African Journal of Microbiology Research Vol. 6(32), pp. 6218-6223, 23 August, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.1035
ISSN 1996-0808

Full Length Research Paper

Study on the identification and bio-corrosion behavior of one anaerobic thermophilic bacterium isolated from Dagang oil field

Xijin Zhao[1,2], Ping Wang[1]*, Guihong Lan[3], Peng Shi[3], Xiuli Chen[3] and Jinyu Wang[3]

[1]College of Life Science and Technology, Central South University of Forestry and Technology, Changsha 410004, China.
[2]Sichuan Academy of Environmental Sciences, Chengdu 610041, China.
[3]School of Chemistry and Chemical Engineering, Southwest Petroleum University, Chengdu 610500, China.

Accepted 2 August, 2012

A slightly curved rod strictly anaerobic, thermophilic bacterium, was isolated from Dagang oil field, China. Its fundamental physiology and corrosion characteristic were analysed. The strain BF4 was Gram-positive. The growth occurred at 45-78°C (optimum was 60°C) and pH 4.5-9.5 (optimum was 7). Substrates could be used involving D-glucose, fructose, sucrose, raffinose, mannose, lactose, lactate, and cellobiose. The 16S rRNA gene sequence analysis indicated that the isolate was affiliated to *Caloramator* (99% similarity to *Caloramator australicus* RC3^T). In the presence of lactate, iron (III) and sulfate were reduced. When the strain was cultivated in modified Baar's medium, black precipitation was found. Weight loss method, electrochemical and surface analysis showed the corrosion behavior of the strain. In the presence of the isolate, the corrosion rate was higher compared to the abiotic control. The results of physiological characteristics and bio-corrosion implicated that the strain BF4 is a special SRB. It might play an important role in the corrosion of equipments of the Dagang oil field.

Key words: Bio-corrosion behavior, thermophilic bacterium, sulfate reduce, hydrogen sulfide.

INTRODUCTION

Since the beginning of commercial oil production, nearly 150 years ago, petroleum engineers have faced problems caused by bio-corrosion or microbiologically influenced corrosion (MIC) (Bastin, 1926; Lata et al., 2012). The MIC is the damage caused or accelerated by the presence of microorganisms and their metabolic activities (Miranda et al., 2006). In petroleum engineering, many types of bacteria are associated with the MIC of metals, including sulfate, iron and CO_2 reducing bacteria, sulfur, iron and manganese-oxidizing bacteria (Beech and Sunner, 2004). Among them, sulfate reducing bacteria (SRB) are recognized as the major group in anaerobic corrosion. They can form biofilms, producing H_2S, accelerating iron corrosion and affecting electrochemical processes (Costello, 1974; Pankhania, 1988). The sulfate reductive activity of anaerobic bacteria is thought to be responsible for >75% of the corrosion in productive oil wells, and for >50% of the failures of buried pipelines and cables (Walch, 1992). In oil fields, mesophilic SRB are merely active in close vicinity to the injection well, and thermophilic SRB mainly responsible for the water/oil mixing zone at temperatures between 45 and 100°C. In recent years, the corrosion behavior of some thermophilic SRB in oil fields had been studied (Anandkumar et al., 2009; Lata et al., 2012). However, no results for corrosion behavior of thermophilic SRB in *Caloramator*.

In this paper, one thermophilic anaerobic bacterium was isolated from Dagang oil field, a sulfate reducing and H_2S producing bacterium. We reported and discussed its physiological characteristics and biological corrosion with

*Corresponding author. E-mail: csfuwp@163.com.

metabolism of sulfate.

MATERIALS AND METHODS

Petroleum reservoir characteristics

The Dagang oil field was exploited by water-flooded. The water for injection was separated from the oil produced fluid and recycled. The formations were situated at 1965-1976 m below the sea floor with temperature of 75°C. The formation water (pH 8.3) was sodium hydrocarbonate type with low salinity (4780 mg/L) and low concentration sulfate (8 mg/L).

