JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

Microbial Influenced Corrosion by Thermophilic Bacteria

Suman Lata, Chhaya Sharma, Ajay K. Singh

Department of Paper Technology, IIT-Roorkee,
Saharanpur Campus, Saharanpur-247001, India,

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

Abstract:

Microbial influenced corrosion (MIC) plays important role in curtailing useful life of steel machinery in many industries. While many investigations have been reported on microbial corrosion due to sulphate reducing bacteria (SRB), most of them are related to mesophilic species. Fewer studies have been done on the corrosion aspects of thermophilic bacteria although these bacteria have been identified frequently in industrial media having moderately high temperatures e.g. condenser in electric power unit, oil wells etc. where corrosion of the metals has been detected. Accordingly, work was undertaken on corrosion due to *Desulfotomaculum nigrificans*. The purpose of the study was to investigate MIC on stainless steels and to correlate it with the growth of the biofilm by monitoring the compositon of its extracellular polymeric substances (EPS). Stainless steel 304L, 316L and 2205 were selected for the test. Modified Baar's media, control and inoculated, were used as test solutions in anaerobic conditions. Electrochemical polarization and immersion tests were performed to estimate the extent of corrosion rate and pitting attack. Extent of corrosion and chemicals present in/outside pit was determined by scanning electron microscopy (SEM)/ energy dispersive spectroscopy (EDS). The composition of EPS, component of biofilm, was determined by UV/visible spectroscopy. This work shows that SRB's degrade own EPS. Correlations of extent of corrosion attack with the composition of the EPS of the biofilm formed on test samples indicate maximum corrosive conditions when bacterial concentration is maximum which in turn minimizes the amount of carbohydrate and protein contents alongwith the increase in fraction of uronic acid in carbohydrate in EPS of the biofilm. Stainless steel 2205 shows maximum corrosion resistance followed by 316L and 304L.

Keywords: *Desulfotomaculum nigrificans;* Stainless steel; electrochemical polarization; Weight loss; Extracellular polymeric substances

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

Introduction

Steel is widely employed in most industries for its low cost, strength, ability to fabricate in various shapes and availability. However, their applications are many times affected by corrosion which necessitates use of stainless steels. Apart from chemical corrosion, the steels may also be affected by microbial influenced corrosion (MIC) which is caused by microbial colonization of metal surfaces and formation of biofilms affecting electrochemical nature of the metal-environment system[1,2]. Most microbial corrosion studies reported in literature are related to sulphate reducing bacteria (SRB's) specially mesophilic type (*Desulfovibrio*)[3-5] due to their prevalence in various industrial media and their reputation as the principal causative organism responsible for localized corrosion on stainless steels causing unexpected failure[6-10]. However, fewer studies have been done on the corrosion aspects of thermophilic SRB (*Desulfotomaculum*), although these bacteria have been identified frequently in industrial media having moderately high temperatures e.g. condenser in electric power unit, oil wells etc. where corrosion of the metals has been detected. Thus, MIC due to thermophilic bacteria was first demonstrated by Torres-Sanchez & Magana-Vazquez[11] in a condenser of a geothermal electric power unit, which operated in the temperature range of 40-150 °C. They exposed 304L stainless steel tubes for several months to the condenser environment. The tubes developed pitting where *Desulfotomaculum nigrificans (DN)* and *Desulfotomaculum acetoxidans* colonies were observed. Almeida et.al.[12] observed corrosion on carbon steel coupons covered by biofilm, after exposure for several weeks at the outlet of a heat exchanger. The biofilm was analyzed to have predominantly thermophilic species of aerobic, anaerobic and sulphate-reducing bacteria (SRB) in planktonic and sessile phases. In another study, mild steel was exposed to four different cultures of SRB including *DN*. Green rust 2 (GR2), ferrous sulfides, γ-FeOOH and super paramagnetic α-FeOOH in different proportions were identified as corrosion products using Mössbauer spectroscopy. The formation of GR2 seems to be the first step for the SRB induced corrosion[13]. Anaerobic corrosion tests were carried out, at 40 and 50^0C, on 316 stainless steel and carbon steel in the two strains of SRB obtained from the condensate fluid of a geothermal electric power station. The microbial activity was observed to influence the overall corrosion process, whereas, pitting and localized attack was found [14]. Effect of iron concentration on corrosion behavior was studied by Ã‡etin et.al. [15] on low alloy steel in the presence of *Desulfotomaculum* isolated from an oil production well. Cetin & Donmez[16], studied corrosion behavior of low alloy steel, in the presence of anaerobic sulfate-reducing *Desulfotomaculum* sp. isolated from an oil production well. They found the corrosion activity depend upon bacterial metabolites, ferrous sulfide, hydrogen sulfide, iron phosphide, and cathodic depolarization effect. They also studied influence of two biocides (formaldehyde and glutaraldehyde) on corrosion behavior. Same group [17], also studied the effect of *D. nigrificans* with electrochemical impedance spectroscopy and scanning electron microscopy (SEM). The incubation of the SRB in culture medium accelerates the cathodic depolarization process of low-alloy steel, but slows down the anodic process. It was observed that the biofilm formation initiates after lapse of a certain incubation period and that the corrosion products (iron sulfides) start affecting the biofilm after a certain incubation period. Anandkumar et al. [18]; Anandkumar & Rajeshkar, [19], investigated the role of *Desulfotomaculum geothermicum* and *Desulfotomaculum kuznetsovii* in mild steel corrosion. Presence of bacteria enhances corrosion by accelerating cathodic reaction and suppressing anodic reaction. Pitting was also observed probably due to cathodic depolarization. The study implicates the importance of *D. geothermicum* in the corrosion of cooling towers of the petroleum refinery. In another study, corrosion was found to enhance under biotic conditions in case of steel containers meant for nuclear waste disposal in a repository, thereby indicating the possibility of SRB growth (including *Desulfotomaculum*) and faster corrosion under the disposal condition, if water is available [20].

Thus the studies performed until now are mostly on c-steel and low alloy steel and relate to (i) MIC under in-plant test conditions (ii) characterization of corrosion products (iii) effect of biocides on MIC (iv) influence of change in anodic and cathodic tafel slopes on corrosion reactions. However, MIC is also expected to depend upon the nature of biofilm since the extracellular polymeric substances (EPS) formed by the bacteria favor attachment of cells to iron metals [1, 21-23] and their macromolecules e.g. carbohydrates and proteins may influence considerably the electrochemical reactions at the metal-biofilm interface [1]. Therefore, investigations were performed on austenitic stainless steel and duplex stainless steel of industrial importance to study microbial induced corrosion in the presence of *Desulfotomaculum nigrificans* through immersion test, electrochemical polarization test and SEM/energy dispersive spectroscopy (EDS) techniques. The biofilms formed on the corroded samples were analyzed for their components in order to see the correlation of their amount with the extent of corrosion attack. Toxic effect of heavy metals on MIC was also observed. Present paper is a report on this work.

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

Experimental Details and Result

Materials

Samples of austenitic 304L, 316L and duplex stainless steel 2205 (Table-1) were tested in the present study. The coupons of these samples were polished progressively from coarse to fine (up to 1000 grit) emery paper and then subjected to 4/0 (equivalent to 2000 grit) ('3M' and 'Premier' make) for final finish. The polished coupons were ultrasonically degreased in acetone and sterilized by exposing to 70% ethanol for 4 hours [24] followed by drying under ultraviolet light in a stream of warm air [25]. Immediately after sterilization, the coupons were submerged in cultures of bacteria. For electrochemical studies, coupons of 1 cm^2 were embedded in a mould of epoxy resin with their electrical connection established via a copper wire. The test material was polished, degreased and sterilized as described above. All the chemicals used were 'Merck' and 'Fisher scientific' make of analytical reagent grade.

