Effect of thermophilic sulphate-reducing bacteria (*Desulfotomaculum geothermicum*) isolated from Indian petroleum refinery on the corrosion of mild steel

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The role of thermophilic sulphate-reducing bacteria (SRB) *Desulfotomaculum geothermicum* in mild steel corrosion was evaluated by electrochemical study and surface analysis technique. In the presence of *D. geothermicum*, the corrosion rate was 0.0698 mmpy at 50°C, which was higher when compared to control. Polarization study revealed that the bacteria enhanced cathodic reaction and suppressed anodic reaction. XRD data revealed that the presence of FeS enhanced the cathodic reaction by the formation of a protective film on the metal surface. Pitting was observed by confocal microscopy, which may be due to cathodic depolarization. The study implicates the importance of *D. geothermicum* in the corrosion of cooling towers of the petroleum refinery.

Keywords: Biocorrosion, mild steel corrosion, petroleum refinery, sulphate reducing bacteria.

MICROBIOLOGICALLY influenced corrosion (MIC) is the damage caused or accelerated by the presence of bacteria and other microorganisms and their metabolic activities on metals and alloys. Many types of bacteria, including sulphate-, iron- and CO_2-reducing bacteria, sulphur-, iron- and manganese-oxidizing bacteria are associated with the MIC of metals and alloys[1]. Among them, sulphate-reducing bacteria (SRB) are recognized as a major group involved in anaerobic corrosion. These latter microorganisms can coexist in naturally occurring biofilms with a wide bacterial community, including fermentative bacteria, often forming synergistic communities (consortia) that are able to influence electrochemical processes through cooperative metabolism[2]. The sulphate reductive activity of SRB is thought to account for >75% of the corrosion in productive oil wells, and for >50% of the failures of buried pipelines and cables[3].

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SRB are strictly anaerobic microorganisms responsible for the terminal mineralization of organic matter in anoxic environments. They are a diverse group of prokaryotes that may be divided into four groups based on rRNA sequence analysis: Gram-negative mesophilic SRB, Gram-positive spore-forming SRB, thermophilic bacterial SRB and thermophilic archael SRB[4]. A majority of studies on SRB in oil-field environments have concentrated on the ecology and physiology of mesophilic microorganisms, which grow optimally between 20°C and 40°C. The cooling towers of the nuclear power plant in Kalpakkam, India, are also facing severe corrosion problem by SRB, where mesophilic bacteria have been reported[5]. Abedi *et al.*[6] observed the influence of stress cracking corrosion (SCC) and SRB-induced cracking propagation in transmission oil products pipeline. Most oil-bearing reservoirs, especially in North Sea fields and Indian Oil wells exist in deep geological strata[7], with temperatures above 60°C.

Miranda *et al.*[8] observed the role of *D. capillatus* isolated from oil field separator in corrosion. Generally, the temperature of condenser tubes in cooling towers is in the range between 50°C and 70°C. The incomplete description of thermophilic SRB species in industrial environments has profoundly hindered the understanding of their bacterial diversity and their role in cycling of matter or nutrient materials. Limited studies have been carried out on thermophilic SRB and their role on corrosion process in heat exchangers[9,10]. No study has been carried out on corrosion behaviour of thermophilic SRB in the Indian process industry. Therefore, the study on thermophilic SRB present in cooling towers/oil field environments is one of the first steps to understand the mechanism of corrosion process at high temperatures. In the present study, sensitive and specific molecular techniques have been employed for the identification of SRB with their functional gene sequences and analysis of corrosion behaviour of the isolate has been carried out.

Materials and methods

Sample collection

Corrosion products and cooling water were collected aseptically from a cooling tower of a petroleum refinery (Chennai, South India) and were transported in an ice-bucket to Sourashtra College Laboratory, Madurai. The physical and chemical characteristics of the cooling water were analysed using standard procedures[11].

