

Monday Afternoon, October 28, 2013

Biomaterial Interfaces

Room: 201 B - Session BI+AI+BA+IS-MoA

Biofouling

Moderator: D.E. Barlow, Naval Research Laboratory

2:00pm **BI+AI+BA+IS-MoA1 Biofouling of Carbon Steel: Effects of Microstructure and Test Media on Initial Bacterial Attachment and Subsequent Corrosion**, M.A. Javed, P.R. Stoddart, S.M. McArthur, S.A. Wade, Swinburne University of Technology, Australia

Biofouling of surfaces causes numerous problems in a wide range of industries such as shipping, health care, oil and gas production and food production. Of specific interest to the current work is the accelerated corrosion of metals that can arise as a consequence of bacterial biofilm formation, which is commonly known as microbiologically influenced corrosion (MIC).

The initial attachment of bacteria to a surface is one of the first steps in the process of biofouling. The attachment is dependent upon a large number of factors, which are broadly related to the properties of the bacteria, substrate/surface and environment. Changes in these properties can not only influence the initial attachment step, but also the interrelated production of extracellular polymeric substances (EPS) by the bacteria and the subsequent corrosion.

A large amount of the work performed to date on bacterial attachment in relation to MIC has focused on stainless steels, possibly due to reports of rapid failures of these materials such as through thickness pitting of piping welds. These studies have highlighted how a range of material properties (e.g. chemical composition, surface roughness, grain size and boundaries) can influence attachment and biofilm formation on steel surfaces. This range of influences means that a high level of care must be taken when designing and carrying out bacterial attachment tests in order to avoid the situation where a number of material variables affect the outcome of a single test. For example one of the criticisms of some of the previous work in this area is the lack of control of surface roughness of the substrates used in the studies.

In this work we will report results of studies of the initial attachment and EPS production of *E. coli* bacteria on highly polished carbon steel samples, with a number of different microstructures, for a number of different test media. We have found that the microstructure and test medium can have a significant effect on the rate of bacterial attachment, the distribution of attached bacteria, the onset of EPS production and the corrosion of samples immersed in *E. coli* inoculated test media.

2:20pm **BI+AI+BA+IS-MoA2 Charged SAMs as Model Surfaces to Understand Anti-fouling Properties of Zwitterionic Coatings**, S. Bauer, University of Heidelberg, Germany, J. Finlay, M.E. Callow, J.A. Callow, University of Birmingham, UK, A. Rosenhahn, Ruhr-University Bochum, Germany

Zwitterionic surfaces are a class of coatings that receive increasing attention due to their good antifouling performance.¹ Since early work on protein resistance of mixed, charged self-assembled monolayers (SAMs), charge neutrality seems to be a prerequisite for their inert properties.^{2,3} Similar to established non-fouling ethylene glycol chemistries, zwitterionic systems rely on a strong hydration of the coating. In this study we attempt a systematic analysis to which extend charge neutrality and the chemical nature of the charged groups affect their antifouling performance. Positively charged trimethylammonium terminated thiols were therefore mixed with sulfonate-, carboxylate- and phosphonate-terminated undecanethiols in varying ratios. Optimized preparation conditions and surface analysis will be presented that demonstrates successful assembly of the coatings and characterizes their physicochemical properties. The antifouling properties were tested against a range of laboratory organisms such as diatoms and spores of algae and compared to protein resistance. The obtained trends will be discussed and correlated with field experiments in the real marine environment.

(1) Chen, S.; Jiang, S. **2008** A new avenue to nonfouling materials. *Advanced Materials*, 20, 335-338.

(2) Holmlin, R. E.; Chen, X. X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. **2001** Zwitterionic SAMs that resist nonspecific adsorption of protein from aqueous buffer. *Langmuir*, 17, 2841-2850.

(3) Chen, S. F.; Yu, F. C.; Yu, Q. M.; He, Y.; Jiang, S. Y. **2006** Strong resistance of a thin crystalline layer of balanced charged groups to protein adsorption. *Langmuir*, 22, 8186-8191.

