



Inhibition of Corrosion of Mild Steel by *Staphylococcus sp*

S.Ponmariappan^{1*}, S.Maruthamuthu¹ and R.Palaniappan²

¹Corrosion Science and Engineering Division, Central Electrochemical Research Institute, Karaikudi 630 006, India

²P.G.Dept.of Microbiology,Sri Paramakalayani College, Alwarkurichi 627 412, India

*Present Address: Division of Biotechnology, Defence Research & Development Establishment, Gwalior 474 003, M.P, India

Received: 29 December 2004 Accepted: 15 January 2005

Abstract

Even though Microbiologically Influenced Corrosion (MIC) has been widely studied and reported it is seldom appreciated that, certain type of microorganisms can effectively contribute to inhibition of corrosion process. In the present study the best biofilm forming bacteria such as *Staphylococcus sp* was isolated from three-month old biofilm on mild steel surface. Mild steel coupons were immersed in cultures of *Staphylococcus sp* at room temperature. Corrosion inhibition behaviour of pure-culture biofilms of *Staphylococcus sp* has been evaluated by conventional weight loss method and electrochemical methods like polarization and impedance spectroscopy. A significant reduction in the corrosion rate was observed in presence of *Staphylococcus sp*. These studies indicate that corrosion inhibition occurs due to the formation of biofilm on the metal surface. Moreover, the mechanism by which the *Staphylococcus sp* biofilm to inhibit corrosion of mild steel due to the secretion of extra cellular polymeric substances (EPS), and will form a thin layer over the metal surface and prevent the metal from further dissolution. The partial chemical characterization of EPS has been carried out by FTIR and the fatty acids content was analyzed by using a Gas chromatogram. The surface topography of the metal along with *Staphylococcus sp* was examined by SEM.

Key words: *Staphylococcus sp*, corrosion control, mild steel, biofilm

Introduction

It is well known that microorganisms can promote corrosion in different ways by forming differential aeration cell,¹ extra cellular polymeric substances²⁻³ or by their binding capability with metal ions.⁴⁻⁵ Biologically inhibiting corrosion is a recent idea which involves the application of microorganisms to inhibit corrosion. Pure culture biofilms prevents diffusion of corrosive species such as oxygen to the metal surface, there by reducing the corrosion rate⁶⁻⁷. Bacterial film and the exopolymers inhibit corrosion.⁸⁻⁹ Soracco *et al*¹⁰ has recorded pitting of mill steel in presence of bacteria than under sterile conditions. Guamet and Videla¹¹ reported the

protective action of *Serratia marcescens* (ASTM90) on aluminium panels. Maruthamuthu *et al*¹² proposed that since most of the bacterial cell wall are negatively charged, it can improve the passivity of stainless steel. Ponmariappan *et al*¹³ reported that the corrosion inhibition of bacterial species such as *Pseudomonas* and *Vibrio sp* reduce the current demand during cathodic protection. Recently Ponmariappan *et al*¹⁴ documented the corrosion inhibition and micro fouling control using *Actinomycetes sp*. These and other observations have lead to a new approach of corrosion protection based on corrosion control using biofilms. The constraints met with the application of conventional classical inhibitors and the increasing advantages offered by the microbiologically inhibiting corrosion concepts are increasing costs of classical methods of protection, diminishing non-renewable resources and less pollution. When compared to the classical methods,

* Corresponding author: S.Ponmariappan

Email: spon@rediffmail.com

biological methods are easy to develop, relatively cheap and involve lower maintenance costs. On this basis, in the present study, pure culture of *Staphylococcus* species was selected and the corrosion rate was evaluated by electrochemical techniques.

Experimental

Specimen preparation

The specimens were fabricated from mildsteel with the following composition: carbon 0.01%, manganese 0.05%, phosphorous 0.04%, and iron balance. Specimen size of 4cmx1cm was used for weight loss studies and a size of 1cm² was used for electrochemical studies. The specimens were cut and polished to mirror finish using emery fine grade paper and cleaned by trichloroethylene and exposed to UV light. All the specimens for weight loss study were autoclaved at 121°C for 15–20 minutes. The weight loss studies were carried out with nine specimens for getting reproducibility and the average values are presented.

Microorganisms used

Locally available pond water in natural condition was used for the formation of biofilm. Mild steel coupons were kept was scrapped using sterile brush. Bacterial species in the biofilm were identified using biochemical tests based on the standard methods described in Bergey's manual of systemic bacteriology^{15& 16}. More than 20 bacterial species have been identified. However, in the present study *Staphylococcus* sp was alone selected for detailed studies. Corrosion studies.

Weight loss measurements

A 100% of nutrient broth medium with the composition of peptone 5g, yeast extracts 3g (Hi-media, Bombay) per liter of water. Based on our previous studies of natural sources, a medium with 3% nutrients is enough to promote the formation of biofilm. 1000 ppm chloride as a threshold value will tend to cause break down of passivity. The medium with 3% nutrients and 1000 ppm chloride was prepared and transferred to the conventional three- electrode cell assembly. The initial weight of the decreased specimens were taken and suspended in the cell containing 3% nutrients. The cells with suspended specimens were sterilized and cooled to room temperature. The cells containing 1000-ppm chloride with 3% nutrients alone were used as control. The corrosion inhibition studies were carried out for individual bacterial strains. 0.6 OD of bacterial cultures was inoculated in pre-

sterilized 3% concentration of nutrient broth (composition: peptone 150mg/l, yeast extracts 30mg/l and sodium chloride 1000mg/l) and the cells were incubated at room temperature for seven days to collect the weight loss data.

