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Effect of Sulphate reducing bacterial-biofilm isolated from refinery cooling water system and sonication on corrosion of carbon steel

Jaya Rawat*, Apoorve Khandelwal, Neha Sharma and Sudha Tyagi

Corporate R&D Center, Bharat Petroleum Corporation Limited,Greater Noida (UP), India

** Corresponding author. E mail: jayarawat@bharatpetroleum.in, Tel : 01202354128*

Abstract

Biofilm formation is one of the major problems of recirculating cooling water systems which detriments the life of equipment, through biocorrosion or microbiologically influenced corrosion (MIC). Sulfate reducing bacteria (SRB) are considered to be the major bacterial group involved in MIC. In the present study, SRB cultures are isolated from aeration basin inlet water sample of cooling water system of refinery and immersion test is done to analyze the effect of SRB-biofilm on the corrosion of carbon steel. Furthermore, the effect of sonication on total microbial count, SRB count and corrosion rate is evaluated. Experimental results concluded that SRB biofilm enhances the corrosion rate, involving a number of general and localized features on the metal surface, which were interpreted by Scanning electron microscopy (SEM). SEM studies, of metal surface showed amorphous and crystalline types of biofilm. Also, the efficacy of sonication is evidenced by a significant decrease in microbial counts of water sample and the corrosion rate of carbon steel.

Keywords: Biofilm; Microbiologically Influenced Corrosion; Sulphate Reducing Bacteria; Sonication; Corrosion Rate; Scanning Electron Microscope

Introduction

Cooling water systems are integral part of any refinery and power plants to dispose off surplus amount of heat generated in many industrial processes. Cooling system provides an ideal aquatic environment (nutrient supply, pH, temperature etc) for the micro organism multiplication [1] (Doğruöz et al. 2009). Most affected regions of a cooling system are cooling water intake tunnels, culverts, pump chambers and heat exchangers. Biofilm formation and microbial corrosion are the major problems of circulating cooling water system that damages expensive equipments causing loss of production and increased maintenance cost [2] (Ilhan-Sungur and Cotuk 2010). Microbiologically influenced corrosion (MIC) can be defined as an electrochemical process in which microorganisms initiate, facilitate, or accelerate the corrosion reaction of metals without changing its electrochemical nature. Microorganisms influence corrosion by changing the electrochemical conditions at the metal–solution interface (Videla and Herrera 2005). Microbial corrosion can accelerate most forms of corrosion; including uniform corrosion, pitting corrosion, crevice corrosion, galvanic corrosion, intergranular corrosion, dealloying, and stress corrosion cracking. Biofilm consist of microbial cells and extra cellular polymeric substances (EPS) which irreversibly attach to metal surfaces, resulting in the formation of complexes with metal ions released by oxidation, which there by accelerates corrosion (Beech 2004; Singh et al 2011).

Biofilm comprises of a complex consortium of aerobic, facultative and anaerobic bacteria, wherein each microbe type occupies a characteristic location. It has been reported that MIC leads to corrosion of stainless steel, carbon steel, aluminum, zinc and copper alloys (Shi et al 2011). MIC is majorly caused by Sulphate reducing bacteria (SRB) under anaerobic conditions. They transform sulfur to hydrogen sulfide which, in the presence of ferrous ionic compounds tend to form ferrous sulfides (Al-Zuhair et al 2008; Castaneda and Benetton 2008). The presence of sulfide plays an important role in corrosive action of SRB. In the presence of SRB, steel and other iron alloys corrode four times faster than with normal oxygen promoted corrosion (Coetser and Cloete 2005). So, it is very important to understand and identify the corrosion pattern formed on the metal

surface due to SRB biofilm, which serves as an evidence of MIC. A key feature of MIC includes presence of several smaller pits (Jack 2002) and micro organisms attached to metal surface. Therefore, in this study microbiologically influenced corrosion of carbon steel surface caused by SRB isolated from cooling tower water was investigated by SEM analysis under laboratory conditions.

