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"ROLE OF BIOFILM IN CORROSION AND SCANNING ELECTRON MICROSCOPY OF SURFACE ATTACHMENT OF BIOFILM FORMING BACTERIA FROM VISAKHAPATNAM COAST ANDHRA PRADESH"

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Abstract:

Metal corrosion is a major global concern in maritime economic sector. The marine environment favors the corrosion processes of different metal alloys, ship hulls, artificial substrates and others. Despite chemical corrosion being the most frequently occurring, the microbial corrosion process also accelerates the corrosion process at a great pace by the formation of complex biofilms. Currently in this paper the rate of corrosion by different biofilm bacteria is estimated and the interactions between the biofilm and metallic surfaces is studied using SEM analysis for understanding the settlement of biofilm on iron substrate.

Key words: Metal corrosion, artificial substrates, complex biofilms, SEM analysis.

Introduction:

Microbially influenced corrosion (MIC) refers to the influence of microorganisms on the kinetics of corrosion processes of metals, caused by microorganisms adhering to the interfaces (usually called biofilms). Prerequisites for MIC are the presence of microorganisms.

In principle, corrosion is an interfacial process. The kinetics of corrosion is determined by the physico-chemical environment at the interface, e.g., by the concentration of oxygen, salts, pH value, redox potential and conductivity. All these parameters can be influenced by microorganisms growing at interfaces. This mode of growth is preferred by most microorganisms on earth (Costerton *et al.,* 1987). Microorganisms implicated in MIC of metals such as iron, copper and aluminum and their alloys are physiologically diverse. Bacteria involved in metal corrosion have frequently been grouped by their metabolic demand for different respiratory substrates or electron acceptors. the capability of many bacteria to substitute oxygen with alternative oxidizable compounds as terminal electron acceptors in respiration, when oxygen becomes depleted in the environment, permits them to be active over a wide spectrum of corrosive metabolic by products over a wide range of environmental conditions makes microorganisms a real threat to the stability of metals that have been engineered for corrosion resistance.

Triorganotin derivatives were extensively used due to their wide-range activity, causing no galvanic corrosion on aluminum hulls and being colorless (Omae I *et al.,* 2003). The preferred TBT derivatives added to both insoluble and soluble matrix paints were the bis-oxide TBTO and the fluoride TBTF, although the biological activity of TBT compounds seems to be independent of the anion (Clare *et al.,* 1992). The fungicide TBTO has the advantages of being an easily handled, solvent miscible liquid toxicant, compatible with many other biologically active

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compounds, thus perfect for fast leaching A/F paints with good control of shell and vegetative fouling (Clare, 1992 & Omae.I, 2003). However, its plasticizing action limits the amount that can be added (Clare, 1992). Furthermore, it behaves as a solvent and migrates to the surface, leading to a rapid depletion (Omae I *et al.,*2003).

Materials and methods:

Isolation of bacteria:

Iron panels of size (2x1) inches were placed in water tied with nylon threads along the berths in the three stations of Visakhapatnam. After 5 days, the iron panels were collected in sterile polythene covers and brought to laboratory for further investigations. The artificial iron panels were washed with sterile physiological saline to remove vegetative cells and the panel is swabbed with loop and mixed in distilled water and are serially diluted and biofilm bacteria were isolated by standard spread plate method using ZoBell marine agar after two days incubation at 28 ± 2^0C . After repeated sub culturing, the pure isolates are obtained.

Estimation of Bio corrosion:

Panels admeasuring 2x2 inch of commercial grade stainless steel were cleaned prior to deployment by following standard methods 10. These panels were weighed accurately up to decimals and then deployed in conical flasks containing bacteria. The investigation was carried out for our weeks. After retrieval the panels were first rinsed gently with sterile de ionized water, cleaned by following standard methods and weighed after desiccation. The difference between initial weight prior to deployment and final weight was used for calculation of corrosion rate by using the following formula:

$$Mdd = W/ (A X T)$$

Where mdd is corrosion rate expressed in terms of metal loss (mg) per decimeter square area per day, w is loss in weight (mg), A is area of panels dm^2, and t is exposure time (days).