Isolation

The corrosion product sample was taken for analysis and it was enriched in sterile Modified Baar's liquid medium kept in Anaerogas Hipack chamber (Himedia Labs, Mumbai) for 7 days. One litre of the medium contained 2 g $MgSO_4$, 5 g sodium citrate, 1 g $CaSO_4$, 1 g NH_4Cl, 0.5 g KH_2PO_4, 1 g yeast extract, and 3.5 g sodium lactate. The enriched culture was inoculated in sterile modified Baar's medium with different carbon sources: lactate and acetate, which were incubated at 50°C for 3 days. The dominant strain grown in lactate medium was examined for its morphology and biochemical characters, including Desulfoviridin test[12].

DNA isolation

Tris-EDTA (10 mM Tris-HCl, 1 mM EDTA; pH 8) buffer and lysozyme (10 mg/ml) were added in the pelleted cells of the dominant isolate and incubated for 30 min at room temperature. 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 was precipitated with ethanol and dissolved in TE buffer[13].

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

Polymerase chain reaction (PCR) was performed with a final volume of 50 µl in 0.2 ml thin-walled tubes. The primers used for PCR amplification of 16S rRNA gene[14] were 8F forward and 1492R reverse primers (Sigma Genosys, Bangalore). The PCR programme 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, primer extension at 72°C for 2 min and a final extension at 72°C for 20 min carried out in Thermal Cycler (Thermo Hybaid). The *dsr*AB gene was amplified with primers DSR1F and DSR4R following the PCR programme used for 16S rRNA gene amplification[15], except primer annealing that was carried out 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 plasmids with insert were isolated and sequenced with M13 sequencing primers using ABI Biosystems automated sequencer. The sequences obtained were matched with previously published sequences available in NCBI using BLAST[16].

Weight loss method

Mild steel coupons of size 1 cm × 5 cm were mechanically polished to mirror finish and then degreased using trichloro ethylene. Three polished coupons were immersed in 250 ml of Modified Baar's medium inoculated with 100 µl of identified SRB isolate and kept at 50°C in an incubator (Cryoscientific Ltd, Chennai). Duplicate systems were maintained for the weight loss study. After 7 days, the coupons were removed and pickled in pickling solutions, washed with water and dried with an air-drier. Final weights of the six coupons in each uninoculated control and culture were taken and the average corrosion rates were calculated.

Polarization study

Mild steel coupons were embedded in araldite with an exposed area of 1.0 cm^2 as a working electrode. The coupons were immersed for 7 days at 50°C in sterile modified Baar's medium and were used as the control. Another set of modified Baar's medium containing coupons was inoculated with *D. geothermicum* isolate. Polarization study with conventional three-electrode cell assembly was done by employing potentiostat (PGP 201, potentiostat with voltammeter-1 software). A three-electrode set-up was used consisting of test coupon as the working electrode, SCE (saturated calomel electrode) 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 towards 200 mV anodically and cathodically with the scan rate of 120 mV/min. IR drop compensation was not needed since this was a high-conductivity electrolyte.

X-ray diffraction and Fourier transform infrared spectroscopy analysis

The dried corrosion products were collected and crushed into a fine powder and used for X-ray diffraction (XRD) analysis for determining the nature of oxides present in the corrosion product. A computer-controlled XRD (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. To verify the adsorption of corrosion products on the metal surface after the electrochemical study in control and inoculated system, the corrosion products formed on the metal surface were carefully removed and dried, mixed thoroughly with potassium bromide (KBr) and made as pellets. These pellets were then analysed using the Fourier transform infrared spectroscopy (FTIR; Perkin–Elmer, Nicolet Nexus-470) to find out the nature of film formed on the surface of the metal coupons.

Scanning electron microscope, confocal laser scanning microscope and atomic force microscope analysis

The surface morphological characteristics of the control and experimental coupons were observed under scanning electron microscope (SEM; Hitachi model S-3000 H) at magnification ranging from 50× to 200×, operated at accelerating voltage of 25 kV.

The mild steel specimen with biofilm of *D. geothermicum* isolate was air-dried, stained using acridine orange and observed under a confocal laser scanning microscope (CLSM; Leica TCS-SP2-RS, Germany) equipped with argon ion laser light source. The excitation wavelength of 488 nm which was used to image the biofilm. The images were obtained in fluorescence mode. The images were generated with an objective magnification of 20× and numerical aperture set at 0.5 (20×/0.5NA), selecting a scan format of 512 × 512 pixels, 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) according to the numbers optimized by the software was generated.