Sample collection and bacterial isolation

One litter of production fluids was collected into sterile carboys. The technique of modified Hungate (Miller and Wolin, 1974) was used, for isolation. 5 mL of sample was injected into a 120 mL serum bottle containing 50 mL of medium. The temperature of cultivation was 60°C. The strain was stored in -20°C with 30% glycerol. The modified Baar's medium was used as isolation medium, containing (g/L): KH_2PO_4 0.5, NH_4Cl 1.0, Na_2SO_4 1.0, $CaCl_2.2H_2O$ 1.0, $MgSO_4.7H_2O$ 2.0, 60% lactate 5.8, yeast extract 1.0, 1 mL trace elements solution SL 10 (Imhoff-Stuckle and Pfennig, 1983), 1 mg/L resazurin and 0.05% $Fe(NH_4)_2(SO_4)_2$.

Physiological studies

Growth of strain BF4 was tested with TYEG medium as described by Patel et al. (1985a, b). Different carbon sources, temperatures and pH were detected (Christopher, 2009). The growth was determined by counting and measuring the increasing optical density at 600 nm. Fe (III) and sulfate reduction were tested referring to Sørensen (1982). The concentration of sulfate and sulfide were detected by EDTA titration method and iodometric method, respectively.

Molecular identification

Total genomic DNA of isolation was isolated using the TIANamp Bacterial DNA kit (Tiangen Biotech (Beijing) CO., LTD. China) according to the manufacturer's instructions. The 16S rRNA gene was selectively amplified from genomic DNA by polymerase chain reaction (PCR) using 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT- 3') (Takara Biotechnology (Dalian) Co., Ltd. China) as the primers (Heuer et al., 1997). The products were recovered by a DNA Recovery Kit (Takara Biotechnology (Dalian) Co., Ltd. China), ligated with pMD-19T cloning Vector (Takara Biotechnology (Dalian) Co., Ltd. China) and transformed into *Escherichia coli* JM109. Three positive clones were selected randomly for sequencing analysis. Sequence analysis and phylogenetic tree constructions were performed using CLUSTAL_X and MEGA 4.1 software. The GenBank accession number was HM228391.

Weight loss analysis

Q235 carbon steel (Cangzhou City Rui Pipe Co., Ltd. China) was used, which composing of the following elements with a mass ratio of 0.22% C, 0.05% Si, 0.48% Mn, 0.012% P, 0.022% S. After being polished, degreased with aceton and air dried, three carbon steels (size of 1 × 5 cm) were immersed in 250 mL triangular flask with 100 mL Modified Baar's medium and incubated at 60°C for 30 days. Duplicate systems were maintained, one was inoculated with 5 mL of separation and the other was blank control. A period of time later, some carbon steels were removed in pickling solutions, washed with water and dried. The average loss weight of the coupons in each culture and uninoculated control was calculated.

Polarization study

Q235 carbon steel was used as a working electrode in this study. It was plugged in araldite with an exposed area of 1.0 cm^2. The specimens were immersed in 250 mL triangular flask with 100 mL Modified Baar's medium and was incubated at 60°C for 30 days in sterile as well as 5 mL of BF4 inoculated in Modified Baar's medium. A platinum plate and a saturated calomel electrode (SCE) were used as a counter electrode and a reference electrode, respectively (Fei et al., 2007). The Tafel polarization curves were obtained according to the reference (Anandkumar et al., 2009).

Scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis

After being cultured, the carbon steel specimens were taken out and dried in cool air. The surface morphological characteristics of metal coupons were surveyed by SEM (Quanta 450). The corrosion products were observed by XRD (X' Pert MPD PRO) analysis.