Polarization and impedance measurements

A conventional three-electrode cell assembly was used for polarization as well as impedance measurements. A mild steel specimen as the working electrode, a large platinum foil as the counter electrode and a saturated calomel electrode as the reference electrode respectively were used. Polarization measurements were carried out potentiodynamically using potentiostat (PAR model 173) in conjunction with potentiostan generator and XY recorder. AC impedance studies were carried out using computer controlled EG&G PAR system model M6310 with software M398. The impedance measurements have been separately carried out at different time intervals (3rd, 5th and 7th day).

Isolation of extra cellular polymer matrix from Staphylococcus sp

The *Staphylococcus* species were inoculated in a sterilized 3% nutrient broth medium. The polished mild steel specimens of size 10x10cm were immersed in the medium and incubated at room temperature for 7 days for the formation of biofilm. After 7 days the biofilm was removed from the specimens using a sterile brush and centrifuged at 10,000rpm for 15 minutes. The cell free broth was collected, filtered through 0.1 to 0.2 µm filter paper by ultra centrifugation and precipitated with methanol. The biopolymer matrix was collected, dissolved in water and subjected to dialysis process overnight. The samples were concentrated at -20°C using a rotavapour water bath again precipitated with methanol and dried at 50°C. After drying, the samples were subjected to chemical characterization.

Gas chromatographic analysis of fatty acids

The polished mild steel specimens of size 5x2cm were immersed to 3% nutrient broth medium and incubated at room temperature for seven days for the formation of biofilm. After seven days incubation period, the specimens were removed from the cell. The biofilm was scrapped using sterile brush, centrifuged at 10,000rpm for 15-20 minutes. The clear supernatant was transferred to another test tube and the pellet was washed with EDTA solution twice. Along with clear supernatant 1ml of saponification reagent was added, vortexed

for 5-10 sec using a cyclomixer and placed under 100°C for 5 minutes. Sample was again vortexed for 5-10 sec, then placed under 100°C for 25 minutes and cooled to room temperature. 20ml of methylation reagent was added and vortexed for 5-10 seconds. Placed in water bath at 80°C for 10 minutes and cooled to room temperature for the formation of methyl esters of fatty acids. 1.25ml of extractive solvent was added and vortexed for 10 minutes. Two layers were formed, i) Aqueous phase, ii) Organic. The top layer was transferred to another eppendorf tube, followed by the addition of 3ml of base wash solution and vortexed for 5 minutes. 2/3 of top phase was transferred to GC vials and the samples were analysed for fatty acids of respective biofilms in a Gas chromatogram model: 6890 plus and the results were compared with standard fatty acids data. From the peak area and the intensity of the peaks, the quantitative data were also calculated and compared with standard test results.

Fourier transform infrared spectroscopy studies (FTIR) for biopolymer analysis

Extra cellular polymeric substances of *Staphylococcus* species were isolated and purified as described earlier. A small amount of dried biopolymer matrix approximately 60 - 100µg was suspended in 50 µl distilled water. An aliquot of 30µl was transferred to a Zinc selenide (ZnSe) optical plate and dried under moderate vacuum between 2.5 and 7.5kpa to a transparent film suitable for absorbance/ transmission FTIR measurements. All spectra were recorded between 4000cm⁻¹ and 400cm⁻¹ wave numbers on a Nexus 670 FTIR spectrometer (Thermo Nicolet) equipped with an DTGS detector and averaging of 128 scans. Spectral resolution was 4cm⁻¹. Data point resolution was approximately 1 point per wave number. The digitized spectra were processed using OMNIC® software. To minimize problems arising from unavoidable base line shifts and to enhance the resolution of superimposed bands, the smoothed first and second derivatives of the original spectra.

pH and Dissolved oxygen measurement

The pH and dissolved oxygen content were measured at various stages of experiments in the bulk solution using the standard pH and DO meter respectively.

SEM studies

Mild steel specimens of size 1x1cm were exposed to different bacterial cultures up to seven days for the formation

of biofilm. After seven days, the specimens were removed from the cell, and immersed in 2-4% cacodylate buffered glutaraldehyde solution for four hours under refrigerated condition. After four hours, the specimens were subjected to series of distilled water washes. Distilled water rinses were followed by a graded series of water/acetone washes (100:0; 75:25; 50:50, 25:75; 0:100 v/v water/acetone). Removal of acetone was accomplished through a series of graded washes of acetone - xylene). Air-dried, coated with gold sputter using Ion sputter Jeol model JFC 1100. Specimens were examined under SEM Hitachi Model S- 3000 H at magnification ranging from 500X to 3000X operated at an accelerating voltage of 25 Kv.

Results

Weight loss data

The weight loss data and the durability factor are given in Table I. The durability factor has been worked out on the basis of reduction in weight loss due to corrosion inhibition and it is compared with the control. The *Staphylococcus* sp inhibit corrosion efficiently and giving a durability factor of about 7.96.