SEM analysis:

The iron panels were treated according to the procedure described by Novitsky and MacSween (1989). Iron panels were fixed in a solution containing (%, v/v): 2.5 glutaraldehyde; filter-sterilized seawater, 85; and distilled water, 15. After fixation the iron panels were washed repeatedly with double distilled sterile water in order to remove salt crystals. The grains were dehydrated in a graded ethanol series (90% for 5 min) and subsequently in HMDS (hydroxyl mexamethyl di silazane). After drying, the iron panels were attached to SEM stubs using double-sided conductive tape and sputter-coated with gold.

Statistical Analysis:

A one way ANOVA was performed with the SPSS statistical software (SPSS Inc., Chicago IL). A $p<0.05$ was considered significant in all occasions. However, and due to the large number of samples compared, if *Ho* was rejected it did not imply that all means were different from one another. Thus, in order to allocate significant differences among treatments the Tukey test was used (Zar 1999).

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Results and Discussion:

In this study the role of biofilm in corrosion is shown in (Figure 1), Marine environment play important role in corrosion (Dang et al. 2011; Rajala et al. 2015) hence prior to the experiment, the hydrographic parameters were studied (Table 1) and the same were maintained during the experimentation to get accurate results. Out of 10 isolates, the sulfate reducing bacteria *Desulfovibrio vulgaris strain GSK1124* caused maximum corrosion expressed in 226.4±0.08 mg mdd followed by *Bacillus sonorensis strain GSK1184* 203.6±0.06 mg and *Bacillus flexus strain GSK1496,* 201.4±0.19 mg for 28 days. The experiment is done in triplicate, the results show significant with p value < 0.05. The iron panels were weighed; the length, breadth and width were calculated prior to the experiment and were represented in the (Table 2). Gamma-Proteobacteria was detected in fishing harbor location. Sulfur reducing bacteria is isolated from naval dockyard canal, which were usually detected in activated sludge biofilms (Santegoeds *et al.,* 1998; Minz *et al.,* 1999).

Much of the current knowledge about biofilms is due to advances in microscopic imaging techniques. Standard optical microscopy, epifluorescence microscopy (EM), and confocal laser scanning microscopy (CLSM) are the most commonly used techniques for biofilm analysis. Nevertheless, scanning electron microscopy (SEM) has been shown to be a suitable tool not only to observe in detail the substratum morphology, but also to follow the bacterial adhesion and biofilm formation on abiotic surfaces. Scanning electron microscopy (SEM) images (Figure 2 a & b) revealed the occurrence of micro-pitting corrosion underneath the biofilm bacteria on the iron surface after the biofilm removal. (Yuan *et al.,* 2007).The presence of sulfate-reducing bacteria (*Desulfovibrio* spp. and *Desulfobacterium* spp.) in the absence of sulfate may be explained by their ability to function as proton-reducing acetogens and/or fermenters by (Lutgarde *et al*., 1996).

Earlier Dexter concluded that synthetic seawater solutions were not free of organics. Instead, the organics were just different from those found in natural seawater. Webster and Newman examined the impact of media constituents on localized corrosion of Fe-15Cr-10Ni stainless steel crevices and it would not readily occur unless chloride (Cl) was the predominant anion in the medium. Anions, including sulfate, hydroxide, phosphate, acetate, carbonate, and nitrate can inhibit pitting corrosion. It is possible that bacterial consumption and fixation of nutrients, including sulfate could render an initially inhibiting solution aggressive by removing non-chloride ions. An additional complication in the interpretation of electrochemical measurements in synthetic media is the effect of culture media on the measurement. Webster and Newman observed interferences in electrochemical measurements when yeast extract was included in the culture medium. The interferences were removed when the yeast extract was removed. Marine SRB experiment can be influenced by de aeration. (Lee *et al*. 2003) demonstrated dramatic changes in the chemistries and micro flora of two natural coastal seawaters as a result of storage and environmental conditions. Exposure to an anaerobic atmosphere containing a mixed gas of nitrogen (N_2), carbon dioxide, and hydrogen generated the highest number of SRB and dissolved sulfide concentrations (McBeth and Emerson 2016; Park et al. 2011; Ramirez et al. 2016; Vigneron *et al.,* 2016; Li et al. 2017). Gamma-Proteobacteria was detected in the old

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biofilms. (Dinh *et al.,* 2004). Sulfur reducing bacteria usually detected in activated sludge biofilms (Santegoeds *et al*., 1998; Minz *et al*., 1999).