Microbes and Test Solution

The anaerobic SRB species, *Desulfotomaculum nigrificans* (*DSM*) were obtained from NCIM in India. The solution meant for cultivating this species and the test solution was nutrient rich modified Baar's medium having magnesium sulphate: 2.0 g; sodium citrate: 5.0 g; calcium sulphate: 2.0 g; ammonium chloride: 1.0 g; *di*-potassium hydrogen orthophosphate: 0.5 g; sodium lactate: 7.0 g; and yeast extract: 1.0 g; all in 1 liter of double distilled water [26]. The pH of the medium was adjusted to 7.5 and anaerobic conditions were maintained using nitrogen gas. The SRB's were cultivated at 55ºC. Simple staining by methylene blue was used for determine the bacteria shape [27]. Motility test was primarily done by Hanging Drop method after that it was confirmed by flagella staining and analyzed under light microscope [27]. The bacterial concentration (planktonic and sessile) was estimated by most probable number (MPN) method [28]. For this purpose, biofilm was removed from the surface of exposed steel coupon by swabbing with sterile cotton. The swab was suspended in 10 ml modified Baar's medium followed by homogenization before undertaking MPN procedure.

Test for bacteria and biofilm characterization

These tests were done up to 144 hours to characterize the biofilm and to study the life cycle of microbes and pH of media with the aim of correlating with the corrosion rate of tested steels. The variation in bacterial count and pH with time are shown in Table 2.

The biofilm formed on the coupons exposed for 72 hrs was analyzed for SRB's. For this purpose, the biofilm on the coupons were immersed for 1 hr in a 2% glutaraldehyde solution, in order to fix the biofilm to the steel surface, and then was dehydrated using four ethanol solutions (15 minutes each): 25, 50, 75 and 100%, successively [29].Thus treated biofilm was rinsed in sterile distilled water, dried and subjected to examination under SEM (Quanta 200 FCG, Netherland). Fig. 1 shows the bacteria during favorable conditions.

For estimating the amount of EPS, the biofilm was removed from the surface of exposed coupon and was treated for isolation [28]. Biofilm was removed from the surface of exposed steel coupon with a sterile cotton swab and suspended in 10 ml distilled water. The suspension was centrifuged at 15000 rpm for 10 minutes. From the resulting liquor, the supernatant part was decanted and the suspended part was added in a 10 ml 8.5% NaCl solution containing 0.22% formaldehyde. For recovery of the capsule bound EPS, the suspension was mixed in a vortex mixer for 1 min. Thus obtained suspension was added to the supernatant decanted earlier and the mixture was made to 20 ml by adding requisite amount of the distilled water. The combined sample was centrifuged at 12000 rpm for 30 minutes at 15^0C. The supernatant was filtered through 0.22 µm cellulose acetate filters to ensure that filtrate was free of cells. This cell free filtrate was analyzed for estimation of carbohydrate (anthrone method [30]), protein (Folin-Lowry's method[30]), uronic acid[31] and lipid[32,33]. For estimating the first three, the spectropohotometer was calibrated using standard solution of glucose for carbohydrates, bovine serum albumin for proteins and D-glucuronic acid for uronic acid. Absorption of 620 nm was measured for estimating carbohydrate, 660 nm for protein and 520 nm for uronic acid. Lipid extraction was done by Bligh and Dyer method [32]. Lipid quantification was done

according to Rouser et al [33]. Standard solution of KH_2PO_4 was used for lipids estimation utilizing absorption of 800 nm wavelength. Variation of EPS components with time is shown in (Fig. 2, 3 & 4).

Corrosion Test

Immersion tests were conducted for duration from 7 to 90 days in control and bacteria inoculated media. Since bacterial concentration reaches maximum after about 96 hours (Table 2), 75% of the test media was replaced by fresh media on every 5^{th} day so as to maintain bacterial and chemical concentration. Carbohydrate and protein are the major components of biofilm in case of this bacterium so they were estimated after the exposure, as described above. The clean and dried coupons were weighed for estimation of corrosion rate as per standard procedure (ASTM G1-72) [34]. Extent of pitting was estimated by measuring maximum pit depth on the surface of cleaned coupons by using stereo microscope (Olympus) and optical microscope (Leica Q500MC). Table 3 shows the corrosion rate and pitting attack data. Fig. 5 shows the relation between corrosion rate and EPS of 304L coupons. SEM analysis was done on corroded coupons (Fig. 6 and 7) while EDS analysis was carried out for finding the chemical composition outside/inside pits (Table- 4).

For estimating corrosion rate and its variation with bacterial growth and EPS, tafel plots were measured. For this purpose, the steel samples were incubated in Barr's media for duration from 12 to 144 hours before they were subjected to the test. Anaerobic conditions were maintained throughout the test. The measurements were done using Radiometer 'Voltalab' Electrochemical Laboratory Model PGZ301. Saturated calomel electrode (SCE) was used as reference electrode, graphite rods as auxiliary and test specimen as working electrode. Variation of corrosion rate with incubation time is shown in Fig.2 & 4. Open circuit potential (OCP) and potentiodynamic polarization measurements were also done as a part of electrochemical tests. For these measurements, the steel samples were put in blank and inoculated Baar's medium for 0, 5 and 10 days under anaerobic conditions before they were subjected to electrochemical tests. Fig. 8 and 9 depicts some of these polarization curves. Electrochemical parameters derived from these curves are shown in Table 5. Values of potential, in this work, are indicated with respect to SCE.

Discussion

Bacteria and Biofilm Characterization

On immersing stainless steel coupons in inoculated Baar's media, biofilm was observed after 12 hours. Fig.1 shows the SEM photograph of bacteria, rod shaped, anaerobic SRB's with the typical morphology of the genus used (*Desulfotomaculum*, size between 0.7 μm – 2.0 μm). From the motility test and bacterial flagella staining it was confirmed that bacteria have peritrichous flagella which are helpful in their motion. Variation of planktonic and sessile bacterial population and pH with time are shown in Table.2. Thus in different cases, the concentration increased from ~ 10^3 to ~ 10^8 cells/ml of media in case of planktonic and cells/cm^2 of biofilm (formed over coupons) in case of sessile bacteria after 96 hrs of incubation (Table 2). Afterwards it decreases. The growth rate of sessile cells was greater than that of the planktonic cells between 12 to 96 hrs after which cell concentration of both planktonic and sessile starts decreasing (Table 2) as also found elsewhere [28,35,36] .

