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Evaluation of inhibition efficiency of Pseudomonas putida over mild steel in the presence of a corrosion inducing microbe

Suma M.S

Department of Chemistry, University of Kerala, Kariavattom
Thiruvananthapuram – 695 581. INDIA

sumasreemangalam@gmail.com

S.M.A. Shibli, Rubina Basheer, Sreelekshmy.B.R, Amina.S., Ardra Ravi

ABSTRACT

Microbial induced corrosion inhibition (MICI) is associated with the formation of biofilm over metal surface which is rather a novel technique that is less exploited so far. Pseudomonas putida is reported as an efficienct microbial corrosion inhibitor for industrial alloys whereas Shewanella putrefaciens as an inducer of corrosion. In this study, the inhibition efficiency of P.putida over mild steel (MS) surface is evaluated in the presence of a microbial corrosion inducer by setting a binary culture of P.putida along with S.putrefaciens in nutrient broth medium. OCP analysis and mass loss determinations were made preliminarily to examine the corrosion pattern of MS in binary culture and the results showed significant reduction in the corrosion rate. In order to understand the mechanism of inhibition, electrochemical analyses were done by Tafel polarization followed by scanning electrochemical microscopic (SECM) techniques. The exposed surface was examined using SEM and AFM and compositional analysis of scraped surface film was also carried out using FT-IR spectroscopy. Phases in corrosion products were analysed by XRD and the results suggested that P.putida suppressed the corrosion causing tendency of S.putrefaciens by instantly forming a vivianite layer ($Fe_3(PO4)_2.8H_2O$) over MS as an initial protection followed by a homogeneous biofilm over it. Variation of pH over a period of seven days showed that nature of bacterial metabolites was less acidic in binary culture. This study provided an insight into the application of P.putida as a versatile inhibitor of mild steel even in the presence of a corrosion-inducing microorganism.

Keywords: Microbial induced corrosion inhibition (MICI); Pseudomonas putida; Shewanella putrefaciens

INTRODUCTION

Corrosion is a universal problem that affects a large variety of industries and services, such as shipping, oil refinery, construction, sewage and drinking water systems, and upkeep of historical buildings and statues.[1] Mild steel, the most important and commonly used industrial alloy is always in the threat of corrosion rather than other commercial alloys and needs effective and lost lasting protection since corrosion costs billions of dollars every year to each and every country in the world. Among the various corrosion control measures, use of corrosion inhibitors draws much the most attention possibility due to its cost effectiveness and practicability. Corrosion mitigation employing microorganisms is more effective in this area due to the simultaneous impact of its ecofriendly and economic nature.

The effect of different single bacterial cultures on the corrosion behaviour of mild steel has been studied by researchers and their reports proved that corrosion inhibition/acceleration depends on the strain and its mode of action on the metal surface.[4] But studies on the corrosion behaviour of metal surface employing binary bacterial culture are hardly been studied. Industrial alloys exposed in environmental conditions are in the circle of different aggressive attacks. In this situation protection of them by effective methods provides durabillity and efficiency.

In this context, evaluation of corrosion inhibition of mild steel using binary bacterial culture is significant. Pseudomonas putida is reported as an effective corrosion inhibitor for mild steel by aerobic respiration.[2,3] At the same time Shewanella putrefaciens is reported as a microbe which induces corrosion. The objective of this study is to examine the inhibition efficiency of P.putida in the presence of S. putrefaciens. As an initial part of the study individual open circuit potenital measurements of both were carried out. Further evaluations were made by OCP and weight loss measurements by the immersion of mild steel in the experimental medium containing both strains followed by impedance measurements and potentiodynamic polarisation measurements. Surface morphology was analysed using SEM, AFM and OSP analysis and composition analysis were carried out by FTIR and XRD analysis.

EXPERIMENTAL PROCEDURE

Substrate preparation and bacterial culture

Mild steel coupons having the following weight percent composition were used for the preent study. The surfaces were polished sequentially using 60, 100, 400, 600, 800, 1000 and 2000 grade emery papers and degreased with acetone, washed with distilled water and sterilized with ethanol before exposure to the experimental media.

Table 1: (Percentage composition of mild steel)

Elements	Mn	Si	P	C Fe
Weight %	0.50	0.05	0.16	0.16 99.13

Bacterial culture

The strain P.putida was isolated from the fresh water of Kerala University premises and S.putrefaciens was collected from the Department of Biotechnology, Kariyavattom. The binary bacterial culture of both strains were made when their optical density became one and 1000µL of both were added to nutrient broth medium for the studies. For all the experiments NB medium without bacteria was taken as the blank and the experiments were conducted in duplicate.
Electrochemical analysis

(a) The OCP measurements were measured during immersion of substrate coupons in cultural medium with respect to a saturated calomel electrode (SCE) using a digital multimeter (Mastech, Model M3900) for a period of 30 days.

