

1 **Influence of surface chemistry in bacterial adhesion to metals and biocorrosion**

2 J.P. Busalmen, M.B. Valcarce, and S.R. de Sánchez*.

3

4 División Corrosión, INTEMA–CONICET. Universidad Nacional de Mar del Plata. Juan B. Justo

5 4302. B7608FDQ, Mar del Plata, Argentina.

6

7 Running title: Adhesion of bacteria and corrosion.

8

9 *Corresponding author:

10 Dr. Susana Rosso de Sánchez

11 División Corrosión – INTEMA

12 UNMdP, Juan B. Justo 4302

13 B7608FDQ, Mar del Plata

14 ARGENTINA.

15 Tel.: 54 223 4816600

16 FAX: 54 223 4810046

17 e-mail: suroso@fi.mdp.edu.ar

18

19

20

20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

Abstract:

The aim of this work is to emphasize the central role of surface chemistry as a determining factor during the process of bacterial adhesion and biofilm formation on metals. Experimental results showing the influence of the presence and composition of an oxide layer, the pH, and the ionic strength of the solution on bacterial adhesion are reviewed and discussed.

Indeed, after a biofilm has been established, the modification of surface chemistry as a result of bacterial activity is considered as a key variable influencing the corrosion process.

Key words: bacterial adhesion, surface oxides, hydrogen bonds, microbiologically influenced corrosion

1. Introduction

Adhesion of bacteria to solid surfaces is a general phenomenon associated with numerous medical, industrial, and ecological problems (6, 7, 11, 17, 19, 22). In particular, the adhesion to metal surfaces is related, for example, to the contamination of prosthetic and medical devices (6), or to the localized corrosion failure of industrial equipment (17) as a consequence of the bacterial surface colonization and biofilm formation. A better understanding of the variables governing bacterial adhesion to metal surfaces will surely contribute to founding solutions to these problems.

A likely rationalization to the interaction of bacteria with a solid surface is often given through the DLVO theory of colloid stability, developed by Derjaguin and Landau (1941), and Verwey and Overbeek (1947)(13). This theory summarizes the electrostatic and van der Waals interactions, yielding the overall interaction energy between surfaces, as a function of separation distance. The suitability of the theory to explain the experimental results has been probed for the adhesion of bacteria to different materials, including polymers, glass and minerals [28, 32, 13, 20]. However, it has been pointed out that there are many aqueous systems where DLVO theory fails to explain the experimental observations (Israel...). The failure has been related to both, the lack of

46 consideration of solvent structuring in relation to surface features [19], or the occurrence of specific
47 interactions that can overcome DLVO barriers [7]. Good examples of systems where a non-DLVO
48 behavior has been found, are metal oxide or hydroxide surfaces on which hydration and the
49 possibility to form hydrogen bonds (H-bonds) are present. Experimental evidences are presented
50 here, showing the participation of surface chemistry in the bacterial adhesion to various metals.

51 Once bacteria are readily adhered to a surface, a biofilm can form as a consequence of
52 bacterial growth and proliferation. The growth of a biofilm on a corrodible metal very often results
53 in an increased corrosion usually referred as MIC (microbiologically influenced corrosion), and
54 includes pitting, dealloying, and crevice corrosion failures. These forms of corrosion are directly
55 related to local changes in chemical equilibria at the metal–electrolyte interface or to changes in the
56 surface oxides composition.

57 In this paper we present a summary of the work we have developed on the study of bacterial
58 interactions with metal surfaces. The project was aimed to determine the influence of bacteria on
59 the stability of metals with industrial applications, focusing the attention on the adhesion of aerobic
60 bacteria belonging to the genera *Pseudomonas* to various materials, including aluminum brass and
61 titanium, frequently used as construction material for condenser tubes with applications in Thermal
62 Central Units, and pure (base) metals as copper and zinc.

63 Results showing the influence of changes in variables like pH and ionic strength, and the
64 presence or not of a passivating oxide layer on the adhesion are discussed. Furthermore, the
65 influence of bacteria on the kinetics of oxides growth, and a possible mechanism for the increased
66 corrosion rates in the presence of bacteria are reviewed.

67

68 **2. The surface of metals in aqueous media.**

69 After immersion of a metal in an electrolyte, a steady potential is reached once the chemical
70 equilibrium between the metal and its ions in solution has established. Depending on the solution
71 parameters in the proximity of the surface, solid oxides and hydroxides may precipitate during

72 metal dissolution leading to the formation of a surface layer. Local pH and ionic strength on the
73 surface determine the kinetics of growth and composition of the layer, which indeed modify the
74 physicochemical interaction between the material and the surrounding environment, including the
75 interaction with water, dissolved compounds, and other elements as colloid particles and
76 microorganisms.

77 Taking this into account we aimed to study the growth and composition of the oxide layer
78 on some of these metals at various pH and ionic strengths in order to evaluate the physicochemical
79 effects on the bacterial adhesion process. The adhesion on pure metals was also evaluated for
80 comparison.

81 The presence of an oxide layer frequently determines a **steady state** at which corrosion is
82 possible, but at a lower rate due to the “passivating” effect of the oxide. **[ver primer trabajo con**
83 **David].**

84 We will refer from here on to oxidized surfaces as “aged surfaces”, in contrast to a recently
85 polished “bare surface” on which an oxide film is assumed to be absent.

86 During our studies water contact angle measurements under air or n-hexane were used to
87 determine surface hydrophobicity of metallic surfaces, as well as those of bacteria. UV-Visible
88 reflectance spectroscopy was used to analyze the composition of bare and aged metal surfaces after
89 different aging treatments. The electrostatic charge of bacteria was estimated from electrokinetic
90 measurements of the Zeta potential at various pH values.

91 The adhesion of bacteria to the surfaces was evaluated counting the adhered cells per unit
92 area at an optical metallographic microscope after classical immersion experiments at various times.

93

94 **3. Surface composition and hydrophilicity**

95 The water contact angle of metal surfaces in the bare and the aged condition for Al brass,
96 Cu and Zn are shown in Table 1. As can be observed in table 1, the higher contact angles for metals
97 surfaces were those corresponding to bare aluminum brass and copper, with values of 51.2° and

98 45.9°, respectively. The ageing treatment results in a strong decrease of the contact angle of
99 aluminum brass up to a value of 29.7°. Meanwhile in the case of copper, the obtained value was just
100 slightly lower (42.5°) than the one measured on the bare surface.

101 On zinc samples the instantaneous oxidation of the surface after polishing hindered the
102 determination of θ_w on a bare surface. On aged zinc on the other hand, the oxide covering the
103 surface was completely hydrophilic and rendered a value of $\theta_w = 0^\circ$.