RESULTS

Isolation and identification

A strictly anaerobic, thermophilic bacterium, designated as strain BF4, was isolated. The bacterium was Gram-positive, slightly curved rods. The growth of the bacterium occurred at 45-78°C (optimum 60°C), pH 4.5-9.5 (optimum 7). Strain BF4 could use many carbon sources as energy sources, including D-glucose, fructose, sucrose, raffinose, mannose, lactose, lactate, and cellobiose. In the presence of lactate, iron (III) and sulfate were reduced, and the product of sulfate reducing was hydrogen sulfide. It indicated that the strain BF4 was one of thermophilic fermentative and sulfate reducing bacteria. 5 mL of the cells were inoculated in 50 mL Modified Baar's medium, and cultivated at 60°C. The concentration of sulfate decreased rapidly from day 1 to 5, without obvious changes after 7 days. While the concentration of sulfide increased rapid from day 1 to 5, and without obvious changes after 7 days (Figure 1).

Nucleotide database was searched with the sequences obtained using NCBI BLAST. The 16S rRNA sequence analysis indicated that strain BF4 was related to the members of the genus *Caloramator*, most closely to *Caloramator australicus* $RC3^T$ (99% similarity to the type strain) (Figure 2).

Weight loss analysis

After being cultivated, a thin layer of loose and black film was found out of carbon steel in inoculated system

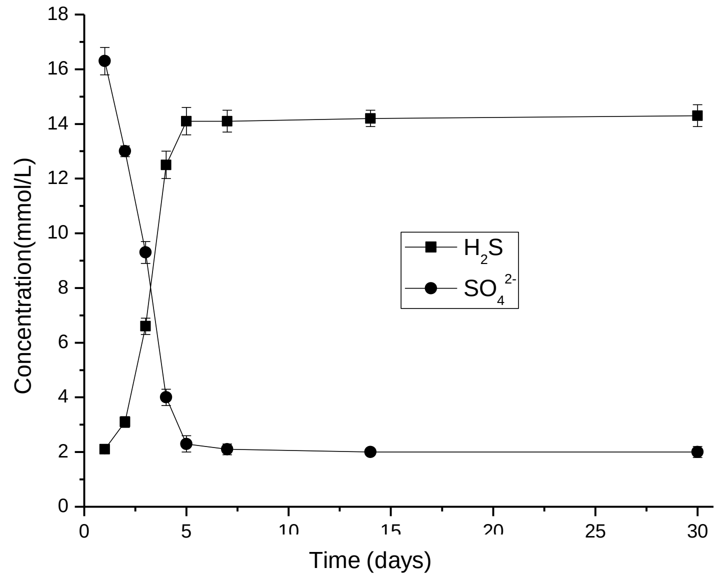

Figure 1. Change of sulfate and sulfide concentration.