Comparison of Fig. 2 and Table 2 shows maximum bacterial concentration when the carbohydrate and protein concentration in the biofilm formed on all the three types of metals is observed to be lowest. Comparison of Fig. 3 and Table 2 shows minimum amount of uronic acid at highest bacterial concentration whereas the lipids concentration continues to increase until the exponential phase of bacteria (96 hrs) after which it increases slightly before tapering off. Increase in lipids' concentration may be attributed to bacterial cells lyses. The decrease in the amount of carbohydrate, protein and uronic acid is an indication of the biodegradation of EPS produced by the bacteria, which was corroborated by performing the test in the media without lactate and yeast. In this test, the coupons with biofilm were transferred into inoculated C-free medium (without lactate and yeast) and the amount of carbohydrate and protein were measured at regular intervals. In pure cultures it has usually been assumed that the bacteria do not degrade their own EPS, but present results showed that *Desulfotomaculum* sp. had the ability to biodegrade the EPS produced by them. This type of property was shown by other bacteria also [28]. Some research shows that mixed cultures or even pure culture degraded their own EPS material when they were in a starved state [37, 38]. The observance of decrease in the amount of carbohydrate, protein and uronic acid when the bacteria is in

the most active stage indicates that nutrients media are not enough and the bacteria is consuming sugar (carbohydrate and uronic acid) and protein by degrading the EPS produced by them. Afterwards, the bacterial concentration starts decreasing while amount of sugar and proteins increase which may be attributed to the death of bacterial cells.

The results show higher amount of proteins than carbohydrate and other components in the EPS produced by SRB (Fig. 2) on respective stainless steel samples, as observed earlier [39] also. However, EPS harvested from SRB's grown in the presence of glass/plastic substrate either shows no or very less protein [1]. This difference could be attributed to the presence of metal centers (iron) in case of hydrogenase enzyme [40] of SRB's and that iron of these enzymes is being supplied due to the corrosion of the stainless steel coupons. This hypothesis is also supported by the comparison of present results with those of the corrosion experiments done in the presence of SRB sp.*Desulfovibrio desulfuricans* [41], which show higher corrosivity and higher amount of protein, in the harvested EPS. Role of protein influencing the composition of the biofilm and hence the biocorrosion has also been suspected earlier [38]. One also observes that the decrease in the concentration of carbohydrates starts decreasing earlier (60 hours after start of the experiment) and its rate is also higher than that of the protein (Fig. 2). Possible use of EPS as substrate by the bacteria and faster utilization of carbohydrate than protein [38] could be the reason for this observation.

Nature of media and Extent of Corrosion

Baar's media after inoculating with SRB shows an increase of pH from 7.23 to 7.7/7.8 in around 4 days. This can be attributed to the metabolic reaction [42]:

$$8\ H^+ + SO_4^{--} + 8\ e^- \rightarrow S^{--} + 4\ H_2O \qquad (1)$$

The reaction occurs due to hydrogenase in SRB's. Another reaction

$$2\ H^+ + S^{--} \rightarrow H_2S \qquad (2)$$

is also responsible for increase in pH of the solution. The e^-s required for the above reactions are available from (i) the presence of ammonium ferrous sulphate and sodium lactate which act as electron donor and/or (ii) oxidation of Fe to Fe^{2+}.

Thus the observance of increase in pH can be assigned to the presence and growth of SRB in the media. The evidence of the presence of bacteria is indicated by the observation of corroded stainless steel coupon under SEM (Fig. 1), the smell of H_2S in the test cell and bacterial counting in the test media (Table 2). The pH of the media is also observed to increase upto 7.7/7.8 in the electrochemical polarization tests and the weight loss test whereas test in control media shows change of pH up to only 7.4. Thus a change of pH to a higher degree in case of inoculated media can be attributed to the presence of microbes. This can be understood since due to anodic polarization of steel samples, more Fe oxidizes, resulting in availability of larger number of e^-'s which in turn enhances the rate of above indicated metabolic reactions.

Fig. 2 shows corrosion rate, obtained from tafel plots, increasing up to 96 hours of exposure and then starts decreasing similar to the change observed for bacterial (planktonic and sessile) concentration (Table 2). This is, however, inverse of the trend observed for components of EPS namely sugar and protein (Fig. 2). This observation suggests a direct correlation between corrosion of steel and SRB activity, as proposed earlier [43] that the dissolved metal concentration can serve as an indicator of the bioactivity of the SRB. A comparison of variation of corrosion rate, carbohydrate and uronic acid content with time (Fig. 4), in case of all the three stainless steel samples, show increase in fraction of uronic acid in carbohydrate as corrosion rate increases with time beyond 60 hours of exposure. This fraction reaches maximum after the samples have got exposed for 96 hours, when corrosion rate is also highest. This observation may be attributed to higher fraction of the acidic part of the sugar i.e. uronic acid which may result in overall decrease in pH of the solution.

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

To further observe the dependence of corrosion on the bacterial presence in media, immersion tests were done. Corrosion rates, calculated from these tests (Table 3), are observed to be higher in case of inoculated media as compared to control, an evidence of enhanced corrosion attack on steels due to SRB's. Pitting attack (Table- 3) is observed to increase in the presence of bacteria and with time. The SEM analysis of metal coupons exposed in sterile medium, exhibited either no or limited localized corrosion (Fig. 6). Tafel plots measurements by Cetin et al [44] show decrease in cathodic and increase in anodic tafel slope with incubation time. However, no such dependence could be clearly observed in present work. During immersion test, concentration of carbohydrates and proteins continues to increase (Fig. 5) which can be attributed to frequent addition of nutrients in the media in these tests.

Corrosion related parameters, obtained from electrochemical tests are given in Table-5. It is observed that OCP in inoculated Baar's media shift towards more negative magnitudes as compared to the respective values in control media. The OCP drop can be attributed to the presence of sulfide in the inoculated media due to the production of H_2S by SRB activity. One observes similar change in OCP with increase of incubation period in inoculated media (Table-5), which may be assigned to increased concentration of sulfides with time. Sulfide solutions are known to be of reducing types hence they lower the OCP values. Anodic polarization curves (Fig. 8) show decrease in pitting potential and passivation range and increase in current density (Table 5) in case of stainless steel samples exposed to inoculated vis-à-vis the control media. In case of measurements in inoculated media, all the three parameters showed similar variations with increase in incubation time (Fig. 8).

The observance of higher degree of corrosion attack as evidenced from increased corrosion rates and deeper pits in immersion test and decreased values of pitting potential and passivation range along with increased corrosion rates in electrochemical tests in inoculated media as compared to control can be understood from the effect on passivity of stainless steel due to the presence of bacteria. The passivity of stainless steel, in abiotic media, is attributed to the presence of oxide and hydroxides of Cr on its surface. In the presence of SRB's, biofilm forms on the metal surface [45] which affects the passive film through bacterially produced sulfides (eqn.1) which results in formation of chromium sulfides and iron/nickel sulfides due to presence of Fe, Ni and Cr in stainless steel (Table-1). These sulfides are better electron conductors, structurally more permeable and unstable; hence make the passive film much less effective in protecting against corrosion attack. This results into enhanced corrosion rate and higher pitting attack in cases where sulfide formation takes place in a localized area [46]. Thus pits observed on the corroded specimens can be analyzed as due to SRB induced corrosion. This is supported by the observation of higher amount of C, S and P inside pit as compared to their respective amount outside the pit from the results of SEM/EDS (Fig. 7 and Table- 4). Higher amount of C inside pits can be assigned to the amount of EPS which form as a result of metabolic activities of bacteria while enhanced bacterial activity of SRB's inside pits leads to conversion of sulfates to sulfide ions with higher rate. Iron phosphide is formed by the reaction of iron with highly active volatile phosphorus compound. According to Iverson and Olson [47], a volatile phosphorus compound is produced by SRB activity or by reaction of hydrogen sulfide with inorganic phosphorus compounds in the environment. When protective FeS layer does not form or break down, volatile phosphorus compound acts on steel surface and causes corrosion of iron [16]. The presence of higher phosphate concentration in present EDS spectra indicates the role of volatile phosphorus compounds in addition to sulfides on corrosion reactions. FeS decreases hydrogen over potential and cause cathodic depolarization [48, 49]. This sulfide, in turn, reacts with iron (main constituent of steel) to form iron sulfide. When one puts the OCP values (Table 5) and pH of the inoculated test solutions (~ 7.5) in E-pH diagram of Fe-S-H_2O system [50] this sulfide appears to be mackinawite ($FeS_{0.943}$). Mackinawite is known to be black in appearance, dissolves easily and is unprotective type. Also, mackinawite deposits are hypothesized to act as large surface area [51] and can act as a cathode in the galvanic couple with steel thus enhancing corrosion [40]. Inoculated test solutions in the present study are also observed to be dark in color after the end of the test indicating the formation of mackinawite and their dissolution. Accordingly, iron inside pits corrode with higher rate in the absence of any protective type of corrosion products leading to deeper pits.