104 The water contact angle for PE was also determined and included for comparison (Table 1).

105 Despite the relative differences of θ_w values measured on the various metals and conditions,
106 it is important to note that with the only exception of PE, contact angles were always below 65°,
107 which is recognized as an indication of the hydrophilic character of the surface of materials (Vogler,
108 1998).

109 The water contact angle is an indirect measurement of the interfacial energy of interaction
110 (γ) of a material or compound, and can be expressed as (Loeb, 1985):

111

$$112 \quad \gamma = \gamma^D + \gamma^{AB}$$

113

114 where γ^D and γ^{AB} are the dispersion component and the acid–base component of the
115 interaction, respectively. The γ^{AB} component for a metal surface can be directly determined
116 measuring the contact angle with water ($\gamma^D = 21.8 \times 10^{-3} \text{ J/m}^2$) under an *n*-alkane with a dispersion
117 component close to that of water. Any changes in the angle in this situation can be attributed to
118 changes in the acid–base component of the surface (Schultz *et al.*, 1977).

119 In order to characterize the acid-base interaction capacity of Al Brass, samples were aged in
120 NaCl 0.1 M at various pH values and their water contact angle was measured under *n*-hexane ($\gamma^D =$
121 $18.4 \times 10^{-3} \text{ J/m}^2$). Results are shown in Table 2. Water contact angles were observed to decrease as
122 pH values of the solution used during the ageing treatment increased (Table 2), indicating the

123 increment of both, the acid–base interaction and H–bond forming capacity of the surface toward
124 basic pH values.

125 In addition to contact angle measurements, oxides grown were also characterized during the
126 ageing treatment in different conditions, using differential reflectance spectroscopy.

127 Results are shown in Fig. 1 a, b, and c for surfaces aged at the corrosion potential in
128 artificial tap water. On copper, the presence of absorption peaks at 237, 314 and 380 nm, and
129 shoulders at 462 and 560 nm is indicating the participation of Cu₂O in the passivating film as a
130 main component (Fig. 1a) (Hummel, 1983, Sánchez *et al.*, 1991). On aluminium brass (Fig. 1b), the
131 occurrence of peaks at the same wavelengths but with different intensities was observed. A broad
132 peak at 462 nm, a minor peak at 360-380 nm and a shoulder at 237 nm suggest the presence of
133 Cu₂O. A peak at 260 nm was also detected. This peak was also found on aged zinc (Fig. 1c), and
134 indicates the formation of zinc oxo-hydroxides (Kim *et al.* 1995).

135 Spectra were also recorded after the ageing of Al brass in NaCl solutions with different pH
136 and ionic strengths in order to evaluate the oxide layer composition changes of this alloy. Results
137 are shown in Figs. 2 to 5.

138 At pH 2 a broad absorption band between 300 and 480 nm was observed, indicating the
139 presence of metallic copper (Kim *et al.* 1995) on surfaces aged at both, 0.1 M and 0.6 M NaCl
140 solutions (Fig. 2). It was also observed that surfaces were etched due to the active dissolution of the
141 metal at this extremely acid pH.

142 At pH 4, absorption peaks at 240, 310 and 560 nm were detected when analysing samples
143 aged in 0.1 M NaCl (Fig. 3). In NaCl 0.6 M, weak absorption peaks were detected at 240, 310 and
144 440 nm on the broad absorption band corresponding to metallic copper (Fig. 2). Peaks at 0.6 M
145 indicate the presence of Cu₂O (Hummel, 1991; de Sánchez *et al.*, 1991), its presence at 0.1 M
146 however needs a further analysis.

147 When working at low pH values (2 and 4) it is possible to find surface dealloying as a
148 consequence of the selective dissolution of the less noble metal in the alloy. In the case of

149 aluminum brass the preferential dissolution of zinc could lead to the formation of a copper rich
150 layer in the metallic lattice surface on which oxides are formed.

151 At pH 6, spectra of the oxides obtained after the ageing in both, 0.1 M and 0.6 M NaCl
152 were markedly different (Fig. 4). In 0.1 M NaCl, absorption peaks at 240 and 310 nm suggest the
153 growth of Cu_2O , whereas peaks at 520 nm and 640 nm could be related to the presence of
154 (hydro)oxides. Pyun and Park (1986) reported that absorption peaks initially found beyond 560 nm
155 shift to wavelengths lower than 460 nm after the ageing of copper in KOH as a consequence of
156 surface dehydration, and assigned the initial absorption to copper (hydro)oxides.

157 In 0.6 M NaCl (pH 6) (Fig. 4), the appearance of peaks related to the presence of CuO (270
158 nm) (de Sánchez *et al.*, 1991), Cu_2O (400–450 nm) (Hummel, 1991; Kim *et al.*, 1995), and ZnO
159 (360 nm) (Kim *et al.*, 1995) was observed. It is important to note the absence of peaks related to the
160 formation of hydroxides (Fig. 4), which indicates the occurrence of oxides dehydration with the
161 raising of the ionic strength.

162 At pH 8, the presence of Cu_2O was evidenced after the ageing in both, 0.1 M and 0.6 M
163 NaCl solutions (Fig. 5). In 0.1 M NaCl the main feature of the spectrum was an absorption peak at
164 640 nm, whereas in 0.6 M NaCl the stronger absorption was around 400 nm (Fig. 5), assigned to the
165 hydrated and dehydrated state of the oxide, respectively.

166 Being bacterial adhesion a physicochemical process, we aimed to study the effects of
167 modify the pH and ionic strength of the electrolyte on it. As shown by results in Figs. 1 to 5 the
168 surface chemistry of copper and Al Brass is readily susceptible to pH and ionic strength, and
169 changes in the oxide composition may interfere in the evaluation of adhesion. Taking this into
170 account we decided to repeat our experiments using titanium as test material.

171 After aging of titanium samples in NaCl solutions at different pH values, the open circuit
172 potential (OCP) was measured and differential absorbance UV-Visible spectra of the oxide were
173 recorded. Mean values of OCP are shown in Table 3. These values are in good agreement with the
174 existence of a TiO_2 layer expected from the Pourbaix diagram [29]. The absorbance spectra of the

175 surface oxide in 0.1 M NaCl at each pH value are shown in Fig.6. The main feature is a broad peak
176 of absorption at 300 nm, which was observed at the different pH values tested. These absorption
177 spectra are generally associated with a charge transfer process [23] which can be assigned, in this
178 case, to the transfer of charge from oxygen to Ti^{4+} ions in TiO_2 [23]. The only change observed in
179 the spectra was the height of the peak, which is related to the thickness of the TiO_2 layer. Similar
180 results were obtained in 0.6 M NaCl solutions (data not shown).

181 It can be assumed from this results that the only changes in the titanium surface with the
182 variations in pH and ionic strength are related to its electrostatic charge due to the differential ions
183 adsorption and hydration, allowing the interpretation of bacterial adhesion results in terms of
184 interfacial electrostatic and van der Waals interactions only.

