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Review – Interactions between diatoms and stainless steel: focus on biofouling and biocorrosion

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There is a considerable body of information regarding bacterially enhanced corrosion, however, this review focuses on diatoms (unicellular algae) whose contribution to biocorrosion is less well studied. The reasons why diatoms have been neglected in studies of biocorrosion in natural waters are discussed and the question whether diatoms should be considered as inert with respect of electrochemical processes is considered. A particular focus is given to the case of stainless steels (SS), which are widely used in variety of applications in natural waters. Basic information on the cell biology of diatoms is included in the review, particularly with respect to their ability to 'sense' and adhere to surfaces. Investigations at the nanoscale are reviewed as these studies provide information about the behavior of cells at interfaces. Recent advances include the use of atomic force microscopy (AFM), although only a few studies have been applied to diatoms. Regarding the electrochemical behavior of SS, the mechanisms by which diatoms influence the potential ennoblement process is discussed. Such studies reveal the association of diatoms, in addition to bacteria, with biocorrosion processes.

Keywords: biofilm; diatom; ennoblement; passive film; atomic force microscopy; electrochemistry

Introduction

Owing to their outstanding resistance to corrosion, stainless steels (SS) are extensively used in many applications involving contact with biological compounds/solutions. They are used in the food industry (Jullien et al. 2003; Whitehead et al. 2011) and in the manufacture of vascular stents, guide wires, or other orthopedic implants (Hanawa 2002; Ratner et al. 2004). In addition, SS are frequently utilized in many structures located in marine and freshwater environments, including port installations, cooling water circuits, and ships and related equipment. When exposed to humid and non-sterile media, SS are usually colonized by a variety of microorganisms, which adhere and grow to form biofilms. This fouling process strongly affects the performance of the material and may cause its deterioration.

Over the two past decades, considerable progress has been made towards understanding the nature and mechanisms relative to (i) the adhesion of microorganisms and (ii) microbiologically influenced corrosion (MIC). Many experiments in natural media, or employing strains isolated from natural sources, have demonstrated the role of bacteria in biocorrosion (for reviews see Beech 2004; Beech and Sunner 2004; Mansfeld 2007; Little et al. 2008). By contrast, diatoms have attracted little interest, either in terms of biofouling, but particularly with respect to biocorrosion, in spite of the fact that they make up the dominant biomass on all wetted and illuminated surfaces (Wetherbee et al. 1998).

Understanding the behavior of diatoms on SS surfaces requires an understanding of the complexity of the interface. In this review, a description of how diatoms interact with SS surfaces in a range of aqueous media is presented, including natural waters (seawater, estuaries, lakes and freshwater) and other waters associated with human activities (dam-water, wastewater, domestic water). Details are presented to illustrate key points: (i) physico-chemical features of SS surfaces, (ii) biochemical properties of the diatom cell surface, including composition, structure and recognition, and (iii) metabolic activities that influence the electrochemical response of SS.

Regarding the adhesion of diatoms, relevant features involved in cell–surface and cell–cell interactions have been gained through the application of atomic force microscopy (AFM) to probe live cells at the nanoscale (Hinterdorfer and Dufrene 2006; Dufrêne 2008; Muller and Dufrene 2008; Dupres et al. 2010). Some studies have reported promising results implicating diatoms in the electrochemical behavior of

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SS upon immersion in aqueous media. However, there is a lack of more basic knowledge of the mechanism by which diatoms, by themselves or *via* their metabolites, influence the free corrosion potential of SS. The following reasons may be relevant: (1) microbiologists are more familiar with the biology of bacteria and tend to favor investigations on this class of microorganisms; (2) bacterial biofilms are a serious cause of persistent infections (eg Costerton et al. 1999), thus research focuses on bacteria of biomedical interest compared to other microorganisms; (3) many bacteria responsible for an electrochemical effect are already well-known (Ismail et al. 1999; Shi et al. 2002; Dumas et al. 2008a; Mansfeld 2007; Parot et al. 2011) while the involvement of diatoms, which may have a key role in these processes, is still not fully understood.

This review aims to point out particular aspects, either experimental or conceptual, which are of primary importance to understanding the behavior of diatoms on SS surfaces, and more generally, at interfaces between materials and aqueous media. An analysis of the different hypotheses reported in the literature indicate a connection between diatoms and the electrochemical response of SS. Considering these aspects is essential in order to make progress in deciphering interfacial mechanisms involved in fouling and biocorrosion processes.

Exposure of SS in natural waters

SS passive film

The surface properties of SS depend strongly on the presence of an oxide passive layer that forms during exposure of the bare alloy to an oxidising medium. Passivity results from the thermodynamic instability of the metal which tends to become covered by a film that insulates the material from the medium (Pourbaix 1963). Passivity occurs by anodic dissolution followed by the formation of a thin layer, typically with a thickness of a few nanometers (Olsson and Landolt 2003). The driving force of passive film growth and stability is the potential gap between the metal and the solution, inducing a high electrical field (up to 10^6 V cm^{-1}) (Baroux et al. 1990). Passive film growth may be controlled by electrochemical polarization, or may occur spontaneously in the presence of an oxidising agent (electron acceptor). Theoretical aspects of the passivation process were reported in detail by Sato (1990). Passive film formation slows down ionic transport and thus metal dissolution, leading to a substantial resistance to corrosion in conditions to which the bare metal would react significantly. Details regarding the properties of passive films (composition, structure, electronic properties and stability have been reviewed elsewhere (see Olsson and Landolt 2003, and references therein). It is now well established that the high corrosion resistance of SS in a wide range of aqueous media is due to the ability of the passive film to adapt to changes induced by physico-chemical parameters (eg ionic strength, pH, potential) or microbiological activities.

Regarding physico-chemical properties, in common with other metals and oxides, SS surfaces exhibit high surface energy, which can be reduced by the adsorption of organic species (Kinloch 1990; Mantel et al. 1995; Caillou et al. 2008). The distribution of surface charge of the passive film is associated with the presence of the electrical double layer that implies the dependence of surface charge on pH (Bockris and Reddy 1970). Accurate measurement of the surface charge of the passive film remains difficult due to experimental considerations (Lefèvre et al. 2006). Values approaching the point of zero charge (PZC) were reported for many oxides using zeta-potential measurements. The PZC value obtained on a standard SS was reported to be around 3–4 (Boulangé-Petermann et al. 1995). Accordingly, a SS surface is negatively charged in natural waters (pH $\sim$6–8).

Surface conditioning and biofilm formation

In the first seconds to minutes that follow the immersion of SS or other metal and alloys in natural waters, the surface becomes covered with inert material present in the liquid phase, namely ions, macromolecules (proteins, polysaccharides, lipids), and inorganic materials. This leads to the formation of a film, commonly called the primary or conditioning film (Loeb and Neihof 1975), which strongly modifies the physico-chemical properties of the SS surface (Characklis and Cooksey 1983; Little and Jacobus 1984; Callow and Fletcher 1994; Taylor et al. 1997; Jain and Boshle 2009). Details of the ways in which the surface physico-chemistry of SS are changed by the adsorbed film have been discussed elsewhere (Schneider 1996; Schneider et al. 1997). In the marine environment, the accumulation of proteins and carbohydrates was observed on SS surfaces (Compère et al. 2001).

Microorganisms interact with the surface and firmly adhere, owing to the secretion of extracellular polymeric substances (EPS). This step, usually considered as irreversible, leads through cell division and further recruitment, to the formation of biofilm, which is a highly hydrated polymeric matrix. The formation of biofilms is detailed in numerous reports (eg Characklis and Marshall 1990; Flemming and Geesay 1991; Geesey et al. 1994; Flemming et al. 2009). The influence of the major biochemical compounds which constitute the conditioning film, ie proteins and

carbohydrates, on the adhesion of microorganisms was investigated by Jain and Bhosle (2009). Although bacteria are considered to be the initial colonizers, followed by diatoms, other algae and invertebrate larvae, this trend should be considered carefully as the relationship may not always be sequential or causally related. For example, diatoms can attach to clean surfaces in the absence of bacteria (Cooksey 1981). The morphology of biofilms has evolved from the uniform representation of Hamilton (1985) to the 3-D 'mushroom-shaped' model described by Costerton et al. (1994). The characterization of biofilms in terms of composition and three-dimensional structure was made possible by the development of 3-D mapping techniques, microanalytical devices, new fluorochrome markers and fiber-optic sensors, which allowed analyses of the liquid phase within the biofilm to be performed with minimal disturbance (Stoodley et al. 1994; Strathmann et al. 2002; Grossmann et al. 2007; Hu et al. 2007; Ganesh and Radhakrishnan 2007). Even so, making generalizations about biofilm structure and physiological activities are difficult, although it is well established that biofilms permit the permeation of nutrients, extracellular enzymes and metabolites that are necessary for the survival of microorganisms and their growth (Lappin-Scott and Costerton 1995; Jenkinson and Lappin-Scott 2001; Sutherland 2001).