Comparison of Metal Performance

Higher concentration of heavy metals (Ni, Cr and Mo) slows down the growth of bacteria and production of EPS [21, 43]. Accordingly, present results show maximum concentration of sessile bacteria and of EPS components in case of 304L (minimum amount of Cr, Ni and Mo) and minimum in case of 2205 (maximum amount of Ni, Cr and Mo) (Table1 and Fig. 2 and 3).

ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

Results of immersion test show extent of corrosion rate and pit depth to be maximum in case of stainless steel 304L followed by 316L and 2205 (Table 3). Electrochemical test shows pitting potential and passivation range; in general, to be highest in case of duplex stainless steel 2205 followed by austenitic stainless steels 316L and 304L (Table 5 and Fig. 9). Thus both the tests predict maximum corrosion resistance of 2205 whereas least resistance of 304L in the studied media. The relative resistance of the studied stainless steels against corrosion may be correlated with their composition through determination of pitting resistance equivalent number (PREN) as given below [52]

$$PREN = \% Cr + 3.3x \%Mo + 16 x \%N$$

Accordingly, the PREN of 2205 is maximum (34.8), that of 316L is 25.2 and 304L is minimum (19.9).

Conclusion

The paper reports the results obtained from the electrochemical polarization and immersion tests, SEM/EDAX analyses conducted on stainless steels 304L, 316L, and 2205 in the Baar's media inoculated with *Desulfotomaculum* sp. Corrosivity of the solution is observed to increase with (i) the addition of bacteria and (ii) the incubation time. Correlations of extent of corrosion attack with the composition of the EPS of the biofilm formed on test samples indicate maximum corrosive conditions when bacterial concentration is maximum which in turn minimizes the amount of carbohydrate and protein contents alongwith the increase in fraction of uronic acid in carbohydrate in EPS of the biofilm. However, as bacterial concentration and corrosion rate decreases, the amount of component in the biofilm continues to increase. Results of the present study show that the toxicity of metals ions affect the bacterial concentration and EPS production and *Desulfotomaculum* sp. had the ability to biodegrade its own EPS. Results show that 2205 is maximum resistant material against MIC followed by 316L and 304L.

Acknowledgement

One of the authors (SL) acknowledges the Research fellowship received from MHRD, Govt. of India.

References

1. Zinkevich, V., I. Bogdarina, H. Kang, M.A.W. Hill, R. Tapper and I.B. Beech, 1996. Characterisation of exopolymers produced by different isolates of marine sulphate-reducing bacteria.Int. Biodeter. Biodegr., 37: 163-172.
2. Zinkevich, V., I. Felhosi and E. Kalman, 2001. Role of redox properties of biofilms in corrosion processes. Electrochimica Acta, 46: 3841-3849.
3. Hamilton, W.A., 1985. Sulfate-reducing bacteria and anaerobic corrosion. Annu. Rev. Microbiol., 39: 195-217.
4. Lee, W., H. P. Nielsen and A. W. Hamilton, 1995. Role of sulfate-reducing bacteria in corrosion of mild steel. Biofouling, 8, 165-194.
5. Yuan, S.J. and S.O. Pehkonen, 2009. AFM study of microbial colonization and its deleterious effect on 304 stainless steel by Pseudomonas NCIMB 2021 and Desulfovibrio desulfuricans in simulated seawater. Corrosion Science, 51: 1372-1385.
6. Sunder, E., T. Thorstenson and T. Torsvik, 1990. Growth of bacteria on water injection additives Society of Petroleum Engineers, 20690: 727-733.
7. Jack, R. F., D. B. Ringelberg and D.C. White, 1992. Differential corrosion rates of carbon steel by combinations of *Bacillus* sp., *Hafnia alvei*, and *Desulfovibrio gigas* established by phospholipid analysis of electrode biofilm. Corrosion Science, 33: 1843-1853.
8. Nilsen, R.K., J. Beeder, T. Thorstenson and T. Torsvik, 1996. Distribution of thermophilic marine sulphate reducers in North Sea oil field waters and oil reservoirs . Applied Environmental and Microbiology, 62: 1793-1398.
9. Beech, I. B., 2003. Sulfate-reducing bacteria in biofilms on metallic materials and corrosion. Microbiol. Today, 30:115-117.

