Mitigation of Microbiologically Influenced Corrosion in Industrial Metals facilitated by Biofilm-Forming *Bacillus* and *Pseudomonas*

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Abstract

Metal is one of the basic need for mankind and metal corrosion causes dramatic problems due to breakdowns in machinery and material leading to loss of man power, material and money. It is estimated that hundreds of thousands crores is lost every year due to corrosion. Currently widely used corrosion control strategies have disadvantages of being expensive, subject to environmental restrictions and sometimes inefficient. Since bacteria can coat metals with a regenerative biofilm, it is becoming evident that they may be used as a means of preventing corrosion. In this present study corrosion behaviour of copper and mild steel (MS) exposed to Luria Bertani Medium has been studied. Tests were performed in sterile media and in the presence of *Bacillus* sp and *Pseudomonas* sp bacterial biofilm. A significant corrosion inhibition was observed in the media in the presence of the biofilms. Biofilms of *Bacillus* sp and *Pseudomonas* sp developed on copper and mild steel was known to inhibit corrosion, that the samples remained untarnished and unattacked. But the samples which were exposed to sterile media was attacked and covered with a dark film of corrosion products. The results suggested that the bacterial biofilms can inhibit corrosion, and it can be a feasible and effective method of natural means of corrosion inhibition.

Key words: Metals, Corrosion inhibition, *Bacillus* sp, *Pseudomonas* sp, Copper, Mild Steel (MS).

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Introduction:

Microbiologically Influenced corrosion (MIC) or Bio corrosion, defined as corrosion damage initiated or aggregated due to the direct or indirect activities of microbiological organisms, example: microorganisms stimulate the cathodic or anodic reaction or microorganisms help to establish the electrolytic environment (Hamilton, 1990). Microorganisms have primarily been characterized as planktonic, freely-suspended cells and described on the basis of their growth characteristics in nutritionally-rich culture media. No bacterium is an island, nearly all bacteria live with, and depend on, other microorganisms for energy, carbon and other nutrients. Scientists have recently realized that in the natural world, more than 99% of all bacteria exists as biofilms (Costerton *et al*., 1987). A biofilm is an organized bacterial community with cells entrapped in a matrix formed by extracellular polymer (Ghannoum & O'Toole, 2003). Biofilm- forming microorganisms have been shown to elicit specific mechanisms for initial attachment to a surface, microcolony formation, development of a three-dimensional community structure and maturation and detachment (Costerton *et al*., 1999).

In general, attachment will occur most readily on surfaces that are rougher, more hydrophobic and coated by surface 'conditioning' films. An increase in flow velocity, water temperature or nutrient concentration may also adequate to increased attachment (Donlan, 2002). After the bacteria adhere to the inert surface or living tissue, the association becomes stable for microcolony formation. The bacteria begin to multiply while emitting chemical signals that 'intercommunicate' among the bacterial cells. Once the signal intensity exceeds a certain threshold level, the genetic mechanisms underlying exopolysaccharide production are activated (Costerton *et al*., 1999).

Biofilm cells may be dispersed either by shedding of daughter cells from actively-growing cells, or detachment as a result of nutrient levels (Baselga *et al*., 1994). As the thickness of EPS increases, anaerobic conditions develop within the biofilm with loci of the biofilm consisting of anaerobic bacteria. Because of film thickness and activity of anaerobic species, the film detaches and sloughs-off from the surface of the substrate (Howell and Atkinson, 1976). Cells in biofilms experience another growth mode different from planktonic growth. Compared to their planktonic counterparts, bacteria in biofilms show distinct physiological characteristics such as significantly enhanced resistance to antibiotics, increased production of exopolysaccharide, changes in cell morphology, different responses to environmental stimuli (O'Toole et al, 2000) and distinct gene expression profile (Watnick & Kolter, 2000 & Ren *et al*., 2004). Biofilm form a transport barrier, which may prevent the penetration of corrosive agents (such as oxygen, chloride, etc.) and decrease their contact with the metal surface, thus reducing corrosion.

This work reports corrosion inhibition of Copper and Mild Steel (MS) by aerobic biofilms of *Bacillus* sp and *Pseudomonas* sp. The protective bio films for corrosion prevention were generated with stationary cultures of *Bacillus* sp and *Pseudomonas* sp in Luria Bertani broth. The main aim of this work is to probe the Microbiologically Influenced Corrosion Inhibition (MICI) of metals Copper and Mild Steel.

Materials and methods:

Isolation of microorganisms from marine water:

The sea water sample was collected in sterile container. And it was brought to the laboratory. Then, the sample was serially diluted. From the dilutions 10^{-4}, 10^{-5}, 10^{-6}, 0.1 ml was taken and spread plated on freshly prepared Nutrient Agar plates. Then, the plates were incubated at 37°C for 24 hours. Based on the colony morphology the isolated colonies were taken from the Nutrient Agar plates and was inoculated onto the Starch Agar plates and *Pseudomonas* Isolation Agar plates. Plates were incubated at 37° C for 24 hours.