(b) Potentiodynamic polarisation measurements were conducted after immersion of metal coupons in bacterial culture for 7 days using EC Lab SP 200-128 electrochemical workstation.

Surface analysis

SEM analysis of bio passivated coupons were carried out by EVO/18 Research ZEISS, USA, AFM by Bruker (Model Dimension edge with scan asyst) was used to observe the sample surface in non contact tapping mode. Optical surface profilometry (OSP) of bio passivated coupons was obtained using scanning electrochemical work station, Uniscan instruments. The vibrational modes in the protective film were studied in the mid IR range (350-4000 cm^{-1}). The FTIR spectra of surface deposits were recorded in an Agilent Cary 630 FTIR spectrophotometer. The phase and crystallinity of the coupons were determined by X-ray diffraction technique in which the coupons were scanned using Cu-K radiation at a voltage of 40 KV and a current of 30mA

RESULTS

Preliminary evaluation of P.putida and S.putrefaciens on the corrosion behaviour of mild steel

Open circuit potential measurements to analyse the trend of potential decay of mild steel coupons immersed in P.putida and S.putrefaciens

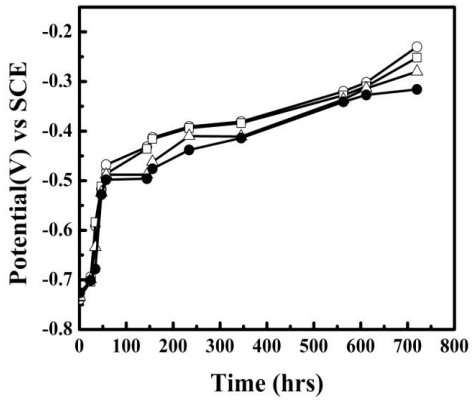


Figure 1: Potential decay curves of MS coupons immersed in NB medium inoculated with Δ- *blank,* ○- *P.putida*, ●- *S.putrifaciens,* □-*P.putida*+ *S.putrifaciens*,

The open circuit potential of MS coupons immersed in all the experimental systems were in the range of -0.720V to -0.650V for the first six days. After that the equilibrium potential was found to get shifted with time. MS coupons inoculated with P.putida shifted equilibrium potential more towards noble side followed by P. Putida+ S.purefaciens whereas in the case of it did not come up to the mark of the former two. MS coupons inoculated with S.putrifaciens markedly showed a decrease in open circuit potential from the very beginning, but shifted to noble side after six days. However, the potential deteriorated towards negative region after 24 days.

P.putida forms the best passive layer followed by P. Putida+ S.purefaciens over the metal coupons than the other ones as evidenced by the potential decay studies. This stable passive layer itself significantly prevents the corrosive agents from attacking the metal coupons by adhering on the metal surface.

Comparison of percentage weight loss and corrosion trends of MS coupons treated with binary culture

System	Initial weight (g)	Final weight (g)	weight loss (g)	Inhibition efficiency (%)	Corrosion rate (mm/year)
Blank	6.0384	6.0349	0.0035	96.09	0.0318
P.putida	6.0103	6.0097	0.0006	99.40	0.0051
S.putrefaciens	5.8074	5.8035	0.0039	96.31	0.0362
P.putida+ *S.putrefaciens*	5.9587	5.9564	0.0023	97.70	0.0212

Mild steel coupons were immersed in blank, P.putida, S.putrifaciens and P.putida+S.putrifaciens system for 7 days. The MS coupons were cleaned before and after immersion and the weight loss measurements were taken using highly accurate SHIMADZU electronic balance.

The inhibition efficiency is very high (99.40%) in the case of *P.putida*, and the corrosion rate is very low (0.0362) and *P.putida+S.putrifaciens* produced an inhibition efficiency 97.70% and the corrosion rate was 0.0212. Compared to *P.putida* and binary culture, *S.putrifaciens* and blank are incapable of biopassivating the MS coupons. Its inhibition efficiencies are 96.31 and 96.01 and corrosion rates are 0.0362 and 0.0318, respectively. As the inhibition efficiency increases, the corrosion rate decreases.