10. Rao, T. S., J.A. Kora, P. Chandramohan, S. B. Panigrahi and V. S. Narasimhan, 2009. Biofouling and microbial corrosion problem in the thermo-fluid heat exchanger and cooling water system of a nuclear test reactor. Biofouling, 25: 581-591.
11. Toress-Sanchez, R., A. Magana-Vazuez and J. M. Sanchez-Yanez, 1997. High temperature microbial corrosion in the condenser of a geothermal electric power unit. Materials Performance, 36: 43-46.
12. Almeida, M. A. N., and F. P. de Franca, 1999. Thermophilic and mesophilic bacteria in biofilms associated with corrosion in a heat exchanger. World J. Microbiol. Biotechnol, 15: 439-442.
13. Kumar, A. V. R., R. Singh and R. K. Nigam, 1999. Moissbauer spectroscopy of corrosion products of mild steel due to microbiologically influenced corrosion. Journal of Radioanalytical and Nuclear Chemistry, 242: 131-137.
14. Alfaro-Cuevas-Villanueva, R., R. Cortes-Martinez, J. J. García-Díaz, R. Galvan-Martinez, and R. Torres-Sanchez, 2006. Microbiologically influenced corrosion of steels by thermophilic and mesophilic bacteria. Materials and Corrosion, 57: 543-548.
15. Ã‡etin, D., S. BilgiÃ§, S. DÃ¶nmez and G. DÃ¶nmez, 2007. Determination of biocorrosion of low alloy steel by sulfate-reducing *Desulfotomaculum* sp. isolated from crude oil field. Materials and Corrosion, 58: 841- 847.
16. Cetin, D., S. Bilgic and G. Donmez, 2007. Biocorrosion of low alloy steel by *Desulfotomaculum sp*. and effect of biocides on corrosion control. ISIJ International, 47: 1023-1028.
17. Cetin D, S. Bilgic, S. Donmez and G. Donmez, 2007. Determination of biocorrosion of low alloy steel by sulfate-reducing *Desulfotomaculum* sp. isolated from crude oil field. Materials and Corrosion 58: 841-847.
18. Anandkumar, B., J.H. Choi, G. Venkatachari and S. Maruthamuthu, 2009. Molecular characterization and corrosion behavior of thermophilic (55°C) SRB *Desulfotomaculum kuznetsovii* isolated from cooling tower in petroleum refinery. Materials and Corrosion, 60: 730-737.
19. Anandkumar, B., A. Rajasekar, G. Venkatachari and S. Maruthamuthu, 2009. Effect of thermophilic sulphate-reducing bacteria (*Desulfotomaculum geothermicum*) isolated from Indian petroleum refinery on the corrosion of mild steel. Current Science, 97: 342-348.
20. Hajj, H. El, A. Abdelouas, B. Grambow, C. Martin and M. Dion, 2010. Microbial corrosion of P235GH steel under geological condition. Physics and Chemistry of the Earth, 35: 248-253.
21. Beech, I. B. and S. W. C. Cheung, 1995. Interactions of exopolymers produced by sulphate reducing bacteria with metal ions. International Biodeterioration & Biodegradation, 35: 59-72.
22. Beech, I. B. and C. C. Gaylarde, 1999. Recent advances in the study of biocorrosion, an overview. Rev. Microbiol., 3: 177-190.
23. Fang, H. H. P., L. C. Xu and K. Y. Chan, 2002. Recent advances in the study of biocorrosion, an overview. Water Res., 36: 4709-4716.
24. Rao, T.S., A. J. Kora, B. Anupkumar,S.V. Narasimhan and R. Feser, 2005. Pitting corrosion of titanium by a freshwater strain of sulphate reducing bacteria (*Desulfovibrio vulgaris).* Corrosion Science, 47: 1071-1084.
25. Obuekwe, C. O., D. W. S. Westlake, F. D. Cook and J. W. Costerton, 1981. Surface changes in mild steel coupons from the action of corrosion-causing bacteria. Applied and Environmental Microbiology, 41:766-774.
26. Antony, P. J., S. Chonmdar, P. Kumar and R. Raman, 2007. Corrosion of 2205 duplex stainless steel in chloride medium containing sulfate-reducing bacteria. Electrochimica Acta, 52: 3985-3994.
27. Aneja, K. R., 2007. New age international publishers, 4th.
28. Sungur, E. I. and N. Cansever, A. Cotuk, 2007. Microbial corrosion of galvanized steel by a freshwater strain of sulphate reducing bacteria (*Desulfovibrio* sp.). Corrosion Science, 49: 1097-1109.
29. Gayosso, H. J. M., Z. G. Olivares, R. N. Ordaz, J. C. Ramirez, Esquivel GR and P. A. Viveros, 2004. Microbial consortium influence upon steel corrosion rate, using polarization resistance and electrochemical noise techniques. Electrochimica acta, 49: 4295-4301.
30. Nigam, A., 2007. Tata McGraw-Hill Publishing Company Limited.
31. Mojica, K., D. Elsey , M. J.Cooney, 2007. Quantitative analysis of biofilm EPS uronic acid content. Journal of Microbiological Method, 71: 61-65.
32. Bligh, E. G. and W. J. Dyer, 1959. A rapid method of total lipid extraction and purification. Biochem. Physiol., 37: 911-917.
33. Rouser, G., S. Fleischer and Yamamoto, 1970. Two dimensional thin layer chromatography separation of polar lipid and determination of phospholipids by phosphorous analysis of spots. Lipid, 5: 494 - 496.
34. ASTM Standard, 1991.Wear and Erosion. Metal Corrosion, 3: 02.

35. Ellwood, C. D., W. C. Keevil, D. P. Marsh, M. C. Brown and N.J. Wardell, 1982. Surface-associated growth. Philos. T. Roy. Soc. B., 297: 517-532.
36. Beech, I. B., C. W. S. Cheung, C. S. P. Chan, M. A. Hill, R. Franco and A. R. Lino, 1994. tudy of parameters implicated in the biodeterioration of mild steel in the presence of different species of sulphate-reducing bacteria. Int. Biodeter. Biodegr., 34: 289-303.
37. Boyd, A. and M. A. Chakrabart, 1994. Role of alginate lyase in cell detachment of *Pseudomonas aeruginos.* Applied and Environmental Microbiology, 60: 2355-2359.
38. Zhang, X. and L. P. Bishop, 2003. Biodegradability of biofilm extracellular polymeric substances. Chemosphere, 50: 63- 69.
39. Chan, K. Y., L. I. C. Xu and H. H. P. Fang, 2002. Anaerobic electrochemical corrosion of mild steel in the presence of extracellular polymeric substances produced by a culture enriched in sulfate-reducing bacteria. Environ. Sci. & Technol., 36: 1720-1727.
40. Silva, D. S., R. Basseguy and A. Bergel, 2004. Electron transfer between hydrogenase and 316L stainless steel: identification of a hydrogenase-catalyzed cathodic reaction in anaerobic mic. J. Electroanal. Chem., 561: 93- 102.
41. Lata, S., C. Sharma, A. K. Singh, 2010. Proc. Microbial Induced Corrosion (MIC) due to *Desulfotomaculum nigrificans.* CORRCON. held at Goa, Sept 7-9 (2010).
42. Kueher, V. W. and I. S. V. D. Vlugt, 1934. Graphitization of cast iron an electrochemical process in anaerobic soil. Water, 18: 147-165.
43. Poulsion, R. S., S. J. P. Colberg and I. J. Drever, 1997. Toxicity of heavy metals (Ni, Zn) to *Desulfovibrio desulfuricans.* Geomicrobiology Journal, 14: 41- 49.
44. Cetin, D. and M. L. Aksu, 2009. Corrosion behavior of low-alloy steel in the presence of *Desulfotomaculum sp.* Corrosion Science, 51: 1584- 1588.
45. Videla, H. A., 2001. Microbiologically induced corrosion: an update overview. International Biodeterioration and Biodegradation, 48: 176- 201.
46. Chen, G. and C. R. Clayton, 1997. Influence of Sulphate reducing bacteria on the passivity of type 304 stainless steel. J.Electrochemical Society, 144: 3140 -3146.
47. Iverson, W. P. and G. J. Olson, 1983. Anaerobic corrosion by sulfate reducing bacteria due to highly reactive volatile phosphorus compound. Proc. Conf. Microbial Corrosion, The National Physical Laboratory and the Metals Society, NPL Teddington, London, 46- 53.
48. Lee, W. and W.G. Charaklis, 1993. Corrosion of mild steel under anaerobic biofilm. Corrosion, 49:186-198.
49. Newman, R. C., B. J. Webster and R. G. Kelly, 1991. The electrochemistry of SRB corrosion and related inorganic phenomena. ISIJ International, 31: 201- 209.
50. Singh, A. K. and A. Pourbaix, 1997. Rapports Techniques Cebelcor., 166, RT.318.
51. Angell, P. and K. Urbanic, 2000. Sulphate-reducing bacterial activity as a parameter to predict localized corrosion of stainless alloys. Corrosion Science, 42, 897 - 912.
52. MunÕz, A. I., J. G. Anton, S. L. Nuevalos, J. L. Guinon and V. P. Herranz, 2004. Corrosion studies of austenitic and duplex stainless steels in aqueous lithium bromide solution at different temperatures. Corrosion Science, 46, 2955- 2974.

