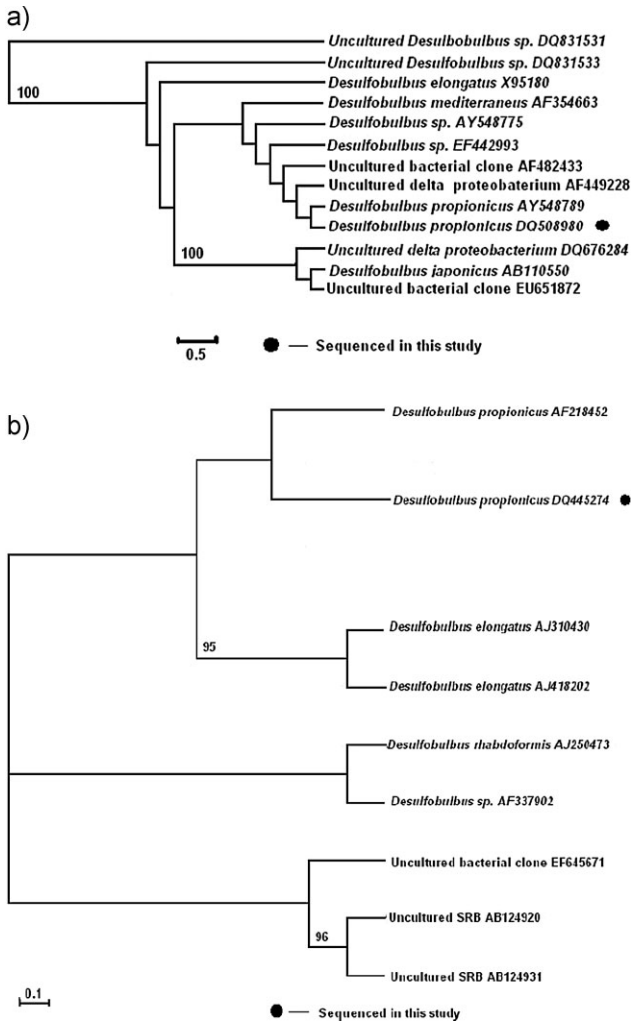

Figure 1. (a) Phylogenetic analysis of 16S rRNA gene sequences of the isolate – the similarity of 16S rRNA gene sequence of SRB isolate with sequences of *D. propionicus* and other species of *Desulfobulbus* deposited in the database. (b) Phylogenetic analysis of *dsr*AB gene sequences of the SRB isolate – the similarity of *dsr*AB gene sequence of SRB isolate with sequences of *D. propionicus* and other species of *Desulfobulbus* deposited in the database

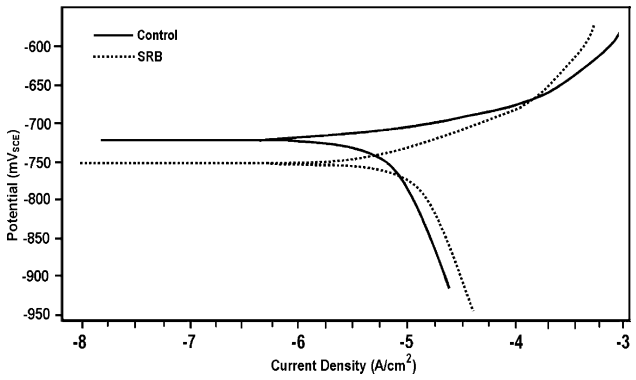

Figure 2. Tafel polarization curves of mild steel coupon exposed to SRB isolate (*D. propionicus*) and in the control system (uninoculated broth) with 2000 ppm Cl

