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Microbiologically influenced corrosion in dairy effluent

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ABSTRACT: In the dairy industry mild steel is used as the construction material for the effluent treatment plants, pipelines, reinforcement in concrete structures etc. The steel exposed to the dairy effluent faces corrosion due to the microbes. In the present study the role of microbes in dairy effluent on the corrosion of mild steel has been investigated. *Pseudomonas* sp., *Streptococcus* sp., *Micrococcus* sp., *Bacillus* sp., *Neisseria* sp. and *Lactobacillus* sp. were identified in dairy effluent. Corrosion rate has been estimated by weight loss measurements and polarization technique. The Fourier transform infrared spectroscopy (FTIR) and X-ray diffractometer (XRD) studies were found helpful in investigating the chemical pathway leading to the formation of corrosion products on the mild steel during fermentation. Initiation of pitting corrosion was noticed on steel specimens by scanning electron microscope (SEM). A mechanism has been proposed for microbiologically influenced corrosion in dairy effluent.

Key words: *Dairy effluent, Lactobacilli spp., Pseudomonas sp, fermentation, iron sulphide, microbiological corrosion*

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INTRODUCTION

Microbiologically influenced corrosion (MIC) of mild steel in wastewater collection and treatment systems is well documented (Tatnall, 1981; Griffiths 1992). The microorganisms affect the corrosion process of the metal by directly influencing the anodic and cathodic reactions, by affecting protective surface films on metals, by producing corrosive substances and producing solid deposits. Various industries like chemical processing industries, nuclear power generation industries, water treatment industries and aviation industries have been affected by microbial corrosion, the most extensively studied microorganisms in relation to biocorrosion are the sulfate-reducing bacteria (SRB), whose participation in microbiologically influenced corrosion (MIC) was evidenced decades ago (Graves *et al.* 1996; Hamilton, 1985; Pope, *et al.,* 1998; Voordouw *et al.,* 1992; Von Wolzogen Kuhr *et al.,* 1934). However aerobic manganese, iron oxidizing and acid producing bacteria and fungi may also participate in the corrosion process (Bento *et al.*, 2001; Shennan, 1988; Videla *et al.*, 1992). Microorganisms influence the corrosion by altering the chemistry at the interface between the metal and the bulk fluid (Jones *et al.,* 2002; Little *et al.,* 2002). Most of the equipment used for effluent treatment processes utilizes steel as a basic material of construction. Steel tanks and pipes are generally used to transport dairy effluent in liquid form from one place to another. Steel structures, tanks, pipes and components of the pipeline like steel valves; pipeline accessories exposed to diary effluent are affected by corrosion. Industrial effluents containing dissolved oxygen, suspended particles and the pH of the solution are the key factors that influence corrosion Gaylarde *et al.*, 1999; Gaylarde, 1989; Buck, *et al.*, 1996; Koch, *et al.*, 2001; Birgitta, *et al.*, 1999; Ternstrom *et al.*, 1993; Kumar *et al.*, 1998). In the present study an attempt has been made to investigate the effect of dairy effluent, collected from Aavin locally available milk processing company, on mild steel corrosion. Samples of dairy effluent were collected on 16th of December 2005 from a local dairy product company, Karaikudi located in Sivaganga district, Tamilnadu, India by using sterilized conical flasks. These samples were transported from the industry to the microbilogical laboratory, Central Electrochemical Research Institute, Karaikudi by using an icebox and the bacterial counts were enumerated within 24 hrs.

MATERIALS AND METHODS

Bacterial enumeration and identification in dairy effluent

Samples of dairy effluent were collected on 3rd. week of December from a local dairy product company located in Sivaganga District, Tamilnadu, India by using sterilized conical flasks. Using an icebox from sites to CECRI, microbiological lab transported these samples. Karaikudi. The collected samples were serially diluted (10 fold) using 9 mL of sterile distilled water-blanks and the samples were