Furthermore, as far as microbial control is concerned, there are different methods suggested to prevent microbial corrosion include, careful selection of metallurgy, removal of bacterial nutrient sources and environmental niches of growth, maintaining a high quantity of make-up water having low bacterial count etc. It is currently a standard practice to use chemical biocides (i.e. free chlorine) which helps in the decrease of corrosion rate. Excess chlorination may lead to (a) reactions with dissolved chemicals to produce harmful by-products, (b) the build-up of resistance to chlorination in micro-organisms, (c) discoloration and the production of unpleasant odour and (d) ineffective killing of micro organisms present inside the agglomerates (Duckhouse et al. 2004). So the environmental concern encourages the replacement of toxic biocides with effective & environmentally safe methods of controlling microbial growth.

Several genetically regulated factors influences biofilm development and structure, however, the physical forces acting on the biofilm can also influence structure of the biofilm (Stoodley et al 2002). Among the physical/non-chemical methods to control microbial growth, sonication treatment has been of immense academic and industrial interest because it eliminates the hazards, costs & complexity associated with chemical disinfection technique. It may also avoid bacteria from becoming resistant to the chemical disinfectants.

Cavitation is the formation, growth, and implosion of vapor bubbles in a liquid. They can be created by sound waves (known as ultrasonic or acoustic cavitation), lasers, or by fluctuations in fluid pressure (known as hydrodynamic cavitation) (Gaines et al 2007). Acoustic cavitation produces a powerful effect by inducing the formation and collapse of micro bubbles, occurring in milliseconds and producing extreme temperature and pressure gradients (Foladoria et al 2007). It can be broadly divided into two types, transient and stable. The former occurs when the cavitation bubbles, filled with gas or vapour, undergo irregular oscillations and finally implode. This produces high local temperatures and pressures that disintegrate biological cells. In contrast, during stable cavitation, bubbles oscillate in a regular fashion for many acoustic cycles (Mason et al 2003). Several processes resulting from the collapse of these cavitation bubbles are responsible for bacterial inactivation:

•Pressure gradients resulting from the collapse of gas bubbles which enter the bacterial solution on or near the bacterial cell wall can cause mechanical fatigue of cells, over a period of time.

•Shear forces induced by micro-streaming that occurs within bacterial cells.

•Chemical attack due to the formation of radicals during cavitation in the aqueous medium can also cause cellular damage

•Amongst the final products of this sonochemical degradation of water is hydrogen peroxide (H2O2), which is a strong bactericide (Joyce et al. 2003). Based on the literature, present study was to investigate SRB isolates from cooling tower water and effect of SRB-biofilm on corrosion of carbon steel coupons under laboratory culture conditions. Furthermore, this study is an attempt to develop sonication as an environment friendly method to mitigate the problem of MIC. Effectiveness of sonication on microbial growth and corrosion rate of carbon steel is also explored.

2. Materials and methods

2.1 Sample collection

Aeration basin inlet water sample was collected aseptically from a cooling tower of a petroleum refinery (Mumbai, South India). Physiochemical analysis of water sample was done using water analysis kit (Merck India Ltd).

2.2 Enumeration of microbes

Microbiological tests of collected water sample were done to enumerate the total bacterial count and the number of sulphate reducing bacteria (SRB), before and after sonication. Viable plate count (CFU/ml) techniques were

used as a measure of microbial activity. The collected sample was serially diluted (10 fold) using 9 ml of sterile distilled water blanks. Serial dilution was done upto 10^{-6} dilution. The sterile nutrient agar media was poured into sterile petridishes and the plates were kept in incubator at 37^0C for 24 hours to check for contamination. One ml aliquot of the each dilution was inoculated using spread plate method and the plates were prepared in triplicate for each dilution. Plates (inoculated and control) were incubated for 24 h at 37^0C and after completion of incubation period, colonies were counted. The plates containing bacterial colonies with 30-300 numbers were selected for calculation of CFU/ml. Sulphate reducing bacteria enumeration was done using Hi-Surba Kit from Himedia.

