Microfouling of Manganese-oxidizing microorganisms in Rameswaram Coastal Waters.

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Abstract

Manganese oxidizing marine microorganisms was studied from the coupons of PVC, Titanium, Brass, Copper and Stainless Steel were immersed one meter below water surface, using wooden rafts. The metal coupons, Brass, Titanium and Brass were exposed for a period of six months (October 2005 to March 2006). The PVC and SS were suspended in the sea for two months (February and March 2006). Sea water samples were also collected from the study area using water sampler to estimate the physiochemical and nutrients were analyzed. The population of HB and MHB on PVC was registered as 3.62 x 10^7 CFU/cm^2 and 2.87 x 10^7 CFU/cm^2, respectively while on Stainless Steel the population density of HB and MHB was recorded as 3.79 x 10^5 CFU/cm^2 and 1.34 x 10^5 CFU/cm^2. The PVC and titanium coupons were recorded relatively higher values comparing with other coupons, and it may be due to the non-toxic nature of the substratum. Brass also recorded higher bacterial population density compared to copper. The least population density observed in copper coupons could be due to it toxic nature. The generic composition of heterotrophic bacterial strains isolated from biofilm samples, both Gram-positive and Gram-negative groups were noted on all the materials studied. The notable thing was that Gram-positive group was fond to be dominant. The genera identified under Gram-positive were *Bacillus* sp., *Staphylococcus* sp. and *Micrococcus* sp. and the Gram-negative strains identified as *Pseudomonas* sp., *Salmonella* sp., *Vibrio* sp. and *Proteus* sp. Among the five types of coupons tested PVC exhibited highest Mn value of 5543 mg/g. Bacterial slim samples generated on the exposed coupons were scrapped and characterized by Bergey's method.

Key words: Manganese; oxidizing; *Micrococcus;* physiochemical; substratum.

When any substrate surface is immersed in seawater, adhesion of many microorganisms is observed within a short time. This initial covering of exposed surface is called the slim, biofilm or primary film. Once this attachment process is initiated, a succession of settling of organisms is observed leading to the development microbial population.