Table 1. Weight Loss Data in presence and in absence of *Staphylococcus* sp

System	Corrosion rate (mppy)	Durability factor
Control	0.3238	—
<i>Staphylococcus</i> sp	0.00405	7.96

Polarization studies

The anodic polarization curves for mild steel immersed in sterile control system and *Staphylococcus* sp inoculated system for different test periods viz 3days, 5days and 7days are shown in Fig 1(a). In the case of control system it can be seen that the anodic polarization is quite steeper and the curve shifts towards right direction with increasing test period indicating that the anodic corrosion current tends to increase with time of immersion. The corrosion potential is almost the same of the order of around -600mV vs SCE. In the case of *Staphylococcus* sp inoculated system the corrosion potential tends to shift in the negative direction by around 120mV after 3days of immersion. After 3 days the potential tends to moves gradually in the positive direction and at the end of 7 days of immersion the shift is around 200mV. It clearly indicates

that the biofilm of *Staphylococcus* sp passivates the metal surface. The corrosion current decreases with time.

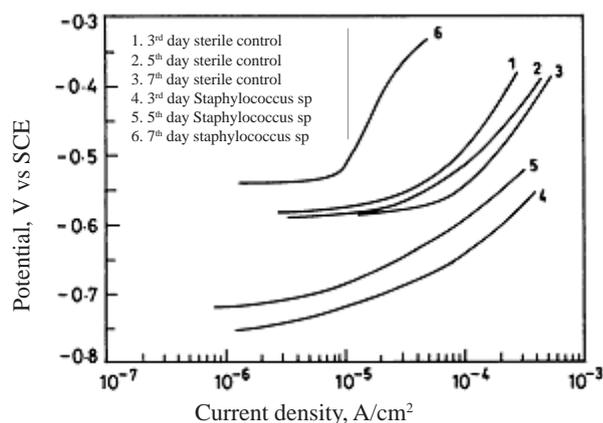


Fig 1(a). Anodic behaviour of mild steel in presence and absence of *Staphylococcus* sp with time

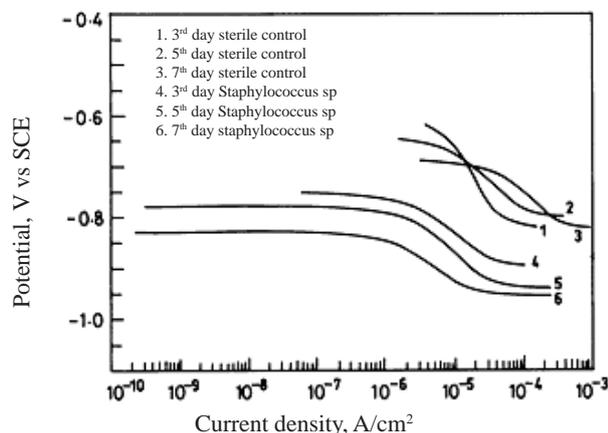


Fig 1(b). Cathodic behaviour of mild steel in presence and absence of *Staphylococcus* sp with time

The cathodic polarization curves for mild steel immersed in sterile control system as well as *Staphylococcus* sp inoculated system for different test periods viz 3 days, 5 days and 7 days are shown in Fig 1(b). In sterile control system the polarization is quite steeper for 3 days test duration and the polarization tends to decrease with increasing time of immersion indicating that the current increases with time. However there is a wide variation in the corrosion potential. *Staphylococcus* sp inoculated system the corrosion potential tends to move towards the negative direction and at the end of 7 days of immersion the shift is around 130 mV when

compared to sterile control system. There is wide variation in the corrosion potential as well as corrosion current. However the corrosion current tends to decrease quite substantially with time of immersion. *Staphylococcus* sp inoculated system appears to be slightly different from sterile control system discussed. Initially the anodic as well as cathodic corrosion potentials are around -750 mV vs SCE, where as the cathodic potential as in earlier system increases with time and reach as a value of -830 mV. Anodic corrosion potential tends to move to the positive direction and reach a value of -540 mV vs SCE.

In sterile control system the anodic corrosion current density tends to increase with time of immersion, where as there is no significant change in the cathodic current density with time of immersion. At the end of 7 days of immersion, cathodic current density is only 7×10^{-5} mA/cm² where as anodic current density is 90×10^{-5} mA/cm² i.e 13 times higher. In this case *Staphylococcus* sp inoculated system also anodic current density is quite high at the end of 7 days of immersion anodic current density is 6920×10^{-9} mA/cm² where as cathodic current density is 10×10^{-9} mA/cm² i.e 690 times higher.

Impedance Studies

The Bode plots and the phase angle for mild steel immersed in sterile control system as well as *Staphylococcus* sp inoculated system are given in Fig 2 (a)&(b). It can be seen from Fig 2 (a) in sterile control system both at low frequency and high frequency regions there is no marked difference. Fig 2(b) reveals that there is a marked difference in the phase angle shift. Initially the shift is as high as 30°. With time it gradually decreases and at the end of 7th day the shift is only 10°. In the case of sterile control system, the R_{ct} values are found to be around 880 ohms.cm² on the 3rd day, it gradually decreases to 520 ohms.cm² on the 5th day and it further reduces to 161 ohms.cm² on the 7th day. The C_{dl} values are around 12063 μ F upto 5th day and 98965 μ F on the 7th day.