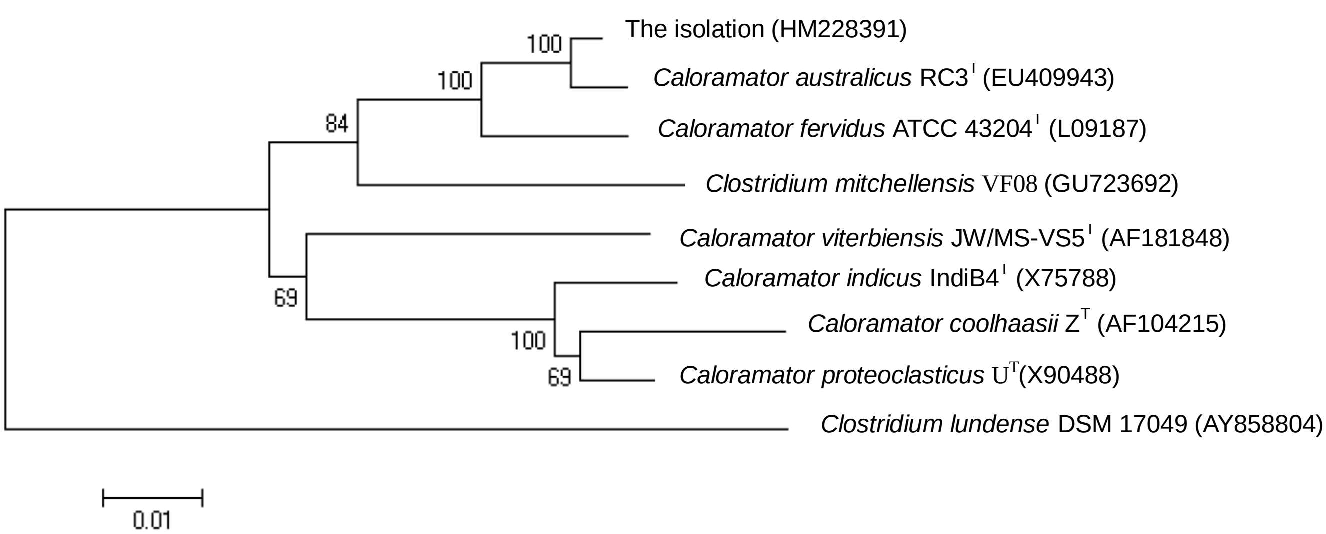

Figure 2. Phylogenetic tree of BF4 and its related bacteria constructed using 16S rRNA sequences.

compared with abiotic control system, the corrosion rate was higher in the presence of strain BF4.

Polarization study

Corrosion currents were increased when cultivated with BF4 (Figure 4). The corrosion current was 1.412e-005 A/cm^2 in the abiotic control, while 3.575e-005 A/cm^2 and 3.910e-005 A/cm^2 after the cultivation for 7 and 30 days with BF4 respectively.

XRD and SEM analysis

The surface morphological characteristics of metal specimens were surveyed by SEM (Figure 5). The SEM analysis showed that the surface of carbon steel was covered by a layer of corrosion film and some bacterial cells (Figure 5a). The bacterial cells were rod-shaped (Figure 5b), uneven gathered on surface of the carbon steel. The corrosion products, thought to be related to microbial activites of strain BF4, on the crabon steel surface were revealed by X-ray diffraction (XRD). Peaks

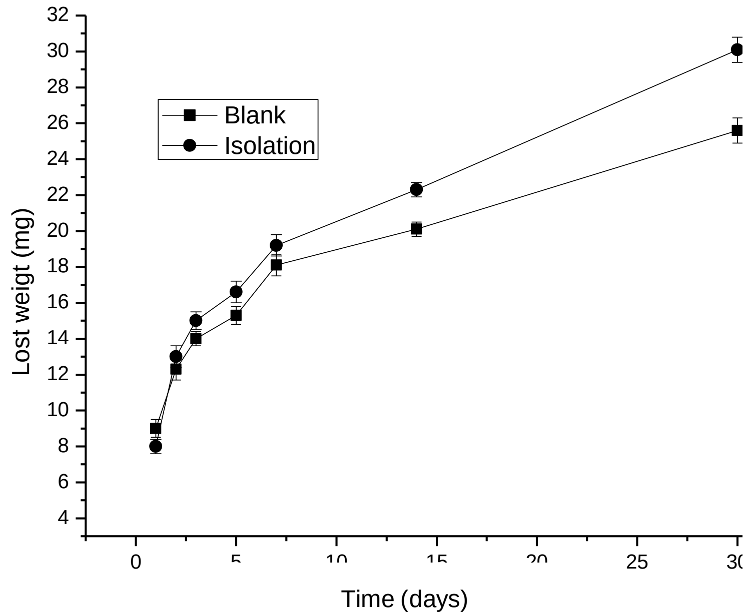

Figure 3. Corrosion rate of carbon steel.