Atomic force microscope (AFM) analyses were carried out to find out the SRB biofilm formed on the metal surface. Briefly, the coupons were removed from the media, lightly rinsed in sterile distilled water, and then left to air-dry. Samples were examined using a Nanoscope IIIA AFM (Digital Instruments, USA) in the tapping mode to capture the images of biofilms on the coupon. The nanoprobe cantilevers were made of silicon nitride (Si_3N_4) with a spring constant of $k = 0.06$ N/m (Digital Instruments, USA).

Results

Isolation and identification

The physical and chemical characteristics of the cooling water are presented in Table 1. The chloride content was 295 mg/kg, total hardness was 160 mg/kg and the sulphate content was 82 mg/kg. The isolate was Gram-positive bacilli having sub-terminal spores. The isolate oxidized lactate, produced H_2S at 50°C and did not contain desulfoviridin (Table 2). The nucleotide database was searched with the sequences obtained using NCBI BLAST (Blastn) tool (http://www.ncbi.nlm.nih.gov/BLAST) and showed 99% similarity with 16S rRNA genes of SRB species in the database sequences. The gene sequences obtained with *dsr* primers showed 99% similarity with dissimilatory sulphite reductase AB genes (*dsr*AB) of the database sequences. Based on these characteristics and sequence analysis, the isolate was identified as thermophilic SRB *D. geothermicum.*

Weight loss method

In the control system (uninoculated) the weight loss was 41 mg, whereas in the presence of *D. geothermicum*, the weight loss was 124 mg. It indicates that the corrosion rate was higher (0.0698 mmpy) in the presence of *D. geothermicum* at 50°C.

Polarization study

In the polarization study (Figure 1), E_{corr} of the control system was –720 mV vs SCE, while in the presence of inoculating bacterial isolate *D. geothermicum*, E_{corr} was –685 mV vs SCE. The corrosion current was 2.0×10^{-5} A cm^{-2} in the control and 1.0×10^{-5} A cm^{-2} in the presence of SRB isolate. This indicates that, bacteria inhibit corrosion by shifting the potential to the positive side. However, i_{corr} was slightly lower in the presence of

Table 1. Physical and chemical characteristics of the cooling water

Factors	Observations
Temperature	65°C
pH	8.1
Total solids	310 mg/kg
Total dissolved solids	235 mg/kg
Total suspended solids	65 mg/kg
Dissolved oxygen	6.1 mg/kg
Chloride	295 mg/kg
Total hardness	160 mg/kg
Sulphate	82 mg/kg
Calcium	74 mg/kg
Magnesium	83 mg/kg

Table 2. Morphological and biochemical characteristics of the isolate

Characteristics	Isolate
Shape	Rod
Gram reaction	Positive
Motility	+
Spore formation	Sub-terminal
Optimal growth temperature	50°C
Oxidation of organic substrates	Lactate
H_2S production	+
Desulfoviridin test	–

bacteria. On the basis of i_{corr} noticed from the polarization study, the corrosion rate calculated was slightly higher in the control system (7.29×10^{-9} mmpy) than the SRB isolate (3.64×10^{-9} mmpy). The nature of the curve indicates that the bacteria enhance cathodic current and suppresses anodic current at 50°C.

XRD and FTIR analysis

Figure 2 presents the details of XRD data corresponding to the phases present in the corrosion product sample collected from the experimental systems. Peaks of higher intensity of ferrous sulphide (FeS), Fe_2O_3, FeO(OH) and Fe_3O_4 in the experimental system were noticed. FTIR spectrum for corrosion product is presented in Figure 3, which shows the amide group of the protein and the C–O group of polysaccharides. A broad band noted in the range 3000–3500 cm^{-1} indicates the presence of adsorbed water molecule and OH/NH groups[17]. The intense peak near 1410 cm^{-1} increases, correspondingly to the symmetric stretch mode of a carboxylate group. Presence of neutral C=O (complexed) and/or iron–polysaccharide complex is indicated by their stretching modes at 1660 cm^{-1}. Bands are mainly due to phosphate stretching vibration in polysaccharides and nucleic acids in the range 1250–1200 cm^{-1} and 1200–900 cm^{-1} spectral region corresponding to C–O–C and C–O–P. These stretching vibrations imply oligo- and polysaccharides present in the bacteria[18].