2.3 Sonication study

Water sample was sonicated for different time intervals at 30°C in the sonicator to evaluate the effect of sonication on microbial counts. Sonicated water samples were analysed for total bacterial count and sulphate reducing bacterial count by the same procedure mentioned above in the section 2.2, for the enumeration of microbes in the water sample before sonication.

2.4 Test coupons

Carbon steel was used as test material since it is frequently preferred as construction material in cooling towers. The coupons (53×11×1 mm) were prepared according to guidelines ASTM G1-72 standards (American Society for Testing Material, 1975). Test coupons were weighed and the total surface area of each coupon was determined.

2.5 Immersion tests for biofilm study

Carbon steel test coupons were mechanically polished to mirror finish and then degreased using trichloroethylene. Polished coupons were immersed in 50 ml of Sulphate reducing medium (Twin pack from Himedia) inoculated with 5% of SRB isolate in anaerobic bottles and kept at 37°C in an incubator (Cryoscientific Ltd, Chennai). Immersion test (control and inoculated) was done in duplicates for each set of experiment as described in table-1. After 48 hours, first set of test coupons were removed from the anaerobic bottle and examined under SEM. Second set of test coupons were examined after 24 days to study biofilm and the effect of SRB biofilm on metal surface. Third set of test coupons exposed to inoculated SRB medium was kept for 40 days to study the biofilm at later stages.

Table: 1 Immersion test under anaerobic conditions for biofim study

Experiment in anaerobic bottle (in duplicates)	Exposure time
SRB medium + test coupon	Control
SRB medium + 5% inoculum + test coupon	48 hours
SRB medium + 5% inoculum + test coupon	24 days
SRB medium + 5% inoculum + test coupon	40 days

2.6 SEM analysis

Scanning Electron Microscopic analysis was done on the corroded coupons after 48 hours, 24 days and 40 days to study morphology of organisms and metal surface before and after removal of biofilm. Coupons were fixed with 2.5% glutaraldehyde, followed by dehydration in a graded series of ethanol and air-drying. The dried samples were coated with a gold layer (30 nm) and imaged with a scanning electron microscope (TESCAN, Vega LSU).

2.7 Weight loss method

The coupons were prepared according to guidelines ASTM G1-72 standards (American Society for Testing Material, 1975). Pre-weighed test coupons were exposed to SRB medium, uninoculated which serves as control

and inoculated with sonicated water samples. Duplicate systems were maintained for the weight loss study. After the exposure for 24 days, the coupons were removed and cleaned in pickling solutions (15% hydrochloric acid solution containing 5% stannous chloride and 5% antimony trioxide as inhibitor), then washed with running water followed by acetone to dry it. Final weights of the coupons in each uninoculated media (control) and inoculated media were taken to evaluate the average corrosion rates as per standard procedure. The difference between the initial and final weight was reported as weight loss. The corrosion rate of the test coupons was calculated in mils per year (MPY) assuming uniform corrosion over the entire surface of the coupon according to the following equation proposed by the ASTM standard G 1-72.

Corrosion rate = 141.616 × W / A T D,

Where: W = Weight loss (g), A =Initial exposed surface area of coupon (mm^2), T =Exposure time, (days), D =Density of coupon metal ($g\ cm^{-3}$).

3. Results and discussion

3.1 Physicochemical properties

Physical and chemical properties of water sample are assessed by water analysis kit from Merck summarized in table 2. Analysis revealed the sulphate contents in water sample which are favourable for SRB growth (Ghazy et al 2011).