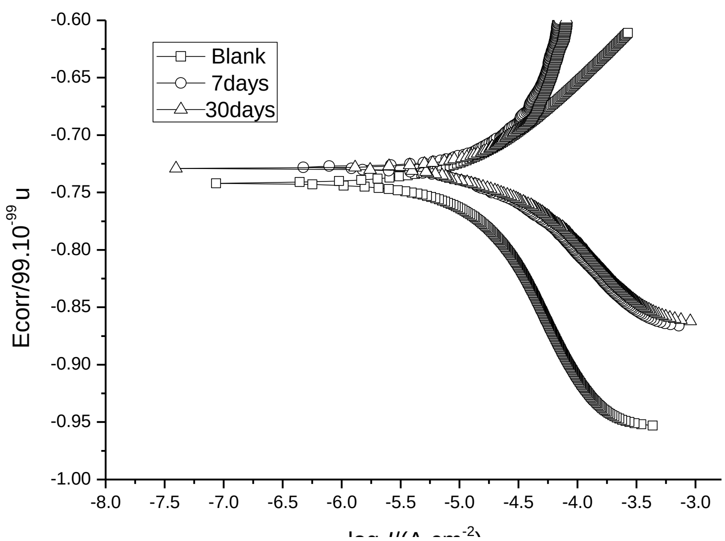

Figure 4. Potentiodynamic polarization curves of Q235 carbon steel in cultivated and control system at different growing stages of BF4.

of higher intensity of Fe_2O_3, FeS, $Fe(OH)_2$ and $FeCO_3$ in the experimental system were observed (Figure 6).

DISCUSSION

An anaerobic, thermophilic fermantive and sulfate reducing bacterium (BF4) was isolated and studied in this paper. The isolate was 99% of 16S rRNA gene sequence similarity to *C. australicus* RC3^T. The genus *Caloramator* have 7 species, most of them are metal-reducing bacteria, and potential biological corrosion bacteria (Christopher et al., 2011), while none of them are known as sulfate-reducing bacteria.

Strain BF4 was able to grow quickly, conserving energy for growth form sulfate reduction, with an optimum temperature and pH value (Figure 1). Compared with control system, higher corrosion current (Figure 3) and corrosion rate of carbon steel (Figure 4) were found in the inoculated system. The corrosion mechanism of SRB