3.6 XRD, FTIR, and SEM analysis

Nature of the surface film was identified with XRD and FTIR analysis given in Figs. 3 and 4, respectively. Figure 3 shows the details of XRD peaks corresponding to the phases present in the corrosion product collected from the mild steel in the presence of *D. propionicus*. Besides peaks of high intensity were noticed with FeO(OH), FeS, Fe_2O_3, and $FeCO_3$ in the corrosion product. A peak at below 20θ indicates the presence of iron and organic complex. In FTIR analysis (Fig. 4), the 3000–2800 cm^{-1} spectral region is dominated by CH_3 and CH_2 stretching vibrations attributed to fatty acid components of membranes, the other peaks at 1685 and 1640 cm^{-1} which was due to free acidic group and iron phosphate, respectively, the band located between 1150 and 1000 cm^{-1} corresponding both to (C–O) stretching vibration of polysaccharide, the 1200–900 cm^{-1} spectral region corresponding to C–O–C and C–O–P stretching vibrations concerns oligo- and polysaccharides present in the bacteria, suggesting the production of extracellular polymeric substances (EPS) by the bacteria and attachment of *D. propionicus* on the metal surface. SEM micrograph (Fig. 5) shows the corrosion products on mild steel coupon exposed to the bacterial isolate.

3.7 CLSM analysis

Confocal laser scanning microscope studies (Fig. 6) indicated the presence of micropits throughout the surface. Several pits of size up to 100 μm diameter and depth were observed. A few pits with 40 μm diameters and 250 μm deep were observed in the mild steel coupons exposed to *D. propionicus*.

4 Discussion

In the present study, a mesophilic SRB, *D. propionicus* was isolated from the cooling tower of a petroleum refinery in South India. The water quality characterization of cooling tower clearly showed the presence of sulfate and chloride ions. *Von Wolzogen Kuhr* and *Van der Vlugt* [27] have clearly suggested that SRB uses sulfate as electron acceptor and reduces it to sulfide. Ferrous sulfides formed act as the cathode reacting with the metal to form an electrochemical cell which may enhance the corrosion rate [1].

Table 3. Corrosion rate of SRB isolate on mild steel; coupons exposed to control and bacteria inoculated systems for 7 days

S. No.	System	Weight loss (mg)	Corrosion rate (mm/year)
1	Control system, uninoculated medium	25.6±2.0	0.14
2	Experimental system *D. propionicus*	38.3±2.5	0.22

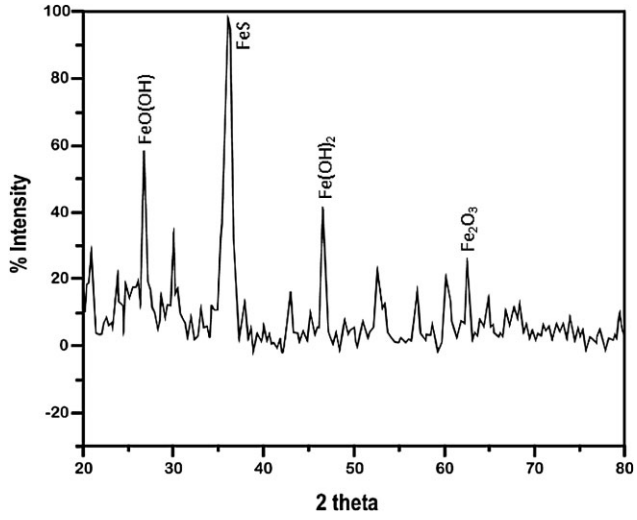

Figure 3. X-ray diffraction pattern of the corrosion product collected from mild steel exposed to SRB (*D. propionicus*) inoculated system

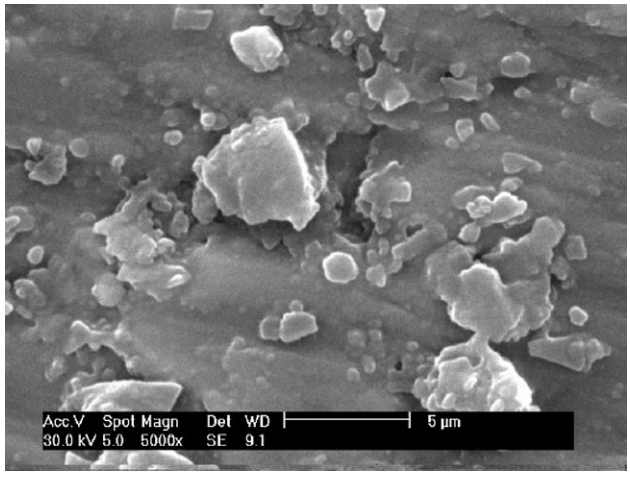

Figure 5. Scanning electro micrograph showing the corrosion products on the mild steel coupon exposed to SRB isolate (*D. propionicus*) for 7 days