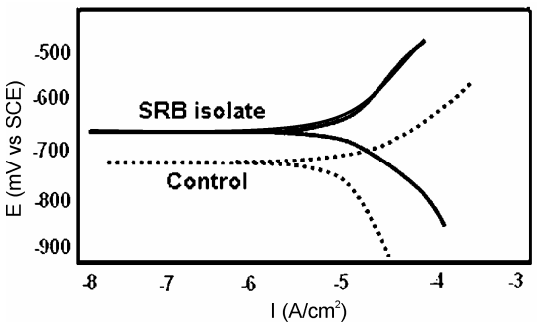

Figure 1. Polarization study with SRB isolate.

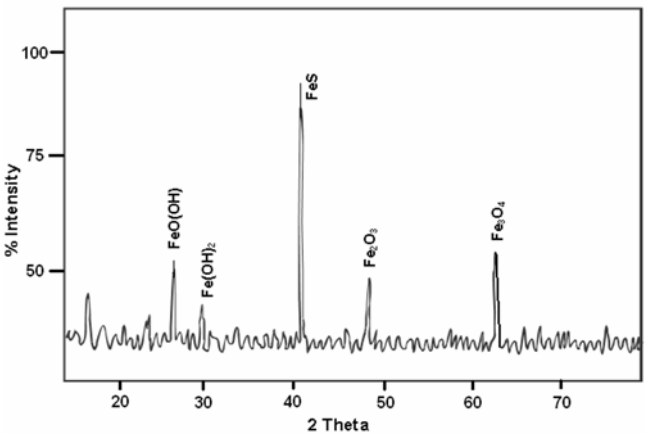

Figure 2. XRD pattern of the corrosion product.

SEM, CLSM and AFM analysis

SEM analysis (Figure 4) showed the presence of corrosion products on mild steel coupon. CLSM studies (Figure 5) indicated the presence of micropits throughout the surface. Several pits of size up to 100 μm diameter were observed. AFM images (two- and three-dimensional) of isolate biofilms after exposure to modified Baar's medium are shown in Figure 6. These AFM images confirm that biofilms are dynamic structural entities in which cell attaches and grows and form micro-colonies on the mild steel surface. Rod-shaped bacterial cells could be observed on the mild steel.

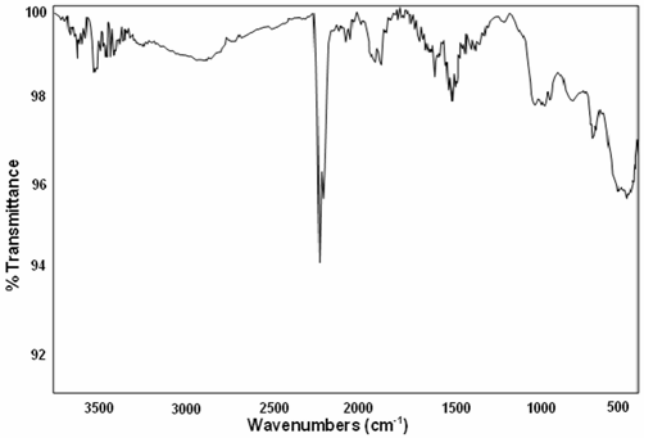

Figure 3. FTIR analysis of the corrosion product.

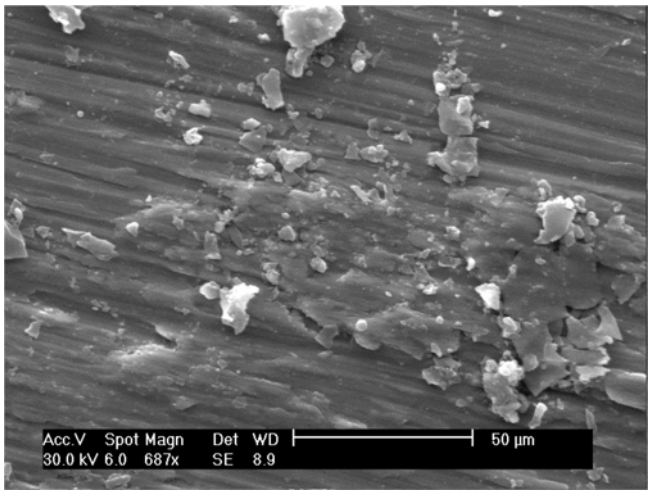

Figure 4. Scanning electron micrograph of mild steel coupon exposed to thermophilic SRB isolate.