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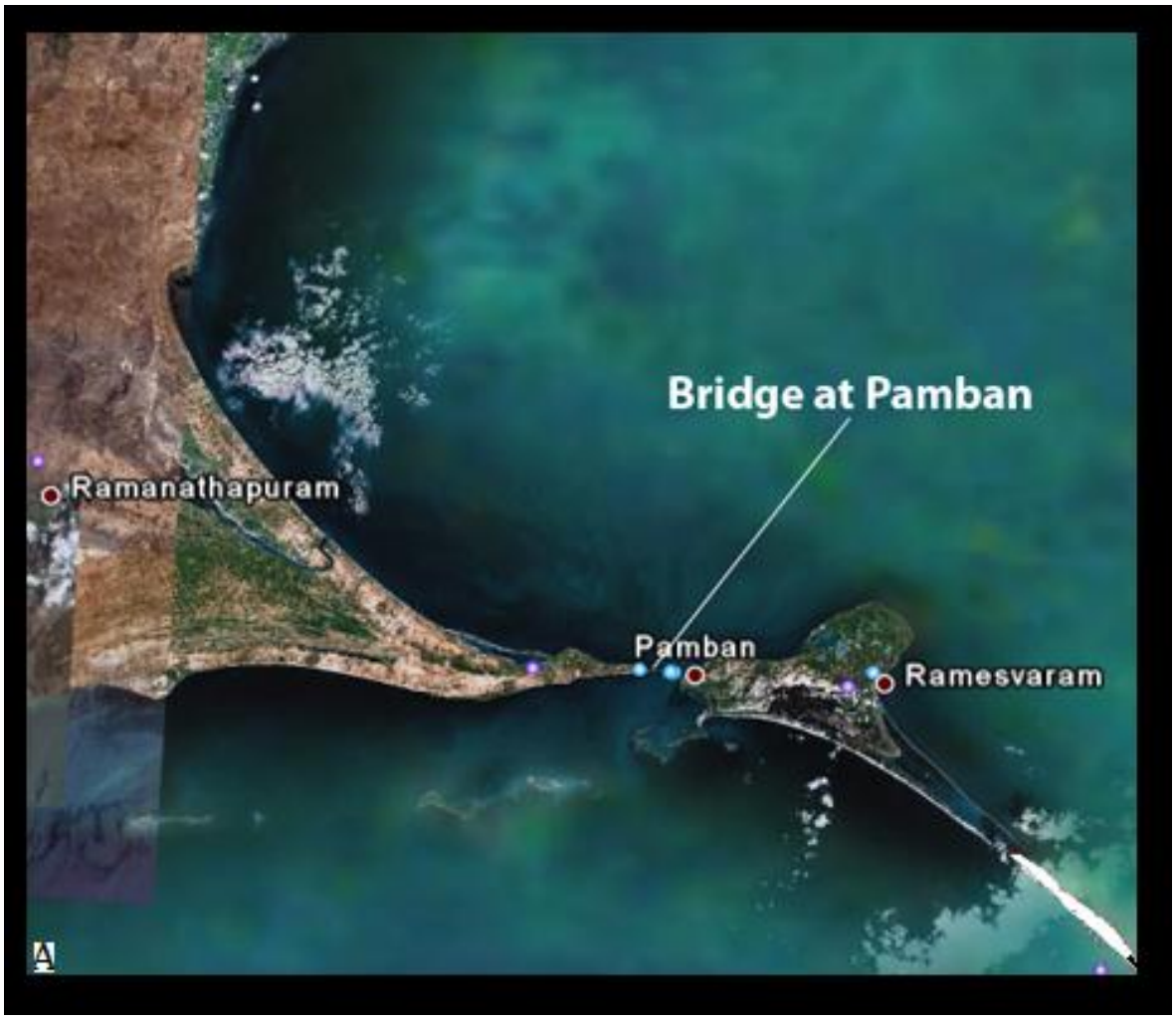
The predominant organisms are bacteria, algae, unicellular fungi and protozoa in a matrix of detritus, which provides a continuous source of nourishment[1]. The epiphytic nature of marine bacteria was first studied by Zobell and this bacterial adhesion leads the surfaces to corrosion of the material[2]. Fouling refers to the undesirable formation of deposits on equipment surfaces, which significantly decreases equipment's performance and/or its useful life.

Several types of fouling and their combinations may occur, including general, more than one type of fouling will be occurring simultaneously. A sequence of discrete events occurs in the development of biological, corrosion, particulate and precipitation fouling. In biofilms on clean surfaces when they are immersed in the marine environment[4]

Figure 1. Rameswram Island (Study area)



1. Adsorption of an abiotic conditioning film.
2. Approach of bacteria to the conditioned surface through water movement, diffusion and/or motility.
3. Reversible adhesion of both motile and non-motile bacteria.
4. Irreversible adhesion of bacteria mediated by bacteria polymers, and
5. Development of a secondary microflora.

Adhesion of microorganisms and subsequent development of surface colonies has been the subject of extensive research[5, 6, 7, 8, and 9]. Subsequently, several workers collected data on this aspect from the important commercial harbors of India[1].

Sulphate-reducing bacteria and iron oxidizing bacteria have long been considered as major contributors of corrosion. Recently manganese oxidizers, have also been identified as major contributors to corrosion[11, 12]. Dickinson *et al*, [13] first to identify the deposition of MnO_2 as the likely causative agent of corrosion it was found that the ennoblement effect on the stainless steel could be reproduced by coating the coupons with an alternative for the cathodic reduction of MnO_2 and such involvement was reported by Linhardt [14]

$$MnO_2 + H_2O + e^- \rightarrow MnOOH + OH^-$$

$$MnO_2 + 2\,H_2O + 2e^- \rightarrow Mn^{2+} + 4\,OH^-$$

Considering the importance of MnOB essentially heterotrophic bacteria and its microbial induced corrosion in different material, and lack of information on the genera involved in manganese deposition/oxidation with analyzing the physical properties of sea water were studied. Present study is aimed to identify HB and MHB bacteria in biofilm of various materials exposed to Rameswaram (Latitude $9^o\,10'$ and $9^o\,20'$ and longitude 79^o 13' 79^o 27' E) coastal waters (Palk Bay) Southeast coast of India.