Identification and characterization of the isolates:

Microscopic and biochemical identification such as Gram's staining, spore staining, motility test, indole test, methyl red test, voges proskaeur test, citrate test, catalase test, nitrate reduction test, triple sugar iron test, starch hydrolysis, gelatin hydrolysis were carried out for *Bacillus* sp and *Pseudomonas* sp.

Screening of biofilm forming ability by the marine isolates:

Tube test method:

Bacteria in biofilms in an aquatic environment are rarely planktonic, instead are associated with surfaces. Luria Bertani broth was prepared under sterile conditions and inoculated with the marine isolates respectively. Incubated at 37^{o}C for 24 hours. After incubation the culture was decanted and washed with phosphate buffered saline and then with distilled water to remove the adhering cells. Then the tubes were stained with Crystal violet. Results were tabulated.

Brain Heart Infusion- Congo red Agar Test:

Brain Heart Infusion agar supplemented with 0.8 % of congo red was prepared and sterilized, dispensed into sterile petri plates under aseptic condition. The plates were inoculated with the marine isolates to confirm the biofilm forming ability. Results were observed and recorded.

Determination of Microbiologically Influenced Corrosion Inhibition of metals (copper and mild steel):

Recent studies have shown that not all bacteria are harmful to metals; many different bacteria can actually protect them from corrosion. This phenomenon is called Microbiologically Influenced Corrosion inhibition (MICI).

Metal coupon preparation:

Copper coupon was collected, polished with emery paper, and washed with distilled water, ethanol and acetone. Coupons were weighed. Mild Steel coupon was collected, polished with emery paper, and washed with distilled water, ethanol and acetone. Coupons were weighed.

Determination of corrosion inhibition in Luria Bertani Broth:

Luria Bertani broth was prepared and sterilized along with the metal coupons. After sterilization, the broth containing the metal coupon was inoculated with both the marine isolates. Incubated under static condition at 37^o C for 7 days. The weight loss of the metal coupons was recorded periodically. The physical changes was also recorded by comparison with the control.

Physical Appearance:

During incubation the metal coupons were examined for the formation of corrosion products periodically in Luria Bertani broth. After incubation, the metal coupons were retrieved and the appearance of the metal coupons was also observed. The test metal coupons were compared with that of the control metal coupons.

Weight loss determination:

During incubation for a period of 7 days, the metal coupons were taken and weighed periodically for days such as 1, 3, 5, 7 etc. The weight loss was recorded and tabulated. The weight loss of test metal coupons was compared with the weight loss of the control metal coupons.

Evaluation of corrosion rate:

After retrieval, the corrosion products from the metal coupons were removed dried in an oven. The cleaned metal coupons were weighed to estimate the weight loss. The corrosion rates (mg. Dm^{-2}. d^{-1}) were then calculated using the formula:

$$C = W_1 - W_2 / At$$

where,

C is the corrosion rate (mg. $dm^{-1}.d^{-1}$).

W_1 and W_2 are the weights in gram of metal coupons before and after immersion.

A is the area of the panels (cm^2).

T is the duration of immersion in days.

Result:

Isolation of microorganisms from marine water:

The collected sea water sample was serially diluted up to 10^{-7} and the dilutions 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} was spread plated on to the Nutrient agar plates. Incubated at 37° C for 24 hours. The results were tabulated in Table1. Based on the colony morphology the isolated colonies in the Nutrient agar plates and two colonies were chosen and were streaked onto the selective agar medium such as Starch agar plates and Pseudomonas Isolation agar respectively. Incubated at 37° C for 24 hours. In Starch agar plates, abundant, opaque, white waxy growth indicated that the isolated organism may be *Bacillus* sp as shown in Figure No. 1. And the biochemical tests was performed to characterize *Bacillus* sp. In *Pseudomonas* Isolation agar plates, abundant, thin, fluorescent colonies indicated that the colonies may be *Pseudomonas* sp as shown in Figure No. 1. After isolation, characterization of *Pseudomonas* sp was performed.

Figure No. 1 - Isolation of *Bacillus* sp and *Pseudomonas* sp

Identification and characterization of *Bacillus* sp and *Pseudomonas* sp*:*

Bacillus sp was identified as gram positive, rod shaped, aerobic, spore- forming, motile bacterium. It can utilize citrate as their carbon source. It can produce an enzyme catalase that break down hydrogen peroxide to water and oxygen, can reduce nitrate and hydrolyze starch, lipid, and gelatin. *Pseudomonas* sp was identified as gram negative, rod shaped, motile, and aerobic bacterium. It can utilize citrate, gelatin, lipid, also it can reduce nitrate to nitrite, produce the enzyme catalase. The results were tabulated in Table. No. 1.