In the case of *Staphylococcus* sp inoculated system are shown in Figs 2(a)&(b). the resistance value in *Staphylococcus* sp gradually increases with time. Where as in the case of sterile control system the resistance value decreases with time. Fig 2(b) reveals that there is a marked shift in the phase angle value of *Staphylococcus* sp with time. At the end of 7th day the phase angle is as high as 55°. In the case of *Staphylococcus* sp containing system, the R_{ct} is around 2.458 Kohm.cm² on the 3rd day, 4.427 Kohm.cm² on the 5th day and 6.921 Kohm.cm² on the 7th day. The double layer capacitance is found to gradually decrease from 2578 μ F (3rd day) to 2300

μF on the 7th day. The surface coverage is around 78% on the 3rd day, 81% on the 5th day and 97% on the 7th day.

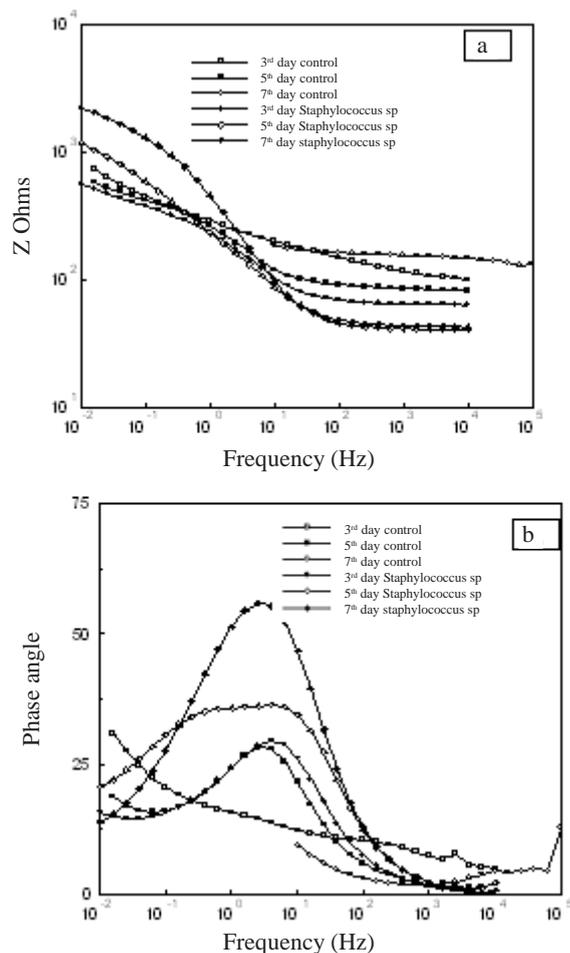


Fig 2 (a) & (b). Impedance behaviour of mild steel in presence and absence of *Staphylococcus sp.* (a - Bode magnitude, b- phase angle)

FTIR analysis of biopolymers of various bacterial species

The FTIR spectrum of extra cellular polymeric substances of *Staphylococcus sp.* is shown in Fig. 3. The extra cellular polymer matrix of *Staphylococcus sp.* shows the presence of various functional groups of organic compounds. The broad peak at 3285 cm^{-1} indicates that the *Staphylococcus sp.* secretes the slime polymers which are hydrogen bonded OH groups of polymeric association. The peak at 2962 cm^{-1} represents the CH_2 absorption of fatty acid region I. The presence of

most of the amino acids is indicated in the peak at 1837 cm^{-1} . The amide I peak is observed at 1629 cm^{-1} and this indicates the presence of ortho, $-\text{CO}-\text{C}_6\text{H}_4-\text{OH}$ or (NH_2) functional group. The amide peak II is observed at 1543 cm^{-1} and this represents the $-\text{CO}=\text{C}=\text{C}-\text{OH}$ or NH_2 functional group.

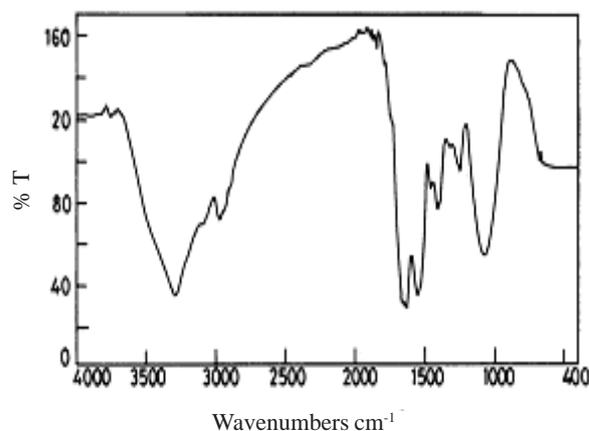


Fig 3. FTIR Spectrum of the extra cellular polymeric substances of *Staphylococcus sp.*

The peak at 1395 cm^{-1} represents the functional group of $\text{C}(\text{CH}_3)_3$ of fatty acid finger print region II. The peak at 1240 cm^{-1} represents the C-O stretching vibrations of dicarboxylic amino acids. The peak at 1068 cm^{-1} represents the S=O stretching vibrations of the functional group R. SO_3H of organic sulfur compounds.

The OMNIC[®] search match analysis suggests the presence of possible compounds in the biopolymers of *Staphylococcus sp.* are as follows: polyamide, benzamide, cephalathin, thiamine and 1-N-6 ethoadenine.

