

Influence of bacteria on silver dissolution from silver-palladium surfaces

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Abstract

Silver-palladium surfaces are potentially used for bacterial and biofilm inhibition by generating micro-electric fields and electrochemical redox processes, and it is desired that the release of any metal ion will be at low concentration. However, in some specific environments, undesired silver dissolution can occur which can be recognized as a result of corrosion or deterioration of the silver-palladium surface, and will reduce the lifetime of the surface and contaminate the surroundings. The effect of bacteria, biofilm and solution contents on silver stability is therefore of interest, if silver-palladium surfaces are used in biologically active systems. In this study, a series of 24- and 72-hour immersion tests using several solutions was performed for the evaluation of silver dissolution and study of the mechanism. The quantitative data on silver dissolution and the correlation between silver dissolution, solution contents, and surface-associated bacteria were obtained. It was not surprising that chemicals aggressive to silver and silver compounds accelerated silver dissolution due to the formations of silver ions and silver complex ions, but a phenomenon of increased silver dissolution was found associated directly with the presence of bacteria. Experiments showed that surface-associated bacteria greatly increased silver dissolution from the silver-palladium surfaces due to the interactions between cell components and the surfaces, and the amount of surface-associated bacteria enhanced this effect.

Keywords: “silver dissolution”, “immersion test”, “silver-palladium surface”, “bacteria”

Introduction

Bacterial contamination can cause many adverse effects, such as deterioration of food products and human diseases. Bacteria in natural, industrial and clinical settings most often live in surface associated communities known as biofilm. Bacteria in biofilm are more tolerant to cleaning, disinfecting operations, and antibiotic therapies than their planktonic phases, making these treatments less effective or ineffective [1-8]. It is of high priority to develop effective and non-toxic methods for combating biofilm formations. Therefore, a biofilm preventing silver-palladium (Ag-Pd) surface has been developed, and the results of microstructure observations, electrochemical tests, and anti-biofilm activity have been published [9-12]. The Ag-Pd surface in focus in this paper can inhibit surface-associated bacteria and prevent biofilm formations by generating micro-electric fields itself and redox processes (electrochemical interactions) with bacteria [9-12]. In this way, it is desired that the release of any metal ion will be at low concentration. However, in our previous experiments, the phenomenon of undesired Ag dissolution from Ag-Pd surfaces has been found [10, 11]. Metal dissolution is a result of corrosion or deterioration of materials [13], and will reduce the lifetime of the material and contaminate the surroundings. It has recently been reported that metallic gold (Au) on medical implants can be dissolved by cells, which is called “dissolucytosis” [14, 15]. These studies demonstrated that whenever metallic Au surfaces are attacked by membrane-bound dissolucytosis, Au ions are dissolved by surrounding cells or

the effects of cell growth on metallic Au surfaces. These observations indicated that even indigestible noble metals can be attacked by the phenomenon of microbiologically influenced corrosion.

In this report we investigated the correlation between solution contents, surface-associated bacteria, and metal dissolution of Ag-Pd surfaces under a condition of high bacterial load. The objective was to decrease the risk of contamination to a surrounding environment due to metal dissolution. We performed immersion tests to obtain quantitative data, and the amounts of Ag dissolved from Ag-Pd surfaces at different test environment were compared. The mechanism of Ag dissolution behind the effect is also discussed based on thermodynamic considerations and experiments carried out in this study.

Experimental

Materials and bacteria

To obtain a test sample with a desired Ag-Pd surface, a Ag (99.9% Ag) plate was treated by immersion plating in palladium chloride solution for 3 minutes at ambient temperature. The palladium chloride solution was prepared from 5 vol. % of the stock solution which is prepared from 0.5 g^l⁻¹ PdCl₂ and 4 g^l⁻¹ NaCl dissolved in water. As-received stainless steel AISI 316 (approximately 68.7% Fe, 16.9% Cr, 10.16% Ni, and 2.02% Mo) was used as a control in this study. The size of the test sample used in this study was 4 mm × 7 mm × 0.5 mm.

In our previous studies [10-12], we employed Ag-resistant bacteria to study the biofilm inhibiting property of Ag-Pd surface. Therefore, Ag-resistant *E. coli* J53[pMG101] [16] and Ag-sensitive *Escherichia coli* (*E. coli*) J53 [17] were used as model organisms in his study. With this setup we can also investigate the correlation between bacterial metal resistance and metal dissolution. Cultivation of a cell suspension of *E. coli* was carried out at 37°C in ABTG solution (bacterial growth medium). The composition of ABTG is shown in Table 1.

Table 1. Composition of ABTG solution.

