**Local Organiser**
Competences Center: Interfaces-Tribocorrosion – Electrochemical Systems (CC-ITES)
Dunarea de Jos University of Galati - Romania
47 Domneasca Street 800008

# COST D33 WG/01/08 Working Group Meeting WG1-WG2 event

D33-0001-06: Development and adaptation and coupling of surface science methods for an improved analysis of the chemical process occurring at the interfaces between materials and micro-organisms.

D33-0002-06: Analyses of chemical and biological processes causing adhesion of macromolecules, (microbial) cells, consortia etc to materials surfaces

**Meeting Place:**
**National Military Circle BUCHAREST - Romania**
Bulevardul Elisabeta nr.2 Bucharest
**Period: November 06-08, 2008**
**Local Organiser: Prof. Dr. Lidia BENEA**

## PROGRAM

**06 of November 2008:**
**Arrivals**

**07 of November 2008:**
**09h00-09h30 Opening- Welcoming: WG1 coordinator**.

***Chair: Prof. Guenter SCHMITT, Prof. Lidia BENEA.***

**09h30-10h30 Invited Speaker: Prof. Pietro CAVALLOTTI:**
*Politecnico di Milano - Metallurgical Coatings and Surface Engineering, **Italy***.
**Title: Electrodeposition of nanodots for improved interaction with organic substances of surfaces**.

**10h30 – 11h00: Prof. Dr. Philippe MARCUS:**

*Laboratoire de Physico-Chimie des Surfaces CNRS-ENSCP (UMR 7045) Ecole Nationale Supérieure de Chimie de Paris - Université Pierre et Marie Curie Paris,* ***France****.*

**Title: XPS and ToF-SIMS investigation of plasma treated stainless steel surfaces for antifouling properties**.

Authors: S. Zanna, A. Seyeux, C. Saulou, M. Mercier-Bonin, B. Despax, P. Raynaud, P. Marcus

**11h00-11h15 Coffee break**

**11h15 – 11h45: Dr. Georg PAPASTAVROU:**

*Department of Applied Chemistry, University of Geneva,* ***Switzerland.***

**Title: Some thoughts about protein adsorption to modified electrodes.**

**11h45 – 12h00: Drd. Alina-Crina CIUBOTARIU:**

*Dunărea de Jos University of Galati – CC-ITES,* ***Romania.***

**Title: Combined Atomic Force and Epifluorescence Microscopy for Visualization of Sulphate Reducing Bacteria on SiC / Ni composite coatings.**

Authors: Alina Ciubotariu, Lidia Benea, Wolfgang Sand

**12h15-12h30: Dr. Paula COJOCARU:**

*Politecnico di Milano Chemical Engineering Faculty,* ***Italy.***

**Title**: **Comparison of the electrochemical behavior of stainless steels in abiotic and biotic seawater**.

**12h30 – 12h45: Prof. Guenter SCHMITT:**

*Institut für Instandhaltung und Korrosionsschutztechnik gGmbH, Iserlohn,* ***Germany****.*

**Title**: **MIC in Stainless Steel Plumbing Systems for Drinking Water.**

Auth: Guenter Schmitt, Hubertus Schlerkmann, Wolfgang Sand, H. Klemp

**12h45 – 13h00: Dr. Andrzej KUKLINSKI:**

*Universität Duisburg-Essen, Biofilm Centre / Aquatische Biotechnologie,*

*Duisburg,* ***Germany****.*

**Title**: **Recent advances in applying combined AFM/EFM**

Andrzej Kuklinski, Wolfgang Sand.

**13h00-15h00: Lunch.**

***Chair: Prof. Dr. Philippe MARCUS, Dr. Georg PAPASTAVROU****.*

**15h00 – 15h30: Dr. Luca OTTAVIANO**

*Università degli Studi dell'Aquila****, Italy.***

**Title**: **A full set of characterization techniques of surfaces and nanoparticles at the Physics department of the Universtiy of L'Aquila.**

Authors L. Ottaviano, P. De Marco, L. Lozzi, M. Passacantando, V. Grossi, S. Santucci.