plated by the pour plate technique. The nutrient agar medium, and lactobacillus medium (Hi-media, Mumbai) were used to enumerate heterotrophic bacteria and lactic acid producing bacteria respectively. The collected samples were further serially diluted up to 10^{-6} dilution. 1 mL of each sample was poured into sterile petridishes. The prepared respective sterile medium was also poured into petridishes. The plates were gently swirled so that the medium might be distributed evenly in the plate. Plates in triplicate were prepared for each dilution. The plates were incubated at room temperature for 24 h. After 24 h. to 48 h. the colonies were counted. The plates containing bacterial colonies with 30-300 numbers were selected for calculation. The bacterial colonies were expressed as colony forming units per mL (CFU/mL). Morphologically dissimilar colonies were selected randomly from all plates and isolated colonies were purified using an appropriate medium by streaking methods. In the streaking method, one loopful of inoculam was placed on the medium near the rim of the plate and spread over a segment in a zigzag horizontal pattern until 1/3 of the plate is covered. The plate was rotated about 60 degrees and spread the bacteria from the first streak in to a second area using the same motion in zigzag horizontal pattern. The lid was replaced and inverted the plate. The plate was incubated at room temperature. The isolated pure cultures were maintained in test tubes as slant culture for further analysis. Six isolates were identified in the dairy effluent samples. The strains were maintained at 40°C to keep the microbial strain viable. The isolated bacterial cultures were identified up to genus level by their morphological and biochemical characterization according to the key described in Bergey's manual of determinative bacteriology (Holt *et al.*, 1994).

Corrosion studies

Mild steel coupons (1x 4 cm^2) were mechanically polished to mirror finish and then degreased using trichloroethylene. A known quantity of dairy effluent was taken in five beakers and coupons were immersed in the effluent and the weight loss values were collected for different periods (5, 10, 15, and 20 days). The effluent was kept at room temperature (25-30 °C) allowed to ferment and the pH was measured for periods of different duration 5, 10, 15, and 20 days. After the different immersion periods, the coupons were removed, washed in distilled water and dried. Final weights of the steel coupons in each system were taken and the average corrosion rates calculated. The Tafel polarization curves were obtained by scanning the potential from the open circuit potential towards 200 mV anodically and cathodically. The scan rate was 120 mV/min. Polarization measurements were carried out potentiodynamically using model PGP201, employing potentiostat with volta master-1-software. A 1 cm^2 mild steel coupon was used for the polarization studies. A saturated calomel electrode (SCE) was used as the reference electrode and a platinum wire as counter electrode. The i_{corr} values were obtained from the plot of E Vs log I curve (Gunasekaran *et al.*, 1997).

Chemical characterization and surface analysis

Chloride, sulphate, magnesium and calcium were estimated in the dairy effluent. Chloride was estimated by Mohr's method and sulphate was estimated by the gravimetric method. Magnesium and calcium present in the dairy effluent sample, were analyzed using atomic absorption spectroscopy (Varian AAS Model: Spectra 220). The biological oxygen demand (BOD) and chemical oxygen demand (COD) of the samples were determined using a BOD analyzer and titration methods respectively. Fourier Transform infrared spectroscopy (Make, Nicolet Nexus 470) was used for the analysis of the biochemical characteristics of the dairy effluent sample and also the biofilm collected from the metal exposed to the dairy effluent. The spectrum was taken in the mid IR region of 400– 4000/cm. The spectrum was recorded using the ATR (Attenuated Total Reflectance) technique. The sample was directly placed on the sample holder (zinc selenide crystal) and the spectrum recorded in the transmittance mode. The mild steel specimens were removed from the dairy effluent and the corrosion product was dried and crushed to a fine powder and used for X-ray diffraction spectroscope (XRD) analysis. A computer controlled XRD system, (JEOL Model JDX – 8030) was used to scan the corrosion products between 10° and 85° with copper K α radiation (Ni filter) at a rating of 40 kV, 20 mA to determine the nature of the film formed on the mild steel. After 20 days exposure the specimens were removed from the dairy effluent sample and examined for the nature of corrosion on the steel in a scanning electron microscope (SEM) Hitachi model S-3000H at a magnification ranging from 500X to 3000X and operated at an accelerating voltage of 25 kV.