2 g^l⁻¹ (NH₄)₂SO₄

6 g^l⁻¹ Na₂HP O₄

3 g^l⁻¹ KH₂PO₄

3 g^l⁻¹ NaCl

1 × 10⁻³ M MgCl₂

1 × 10⁻⁴ M CaCl₂

1 × 10⁻⁵ M FeCl₃

6.25 g^l⁻¹ glucose

25 mg^l⁻¹ methionine

25 mg^l⁻¹ proline

2.5 mg^l⁻¹ thiamine

1000 ml H₂O

Immersion test and determination of dissolved Ag in solution

The purpose of immersion tests was to investigate the influence of different bacteria-containing ABTG solutions on the metal dissolution from Ag-Pd surface under a condition of high bacterial load. In cold tap water, the amount of naturally occurring bacteria is approximately 10^1 CFUml⁻¹ (colony forming units) [9, 11]. For immersion tests in bacteria-containing solutions, the initial bacterial concentration was approximately 10^6 CFUml⁻¹. Each test was three replicates. As shown in Table 1, the ABTG solution used in this study contains ammonium sulfate ((NH₄)₂SO₄). It is well-known that the ammonium ion (NH₄⁺) is aggressive to Ag and its compounds, e.g. AgCl [9]. Therefore, tests in bacteria-free ABTG solutions with different ammonium contents were performed to investigate the influence of NH₄⁺ content on Ag metal dissolution, and also to be used as a control. Table 2 shows test conditions used in this study. Each sample was placed into a dish with 5 ml solution at 37 °C with shaking at 60 rpm for 24 and 72 hours respectively. For each test condition, a total of three independent tests were conducted.

Table 2. Bacteria-containing and bacteria-free solutions used in the immersion tests.

| Solution | Model organism | Test duration |
|--|---|-----------------|
| ABTG* | - | 24 and 72 hours |
| ABTG* | Ag-sensitive <i>E. coli</i> J53 | 24 and 72 hours |
| ABTG* | Ag-resistant <i>E. coli</i> J53[pMG101] | 24 and 72 hours |
| ABTG (25% NH ₄ ⁺)** | - | 24 hours |
| ABTG (1% NH ₄ ⁺ ***) | - | 24 hours |

* The composition of the ABTG solutions used is shown in Table 1.

** The content of (NH₄)₂SO₄ in ABTG is reduced from 2 to 0.5 gl⁻¹.

*** The content of (NH₄)₂SO₄ in ABTG is reduced from 2 to 0.02 gl⁻¹.

After 24- and 72-hour immersion tests, the spent solutions were collected for the measurements of Ag concentrations to evaluate the levels of Ag dissolution from Ag-Pd surfaces under different test environments. The measurements of Pd concentrations were also conducted. The collected solutions were diluted, and then acidified (nitric acid) to make a clear solution for analyses. The concentration of total Ag in medium was analyzed by the use of a PerkinElmer SIMA 6000 graphite furnace atomic absorption spectrometry (GFAAS), and the concentration of total Pd in medium was analyzed by the use of a JOBIN YVON JY38S inductively coupled plasma optical emission spectrometry (ICP-OES). A total of three independent analyses were conducted for each solution, and the average concentration was calculated.

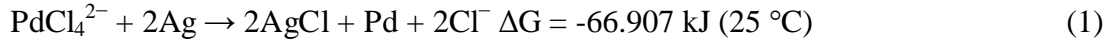
Bacterial activity associated with Ag-Pd surface

The purpose of this study was to use microscopic techniques to directly observe bacterial activity associated with Ag-Pd surface under a condition of high bacterial load, and furthermore study the mechanism of metal dissolution. After 24- and 72-hour immersion tests, the bacterial activity on the Ag-Pd surface were observed by the use of a Zeiss LSM 510 META confocal laser scanning microscope (CLSM), and staining with Molecular Probes LIVE/DEAD BacLight Bacterial Viability Assay, which utilizes the green fluorescent SYTO 9 for staining of cells, and the red fluorescent propidium iodide for staining of membrane-compromised cells. A 488-nm argon laser was used to excite the SYTO 9-stained cells, and a 543-nm helium/neon laser was used to excite the propidium iodide-stained cells. Simulated 3-D images were generated by the use of Bitplane IMARIS software.

Results and discussion

Silver stability

The microstructure of the Ag-Pd surface has been described previously [9-11]. Pd was incompletely deposited as a microhole-structured layer upon Ag. Ag was partially exposed through these microholes. In these microholes, some Ag can react to form silver chloride (AgCl) during Pd deposition. The calculated reaction of AgCl formation at room temperature during Pd deposition is as follows [19-21]:



The resulting surface can therefore release Ag ions or compounds like AgCl and theoretically also Pd, if the surface is degraded.