Table: 2 Physiochemical analysis of aeration basin inlet water sample

Test parameter	Result (mg/L)
pH	8.64
BOD	20
COD	110
TSS	<25
Iron	0.66
Chloride	150
Ammonia(NH^{4+})	46
Sulphide	0.025
Phenol	14.5
Sulphate	580
Total nitrogen	54

3.2 Sonication study

Microbiological studies revealed that the total microbial count and sulphate reducing bacterial count in water sample was found to be 1.1 X 10^6 CFU/ml and 10^5/ml respectively. Water sample was sonicated for different time intervals and plated in order to monitor the effect of sonication on total bacterial count and SRB count. Sonication significantly affected the total microbial count and SRB count as shown in figure 1, 2. These results indicate that sonication initially gives a rise in cell numbers suggesting de-clumping of the bacteria. Sonication is able to deagglomerate bacterial clusters or flocs through a number of physical, mechanical and chemical effects arising from acoustic cavitation. Initially sonication contributes in declumping of bacteria which breaks up bacterial clumps into a greater number of individual bacteria and later on results in continuous reduction in viable cell count i.e. after few minutes of sonication declumping finishes and killing predominates. The initial rise in cell number falls with further increase in duration of sonication.

3.3 Immersion test

Immersion tests are done by exposing test coupons to SRB medium (control and inoculated). The SRB growth is confirmed by smell of H_2S in the anaerobic bottle and blackening due to formation of biogenic sulfide (Ghazy et al. 2011). The test is done for 48 hours, 24 and 40 days with the aim of characterizing the SRB biofilm and its effect on metal surface. SEM provides information about the morphology of microbial cells and colonies, the

distribution of microbial colonies on the surface, presence of EPS and the nature of corrosion products (Beech and, Gaylarde 1999).

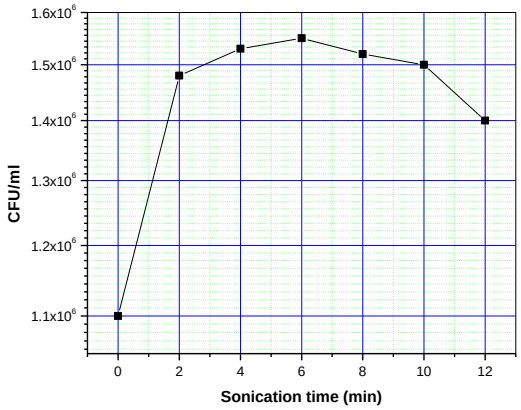

Figure 1 Effect of sonication on microbial count of aeration basin inlet sample

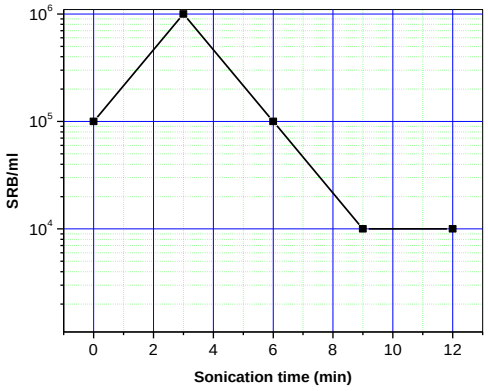

Figure 2 Effect of sonication on SRB count of aeration basin inlet sample

3.4 SEM analysis of biofilm formed on metal surface after immersion test

Immersion test for 48 hours

In the SEM analysis of metal coupons exposed to the sterile medium (control) for 48 hours no biofilm formation was observed (figure 3B).The biofilm formed on the metal surface exposed to inoculated media for 48 hours were analyzed for the presence of SRB (figure 3C, D). Bacterial adhesion on the coupons during the initial stages of exposure was confirmed by SEM studies. After 48 hours biofilm was found to be less dense and rod shaped bacterial morphology was also observed.