Table. No. 1 Identification and Characterization of *Bacillus* sp & *Pseudomonas* sp:

Characteristics	*Bacillus* sp	*Pseudomonas* sp
Microscopic characteristics		
Gram's staining	Gram positive rod	Gram negative rod
Spore staining	Spore former	Not a spore former
Motility	Motile	Motile
Biochemical characteristics		
Indole Test	- ve	- ve
Methyl red Test	- ve	- ve
Voges Proskauer Test	- ve	- ve
Citrate Test	+ ve	+ ve

Catalase Test	+ ve	+ ve
Nitrate Reduction Test	+ ve	+ ve
Triple Sugar Test	- ve	+ ve (Alkaline butt)
Starch Hydrolysis	+ ve	- ve
Gelatin Hydrolysis	+ ve	+ ve
Lipid Hydrolysis	+ ve	+ ve

Confirmation of biofilm formation by *Bacillus* sp and *Pseudomonas* sp*:*

The tube test method was performed for both *Bacillus* sp and *Pseudomonas* sp*.* The Luria Bertani tubes which was inoculated individually with *Bacillus* sp and *Pseudomonas* sp showed less ring formation, when compared to tubes inoculated with both *Bacillus* sp and *Pseudomonas* sp. The ring formation at the liquid interface indicated the biofilm formation. The mixed culture of both *Bacillus* sp and *Pseudomonas* sp showed high biofilm formation than the single culture of *Bacillus* sp and *Pseudomonas* sp as shown in Fig. No. 2*.* Black precipitated colonies were formed in the Brain Heart Infusion - Congo Red Agar plates as shown in Fig. No. 3. This showed the biofilm forming ability of *Bacillus* sp and *Pseudomonas* sp*.*

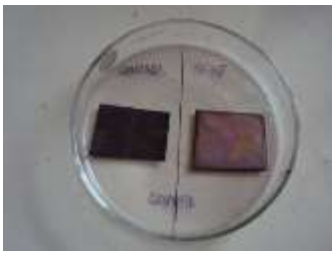
Fig. No. 2 Test tube method **Fig. No. 3 Brain heart infusion – Congo red agar method**

Determination of microbiologically influenced corrosion inhibition of copper and mild steel in Luria Bertani broth:

Microbiologically Influenced corrosion Inhibition (MICI) was determined in Luria Bertani medium with the biofilm forming organisms such as *Bacillus* sp and *Pseudomonas* sp*.*

Copper and Mild Steel (MS) coupons were used for the present work. During incubation the physical appearance was observed as shown in Fig. No. 4. After incubation for a period of 7 days of Copper and Mild Steel (MS) with biofilm forming isolates (*Bacillus* sp and *Pseudomonas* sp) the metal coupons were analyzed for weight loss, corrosion rate.

Fig. No. 4 Physical appearance of copper & mild steel after incubation



Evaluation of weight loss and corrosion rate:

The weight of the metal coupons before and after incubation was noted and weight loss was determined. Using the weight loss of the metal coupons, the corrosion rate was also calculated. The corrosion rate was tabulated a shown in Table. No. 2. There is a significant decrease in corrosion rate in the test coupons as compared with the control metal coupons

Table. No. 2 Evaluation of weight loss and corrosion rate

S. No	Metal coupons	Weight loss (g)	Corrosion rate (mg. $dm^{-1}.d^{-1}$)
1.	MS control	0.6	0.009
2.	MS test	0.03	0.0004
3.	Copper control	0.3	0.004
4.	Copper test	0.06	0.0008

Conclusion:

The present study underscores the significant potential of biofilm-forming *Bacillus* and *Pseudomonas* species in mitigating Microbiologically Influenced Corrosion (MIC) in industrial metals. Through their biofilm-forming capabilities, these bacteria create a protective barrier that limits the interaction between the metal surface and corrosive environmental factors, thereby reducing corrosion rates. The experimental results reveal that both genera, exhibit corrosion-inhibiting properties without introducing harmful by-products to the environment—positioning them as sustainable and eco-friendly alternatives to conventional chemical inhibitors. Furthermore, the synergistic effect observed in co-culture systems highlights the importance of microbial consortia in enhancing bio protection efficacy. This biological approach opens avenues for the development of innovative biotechnological strategies in corrosion management, particularly in industries where metal infrastructure integrity is critical. In conclusion, biofilm-forming *Bacillus* and *Pseudomonas* offer a promising and sustainable solution for MIC mitigation, marking a progressive step toward green corrosion control technologies in industrial settings.

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