PVC coupons (15 cm x 6 cm), Stainless steel coupons (30mm x 15 mm x 2mm), Titanium coupons (15 cm x 3 cm), Brass coupons (15 cm x 10 cm) and Copper coupons (15 cm x 10 cm). All the coupons were polished, washed with detergents and rinsed with distilled water and stored under sterile conditions until use. The coupons of PVC, Titanium, Brass, Copper and Stainless Steel were immersed one meter below water surface, using wooden rafts. The metal coupons, Brass, Titanium and Brass were exposed for a period of six months (October 2005 to March 2006). The PVC and SS were suspended in the sea for two months (February and March 2006). Sea water samples were also collected from the study area using water sampler to estimate the physiochemical parameters (Table: 1) such as salinity, air and water temperature, hydrogen-ion concentration (pH) and dissolved oxygen and the sea water nutrients were analysed.

Table 1. Physiochemical characteristics of sea water

Parameters	Maximum	Minimum
Atmospheric temperature	30 ° C	28.2 ° C
Surface water temperature	29.8° C	27.9 ° C
Salinity(ppt)	34.6 ppt	32.9 ppt
pH	8.5	8.1
Dissoved oxygen (mg/L)	5.26	4.23
Sulfate(mg/L)	1231	987
Bi-carbonate (mg/L)	124	88
Calcium(mg/L)	512	402
Magnesium(mg/L)	1187	988
Nitrite(µg/L)	0.26	0.14
Nitrate(µg/L)	1.88	1.32
Phosphate(µg/L)	2.80	1.84
Reactive silicate (µg/L)	9.06	6.88

The average population of Heterotrophic bacterial (HB) and Manganese-oxidizing heterotrophic bacterial (MHB) count in immersed coupons like, PVC, Stainless Steel, Brass, Titanium and Copper. The population of HB and MHB on PVC was registered as 3.62 x 10^7 CFU/cm^2 and 2.87 x 10^7 CFU/cm^2, respectively while on Stainless Steel the population density of HB and MHB was recorded as 3.79 x 10^5 CFU/cm^2 and 1.34 x 10^5 CFU/cm^2. The lower population density of Manganese-oxidizing heterotrophic bacteria at Stainless Steel was due to the nature of the surface of the material[11]. On titanium coupons the count of HB was 3.86 x 10^5 CFU/cm^2 and MHB was 2.86 x10^5 CFU/cm^2, while the counts HB and MHB of copper were recorded as 1.42 x 10^3 CFU/cm^2 and 1.10 x10^3 CFU/cm^2. Brass coupons recorded the population density of HB and MHB were 4.03 x 10^6 CFU/cm^2 and 2.02 x 10^6 CFU/cm^2. The PVC and titanium coupons were recorded relatively higher values comparing with other coupons, and it may be due to the non-toxic nature of the substratum. Brass also recorded higher bacterial population density compared to copper. Videla *et al.*[3] reported that the maximum number of bacterial colonization were found in titanium coupons and it served as an ideal substratum for bacterial colonization this may be due to the corrosion resistant nature of the material. The least population density observed in copper coupons could be due to it toxic nature. Ponmariappan *et al*[15] reported less HB population density on copper than Monel in Tuticorn harbour waters. Venugopal *et al.*[16] studied that bacteria and diatoms constitute two major groups of microorganisms that colonize solid surface immersed in Kalppakkam coastal waters. The population density of Manganese-oxidizing heterotrophic bacteria was more or less same as heterotrophic bacteria in all coupons tested. It indicates that most of the heterotrophic bacterial strains isolated were act as manganese depositors after oxidation.