Gas chromatographic analysis

The chromatographic profiles of the fatty acid of *Staphylococcus sp.* are shown in Fig. 4. It can be seen that the extra cellular polymer of *Staphylococcus sp.* contains nine different types of fatty acids. The peak at 0.75 minutes represents the presence of caprylic acid and it is found to be around 65 $\mu\text{g}/\text{gm}$ of lipid content. Another peak at 2.47 minutes shows the presence of a saturated fatty acid called myristic acid, which is found to be around 283 $\mu\text{g}/\text{gm}$ of lipid. A strong peak at 4.14 minutes indicates the presence of a palmitic acid, and it is found to be around 1386 $\mu\text{g}/\text{gm}$ of lipid content. A peak at 4.79 minutes shows the presence of a monoenoic fatty acid identified as palmitoleic acid and its

content is found to be 543.3 µg/gm of lipid. The peak at 8.34 minutes indicates the presence of another monoenoic fatty acid identified as oleic acid whose content is 727.8 µg/gm of lipid. At 30.89 minutes, a peak is noticed which represents the presence of 5,8,11,14,17-eicosa pentaenoic acid with 20 carbon atoms and 3 double bonds from the double bonds to the terminal functional group. Its content is found to be 146.4 µg/gm of lipid. The peak at 39.59 minutes shows the presence of lignoceric acid whose content is 32.5 µg/gm of lipid. The peak at 44.44 minutes indicates the presence of cis-15-tetra cosanoic acid with 24 carbon atoms and one double bond, 9 carbon atoms from the double bonds to the terminal methyl esters functional groups. Content of cosanoic acid is 153.3µg/gm of lipid. The last peak at 54.50 minutes shows the presence of penta cosanoic acid with 25 carbon atoms and its content 146.4 µg/gm of lipid. Among the 9 fatty acids of *Staphylococcus sp*, palmitic acid and oleic acid are found to be dominant.

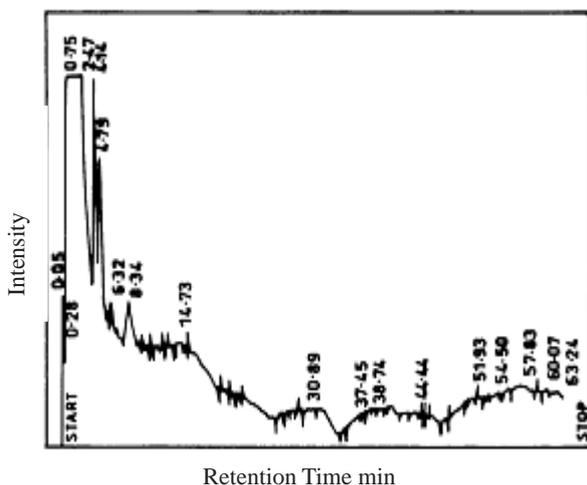


Fig 4 . Gas chromatogram of Fatty acid profiles of *Staphylococcus sp* on mild steel

Dissolved Oxygen

The Dissolved oxygen content in the control was in the range of 4.40-4.50 mg/l. around the entire study period. But in the presence of *Staphylococcus sp* 4.46mg/l on the 1st day and it gradually decreased to an extent of 0.50mg/l on the 7th day. The dissolved oxygen content with time in presence and in absence of *Staphylococcus sp* are shown in Fig. 5.

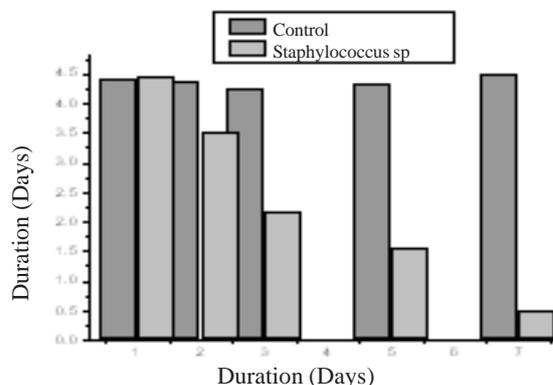


Fig 5. Dissolved oxygen in presence and in absence of *Staphylococcus sp* with time

PH

The negative logarithm of hydrogen ion concentration of control lied in the range of 7.36 –7.30 during the entire study period. But in the case of *Staphylococcus sp* the pH was around 7.29 on the 1st day it was slightly reduced to about 6.90 on the 5th day, at the end of 7th day it was around 6.56. The pH in presence and in absence of *Staphylococcus sp* is presented in Fig. 6.

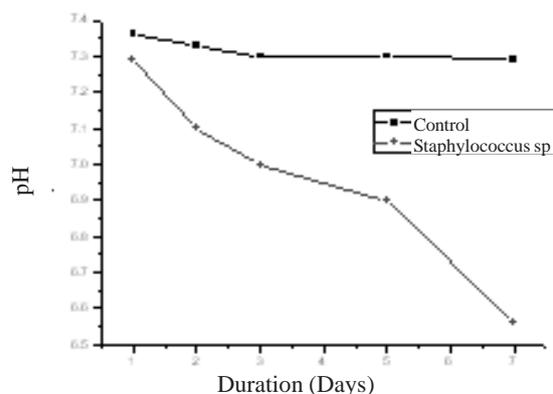


Fig 6. pH in presence and in absence of *Staphylococcus sp* with time

SEM Studies

The typical scanning electron micrograph of the surface of mild steel coupon immersed for 7 days in nutrient broth medium containing 1000ppm of chloride (sterile control) system is shown in Fig -7(a)&(b). Fig- 7 (a) shows the surface

at a lower magnification of $\times 70$. It can be seen that several corrosion pits have developed on the metal surface. Fig 7(b) shows that the corrosion products are peeling off from the metal surface. Obviously the corrosion products are non adherent and non protective.