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

Table 1 Composition of steel samples

Sample	C	Si	Mn	P	S	Cr	Ni	Mo	N	Cu	Ti	Co
304L	0.036	0.44	1.84	0.024	0.001	18.11	8.01	0.26	0.058	0.46	0.002	-
316L	0.020	0.69	1.69	0.03	0.03	17.44	10.8	2.16	0.04	0.31	-	0.18
2205	0.02	0.52	1.45	0.02	0.002	22.25	5.48	3.08	0.15	-	-	-

Balance % - Fe

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

Table 2 Bacterial counting of *Desulfotomaculum nigrificans* (*DSM*) by MPN Method

Time (hrs)	pH	Planktonic Cells (per ml)	Sessile cells (per cm^2) 304L	316L	2205
0	7.23	2×10^3	-	-	-
12	7.32	21×10^2	24×10^2	22×10^2	2×10^3
24	7.48	7×10^3	84×10^2	78×10^2	75×10^2
36	7.59	54×10^3	75×10^3	69×10^3	60×10^3
48	7.69	73×10^4	98×10^4	82×10^4	75×10^4
60	7.73	32×10^5	49×10^5	42×10^5	36×10^5
72	7.79	23×10^6	45×10^6	36×10^6	30×10^6
84	7.8	28×10^7	43×10^7	35×10^7	32×10^7
96	7.79	15×10^8	35×10^8	29×10^8	22×10^8
120	7.76	1×10^8	32×10^8	25×10^8	19×10^8
144	7.74	67×10^6	87×10^7	7×10^8	6×10^8

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

Table 3: Corrosion rate for long term immersion test in Baar's media with/without *DSM*

Metals	Exposure time (days)	Baar's media (Control) Corrosion Rate(mpy)	Baar's media (Control) Max.Pit Depth (µm)	Baar's media (inoculated) Corrosion Rate (mpy)	Baar's media (inoculated) Max.Pit Depth (µm)
304L	7	0.08	ND	1.04	ND
	21	0.04	ND	0.58	38
	35	0.03	35	0.36	62
	50	0.02	40	0.34	83
	90	0.02	69	0.31	102
316L	7	0.04	ND	0.69	ND
	21	0.03	ND	0.36	32
	35	0.02	20	0.27	57
	50	0.02	35	0.24	72
	90	0.01	48	0.22	83
2205	7	0.04	ND	0.56	ND
	21	0.02	ND	0.30	ND
	35	0.02	ND	0.25	11
	50	0.01	10	0.19	28
	90	0.01	22	0.15	35

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

Table 4: Elements Identified by SEM/EDAX in/outside pit on corroded Stainless Steel 304L (Fig. 7)

Elements	C		P		S		Fe	
	Wt%	At%	Wt%	At%	Wt%	At%	Wt%	At%
Inside Pits	38.58	50.11	00.84	00.88	13.09	13.12	43.65	31.12
Outside Pits	11.97	37.98	00.18	00.23	1.51	1.79	48.48	43.34

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

Table 5: Electrochemical Parameters of corrosion study for *DSM*

Metals	Baar's media (Control)			Baar's media (Bacteria inoculated)		
	0 DI	5 DI	10 DI	0 DIP	5 DIP	10 DIP
Open circuit potential (mV)						
304L	-251.9	-265.4	-283.3	-297.3	-307.6	-413.2
316L	-310.2	-318.2	-322.6	-321.3	-354.9	-434.4
2205	-327.3	-332.7	-335.1	-338.2	-366.9	-467.8
Passivation range (mV)						
304L	1381.4	1377.9	1370.5	1379.4	1134.9	1119.7
316L	1385.6	1381.2	1375.4	1382.2	1321.7	1290.2
2205	1486.6	1485.8	1482.1	1409.1	1400.3	1365.0
Pitting Potential (mV)						
304L	949.3	945.4	940.8	940.3	672.2	619
316L	983.3	980.1	978.3	950.1	789	720.1
2205	991	989.1	988.9	1019	987.7	734.6
Corrosion rate (mpy)						
304L	0.08	0.07	0.06	0.09	0.08	0.07
316L	0.06	0.06	0.06	0.07	0.06	0.06
2205	0.05	0.05	0.05	0.07	0.06	0.05

DI- Day immersion DIP- Day incubation period

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

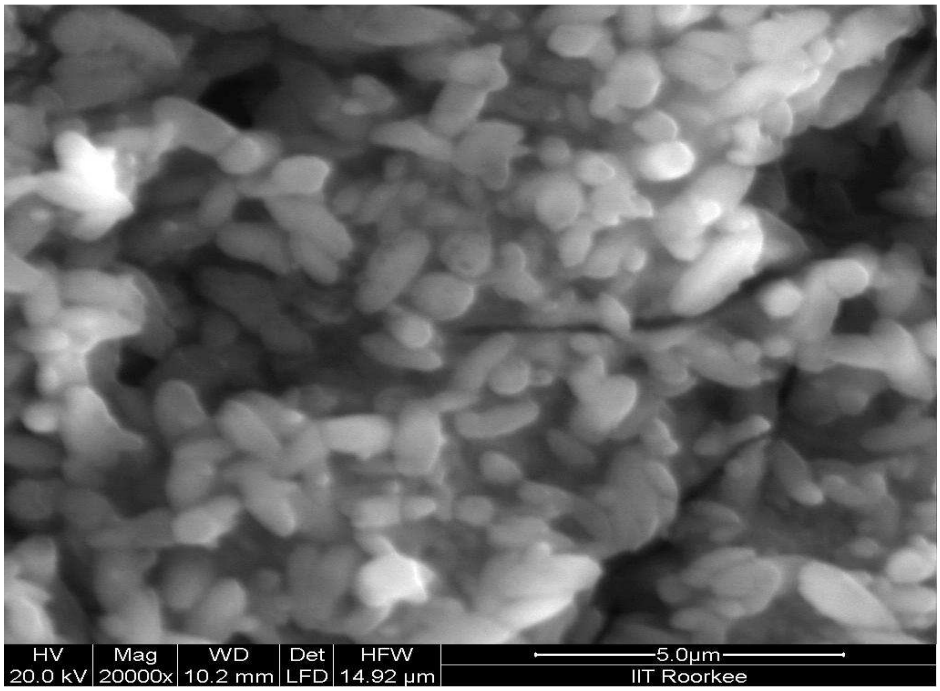

Fig. 1 Scanning Electron Micrograph of *Desulfotomaculum nigrificans* bacteria on SS 304L after 72 hrs

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

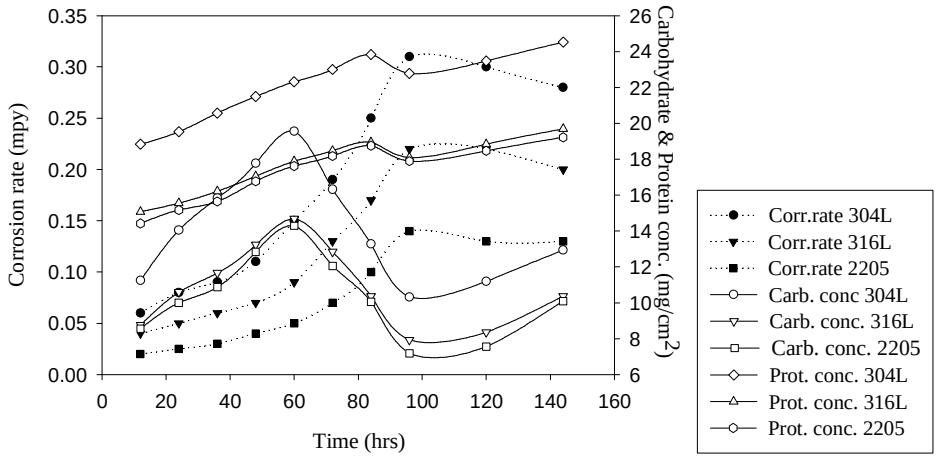

Fig. 2 Corelation between corrosion rate (mpy) and EPS component (Carbohydrate & Protein) of various metal samples with DSM during immersion tests when media replenishing