.Table 2: Comparison of percentage weight loss of MS in different systems

Electrochemical analysis for examining the stability of biofilms coated on MS coupons by different bacterial culture

Potentiodynamic polarization studies for measuring the corrosion rate of bio passivated MS coupons

Tafel polarization

The mild steel coupons were inoculated with P.putida, S.putrifaciens and P.putida+S.putrifaciens for a period of 24hrs. The corrosion parameters (E_{corr} and I_{corr}) were estimated from the Tafel polarization curves

Table 3: Tafel parameters of binary bacterial culture for a period of 24 hrs

System	E_{corr}(mV)	I_{corr}(µA)
Blank	-799.995	71.432
P.putida	-49.986	0.073
S.putrifaciens	-808.408	143.393
P.putida+S.putrifaciens	-776.619	419.696

The electrochemical parameters such as corrosion potential (E_{corr}) and corrosion current (I_{corr}) for a period of 24 hrs, 96 hrs and 168 hrs were summarized.

Table 4: Tafel parameters of MS coupons in bacterial culture for a period of 96hrs

System	E_{corr}(mV)	I_{corr}(µA)
Blank	-714.546	139.189
P.putida	-548.169	0.132
S.putrifaciens	-818.428	206.276
P.putida+S.putrifaciens	-72.872	0.770

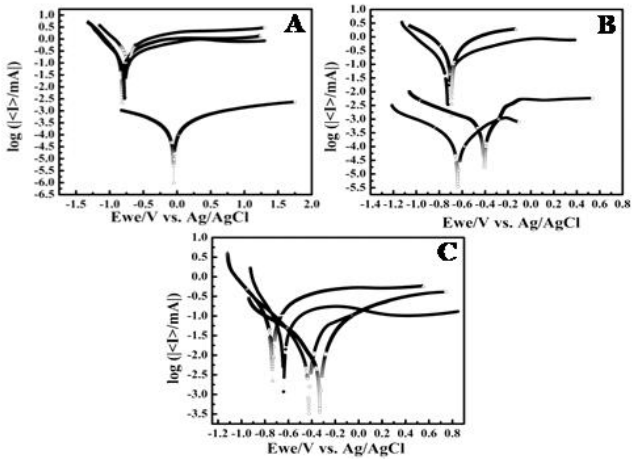


Figure 2: **Potentiodynamic polarization curves of MS coupons after immersion in ○- Δ- *blank,* ○- *P*.putida, ●- *S.putrifaciens,* □-P.putida+ *S.putrifaciens*, for a period of A-24hrs, B-96hrs, C-168hrs at temperature 35±2˚C**

Table 5. Tafel parameters of MS coupons in bacterial culture for a period of 168hrs

System	E_{corr}(mV)	I_{corr}(μA)
blank	-605.055	74.939
P.putida	-444.401	12.547
S.putrifaciens	-757.120	80.550
P.putida+S.putrifaciens	-495.076	14.678

From the Tafel parameters, P.putida had better corrosion potential and lower corrosion current which indicates high resistance to corrosion. However the high corrosion current and the low corrosion potential shown by S.putrifaciens clearly indicated the poor corrosion inhibition capacity of the same. It is interesting to note that the action of corrosion causing bacteria S.putrefaciens along with the action of P.putida brings miraculous results compared to the individual culture of S.putrefaciens.

Analysis of surface of MS coupons after exposition to binary bacterial culture at the end of 7 days Compositional analys FTIR analysis to identify the functional groups present and to study the mechanism of biopassivation

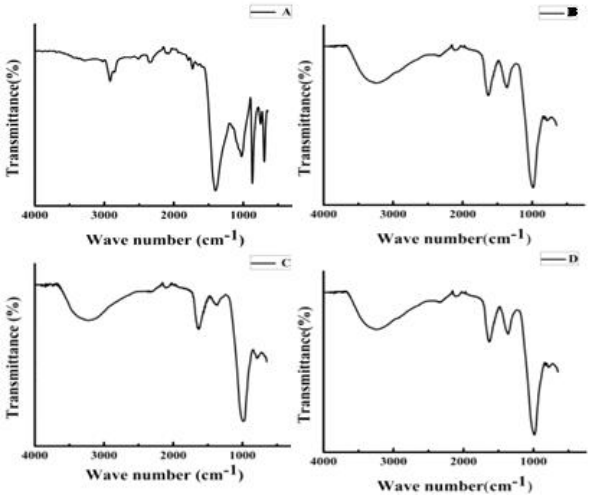


Fig 3: FTIR spectra of surface deposits of MS coupons after 7 days exposition to A-*blank*, B-*P.putida,* C- *S.putrifaciens* and D-*P.putida+S.putrefaciens*.