185 For the analysis of the bacterial adhesion results the interfacial features of interacting
186 surfaces are needed. A theoretical analysis is presented below for the estimation of electrostatic net
187 charge on titanium in the different experimental conditions, and the electrostatic charge of bacterial
188 cells is estimated from electrokinetic measurements.

189

190 **4. Acidic and basic properties of hydroxylated metal surfaces.**

191 Metal and metalloid oxides are know to sustain hydroxylation in the presence of adsorbed
192 water molecules forming two types of co-existing hydroxyl groups which differ in their
193 coordination with Me^{n+} ions: the basic-type (single coordinated), and the acid-type (double
194 coordinated) [5]. Basic sites accept a proton as a base and acid sites donates a proton as an acid
195 yielding positively and negatively charged sites, respectively. It follows that the net charge of the
196 hydroxylated surface will change with the pH of the electrolyte due to changes in the ionization of
197 these groups. There is a pH at which the number positively charged (basic) hydroxyls equals the
198 number of negatively charged (acidic) ones in the absence of other adsorbed ions [30], this is called
199 the point of zero charge (*pzc*) of the oxide. For a typical hydroxylated surface, the charge of the
200 oxide changes from positive at $pH < pzc$, to negative at higher values.

201 The pH of the *pzc* depends on the value of the proton affinity constant of the adsorbed ions,
202 which in turn depends on the acidity of the metal ion involved, and its coordination number with
203 oxygen.

204 Based on the protonation of the surface adsorbed ions cited above, the relation between
205 neutral and charged OH species on the TiO₂ surface was obtained from the expression of the proton
206 affinity constants, pK_{a1} and pK_{a2} , for the basic and acidic sites respectively [30]. Its dependence on
207 the pH of the solution is:

208

$$[1] \quad \{ \text{TiOH} \} = \frac{10^{-pK_{a1}}}{10^{-\text{pH}}} \{ \text{TiOH}_2^+ \} \quad \text{for the basic species}$$

and

$$[2] \quad \{ \text{TiOH} \} = \frac{10^{-\text{pH}}}{10^{-pK_{a2}}} \{ \text{TiO}^- \} \quad \text{for the acidic species}$$

209

210

211 where { } indicates concentrations of the species at the surface.

212 The charged site densities (N_{a1} and N_{a2}) can be obtained as:

213

$$[3] \quad N_{a1} = \{ \text{TiOH}_2^+ \} = N_s - \{ \text{TiOH} \}$$

and

$$[4] \quad N_{a2} = \{ \text{TiO}^- \} = N_s - \{ \text{TiOH} \}$$

214

215

216 where N_s is the number of surface sites of every type (either acidic or basic) per square nanometer.

217 Replacing from eqn. 1 and 2 and rearranging:

218

$$[5] \quad N_{a1} = \{ \text{TiOH}_2^+ \} = \frac{N_s}{1 + \frac{10^{-pK_{a1}}}{10^{-\text{pH}}}}$$

and

$$[6] \quad N_{a2} = \{ \text{TiO}^- \} = \frac{N_s}{1 + \frac{10^{-\text{pH}}}{10^{-pK_{a2}}}}$$

219

220

221

222

223

224

225

226

227

228

229

230

231

232

Calculations were made using equations 5 and 6 to determine the number of charged sites and the overall charge condition of the surface at every pH value, taking $pK_{a1}= 4.4$, $pK_{a2}= 7.5$ [18], $N_s = 8$ sites nm^{-2} (estimated for rutile on the basis of crystallographic data [22]) and a 1:1 ratio of acidic to basic sites on the TiO_2 surfaces [5]. Results are shown in Fig. 7a and b. The *pzc* is at pH 6.0, where $N_{a1} = N_{a2}$ as described above. This value agrees with previous experimental determinations [2]. It is not possible from the results of this simple model to obtain the magnitude of the charge at every pH. However, a positive charge value at pH values below 6 and a negative one at pH values above 6 can be predicted (Fig. 7a). The fraction of charged sites changes from 0.5 to almost 1 at pH 6 indicating that at this pH all the surface sites have a positive or negative charge (Fig. 7b).

5. Electrophoretic mobility and bacterial cells charge.

233

234

235

236

237

The zeta potential (ζ) of bacterial cells was determined by electrophoretic migration in 0.01 M NaCl at a final cell number of 10^5 cells ml^{-1} . A Rank Bros. Ltd. Mark II Particle Microelectrophoresis Apparatus (Bottisham- Cambridge, England.) was used, and the applied potential was 80 V. The value of ζ was calculated using Smoluchowski's equation as $\zeta = 12.87 \mu$, where μ is the measured electrophoretic migration.

238

239

The ζ values for bacterial cells at the different pH values are listed in Table 4. The isoelectric point occurred at pH = 2, with the bacteria bearing a negative charge above this value.

240

241

6. Bacterial adhesion results.

242

Metal samples were exposed during different time periods to bacterial suspensions in order to determine the adhesion of cells to the various kind of surfaces.

244

The results for Cu, Zn, and their alloys as well as for Al Brass and titanium at different pH and salinity are shown in Figs. 8 to 10.

246

As can be observed in Fig. 8 there was almost no bacterial adhesion on bare metal samples, on which the number of adhered cells was negligible even after 120 min of exposition. Bare zinc was not included in these tests since, as discussed above, it was not possible to obtain a bare surface in contact with the electrolyte due to its high susceptibility to oxidation.

250

The number of bacteria adhering to aged metals on the other hand, was increasing at different rates with the time of exposition. Results were expressed as percent of adhesion taking the number of bacteria adhered to zinc at 120 min (325 adhered bacteria / 100 μm^2) as 100%. Adhesion was found to increase faster following the sequence zinc > Al-brass > copper (Fig. 8). Final adhesion values of 16.3% and 77.5% were obtained for copper and aluminum brass, respectively.

255

On the PE reference surface the adhesion was negligible throughout the experiments (Fig. 8).

257

Variations in the cell density (adhered bacteria per unit area) of irreversible adhered bacteria found on aluminum brass samples at the various pH values tested can be observed in Fig. 9. When experiments were done at a constant ionic strength of 0.1 M NaCl, bacterial adhesion was clearly higher at pH values of 6 and 8, as compared to that at pH 2 and 4, despite the marked dispersion of results through the various repetitions (Fig. 9). When determinations were done at a higher ionic strength (0.6 M), the adhesion of bacteria was strongly reduced to negligible levels throughout the pH interval tested (Fig. 9).

264

Bacterial adhesion to aged titanium surfaces was minimal at pH 2 and increased with pH until pH 6. At pH 8, the number of adhered bacteria declined to a value close to that at pH 4.