Discussion

The morphological and biochemical characteristics observed in Table 2, having identical characters to that of *Bacillus* and *Clostridium* species, except the sulphate reduction character (H_2S production), indicate the presence of thermophilic SRB *Desulfotomaculum* species in the sample. Desulfoviridin mainly found in *Desulfovibrio* species[19] (Table 2) also confirms the isolate as thermophilic sulphate reducer. The metabolism of carbon sources by several SRB species has been well documented[20] and suitable electron donors for sulphate reduction have been reviewed[21]. The oxidation of organic substrates in SRB may be complete, leading to CO_2 with lactate usually being the end-product[20]. The identified isolate infers the complete oxidation of lactate and the presence of a pathway for acetyl-CoA oxidation that is usually associated with the ability to use free lactate as a growth substrate. Widdel[20] also assessed lactate as an organic substrate for enrichment of sulphate reducers. Sulphate reduction using lactate as an electron donor can be described as follows:

$$CH_3CHOHCOOH + 1/2H_2SO_4 \rightarrow CH_3COOH + CO_2 + 1/2H_2S + H_2O \ (\Delta G = -34.2 \text{ kJ/mole})^{20}.$$

The isolate in Table 2 showed similar characters and exhibited sequence similarity (99%) to the *D. geothermicum* sequence in the GenBank database, which was also identified by 16S ribosomal RNA genes and comparative analysis done with the closest representatives of the genus *Desulfotomaculum* by phylogenetic tree. 16S rRNA gene-targetted PCR primer sequences specific for SRB subgroups have been designed and used to detect phylogenetic subgroups of SRB[22]. However, 16S rRNA-based analysis does not provide an unambiguous link to the 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 has been used to identify the bacteria responsible for biogeochemical processes in the environment, as done by Minz *et al.*[24]. The functional gene encoding dissimilatory sulphite reductase (EC 1.8.99.3) was found in all SRBs[25], which was taken for confirming the isolate. A molecular approach based on the dissimilatory sulphite reductase genes had been used to characterize SRB in a variety of environmental settings[24–26]. The present work shows 99% similarity of gene sequences with dissimilatory sulphite reductase AB genes of database sequences and confirms the presence of *D. geothermicum.*

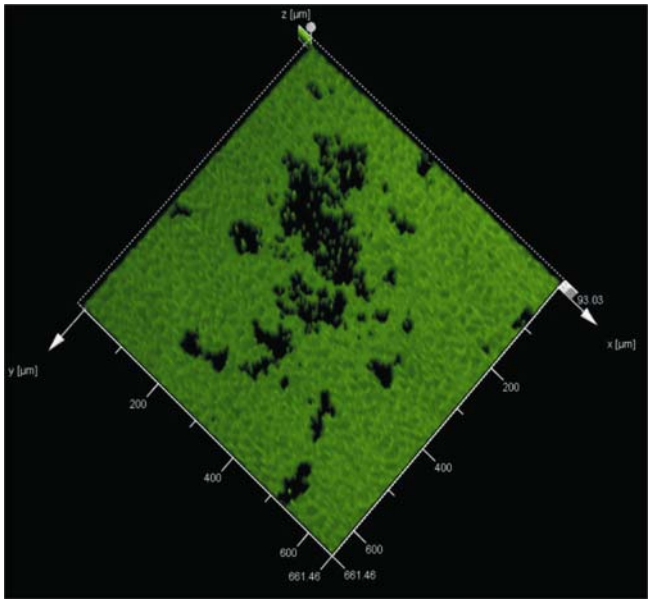

Figure 5. Confocal microscopy showing pits on mild steel exposed to thermophilic SRB isolate.