The development of biofilms on a SS surface creates a complex SS/biofilm interface where multiple and diverse processes take place, including: (1) modification of the SS passive film in terms of composition, morphology and physico-chemical properties as a function of the medium, in particular in terms of the range of microorganisms and related biomacromolecules (Ismail et al. 1999; Yuan and Pehkonen 2007; Landoulsi et al. 2008b); (2) biofilms may be considered as a multi-compartment system involving numerous chemical reactions and mass transport processes and include: (i) a semi-continuous liquid phase, containing ions, other chemical compounds, and macromolecules, (ii) microorganisms that may be aggregated, (iii) solid particles, including cellular debris with a variable level of dispersion and reactivity, (iv) a macromolecular gel, composed largely of sugar polymers (eg polymers of glucose, galactose and mannose) (Christensen and Characklis 1990; Bhosle et al. 1995) and (v) one or several interfaces in contact with the metal surface where adsorbed substances and compounds, originating from metal dissolution, accumulate. Studying the SS/biofilm interface is thus a challenge. The most promising method adopted consists of monitoring the electrochemical behavior of SS during immersion in natural waters. This approach allows information to be acquired *in situ* without noticeable disturbance of the interface. Recent progress regarding the electrochemical behavior of biofouled SS is detailed below.

Potential ennoblement

The free corrosion potential (E_{corr}), also called open circuit potential (OCP), has been recognized as a relevant parameter to characterize the electrochemical behavior of SS in natural waters *in situ*. Mollica and Travis (personal communication) were the first to report that E_{corr} shifted towards anodic values upon immersion of SS in natural waters. This potential shift considerably exceeds the one related to SS surface passivation and reaches values higher than +200 mV/SCE in most cases. The term 'ennoblement' was used to describe this phenomenon, but it does not mean that the surface becomes more resistant against corrosion. When the potential increases towards anodic values it could come close to the pitting potential (E_p) and affects the stability of the passive film.

Ennoblement has been observed in seawater, independent of parameters related to the composition (eg geographic location, season, immersion depth, hydrodynamic factors) or to the SS material (SS composition and microstructure, surface roughness, geometrical sample form) (Scotto et al. 1985; Bardal et al. 1993; Scotto and Lai 1998; Feron et al. personal communication; Fischer et al. personal communication). It appears that the various parameters only influence the time which precedes ennoblement and/or the rate of increase in potential. In contrast to seawater, a generalization appears to be more difficult to make for natural freshwaters, including estuaries, rivers and lakes. This is due to a high variability related to the composition of the water and microbial activity as a function of location. Nevertheless, ennoblement has been reported to occur systematically in natural rivers (Dickinson and Lewandowski 1996; Dickinson et al. 1996a; Marconnet et al. 2008; Landoulsi et al. unpublished data). Ennoblement was also observed in other low chloride media such as domestic waters (Percival et al. 1998a, 1998b) and dam-waters (Liao et al. 2010).

Diatoms: the predominant biofouling community

Although the literature on biofouling and resulting issues related to biocorrosion is dominated by studies on bacteria, biofilms formed on SS surfaces and other metal and alloys are typically dominated by diatoms, especially when SS surfaces are illuminated. Many authors have recorded diatoms on a SS surface when studying ennoblement in a wide range of media. The main results, summarized in Table 1, show the

Table 1. The main diatom species identified on stainless steel surface after immersion in natural waters at different locations.

Species	Medium	Location	SS Type	Reference
Gomphonema spp.	Freshwater	(Tasmania, Australia)	Not specified	Andrewartha et al. (2010)
	Freshwater		Not specified	Rao et al. (1997)
Cymbella	Freshwater	(Tasmania, Australia)	Not specified	Andrewartha et al. (2010)
	Estuary	(India)	Not specified	Mitbavkar and Anil (2000, 2008)
	Freshwater		Not specified	Rao et al. (1997)
	River	(Oise, France)	304L, 316L, 254SMO	Landoulsi et al. (unpublished data)
T. flocculosa	Freshwater	(Tasmania, Australia)	Not specified	Andrewartha et al. (2010)
Synedra	Freshwater	(Tasmania, Australia)	Not specified	Andrewartha et al. (2010)
Cylindrocyst	Freshwater	(Tasmania, Australia)	Not specified	Andrewartha et al. (2010)
Diploneis	Seawater	(Indian coast)	316	Eashwar et al. (2009)
	Estuary	(India)	Not specified	Mitbavkar and Anil (2000, 2008)
Navicula	Seawater	(Indian coast)	316	Eashwar et al. (2009)
	Seawater	(Brazil)	904L, super duplex	de Messano et al. (2009)
	Estuary	(India)	Not specified	Mitbavkar and Anil (2000, 2008)
	Freshwater		Not specified	Rao et al. (1997)
Climacosphenia	Seawater	(Indian coast)	316	Eashwar et al. (2009)
	Seawater	(Brazil)	904L, super duplex	de Messano et al. (2009)
Cocconeis	River	(Seine, France)	304L, 316L, 254SMO	Marconnet et al. (2008)
	Freshwater		Not specified	Rao et al. (1997)
	Estuary	(India)	Not specified	Mitbavkar and Anil (2000, 2008)
	River	(Oise, France)	304L, 316L, 254SMO	Landoulsi et al. (unpublished data)
Amphora	Seawater	(Brazil)	904L, super duplex	de Messano et al. (2009)
	Estuary	(India)	Not specified	Mitbavkar and Anil (2000, 2008)
	Freshwater		Not specified	Rao et al. (1997)
	River	(Oise, France)	304L, 316L, 254SMO	Landoulsi et al. (unpublished data)
Bleakeleya notata	Seawater	(Brazil)	904L, super duplex	de Messano et al. (2009)
Striatella unipunctata	Seawater	(Brazil)	904L, super duplex	de Messano et al. (2009)
Nitzschia	Seawater	(Brazil)	904L, super duplex	de Messano et al. (2009)
	Freshwater		Not specified	Rao et al. (1997)
	Estuary	(India)	Not specified	Mitbavkar and Anil (2000, 2008)
	River	(Oise, France)	304L, 316L, 254SMO	Landoulsi et al. (unpublished data)
Manguinea rigida	Seawater	(Brazil)	904L, super duplex	de Messano et al. (2009)
Navicula spp.	Seawater	(Brazil)	904L, super duplex	de Messano et al. (2009)
Licmophora sp.	Seawater	(Brazil)	904L, super duplex	de Messano et al. (2009)
	Freshwater		Not specified	Rao et al. (1997)
Cylindrotheca	Seawater	(Brazil)	904L, super duplex	de Messano et al. (2009)
	Estuary	(India)	Not specified	Mitbavkar and Anil (2000, 2008)
Pleurosigma spp.	Seawater	(Brazil)	904L, super duplex	de Messano et al. (2009)
Achnanthes	Estuary	(India)	Not specified	Mitbavkar and Anil (2000, 2008)
	Freshwater		Not specified	Rao et al. (1997)
Fragilaria	Freshwater		Not specified	Rao et al. (1997)
Rhoicospheria	Freshwater		Not specified	Rao et al. (1997)
	River	(Oise, France)	304L, 316L, 254SMO	Landoulsi et al. (unpublished data)
	River	(Oise, France)	304L, 316L, 254SMO	Landoulsi et al. (unpublished data)
Gomphoneis olivaceum	Freshwater		Not specified	Sekar et al. (1998)
Melosira varians	River	(Oise, France)	304L, 316L, 254SMO	Landoulsi et al. (unpublished data)
Gyrosigma	River	(Oise, France)	304L, 316L, 254SMO	Landoulsi et al. (unpublished data)

diversity of diatoms, independent of immersion conditions and SS type. In other reports, some authors have mentioned the presence of diatoms on SS surfaces without identification of the species (Scotto et al. 1986; Motoda et al. 1990; Mansfeld et al. 1994; Videla 1994; Mattila et al. 1997). Cooksey et al. (1980) showed that the initial colonization of SS coupons by diatoms exposed in Biscayne Bay (Florida) was light dependent, but after the first cells attached, it was not possible to distinguish between further colonization and the division of attached cells that had adhered to the substratum first. In any event, the number of cells on the surfaces increased logarithmically during each light period over 1 week. There was no increase in cell density at night. In short-term laboratory-based experiments, adhesion of diatoms in the dark was far less than in the light. Figure 1 shows SS samples after immersion in a natural river using environmental scanning electron microscope (ESEM). The dominating presence of diatoms either in close contact with the SS surface (Figure 1A) or when the surface is well covered with biofilm (Figure 1B) is apparent. In these cases, E_{corr} was observed to reach values ranging from +200 to +400 mV/SCE (Landoulsi et al.

