World Applied Sciences Journal 10 (2): 169-172, 2010
ISSN 1818-4952

Scanning Electron Microscopy and Elemental Studies on Primary Film

S. Bragadeeswaran, S.T. Balasubramanian, S.M. Raffi and S. Sophia Rani

CAS in Marine Biology, Annamalai University, Parangipettai-608 502

Abstract: Biofilm development on a surface is the net result of several physical, chemical and biological processes and also it is a surface phenomena resulting in biocorrosion and biofouling process. Use of the Scanning Electron Microscope (SEM) has also allowed an investigation of the C, O, Na, K, Al, Si, Ca and Fe intimate relationship between an antifouling paint matrix and it attached microbial community. Two representative fibre glass panels were analysed for elemental oxides using scanning electron microscope and energy dispersive spectrum. In the Laboratory condition Oxygen and Si are the dominated percentage of element and it showed 44.98% and Si 19.54% and in the natural biofilm formation Si was showed at maximum 46.65%. Si concentrations have a variable association with Fe possibly as corrosion initiation.

Key words: Biofilm · SEM · EDX Analysis · Fibre glass

INTRODUCTION

Biofouling in the marine environment is initiated by a conditioning film consisting of organic compounds followed by the initial colonizers-bacteria and microalgae-on which macroscopic algae and invertebrates settle and develop a complex community [1]. Biofilm development on a surface is the net result of several physical, chemical and biological processes and also it is a surface phenomena resulting in biocorrosion and biofouling process. Surface-associated growth leads to the formation of biofilms, which can be defined as a consortium of microorganisms which are immobilised at a substratum surface and embedded in an organic polymer matrix of microbial origin [2].

The biofilm is rich source of nutrients to another trophical level. It serves as food for growth of invertebrate larvae on the surface of submerged substratum like underwater cable and other maritime structure. However, this is a potential risk for increased biocorrosion events.

Very often biofilms are composed of mixed communities of microorganisms and their metabolic products. The predominant component of biofilms is extracellular polysaccharide (s) (EPS), which accounts for approximately 90% of the enveloping matrix polymers. Exopolymer complexation with trace metals may impact strongly on the availability of these micronutrients to marine organisms and may be important in the downward transport of trace metals and micronutrients in the ocean [3]. Microbial EPS may also be a major component of the colloidal matter that has been proposed to bind trace metals within an onion-like matrix of metal oxides or hydroxides and organic compounds [4]. The polymer gels are network of molecules interconnected by chemical or physical crosslink's. Electron probe microanalyses reveal that microgels resulting from DOM polymer assembly are generally rich in Ca and low in Mg and P [5]. The EPS of the contributing microbial flora provide a major part of the dry matter of biofilms. The EPS are usually acidic heteropolysaccharide. Functional groups (e.g. hydroxyl, carboxyl and phosphoric acid) associated with EPS exhibit a high affinity towards certain metal ions [6]. Calcium ions and the pH of the medium appear to play an important role in the adhesion of EPS to surfaces [7].

MATERIALS AND METHOD

Cleaned panel of fibre glass (15x10cm), was deployed, in duplicate, in surface waters (depth ~1m) at Parangipettai boat jetty (Latitude 11°29' N; Longitude 79°46' E) and the control was maintained in the laboratory condition for one month. On retrieval, the biofilm sample in the panels were scraped individually using a nylon brush and filter sterilised (0.2µm) seawater. Later the sample was treated with HCl to remove organic material. After several washings the sample was treated with HNO_3. Finally, the sample was washed and spread on glass cover slips for SEM observation and elemental analysis [8].

Corresponding Author: Dr. S. Bragadeeswaran, Centre of Advanced study in Marine biology, Annamalai University, Parangipettai-608 502

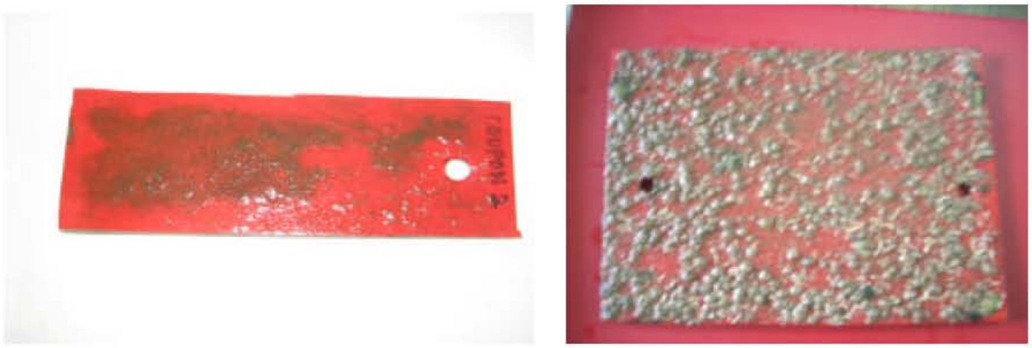

Fig. 1: Fibre glass panels from the laboratory and the Environmental conditions Energy Dispersive X-rays analysis