**15h30 – 15h45: Prof. Dr. Geta CARAC:**

*Dunărea de Jos University of Galati,* ***Romania.***

**Title**: **Electrochemical investigations of the fungi behavior in the disinfectants medium.**

Authors: Maricica Stoica, Geta Carac

**15h45 – 16h15: Invited Speaker: Dr. Fraddry D`SOUSA:**

*TNO Science and Industry, TNO Laboratory,* ***Nederland.***

**Title**: Electrochemical sensing of Biofilm and MIC:

**16h15 – 16h30: Prof. Dr. Lidia BENEA**

*Dunărea de Jos University of Galati CC-ITES,* ***Romania.***

**Title**: **Chemical and structural factors influencing biofilm formation on material surfaces**.

**16h30 – 16h45: Coffee break**

**16h45 – 18h15 ROUND TABLE:**

Closing Remarks.

Discussions and proposals for new COST Actions and others Collaborative Projects.

Management aspects.

STSM proposals.

**19h00: Dinner**

**08 of November 2008:**
**Social: Excursion to Brasov (Bran Castle, Sinaia Castle, etc).**
**Departures.**

**Prof. Dr. Lidia BENEA**
COST D33 Vice-chair, WG 01 coordinator

# Electrodeposition of nanodots for improved interactions with organic substances of surfaces

**Pietro Luigi Cavallotti**

*Dip. CMIC Politecnico Via Mancinelli,7 20131 Milano Italy*
*pietro.cavallotti@polimi.it*

Very qualified processes with a strong control of the results at the nano level are the future of electrodeposition. A process can be developed employing a porous anodic alumina (PAA) mask as a template to electrochemically grow supported metal nanodots and nanowires without the need of aluminum evaporation. The conditions to obtain highly ordered PAA structures and to control the growth are reviewed. A PAA film can be fabricated from a high purity Al foil and transferred onto a copper-coated silicon wafer. Nickel was plated with direct current inside the pores of the PAA and silicon supported arrays of Ni nanodots and nanowires were obtained. A particular care is dedicated to the obtainment of highly ordered arrays of Ag nanodisks on Si for surface-enhanced Raman scattering applications. An interesting process regards the transfer of a porous alumina template mask onto a metal-coated Si wafer and its use to electrodeposit hexagonal patterns of Ag nanoparticles with tunable diameters and spacing. The SERS activities of well-ordered nanodot arrays with different ratios of particle diameter to interparticle spacing are measured using phenylethyl mercaptan as the probe molecule. By increasing the particle diameter to interparticle spacing ratio, the SERS enhancement is augmented by nearly one order of magnitude. The excellent uniformity of these nanoarrays and the reproducibility of the resulting SERS intensities offer a powerful tool with high sensitivity for the detection of chemical and biological species. Composite coatings with nano ceramic powders can be also an interesting method to codepositive sensitive phases. Nano $TiO_2$ particles, of anatase structure, can be codeposited in a nickel matrix increasing their oxidation effect with respect to biomaterials and organic substances.

**Plasma deposition of silver nanoparticles onto stainless steel for the prevention of biofilms: an XPS and ToF-SIMS investigation.**

S. Zanna[1], B. Despax[2], P. Raynaud[2], C. Salou[3], M. Mercier-Bonin[3], A. Seyeux[1], **P. Marcus[1]**

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A silver nanoparticle composite thin film was deposited onto stainless steel to prevent fungal biofilm formation by limiting initial microbial adhesion. The film deposition was carried out under cold plasma conditions, combining RF glow discharge fed with argon and hexamethyldisiloxane and simultaneous silver sputtering. The coatings were characterized by XPS and ToF-SIMS. The efficiency of the plasma-modified stainless steel was evaluated on a yeast model system (*Saccharomyces cerevisiae*) under laminar flow conditions in a shear stress flow chamber. Shear flow-induced detachment experiments clearly demonstrated that the silver-nanocomposite film was successful in drastically reducing yeast adhesion. However, the surface tended to undergo some changes, due to prolonged contact with the aqueous media. Aging in a physiological media was studied by XPS and ToF-SIMS for silver nanocomposite films with different silver concentrations. The surface analyses confirmed the presence of metallic silver for the native plasma-modified surface and some modifications of the extreme surface layer after use. The silver content at the surface was lower after contact with the solution, which was due to release of ionic

silver from the composite into the aqueous medium. After 60 days of immersion and for the low silver concentration nanocomposite film, the deposit thickness was not modified by aging treatment. On the contrary, the thickness of the plasma deposit decreased with the immersion time for the high silver concentration deposit. This result is explained by a delamination of the high silver content nanocomposite film in the aqueous media.