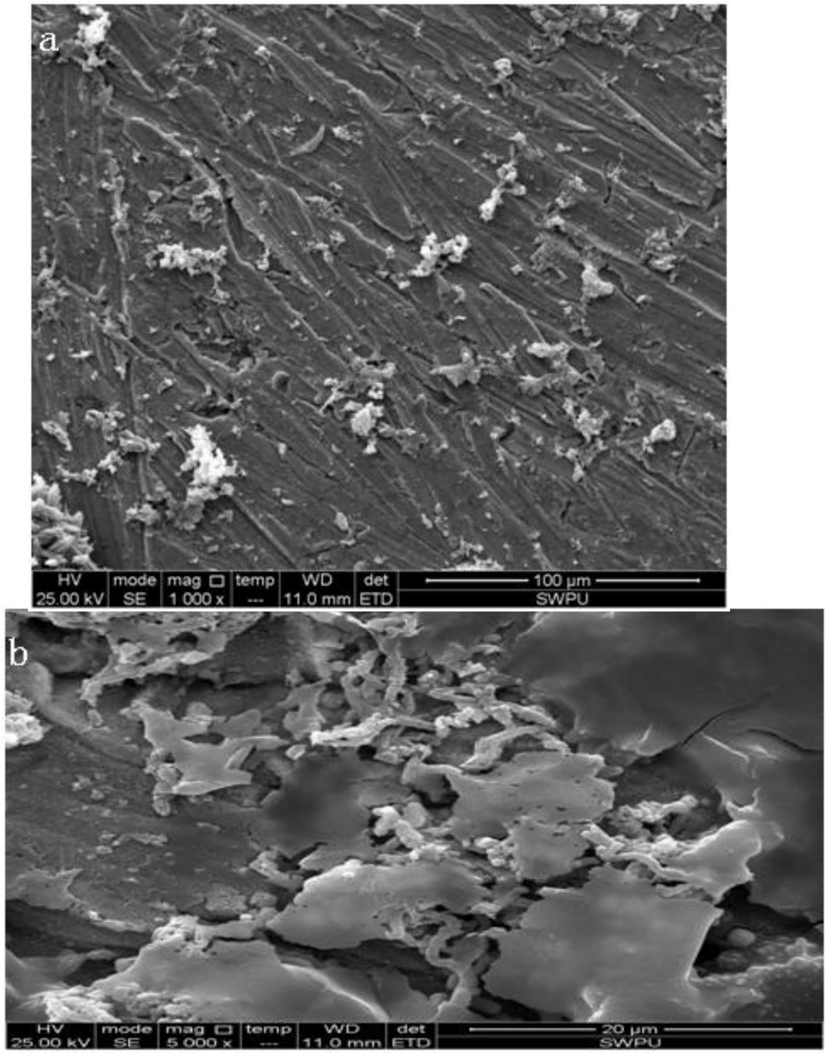

Figure 5. Scanning electron micrograph of carbon steel coupon exposed to BF4 (a, b).

mainly includes H_2S chemical corrosion and corrosion polarization, the cathodic depolarization theory is thought as the mainly mechanism of corrosion caused by SRB and frequently discussed (Von and Van der, 1934). In anaerobic and moist environment, metal is polarized with positive metal ions lost (anodic reaction).

Proton is derived from reducing of the free electrons (cathodic reaction), and remained on the surface of metal, where a dynamic equilibriums hypothesis was set up (Dinh, 2003). The formed hydrogen is supposed as SRB surface of metal, a net oxidation of metal takes place. Only one fourth of the dissolved Fe^{2+} would be changed stoichiometrically into FeS. At the anodic site, $FeCO_3$ or $Fe(OH)_2$ usually acts as the main precipitates, depending on the existence of CO_2 and bicarbonate (Dinh, 2003). The net reaction of SRB corrosion is shown as follows;

Equation 1:

$$4Fe + SO_4^{2-} + 3HCO_3^{-} + 5H^{+} \longrightarrow FeS + 3FeCO_3 + 4H_2O \quad (1)$$

The results of SEM (Figure 5) and X-ray (Figure 6) supported the corrosion mechanism of SRB, since bacterial films and corrosion products were stick to the surface of carbon steel, involving Fe_2O_3, FeS, $Fe(OH)_2$ and $FeCO_3$.

Conclusion

The main objective of this study is to isolate new sulfate reducing bacteria from oil reservoir and reveal their bio-corrosion process. The results suggest the isolation is a new SRB, that can survive in the subsurface floor of the

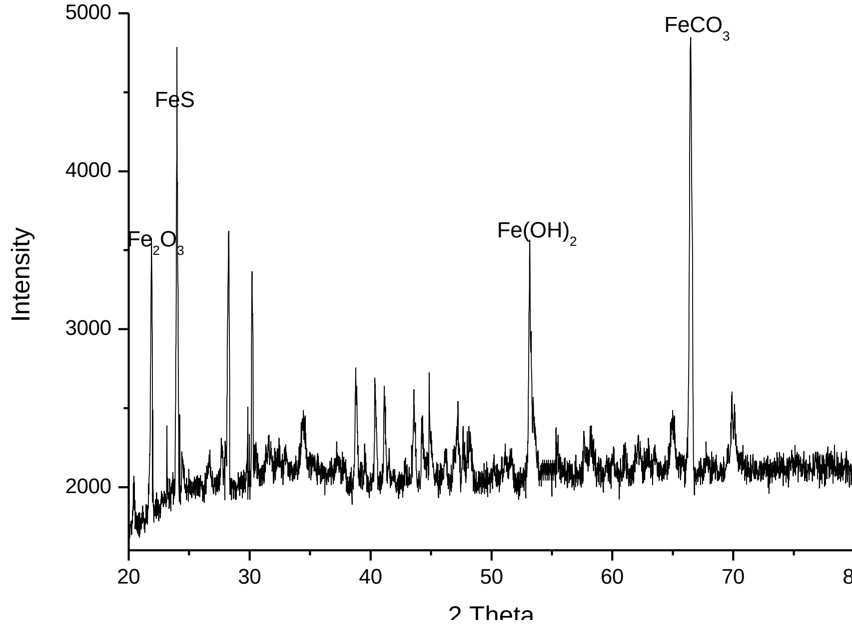

Figure 6. XRD pattern of the corrosion product.