To verify the extent of deposition of EPS over the mild steel coupons immersed in blank, *P.putida*, and *P.putida+S.putrifaciens* system separately for 7 days the deposition formed were scraped with a non-metallic scraper and subjected to FTIR spectroscopy.

A broad band is noted in the range of 3000-3500cm^{-1} which is assigned to the presence of adsorbed water molecules and O-H/N-H groups [5,6] in all the 4 graphs. The presence of neutral C=O and or iron-EPS complex are indicated by their stretching modes at 1660cm^{-1}. The band at 450-700cm^{-1} probably originated from γ-Fe_2O_3. The peak at 793cm^{-1} also indicated the presence of iron oxide.[7]

It showed that mild steel surface immersed in *blank and S.putrefaciens* contained iron oxides and EPS having acidic groups. However in *P.putida* and *P.putida+ S.putrifaciens*, the steel surface contained iron oxides and EPS having neutral carbonyl groups which complex with metal ions.

XRD analysis to examine phases present in the corrosion product

To examine the phases present in the corrosion products, the mild steel surfaces were inoculated with blank,P.putida,S.putrefaciens and P.putida+S.putrefaciens.

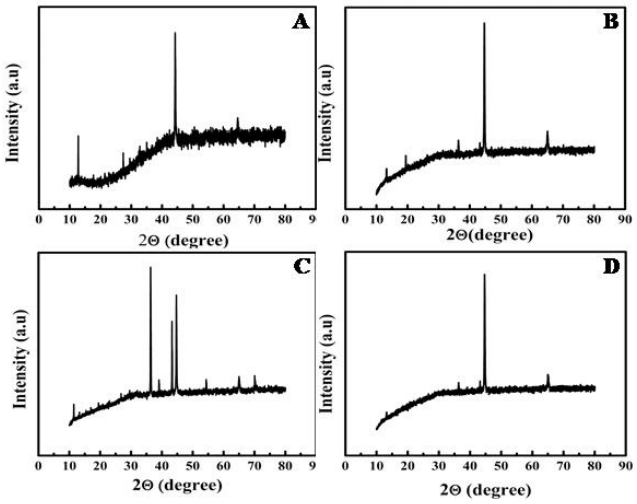


Fig 4: XRD pattern of corrosion products generated on MS coupons after inoculation with A--P.putida, B-P.putida+ S.putrefaciens, C- S.putrefaciens, D-blank
It is observed that the surface of the metal immersed in P.putida and P.putida+S.putrefaciens contained iron oxides of Fe_3O_4 ,FeOOH and vivianite $(Fe_3(PO_4)_2.8H_2O)$[8] It is observed that the peaks due to vivianite were absent in the case of S.putrefaciens and also there was presence of Fe_2O_3. The peaks due to iron alone are observed at $2\Theta = 43.6^0$, 50.7^0 and 74.3^0.

Surface analytical studies to ascertain the cause of the observed polarisation resistance values SEM analysis of binary bacterial culture after 7 days immersion

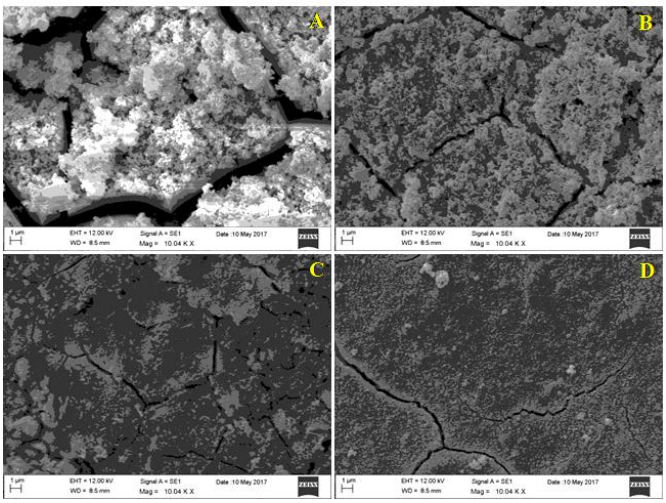


Fig 5: SEM images of MS in bacterial culture after 7days of exposition a-*P.putida*,b-*P.putida+S.putrefaciens*,c-*S.putrefaciens* d-*blank*

The SEM micrographs of the MS coupons were taken after immersion in P.putida,P.putida+S.putrefaciens,S.putrefaciens and blank after a period of 7 days.
In P.putida, the mild steel showed EPS deposition over mild steel surface and in case of P.putida+ S.putrefaciens, good deposition of EPS layer was noticed and no: of pores was less compared to others.