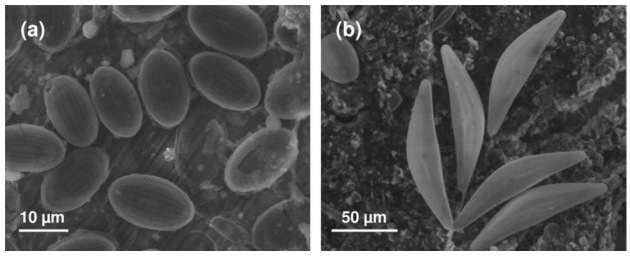

Figure 1. SEM images of diatom fouling of SS surfaces after exposure in natural river (Oise, France), showing the presence of (A) *Cocconeis* and (B) *Cymbella* sp.

unpublished data). The presence of bacteria is not obvious from the images, but cannot be ruled out as samples were immersed in natural river water. A heterotrophic bacterial film requires a source of organic carbon for growth and since the level of free organic material is relatively low in natural waters, the initial bacterial film is probably carbon-limited. Diatoms, however are autotrophic and thus require only carbon dioxide and nutrients for growth and these are usually not limiting. Once the diatom film is established, a mutualistic relationship between diatoms and bacteria will be developed. Although the primary film is generally dominated by bacteria, especially after immersion for $\sim$1 day, the first major accumulation of biomass is attributed to diatoms (Cooksey et al. 1980, 1981).

General biology of diatoms

Diatoms are eukaryotic microalgae that form brown coloured 'slimes' on wet illuminated surfaces. They vary in size from about 2 μm to several hundred μm, but are most commonly in the range 10–100 μm. The cell wall (frustule), composed of silicon dioxide, consists of a top and bottom (hypotheca and epitheca) valve, the two valves being held together by girdle bands. Some diatoms have one or two slits (raphes) in the cell wall. Traditional taxonomists use, among other criteria, the shape, size and 'decoration' of the silica frustules (eg number of ridges and pores) and presence/absence of a terminal pore(s) to speciate diatoms (Figure 2). Since diatom cells respond to stress by altering their shape, and even in unstressed situations they change their size when they divide, distinguishing between diatoms at the species level is challenging. When compared to similar technology used for bacteria, molecular taxonomy using 18s-RNA is not well developed. Although RNA extraction of cells can be made, the database available for comparisons is still scarce. Two main groups of diatoms can be designated viz. centric and the pinnate diatoms. The former shows radial symmetry of the frustules and the latter exhibits bilateral symmetry. Diatoms are found in all aquatic

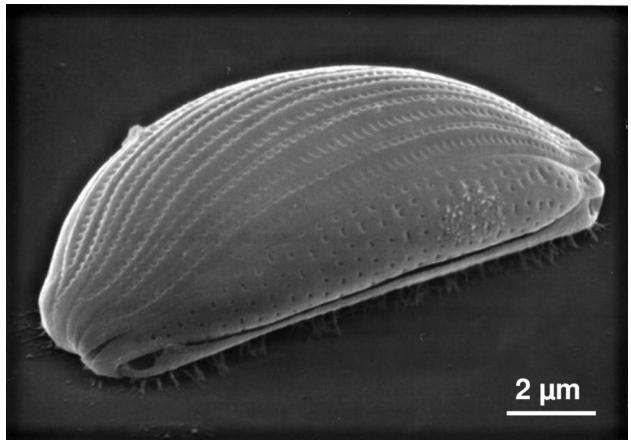

Figure 2. Micrograph of *Amphora* sp., showing the presence of EPS on a solid surface. This organism has two raphes (one is visible) both of which are on the ventral surface.

environments either in the water column (planktonic) or attached to surfaces (episammic or more generally, benthic). It is the attached organisms that cause biofouling. Attachment and motility are achieved *via* EPS secreted through the raphe slit(s), thus only raphid diatoms have these attributes (see review by Molino and Wetherbee 2008). Diatoms are most often obligately autotrophic, but some are facultative heterotrophs, many are mixotrophic and a few, having no chloroplast, are obligately heterotrophic (Chansang and Cooksey 1977; Werner 1977).

Diatoms at the nanoscale

Diatoms exhibit unusual cell surfaces, compared to other common fouling microorganisms, which differentiates them when adhered in biofilm. Investigating diatoms at the nanoscale may help to decipher how diatoms interact and adhere to SS surfaces. Different techniques have been successfully used for the characterization of diatoms including scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (De Stefano et al. 2003; Hildebrand et al. 2008), X-ray photoelectron spectroscopy (XPS) (Tesson et al. 2009), small angle X-ray scattering (SAXS) (Vrieling et al. 1999), confocal microscopy (Groger et al. 2008), Fourier transform infrared spectroscopy (FTIR) (Kiefer et al. 1997) and Raman mapping (Kammer et al. 2010). Traditional SEM and TEM are the most frequently used and high resolution images have provided information about the ultrastructure of diatom surfaces. However, such techniques are performed on dried samples and only provide limited information regarding adhesion. Recently, a quartz crystal microbalance with dissipation monitoring (QCM-D) has been used to this end (Molino et al.

2006, 2008). QCM-D allows the adhesion of diatoms to solid surfaces to be investigated, but spatial heterogeneity of the secreted adhesives is difficult to take into account. The use of atomic force microscopy (AFM) overcomes the limitation of the aforementioned methods by allowing a single living cell to be imaged (Muller and Dufrêne 2008; Dupres et al. 2010). Although the pioneering AFM experiments on diatoms were carried out on dried samples (Linder et al. 1992), imaging of cells in the native and hydrated state was quickly exploited (Crawford et al. 2001; Higgins et al. 2002, 2003a; Gebeshuber et al. 2003). In addition to not requiring a conductive layer that is required for SEM, AFM enables experiments with minimal preliminary sample preparation. Losic et al. (2007a) used AFM to reveal details of frustule structure of *Thalassiosira eccentric* at the nanoscale, showing that the frustules are built from silica nanoparticles, with sizes varying from 20 to 70 nm. In another study, AFM was used to image the surface structure of *Phaeodactylum* (Francius et al. 2008a), a pennate diatom possessing three different morphotypes (ovoid, fusiform and triradiate). Fusiform cells were of an elongated shape in which the girdle region resulting from the valve overlapping was resolved (Figure 3B). Examination of the triradiate forms confirmed previous SEM images and revealed cells with three arms emerging from a central core and forming a star (Figure 3D, E). The ovoid morphotype was two to three times smaller than the two other morphotypes (Figure 3G). High resolution images revealed a rougher surface and 'streaks' following the scanning direction (Figure 3I). The authors suggested the presence of secreted polymers involved in adhesion and gliding motility, as reported elsewhere (Chiovitti et al. 2003; Dugdale et al. 2006a). Gebeshuber et al. (2003) determined the thickness of the layer of EPS covering the siliceous frustules to be about 10 nm for benthic species, while more accurate measurements showed a thickness between 9–24 nm, depending on the species (Hildebrand et al. 2009).

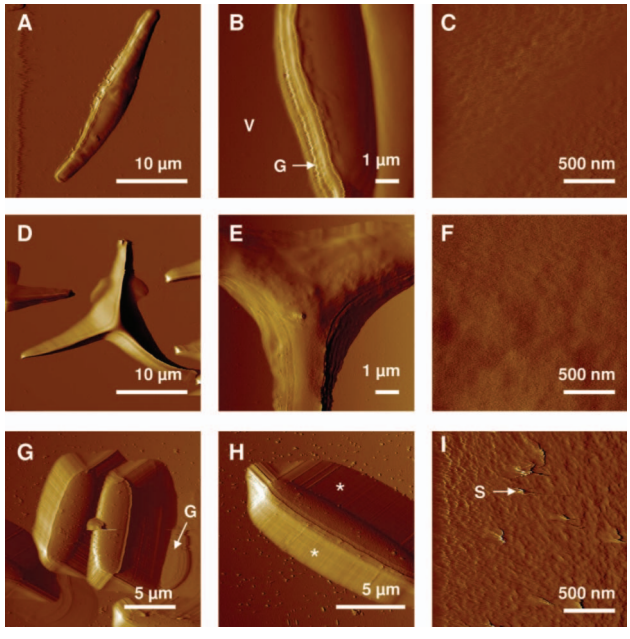

Figure 3. AFM deflection images recorded in aqueous solution for the fusiform (A–C), triradiate (D–F) and ovoid (G–I) morphotypes of *Phaeodactylum tricornutum*. Labels V, G and S correspond to the following features: valve, girdle region and streaks. The features highlighted by the asterisks in (H) reflect tip convolution artifacts. Reproduced with permission from Francius et al. (2008a).