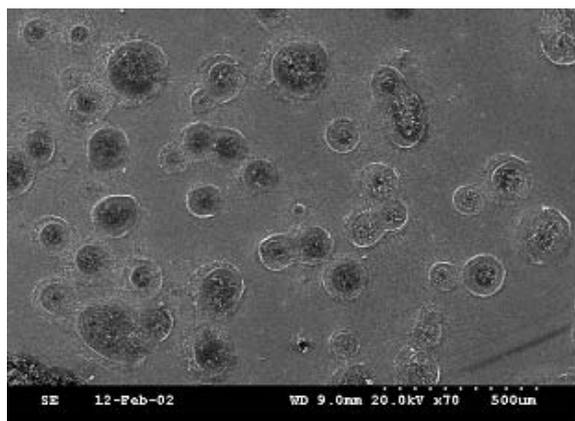


Fig 7(a). SEM photograph of mild steel in sterile control (without bacteria)

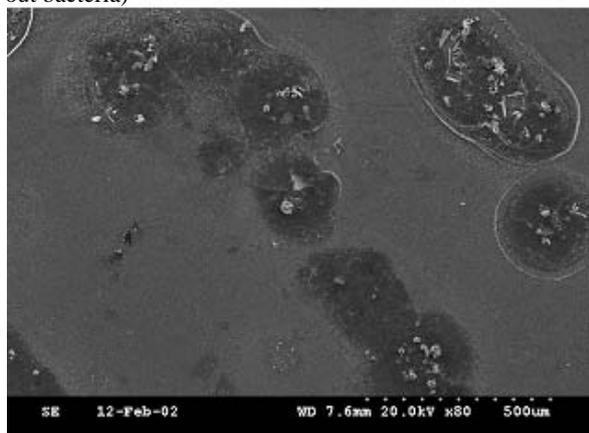


Fig 7(b). SEM photograph of mild steel in sterile control (without bacteria)

Fig .8 (a) shows a single cluster in greater detail it is approximately in $1\mu\text{m}$ diameter arranged characteristically in a bunch of grape clusters. The slime of *Staphylococcus* sp is able to provide a thin protective layer over the metal surface. The attachment of *Staphylococcus* sp on steel is heterogeneous in nature. Besides, it assumed that the EPS of *Staphylococcus* sp will uniformly cover the entire steel surface i.e there is no pitting were observed on the surface. Fig.8(b) shows the biofilm of *Staphylococcus* sp at $\times 3000$ magnification. They are spherical cocci that occur in grape like clusters. They are densely distributed throughout the metal surface.

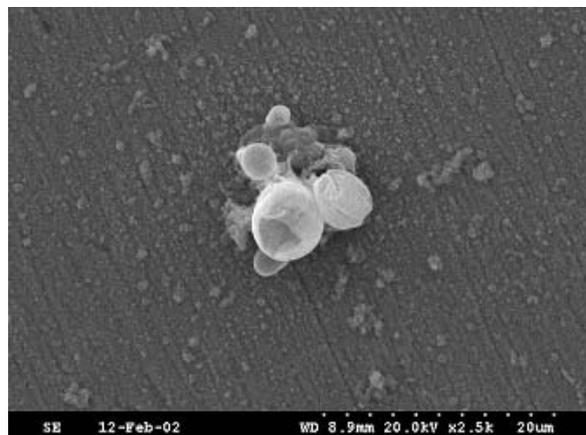


Fig 8(a). SEM of *Staphylococcus* sp on mild steel at 2.5k magnification

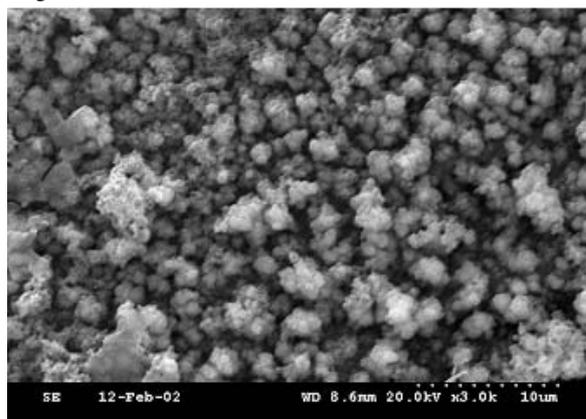


Fig 8(b). SEM of *Staphylococcus* sp on mild steel at 3.0k magnification

Discussions

The thought held by the corrosion engineers is that the microbes play an important role in enhancing corrosion. The mechanism of anaerobic corrosion was reported by several authors^{8, 17-20}. But till now there is no study for the effect of *Staphylococcus* sp on corrosion inhibition process. So the present investigation will fulfill the above gap. The weight loss studies reveal that the *Staphylococcus* sp will inhibit corrosion more efficiently (durability factor 7.96).