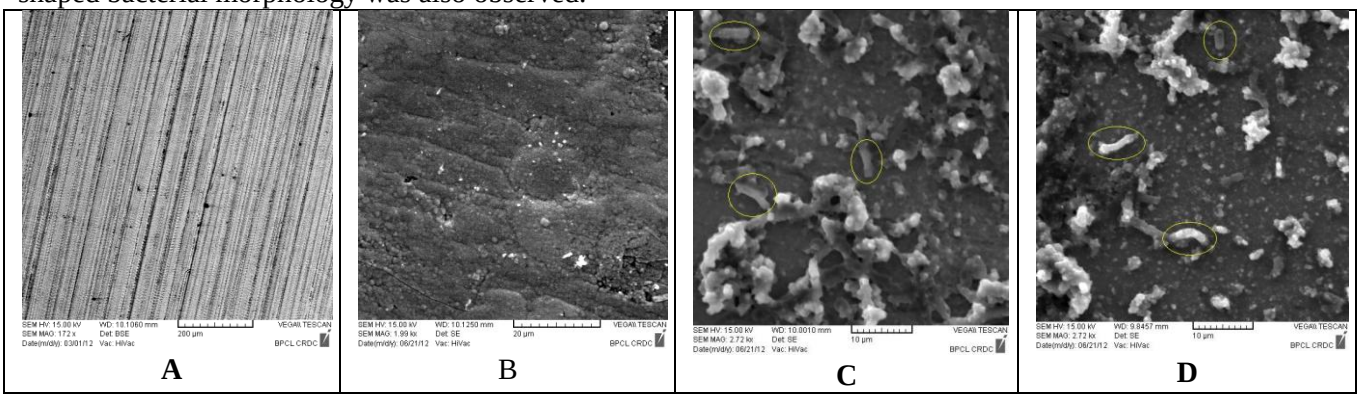

Figure 3- (A) Carbon steel metal surface before immersion test; (B) Metal surface exposed to sterile media showing no biofilm formation after 48 hours; (C, D) SRB attached on the surface of carbon steel after 48 hour exposure

3.5 Immersion test for 24 days

After 24 days of Immersion test, two types of biofilm (amorphous and crystalline) were observed on the metal surface (figure 4). Extra cellular polymeric substance (EPS) and voids within biofilm were also observed. One of the most important properties of EPS is its ability to bind with the metal surface. EPS helps in determining the architecture and also provides strength to the biofilm (Stoodley et al 2002). Also, EPS contributes to the formation of corrosion cells and promotes galvanic coupling, thus influencing the electrochemical behavior of m the metal (Ford et al. 1988; King R A 2008). It helps in survival of fragile bacterium from the external adverse conditions by the formation of thick outer layer (King R A 2008).

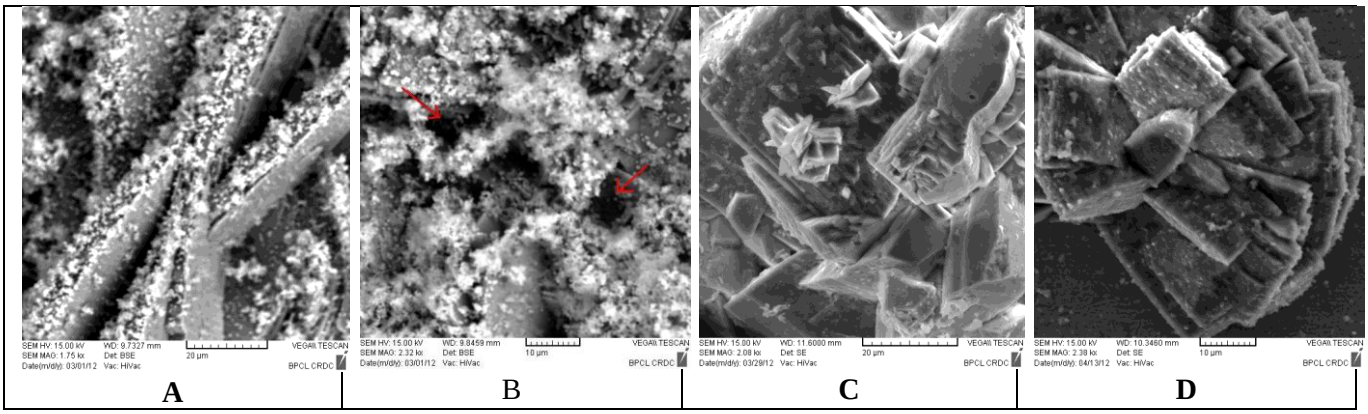

Figure 4-(A, B): Amorphous biofilm (arrows pointing voids within the biofilm) (C, D): crystalline biofilm formation on metal surface