However in this study the corrosion behavior of all the isolates were studied, of which the SRB *desulfovibrio vulgaris strain* showed in (Table 3) maximum corrosion of 226.4 ± 0.08Mdd for 4 weeks.

Conclusion: Biocorrosion is an immensely complex process, involving microbial agents, chemical agents and also environmental factors which accelerate the corrosion process. In this study it is evident that the role of microorganisms is significant in the process of corrosion and the same can be visualized by the scanning electron microscopy.

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Table 1: Hydrographic parameters of sea water during sample collection

	pH	Temp ^{o}C	Salinity	DO mg/l	TDS mg/l
Station 1	7.86	31.1	35.5	5.29	147.56
Station 2	7.85	30.9	35.3	5.34	150.42
Station 3	7.73	31.5	35.5	5.47	149.97

Table 2: Length in millimeter, width in millimeter area in decimeter

	Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6	Panel 7	Panel 8	Panel 9	Panel 10
Length	20.8	23.36	23.8	23.2	23.7	23.6	21.8	21.2	22.7	23.1
Width	18.933	17.633	17.266	18.033	16.533	17.766	17.266	18.266	18.226	17.633
Area	0.0393	0.0411	0.0410	0.0418	0.0391	0.0419	0.0376	0.0387	0.0413	0.0407

Table 3: Overview of corrosion

Overview of Corrosion rate(*mdd*) by 10 bacterial isolates of Visakhapatnam Harbour

Time In days	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
2	90.6 ± 0.15	106.7 ± 0.18	82.4 ± 0.10	63.5 ± 0.09	119.6 ± 0.10	99.4 ± 0.10	78.5 ± 0.08	51.8 ± 0.07	102.6 ± 0.12	122.7 ± 0.11
7	157.2 ± 0.14	174.9 ± 0.12	132.4 ± 0.12	131.7 ± 0.07	166.6 ± 0.06	152.8 ± 0.10	143.6 ± 0.17	110.5 ± 0.10	163.7 ± 0.28	198.6 ± 0.12
28	183.2 ± 0.15	201.4 ± 0.19	163.3 ± 0.14	153.5 ± 0.16	203.6 ± 0.06	173.5 ± 0.13	165.7 ± 0.22	128.6 ± 0.07	194.4 ± 0.23	226.4 ± 0.08

Mdd= W/(A X T) is corrosion rate expressed in terms of metal loss(mg) per decimeter square area per day, w is loss in weight (mg), A is area of panels dm^2, and t is exposure time (days)

