Marine Biofouling Focus Topic
Room: 105 - Session MB-MoM

Interfacial Aspects of Marine Biofouling
Moderator: D. Barlow, Naval Research Laboratory

8:20am **MB-MoM1 Fouling in the Face of a "Little" Surface Roughness**, ***R. Lamb***, *A. Wu, K. Cho, H. Zhang*, The University of Melbourne, Australia **INVITED**

Nano-engineered superhydrophobic surfaces have been investigated for potential fouling resistance properties. Integrating hydrophobic materials with nanoscale roughness generates surfaces with superhydrophobicity that have water contact angles (θ) in excess of 160° and low hysteresis (< 10°).

Small angle x-ray scattering (SAXS) was used to investigate the presence of air incursions at immersed superhydrophobic interfaces with varying nano/microscale architecture. This technique, sensitive to local changes in electron density, looks at the nanoscale wetting of the rough interface.

Three superhydrophobic coatings differing in their chemical compositions and architecture were analyzed using SAXS and tested against major fouling species (*Amphora sp., Ulva rigida, Polysiphonia sphaerocarpa, Bugula neritina, Amphibalanus amphitrite*) in settlement assays.

Varying extents of attachment-inhibiting properties were observed across the tested coatings and appeared to correlate with the resistance to nanowetting rather than macroscopic contact angle measurements.

9:00am **MB-MoM3 Chemistry Depending Surface Conditioning and its Implication for Colonization by Microorganisms**, ***I. Thomé***, Karlsruhe Inst. of Tech. (KIT), Germany, *M.E. Pettitt,* University of Birmingham, UK, *S. Kirchen, T. Schwarz, S. Heissler,* Karlsruhe Inst. of Tech. (KIT), Germany, *M.E. Callow, J.A. Callow,* Univ. of Birmingham, UK, *G. Swain,* Florida Inst. of Tech., *M. Grunze, A. Rosenhahn*, Karlsruhe Inst. of Tech. (KIT), Univ. of Heidelberg, Germany

Biofouling is a ubiquitously occurring phenomenon in tidal zones worldwide [1]. To prevent unwanted effects caused by biofouling, suitable non-toxic coatings for these environments are required. Changing the surface chemistry and the composition of a coating changes not only its properties but also the formation and composition of a conditioning layer. We use self-assembled monolayers (SAMs) on gold as highly controlled surface chemistries which allow to fine tune the physicochemical surface properties. In order to correlate colonization with surface conditioning, we varied the surface chemistry and thus their wetting properties. In agreement with previous work, chemical termination of the surface affects not only the settlement kinetics of spores of the macrofouler *Ulva linza* [2] but also the settlement of other species. As different SAMs have different affinity towards macromolecules, settlement is controlled by both, surface chemistry itself and an adsorbed conditioning layer. To disentangle both effects, formation of conditioning layers depending on the surface chemistry was investigated in greater detail by spectral ellipsometry and IRRAS. Organism settlement is significantly changed if pristine chemistries are compared to conditioned surfaces.

[1] M.E. Callow, J.A. Callow, J.D. Pickett-Heaps, R. Wetherbee, "Primary Adhesion of Enteromorpha (Chlorophyta, Ulvales) Propagules: Quantitative Settlement Studies and Video Microscopy", *J. Phycol*., **1997**, *33*, 938.

[2] M. E. Callow, J. A. Callow, L. K. Ista, S. E. Coleman, A. C. Nolasco, G. P. López, "Use of self-assembled monolayers of different wettabilities to study surface selection and primary adhesion processes of green algal (*Enteromorpha*) zoospores", *Appl. Environ. Microbiol*., **2000**, *66*, (8), 3249-3254.

9:40am **MB-MoM5 Probing Molecular Details of Marine Bioadhesion with In Situ Infrared Spectroscopy**, ***A.J. McQuillan***, University of Otago, Dunedin, New Zealand **INVITED**

The critical step which triggers biofouling at interfaces is the initial adhesion of an invading species to a solid substrate. Of less importance in what occurs thereafter is growth of the species under the infuence of nutrients and with the protection of its more sheltered environment. Understanding the factors determining the propensity of species to adhere to substrates is the key to developing new strategies aiming to more effectively inhibit the development of biofouling in many contexts.