Atomic Force microscopic analysis to investigate the process of inhibition at nano to micro scale at the metal/solution interface

To verify the surface topography of binary bacterial culture, the mild steel coupons were immersed in *P.putida*,*P.putida+S.putrefaciens*,S.putrefaciens and blank separately for 7 days and subjected to atomic force microscopy.

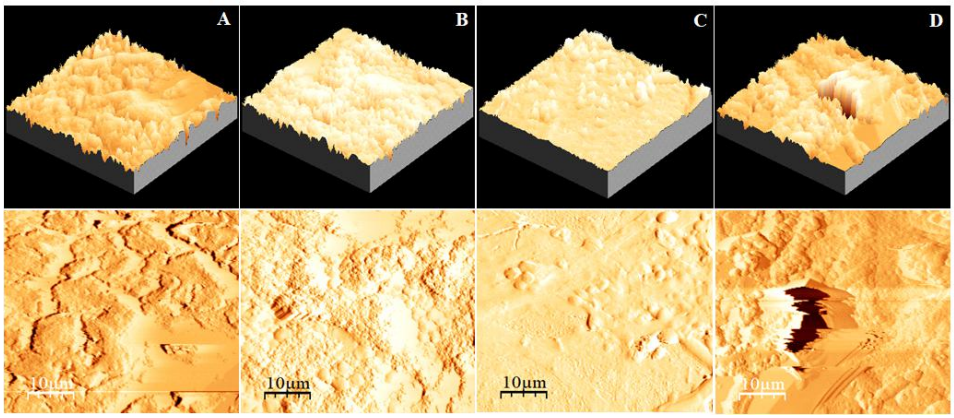


Fig 6: 3D and 2D images of MS coupons inoculated with A-P.putida, B-P.putida+ S.putrifaciens, C-blank, D- S.putrifaciens

It was observed that some pits were formed on the mild steel surface inoculated with S.putrifaciens.P.putida has large EPS production on mild steel surface.

The 2D and 3D images of P.putida+S.putrefaciens revealed the EPS production on the mild steel surface. The pitting corrosion was clearly seen in the images of S.putrifaciens

OSP images of MS coupons after immersion in bacterial culture

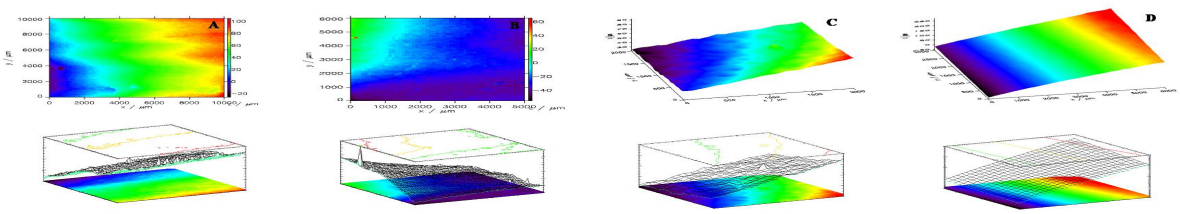
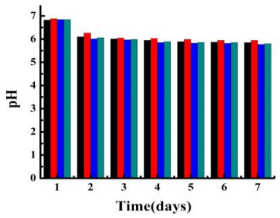


Figure 7: OSP images of MS after immersion in A-P.putida, B-blank, C-P.putida +S.putrefaciens, D- S.putrefaciens

The OSP images of MS coupons developed after immersion in bacterial culture revealed the surface porosity and topography of the coupons. MS in P.putida and in P.putida+S.putrefaciens showed the presence of uniform layer over of EPS over the metal surface with less porosity. But in the case of the other two no signals of preventive layer formation which implied that surface homogenity is superior in the case of P.putida based culture.

Variation of pH of experimental solution containing MS inoculated with binary bacterial culture



Lowering of pH is most observed in the case of *S.putrefaciens* and in the blank whereas in the case of *P.putida* and *P.putida+S.putrefaciens* it is only slightly lowered which indicated that more acidic groups are liberated in the former case compared to the latter.

Lowering of pH is an indication of corrosion due to the release of some acidic metabolites in the experimental system. This is in good agreement with FTIR spectroscopy.

CONCLUSION

Pseudomonas putida provides better protection for mild steel since it is a corrosion inhibiting bacteria. But S.putrefaciens, which is reported as a corrosion inducing bacteria in the presence of P.putida is found to be passive in its corrosion action due to the suppression by P.putida which seems to dominate in this case. Formation of vivianite is seen in the case of P.putida which itself acts as a protection layer along with strong EPS formation

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