2:40pm **BI+AI+BA+IS-MoA3 The Role of Bacterial Physiology in Biodeterioration of Polyurethane Coatings**, S. Zingarelli, Air Force Research Laboratory, D.E. Barlow, J.C. Biffinger, Naval Research Laboratory, L.J. Nadeau, Air Force Research Laboratory, D. Babson, Naval Research Laboratory, B.W. Stamps, University of Oklahoma, R.K. Pirlo, Naval Research Laboratory, C.N. Drake, Air Force Research Laboratory, B.S. Stevenson, University of Oklahoma, J.N. Russell, Jr., Naval Research Laboratory, W.J. Crookes-Goodson, Air Force Research Laboratory
INVITED

Microbial biofilms frequently contaminate surfaces and can cause degradation of polyurethane coatings that are intended to protect against environmental degradation. Historically, investigations of polyurethane biodeterioration have focused on identification and characterization of the organisms and 'polyurethanase' enzymes involved in the degradation process. However, many questions remain unanswered. For example, microbes capable of polymer degradation are ubiquitous in the environment, yet only affect polymers under some circumstances. What controls the production of polyurethanases? What is the role of planktonic vs. biofilm populations in the biodeterioration process? The goal of our research is to define the parameters and regulatory mechanisms that result in polyurethane biodeterioration by *Pseudomonas protegens* Pf-5, with a focus on environmental conditions (nutrients, pH, oxygen) and microbial 'lifestyles' (planktonic vs. biofilm populations). First, we screened a variety of carbon sources with a polyurethane agar plate-clearing assay using the polyester polyurethane Impranil DLN. Results showed that strain Pf-5 could grow on a variety of carbon sources but that degradation of polyurethane varied depending on the carbon source. We observed strong polyurethane degradation in the presence of M9-citrate medium but severely reduced clearing of polyurethane when glucose was provided as a carbon source. Subsequent studies with planktonic cultures of *P. protegens* Pf-5 verified the inhibitory effect of glucose on polyurethanase activity. Using proteomic tools, activity in citrate-grown planktonic culture supernatants was ascribed to two esterases, polyurethane esterases A and B. Currently the regulation of these enzymes is being investigated through a combination of genetic and transcriptomic approaches. Biofilms were grown on Impranil DLN in M9-citrate or -glucose to determine if these nutrients also regulated polyurethanase secretion in biofilms. Micro ATR-FTIR surface chemical analysis of the coatings after biofilm removal showed that degradation proceeds through preferential loss of the ester component. However, optical microscopy and profilometry clearly show that subsequent bulk coating loss can occur under certain conditions, resulting in complete loss of the original coating surface, and eventually complete loss of the coating. Transmission FTIR microscopy was also used to detect bulk coating degradation in a biofilm culture plate assay we developed to complement the Impranil clearing assay. This assay demonstrated significant Impranil coating degradation from citrate-grown biofilms versus minor degradation for glucose-grown biofilms.

3:40pm **BI+AI+BA+IS-MoA6 Multifunctional Active Nano and Microstructured Surfaces for Biofouling Management**, G.P. López, Duke University
INVITED

This talk will present (i) recent developments of stimuli responsive surfaces that exhibit dynamic structure on lateral length scales of the order of 10 microns and below, (ii) a prospectus for the formation of multifunctional bioactive surfaces based on such dynamic micro- and nanostructured materials, and (iii) results from study of bioadhesion and biorecognition on these surfaces. Stimuli responsive polymer surfaces include patterned polymer brushes and elastomers; biological systems of interest include protein solutions, adherent mammalian cell lines, as well as marine and infectious bacteria. Our previous studies have demonstrated that stimuli responsive polymers can be used to control the adhesion of such systems and, in this presentation, we will provide our latest advancements in this line of study, as regards to both molecular and cellular biointerfacial phenomena. Methods for preparing dynamic micro- and nanopatterns of stimuli responsive polymers will be presented, along with characterization of their structure, dynamic behavior and bioadhesion resistant character.