Dagang oil field (75°C), and it would cause serious corrosion of the oil reservoir equipments. The results also reveal that more new SRB and mechanism of SRB would be found, if more isolation and researches are done.

ACKNOWLEDGEMENT

This work was supported by Research Project (2010HBY002) of Sichuan Environmental Protection of Sichuan.

REFERENCES

Anandkumar B, Choi JH, Venkatachari G (2009). Molecular characterization and corrosion behavior of thermophilic (55°C) SRB *Desulfotomaculum kuznetsovii* isolated from cooling tower in petroleum refinery. Mater. Corros. 60(9):730-737.

Bastin ES (1926). The problem of the natural reduction of sulphates. Bull. Am. Assoc. Petrol. Geol. 10:1270–1299.

Beech IB, Sunner J (2004). Biocorrosion: towards understanding interactions between biofilms and metals. Curr. Opin. Biotechnol. 15: 181-186.

Christopher D, Bharat OGG, Patel KC (2009). *Caloramator australicus* sp. nov., a thermophilic, anaerobic bacterium from the Great Artesian Basin of Australia. Int. J. Syst. Bacteriol. 59:95–101.

Christopher D, Bharat OGG, Patel KC (2011). Draft Genome Sequence of *Caloramator australicus* strain $RC3^T$ a thermoanaerobe from the Great Artesian Basin of Australia. J. Bacteriol. 193(10):2664-2665.

Costello JA (1974). Cathodic depolarization by sulfate-reducing bacteria. S. Afr. J. Sci. 70:202-204.

Dinh THH (2003). Microbiological of the anaerobic corrosion of iron. PhD thesis, University of Bremen, Bremen, Germany. pp. 11-13.

Fei K, Jia W, Li Y (2007). Effects of sulfate-reducing bacteria on the corrosion behavior of carbon steel. Electrochim. Acta. 2:6084-6088.

Heuer H, Kresk M, Baker P, Smalla K, Wellington EM (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic searation in denaturing gradients. Appl. Environ. Microbiol. 63:3233-3241.

Imhoff-Stuckle D, Pfennig N (1983). Isolation and characterization of a nicotinic acid-degrading sulfate-reducing bacterium, *Desulfococcus niacini* sp. nov. Arch. Microbiol. 136:194-198.

Miller TL, Wolin MJ (1974). A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. Appl. Microbiol. 27:985-987.

Miranda E, Bethencourt M, Botana FJ, Cano MJ, Sánchez-Amaya MJ, Corzo A, García de Lomas J, Fardeau ML, Ollivier B.(2006). Biocorrosion of carbon steel alloys by an hydrogenotrophic sulfate-reducing bacterium *Desulfovibrio capillatus* isolated from a Mexican oil field separator. Corros. Sci. 48:2417-2431

Pankhania IP (1988). Hydrogen metabolism in sulfate-reducing bacteria and its role in anaerobic corrosion. Biofouling. 1:27-47.

Patel BKC, Morgan HW, Daniel RM (1985a). A simple and efficient method for preparing and dispensing anaerobic media. Biotechnol. Lett. 7:277-288.

Patel BKC, Morgan HW, Daniel RM (1985b). *Fervidobacterium nodosum* gen. nov. sp. nov., a new chemoorganotrophic, caldoactive, anaerobic bacterium. Arch. Microbiol. 141:63-69.

Sørensen J (1982). Reduction of ferric iron in anaerobic, marine sediment and interaction with reduction of nitrate and sulfate. Appl. Environ. Microbiol. 43:319–324.

Lata S, Sharma C, Singh AK (2012).Microbial influenced corrosion by thermophilic bacteria, Cent. Eur. J. Eng. 2(1):113-122

Von WKC, Van der VIS (1934). The graphitization of cast iron as an electrobiochemical process in anaerobic soil. Water. 18:147-165.

Walch M (1992). Microbial corrosion. Lederberg J. (eds) Encyclopaedia of Microbiology, Academic Press, New York, pp. 585–591.