Table: 2 shows the generic composition of heterotrophic bacterial strains isolated from biofilm samples scrapped from various coupons. The notable thing was that Gram-positive group was fond to be dominant than Gram-negative on all materials studied. When comparing with other coupons the Brass showed higher population density of Gram-negative group than Gram-positive. The similar findings were reported earlier by Palanichamy *et al*[11]. The generic composition and microbial load were found to be varying from material to material. The genera identified under Gram-positive were *Bacillus* sp., *hlococcus* sp. and *Micrococcus* sp. and the Gram-negative strains identified as *Pseudomonas* sp., *Salmonella* sp., *Vibrio* sp. and *Proteus* sp. In PVC, the genera identified under Gram-positive included *Bacillus* sp., *Stpahylococcus* sp. and *Micrococcus* sp., while the Gram-negative groups were identified as *Pseudomonas* sp., *Salmonella* sp., *Vibrio* sp. and *Proteus* sp. In stainless steel coupons, the identified genera of Gram-positive bacteria were *Bacillus* sp., *Staphylococcus* sp. and *Micrococcus* sp., while in Gram-negative group showed all the isolates except *Salmonella* sp. In brass Gram-negative strains were found to dominate over the Gram-positive by the genre *Pseudomonas* sp., *Salmonella* sp., *Vibrio* sp. and *Proteus* sp., while Bacillus was the only Gram-positive group on brass. Titanium showed all the Gram-positive isolates like, *Bacillus* sp., *c*sp. and *Micrococcus* sp., and all the Gram-negative isolates like. *Pseudomonas* sp., *Salmonella* sp, *Vibrio* sp. and *Proteus* sp. In copper coupons, the genera identified under Gram-positive included *Bacillus* sp., and *Staphylococcus* sp. Micrococcus was not registered from copper. In Gram-negative isolates were identified as *Pseudomonas* sp., and *Vibrio* sp., in copper coupons *Salmonella* sp. and *Proteus* sp. will absent.

The generic composition of manganese-oxidizing heterotrophic bacteria isolated from the coupons of PVC, stainless steel, brass, titanium and copper was shows in the Table: 3. all the tested coupons were recorded with both Gram-positive and Gram-negative groups of bacteria. The common contaminant Bacillus sp. was commonly encountered in all the tested materials. *Staphylococcus* sp. and *Micrococcus* sp. were the other Gram-positive isolates from the coupons. Copper coupons did not show the presence of *Staphylococcus* sp. The Gram-negative MHB isolates from different coupons contains *Pseudomonas* sp., *E. coli* and *Vibrio* sp. When comparing HB isolates *Salmonella* sp. and *Proteus* sp were absent in MHB and *E. coli* is present. The presence of *E. coli* registered only from PVC and SS coupons. In general the bacterial strain isolated from all the coupons showed that Gram- positive groups were dominant.

The Manganese (Mn) concentration in sea water and biofilm formed in different material were showed in Table: 4. Among the five types of coupons tested PVC exhibited highest Mn value of 5543 ng/g, while lowest Mn value 309 ng/g was recorded from brass coupons. Sea water showed 0.78µg/l concentration of Mn.

Table 2. Average counts of total heterotrophic bacteria and manganese-oxidizing heterotrophic bacteria in immersed coupons PVC, Stainless Steel, Brass, Titanium and copper

Material immersed	**Duration (in months)**	**Total heterotrophic bacteria CFU/cm$_2$**	**Manganese-oxidizing heterotrophic bacteria CFU/ cm$_2$**
PVC	3	3.92×10^7	2.89×10^7
Stainless Steel	3	3.79×10^5	1.34×10^5
Brass	6	4.03×10^6	2.02×10^6
Titanium	6	3.86×10^5	2.86×10^5
Copper	6	1.42×10^3	1.10×10^3

Table 3. Generic composition of heterotrophic bacterial isolates and their present occurrence in immersed coupons like, PVC, Stainless Steel, Brass, Titanium and Copper.