## Some thoughts about protein adsorption to modified electrodes

**Georg PAPASTAVROU**

*Department of Applied Chemistry, University of Geneva Switzerland*

The modification of an electrode surface by self-assembled monolayers allows probing the influence of different surface contributions on the adsorption of charged proteins. Due to the external potential applied to the electrode the electrostatic interaction with charged proteins can be varied over a wide range. These long-ranged electrostatic forces can be probed by direct force measurements with suitable colloidal particles and the dependence of electrode diffuse layer properties on the external potential can be determined with high accuracy. Additionally, the chemical surface properties (e.g hydrophilic/hydrophobic) can be varied by the functional groups terminating the thiol-layer. In a set of first preliminary measurements we studied the adsorption of ferritin to such modified gold electrodes. The adsorbed amount has been determined by measuring the number density of ferritin on the surface by AFM-imaging. The adsorption behavior can be described in terms of the random-sequential-adsorption (RSA-) model.

# Combined Atomic Force and Epifluorescence Microscopy for Visualization of Sulphate Reducing Bacteria on SiC / Ni composite coatings

**Alina CIUBOTARIU[1],** Lidia BENEA[1], Wolfgang SAND[2]

*[1]Dunarea de Jos, University of Galati, Metallurgy and Materials Science Faculty,*
*47 Domneasca Street, 80008, GALATI, Romania*
*[2]University of Duisburg Essen, Biofilm Centre, Aquatic Biotechnology,*
*Geibelstraße 41, 47057, Duisburg, Germany*

Bacterial adhesion on the surfaces of medical devices, food processing equipment, heat exchangers and ship hulls has been recognized as a widespread problem. Bacterial adhesion mechanism is complex and many factors affect cell adhesion. SRB are anaerobes that are sustained by organic nutrients. SRB reduce sulphate to sulphide, which usually shows up as hydrogen sulphide or, if iron is available, as black ferrous sulphide. In the absence of sulphate, some strains can function as fermenters and use organic compounds such as pyruvate to produce acetate, hydrogen, and carbon dioxide. SRB have been implicated in the corrosion of cast iron and steel, ferritic stainless steels, 300 series stainless steels (also very highly alloyed stainless steels), copper nickel alloys, and high nickel molybdenum alloys. The work was focussed on performing AFM coupled with epifluorescence studies in order to observe the influence of materials structure (nano and micro-structured coatings prepared by electrolytic co-deposition) on bacteria cells (Sulphate Reducing Bacteria) attachment. Sessile bacteria on coupons were stained with 4, 6-diamidino-2- phenylindol (DAPI) and visualized by EFM as well as AFM. The best imaging conditions for AFM were assessed. Scans of bacteria attached to surfaces were performed in contact mode in air.

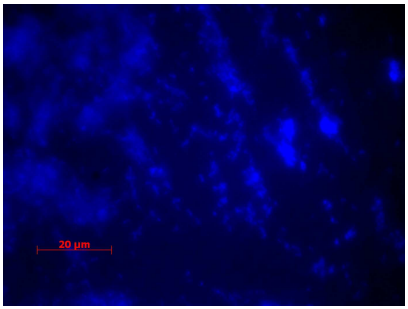


**Figure 1.** Fluorescence microscope image of SRB on nickel surface (current density 2 A/dm$^2$ for 1h )

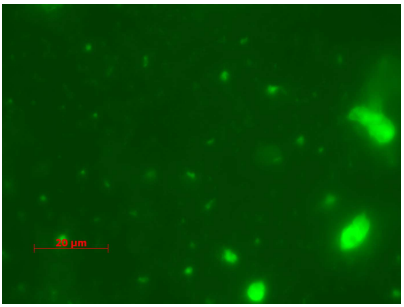


**Figure 2.** Fluorescence microscope image of SRB on Ni/SiC (nano) composite coatings (current density 2 A/dm$^2$ for 1h )