Much of the thinking about biofilm formation and the adhesion of biological species to surfaces has been extrapolated from macroscopic observations about the aggregation of colloids. This has been largely based on the interplay between attractive dispersion forces and repulsive electrostatic forces and has given rise to adhesion descriptions in terms of reversible and irreversible stages. A major advance has been the use of in situ atomic force microscopy (AFM) to measure forces during adhesion of microbes to substrates and evaluate their environmental influences. Nevertheless, this approach is unable to provide in situ molecular details of the chemical components which are suspected to play major roles in adhesion processes.

Vibrational spectroscopy is powerful to reveal the identity and environmental details of molecules in wet interface environments. Vibrational sum frequency spectroscopy and infrared spectroscopy are increasingly thus employed. However, attenuated total reflection infrared (ATR-IR) spectroscopy has the advantages of relative simplicity and greater general familiarity in spite of its use for wet surface situations having only recently been recognised. The ATR-IR approach employs total internal reflection at a high index refraction crystal such as ZnSe or diamond resulting in an evanescent wave sampling a few micrometers of material.

In this talk I will outline the principles of the ATR-IR method and how we have adapted them for studies of initial settling of live marine organisms settling onto surfaces under controlled temperature and environment conditions. Observations from recently published work on the settling of Perna canalicula mussel larvae and Undaria pinnatifida kelp spores will be presented and prospects for breakthrough studies of the settling and propagation of the feshwater diatom Didymosphenia geminata, invasive to New Zealand, will also be discussed.

10:40am **MB-MoM8 Relationships between Cement Production Cycles and Adhesive Strength of the Barnacle Balanus Amphitrite**, ***D.K. Burden***, *D.E. Barlow,* U.S. Naval Research Laboratory, *B. Orihuela, D. Rittschof,* Duke University Marine Laboratory, *K.J. Wahl*, U.S. Naval Research Laboratory

Marine organisms attach themselves to a wide variety of submerged surfaces. The barnacle is one of the most pervasive and persistent species to do so, securing itself by forming a thin film of permanent proteinaceous adhesive. For hard-shelled acorn barnacles like *Balanus amphitrite*, this process involves the recurring sequential release of two major secretions at the adhesive interface. We show that as the barnacle grows laterally, one of these cement precursor solutions, CPS1, is released on a fairly continuous basis, while the other, CPS2, is released cyclically. By utilizing the differences in secretion patterns, we have begun to deconvolve the contributions of these cement precursors to adhesion. Barnacles were resettled on CaF_2 substrates and release of the components at the interfaces was distinguished by optical and fluorescence microscopy. Shear detachment measurements of resettled barnacles showed that the release of CPS2 into the interface corresponded with a roughly twofold increase in adhesion versus CPS1 alone. AFM and FTIR also showed distinct differences in morphology, protein conformation, and chemical functionality for the CPS mixture versus CPS1. Possible ways in which the two components contribute to barnacle adhesion will be discussed based on these results.

11:00am **MB-MoM9 Micro to Nanostructured Stimuli-Responsive Surfaces for Study and Control of Bioadhesion**, ***G.P. Lopez***, Duke University

This contribution will present recent results on the development and study of bioadhesion on stimuli responsive surfaces that are patterned on lateral length scales of the order of 10 microns and below. These length scales are commensurate with the sizes of the smallest creatures known to be problematic in marine biofouling. Model stimuli responsive surfaces include patterned polymer brushes and model marine organisms include marine bacteria grown in culture. Our previous studies have demonstrated that stimuli responsive materials can be used to control the adhesion of model marine organisms and this presentation will provide our latest advancements in this line of study, as regards to both molecular and cellular biointerfacial phenomena. Methods for preparing nanopatterns of stimuli responsive polymer brushes over areal scales necessary for biofouling studies will be presented, along with characterization of their structure and dynamic behavior.