SRB mostly distinguished in MIC research by their sulfate reduction capacity [4] are a complex physiological bacterial group, and various properties have been used in traditional classification schemes. The most important of these properties are cell shape, motility, GC content of DNA, presence of desulfoviridin and cytochromes, optimal temperature, and complete and incomplete oxidation of carbon sources. *LeGall* and *Fauque* [28] have established that desulfoviridin is present only in *Desulfovibrio* sp. In the present study, the absence of desulfoviridin in the isolate confirmed that this might be a different genus of mesophilic SRB group.

Isolation and enumeration were done with different carbon sources and extensive morphologic and biochemical characters were done to identify this different genus of mesophilic SRB. Our isolate in Table 2 is a non-spore former capable of oxidizing propionate. This inferred the presence of mesophilic SRB, *D. propionicus* in the sample. In the absence of sulfate, *D. propionicus* ferment ethanol + CO_2, pyruvate, or lactate to propionate and acetate via a succinate pathway involving a methylmalonyl-CoA:pyruvate transcarboxylase [29]. H_2S is formed with propionate in the presence of sulfate transported into the cell [30] in accordance with a randomizing route such as the methylmalonyl-CoA pathway for propionate [31]. Results of morphological and biochemical characterization studies clearly showed H_2S production by this species.

16S rRNA-targeted PCR primer sequences specific for SRB subgroups have been designed and used to detect phylogenetic subgroups of SRB [32]. SRB species were identified in fresh water and saline oil field environment by reverse sample genome probing and characterized by Southern blotting; using both 16S rRNA and hydrogenase gene probes [33]. However 16S rRNA based analysis does not provide an unambiguous link to the

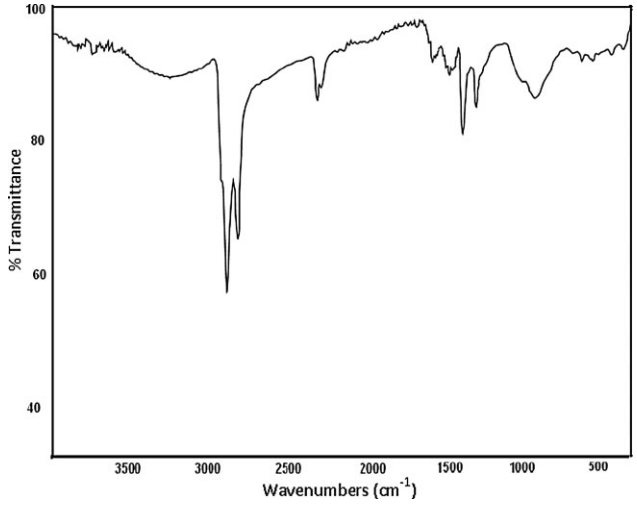

Figure 4. FTIR analysis – spectral peaks of the corrosion product collected from mild steel exposed to SRB isolate (*D. propionicus*)