3.6 Immersion test for 40 days

After 40 days of exposure to inoculated media, the entire metal surface was found to be covered by biofilm. Although the cell bodies were not clearly visible on the biofilm (figure 5a), it is quite possible that cells were embedded within the EPS matrix. Later on bacterial spores were also seen which indicates the presence of spore forming species of SRB in the water sample (figure 5b). The interaction of spores with metal surface is a challenge in biofilm control owing to its stronger attachment and persistence when the spores are formed under adverse environmental conditions (Harimawan et al, 2013). Further studies can be done to identify the spore forming species in the water sample of cooling systems in refinery and the role of spore forming bacteria in MIC can be explored.

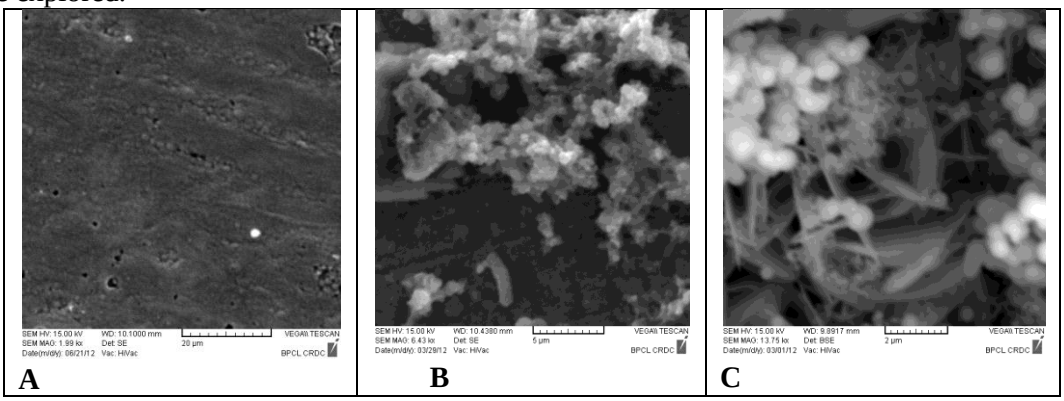

Figure 5 (A) Control showing no biofilm formation after 40 days; (B) Dense biofilm with SRB embedded and (C) Spores are observed in biofilm after 24 days exposure

3.7 SEM analysis of metal surface after removal of biofilm

After completion of immersion test for 24 days, the deterioration of metal surface due to microbial biofilm was qualitatively assessed using scanning electron microscopy (SEM). The coupons were cleaned according to the

procedure described under the ASTM G1-72. SEM analysis, of metal surface after removal of biofilm, reveals the type of attack by microbes (e.g. pitting or uniform corrosion) by visualizing changes in microstructure and surface features as also observed by Beech and Gaylarde (1999). It has been suggested that the mechanism through which these bacteria could act involves the formation of hydrogen sulfide leading to precipitation of iron sulfides and the formation of elemental sulfur (Castaneda and Benetton 2008). Under these conditions, SRB are likely to promote the formation of pits beneath the sulfide deposits. After removing the biofilm, cracks (crevice corrosion), oxide film and micropits (Fig-6 a, b) were observed on the surface. Also local areas of black colored surface were noticed as also observed by Antony et al (2007).