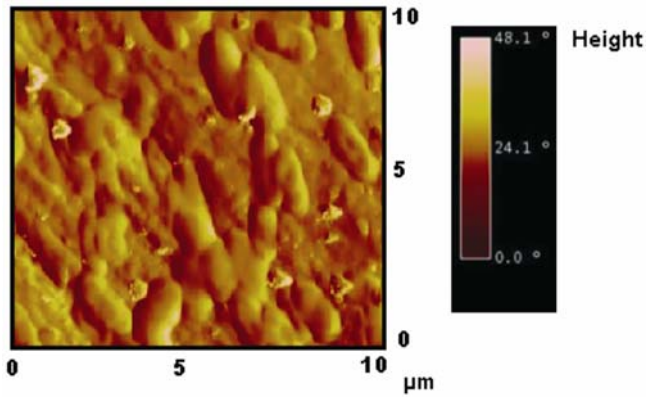

Figure 6. Atomic force microscopy analysis showing the biofilm of SRB isolate on metal surface.

The polarization study reveals that the enhancement of cathodic current in presence of the bacteria at 50°C is due to the formation of FeS film on the metal surface, as noted by Cetin *et al.*[27]. Reduction in the anodic current was due to the adsorption of organic film on the metal surface noticed in FTIR analysis, where the biogenic product may improve the passivity of steel. Though passivity was noticed in electrochemical behaviour of steel, it is assumed that the heterogeneity of bacterial attachment and that of temperature encourage the pitting corrosion on the metal surface. XRD results reveal the presence of ferrous sulphide, indicating the role of *D. geothermicum* in iron sulphide deposition during the formation of corrosion product, thereby accelerating the microbial corrosion directly on the pipeline. Kuang *et al.*[28] suggested that the electrochemical corrosion behaviour of carbon steel is influenced mainly by the redox quality, which depends on the concentration of sulphide generated by the metabolisms of SRB.

SRB grow best at temperatures from 25°C to 40°C and that for thermophilic species is 45–65°C or higher. The effect of some thermophilic SRB on pitting behaviour has

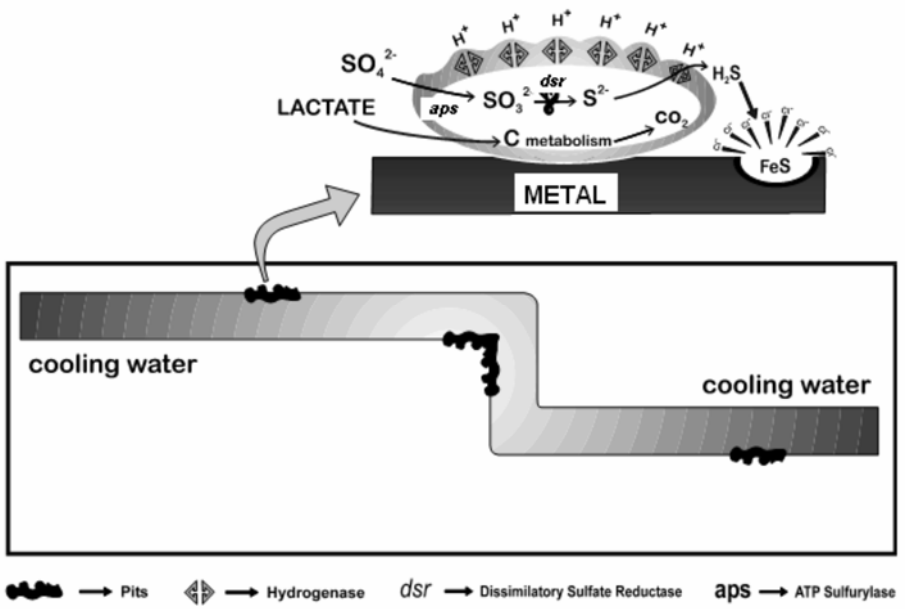

Figure 7. Pitting corrosion mechanism of SRB on metal of cooling towers.