4:40pm **BI+AI+BA+IS-MoA9 Roles of Extracellular DNA in the Development and Expansion of Bacterial Biofilms**, C.B. Whitchurch, University of Technology, Sydney, Australia
INVITED

Biofilms are multicellular communities of bacteria that are often found attached to surfaces and cause significant problems in medical, industrial, and marine settings. Cells within biofilms are enmeshed in an extracellular polymeric matrix comprised of polysaccharides, proteins, lipids, and nucleic acids. Over the past decade, extracellular DNA (eDNA) has been found to be essential for biofilm formation by many species of bacteria where it is

thought to function as an intercellular “glue” that binds cells together. Interestingly, whilst it has been known for over a decade that eDNA is essential during the early stages of biofilm development by the opportunistic pathogen *Pseudomonas aeruginosa*, the precise roles of eDNA in this process have yet to be elucidated. We have used advanced techniques in microscopy, computer vision and image informatics to explore the roles of eDNA during early biofilm development and during active expansion of biofilms formed by *P.aeruginosa*. Many species of bacteria, including *P. aeruginosa* utilize type IV pili mediated twitching motility to actively translocate across solid and semi-solid surfaces. Twitching motility can manifest as a complex, multicellular behavior that enables the active expansion of bacterial biofilms. Under appropriate conditions, such as those encountered at the interface of a glass coverslip and semi-solid nutrient media, the expanding biofilm can develop dramatic networks of intersecting trails. Our analyses reveal that at the leading edge of the interstitial biofilm, highly coherent groups of bacteria migrate across the surface of the semi-solid media, and in doing so, create furrows along which following cells preferentially migrate. This leads to the emergence of a network of trails that guide mass transit toward the leading edges of the biofilm. We have determined that eDNA facilitates efficient traffic flow throughout the expanding biofilm by maintaining coherent cell alignments, thereby avoiding traffic jams and ensuring an efficient supply of cells to the migrating front. Our analyses reveal that eDNA also co-ordinates the movements of cells in the leading edge rafts and is required for the assembly of cells into aggregates that forge the interconnecting furrows. Our observations have revealed that large-scale self-organization of cells in actively expanding biofilms of *P. aeruginosa* occurs through construction of an intricate network of furrows that is facilitated by eDNA.

5:20pm **BI+AI+BA+IS-MoA11 Sample Preparation and Optimization for Bacterial Identification by Raman Spectroscopy**, *M.M. Hlaing, M. Dunn, S.M. McArthur, P.R. Stoddart*, Swinburne University of Technology, Australia

The characterisation and identification of individual bacteria using Raman spectroscopy can aid in rapid, in situ microbiological diagnosis and hence timely, appropriate treatment and control measures [1, 2]. Appropriate sample preparation methods and experimental conditions are crucial to avoid some potential difficulties in analysing the information-rich Raman spectra from bacterial cells. In this study, the Raman spectra of fresh and stored samples of bacterial isolates (*Escherichia coli*) were analysed to determine any variations caused by sample processing. Analysis based on principal components suggests that different methods of sample preparation and storage affect the spectral components associated with different biochemical compounds in bacterial cells. The effect of long term storage in glycerol stock at freezing temperatures on the Raman spectrum of cells from the early exponential phase was observed in this study and found to modify the bacteria cells. Furthermore, the presence of extracellular polymeric substance (EPS) matrix around bacterial cells at later stages of the growth cycle provide higher resistance to environmental stress compared with other phases. Based on these results, a specific experimental protocol has been developed in order to obtain interpretable, comparable and reliable Raman data from bacterial samples.

Keywords: Raman spectroscopy; Bacterial identification; Sample preparation.

References

- [1] W. E. Huang, R. I. Griffiths. *Anal. Chem.* 2004, **76**(15): 4452-4458.
- [2] T. J. Moritz, S. T. Douglas. *J. Clin. Microbiol.* 2010, **48**(11): 4287-4290.

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