265

266 At a higher ionic strength (0.6 M NaCl) the adhesion of bacteria was, as in the case for Al
267 brass, reduced to negligible levels throughout the range of pH tested (Fig. 10).

268

269

7. Discussion.

270 The DLVO theory of colloid stability summarizes the electrostatic and van der Waals
271 interactions between surfaces, yielding the overall interaction as a function of separation distance
272 (Norde and Lyklema, 1989). In close agreement with this theory, hydrophobicity and charge have
273 been identified as the overall non-specific parameters determining the fate of the adhesion process
274 (van Loosdrecht *et al.* 1987).

275 In addition to these non-specific interactions, specific interactions have been described
276 participating at short separation distance, including the formation of ionic, hydrogen, and chemical
277 bonds (Busscher and Weerkamp, 1987).

278 Adhesion of bacteria to the surface of different materials has been extensively studied
279 during last twenty-five years. The emerging conclusion from these studies, mainly those related to
280 polymeric surfaces, was the correlation originally found by Absolom *et al.* (1983) between the
281 interfacial surface tension of both, the material (γ_{sv}) and the bacterial surface (γ_{bv}), as related to the
282 interfacial surface tension of the surrounding medium (γ_{lv}). For hydrophilic bacteria ($\gamma_{lv} < \gamma_{bv}$) the
283 adhesion decreases as the material surface hydrophobicity increases. In the case of hydrophobic
284 bacteria ($\gamma_{lv} > \gamma_{bv}$) on the other hand, the adhesion increase with the increment of the surface
285 hydrophobicity. The measurement of the water contact angle by the sessile drop method showed
286 that bacterial cells used in our experiments work are hydrophilic (Table 1). As shown in Fig. 11, the
287 adhesion of this strain to various metal surfaces was observed to decrease with the diminution of
288 surface hydrophilicity in the order: aged Zn > aged Al-Brass > aged Cu > Al-Brass > Cu in
289 agreement with the previously cited statement.

290

291 **7.1.The influence of surface composition on bacterial adhesion**

292 Even when metal and metal oxides are typically hydrophilic (Kinloch, 1987) (Table 1),
293 there are differences between them, particularly in their relation with water, depending on the
294 surface chemistry. The interaction force between a metallic ion and other ion or molecule depends
295 on the ion co-ordination capacity (Cotton and Wilkinson, 1993). Differences observed in both, the
296 water contact angle, and the adhesion of bacteria to the various metal surfaces can be related to
297 variation in the interaction force due to changes in the oxide layer composition.

298 As can be observed in the reflectance spectrum of aged Cu (Fig. 1a), the oxide film on this
299 surface is mainly composed by Cu₂O. Aluminum brass on the other hand, shown (fig. 1b) the
300 presence of both, Cu₂O and ZnO. ZnO was the only component on the oxidized surface of metallic
301 Zn (Fig. 1c).

302 Whereas little differences exist between contact angle values on bare and oxidized copper
303 (Table 1), the presence of ZnO in aluminum brass introduce a strong variation on the contact angle
304 of the alloy after the ageing treatment (Table 1), which results in a value significantly lower than the
305 one for bare aluminum brass. On oxidized zinc, the measured contact angle was null, showing a
306 completely hydrophilic surface (Table1).

307 The adhesion of bacteria to the various surfaces showed changes similar to those observed
308 in contact angle values, and were also related to the presence of ZnO in the oxide layer. The
309 adhesion to bare copper and aluminum brass was very low, and increased on oxidized samples,
310 being the increment higher on aluminum brass. The highest adhesion was observed on zinc where
311 the film was only composed by ZnO (Fig.1c).

312 These results can be explained considering the reactivity of an oxidized surface on which
313 Mⁿ⁺ ions have an unsatisfied co-ordination capacity (Cotton and Wilkinson, 1993). Metallic ions
314 with a high charge-volume relation are acid cations which are able to accept electron “pairs” from,
315 and establish strong co-ordination with groups such as O²⁻, CO, NH₂, OH⁻ and COO⁻. Several of
316 these groups can be found in the outermost layer of bacteria (Jucker *et al.*, 1997). Among these

317 metallic ions, Zn^{2+} has the strongest acid force and exhibit a high co-ordination capacity with an
318 also high reaction rate. A ligand as water can easily get in and out from the co-ordination sphere of
319 Zn^{2+} , allowing an easy exchange with others ligands as those found on the bacterial outermost layer.
320 Cu^{1+} on the other hand, is a cation with a very low co-ordination capacity due to its low acidity.

321 Considering very short interaction distances, ligands exchange is connected to the
322 possibility to form specific bonds. Between them, the formation of hydrogen bonds as a
323 consequence of the acid-base properties of the metal surface is of main importance.

324 Materials surface hydrophobicity has been interpreted as a macroscopic manifestation of the
325 self association of water at the interface (Vogler, 1998), and has been shown to depend on the
326 density of acid–base Lewis sites on the surface, being in consequence closely related to the
327 formation of specific bonds. On hydrophilic surfaces, where the density of sites is high enough, the
328 self association of water is disrupted an water molecules interact with the surface (Vogler, 1998).
329 This is the case for metals and metal oxides. Ions in a metallic structure are acid sites capable to
330 form covalent bonds with water (Sato, 1998), whereas oxides are generally covered by hydroxyl
331 ions derived from the dissociation of adsorbed water (Bohem, 1971), and have an increased
332 capability to form hydrogen bonds.

333 As more acid-base sites are present on the surface, and as more acidic are these sites, the
334 interaction of the surface with water and other molecules is expected to increase yielding a more
335 hydrophilic surface on which hydrophilic bacteria should easily adhere.

336

337 **7.2. The influence of pH on bacterial adhesion**

338 Results shown in Table 3 and Fig. 6 are in good agreement with the presence of a TiO_2 film
339 on the titanium surface over the range of pH values evaluated [29]. Therefore, it can be assumed
340 that the physicochemical properties of the titanium surface were always defined by the properties of
341 the TiO_2 layer.

342 Results in Figs. 7 and 10, indicate that the maximum number of adhered bacteria was in

343 agreement with the *pzc* of the titanium oxide at pH 6. As the overall charge of the oxide at this pH is
344 equal to zero neither attractive nor repulsive electrostatic forces existed in this situation. Bacterial
345 adhesion should then be the result of van der Waals attractive forces.

346 The overall charge of the oxide surface changed from positive at $\text{pH} < 6$ to negative at
347 higher pH values due to the variations in the number of charged sites (Fig. 7a). Consequently,
348 attractive and repulsive forces are expected to act on negatively charged bacteria (Table 4) at pH 4
349 and pH 8, respectively. This should lead to differences in bacterial adhesion in these extreme
350 situations, with an increased adhesion at pH 4 and a reduced one at pH 8. In contrast, similar
351 degrees of adhesion were observed upon departure from the *pzc* of the oxide in either direction (Fig.
352 10) indicating that a variable other than electrostatic interactions was involved.