RESULTS

Enumeration and identification of bacteria

The total viable bacterial activity of hetrotrophic bacteria (HB) is 7.2 X 10^{14} CFU/mL while *Lactobacilli* (LB) is 4.7

X10^8 CFU/mL. Though several isolates have been identified in the effluent, only six predominant bacterial species were characterized up to generic level using standard biochemical characterization methods. The identified genus types are as follows: *Pseudomonas* spp. *Streptococcus* spp. *Micrococcus* spp., *Bacillus* spp. *Neisseria* spp. and *Lactobacillus* spp. It can be seen that out of the six genuses three are rod shaped while three are coccus shaped. Four are gram positive while two belong to gram-negative strains. Among six isolates, four have the ability to move from one place to other. The entire six genus have the ability to secrete the enzyme called oxidase, and to carryout carbohydrate fermentation. In addition, four genuses are able to reduce nitrate and five genuses have the ability to consume citrate. It can also be seen that the entire six genus can produce acid through the consumption of sugar, where as five strains have the ability to produce gas. *Micrococcus* is a chemoorganotroph with a respiratory metabolism often producing little or no acid from carbohydrates. *Streptococcus* is chemo an organotrph requiring nutritionally rich media for growth and 5% carbon dioxide. The cell metabolism is fermentative, producing lactate but no gas. *Bacillus* is a chemolithio organotroph with a fermentative or respiratory metabolism. *Lactobacillus* is also a chemoorganotroph, which requires rich complex media and metabolism is fermentative and saccharoclastic; at least half of the end product carbon is lactate. *Niesseria* is chemoorganotroph, which produces carbonic anhydrase. *Pseudomonas* is also chemolitho organotrophic, which is able to use hydrogen or carbon monoxide as an energy source.

Corrosion studies

The corrosion rate of mild steel obtained from the weight loss study is shown in Table 1. The corrosion rates of mild steel were 0.040, 0.047, 0.048, 0.049 mmpy at 5, 10, 15 and 20 days respectively. Table 2 shows the corrosion current observed using the Tafel polarization method. The instantaneous corrosion current was found to be 0.51 μA/cm whereas on the second day the corrosion current was found to be 0.27 μA/cm. After the 14$^{th.}$ day the corrosion current was 5.00 μA/cm, where as 9.72 μA/cm was observed on the 20$^{th.}$ day (Fig. 1).

Table 1: Corrosion rate of mild steel by weight loss method

Systems	Immersion Period (days)	Weight loss (mg)	Corrosion rate (mmpy)
1	5	14 ± 2	0.040
2	10	33 ± 3	0.047
3	15	50 ± 2	0.048
4	20	69 ± 3	0.049

Chemical characterization and surface analysis

Table 3 shows the chemical analysis of the dairy effluent. It can be seen that the sulphate and chloride contents were 40 and 70 ppm respectively. The values of BOD and COD before and after corrosion were 25 ppm, 320 ppm and 9 ppm, 360 ppm respectively. pH values were in the range between 7.2 and 7.5. Fig. 2 shows the FTIR spectrum of dairy effluent over a period of 20 days. The FTIR spectrum shows a peak at 3317/cm indicates the presence of OH peak. The other peaks at 2918/cm and 2849/cm indicate the presence of CH aliphatic stretch. The carbonyl peak (C=O) in the range between 1722/cm and 1737/cm and C=C conjugated diene at 1631/cm and 1657/cm are noticed. The Carboxylate ion (COO^-) could be seen in the range between 1548/cm and 1480/cm. The C-Cl bond is seen at about 626/cm. Fig. 3 shows the FTIR spectrum of biofilm on mild steel immersed in dairy effluent. The IR spectrum analysis shows that the peaks at 3316/cm indicate the presence of OH bond. The other peaks in the range between 1754/cm and 1776/cm indicate the presence of the carbonyl group (C=O) and C=C conjugated diene at 1632/cm, carboxylate anion (COO^-) at 1555/cm and 1468/cm. Another peak at 743/cm indicates the iron oxide bond and the peak at 626/cm indicates the carbon chloride (C-Cl) bond. The XRD pattern of corrosion product is shown in Fig. 4. A predominant iron sulphide peak could be noticed, indicating the role of microaerophilic in the conversion of sulphate to sulphide on steel corrosion. An SEM photograph shows the initiation of pitting on a mild steel coupon (Fig. 5).

