



Multi metal assessment on biofilm formation in offshore environment



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ABSTRACT

Eight metal and non-metal coupons were exposed to seawater in offshore platform laboratory on biofilm formation in seven days of every month interval time for one year period. Among metal coupons, the highest mean heterotrophic biofilm bacteria population (HBBP) ranges were observed in mild steel (MS) coupon (33.03 to 40.00 [$\times 10^4$] CFUs cm^{-2}) and lowest in copper (Cu) coupon (3.0 to 5.1 [$\times 10^4$] CFUs cm^{-2}). Non-metal coupons show, highest mean HBBP ranges in glass (GL) coupon (29.6 to 66.0 [$\times 10^4$] CFUs cm^{-2}) and lowest in polyvinyl chloride (PVC) coupon (34.4 to 52.5 [$\times 10^4$] CFUs cm^{-2}). Within the studied coupons, high HBBP level was witnessed in GL and simultaneously low in Cu due to its toxic leaching product of Cu_2O . Gram-positive bacterial deposit show dominated trend in all coupons with increasing order of $\text{Cu} > \text{SS} > \text{MS} > \text{PVC} > \text{GL} > \text{Cu-Ni 70/30} > \text{Cu-Ni 90/10} > \text{Br}$ and Gram-negative show different in the order of $\text{Br} > \text{Cu-Ni 90/10} > \text{Cu-Ni 70/30} > \text{GL} > \text{PVC} > \text{MS} > \text{SS} > \text{Cu}$, respectively. The 16S rDNA sequence confirmed, heterotrophic biofilm *Bacillus* sp. (Gram-positive) were dominated in all coupons for all months.

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1. Introduction

Bacteria live predominantly on surfaces and develop as biofilm communities in natural and man-made environments in both moist terrestrial and aquatic settings [1]. When immersed in the natural marine environment man-made substrata typically get colonized by successive overgrowth of epibiotic organisms/microorganism by a process known as biofilm formation [2,3]. Ballast water discharge, shipping waste and other polluted aquatic systems typically contain a variety of biological materials which adhere on wet surfaces and hence are forming biofilms [4]. Natural biofilms consist of multiple species of bacteria that may provide each other's nutritional needs such as those comprising syntrophic consortia. Biofilms may moderate the settlement/recruitment of algal spores and invertebrate larvae of macrofouling organisms in a process known as biofouling [5].

Biofilm formation is a complex biological phenomenon and has been generally described as a temporal process involving a succession of

distinct stages: a reversible and irreversible attachment of planktonic organisms on submersed surfaces [6]. Within minutes, surfaces can be covered by conditioning films composed of organic and inorganic molecules [5]. Subsequently, the physico-chemical properties of the surfaces will be modified, potentially affecting the settlement of a variety of microfoulers (marine bacteria, protozoans, and microalgae) that may develop extensively within days. This way surface colonization may follow a 'successional' model where bacterial biofilm formation is succeeded by diatoms, microalgae spores of macroalgae (seaweed), protists, and fungi [7].

Larvae of invertebrates such as barnacles follow this initial settlement typically within several days [8]. Biofouling is a serious global problem in marine systems, causing extensive economic losses due to rising maintenance costs. Natural and man-made substrata immersed in the marine environment could develop biofilm effects which include cells and tissues of organisms, soils, sediments, hydrothermal vents, pipelines, heat exchangers, separation membranes, and filters. In the marine environment, biofilms cover most sub-tidal and intertidal solid surfaces such as rocks, ships, loops, marine animals, and algae [9]. Biofouling takes place also at the surface of virtually all structural metals and an alloy immersed in aquatic environments and mediates corrosion/biocorrosion of metals and alloys [10,11]. As more and more effort is made in areas of offshore drilling, ocean mining, shipping

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industries, industrial equipment, offshore platforms, underwater pipelines and desalination plants, the best possible materials must be developed for the protection of such materials [12].

Seawater quality can differ substantially when retrieved from open sea and this can produce changes in biofilm composition and its effect on common engineering alloys. There is also some evidence in the literature that local pollution can affect the type and extent of fouling [13] which, in turn, can affect the performance of engineering alloys [14]. Data on alloy performance in Indian waters are mainly limited to open ocean conditions. Although data on material performance of engineering alloys in polluted waters are limited, the few published records strongly suggest that biofilm data from open ocean environments are not very much relevant for service of the alloys in coastal seawater locations that receive significant amounts of pollutants. Engineering alloys are used in most metal water front structures because it is strong, readily available, easily fabricated and less costly. The growth on associated natural and man-made substrata leads to substantial economic losses in these sectors mainly due to increased fuel consumption, maintenance costs and the renewal of existing infrastructure [15].

The chemical agents that are generally used to prevent/protect metallic structures from biofouling/biocorrosion are commonly highly toxic and can have negative impacts on the aquatic environment. So far, the most effective methods of biofouling control are based on the application of man-made engineering alloys. Hence, the present study was focusing on marine biofilm forming communities which were developing on eight different engineered coupons (PVC, GL, MS, SS 304, Cu/Ni 70/30, Cu/Ni 90/10, Br and Cu), submerged at the offshore platform laboratory Tuticorin harbour. The objective of this study was to investigate substrate depending biofilm development, diversity and abundance of biofilm bacteria from different engineering alloys, as well as the pattern of bacterial succession on such materials.