been studied[29]. In the present study, the thermophilic strain is able to survive in the 50°C temperature range by forming spores. The vegetative forms might develop and multiply when the optimum temperature of growth is encountered. The conversion of sulphate to sulphide is temperature-specific at 50°C, whereas at lower temperature sulphide production could not be noticed. On the basis of influence of sulphate and lactate metabolism of SRB, a mechanism has been proposed for the pitting corrosion by SRB on the metal surface. The influence of adenosine phospho sulphate reductase, dissimilatory sulphite reductase in sulphate reduction for energy metabolism with electron transfers, carbon source (lactate) metabolism for biomass production, role of hydrogenase in the production of H_2S and corrosive nature of H_2S on metal surface are depicted schematically in Figure 7.

Conclusion

A thermophilic SRB strain, *D. geothermicum* was isolated from the corrosion product of cooling towers, identified with 16S rRNA and functional gene *dsrAB* sequences, and the corrosion behaviour was analysed. A high corrosion rate, enhancement of cathodic current and suppression of anodic current due to biogenic product were observed at 50°C. It can be assumed that chloride may encourage breaking the passivity and enhance the pitting corrosion. This is due to the proliferation of thermophilic SRB at higher temperature (about 50°C) present in the heat exchanger tubes. It is also possible that SRB could be involved in the corrosion process in cooling towers of petroleum refineries. The present study suggests that in-depth investigation on corrosion behaviour of thermophilic bacteria will be useful for the selection of biocides in the petroleum industry to combat corrosion.

Sequences submitted in GenBank (NCBI) under accession numbers: DQ155285 and DQ403855.

1. Beech, I. B. and Sunner, J., Biocorrosion towards understanding interactions between biofilms and metals. *Curr. Opin. Biotechnol*., 2004, **15**, 181–186.
2. Beech, I. B. and Gaylarde, C. C., Recent advances in the study of biocorrosion – an overview. *Rev. Microbiol*., 1999, **30**, 177–190.
3. Walch, M., *Microbial Corrosion*. *Encyclopaedia of Microbiology* (ed. Lederberg, J.), Academic Press, New York, 1992, vol. 1, pp. 585–591.
4. Castro, H. F., Williams, N. H. and Ogram, A., Phylogeny of sulphate-reducing bacteria. *FEMS Microbiol. Ecol*., 2000, **31**, 1–9.
5. Rao, T. S., Sairam, T. N., Viswanathan, B. and Nair, K. V. K., Carbon steel corrosion by iron oxidizing and sulfate reducing bacteria in a fresh water cooling system. *Corros. Sci*., 2000, **42**, 1417–1431.
6. Abedi, S. Sh., Abdolmaleki, A. and Adibi, N., Failure analysis of SCC and SRB induced cracking of a transmission oil products pipeline. *Eng. Failure Analysis*, 2006, **14**, 250–261.
7. Barth, T. and Riis, M., Interactions between organic acid and anions in formation waters and reservoir mineral phases. *Org. Geotherm*., 1992, **19**, 455–482.
8. Miranda, E. *et al*., Biocorrosion of carbon steel alloys by an hydrogenotrophic sulphate-reducing bacterium *Desulfovibrio capillatus* isolated from a Mexican oil field separator. *Corros. Sci*., 2005, **48**, 2417–2431.
9. Almeida, M. A. N. and de Franca, F. P., Thermophilic and mesophilic bacteria in biofilms associated with corrosion in a heat exchanger. *World J. Microbiol. Biotechnol*., 1999, **15**, 439–442.
10. Villanueva, R. A.-C., Martinez, R. C., Garcia-Diaz J. J., Galvan-Martinez, R. and Torres-Sanchez, R., Microbiologically influenced corrosion of steels by thermophilic and mesophilic bacteria. *Mater. Corros.*, 2006, **57**, 543–548.
11. Ehrhardt, M. and Burns, K. A., Determination of petroleum residues dissolved and/or finely dispersed in surface seawater. In