From the epifluorescence microscopy and atomic force microscopy images it was observe that attachment of SRB bacteria on composite coatings is lesser than pure nickel. Imagines obtained indicate an adherence process of the microorganisms on the surfaces studied. It shows that the new system for combined imaging with AFM and EFM on nickel and nano and micro composite coatings SiC/Ni is feasible for the application to Sulphate Reducing Bacteria on their surfaces. In addition, combined AFM and EFM can now be applied to the investigation of composite coatings. Bacterial adhesion is a complicated process that is affected by many factors including the bacteria (hydrophobisity, surface charge), the material surface (chemical composition, roughness, configuration, wet ability) and environmental factors (temperature, bacterial concentration, time of exposure, flow conditions). Surface modification enables the evaluation of the effect of these parameters on bacterial adhesion.

## Comparison of the electrochemical behavior of stainless steels in abiotic and biotic seawater

**P. Cojocaru,** L. Magagnin, G. Salvago, P.L. Cavallotti

*Dipartmento di Chimica, Materiali e Ingegneria Chimica, Politecnico di Milano, 20131 Milan, Italy*

Since LaQue has pointed out that seawater, directly provided from ocean, is more aggressive than seawater carried to laboratory and than the artificial one, the influence of bacteria on corrosion phenomena and of corrosion phenomena on microbiology were recognized. Engineers, chemists, physics, biologists and traders are now collaborating in lots of works to try to understand, to monitor and to control interactions between corrosion and microbiology.
Corrosion potential of passive materials exposed to water environment and in absence of localized corrosion is characterized by values distributed in wide ranges. Moreover, ennoblement of the corrosion potential vs. exposure time has been observed in different environment, e.g. natural and artificial seawater or hydrochloric acid solution.
In this work, the evolution in time of the anodic behavior of passive materials and particularly its effect on the corrosion potential in natural waters are investigated. Different hydrodynamic and biological conditions are considered. The distinction between the kinetics of the cathodic and anodic process and experimental polarization curves is highlighted. A careful analyses on the equilibrium between cathodic and anodic current in the experimental polarization curve is carried out to relate the ennoblement of the corrosion potential to the aging of the passive film. A weak inhibition of the anodic process rather than an enhancement of the cathodic one can justify the evolution of the corrosion potential.

# MIC in Stainless Steel Plumbing Systems for Drinking Water

**Guenter Schmitt**[1], Hubertus Schlerkmann[2], Wolfgang Sand[3], Helmut Klemp[4]

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## Abstract

Stainless steel (SS) experiences potential ennoblement when biofilms form on its surface when contacted with sea water, brackish water or river water. Recently is has been observed that potential ennoblement is also encountered at SS in drinking water causing crevice corrosion in pressfitting joints of SS plumbing systems. However, it appeared that not all drinking waters exhibit the ability for SS ennoblement.

This case was investigated with microbiological and electrochemical methods. It was found in long term experiments with once-flow-through test rigs that biofilms are formed on SS in drinking water distribution systems and that chlorination can prevent potential ennoblement of SS in drinking waters which are active to cause SS ennoblement. Waters which generally are ineffective to promote SS ennoblement can be activated to cause ennoblement by filtering the water with carbon black. Chlorination of carbon black filtered drinking water inactivates the water again for promoting potential ennoblement.

The mechanism of this effect is discussed with respect to interphase phenomena between the SS surface and an attached biofilm. The kind of water treatment in the water works significantly influences the activity of a drinking water to cause potential ennoblement of SS in tap water. The crevice corrosion attack in pressfitting joints of plumbing systems for active drinking water can be mitigated by impregnating the O-rings in the fittings with silicone grease.