353 A hydrogen bond has partially covalent – partially ionic character and its energy increases
354 when the ionic contribution to the bond increases [15]. Depending on the pH of the solution,
355 hydroxyl ions on the oxide surface can be in its neutral state or have a negative or positive net
356 charge (equations 1 and 2; Fig. 7). Since the strength of the H-bond is related to the electrostatic
357 force of the intervening ion [3], the presence of a net charge on the surface-active groups gives an
358 strong ionic character to its H-bonding with other molecules. This may affect the energy of the
359 bonds, reinforcing the interaction with water or other H-bond forming molecule.

360 The formation of hydrogen bonds between adsorbed OH groups, water molecules, and
361 lipopolysaccharides (LPS) of bacteria was proposed by Jucker et.al. (1997) as a way of adhesion of
362 bacteria to oxide surfaces. A two-step adsorption mechanism was described, involving the
363 formation of H-bonds between LPS and the hydration water molecules on the surface in the first
364 step, and the displacement of water with the formation of direct H-bonds between adsorbed OH and
365 the LPS in the second step.

366 Considering the establishment of reinforced H-bonds between charged OH groups on
367 titanium surface and the LPS outermost ends, and taking into account the variation of the total
368 number of this sites ($Na_1 + Na_2$), it can be observed that at the *pzc* of the oxide, the surface is

369 exposing the maximal number of reinforced bonding sites to interact with bacterial LPS (Fig. 7b).
370 This can explain the high number of irreversible adhered cells at this point (Fig. 10).

371 At either higher or lower pH values, the number of reinforced bonding sites is reduced due
372 to the neutralisation of Na_1 or Na_2 sites respectively (Fig.7a). Both at, pH 4 and 8 the total number
373 of sites is reduced (Fig. 7b) in agreement with a proportional decrease in bacterial adhesion at the
374 same pHs (Fig. 10).

375 It is assumed in this interpretation of the adhesion results that the possibility to form a bond
376 with the bacterial LPS is the same for acidic than for the basic sites on the oxide.

377 As shown above bacterial adhesion results can be better explained if the number of acid-
378 base binding sites on the metal surface are considered.

379 This information can now be extrapolated to the situation on a surface with changing
380 composition.

381 As evidenced by reflectance spectroscopy results in Figs. 2-5, differences in the solubility
382 of oxides and hydroxides included in the surface layer of aluminum brass lead to changes in the
383 layer composition when parameters like pH and ionic strength are modified.

384 A schematic representation of the surface layer composition of aluminum brass constructed
385 taking into account the potential-pH diagrams for copper in seawater (Bianchi and Longhi, 1973),
386 and for zinc in pure water (Pourvaix, 1965) is shown in Fig. 12. The pH domain of every soluble or
387 solid compound whose presence is possible considering thermodynamic information is indicated.
388 Since potential domains cannot be extrapolated from the data from pure metals, they were not
389 included in the figure.

390 The reflectance results obtained for Al brass at every pH and ionic strength value will be
391 contrasted to thermodynamic information in Fig. 12 in order to explain the bacterial adhesion
392 results.

393 Data in Fig. 2 showed the absence of an oxide layer after the ageing of samples in both, 0.1
394 M and 0.6 M NaCl solutions (pH 2). This is in agreement with the thermodynamic information in

395 Fig. 12, where the presence of Cu^{2+} and Zn^{2+} ions is predicted together with the formation of CuCl .
396 Although a bare metal surface has Lewis acid–base sites on its surface (Sato, 1998), bacterial
397 adhesion at this pH was very low (Fig.9). The low adhesion can be related to both, the continuous
398 dissolution of the base metal at this pH, and the dependence of bacterial adhesion on the presence of
399 an oxide layer.

400 At pH 4, weak absorption peaks at wavelengths related to the presence of Cu_2O were
401 detected (Fig. 3) in contrast to predictions in Fig. 12. To investigate the presence of these
402 compounds, reflectance results were analyzed considering the influence of surface dealloying.
403 Taking into account that optical properties of electro–deposited Cu films thicker than $\sim 1 \mu\text{m}$ are
404 those corresponding to bulk copper (Pyun and Park, 1986) and assuming that surface dealloying
405 was deep enough, the absorption spectrum in Fig. 3 was considered to correspond to the oxides
406 absorption plus the differential absorption between base metals (Hummel 1983). After subtraction
407 of the latter the presence of Cu (I) compounds, presumably hydrated Cu_2O , was confirmed. Cu (I)
408 solid compounds has also been observed by others on Cu electrodes oxidized at pH 3 using XPS
409 and Auger spectroscopies (Feng *et al.*, 1997).

410 Bacterial adhesion was relatively low at pH 4, but a wide dispersion of results was observed
411 from one experiment to the other (Fig. 9). This can be due to the formation of a variable amount of
412 oxide irregularly distributed as a consequence of local changes in pH and potential during the
413 corrosion process. The presence of the oxide determines the increment of the H–bond forming sites
414 density on the surface and consequently the increased adhesion of bacteria as compared to the
415 situation at pH 2 (Fig. 9).

416 Similarly, the formation of a uniform layer composed by oxides and (hydro)oxides (Figs. 4
417 and 5) which strongly increment the number of H–bond forming sites on the surface can explain the
418 incremented bacterial adhesion observed at pH values higher than 6 (Fig. 9) at low ionic strength
419 (0.1 M). Besides the presence of Cu (I) and Cu(II) compounds, the formation of zinc oxide was

420 observed (Fig. 4), which as explained above determines an increment in interaction capacity of the
421 oxide layer with water and bacteria.

422 The explanation based on the increased interaction by specific bonds can be reinforced
423 considering the results of the water contact angle measurements (Table 2).

424 The decrease in the water contact angle measured under *n*-hexane with the raising of pH
425 (Table 2) indicates an increment of the acid–base interaction capability of the aluminum brass
426 surface. This increased interaction can account for both, the oxides hydration denoted by the
427 presence of (hydro)oxides (Figs. 4 and 5) and the increment of bacterial adhesion (Fig. 9).