The elastic properties of the cell surface can also be obtained from AFM nanoindentation measurements performed on the siliceous cell walls. For example, *Navicula pelliculosa* has an elastic modulus varying from 7 to hundreds of GPa, depending on the location on the frustules (Almqvist et al. 2001). These values are similar to those found for silica. Other results showed that the elastic modulus varied at different parts of the frustules of *Coscinodiscus* sp. ranging from ~2 GPa for the cribrum to ~15 GPa for the internal plate (Losic et al. 2007b). By comparison, the elastic modulus of EPS secreted from the cell was found to be much lower, varying from 250 to 750 kPa (Higgins et al. 2003a, 2003b). Francius et al. (2008a) investigated the cell wall elastic properties of different morphotypes of *P. tricornutum*. Elastic modulus values for the three morphotypes were lower than the GPa values reported for the walls of siliceous diatoms (Almqvist et al. 2001; Losic et al. 2007b) and differed from one morphotype to another. Indeed, the cell wall of the silicified ovoid form was found to be around five-fold stiffer (elastic modulus of ~500 kPa) than that of the two non-silicified forms (~100 kPa). In some situations, elasticity maps revealed heterogeneous contrast, as observed in the fusiform cell in Figure 4. The girdle region appeared softer (~80 kPa) than the valve (~320 kPa), suggesting that the girdle has a lower silica content and is enriched in organic material.

Diatoms in biofilms

Comprehensive reviews of the involvement of diatoms in marine microfouling have been published elsewhere (eg Cooksey and Wigglesworth-Cooksey 2001; Cooksey et al. 2009). Diatoms form the bulk of the initial colonizing biomass on surfaces immersed in the marine environment (Cooksey 1981; Callow 1986; Wetherbee et al. 1998) and diatom biofilms generate hydrodynamic drag on vessels (Bohlander 1991; Schultz et al. 2011). A detailed description of the diatom community

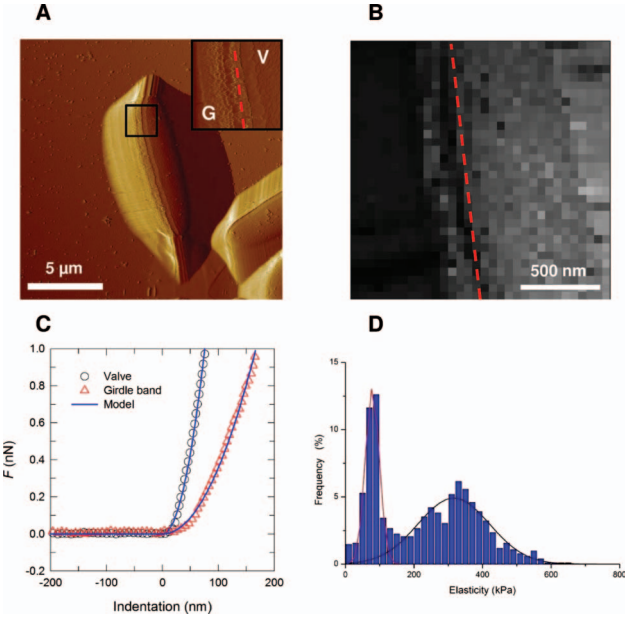

Figure 4. Mechanical properties of the ovoid girdle (G)/valve (V) interface of *Phaeodactylum tricornutum* diatom. (A) Deflection image (dashed line indicates the interface); (B) elasticity maps (z-range = 1000 kPa) corresponding to the inset image in (A); (C) typical force-indentation curve; (D) distribution of elasticity values ($n = 1024$ force curves). Reproduced with permission from Francius et al. (2008a).

which adheres to a range of ship hull coatings can be found in a recent report by Zargiel et al. (2011). Although there is more information appertaining to marine than freshwater biofilms, the biology of cell adhesion is likely to be similar. Attachment of all cells to surfaces (Berridge et al. 1998) is controlled by intracellular calcium levels (Cooksey et al. 1980; Cooksey 1981; Wetherbee et al. 1998). The intracellular calcium concentrations that invoke metabolic responses in all cells are changes in concentration in the range 10^{-7}–10^{-6} M. A tenfold difference in environmental calcium levels between freshwater (*ca* 1 mM) and marine water (*ca* 10 mM) is not likely to be significant since intracellular calcium levels are 1000 times smaller. Thus, conclusions based upon studies of marine organisms are likely to be generally applicable.

Early work on the design of antifouling (AF) surfaces can be found in the publications of Baier (1980), Characklis and Cooksey (1983) and Wigglesworth-Cooksey et al. (1999). Basic conclusions were that surfaces of intermediate surface energy (*ca* 25 dynes cm^{-1}) were the least hospitable to cells in general and diatoms in particular, although cells attached to all substrata whatever their surface properties. Since this early work, there have been major advances stimulated by the need for fouling protection of marine structures without the release of toxic materials into the environment. Thus, AF coatings have become more complex. Recent studies using different strategies to control slimes dominated by diatoms can be found in the literature (eg Molino et al. 2009; Dobretsov et al. 2011; Patil and Jagadeesan 2011; Zargiel et al. 2011). Whereas earlier efforts used simple chemistries to generate differences in wettability of surfaces, more recent efforts have focused on mixed polymers. For example, Sommer et al. (2010) used siloxane-polyurethane coatings based on aminopropyl terminated polydimethylsiloxane (PDMS). Urethane polymers alone have little fouling resistance, but provide mechanical strength whereas siloxanes, which are not mechanically strong, have fouling-release (FR) properties. The layering of these two components provided a coating with the positive properties of each component resulting in lower adhesion of bacteria, diatoms and macroalgae. The AF properties of polysiloxane polymers were also improved by the inclusion of tethered biocides (quaternary ammonium compounds (quats)), which were not released from the coating (Majumbar et al. 2008). Whilst coatings with 18 carbon length quats were effective in inhibiting bacterial biofilm formation, 14 carbon quats were most effective in inhibiting growth of the diatom *Navicula* sp. This technology demonstrates a two pronged attack on fouling control; the quat has AF properties, while the low surface energy surface reduces adhesion strength ie enhances FR. It has been shown that more hydraulic force is required to remove diatoms from a hydrophobic siloxane FR surface than to remove young plants of the macroalga *Ulva* (Cassé et al. 2007); the same relationship has been shown for other coating systems (see Bennett et al. 2010; Finlay et al. 2010). Since the extracellular adhesives of potential fouling organisms are diverse, it may not be possible to design a universal FR coating (Cooksey et al. 2009).

There is little information about AF coatings for application to SS that are specifically designed to resist diatoms. However studies performed on other surfaces, as described above, are expected to be generally applicable to chemically-modified SS surfaces. A widespread procedure to modify SS surfaces consists of grafting silane coupling agents onto the passive oxide film to form an anchoring layer (Landoulsi et al. 2011), and to use the amino-end groups to attach various molecules of AF interest, especially polymers. Other procedures of surface functionalistion have been also applied on SS, including the self-assembly of long chain aliphatic molecules with different headgroups, such as carboxylic acids, phosphonic acids and thiols (Shustak et al. 2004; Mahapatro et al. 2006; Raman and Gawalt 2007; Raman et al. 2010; Kruszewski and Gawalt 2011).

Since it appears that modifications to the surface energy of substrata, aimed at reducing adhesion do not prevent the adhesion of all fouling organisms, the

question arises what properties of the surface could be altered to discourage/reduce cellular adhesion, ie what would be an ideal AF surface? One possible approach would be to alter the surface chemistry of a coating in order to induce a specific response by the potential fouling cell or larva. A number of recent papers have reported the benefits of using amphiphilic coating systems that present both hydrophobic and hydrophilic domains on the surface; such coatings show excellent AF and FR properties for both diatoms and macroalgae (eg Dobretsov and Thomason 2011; Martinelli et al. 2011; Sundaram et al. 2011).