# RECENT ADVANCES IN APPLYING COMBINED AFM/EFM ON OPAQUE SUBSTRATA

**A. Kuklinski**[1], W. Sand

Aquatic Biotechnology, Biofilm Centre, University of Duisburg-Essen, Duisburg, Germany

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For the visualization of interfacial processes on opaque substrata, such as metals or metal sulfides we are applying a newly developed combination of atomic force and epifluorescence microscopy (AFM and EFM, respectively). This novel system allows the imaging of the same sample location with both instruments. The sample, fixed on a so-called shuttle stage, is transferred between the – otherwise independent – AFM and EFM instruments. Fluorescence staining allows the proper identification of all bacterial cells (e.g. by DAPI), single species (FISH) or other distinct structures like EPS (by e.g. lectin-staining). AFM then allows the high-resolution examination of these structures or features of the substratum. We will present recent results of the successful examination of different biofilms and single microbes on several varying substrata, ranging from pyrite or polished metal coupons to stainless steel meshes. The biological structures were thereby identified e.g. by employing DAPI-staining and FISH, as well as lectin-staining for visualizing EPS or nile red for lipids. These results demonstrate that the combination of AFM and EFM in general seems to be a powerful tool for investigations on biofilms on opaque materials and will help to advance our knowledge about biological interfacial processes.

## A Full set of characterization techniques of surfaces and nanoparticles at the Physics department of the University of L'Aquila

**L. Ottaviano**, P. De Marco, L. Lozzi, M. Passacantando, V. Grossi, and S. Santucci.

*Dipartimento di Fisica Università dell'Aquila, Via Vetoio 10 I67100 L'Aquila Italy*

A brief review will be given of the potential of surface analytical techniques in the investigation of nano-structured and nano-bio structured materials. Results will be shown from core level and valence band photoemission spectroscopy, Scanning Electron Microscopy, Scanning Tunneling Microscopy and Spectroscopy, Atomic Force Microscopy, and X-ray diffraction. As case system will be illustrated the investigation of the adhesion modes and the rich morphology of ultrathin films of the microperoxidase MMP5 molecule (the heme containing fragment of a larger protein) deposited on inert substrates like gold and HOPG.

**Electrochemical investigations of the fungi behavior in the disinfectants solution**

Maricica STOICA[1] and **Geta CÂRÂC[2]**

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Microorganisms, such as bacteria, yeast and mould fungi, attach readily tovarious surfaces. If biofilms are allowed to form in food and process industry, the microbes they contain may pollute large amounts of product. Therefore hygienic monitoring is, or at least should be, used in most production plants. Often, however, the biofilm is found only when it's become visible, and problems have already appeared, such as a functional disorder in production unit, clogging of tubing or valves, or corrosion. Unlike biocides are multitargeted antimicrobial agents. Those factors that affect antimicrobial activity, namely period of contact, concentration, pH and type of microorganism influence the manner in with biocides are used for whatever purpose and against a variety of microorganism.

The present study is based on the concept that the microorganisms are removed from the desired surface by disinfecting of the surface. The behavior of the yeast and fungi in the chemical disinfectants medium with stainless steel AISI 304 surfaces of different finishing was presented. Chemical disinfectants with different composition into wide pH medium (e.g. hydrogen peroxide, sodium dichloroisocyanurate, mixture as athanol, alkyldimethyl benzyl ammonium, dodecylpropyltriamine) were used in according to purveyor and compared their efficacy against the food spoilage microorganisms (*Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida species*).

The investigation were made into adapted cell to ensure the correct steps. Electrochemical parameters (pH, conductivity and open circuit potential) have been influenced on the immersion time of injected microbial suspension in the disinfectant solution.

## Electrochemical sensing of biofilm and MIC

**Fraddry D'Souza**, Mohamed Miqyass, Glen Donnelly, Job Klijnstra

*TNO Science & Industry, Bevesierweg (Harssens), 1785 AM, Den Helder, The Netherlands.*

In this study, we present two different electrochemical techniques, Electrochemical Impedance Spectroscopy (EIS) and Electrochemical Noise Measurement (ENM), for detection of biofilm and discrimination of MIC from non-MIC, respectively. EIS involves the application of well-defined external perturbation of known frequencies in order to obtain information on the measured system. For the biofilm detection of *E. coli*, impedance was measured at different frequencies ($10^{-2}$ to $10^{5}$ Hz) using interdigitated microelectrodes (IMEs). At very low frequencies, the interaction of biofilm bacteria and surface results in the shift in the phase angle. This change in phase angle is presently the criterion for biofilm detection.