Microorganisms	**PVC**	**SS**	**Brass**	**Titanium**	**Copper**
Total no. of isolates	**35**	**25**	**20**	**35**	**10**
Gram-Positive	**25**	**20**	**5**	**25**	**6**
Gram-Negative	**10**	**5**	**15**	**10**	**4**
***Bacillus* sp.**	**15**	**10**	**5**	**14**	**4**
***Micrococcous* sp.**	**4**	**5**	**-**	**6**	**2**
***Staphylococcous* sp.**	**6**	**5**	**-**	**5**	**-**
***Pseudomonas* sp.**	**3**	**2**	**8**	**4**	**2**
***Salmonella* sp.**	**2**	**-**	**2**	**1**	**-**
***Vibrio* sp.**	**3**	**1**	**3**	**4**	**2**
***Proteus* sp.**	**2**	**2**	**2**	**1**	**-**

Table 4. Heavy metal concentration in sea water (µg/l) and biofilm (ng/g) on PVC, Stainless Steel, Brass, Titanium and Copper coupons

Coupons	Duration (days)	Manganese Concentration.
Sea water	-	0.78
PVC	60	5543
Stainless Steel	60	2412
Brass	180	309
Titanium	180	1432
Copper	180	867

Table 5. Generic composition of Manganese-oxidizing heterotrophic bacterial isolates and their present occurrence in immersed coupons like, PVC, Stainless Steel, Brass, Titanium and Copper

Microorganisms	PVC	SS	Brass	Titanium	Copper
Total no. of isolates	**22**	**20**	**16**	**18**	**9**
Gram-Positive	**12**	**14**	**9**	**12**	**6**
Gram-Negative	**10**	**6**	**7**	**6**	**3**
Bacillus sp.	**8**	**10**	**6**	**6**	**5**
Micrococcous sp.	**2**	**2**	**2**	**2**	**4**
Staphylococcus sp.	**2**	**2**	**1**	**4**	**-**
Pseudomonas sp.	**6**	**3**	**5**	**3**	**2**
E. coli	**2**	**1**	**-**	**-**	**-**
Vibrio sp.	**2**	**2**	**2**	**3**	**1**

Bacterial slim samples generated on the exposed coupons were scrapped using sterile brush and immediately transferred to sterile saline water. All the samples were serially diluted. For quantitative examination of the bacterial colonies, the samples were inoculated by spread plate method. The Zobell Marine Agar medium (2216E) was used to enumerate the heterotrophic bacteria (HB) and K-medium was used to enumerate the Manganese-oxidizing heterotrophic bacteria (MHB) Table 5. The pure cultures were maintained in slants for bacterial characterization. The isolated bacterial strains were characterized up to generic level was done to the key described in the *Bergey's manual of determinative bacteriology* (8^{th}edition and other developed schemes)[17]. The identification of isolated bacterial strain was done by inoculating each strain in to nutrient broths and nutrient agar plates and incubated for 24-48 hours at 37 ^{o}C. The developed cultures were subjected to further microscopic, physiological and biochemical analysis was shown on Table: 6.

Table 4. Morphology and biochemical characterization of isolated bacterial strains from various materials.

Identification Tests (Biochemical)	Gram Staining	Shape	Motility	Indole	MR	VP	Citrate	Oxidase	Urease	Catalase	H_2S production on	Starch Hydrolysis
Gram-Positive												
Bacillus sp.	+	Rod	NM	-	+	-	-	-	-	+	-	+
Micrococcous sp.	+	Cocci	NM	-	-	-	-	-	+	+	-	-
Staphylococcus sp.	+	Cocci	NM	-	+	+	-	-	+	+	-	-
Gram-Negative												
Pseudomonas sp.	-	Rod	M	-	-	-	-	+	+	+	-	-
E. coli	-	Rod	M	+	+	-	-	-	-	+	-	-
Vibrio sp.	-	Coma	M	+	*v*	*v*	+	+	+	+	-	-
Salmonella sp.	-	Rod	M	-	+	-	+	-	-	+	+	-
Proteus sp.	-	Rod	M	-	+	-	-	-	-	+	+	-

v: variable(Species)
NM: Non Motile.,
M: Motile
'+' denotes positive activity,
'-' denotes negative activity

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