There are also a number of concepts that moderate diatom adhesion. It has been shown that diatoms can sense sugars when presented as a concentration gradient (Wigglesworth-Cooksey and Cooksey 1992) and that an intracellular calcium concentration flux may be involved. The ability to sense the presence of a sugar was investigated using *Amphora coffeaeformis* and positive and negative taxis was found, depending on the sugar used. The 'conditions' for sensing involved orientation of a hydroxyl group at position 2 of the pyranose ring as well as the diatom being able to move towards a sugar gradient, suggesting the diatom cell has a sophisticated array of cell surface receptors. Support for the idea of sensing is found in the work of Wetherbee et al. (1998), who showed that cells of *Stauroneis decipiens* were able to re-orientate so that the raphe slit in the cell wall is on the ventral side of the cell, instead of being uppermost. They postulated that surface recognition allowed the cell to 'search' for the substratum by strands of polymer secreted through the raphe slit. The strands then contract allowing the cell to turn so that motility is possible. The involvement of calcium transients in sensing has been shown in *Phaeodactylum tricornutum* and *A. coffeaeformis* (Falciatore et al. 2000; Cooksey et al. 2009). Further information was provided by Thompson et al. (2008) who investigated the ability of diatom cells to detect and respond to the surface energy of the substratum. Cells adhere more strongly to hydrophobic surfaces and it would be reasonable to assume that the adhered state is preferable for survival. Thompson et al (2008) measured the cellular level of nitric oxide, a general stress indicator found across the biological kingdom, in diatom cells on hydrophobic and hydrophilic surfaces. The level of nitric oxide was four-fold higher in cells on a hydrophilic surface (glass) than those on a hydrophobic surface (silicone) indicating that hydrophobic surfaces were less stressful. Molecules that induce stress in fouling organisms are candidates for inclusion in AF coatings, especially if they can be incorporated covalently into the coating. One such molecule is trans-trans-2,4- decadienal (DD) which has been implicated as a chemical defense molecule in that it inhibits invertebrate grazing of phytoplankton (Ianora et al. 2004, 2006). DD generates nitric oxide bursts which produce apoptosis, ie programmed cell death. As DD is produced by diatoms, it could be the trigger that causes clumps of diatoms to disperse (Wigglesworth-Cooksey et al. 1999). DD caused a rapid loss in motility and cells became permeable to Sytox Green 1 (a vital dye) soon afterwards (Cooksey et al. 2009). Based on the finding of Thompson et al. (2008), the inhibition of cellular sensing may be a promising strategy. Since such molecular control mechanisms are often similar across biology, sensory inhibition may be a general AF strategy for organisms from both the plant and animal kingdoms. Such an approach would be applicable to metal and alloys, including SS and could provide a new way to design an efficient AF surface to prevent the adhesion of diatoms in natural waters. In a review on diatom adhesion, Molino and Wetherbee (2008) concluded that 'many questions remain unanswered'. Research is especially needed regarding the interaction between biofilm bacteria and diatom adhesion. It has been suggested that photorespiration in diatoms caused by a reduced oxygen diffusion in the biofilm matrix can be controlled by its utilization by heterotrophic bacteria (Wigglesworth-Cooksey et al. 2001). Diatom-bacterial interactions have been investigated by (Murray et al. 1986; Wigglesworth-Cooksey et al. 2001, 2005), but more work is needed in this area.

Diatom adhesion: guidelines for the future

Upon contact with a surface, adhesion forces are mediated by the physico-chemical properties of the substratum and those of the microorganism, eg hydrophobicity and surface charge. Although substratum properties are easily measured using traditional techniques of surface characterization, knowledge of cell surface properties at the single cell level remains challenging.

It is now established that the siliceous cell wall of diatoms is covered by an organic envelope composed of polysaccharides, proteins, and glycoproteins (Hecky et al. 1973; Staats et al. 1999; Chiovitti et al. 2003) and that adhesion of diatoms on surfaces is associated with the secretion of mucilaginous material (EPS) (Hoagland et al. 1993). Diatom EPS have some common attributes; most are carbohydrate-based polymers with some protein content, which provides the ability to bind to both hydrophilic and hydrophobic substrata. However, analyses of extracted polymers, eg by time of flight mass spectroscopy, provides only limited information compared to *in situ* sampling because extraction techniques may introduce artifacts (de Brouwer et al. 2006).

AFM has been used to determine the adhesive and mechanical properties of individual proteins secreted by live diatoms cells (Dugdale et al. 2005, 2006a, 2006b). Force curves recorded for the benthic diatom *Toxarium undulatum* revealed a regular sawtooth-like pattern, which is a reliable signature of modular protein unfolding. Dugdale et al. (2005) hypothesized that single adhesive nanofibers were each made of a specific number of modular proteins aligned in parallel, forming a cohesive unit. The modular and flexible nature of these proteins conveys both strength and toughness, making it ideally suited for adhesion to the substratum. However, one question remains: what is the contribution of each of these macromolecules in the attachment of diatoms to a surface? The use of force spectroscopy with modified tips will provide insight into the distribution of specific sugar moieties on live diatoms, while AFM tips functionalized with specific antibodies should resolve protein mapping. Such studies will aid understanding of the physical properties of diatom EPS.

Cell probe

Diatoms can be used as probes to investigate cellular adhesion. Bowen et al. (1998) were the first to use a single, living, immobilised cell as a 'cell probe' for the study of cell-surface adhesion. Following this study, a large variety of cell probes from different microorganisms have been used, including fungal spores (Bowen et al. 2002; Wargenau and Kwade 2010), yeast cells (Bowen et al. 2001) and bacteria (Dague et al. 2010). In the biofouling and biocorrosion contexts, recent experiments have been performed to probe the interaction between bacteria immobilized on an AFM tip and different metal surfaces, including SS (Sheng et al. 2007, 2008). Despite the interest in this approach to probe interactions between cells and surfaces, very few studies have been reported using diatoms. Arce et al. (2004) used AFM to compare the adhesion of *Navicula* sp. to surfaces of different physico-chemical properties. Live diatom cells were immobilized at the end of tipless cantilevers and both hydrophobic and hydrophilic surfaces were tested with the same diatom probe to avoid artefacts (Figure 5A). Force *vs* distance curves revealed comparable cell adhesion strengths on Intersleek® and mica, indicating that *Navicula* secretes EPS with both hydrophobic and hydrophilic properties (Figure 5B).

Chemical properties of the cell surface

Ahimou et al. (2002) used AFM tips functionalized with ionizable carboxyl groups (COO^-/COOH) to probe the surface charges of *Saccharomyces cerevisiae*

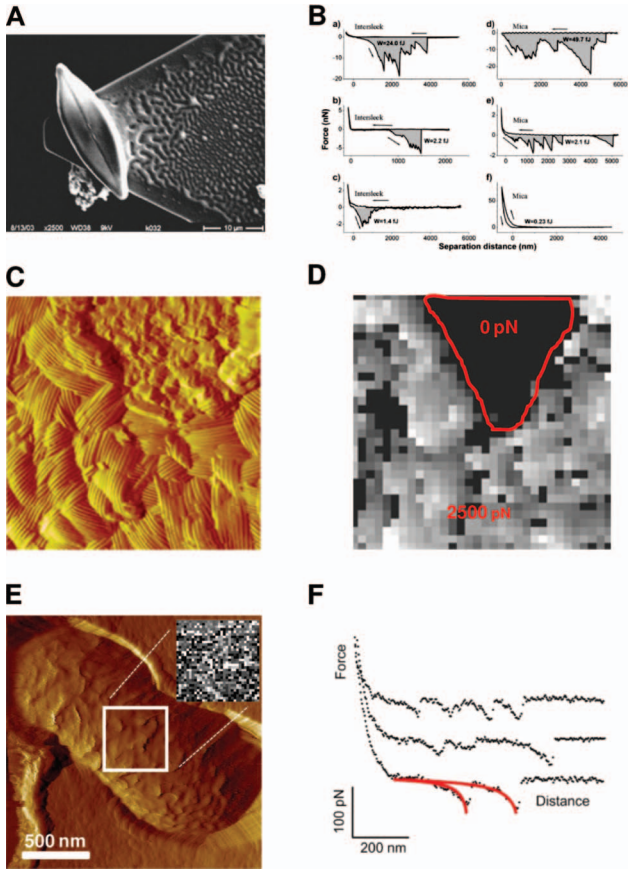

Figure 5. Cell probe experiments (A and B). (A) SEM micrograph of a single diatom cell attached with epoxy glue to an AFM tipless cantilever; (B) representative force *vs* distance curves obtained with bioprobe diatoms in the stationary phase on Intersleek (a–c) and mica (d–f) surfaces. The work of detachment, W, is given in fJ units (10^{-15} J) for each curve. The arrow represents the approach and retraction directions. Reproduced with permission from Arce et al. (2004). Nanoscale structure and hydrophobicity of *Aspergillus fumigatus*. (C) Deflection image and (D) adhesion force map obtained with a hydrophobic tip on SDS-treated conidia, revealing highly correlated structural and hydrophobic heterogeneities. Reproduced with permission from Dague et al. (2007). Detecting individual galactose-rich polysaccharides on LGG bacteria (E and F). (E) AFM deflection image of single LGG bacteria trapped into porous polymer membrane and adhesion force map (inset, gray scale: 200 pN) and (F) representative force curves recorded with PA–1 tip on LGG wild-type. Reproduced and adapted with permission from Francius et al. (2008b).