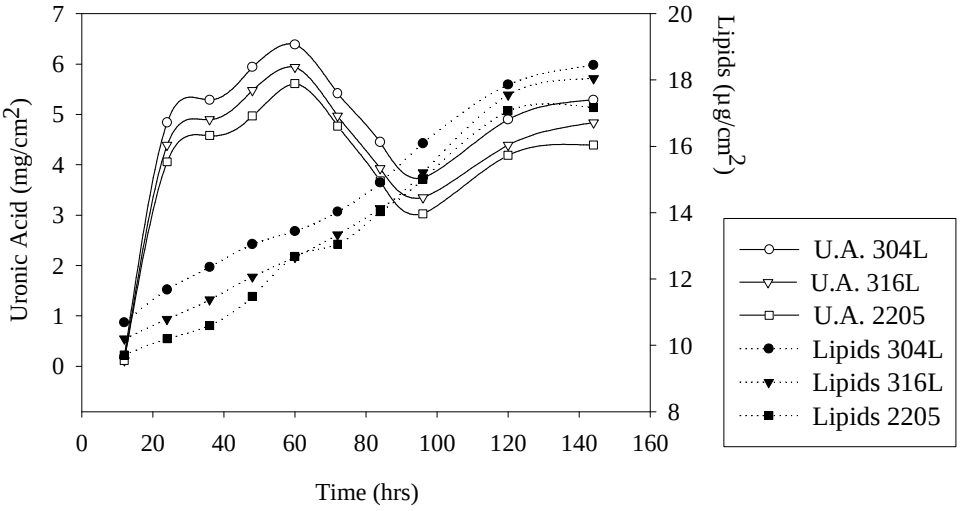

Fig. 3 Corelation in conc. of uronic acid and lipids of EPS with time on various metals with DSM during immersion tests when media replenishing

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

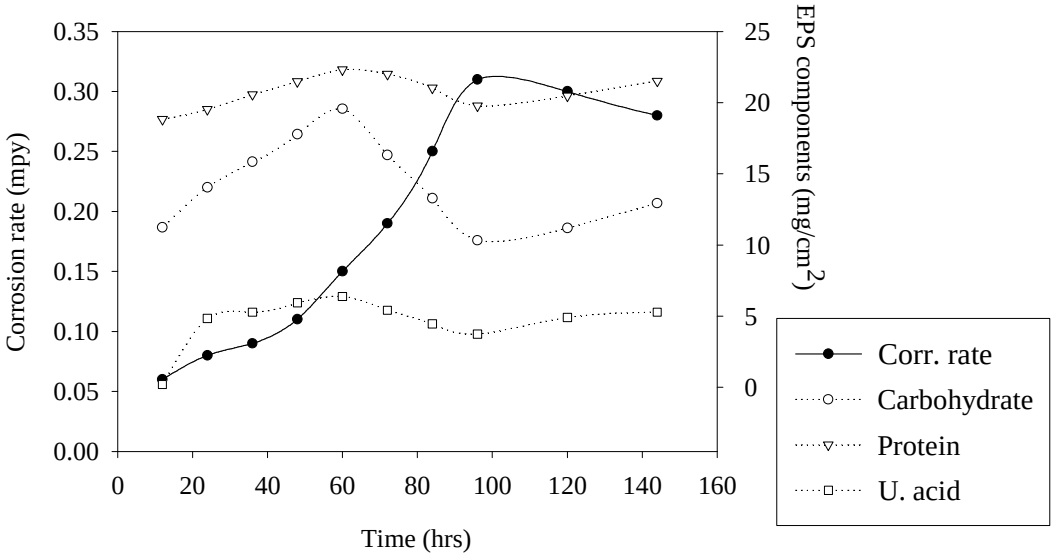

Fig. 4 Corelation between corrosion rate (mpy) and EPS components (carb., protein and U.acid) of 304L with DSM during immersion tests without media replenishing

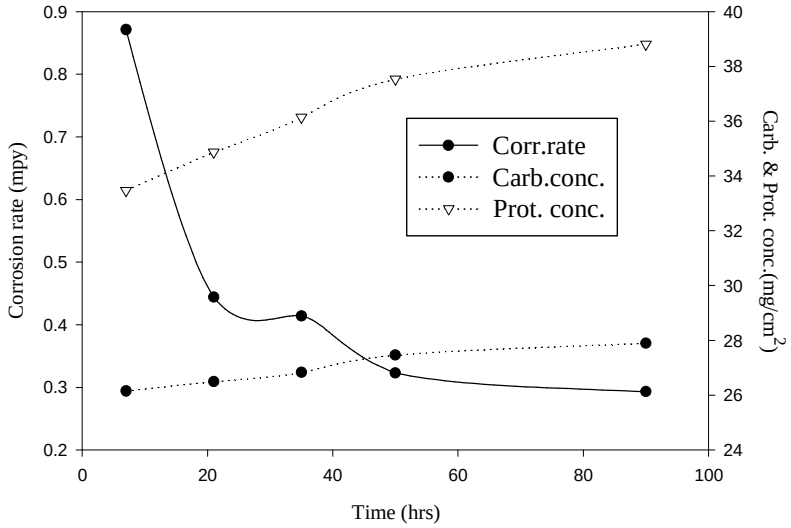

Fig. 5 Relation between corrosion rate and EPS component in case of stainless steel (with media replenishment) 304L exposed to DSM in immersion test

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

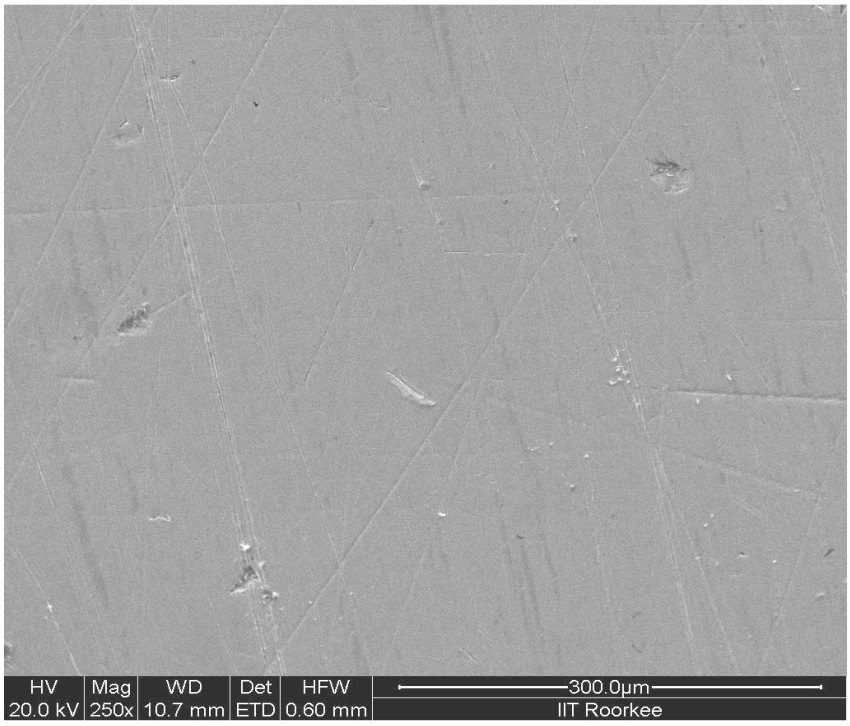

Fig. 6 Scanning electron micrograph of 304L after 50 days Immersion test in Baar's Media (Control)