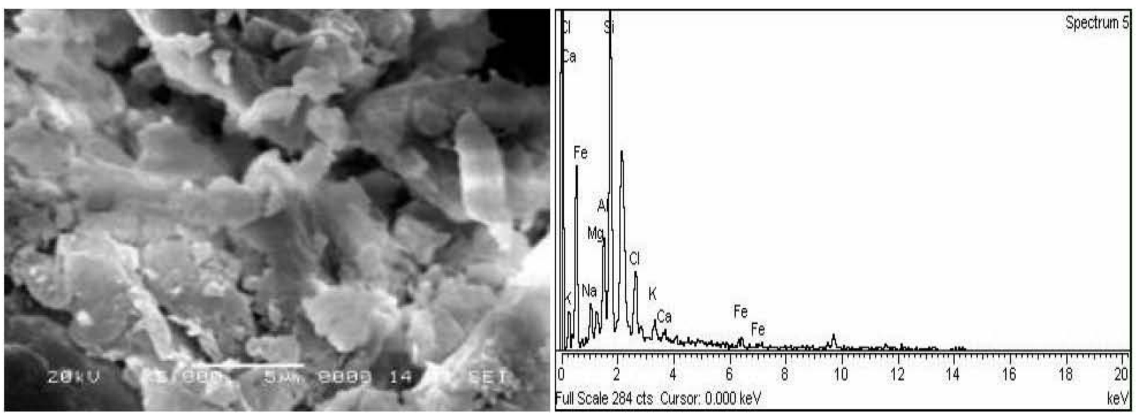

Fig. 2: SEM study of primary film formation in laboratory condition and its elemental composition

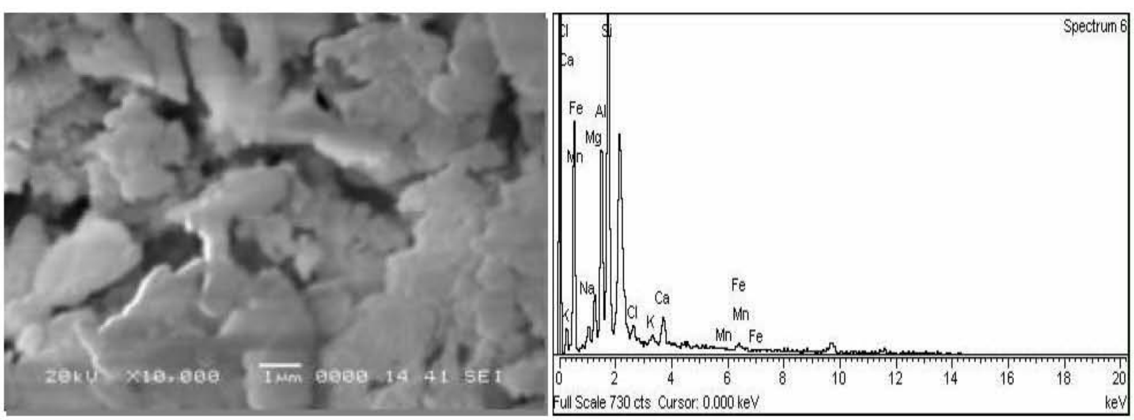

Fig. 3: SEM study of primary film formation in environmental condition and its elemental composition

RESULT AND DISCUSSION

SEM coupled with EDX has been extensively used in biodeterioration studies [9-12]. Elemental chemistry of the base material, attacked areas, abiotic deposits and biofilms can be identified. In addition, presence, morphology and distribution of microorganisms within biofilms can also be determined. The Fibre glass test panel was treated for Scanning Electron Microscopy (SEM) and Energy Dispersive X-rays analysis (EDX). The test panels was washed and stored in a dehumidifying chamber. Each test panel was viewed in the JOEL Electron Scanner-2000 by spluttering of platinum metal atoms after the samples were air dried and metal coated at 15KV. The spectral mapping was performed at 1000x or above way EDX Genesis software to detect the elemental composition such as C, O, Na, Al, Si, K, Ca and Fe and provide relative concentrations.