Figure 1: showing the graphical representation of rate of corrosion

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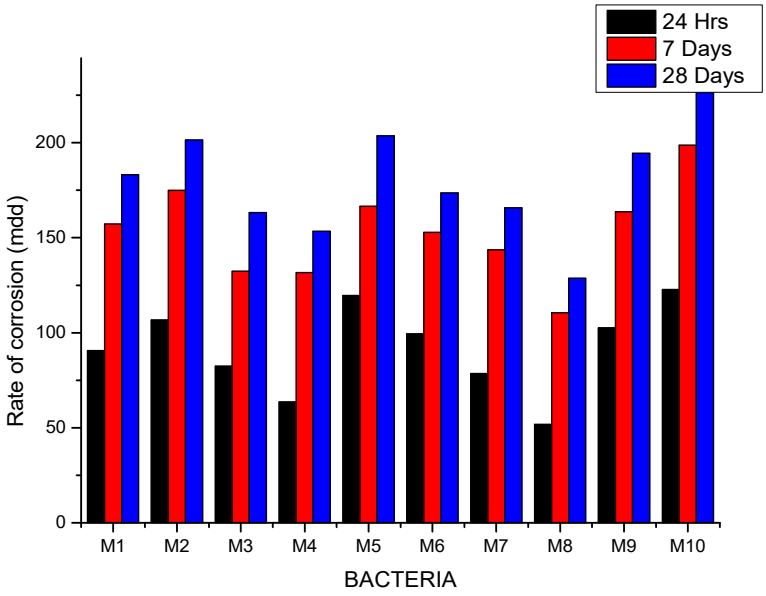
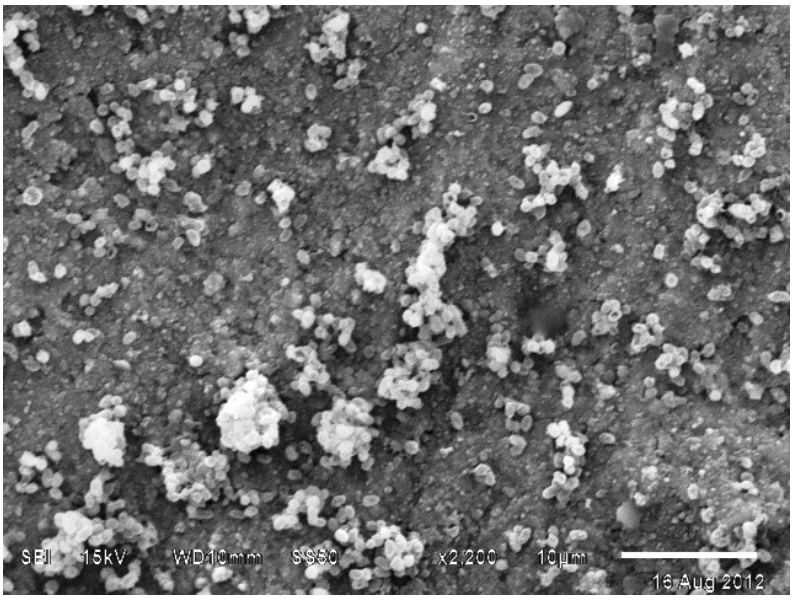
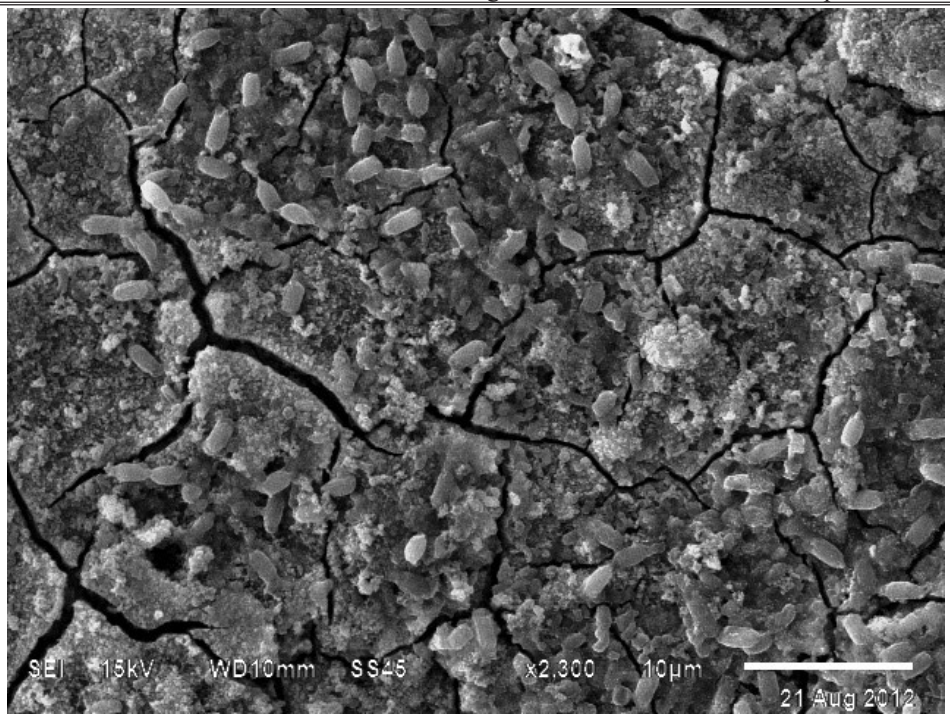


Graphical representation of rate of corrosion by all isolates at pH 7.0 Temperature 29°C in time period of 48 hrs, 7 days and 28 days, the graphs clearly indicate the maximum corrosion occurred in M10 Desulfovibrio vulgaris strain with 226mg of metal loss for 28 days.

Figure 2: Biofilm formation on metal surface.



a) The formation of *Staphylococcus haemolyticus biofilm* on Iron



(b) Pitting of metal under the colonies of bacteria.

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