428

429 **7.3. The Influence of ionic strength on adhesion**

430 The presence of surface charge on solids in electrolyte solutions leads to the formation of an
431 electric double layer. In interfacial interactions, this double layer is the closest limit of approach due
432 to electrostatic repulsion for two surfaces charged alike [30]. The position of this limit, i.e., the
433 thickness of the double layer ($1/\kappa$), is dependent of the ionic strength according to [4]:

434

435 [7]
$$\kappa = \left(\frac{e^2 n^\infty z^2}{\epsilon k T} \right)^{1/2}$$

436

437 where κ is the Debye-Hückel parameter, e the elementary electrical charge, ϵ the dielectric constant,
438 and z and n^∞ the electrolyte solution valence and concentration, respectively. The influence of the
439 double layer thickness on bacterial adhesion was proposed by Marshall et al. (1971) in accordance
440 with the predictions of the DLVO theory. As shown by these authors, incremental changes in ionic
441 strength result in a reduction of the double layer thickness, allowing bacteria to approach closer to
442 the substratum and facilitating interaction and subsequent irreversible adhesion. These results were
443 confirmed by several authors [32, 7, 20]. Nevertheless, as much of the work done to date involved

444 ionic strength at or below 0.1 M, it is not clear if these concepts are applicable at higher ionic
445 strength, such as that of seawater.

446 Bacterial adhesion to TiO₂ as well as that to Al Brass were strongly reduced, instead of
447 increased, in NaCl 0.6 M as compared with NaCl 0.1 M (Figs. 9 and 10). This was in agreement
448 with the results reported by Gordon et al. (1984) that show an inflection point at 0.1 M, with
449 bacterial adherence decreasing exponentially with an increase in the ionic strength above this point.

450 Calculations using eq. 7 show the strong decrease in the thickness of the double layer with
451 an increase in the ionic strength up to 0.1 M NaCl (Fig. 13). Additional compression of the double
452 layer above this limit was in the order of 0.5 nm, which, taking into account the bacterial
453 dimensions, seems of minor importance for bacterial adsorption. For this reason, and as pointed out
454 by Gordon et al. (1984), changes in bacterial adherence at ionic strengths higher than 0.1 M can not
455 be explained by a double layer effect and must be a consequence of some other mechanism.

456 As the result of the high ionic strength (0.6 M), the specific adsorption of Cl⁻ and Na⁺ ions
457 is expected to occur on both interacting surfaces [4]. Adsorbing cations may adsorb at the same site
458 as protons, or on independent sites directly on the surface [22]. Anions as chloride on the other hand
459 can replace surface OH ions in oxide films, and its concentration in the film is governed by a
460 chemical equilibrium [25].

461 Due to the low polarising effect of Cu⁺ ions (Cotton and Wilkinson, 1993), hydroxyl ions
462 on the oxide layer of Al Brass could be replaced by Cl⁻ as the NaCl concentration increases (Stumm
463 and Morgan, 1981; Marcus and Herbelin, 1993). This replacement strongly decreases the surface
464 capacity to form hydrogen bonds and shifts the isoelectric point of the oxide to lower pH values
465 (Stumm and Morgan, 1981), imposing a negative charge to the surface. It needs to be stressed based
466 on this considerations, that the low number of binding sites on the surface, together with the
467 electrostatic repulsion against negatively charged bacteria can account for the suppression of
468 bacterial adhesion observed at high ionic strength (Fig. 9).

469 Ti^{4+} ions on the other hand, induce a hard polarization on adsorbed OH enabling the
470 formation of H-bonds with water and other molecules, and reducing the possibility to replace these
471 ions by Cl⁻.

472 Even when the reduction in bacterial adhesion shown in Fig. 10 at high ionic strength can
473 be due to the reduction in the number of binding sites by OH replacement to some extent, some
474 additional elements are needed to fully explain these results. A possibility that consider hydration
475 repulsive forces has been presented previously (Busalmen and de Sánchez, 2001), although will not
476 be discussed here.

477

478 **7.4. The importance of surface chemistry in microbial corrosion**

479 The relevance of results presented above should be analyzed taking into account that the
480 composition of the surface oxide layer and its acid-base properties may influence not only the initial
481 bacterial adhesion to the metal surface, but also the bacteria–metal interactions during the
482 development of biofilms.

483 The surface of some metals as those used in this work become unstable due to the
484 localization of corrosion reactions as a consequence of bacterial colonization.

485 Considering changes in pH due to the spatial separation of anodic and cathodic reactions
486 during corrosion on a patchily covered surface, it can be proposed that the biofilm tri-dimensional
487 structure could be in some way influenced by the differential composition of the surface oxide layer
488 in cathodic and anodic areas. The increase in pH due to oxygen reduction at cathodic areas
489 surrounding pioneering bacterial micro–colonies for example, could increase the interaction of
490 daughter cells with the surface favoring the colony spreading and the biofilm formation.

491 Although not determined experimentally, a complex balance between bacteria-surface
492 physicochemical interactions and metabolic requirements of cells will probably determine the final
493 3D structure of bacterial clusters, channels and voids during the growth of biofilms on a corroding
494 metal surface.

495 In addition, surface chemistry also influence the way in which electrochemical corrosion
496 reactions take place. It is commonly accepted that oxygen reduction can proceed through a parallel
497 mechanism involving the direct reduction via four electrons and a pathway where intermediate
498 peroxide is formed. In this last case, oxygen electroreduction proceeds by the transference of two
499 electrons, with the production of H₂O₂ as an intermediate compound, and then to hydroxyl ions by
500 the transference of two more electrons [18]. On copper, Vázquez *et al.* [18] demonstrated that the
501 second two electrons are readily transferred ($k_{III} \gg k_{IV}$ in Fig. 1), yielding low levels of H₂O₂ in
502 solution. However, the same authors showed that, depending on the composition of the surface
503 oxides, the electroreduction of peroxide can be inhibited, favouring its desorption to the solution
504 ($k_{III} \ll k_{IV}$) [18]. The coupling of electrochemical hydrogen peroxide production with the activity
505 of microbial catalase has been proposed by Busalmen et al (2002) as an explanation to the catalysis
506 of corrosion reactions by microorganisms in aerobic biofilms.

507 The catalase mechanism is based on the enzymatic conversion of electrochemically
508 produced H₂O₂ to water and oxygen in the proximity of the surface. The oxygen generated by the
509 enzyme can be electrochemically reduced in addition to the oxygen reaching the surface by
510 diffusion from the bulk, resulting in higher cathodic limiting currents that can be attributed to the
511 increase in the oxygen concentration [13].

512 Since the peroxide production depends on the surface chemistry, the extent to which the
513 enzymatic mechanism can influence corrosion does so.

