Biofilms and Biofouling: Marine Medical Energy Focus Topic
Room: 23 - Session MB+BI-ThA

Marine Biofouling
Moderator: D.E. Barlow, Naval Research Laboratory

2:00pm **MB+BI-ThA1 The Role of Oxygen in Microbiologically Influenced Marine Corrosion**, ***B.J. Little***, *J.S. Lee, R.I. Ray*, Naval Research Laboratory **INVITED**

Two microbiologically mediated processes dominate the literature on microbiologically influenced corrosion (MIC) in natural marine environments – ennoblement and sulfate reduction that leads to sulfide derivitization. Both are global phenomena and both depend on the presence of oxygen for aggressive attack. Marine biofilms cause a noble shift, or ennoblement, in corrosion potential (E_{corr}) for most passive alloys. E_{corr} ennoblement increases the probability for pitting and crevice corrosion initiation and propagation for those passive alloys where E_{corr} is within a few hundred millivolts of the pitting potential (E_{pit}) (e.g. 304L and 316L stainless steels). Numerous researchers have shown that increased cathodic oxygen reduction reaction (ORR) rates accompany ennoblement of E_{corr}, but do not agree on a universal mechanism for acceleration of ORR by biofilms. The role of oxygen in accelerating marine sulfide influenced corrosion has not been precisely defined. Past experiments have demonstrated that dissolved oxygen (DO) in stagnant natural seawater (8 ppm) exposed to corroding carbon steel will be depleted to the detection limits of an electrochemical probe (100 ppb) within 48 h due to aerobic microbial respiration and corrosion reactions. Furthermore, most solid surfaces in contact with seawater are anaerobic because the rate of microbial respiration within a biofilm is greater than the rate of oxygen diffusion. Even in an oxygenated bulk environment, sulfate-reducing bacteria (SRB) dominate anaerobic niches in marine biofilms and produce aqueous sulfides that can derivitize some metals and alloys (e.g., carbon steel and copper-based alloys). In the absence of oxygen, corrosion will slow or cease as bare metal, surface oxides and metal ions are derivatized forming protective surface metal sulfides. Whereas in the presence of oxygen, the protective surface-bound sulfides are oxidized thus allowing more corrosion reactions can take place. In addition, introduction of oxygen (e.g., flow after stagnation) dramatically increases instantaneous corrosion rates owing to metal sulfides being more efficient oxygen reduction cathodes compared to metal oxides. The end result is deeper metal penetration when compared to strictly anaerobic environments. More recent experiments have demonstrated that even transient DO in the bulk medium can influence the microflora and corrosion rates of carbon steel. Using optical DO probes (detection limits 4 ppb) bulk concentrations of DO in the bulk medium are being correlated with corrosion rates and pit depths in carbon steel. Persistence of aerobic bacteria at ppb DO is being followed.

2:40pm **MB+BI-ThA3 3D-Tracking of Biofouling Microorganisms with Digital In-Line Holographic Microscopy**, ***S.M. Stuppy***, University of Heidelberg, Germany, *A. Rosenhahn, T. Schwartz,* Karlsruhe Institute of Technology, Germany, *T. Ederth,* Linköping University, Sweden, *J.A. Callow, M.E. Callow,* University of Birmingham, UK, *B. Liedberg,* Linköping University, Sweden, *G.W. Swain,* Florida Institute of Technology, *M.H. Grunze*, Karlsruhe Institute of Technology, Germany

Digital in-line holographic microscopy, based on Gabor´s initial idea of a lensless microscope[1], is an imaging technique which allows to track microorganisms in three dimensions. A so-called “source wave” interferes with the wave scattered off the swimming objects and forms an interference pattern (Hologram) on the detector which contains three-dimensional information of the objects investigated. To obtain real space information from the Hologram, a reconstruction algorithm is applied[2]. The reconstructed data provides 3D trajectories of single spores with a 10 Hz time resolution and thus allows a qualitative and quantitative analysis of swimming behavior and settlement kinetics of microorganisms such as marine biofoulers or pathogen bacteria down to a lenght of 1-2 µm.

In our recent work the swimming and settlement behavior of *Ulva linza* zoospores as a common motile biofouling organism was investigated in the vicinity of surfaces with different chemistry[3-5]. We analyzed the effect of fast and abnormal settlement of *Ulva* spores on a charged Arginin containing oligopeptide surface[6] by digital holography to study the exploration behavior and kinetics of the colonization of the surfaces. As step towards application of holography at ocean test sites in the field, we constructed a compact holographic setup and tested it at the FIT test site and studied the swimming behavior of small marine organisms in their native environment.