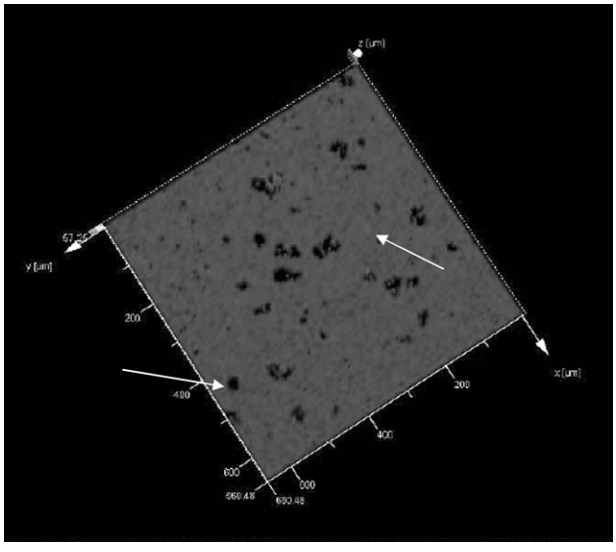

Figure 6. Confocal micrograph showing few pits on mild steel exposed to SRB isolate (*D. propionicus*)

physiology or metabolic capacities of a bacterium, particularly in newly discovered phylogenetic lineages without cultured isolates and known phenotypes [23]. Hence, in the present study, the functional gene approach for dissimilatory sulfate reductase has been used to confirm the isolate.

The dissimilatory sulfate reduction is the major component of their bioenergetics processes and the high concentration of H_2S produced often indicates the activity and the presence of sulfate reducing microorganisms in natural habitats [34]. The key gene coding for this dissimilatory sulfite reductase (EC 1.8.99.3) was found in all SRBs. The 1.9 kb DNA fragment encoding most of the α and β subunits of dissimilatory sulfite reductase have been amplified and analyzed by PCR from all recognized lineages of SRBs and to detect SRB in different environments [34, 35].

Thus molecular approach based on the dissimilatory sulfite reductase genes (*dsr*AB) had been used in this study to characterize the isolated SRB. The present work showed the similarity of gene sequences with dissimilatory sulfite reductase AB genes (*dsr*AB) and confirmed the presence of *D. propionicus*.

Weight loss studies in SRB medium and polarization studies were established methods [36] used to identify the corrosion character of *Desulfobulbus* species in anaerobic environments. In the present study, weight loss analysis clearly showed that corrosion rate was high in the presence of bacteria and triplicate analysis with standard deviation confirmed the significance of results.

Polarization study showed the lowering of OCP due to biogenic H_2S present on the mild steel. The enhancement of anodic current and OCP lowering clearly indicated that this SRB isolate enhanced the corrosion on the metal surface. The effect of ferrous ion availability on growth of a corroding SRB and the crucial role of Fe^{2+} availability on the physiologic properties of SRB and its relevance in the process of anaerobic biocorrosion was reported [14]. Biocorrosion mechanism was proposed in which the excretion of acidic products can promote Fe^{2+} availability for SRBs. Metabolism of propionate [29] shown in Equation (1) forming acetate as acidic product may promote Fe^{2+} availability for SRB corrosion.

$$\text{Propionate} + SO_4^{2-} + H_2 \rightarrow HS^- + HCO_3^- + \text{Acetate} + H_2O, \quad \Delta G = -75.8\,\text{kJ/reaction} \tag{1}$$

The degradation of propionate is substantially enhanced by the presence of SRB. SRB play an important role in the breakdown of propionate either through direct utilization or through interspecies transfer (e.g., H_2). Propionate-degrading SRB, *D. propionicus*, was reported to be able to breakdown propionate efficiently to acetate and also stated that propionate oxidation by SRB becomes more efficient at high sulfate concentrations [37]. The pH (7.2) of the culture media was stable during corrosion experiment, this might be the due to presence of HCO_3 as a buffering agent [38]. The SRB could have induced the electron transfer by consuming the cathodic hydrogen produced on the metal while using propionate as an energy source. Thus use of hydrogen agrees with the theory of cathodic depolarization [27]. H_2S produced during bacteria growth have been thought to have effect on the corrosion and dissolution potential of metals. Then iron sulfides (FeS_x) are formed by the precipitation of ferrous ions with the sulfide ions and this can act as an additional cathode which can enhance corrosion. Biochemical tests showed H_2S production by the SRB isolate and XRD patterns of corrosion products clearly showed the presence of FeS. Thus it can be concluded that *D. propionicus* enhanced anodic and cathodic current by the formation of iron sulfide, which formed an additional cathode to parent metal. The interaction of exopolymers produced by SRB with metals enhanced the process of complexation of metal ion species [39]. *Miranda* et al. [15] and *Villanueva* et al. [40] have also noticed increased corrosion current in presence of bacteria. They also presented an explanation that sulfides or some other metabolic product of bacteria accelerated the anodic reaction. Lowering of the pH by metabolic products, such as acetic acid, increases the level of corrosive sulfides (HS^-), which in turn, promote the attack of metallic iron, which produces Fe^{2+}. The acidic conditions ensure Fe^{2+} for SRB growth and can account for the persistency of corrosion.