at the nanometer level. Force–distance curves were strongly influenced by pH: no adhesion was measured at neutral/alkaline pH, while multiple adhesion forces were recorded at acidic pH. The change of adhesion force as a function of pH was interpreted as resulting from a change of cell surface electrostatic properties. Using a similar approach, it has been shown that hydrophilic (OH) and hydrophobic (CH_3) tips can be used to map cell surface hydrophobicity (Dufrêne

2000). Moreover, this technique, called chemical force microscopy (CFM) now makes it possible to map the spatial arrangement of chemical groups on live cells (Alsteens et al. 2007; Dague et al. 2007; Hu et al. 2011). Using CFM with hydrophobic tips Dague et al. (2007) demonstrated large adhesion forces on the surface of *Aspergillus fumigatus* conidia, reflecting strong hydrophobic properties, in agreement with the presence of hydrophobins in the outer rodlet layer. Variations in hydrophobicity on a single cell could also be resolved, revealing contrasted hydrophobicity between rodlet and polysaccharide regions (Figure 5C, D). These studies demonstrate that chemically functionalized tips enable quantitative measurement of surface properties at the subcellular level and could be of interest to probe the distribution of EPS on live diatoms.

Identifying cell surface proteins and polysaccharides

Force spectroscopy experiments using biospecific tips, ie tips in which specific biological molecules are immobilized, have been shown to be particularly useful in identifying individual polysaccharides and proteins on living cells, and to measure their adhesion (Dupres et al. 2005; Dufrêne 2008). Notably, force spectroscopy offers a means of probing the conformational properties of microbial polysaccharides (Camesano and Abu-Lail 2002; Abu-Lail and Camesano 2003; Camesano et al. 2007). For example, AFM tips modified with lectins were used to specifically detect, localize and analyse individual polysaccharides on live *Lactobacillus rhamnosus* GG (LGG) (Francius et al. 2008b, 2009). Two types of polysaccharides were identified using AFM tips functionalized with two polysaccharide-specific lectins (Figure 5E, F). Additionally, the properties of the polysaccharide (distribution, adhesion, extension) of LGG wild-type were markedly different from those of a derived mutant impaired in terms of adhesion, biofilm formation and exopolysaccharide production.

Implication of diatoms in electrochemical processes

Oxygen plays a pivotal role in processes associated with biocorrosion of SS as it is involved in both abiotic and biotic mechanisms, which influence the electrochemical behavior of these alloys (Landoulsi et al. 2008a). The involvement of diatoms in these processes may be mediated by photosynthetic activity, which produces O_2 at the SS/biofilm interface. Though this has not been shown directly in biocorrosion studies, many reports in the literature suggest that diatoms are involved. The role of aerobic activities within biofilms on the electrochemical behavior of SS is detailed below.

Mechanism of ennoblement involving aerobic activities

Since early observations on the potential ennoblement of SS in natural seawater (Mollica and Trevis 1976), many hypotheses have been proposed to explain the interfacial processes involved in ennoblement. However, progress which has been gained regarding the structure and properties of biofilm changed the vision of researchers regarding its role. Taking into account the high level of biofilm heterogeneity and thus of the SS/biofilm interface, some hypotheses have been revised (for recent reviews see Beech et al. 2005; Mansfeld 2007; Landoulsi et al. 2008a).

Within the biofilm, oxygen is involved in the metabolic pathways of many microorganisms. It acts as a final electron acceptor in the oxidation process of organic molecules, eg lipids and sugars, or inorganic species such as manganese. Due to energetic considerations, the reduction reaction of oxygen leads to the formation of highly reactive free radicals or molecular species. Such intermediate products, commonly called reactive oxygen species (ROS), are involved in biocorrosion because their reactivity is higher than that of oxygen itself.

Biogenic formation of H_2O_2

Hydrogen peroxide (H_2O_2) is one of the main intermediates of the oxygen reduction reaction. The presence of H_2O_2 has been reported within biofilms formed on SS surfaces immersed in natural seawaters (Dickinson et al. 1996b; Xu et al. 1998; Washizu et al. 2004) and freshwaters (Marconnet et al. 2008; Liao et al. 2010; Landoulsi et al. unpublished data). In these studies, the concentration of H_2O_2 was detected in the range of several mM. The generation of H_2O_2 is governed by two antagonist processes: (i) production by enzymes using O_2 as electron acceptors (oxidases) and (ii) degradation by enzymes involved in the defense of microorganisms against oxidative stress (catalases, peroxidases).

The generation of H_2O_2 in biofilms has attracted much interest in biocorrosion studies, owing to the ability of H_2O_2 to influence the electrochemical behavior of SS. In natural waters, cathodic processes on SS are mainly due to the oxygen reduction reaction. However, H_2O_2 exhibits a redox potential ($E^\circ = 1.776$ V/SHE), significantly higher than that of oxygen ($E^\circ = 1.228$ V/SHE), making it a good candidate to initiate ennoblement.

Enzymatic system

Previously, it has pointed out that the biogenic generation of H_2O_2 is at the crossroads of many

enzymatic reactions and plays a key role in the ennoblement of SS. Hence, there is growing interest in using purified enzymes in electrochemical tests to study SS ennoblement (Landoulsi et al. 2008a). In particular, an enzymatic system mimicking the generation of H_2O_2 in biofilms has been used. To this end, glucose oxidase (EC. 1.1.3.4) was used, which catalyzes the formation of H_2O_2 by converting glucose into gluconolactone, then spontaneously decomposed in gluconic acid (Equation (1)):

$$\begin{aligned} &D\text{-glucose}\,(C_6H_{12}O_6) + O_2 + H_2O \rightarrow \\ &D\text{-glucono-1, 5-lactone}(C_6H_{12}O_7) + H_2O_2 \end{aligned} \quad (1)$$

In addition to practical experimental considerations, the choice of this enzyme was justified by the fact that glucose, the substrate of the enzyme, is the major sugar in polysaccharides present in natural waters and glucose has also been detected in biofilms formed on SS surfaces (Bhosle et al. 1990). Furthermore, the amount of H_2O_2 produced may be adjusted to be in the range of few mM, as observed in natural biofilms. Electrochemical tests using this enzyme have been performed in natural sterilized seawater (Amaya and Miyuki 1995, 1997, 1999; Dupont et al. 1998), in artificial seawater (Amaya and Miyuki 1995, 1997, 1999) and artificial freshwater (Landoulsi et al. 2008c; Marconnet et al. 2008). Experimental parameters relating to enzymatic activity, including pH and the ratio of enzyme and substrate, were optimized to be relevant to biocorrosion studies. Ennoblement occurred similar to that observed in natural waters reaching values ranging from +250 to +350 mV/SCE. In synthetic freshwater, simulating natural rivers, ennoblement was observed on SS type 316L whether H_2O_2 was generated *in situ* (ie produced by the enzymatic reaction) or added to the solution (Figure 6A). By combining electrochemical measurements and detailed surface characterization by XPS, it was shown that ennoblement was due to the electrochemical effect of H_2O_2. Furthermore, modification of the passive film during immersion was not sufficient to initiate such ennoblement (Landoulsi et al. 2008c), even if it influenced cathodic processes, especially the oxygen reduction reaction (Le Bozec et al. 2001). These findings were reinforced by further electrochemical measurements, which showed an increase in cathodic current density in the vicinity of E_{corr}, when H_2O_2 was present in the solution (Figure 6B).