DISCUSSION AND CONCLUSION

On the basis of biochemical characteristics of microbes it can be concluded that the above-mentioned chemoorganotrophs utilize energy from dairy effluent and chemolithotrophs accelerates the corrosion process by converting ferros ion to ferric and its oxides (Muthukumar *et al.*, 2003, Dawood *et al.*, 1998, Jayaraman *et al.*, 1998, Rajasekar *et al.*, 2005). The corrosion rates of mild steel were 0.040, 0.047, 0.048, 0.049 mm/y at 5, 10, 15 and 20 days respectively. The corrosion current increased with time except on the second day while b_c (cathodic tafel slope) values for the initial and on the second day were not

Table 2: Corrosion rate of mild steel in dairy effluent by polarization technique

Immersion period (days)	I_{corr} (µA cm^{-2})	b_a mV $decade^{-1}$	b_c mV $decade^{-1}$	Corrosion rate (mmpy)
0	0.51	164	157	0.006
2	0.27	135	137	0.003
14	5.01	189	614	0.059
20	9.72	167	273	0.114

Table 3: Chemical analysis of dairy effluent

Chemical characteristics	Concentration (ppm)
Sulphate	40.0
Chloride	70.0
Magnesium	7.0
Calcium	56.0
BOD	25*
COD	320*

After corrosion studies BOD is 9 ppm and COD is 360 ppm

significantly changed, but some significant change in b_a (anodic tafel slope) value was noted. These data indicate that there is not much influence of cations on steel up to the 2nd. day. On the 14th. and 20th. days both the curves (anodic and cathodic) were shifted to right and indicate a higher corrosion rate (Fig. 1). It demonstrates the influence of both anionic and cationic species on corrosion. It can be assumed that the presence of calcium and magnesium ions in the dairy effluent and the formation of ferric sulphide (cathodic to parent metal) during fermentation (decay), may influence the changes in the cathodic curves. The formation of hydrogen sulphide (HS^-) ions in the dairy effluent may enhance the anodic reaction. Further the reduction of oxygen during fermentation also influences the cathodic reaction and accelerates the corrosion current. The values of BOD and COD suggest that the decreasing of BOD may be due to the fermentation of dairy effluent. During decay (fermentation) the microbes present consume organic compounds, degrade the effluent, converting them in to organic acids with the creation of anaerobic environment**.** The metal immersed in dairy effluent FTIR shows a new peak at 743/cm which indicates the presence of a carbon chloride bond (Fig. 3). It may be due to the presence of chloride in the dairy effluent. CH aliphatic stretch (2918/cm and 2849/cm) could not be observed in biofilm on metal steel immersed in dairy effluent. It reveals that the carbon hydrogen bond is converted as carbon-chloride bond during the fermentation of dairy effluent. The XRD pattern indicates the presence of iron sulphide peaks on steel surface. It can be assumed that the assimilated sulphate conversion into sulphide by a micro aerophilic organism, namely *Lactobacillus* influences the corrosion rate. The sulphide combines with Fe^{2+} to form FeS. The FeS may then combine with an organic molecule and influence the corrosion process both anodically and cathodically (Williams, 2004). The FTIR spectrum reveals the formation of carbon chloride compounds on the coupon during fermentation in the dairy effluent. It can be claimed that the adsorption of chloride ions and the formation of FeS is the causative factor for corrosion.

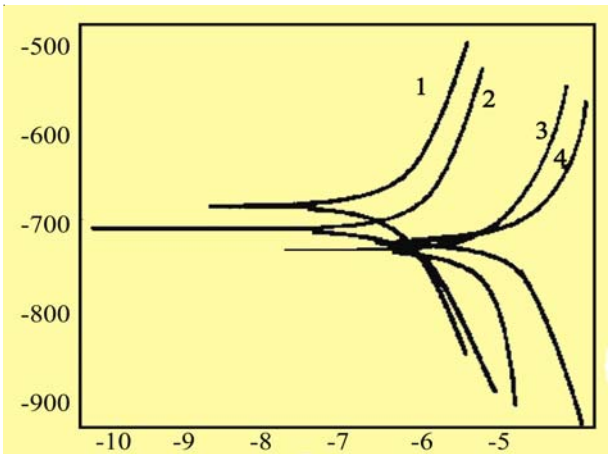


Fig 1. Polarization beaviour of mild steel in presence of dairy effluent at various periods. 1 = 0 day , 2 = 1st day, 3 = 14th day, 4 = 20th day