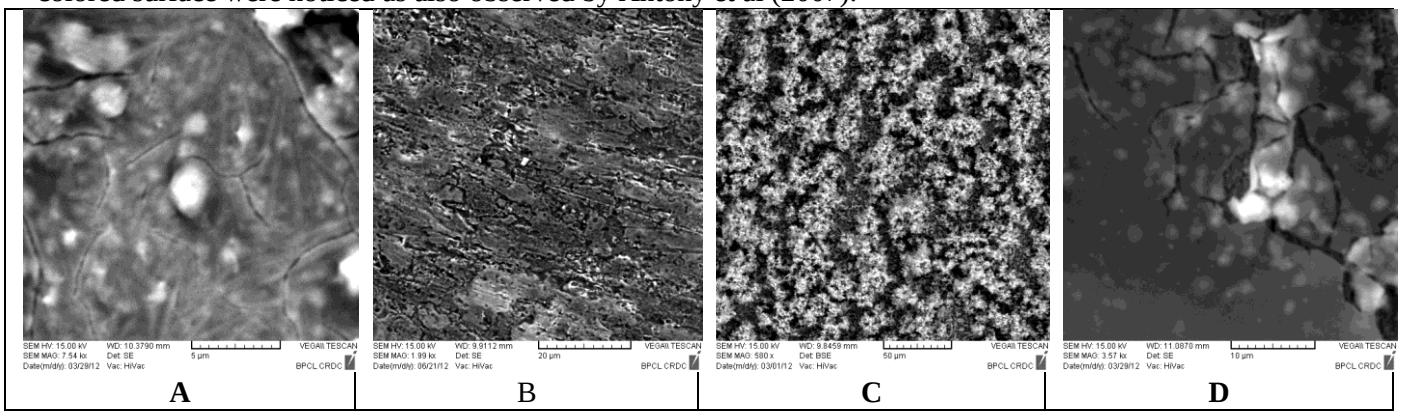

Figure 6 (A, B) Micrographs of metal surface after removal of Biofilm showing Oxide film and rough surface; (C, D) tubercle surface and biofilm in tubercle surface

Corrosion product on the metal surface was detected as tubercles (figure 6c, 6d). Corrosion product was relatively porous within the tubercle, whereas inside the bulk material it was much denser and cracked. Similar finding were also stated by Huttunen-Saarivirta et al (2012). Metal coupons exposed in a sterile medium exhibited either no or limited localized corrosion (figure 7a) whereas metal coupons exposed to inoculated media exhibited major modifications on metal surface due to biofilm formation (figure 7b, 7c, 7d).

Pitting attack was observed to increase in the presence of the bacteria and with time. Big pits without regular edges were observed on the surface of the carbon steel with SRB (figure 7c). Whereas on the surface of the carbon steel exposed to sterile media, much smaller pits with regular edges were observed (figure 8a). The average area of the pits on the carbon steel surface exposed to SRB was much bigger than the coupon exposed to media without SRB. These studies are in agreement with the result of other studies (Xu et al 2011).