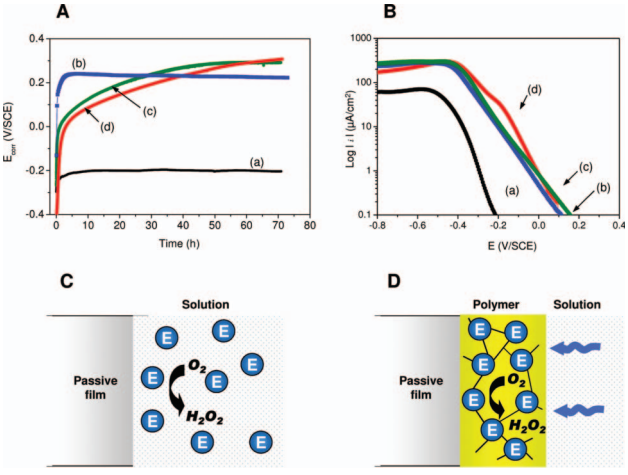

Figure 6. Panel (A) and (B). Electrochemical measurements in laboratory controlled model (E_{corr} evolution and cathodic polarization curves, respectively). H_2O_2-induced ennoblement obtained in synthetic freshwater, simulating natural rivers, (a) before and after the addition of (b) H_2O_2 (2 mM, pH ~ 8), (c) free or (d) immobilized enzymes. Panels (C) and (D). Schematic representation of the enzymatic system used to generate H_2O_2. When enzymes (designated 'E') are free (C), the formation of H_2O_2 occurs randomly in the solution, while immobilized enzymes (D) catalyze the reaction near the SS surface, leading to an enrichment of H_2O_2 and depletion of O_2.

Landoulsi et al. (2008d) elaborated a SS-modified electrode based on an enzyme immobilization method, to concentrate enzymatic activity near to the SS surface. This strategy was aimed at mimicking the physico-chemical conditions of the SS/biofilms interface (ie depletion of oxygen and production of oxidant species). Moreover, it allowed the activity of the immobilized enzymes to be preserved longer, since the polymer film confined the enzyme in a stable configuration and thus avoided its inactivation. When glucose oxidase was immobilized in a polymeric film coated onto a SS surface (Figure 6C and D) H_2O_2 was mainly produced within the polymeric film according to Equation (1), leading to local accumulation. This was accompanied by a strong depletion of oxygen near the SS/film interface, owing to the fact that (i) the polymeric film partially hindered access of dissolved oxygen to the SS surface and (ii) the oxygen was consumed by the entrapped enzymes (Figure 6D). These experiments provided information about the cathodic processes and demonstrated the separate roles of O_2 and H_2O_2 in ennoblement. The same approach was applied on SS, based on the use of a wire beam electrode to mimic the heterogeneity of the SS/biofilms interface (Wang et al. 2009). These authors showed a heterogeneous distribution of potential and current due to the generation of H_2O_2 catalyzed by glucose oxidase.

Consequences on corrosion behavior

Although the mechanism of ennoblement involving H_2O_2 and related species is now known, one issue of

primary importance in biocorrosion studies still remains poorly understood, viz. does ennoblement lead to localized corrosion of SS?

In natural waters, although the systematic feature of ennoblement is well established, pitting corrosion or other forms of localized corrosion, were not always observed. The correlation between ennoblement and corrosion is still a topic of debate. On the one hand, some authors have observed a beneficial effect of biofilms against corrosion and reported the notion of inhibition of MIC. This observation stems from the presence of EPS secreted by bacteria or other microorganisms (Mansfeld 2007; Videla and Herrera 2009), leading to protection against corrosion for several metal and alloys (Chongdar et al. 2005; Stadler et al. 2008). On the other hand, the pitting corrosion of SS has been investigated using the enzymatic generation of H_2O_2 to mimic aerobic activity of biofilms (Landoulsi et al. 2009). The results showed that the presence of H_2O_2 may limit pit propagation, leading to a noticeable shift of the pitting potential. From the electrochemical point of view, the involvement of H_2O_2 both in ennoblement and in the pitting corrosion behavior of SS may be explained on the basis of anodic and cathodic branches, as depicted in Figure 7. All these findings enable reappraisal of the commonly acknowledged hypothesis that ennoblement increases the risk of localized attacks. Both EPS and dissolved compounds, such as H_2O_2 and related species, may play a beneficial role in protecting SS against localized corrosion. Hence, ennoblement does not necessarily increase the susceptibility of the passive film to pitting.

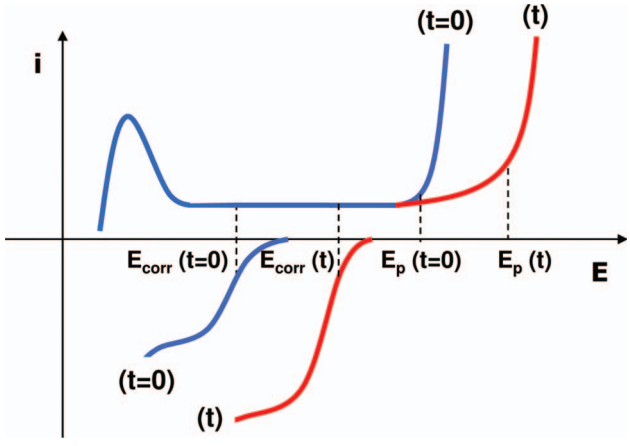

Figure 7. Hypothetical polarization curves of the cathodic and anodic processes on SS under MIC conditions: before ($t = 0$) and after (t) the formation of H_2O_2, causing a cathodic and an anodic response. These mechanisms result in the shift of both the corrosion potential (E_{corr}) and the pitting potential (E_p) towards anodic values.

Light-dependent ennoblement

The effect of light on E_{corr} evolution has been investigated by exposing SS to dark conditions (Dexter and Zhang 1991; Little et al. 1991). Little et al. (1991) observed that immersion of SS in natural seawater lead to the formation of biofilms which were dominated by diatoms. However, the presence of the biofilms did not result in an ennoblement of E_{corr}. On the basis of dissolved oxygen profiles through the biofilm and microprobe pH measurements, the authors suggested that diatoms modify the interfacial chemical properties by influencing the local oxygen concentration and the pH.

In a later report, periodic fluctuations of E_{corr} were observed on SS immersed in natural waters (Maruthamuthu et al. 1993). Interestingly, these variations were concomitant with the day/night cycle, suggesting a light-dependence of E_{corr} evolution. These findings implicate diatoms through their photosynthetic metabolism. The authors suggest that the 'loss' of ennoblement is due to a decrease in the pH induced by a significant proportion of acidophilic sessile bacteria ($\sim$50% of the total aerobic bacteria), creating an unfavorable pH for enzymatic reactions. Ennoblement is restored because photosynthesis by diatoms produces alkalization within the biofilm (Maruthamuthu et al. 1993).

An alternative hypothesis to explain ennoblement through the photosynthetic activity of diatoms was reported by Eashwar and Maruthamuthu (1995). The authors proposed a hypothetical model, based on the work of Little et al. (1991) involving a change in pH and dissolved oxygen within the biofilm near the SS/biofilm interface. However, their interpretation is not straightforward because the heterogeneity of the biofilms was not taken into account. The authors used a homogenous layer to describe the microbial biofilm present on the SS surface, which is now accepted as too simplistic as the high heterogeneity of biofilms is now well known. For instance, the use of microelectrodes demonstrated that the concentration of oxygen decreased with increasing depth into the biofilm (Little et al. 1991; Xu et al. 1998). However, the spatial distribution of oxygen inside the biofilm is difficult to determine with accuracy. Recently, a three-dimensional map of oxygen concentration revealed the existence of some highly concentrated pockets of oxygen within the biofilm (De La Rosa and Yu 2005).

In more recent studies, the day/night cycle-dependance of E_{corr} upon immersion of SS in natural river and in fresh-dam water has been reported (Marconnet et al. 2008; Liao et al. 2010). In both studies, the authors reported the dominating presence of diatoms on the SS surfaces. It was shown that E_{corr} increased at night and decreased during daytime (Figure 8). The potential values fluctuated as a function of the day/night

cycle with an amplitude $<+200$ mV. In contrast, without light, the diurnal fluctuations were reduced and the E_{corr} was kept at a value $\sim+400$ mV/SCE.

The correlation between light-dependent ennoblement and corrosion remains obscure. As mentioned above, the major issue relates to the complexity of the interface. Furthermore, it must be kept in mind that sunlight may influence the physical properties of the passive film, as it behaves as a semiconductor. In the marine environment, Eashwar et al. (2011) have demonstrated the influence of exposure to sunlight on the susceptibility of SS to localized corrosion.

How may diatoms be involved in ennoblement?