In the Laboratory condition oxygen and Si are the dominated percentage of element and it showed 44.98% and Si 19.54% respectively and followed by carbon 17.71%, Mg 7.11%, Ca 3.93%, Al 3.42% and K 1.32% Fig. 1. In the natural biofilm formation Si was showed at maximum 46.65% followed by Cl 17.70%, Al 12.63%, Na 6.82%, Fe 5. 57%, K 4.94% and Ca 2.11%. In the natural biofilm formation Fe was formed in addition to laboratory biofilm formation. Fig. 2. Si concentrations have a variable association with Fe possibly as corrosion initiation. Therefore, similar studies on biofilm will help to understand the divergence in the biofilm formation on different test panels.

Whereas the highest concentrations of SiO2 and SO2 were recorded associated with conidia. The chemical composition associated with the fungal structures is completely different from the initial composition of the glass and the strongly deteriorated glass [13], although the elements detected could be either remains of the initial glass network or part of the hyphal composition. In the present study the highest concentrations of SiO2 were recorded associated with diatom in the fibre glass test panels in both conditions.

ACKNOWLEDGMENT

We are grateful to the Department of Biotechnology for financial support to undertake this research project.

REFERENCES

1. Abarzua, S. and S. Jakubowski, 1995. Biotechnological investigation for the prevention of biofouling I. Biological and biochemical principles for the prevention of biofouling. Marine Ecology Progress Series, 123: 301-12.
2. Marshall, K.C., 1992. Biofilms: an overview of bacterial adhesion, activity and control at surfaces, ASM New, 58: 202-207.
3. Decho, A.W., 1990. Microbial exopolymer secretions in ocean environments: their roles(s) in food webs and marine process. *In: Oceanogr Mar Biol Annu Rev, Barnes M, (Ed)*. (Aberdeen, UK: Aberdeen Univ Press), pp: 73-153.
4. Mackey, D.J. and A. Zirino, 1994.comments on trace metal speciation in seawater or do onions from in the sea. Anal. Chim. Acta., 284: 35-647.
5. Chin, W.C., M.V. Orellana and P. Verdugo, 1998. Formation of microgels by spontaneous assemble of dissolved marine polymers. Nature, 391: 568-572.
6. Mittelman, M.W. and G.G. Glesey, 1985. Copper binding characteristics of exopolymers from a fresh water sediment bacterium. Appl. Environ. Microbiol., 49: 846-851.
7. Bhosle, N.B., P.A. Suci, A.M.Baty, R.M. Weiner and G.G. Glesey, 1998. Influence of divalent cations and pH on adsorption of a bacterial polysaccharide adhesion. J. Colloid. Interf. Sci., pp: 205: 89.
8. Tomas, C.R., 1997. Marine diatoms. In: Tomas CR, editor. Identifying marine phytoplankton. *Acedemic press*, New York, USA.
9. Ascaso, C., J. Wierzchos, V. Souza-Egipsy, A. De Los Rıos and J.D. Rodrigues, 2002. In situ evaluation of the biodeteriorating action of microorganisms and the effects of biocides on carbonate rock of the Jeronimos Monastery (Lisbon). International Biodeterioration and Biodegradation, 49: 1-12.
10. Herrera, L.K., 2003. Materials Characterization and Surface Analysis, Key Note Lecture, RILEM TC 183-MIB Conference, Lisbon, Portugal, CD Rom. *RILEM Publications SARL*, Bagneux, France.
11. Laiz, L., M.C. Portillo, M. Gonzalez-del Valle, J.M. Gonzalez, H.A. Videla, L.K. Herrera, S. Le Borgne and C. Saiz-Jimenez, 2004. A preliminary assessment of the microbial communities of limestone in the archaeological site of Palenque, Mexico. In: Ribas Silva, M. (Ed.), *Proceedings of the II International RILEM Workshop on Microbial Impact on Building Materials* (TC 183-MIB). RILEM Publications, Bagneux, France, pp: 110.

12. Videla, H.A., P.S. Guiamet, S. Go´mez de Saravia and L. Maldonado, 2001. Mechanisms of Microbial Biodeterioration of Limestone in Mayan Buildings. Paper 01250, Corrosion/2001. NACE International, Houston, TX.
13. Muller, E., U. Drewello, R. Drewello, R. Weissmann and S. Wuertz, 2001. In situ analysis of biofilms on historic window glass using confocal laser scanning microscopy. Journal of Cultural Heritage, 2: 31-42.