514

515

References

- 516 1. **Absolom, D.R., Lamberti, F.V., Policova, Z., Zingg, W., van Oss, C.J. and Neumann W.**
517 1983. Surface Thermodynamics of Bacterial Adhesion. Applied Environmental. Microbiology
518 **46(1):90-97.**
- 519 2. **Bérubé, Y.G. and P.L. de Bruyn.** 1968. Adsorption at the rutile-solution interface I:
520 thermodynamic and experimental study. J.Colloids Interf. Sci. **27:305-318.**

- 521 3. **Bianchi, G. and P. Longhi.** 1973. Copper in sea-water, potential-pH diagrams. *Corrosion*
522 *Science* **13**:853-864.
- 523 4. **Bockris, J.O'M.** 1990. The double layer. In: *Comprehensive treatise of electrochemistry.*
524 (Bockris, J.O'M, Conway, B.E. and Yeager, E. ed), vol. I. pp 203. Plenum Press, NY.
- 525 5. **Boehm, H.P.** 1971. Acidic and basic properties of metals oxides and hydroxides. *Disc. Faraday*
526 *Soc.* **52**:264-275.
- 527 6. **Busalmen J.P., Frontini M.A. and S.R. de Sánchez.** Proc. 9th Int. Congress on Marine
528 Corrosion and Fouling, Portsmouth, UK, 17-21 Jul. 1995. In: S.A. Campbell, N. Campbell and
529 F.C. Walsh (Ed.), *Developments in marine corrosion*, Royal Society of Chemistry, (1998) 19.
- 530 7. **Busalmen, J.P., and S.R. de Sánchez.** 2001. Influence of pH and ionic strength on the
531 adhesion of a wild strain of *Pseudomonas sp.* to titanium. *Journal of Industrial Microbiology*
532 *and Biotechnology* **26**:303-308.
- 533 8. **Busscher, H.J. and A.H. Weerkamp.** 1987. Specific and non-specific interactions in bacterial
534 adhesion to solid substrata. *FEMS Microbiol. Rev.* **46**:165-173.
- 535 9. **Cotton F.A., and Wilkinson G.,**1993. *Química Inorgánica Avanzada.* Ed. Limusa, México, DF.
- 536 10. **Feng, Y., K.S. Siow, W.K. Teo, K.L. Tan and A.K. Hsieh.** 1997. Corrosion mechanisms and
537 products of copper in aqueous solutions at various pH values. *Corrosion* **53**:389-398.
- 538 11. **Frymier, P. D., R.S. Ford, H. C. Berg, and P.T. Cummings.** 1995. Three-dimensional tracking
539 of motile bacteria near a solid planar surface. *Proc. Natl. Acad. Sci. USA.* **92**: 6195-6199.
- 540 12. **Gristina, A.G.** 1987. Biomaterial-centered infection: microbial adhesion versus tissue
541 integration. *Science* **237**:1588-1595.
- 542 13. **Gordon A.S., and F.J. Millero.** 1984. Electrolyte effects on attachment of an estuarine
543 bacterium. *Appl. Environ. Microbiol.* **47**:495-499.
- 544 14. **Gotowski, W.** 1991. Thermodynamics of adhesion. In: *Fundamentals of adhesion.* (L.-Huang
545 Lee, ed) Chap. 2, pp. 87-135. Plenum Press, NY.
- 546 15. **Hummel, R.E.** 1983. Differential Reflectometry and Its Application to the Study of Alloys.
547 *Physics State Solid (a)* **76**(11): 12-43.

- 548 16. **Isrealachvili, J.N.** 1992. Adhesion forces between surfaces in liquids and condensable vapors.
549 *Surf. Sci. Rep.* **14**:109-160.
- 550 17. **Jucker, B.A., H. Harms and A.J.B. Zehnder.** 1996. Adhesion of the positively charged
551 bacterium *Stenotrophomonas (Xanthomonas) maltophilia* 70401 to glass and teflon. *J.*
552 *Bacteriol.* **178**:5472-5479.
- 553 18. **Jucker, B.A., Harms, H., Hug, S.J. and Zehnder, A.J.B.** 1997. Adsorption of Bacterial
554 Surface Polysaccharides on Mineral Oxides is Mediated by Hydrogen Bonds. *Colloid and*
555 *Surfaces B: Biointerfaces* **9**:331-343.
- 556 19. **Kim, B.S., Piao, S.N., Hoier, S.N. and Park, S.M.** 1995. In Situ Spectro-Electrochemical
557 Studies on the Oxidation Mechanism of Brass. *Corrosion Science* **37**(4):557-570.
- 558 20. **Kinloch, A.J.** 1987. *Adhesion and Adhesives.* Ed. Chapman and Hall. London. U.K.
- 559 21. **Koopal, L.K.** 1996. Mineral hydroxides: from homogeneous to heterogeneous modelling.
560 *Electrochim. Acta* **41**:2293-2306.
- 561 22. **Lever, A.B.P.** 1984. *Inorganic electronic spectroscopy*, vol. 33 *Physical and Chemical*
562 *Theoretical Studies.* Sec. 5.12, pp. 320-323. Elsevier, NY.
- 563 23. **Loeb, G.I.** 1985. The properties of non-biological surfaces and their characterization. In:
564 "Bacterial adhesion: mechanisms and physiological significance". Chap. 5, pp. 111-127. D.C.
565 Savage and M. Fletcher (Eds.). Plenum press, NY, USA.
- 566 24. **Marcus, P. And, J.M. Herbelin.** 1993. The entry of chloride ions into passive films on nickel
567 studied by spectroscopic (ESCA) and nuclear (³⁶Cl radiotracer) methods. *Corr. Sci.* **34**:1123-
568 1145.
- 569 25. **Marshall K.C., R. Stout and R. Mitchell.** 1971. Mechanism of the initial events in the
570 sorption of marine bacteria to surfaces. *J. Gen. Microbiol.* **68**: 337-348.
- 571 26. **Marshall, K.C.** 1994. Microbial adhesion in biotechnological processes. *Curr. Opinion*
572 *Biotechnol.* **5**:296-301.
- 573 27. **Miller, M.J. and D.G. Ahearn.** 1987. Adherence of *Pseudomonas aeruginosa* to hydrophilic