Because of the dominant presence of diatoms on SS surfaces, considerable care in interpreting the electrochemical behavior of SS, namely potential ennoblement, is necessary. The light-dependence of E_{corr} evolution suggests the involvement of diatoms on the ennoblement process. The question of the mechanism by which diatoms, directly or *via* their metabolism, influence the potential of SS potential is difficult to answer because few studies based on electrochemical tests on SS using pure cultures of diatoms are documented. Furthermore, as mentioned above the biofilm ensemble is a heterogeneous complex. An analysis of data reported in the literature reveals the possibility of different mechanisms, as follows: (1) Direct action on E_{corr} *via* photosynthetic metabolic activity in various aqueous media (Maruthamuthu et al. 1993; Ishihara and Tsujikawa 1998; Marconnet et al. 2008; Liao et al. 2010) although photosynthetic metabolism did not inhibit potential ennoblement (Liao et al. 2010). The latter was deduced from observations under reduced illumination (Figure 8A) and may explain why ennoblement occurred in dark conditions. Furthermore, the electrochemical response time of the SS electrode indicated that the variation in day/light potential could be attributed to enrichment/depletion cycles of oxygen at the SS/biofilm interface (Figure 9, process a). It is easily understandable if the Nernst equation is considered, which predicts that production of oxygen would increase the electrode potential and *vice versa*. A future challenge is to examine this mechanism by means of real-time measurements of dissolved oxygen close to the SS/biofilm interface. (2) Direct action mediated by diatom metabolites, in particular ROS, that react with the SS

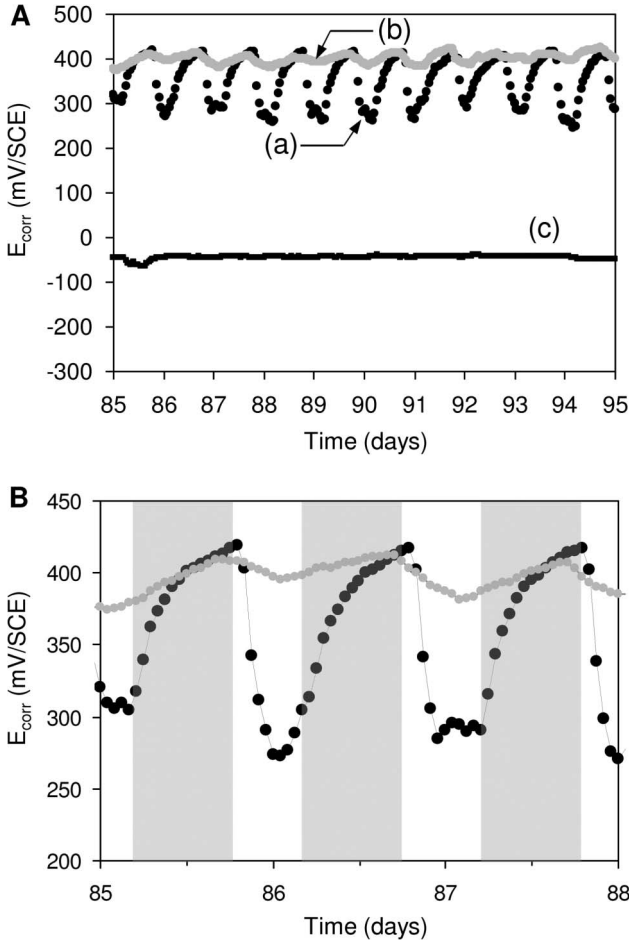

Figure 8. Light-dependent evolution of E_{corr} on SS samples. Panel (A). Potential variation recorded in (a) natural exposure conditions (dam-water), (b) the same without light and (c) the same after addition of filter. Panel (B). Detailed E_{corr} variation in a short period: the grey shaded regions indicate the night periods. Reproduced and adapted with permission from Liao et al. (2010).

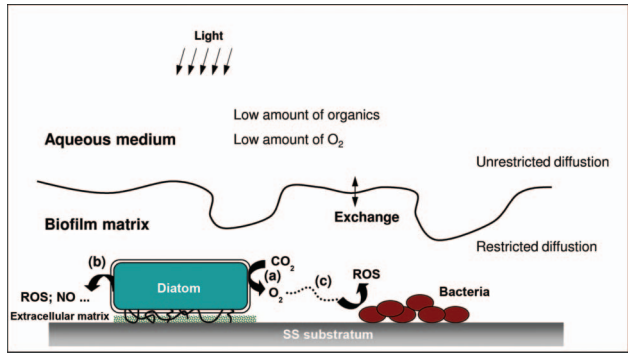

Figure 9. Proposed metabolic pathways to explain the possible involvement of diatoms in influencing the electrochemical behavior of SS. (a) Direct action *via* photosynthetic metabolic activity, influencing physico-chemical conditions of the SS/biofilm interface; (b) direct action *via* the effect of other diatom metabolic substances: production of reactive oxygen species (ROS) due to oxidative stress; (c) Indirect action by providing metabolic products, namely oxygen, to other microorganisms: potential metabolic interactions within the biofilms between diatom (phototrophic) and bacteria (heterotrophic).

surface. Indeed, as observed in other microorganisms, the oxidative stress of diatoms may lead to the production of H_2O_2 or other ROS (Figure 9, process b). These processes were observed in diatoms and other algae that were exposed to various forms of stress including mechanical stress, variation of light or temperature, addition of herbicides (Collén et al. 1994; Sundström et al. 1996; Abrahamsson et al. 2003). Although the mechanism remains poorly understood, it was shown that stress induced H_2O_2 may be related to the formation of volatile halocarbons involving haloperoxidase-catalyzed reactions (Wever et al. 1991). This process was recorded for the diatom *Pleurosira laevis* (Abrahamsson et al. 2003). The generation of ROS was also observed for *Nitzschia* in response to the toxic effect of redox-active compounds and their copper complexes. (Stauber and Florence 1985; Florence and Stauber 1986). The effect appears to be due to inhibition of the enzyme that breaks down H_2O_2 formed during oxidation of copper compounds. H_2O_2 may react with lipids to form hydroxyl radicals or diffuse into the extracellular space. $OH^{\bullet}$ and superoxide radicals ($O_2^{\bullet}$) are also generated extracellularly (Florence and Stauber 1986), but they did not influence the growth of diatoms. The production of H_2O_2 and related species in biofilms was reinforced by recent studies, in which the presence of diatoms on ennobled SS samples was accompanied by the production of a significant amount of H_2O_2 (Marconnet et al. 2008; Liao et al. 2010; Landoulsi et al. unpublished data). (3) Indirect action by providing metabolic products, namely oxygen, to other heterotrophic microorganisms present in the biofilm. Ishihara and Tsujikawa (1998, 1999) examined the potential for ennoblement by incubating SS samples in two stages: in 'stage I', SS was immersed in natural seawater for several days in a way that potential ennoblement did not exceed $\sim +100$ mV/SCE. In 'stage II', SS samples were transferred to a diatom-enriched solution in which ennoblement reached $\sim +400$ mV/SCE. The authors observed that 'stage II' alone could not lead to significant ennoblement and that 'stage I' was needed. These findings may imply two processes: (i) without 'stage I', diatoms are not able to adhere to the SS surface, possibly due to the physico-chemical properties of the interface, (ii) ennoblement is the result of the combined activities of diatoms and bacteria, based on the production of oxygen by diatoms and its consumption by heterotrophic bacteria as described above (Figure 9, process c). That heterotrophic bacteria may influence photorespiration in diatoms supports this scenario (Wigglesworth-Cooksey et al. 2001). Other investigations on diatom-bacterial interactions have also been reported (Murray et al. 1986; Wigglesworth-Cooksey and Cooksey 2005).

Prospects

It is obvious that diatoms are important in the biofouling community that develops on SS and other metals and alloys in natural waters. While the effect of bacteria in potential ennoblement has been widely discussed in the literature, diatoms are usually neglected in investigations of the electrochemical processes that may lead to biocorrosion. In the present review, it has been shown that the role of diatoms in such processes cannot be excluded. The ways in which diatoms may be involved in the potential ennoblement of SS have been identified, thereby opening new possibilities to gain an understanding of the ability of diatoms to initiate an electrochemical effect on SS electrodes. Investigating diatoms at the nanoscale will provide unique insights into how diatoms 'sense' surfaces and how they are involved in cell–surface and cell–cell interactions. Biomimetic systems, based on the use of either cultured axenic diatoms or in the presence of both mixed consortia of diatoms and bacteria, should be used in electrochemical tests. Furthermore, experiments based on the combination of AFM and electrochemical tests may pave the way for new comprehensive approaches to understanding features regarding diatom–SS and diatom–bacteria interactions. The ability of diatoms to induce an electrochemical response when they are in close contact to an SS electrode may be exploited in many areas of research, especially in the design of new microbial fuel cells. Indeed, studies have reported the construction of microbial fuel cell prototypes based on the use of marine biofilms and SS electrodes as the anode or cathode (Bergel et al. 2005; Dumas et al. 2007; Dumas et al. 2008b).

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