11:20am **MB-MoM10 Surface Topographic Features to Control Biofouling**, ***L. Xiao***, University of Heidelberg, Germany, *M. Röhrig,* Karlsruhe Institute of Technology, Germany, *S.E. Thompson, M.E. Callow, J.A. Callow,* University of Birmingham, UK, *A. Rosenhahn, M. Grunze*, University of Heidelberg, Germany

Marine biofouling is the undesirable accumulation of microorganisms, plants and animals on artificial surfaces immersed in the sea [1]. The increased hydrodynamic drag caused by fouling leads to higher operating costs of vessels. Studying the interaction between marine organisms and surfaces enhances the development of environmentally compatible approaches to control fouling [2]. Surface microtopography has been found to influence the settlement of cells and larvae [3]. We have studied the influence of surface topographic features on the biofouling process. Honeycomb gradient structures, inspired by the pattern found on the skin of the pilot whale [4], were obtained by a hot embossing process, and the effect on the density of spores of the green alga *Ulva* that attached in laboratory assays was quantified. Spore settlement density was higher on the microstructured gradients than the smooth background. The highest density of spores was found when the size of the microstructures was similar to or larger than the size of a spore. With decreasing size of the honeycombs, spore settlement decreased to a level similar to that on the smooth background. In line with the results from the Brennan group [5], spore settlement correlated with Wenzel roughness.

[1] D. M. Yebra, S. Kiil, K. Dam-Johansen, “Antifouling technology – past, present and future steps towards efficient and environmentally friendly antifouling coatings”, *Progress in Organic Coatings,* **2004**, *50*, 75-104.

[2] M. E. Callow, J. A. Callow, "Marine biofouling: a sticky problem", *Biologist,* **2002**, *49,* (1), 1-5.

[3] A. J. Scardino, R. de Nys, "Mini review: Biomimetic models and bioinspired surfaces for fouling control", *Biofouling*, **2011**, *27,* (1), 73-86.

[4] X. Cao, M. E. Pettitt, F. Wode, M. P. Arpa Sancet, J. Fu, J. Ji, M. E. Callow, J. A. Callow, A. Rosenhahn, M. Grunze, “Interaction of zoospores of the green alga *Ulva* with bioinspired micro- and nanostructured surfaces prepared by polyelectrolyte layer-by-layer self-assembly”, ***Adv. Funct.*** *Mater*., **2010**, *20*, 1984–1993.

[5] M. L. Carman, T. G. Estes, A. W. Feinberg, J. F. Schumacher, W. Wilkerson, L. H. Wilson, M. E. Callow, J. A. Callow, A. B. Brennan, “Engineered antifouling microtopographies – correlating wettability with cell attachment”, *Biofouling*, **2006**, *22*, (1-2), 11–21.

11:40am **MB-MoM11 Biofouling: It's a Rough Business***, **A. Wu**, R. Lamb, A. McDonald*, The University of Melbourne, Australia

The effect of minute changes in nanoengineered superhydrophobic surfaces on the attachment behaviour of several fouling species (*Amphora sp., Ulva rigida, Bugula neritina*) was investigated.

Superhydrophobic surfaces were fabricated from latex-templated silica sol-gels[1]. Nanoscale features of the surfaces were varied using two building blocks; silica nanoparticles ranging from 7 – 40 nm and PMMA templating latex ranging from 400 – 800 nm. The combination of these building blocks affords various roughness changes at the nanoscale. In maintaing surface chemistry identical, all fabricated surfaces exhibited superhydrophobic characteristics.

Attachment assays of each surface were conducted and a large variation in attachment-inhibition behaviour was observed. Correlating this behaviour against measured AFM roughness suggests that an intricate relationship exists between surface roughness and attachment behaviour. Data indicate that surface fabricated using small nanoparticles (7 nm) coupled with large templating latexes and high RMS roughness (> 130 nm) exhibited significant attachment inhibiting behaviour. This set of criteria also conforms to the definition of a fractal surface, where self-similarity is present different length scales.

Coupled with previous work[2] that linked pseudo-fractal dimension with nanowetting, this work strongly suggests that resistance to nanowetting has a key influence on attachment of marine organisms.

1 Cho, K. L., Wu, A. H. F., Lamb, R. N. & Liaw, I. I. *The Journal of Physical Chemistry C* **114**, 11228-11233, (2010).

2 Zhang, H., Lamb, R. N. & Cookson, D. J. *Applied Physics Letters* **91**, 254106, (2007).

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