This conclusion is corroborated by the studies of Ford et al⁹ who found that bacterial films inhibited corrosion, and the attached cells form a diffusion barrier for the corrosive species to the metal surface. The films are able to inhibit corrosion and simultaneously improving the passivity of the metal and it also lowers the reactivity of the metal with the

corrosive species. In the electrochemical studies, from the charge transfer resistance (R_{ct}) we can confer that the *Staphylococcus* sp will inhibit corrosion because the charge transfer resistance was around $2.458 \text{ K}\Omega \cdot \text{cm}^2$ in initial periods, but later periods the resistance slowly increased to a maximum of $6.921 \text{ K}\Omega \cdot \text{cm}^2$ on the 7th day. These findings are in line with the observation of Soracco et al ¹⁰.

The graphical illustration (Figs.1&2) of the polarization and impedance indicates that electrochemical kinetic charge varies greatly with time. Such charges are attributed to the formation of a protective film and confirmed improvement in the percentage and ability of this film. The θ values are low in presence of *Staphylococcus* sp in the initial periods and it increases in later periods. The series of θ values increase will coincide with the R_{ct} values. The C_{dl} values are very low in presence of *Staphylococcus* sp when compared with the control system.

Neumann et al²¹ have discussed in detail about the pre-selection of spectral windows and their assignment to various species and their discrimination power in biological system. The peak between 3000 and 2800 cm^{-1} are assigned to fatty acid region I, dominated by $-\text{CH}_3$, $>\text{CH}_2$ and Triple bond CH stretching vibrations of the functional groups usually present in the fatty acids. Peaks between 1800 and 1500 cm^{-1} are assigned to amide region, dominated by amide I and amide II peaks of protein and peptides. The peak between 1500 and 1200 cm^{-1} are assigned as mixed region, these spectral region containing information on proteins, fatty acids and phosphate carrying compounds. Peaks between 1500 and 1400 cm^{-1} are assigned to fatty acid region II, dominated by $-\text{CH}_3$ and $>\text{CH}_2$ bending vibrations. The peaks between 1200 and 900 cm^{-1} are assigned to polysaccharide region, dominated by the finger print like absorption peaks of the carbohydrates present in the sample. Peaks between 900 and 700 cm^{-1} are remarkably specific spectral patterns, which are not yet assigned to functional groups. Based on the above classification nine fatty acids have been identified from the EPS of *Staphylococcus* sp, among the nine fatty acids palmitic acid and oleic acid are found to be dominant.

Majumdar et al²² suggested that biofilm microorganisms produce EPS, which serve as a corrosion inhibitor for mild steel. A number of bacteria isolated from the corrosion products showed promise for EPS production. The isolated polysaccharide appears to be sulphated acidic polysaccharide. Further in-depth studies are needed to evaluate the potential of the biofilm exopolysaccharide as anticorrosive agents. The

suggested results have good agreement with the present investigation.

The chemical composition of the EPS reveals that, all the bacterial species released enough quantity of polysaccharides, protein and fatty acids. Most of these compounds generally help to inhibit the corrosion process. Probably these compounds get absorbed on the metal surface and formed a thin layer over the metal surface and inhibited the corrosion process. From the dissolved oxygen measurements studies we can conclude that *Staphylococcus* sp scavenging the oxygen for their respiration, metabolism and growth etc., which in turn completely removes the oxygen or lowers the oxygen concentration of the same to a sub minimal value which is not sufficient to cause corrosion. This hypothesis is supported by the fact found by Pederson et al⁶ suggested that protection is a result of metabolic activity including decrease of oxygen content. In the present study the organisms used are aerobic, it might most effectively utilize oxygen for their respiration and metabolism and it implicates that the above hypothesis may be valid for this case. Jayaraman et al⁷ have reported that oxygenic *Pseudomonas fragi* biofilms on SAE 1018 steel decreased the corrosion rate compared to sterile controls by two-to-ten fold over a period of four weeks in batch reactors. The mechanism for the general reduction in corrosion was found to be due to oxygen depletion at the metal surface by respiring cells.

The hydrogen ion concentration (pH) indicates that the bacterial species may release some organic acids during metabolism. Because of the pH was changed to slightly acidic in nature.

Any solid surface either metal or non-metallic substance when immersed in aqueous environment, immediately the ions and the nutrient molecules present in the environment get adhered on the metal surface first. By means of electrostatic attraction the pioneering microorganisms get colonized on the metal surface and form an interface between the metal surface and the environment. An interface is defined as a phase boundary between two phases in a heterogeneous system. For all interfacial systems, it is known that organic molecules form the non solid phase immobilized at the solid interface. There they eventually form a film known as conditioning film which will change the original properties of the original surface.

In the above said way, the conditioning film may influence the interaction of bacteria with the interface. The molecules comprising the conditioning layer include small and polymeric compounds such as lipids, proteins and complex polysaccharides.

In the meantime, the chloride ions present in the medium will initiate the corrosion process. The initial corrosion of the metal surface favours the roughening of the metal surface.

After few hours, the succeeding microbes make a strong binding between the metal surface and their cell walls by means of electrostatic forces and physical enlargement process. The bacterial species are densely distributed on the entire metal surface.