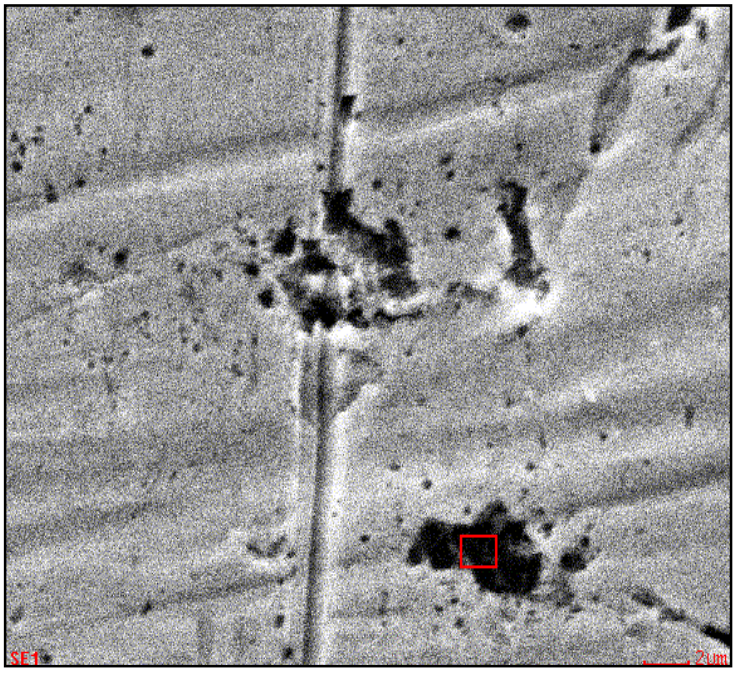

Fig.7 Scanning electron micrograph of Pit on SS 304L exposed to inoculated Baar's media after 50 day's immersion test.

JCSE The Journal of Corrosion Science and Engineering
ISSN 1466-8858 Volume 14, Preprint 25 submitted 17 June 2011

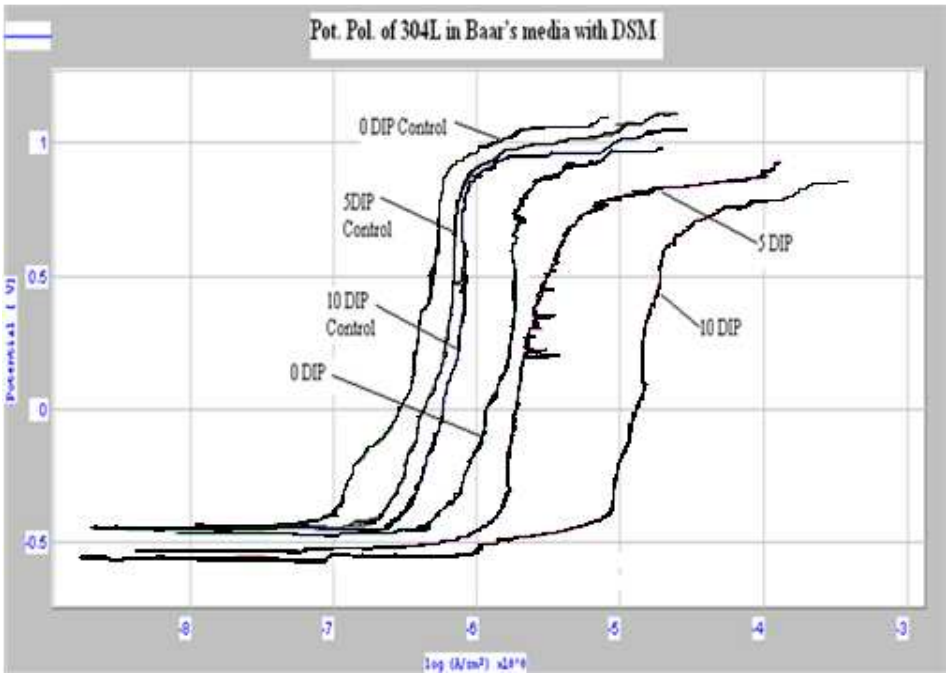

Fig. 8 Anodic polarization test of 304L in Baar's media at various time periods

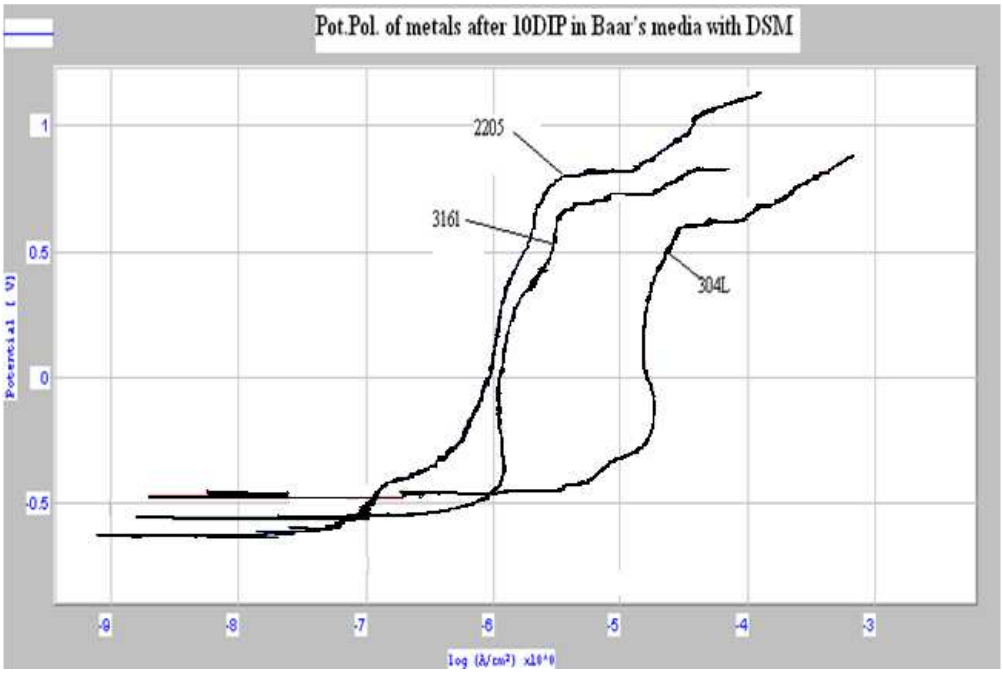

Fig. 9 Anodic polarization of various SS after 10 days incubation periods

DI- Day immersion, DIP- Days incubation period, 0 DIP – Zero day incubation, 5 DIP - Five days' incubation, 10 DIP - Ten days' incubation