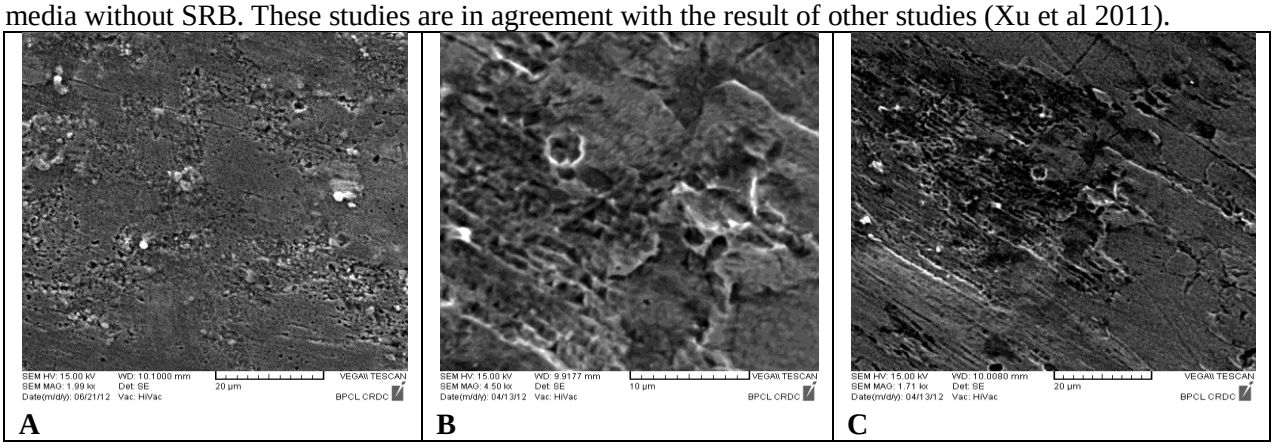

Figure 7 (A) Carbon steel metal surface after exposure to sterile media
(B, C,) Micrograph of pits on the metal surface exposed to SRB medium for 24 days

3.8 Corrosion Rate calculation by Weight loss method

Weight loss studies were done using control (media without inoculum), media inoculated with non-sonicated water sample and the media inoculated with water samples sonicated for different time intervals. In this study, a decrease in corrosion rate of carbon steel with an increase in duration of sonication of water sample was

observed as shown in the figure 8. The reduced corrosion rate can be co-related with reduction in number of SRBs due to sonication of water sample which in turn, lessen the corrosion rate of the metal. No further significant decrease was observed in corrosion rate after 10 minutes of sonication.

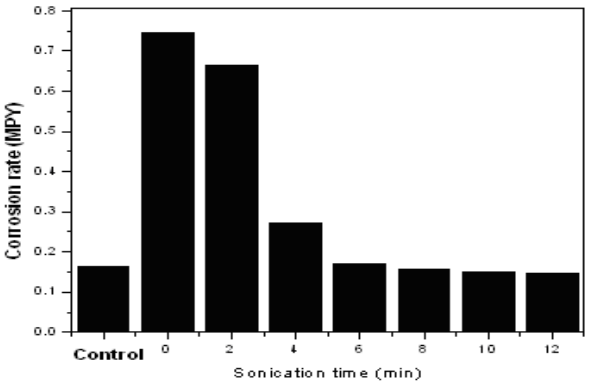

Figure 8 Effect of sonication on corrosion rate

The coupon which was immersed in the medium inoculated with non-sonicated water sample (control) exhibited the highest corrosion rate, which is the evidence of enhanced corrosion attack on carbon steel due to SRBs under anaerobic conditions. This is usually explained by corrosiveness of H2S formed by SRBs (Enning 2012). Also, according to one of the mechanisms proposed for corrosion due to SRB, enhanced corrosion of metal in presence of SRB can be due to the formation of biogenic sulfide (FeS) on metal surface can induce a local decrease in pH enhances the breakdown of passive film further may lead to activation of corrosion cells between the steel surface as anode and the FeS as cathode (Kakooei et al 2012).
Detection of SRB in the biofilm formed on metal surface cannot be considered the only evidence for its role in MIC. For detailed analysis, specific activities of the microbes at the site where corrosion is occurring should be studied. It can be done by means of SEM by visualizing changes in microstructure and surface features after removal of the biofilm and corrosion products. Immersion tests followed by SEM analysis for the qualitative assessment biofilm formed on carbon steel metal coupons, exposed to the SRB medium, showed that the corrosivity of the solution increased with the presence of SRB and increase in incubation time.

Conclusion

In conclusion, carbon steel can undergo enhanced corrosion in the presence of SRB under anaerobic conditions. SEM analysis of metal surface showed the pitting attack, a key feature of MIC. SEM studies of metal surface and microstructure can be helpful in understanding the interaction of metal and microbes and its effect on the metallurgy. Sonication of water sample significantly decreased the total microbial count, SRB count and the corrosion rate of carbon steel in the SRB media. It may be concluded that the main benefit of this study is to understand the corrosion of carbon steel in the presence of SRB-biofilm and potentiality of sonication to control MIC. Furthermore, implementation of sonication as a tool for preventing microbial corrosion can be explored.

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