We also applied holography to study motile biofilm forming bacteria. Using a large CMOS sensor, we were able to resolve and track rod shaped bacteria with a length of 2 µm, namely the pathogen *Pseudomonas aeruginosa*. We will show the first three-dimensional trajectories for a free swimming *Pseudomonas aeruginosa*.

1 Gabor, D. *Nature***161**, 777 (1948).

2 Xu, W. B., Jericho, M. H., Meinertzhagen, I. A. & Kreuzer, H. J.. *Proceedings of the National Academy of Sciences of the United States of America***98**, 11301-11305 (2001).

3 Heydt, M., Divós, P., Grunze, M. & Rosenhahn, A. *The European Physical Journal E***30**, 141-148, doi:DOI 10.1140/epje/i2009-10459-9 (2009).

4 Heydt, M. *et al.Journal of Adhesion***83**, 417-430 (2007).

5 M. Heydt, M. E. Pettitt, X. Cao, M. E. Callow, J. A. Callow, M. Grunze, A. Rosenhahn, *Biointerphases***2012**, in press

6 Ederth, T. *et al.Biofouling***24**, 303-312, doi:Doi 10.1080/08927010802192650 (2008).

3:00pm **MB+BI-ThA4 A Multidisciplinary Approach to Tackling Microbiologically Influenced Corrosion**, ***S.A. Wade***, *P.R. Stoddart, E. Palombo, M.M. Hlaing, M.A. Javed, D. Marić, D. Eldridge, S.L. McArthur*, Swinburne University of Technology, Australia

Microbiologically influenced corrosion (MIC) can lead to localized material degradation rates that are orders of magnitude higher than would normally be expected from standard, abiotic corrosion. This can lead to the premature failure of a wide range of important structures that can not only be costly to repair, but in some cases can have fatal consequences.

Studies of MIC require expertise from a wide range of fields such as material science, microbiology, chemistry and engineering. However, much of the past work that has been undertaken on MIC has been performed with a discipline-specific focus. This is somewhat understandable in a historical research context and may help to explain some of the observed discrepancies between MIC studies undertaken in the laboratory and field observations. In order to overcome some of these issues and develop solutions to the problems caused by MIC a multidisciplinary approach is required.

We have assembled a multidisciplinary team to investigate two specific aspects of MIC, namely the composition of bacterial consortia implicated in MIC and the associated physicochemical processes that drive MIC.

With respect to bacterial identification work is being carried out using a variety of techniques, including the relatively novel application of MALDI-TOF and Raman spectroscopy to MIC. The latter technique is particularly attractive as it potentially allows single bacteria to be identified at different stages of their life cycle, as well as in biofilm. Initial work in this area has required the development of data analysis techniques in order to remove background fluorescence signals in a consistent manner. MALDI-TOF potentially allows rapid routine identification from large numbers of samples. Initial results obtained with this technique will be presented.

The second area of interest includes work undertaken to look at how changes in field conditions can affect the likelihood of MIC. Metal coupon corrosion tests using seawater samples obtained from different field locations have been performed. A range of metallurgical, chemical and microbiological measurements were made to investigate differences observed for samples tested in two different seawater solutions and also for replicate samples tested using seawater from the same location.

Although progress remains challenging, the multidisciplinary approach reported here is showing great promise, with chemists, metallurgists and physical scientists working closely with microbiologists to understand the full complexity of the underlying biological processes.

3:40pm **MB+BI-ThA6 Bioinspired Surfaces with Dynamic Topography for Active Control of Biofouling**, ***X.H. Zhao***, *G.P. Lopez, D. Rittschof*, Duke University

Biofouling of ship hulls and propellers increases drag and power usage and decreases fuel efficiency. Biofouling costs the US Navy alone approximately one billion dollars per year, and the decreases in fuel efficiency further increase green-house gas emissions. Traditional antifouling coatings, relying primarily on biocidal organics and metals, have negative environmental impacts, while newer polymer-based coatings are easily damaged and ineffective in long-term applications.

On the other hand, nature has created an enormous number of biological surfaces that can effectively clean themselves via active deformation and motion. For example, tiny hairs called cilia on the surfaces of respiratory tracts constantly move back and forth, pushing inhaled foreign particles out of our lungs. The ciliary cleaning has also been used by molluscs, corals and many other marine organisms for active antifouling.