Pitting of passivated steel in saturated H_2S solutions was observed in the presence of chlorides [41]. The chloride concentration had a marked effect on the pitting potential of SS electrode. The breakdown potential shifted to more negative values as the chloride content increased in the solution. A comparison of the results obtained in SRB media and those in NaCl solutions, suggests that iron pitting is induced by the

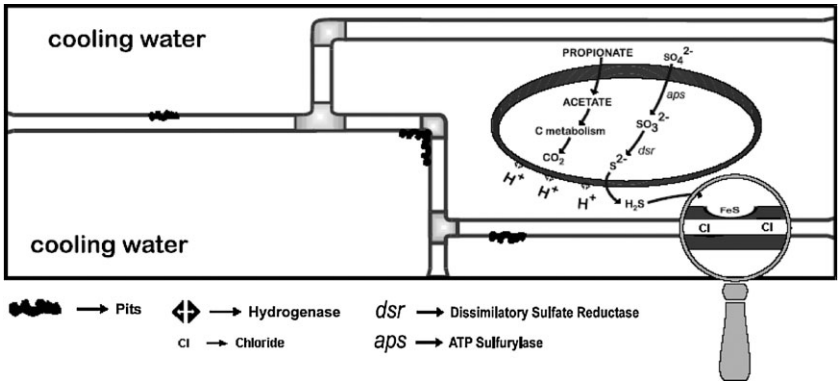

Figure 7. Schematic representation of carbon source metabolism, sulfate reduction pathway, and corrosion mechanism of SRB on metal surface of cooling towers depicting the propionate metabolism, influence of metabolic enzymes like hydrogenase, dissimilatory sulfite reductase, ATP sulfurylase, and corrosive metabolite H_2S, FeS, and also chloride in the corrosion

presence of chloride ions [42]. In the present study, polarization studies with Cl concentration showed the influence of chloride ions on corrosion of the steel. It can be concluded that the presence of chloride might have enhanced the pitting corrosion on mild steel surface under the biofilms of this SRB. Confocal micrograph images confirmed the pitting on the steel surface.

A mechanism proposed for the physiology and corrosion of SRB isolate is given in Fig. 7 on the basis of influence of dissimilatory sulfite reductase and propionate metabolism on SRB in sulfide and biomass production, respectively. Future studies are proposed to explore genes of SRB, which are getting expressed during corrosion process, which will provide more insight to control of this corrosion causing species.

5 Conclusions

1. Mesophilic SRB *D. propionicus* was isolated for the first time from cooling tower of a petroleum refinery and identified by analyzing 16S rRNA and functional gene *dsr*AB sequences. The 16S rRNA and *dsr*AB gene sequences reported in this study have been deposited in Genbank under accession numbers DQ508980 and DQ445274, respectively.
2. The corrosion rate of mild steel was higher in the presence of bacteria than in uninoculated system.
3. The E_{corr} value of mild steel shifted to more negative values of −750 mV *versus* SCE in the presence of *D. propionicus* and the i_{corr} value of mild steel in the presence of bacteria, was high (9×10^{-5} A/cm^2) confirming the corrosive effects of this SRB species on mild steel.
4. Surface films characterized by XRD studies confirmed presence of FeS and FTIR analysis confirmed the production of EPS in the corrosion products. Further CLSM studies confirmed the presence of micropits on the metal surface.

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