ENM on the other hand involves the measurement of self-generated potential and current fluctuations in the ongoing corrosion process. ENM measurements were made in small bottles with acid producing (*Acidithiobacillus thiooxidans*) and iron oxidising (*Acidithiobacillus ferrooxidans*) bacteria. Clear cut differences in noise signals between MIC and non-MIC corrosion were found in both bacteria. ENM signals were further analysed in time and frequency domain. The frequency domain analysis (FFT; Fast Fourier Transform) showed a large difference between MIC and non-MIC corrosion at very low frequencies. Interestingly, these low frequency changes compared well with that of shift in the phase angle in biofilm detection with EIS. In conclusion, very low frequencies response indicates the interactions of bacteria with the surface, which are or could be used for the sensing of biofilm and MIC.

**Chemical and structural factors influencing biofilm formation on material surffaces.**

**L. BENEA**[1], B. TRIBOLLET[2], W. SAND[3], F. WENGER[4], P. PONTHIAUX[4]

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[4]*Laboratoire L.G.P.M., Ecole Centrale Paris, F-92290 Châtenay-Malabry, France.*

Bacteria adhesion is a very complicated process affected by many factors: bacterial/material properties and environment. Surface modification enables the evaluation of the effect of these parameters on bacterial adhesion. A rigorous study of the effects of surface chemistry / topography on bacterial adhesion and protein adsorption requires a model system that allows precise control of the type and the configuration of functional groups at the substrate surface under dynamic conditions. Regarding the environment it was observed that bacterial concentration versus time of exposure increases with increasing bacterial concentration and time up to a saturation level, specific for each type of surface-bacterial strain. Concentration of electrolytes, $CO_2$, pH and ionic strength depending on bacteria and material surface characteristics presence of Antibiotics decreases, depending on bacterial susceptibility and antibiotics concentrations.
All these factors may influence bacterial adhesion by either changing physical interactions in phase one of adhesion or by changing surface characteristics of bacteria or materials.
Bacterial characteristics influence also biofilm formation and growth. Hydrophobic bacteria prefer hydrophobic material surfaces. Materials Characteristics and Chemistry of surfaces are the most

important factors in bacterial adhesion and biofilm growth. Representation of cyclic steps involved in the formation of an active biofilm are presented in Fig. 1.

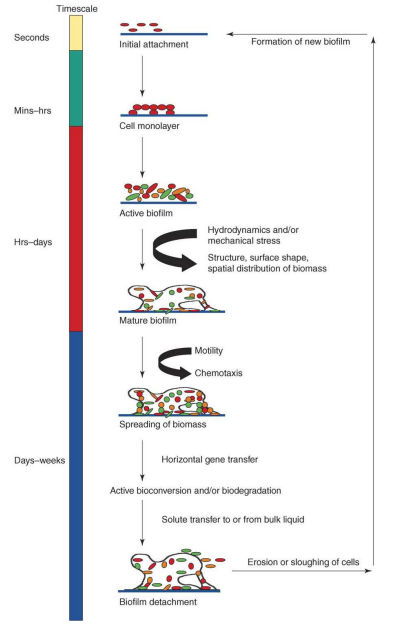


Fig. 1. Steps in the formation of an active biofilm

Cells initially attach by physico–chemical interactions or extracellular matrix protein secretion to form a cell monolayer, in which cells express pili and have twitching motility and/or the ability to undergo chemotaxis. Cells proliferate in the monolayer and other microbes attach to form an active biofilm, the development and distortion of which is influenced by environmental factors such as hydrodynamic and mechanical stress. Cells in the mature biofilm are motile and undergo chemotaxis, which leads to spreading of biomass and an increased rate of horizontal gene transfer. As cells die, active bioconversion and/or biodegradation leads to solute transfer to or from the bulk liquid which results in eventual biofilm detachment.

The processes of formation and detachment of cells are repeated in a cycle, thereby enabling further development of similar biofilms, which can subsequently attain new dimensions as a result of environmental influences.

# List of Participants

| *Nr crt* | *Family Name / First Name* | *COST D33* | | *e-mail address* |
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**Other participants not to be reimbursed:**
7 Local experts and researchers.