Methods of Sea Water Analysis (eds Grosshoff, K., Kremling, K., and Ehrhardt, M.), Wiley VCH, Weinheim, Germany, 1999, pp. 164–188.
12. Pfennig, N., Widdel, F. and Truper, H. G., The dissimilatory sulphate reducing bacteria, In *The Prokaryotes: A Handbook of Habitat, Isolation and Identification of Bacteria* (eds Starr, M. P. *et al.*), Springer Verlag, Berlin, 1986, pp. 926–942.
13. Wawer, C. and Muyzer, G., Genetic diversity of *Desulfovibrio* spp. in environmental samples analyzed by denaturing gradient gel electrophoresis of [NiFe] hydrogenase gene fragments. *Appl. Environ. Microbiol*., 1995, **61**, 2203–2210.
14. Teske, A. *et al.*, Microbial diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. *Appl. Environ. Microbiol*., 2002, **68**, 1994–2007.
15. Wagner, M., Roger, A. J., Flax, J. L., Brusseau, G. A. and Stahl, D. A., Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate reduction. *J. Bacteriol*., 1998, **180**, 2975–2982.
16. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J., Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res*., 1997, **25**, 3389–3402.
17. Cornell, R. M. and Schwertmann, U., Crystal morphology and size. In *The Iron Oxides, Structure, Properties, Reactions, Occurrence and Uses*, Wiley-VCH, New York, 1996, pp. 58–94.
18. Rubio, C., Ott, C., Amiel, C., Dupont-Moral, I., Travert, J. and Mariey, L., Sulfato/thiosulfato reducing bacteria characterization by FT-IR spectroscopy: a new approach to biocorrosion control. *J. Microbiol. Methods*, 2006, **64**, 287–296.
19. LeGall, J. and Fauque, G., Dissimilatory reduction of sulfur compounds. In *Biology of Anaerobic Microorganisms* (ed. Zehnder, A. J. B.), John Wiley, New York, 1988, pp. 587–639.
20. Widdel, F., Microbiology and ecology of sulphate- and sulfur reducing bacteria. In *Biology of Anaerobic Microorganisms* (ed. Zehnder, A. J. B.), John Wiley, New York, 1988, pp. 469–585.
21. Liamleam, W. and Annachhatre, A. P., Electron donors for biological sulphate reduction. *Biotechnol. Adv*., 2007, **25**, 452–463.
22. Daly, K., Sharp, R. J. and McCarthy, A. J., Development of oligonucleotide probes and PCR primers for detecting phylogenetic subgroups of sulphate reducing bacteria. *Microbiology*, 2000, **146**, 1693–1705.
23. Stams, A. J. M., Kremer, D. R., Nicolay, K., Weenk, G. H. and Hansen, T. A., Pathway of propionate formation in *Desulfobulbus propionicus*. *Arch. Microbiol*., 1984, **139**, 167–173.
24. Minz, D. *et al.*, Diversity of sulfate reducing bacteria in oxic and anoxic regions of microbial mat characterized by comparative analysis of dissimilatory sulfite reductase genes. *Appl. Environ. Microbiol*., 1999, **65**, 4666–4671.
25. Klein, M. *et al.*, Multiple lateral transfers of dissimilatory sulfite reductase genes between major lineages of sulphate reducing prokaryotes. *J. Bacteriol*., 2001, **183**, 6028–6035.
26. Stahl, D. A., Fishbain, S., Klien, M., Baker, B. J. and Wagner, M., Origins and diversification of sulfate respiring microorganisms. *Antonie van Leeuwenhoek*, 2002, **81**, 189–195.
27. Cetin, D., Bilgic, S., Donmez, S. and Donmez, G., Determination of biocorrosion of low alloy steel by sulphate-reducing *Desulfotomaculum* sp. isolated from crude oil field. *Mater. Corros*., 2007, **11**, 841–847.
28. Kuang, F., Wang, J., Yan, L. and Zhang, D., Effects of sulphate-reducing bacteria on the corrosion behaviour of carbon steel. *Electrochim. Acta*, 2007, **52**, 6084–6088.
29. Sanchez, R. T., Vargas, J. G., Alonso, A. A. and Gonez, L. M., Corrosion of AISI 304 stainless steel induced by thermophilic sulphate reducing bacteria (SRB) form a geothermal power unit. *Mater. Corros*., 2001, **52**, 614–618.

Received 13 November 2008; revised accepted 1 June 2009