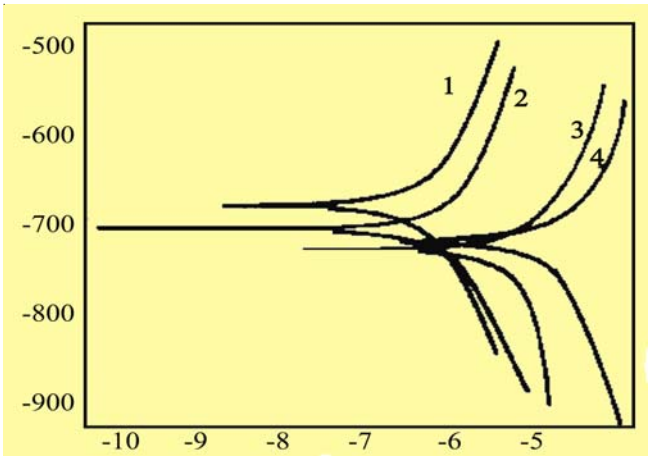


Fig. 2: FTIR spectrum of diary effluent

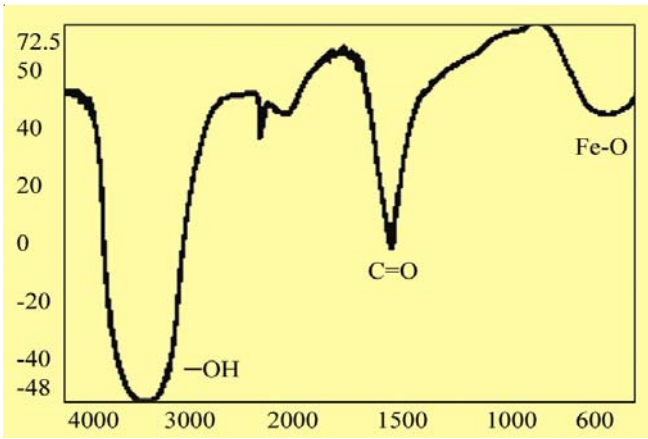


Fig. 3: FTIR spectrum of biofilm on mild steel immersed in dairy effluent

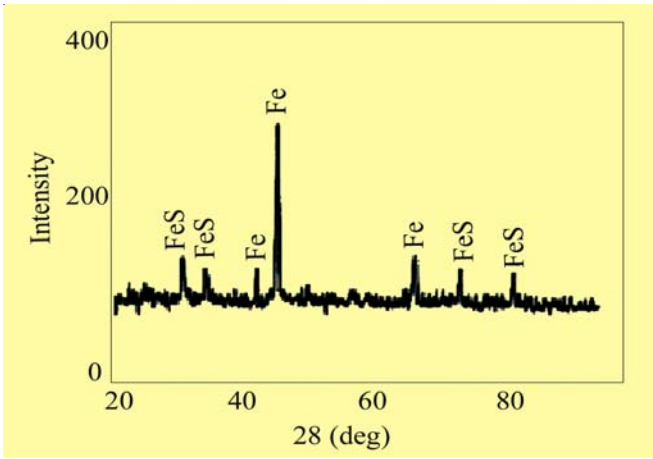


Fig. 4: XRD pattern of corrosion product on mild steel

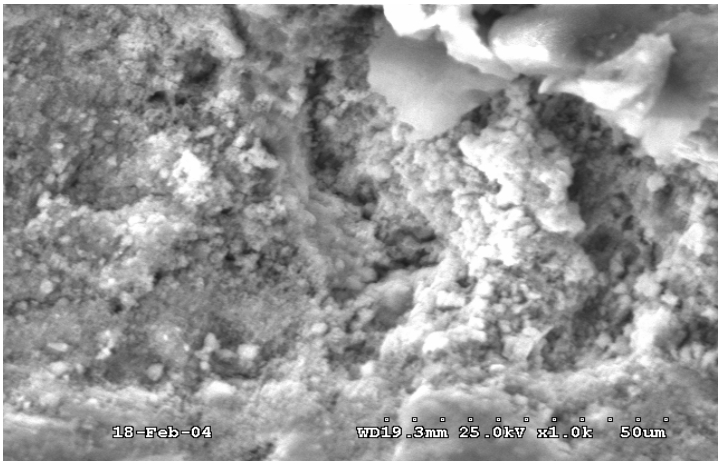
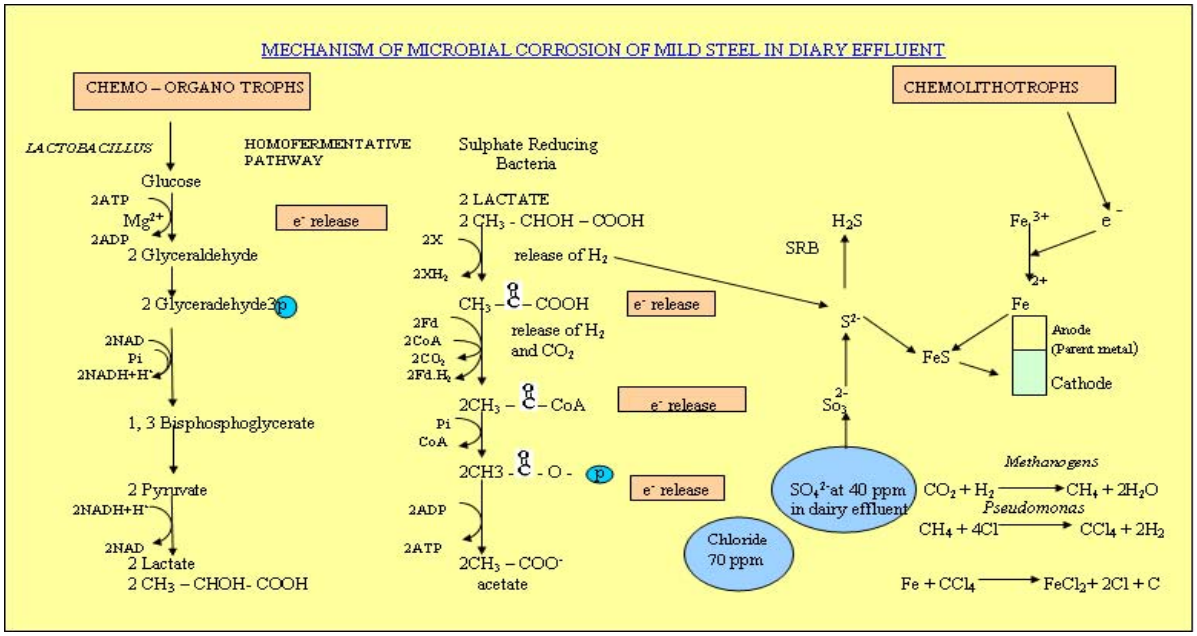


Fig. 5: SEM micrograph of mild steel after immersion in dairy effluent



* The formed hydrogen and carbon are taken up by the chemolithotrophs and helps in the further reduction leading to the corrosion process.

Fig. 6: Corrosion model for mild steel in dairy effluent

The presence of FeS was noticed in the XRD studies. SEM studies also showed some pitting initiation in the coupon immersed in the dairy effluent. It can also be claimed that during the fermentation process bacteria consume oxygen from the metal surface (O_2 reduction) and take energy from the organic content in the dairy effluent. Subsequently the microbes convert sulphate into sulphide with the acceptance of electrons from the organic content (Sass and Cypionka, 2004) (Lactate-electron donor; sulphate-electron acceptor). *Pseudomonas* chemolithitrophic species may reduce ferric to ferrous (Weslake, 1986) and favour the formation of FeS. Hence sulphide formation has been noticed on the metal surface and FeS corrosion product appeared. It can be concluded that microbiologically influenced corrosion is responsible for corrosion of steel in dairy effluent and a possible mechanism has been explained in Fig. 6. The following

observations have been made from the work reported in the present study

1. *Bacillus, Pseudomonas, Micrococcus, Niesseria, Streptococcus* and *Lactobacillus* were found in dairy effluent.
2. Corrosion rate was found to increase with time by weight loss measurements and the values of I_{corr} increases with time in polarisation technique.
3. It was clearly noted that the microbes influence the corrosion by oxygen reduction and fermentation processes.
4. The presence of sulphide ions on the mild steel coupon indicates that the microbes take energy from the organic content and convert sulphate into ferrous sulphide (FeS), which can act as cathode and the parental metal as anode.
5. An SEM study showed the initiation of pitting corrosion of mild steel.

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