2. Materials and methods

2.1. Study site

Tuticorin Port or V.O. Chidambaraner (VOC) Port is one of the 12th major sea ports in India and the second-largest port in Tamil Nadu, fourth-largest container terminal in India and size of the harbour 960 acres (388.8 ha) located at the Gulf of Mannar region (longitude 8.4730° north and latitude 78.1215° east), southern India. The present study was conducted at the Offshore Platform and Marine Electrochemistry Center (OPMEC), unit of the Central Electrochemical Research Institute (CECRI), a CSIR organization Tuticorin, (longitude 8.45° north and latitude 78.13° east) located at the ocean front in the new harbour area of the Gulf of Mannar region, southern India.

2.2. Physico-chemical analysis of seawater

The physico-chemical characteristics of seawater samples were collected between summer 2013 to post-monsoon 2014 [summer (March, April & May) 14 and 15; pre-monsoon (June, July & August) 14 and 15; monsoon (September, October & November) 17 and 18; and post-monsoon, (December, January & February) 17 and 18] by sterile containers from 0 to 20 cm below the surface. All samples were kept in ice boxes and processed within 10 h of collection. In situ parameters, like temperature (°C), pH, electrical conductivity (EC), total dissolved solids (TDS) were measured using a field kit (Thermo Orion 5-Star pH Multi-Meter, India) at the site. Salinity, dissolved nutrients and major ions were measured in the laboratory using a standard procedure [16,17].

2.3. Heavy metal analysis of seawater

Heavy metals from water samples were extracted from one liter of seawater filtered through a 0.45 µm nitrocellulose membrane filter paper and adjusted to pH 2, with HNO₃ taken in a separation funnel. A freshly prepared solution of 10 ml (3% w/v) amino-pyrolidine dithiocarbamate (APDC) was added to the funnel, and the mixture was shaken by a mechanical shaker for 10 min. Furthermore, 25 ml of methyl-isobutylketone (MIBK) was added to this mixture and shaken for 15 min. The phases were allowed to separate and the upper organic phase was collected. The bottom aqueous phase was shaken again with 25 ml of MIBK, and the organic phase was obtained and pooled with the previous phase. The pooled organic phase was mixed with 2 ml of 50% HNO₃, and shaken vigorously for 10 min to separate the bottom acid layer [18,19]. The separated samples were analyzed for heavy metals such as Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Nickel (Ni), Lead (Pb), and Zinc (Zn) by Atomic Absorption Spectrometer (GBC-SensAA, Australia).

2.4. Preparation of (metal and non-metal) coupons and their exposure in seawater

The metal coupons such as Mild steel (MS), Stainless steel (SS) 304, Copper and Nickel based alloys (Cu/Ni 90/10), Copper and Nickel based alloys (Cu/Ni 70/30), Brass (Br) and Copper (Cu) were prepared from the metal sheets supplied by M/s. Lawrence Metal Industries, Chennai. For non-metal Polyvinyl chloride (PVC) and Glass (GL) coupons were used. The coupons were cut into pieces with the size of 1" × 3" dimension and analyzed in Energy Dispersive X-Ray Spectroscopy (EDS) (Thermo, Electron Corporation, Noran System Six Software) in combination with Scanning Electron Microscope (Model:S-3000 H, Hitachi) measuring at, 20 kV acceleration at the CSIR-CECRI Laboratory, in Karaikudi, Tamil Nadu, India for the chemical compositions. The results were showed in Table 1.

Table 1
Chemical composition of the tested (metal & non-metal) alloys.

Alloys	Composition															
	Fe	Cr	Ni	Mo	Cu	Mn	C	S	N	Si	P	Zn	O	Na	Ca	Others
PVC	–	–	–	–	–	–	98.87	–	–	0.67	–	–	–	0.43	1.38	Mg - 0.39, Al - 0.23, K - 0.22 & Ti - 0.35
GL	–	–	–	–	–	–	1.97	–	4.22	29.73	–	–	50.94	8.33	3.93	Mg - 2.01 & Al - 0.63
MS	Bal	–	–	–	–	0.46	0.1	0.03	–	–	0.07	–	–	–	–	–
SS 304	Bal	18.28	8.13	0.17	0.19	1.48	0.047	0.01	0.076	0.49	0.019	–	–	–	–	Co - 0.14
70/30 Cu/Ni	0.53	–	30	–	68.81	0.51	0.02	–	–	0.01	0.003	0.08	–	–	–	Pb - 0.01
90/10 Cu/Ni	1.41	–	9.93	–	88.18	0.273	0.001	0.002	–	–	0.002	0.155	–	–	–	Pb - 0.004
Br	3.22	–	–	–	69.49	–	–	–	–	–	–	27.29	–	–	–	–
Cu	Bal	–	–	–	99	–	–	–	–	–	–	0.008	–	–	–	Pb - 0.01

PVC - Polyvinyl chloride; GL - Glass; MS - Mild steel; SS 304 - Stainless steel; Cu-Ni 70/30 - Copper and Nickel based alloys; Cu-Ni 90/10 - Copper and Nickel based alloys; Br - Brass; Cu - Copper.

Fe - Iron; Cr - Chromium; Ni - Nickel; Mo - Molybdenum; Cu - Copper; Mn - Manganese; C - Carbon; S - Sulfur; N - Nitrogen; Si - Silicon; P - Phosphorus; Zn - Zinc; O - Oxygen; Na - Sodium; Ca - Calcium; Mg - Magnesium; Al - Aluminum; K - Potassium; Ti - Titanium; Co - Cobalt; Pb - Lead (- Not present, Bal - Balance).

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