The bacterial species consume the nutrients present in the metal surface and surrounding environment for their metabolic activity. During the metabolic process, it oozes out the extra cellular polymeric substances as excreta. The extra cellular polymeric substance covers a thin layer over the metal surface. For initial days the pattern of distribution of the extra cellular polymeric substances depends on the quantity of the electropositive charges present on the metal surface. With time the biopolymer substances gradually build up a considerable thickness of the biofilm over the metal surface.

First few hours of immersion corrosion was taking place, it was favouring the roughening of the metal surface which also facilitates better adherence and anchoring capacity for biopolymer substances. Besides, the free ions and charges present in the metal surface favour the formation of organometallic complex. Moreover the extra cellular polymeric substances also act as a physical barrier over the metal surface and prevent the penetration of corrosive species to the metal surface.

Naturally the biopolymers are composed of different types of fatty acids, different types of amino acids, and different types of polysaccharides which give better corrosion inhibition efficiency and decrease the chloride ion activity. It is also possible that, the biofilms have negative charges that would repel chloride ions and reduce the chloride ion activity at the metal surface.

Conclusions

The Staphylococcus sp efficiently inhibits corrosion. The inhibition efficiency was relatively higher in the case of Staphylococcus sp when compared with other reported species as reflected by the weight loss studies. This inhibition may be due to the protective biofilm formation, rapid oxygen consumption and release of extra cellular polysaccharide substances etc. from this study results it concluded that the chemical nature of biopolymers will decide whether the bacteria going to inhibit /accelerate corrosion process.

Acknowledgements

One of the authors S.Ponmariappan expresses his sincere thanks to CSIR for the award of Senior Research Fellowship (SRF).

References

1. W. P. Iverson, *Advances in Applied Microbiology*, 32 (1987) 1
2. G. Geesey, L. Jang, J. G. Jolley, M. R. Hankins and P.R. Lovaokat Griffiths, *Water and water microbiology*, (1988) 20
3. F.L. Roe, Z. Lewondowski and T. Funk, *Corr Sci*, 52 (1996) 744
4. G. Geesey and L. Jang, *Binding of metal ions and bacteria*, eds. T. Bettridge, R. Doyle New York. NY: John Wiley and Sons (1988) 325
5. A. Pedersen and M. Hermansson, *Biofouling*, 1 (4) (1989) 313
6. A. Pedersen, and M. Hermansson, *Biofouling*, 3 (1) (1991) 1
7. A. Jayaraman, E.T. Cheng, J.C. Earthman and T.K. Wood, *Appl. Microbiol. & Biotechnol*, 11 (1997) 48
8. C. J. Thomas, R. G. J. Edyvean and R. Brook, *Biofouling*, 1 (1988) 63
9. T. Ford, J.S. Maki and R. Mitchell, *Involvement of bacterial exopolymer in Biodeterioration of metals*, In *biodeterioration 7*, Houghton, D.R. Smith, R.N and Eggins H.O.W. Eds, Elsevier Applied science, London, (1998) 378
10. R. Soracco, L. Berger, L. Mayak, D. Pope and E. Wild, *Corrosion*, 84 (1984) Paper No.98
11. P.S. Guimet, and H. A. Videla, (1987). *Protective action of Serratia marcescens in relation to the corrosion of aluminium and its alloys*; In *Biodeterioration Research I*, Llewellyn, G.C and O'Rear, C.E, Eds, Plenum Press, New York: 275
12. S. Maruthamuthu, G. Rajagopal, S. Sathyanarayanan, M. Eashwar and K. Balakrishnan, *Biofouling*, 8 (1995) 223

13. S. Ponmariappan, P. Kandaswamy, J. Mathiyarasu, S. Mohanan, S. Maruthamuthu, R. Palaniappan, and N. Palaniswamy, Effect of Pseudomonas and Vibrio sp on cathodic protection. *Proc. of 4th Int. EFC workshop on Microbial corrosion*, Eds C.A.C. Sequeira, Portugal, EFC No: 29, (1999) 219
14. S. Ponmariappan, S. Maruthamuthu, R. Palaniappan, and N. Palaniswamy, Control of corrosion and biofouling on mild steel by Actinomycetes sp. *Int. cong. On emerging corrosion control strategies for the New Millennium*, New Delhi, India. (2002) 28
15. Bergey's manual of systemic Bacteriology, Hole (edn) Waver press Inc (1984-1989) 1
16. R.K.Jain, *World journal of microbiology and biotechnology*, 6 (1990) 356
17. V. Scotto and M. E Lai, *Corr Sci*, 40 (1998) 1007-1018
18. G. A. H. Von Wolzogen Kuhr and L. R. Van der Vlugt, *Corrosion*, 17 (1961) 293
19. J. P. Black, T. E. Ford and R. Mitchell, *Corrosion*, 88 (94) (1998) 1
20. B. Kinsella, Y. J. Tan and S. Bailey, *Corr J*, 54 (10) (1998) 835
21. D. Neumann, H. Labischinski and P. Giesbrecht, In modern techniques for rapid microbiological analysis, Edited by W.H. Nelson, Weinheim, New York, VCH verlag chemie. (1990)
22. I. Majumbar, N.B. Bhosle, and F.D. Souza, *J. Indian Inst. Sci.*, 79 (6) (2000) 539