- 574 contact lenses and other substrata. *J. Clin. Microbiol.* **25**:1392-1397.
- 575 28. **Norde W. and Lyklema J.** 1989. Protein Adsorption and Bacterial Adhesion to Solid Surfaces:
576 A Colloid-Chemical Approach. *Colloid and Surfaces.* **38**:1-13.
- 577 29. **Pourbaix, M.** 1965. Atlas of electrochemical equilibria in aqueous solutions. Sec. 8.1, pp: 213-
578 222. Pergamon Press, Oxford, UK.
- 579 30. **Pyun Ch-H and S. Park.** 1986. In situ spectroelectrochemical studies on anodic oxidation of
580 copper in alkaline solution. *Journal of the Electrochemical Society* **133**:2024-2030.
- 581 31. **Sánchez, S.R. de, Berlouis E.A. and Schiffrin D.J.** 1991. Difference Reflectance
582 Spectroscopy of Anodic Films on Copper Base Alloys. *Journal of Electroanalytical Chemistry*
583 **307**: 73-86.
- 584 32. **Sánchez, S.R. de, and D.J. Schiffrin.** 1985. The effect of pollutants and bacterial microfouling
585 on the corrosion of copper base alloys in seawater. *Corrosion* **41**:31-38.+
- 586 33. **Sato, N.** 1998. Electrochemistry at metal and semiconductor electrodes. Chap. 5, pp. 119.
587 Elsevier Sci. Amsterdam, The Netherlands.
- 588 34. **Schultz, J., K. Tsutsumi and J.B. Donnet.** 1977. Surface properties of high energy solids II:
589 determination of the non-dispersive component of the surface free energy of mica and its
590 energy of adhesion to polar liquids. *Journal of Colloid and Interface Science* **59**:277-282.
- 591 35. **Shabtai, Y. and G. Fleminger.** 1994. Adsorption of *Rhodococcus Strain* GIN-1 (NCIMB
592 40340) on titanium dioxide and coal fly ash particles. *Appl. Environ. Microbiol.* **60**:3079-3088.
- 593 36. **Stumm, W. and J.J. Morgan.** 1981. The solid-solution interface. In: *Aquatic chemistry.* (J.Wiley
594 & Sons, ed), pp. 599-682. NY, USA.
- 595 37. **Valcarce, M.B; J.P. Busalmen; M.A. Frontini and S.R. de Sánchez.** 1998. Comparative
596 evaluation of bacterial adherence to surfaces. Third Latin American biodegradation and
597 biodeterioration symposium, Florianopolis, Brazil.
- 598 38. **Van Loosdrecht, M.C.M., J. Lyklema, W.Norde, G. Schraa and A. J.B. Zehnder.** 1987 a.
599 Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of

- 600 bacterial adhesion. Appl. Environ. Microbiol. 53, 1898-1901.
- 601 39. van Loosdrecht, M.C.M., Lyklema, J., Norde, W., Schraa, G. and Zehnder, A.J.B. 1987
- 602 b. The Role of Bacterial Cell Wall hydrophobicity in Adhesion. Applied Environmental
- 603 Microbiology **53**(8):1893-1897.
- 604 40. Vázquez M.V., S.R. de Sánchez, Calvo E.J., and Schiffrin D.J. 1994. J. Electroanal. Chem.
- 605 **374**: 189.
- 606 41. Vogler, E.A. 1998. Structure and Reactivity of Water at Biomaterial Surfaces. Advances in
- 607 Colloid and Interface Science. **74**:69-47.
- 608 42. Wang, I., J.M. Anderson, M. R. Jacobs and R.E. Marchant. 1995. adhesion of
- 609 *Staphylococcus epidermidis* to biomedical polymers: contributions of surface thermodynamics
- 610 and hemodynamic shear conditions. J. Biomed. Mater. Res. **29**:485-493.

611

612

Captions to figures

613 **Table 1:** Water contact angle (θ_w) of the different surfaces under study. Results are indicated as

614 mean value \pm standard deviation (n=3).

615 **Table 2:** Water contact angle (θ_w) measured under *n*-hexane on aluminium brass surfaces aged

616 during 24 h in 0.1 M NaCl solutions at various pH values. Values are shown as mean \pm standard

617 deviation of at least three determinations.

618 **Table 3:** Open circuit potentials (OCP) measured for titanium electrodes after 24 h in NaCl

619 solutions of various pH and ionic strength. NHE: normal hydrogen electrode. Values are indicated

620 as mean \pm standard deviation.

621 **Table 4:** Zeta potential (ζ) of a wild strain of *Pseudomonas sp.* in 0.01 M NaCl at various pH

622 values. Values are indicated as mean \pm standard deviation.

623 **Figure 1:** Differential absorbance spectra of a) Cu, b) Al-brass, and c) Zn, after 24 h of immersion

624 in ATW at the corrosion potential.

625 **Figure 2:** Differential absorbance spectra of the aluminium brass surface aged during 24 h in (○)
626 0.1 M and (●) 0.6 M NaCl, pH 2. Absorbance is given in arbitrary unit.

627 **Figure 3:** Differential absorbance spectra of the aluminium brass surface aged during 24 h in (○)
628 0.1 M and (●) 0.6 M NaCl, pH 4. Absorbance is given in arbitrary unit.

629 **Figure 4:** Differential absorbance spectra of the aluminium brass surface aged during 24 h in (○)
630 0.1 M and (●) 0.6 M NaCl, pH 6. Absorbance is given in arbitrary unit.

631 **Figure 5:** Differential absorbance spectra of the aluminium brass surface aged during 24 h in (○)
632 0.1 M and (●) 0.6 M NaCl, pH 8. Absorbance is given in arbitrary unit.

633 **Figure 6:** Differential absorbance spectra of the titanium surface aged during 24 h in 0.1 M NaCl at
634 various pH values. (·····) pH 2; (-----) pH 4; (——) pH 6 and (---) pH 8.

635 **Figure 7:** a) Estimated variation of charged site density (N_s) for (●) basic ($\text{Na}_1, \equiv\text{TiOH}^{2+}$) and, (○)
636 acidic ($\text{Na}_2, \equiv\text{TiO}^-$) OH groups on TiO_2 with the pH of the solution. b) Fraction of charged sites on
637 the TiO_2 surface. $N_t = 16$ sites nm^{-2} .

638 **Figure 8:** Bacterial adhesion (%) to various materials as a function of time. (◆) aged Zn, (■) aged
639 aluminium brass, (●) aged Cu, (○) bare Cu, (□) bare aluminium brass and (+) PE. 100% of adhesion
640 = 325 adhered bacteria / 100 μm^2 . Results are indicated as mean value \pm standard deviation (n=10).

641 **Figure 9:** Density of adhered bacteria on aluminium brass electrodes exposed during 1h to bacterial
642 suspensions in, (open symbols) 0.1 M, and (close symbols) 0.6 M NaCl solutions at various pH
643 values. Symbols correspond to three replicate experiments. Bars indicate SD of 10 different field on
644 each sample.

645 **Figure 10:** Number of adhered bacteria on titanium electrodes exposed during 1h to bacterial
646 suspensions in (open symbols) 0.1 M and (close symbols) 0.6 M NaCl solutions, at various pH
647 values. Symbols correspond to three replicate experiments. Bars indicate SD of 10 different field on
648 each sample.

649 **Figure 11:** Dependence of bacterial adhesion (%) on the water contact angle (θ_w) of the surface. 1)
650 aged Zn, 2) aged aluminium brass, 3) aged Cu, 4) bare Cu, 5) bare aluminium brass and 6) PE.

651 **Figure 12:** Schematic representation of the aluminium brass surface layer composition at various
652 pH values.

653 **Figure 13:** Double layer thickness as a function of electrolyte ionic strength calculated for NaCl
654 solutions using equation 7 (see text).

655