Inspired by active biological surfaces found in nature, we have developed a novel active-antifouling technology by harnessing dynamic deformation of polymer coatings in response to external stimuli. We discover that the surfaces of silicone-based coatings can be significantly deformed by applying a direct-current voltage across the coatings. The deformation is on-demand, dynamically switching the coating surfaces between patterned and flat states as the applied voltage is on and off. The on-demand deformation can actively and effectively detach various biofouling organisms such as bacterial films and barnacles adhered on the polymer coatings. The new technology can be readily integrated with existing or newly-developed polymer coatings, combining the advantages of various state-of-the-art antifouling technologies. This new active-antifouling system is environmentally friendly, autonomous, highly effective, and potentially durable over long-term applications. Next, we will further discuss the fundamental effect of active surface deformation on marine organism-surface interactions. A new theory for biofouling detachment caused by substrate deformation, instead of external forces, will be presented.

4:00pm **MB+BI-ThA7 Seasonal Study of Cathodic Current and Elucidation of Oxygen Reduction Enhancement Mechanism in Marine Biofilms**, ***M.J. Strom***, Naval Research Laboratory, *S.C. Dexter*, University of Delaware

The ability of a biofilm to influence the corrosion rates through the enhancement of cathodic currents is well known but what mechanisms cause this enhancement and how sustainable is it during seasonal variation? Enhancement of the oxygen reduction reaction has been shown to occur in Delaware Bay waters. Historically, enhancement of the oxygen reduction reaction by biofilms has been attributed to the presence of catalase in biofilms. However, recent work has indicated that manganese oxides may also provide a means for oxygen reduction enhancement in. The following investigation looks at the effect of seasonal variation of the sustainability of oxygen reduction enhancement and distinguishes between manganese and catalase based mechanisms

The following work used sacrificial anodes to provide a long-term cathodic current to biofilm-coated cathodes in Delaware Bay waters, in order to monitor seasonal variation of biofilm-coated cathodes under varying polarization intensities over a year. Manganese and catalase based oxygen reduction enhancement mechanisms were evaluated through the addition of glutaraldehyde or formaldoxime (FAD) treatments to the bulk solution of immersed galvanic couples.

Varying the polarization intensities of 6XN cathodes in a galvanic couple with a sacrificial anode has provided further evidence that the sustainable cathodic current enhancement found by biofilms of Delaware Bay is a result of oxygen reduction enhancement. Glutaraldehyde treatment experiments indicate that a catalase mechanism of oxygen reduction enhancement is not likely in at this location. FAD treatment experiments support the hypothesis that manganese oxides are the dominant catalysts in oxygen reduction enhancement in these waters. Seasonal studies of cathodic current enhancement show that cathodic current enhancement in Delaware Bay is seasonally dependent, with higher cathodic currents in the late spring to early fall. It is suggested that this variation is the result of the biological activity of the surrounding sediments providing a manganese resource into the water column during the warmer seasons.

4:20pm **MB+BI-ThA8 Tailoring Anode and Cathode Biofilms for Higher Current Production in Bioelectrochemical Systems**, ***J. Regan***, Penn State University **INVITED**

Bioelectrochemical systems (BESs) exploit the ability of some microbes to reduce an anode (exoelectrogenesis) or oxidize a cathode (exoelectrotrophy) for the generation of electrical current coupled with some biotransformation. There has been a lot of research in the past decade on improving the performance of BESs, primarily by addressing system features that allow reduced internal resistance. These design advancements have led to more than a six order of magnitude increase in power densities in that short time period. Moreover, a growing number of potential BES applications are being developed, including electricity production from wastes and sediments in microbial fuel cells for remote or centralized power, the production of fuels such as hydrogen and methane in microbial electrolysis cells, the recovery of value-added chemical products such as caustic and hydrogen peroxide, water desalination in microbial desalination cells, and microbial electrosynthesis for the production of organic products. Some design and operation parameters can have significant effects on anode and cathode biofilm architecture, composition, and functionality. For a given system configuration (e.g., electrode material, electrode spacing, membrane), there are only a few parameters that can be manipulated during operation. One of these operational variables is the external load or the applied potential in a potentiostatically operated system, which can significantly affect the microbial ecology of BESs as it influences the availability of the anode to serve as an electron acceptor for exoelectrogens and thereby controls the cooperation and competition among various community members in mixed-culture systems. This directly translates into performance effects, not only with respect to the time required to achieve a desired electron donor removal efficiency, but also with electron losses to competing metabolisms such as methanogenesis and aerobic respiration in an air-cathode system. This presentation will cover the mechanics of BESs, including some of the emerging designs and applications, as well as some of the parameters than can be manipulated to include microbial function, density, and productivity.

Authors Index

Bold page numbers indicate the presenter