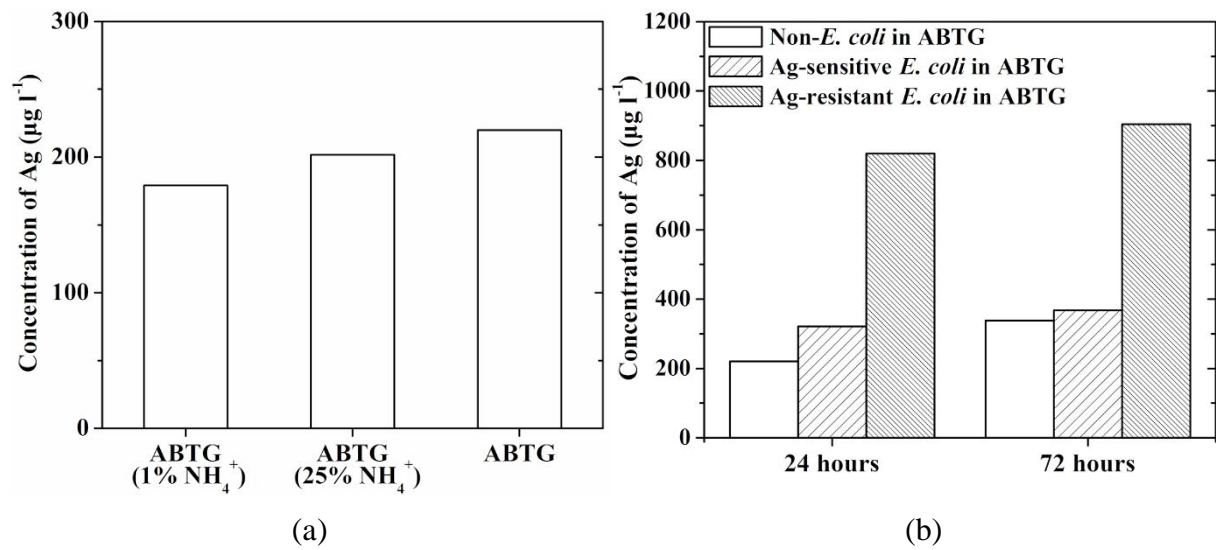
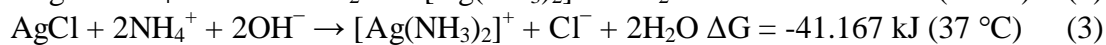
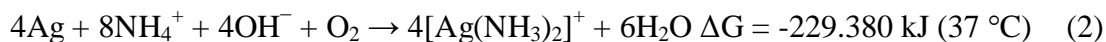


Fig. 1. Concentration of Ag in different immersion environments. (a) Effects of NH₄⁺ content in bacteria-free ABTG medium on Ag dissolution after 24-hour immersion, and (b) effects of bacteria in ABTG medium on Ag dissolution after 24- and 72-hour immersion.

Fig. 1 shows the concentration of Ag dissolved from Ag-Pd surfaces into solutions. The concentration of Pd dissolved from Ag-Pd surfaces was $\leq 2 \mu\text{g l}^{-1}$ for all tests, thus indicating no dissolution of this metal. In the following it is assumed that all measured Ag were present as dissolved ions in solutions, although the analyses measured Ag compounds in total and not only dissolved ions. In Fig. 1 (a), it is observed that the dissolved Ag concentration slightly increased with increasing NH₄⁺ content in medium. The data also show that ABTG solution with only a small amount of NH₄⁺ content has a strong tendency for Ag dissolution. When NH₄⁺ is present, the process of increased Ag dissolution can occur by Ag complex (diammine silver ion) formations. The calculated reactions are as follows [22, 23]:



Since the Gibbs free energy (ΔG) for these reactions is negative, these calculated reactions are thermodynamically favorable. The test media in itself therefore facilitates Ag dissolution.

In Fig. 1 (b), surprisingly high Ag concentrations were found in the tests of Ag-resistant *E. coli* compared to those from the tests of Ag-sensitive *E. coli* and *E. coli*-free solutions. It was also found that bacteria-containing solutions had a higher rate of Ag dissolution than that of bacteria-free ABTG solutions.

Biofilm prevention

Fig. 2 and Fig. 3 show bacterial activities associated with the test surfaces, which were LIVE/DEAD stained and visualized by the use of CLSM. Samples with 316 steel were included as controls along with the Ag-Pd surface. As shown in Fig. 2, Ag-sensitive and Ag-resistant bacteria both can form biofilm on the 316 surfaces. It can also be observed that the thickness and density of biofilm increased with increasing incubation time from 24 to 72 hours.

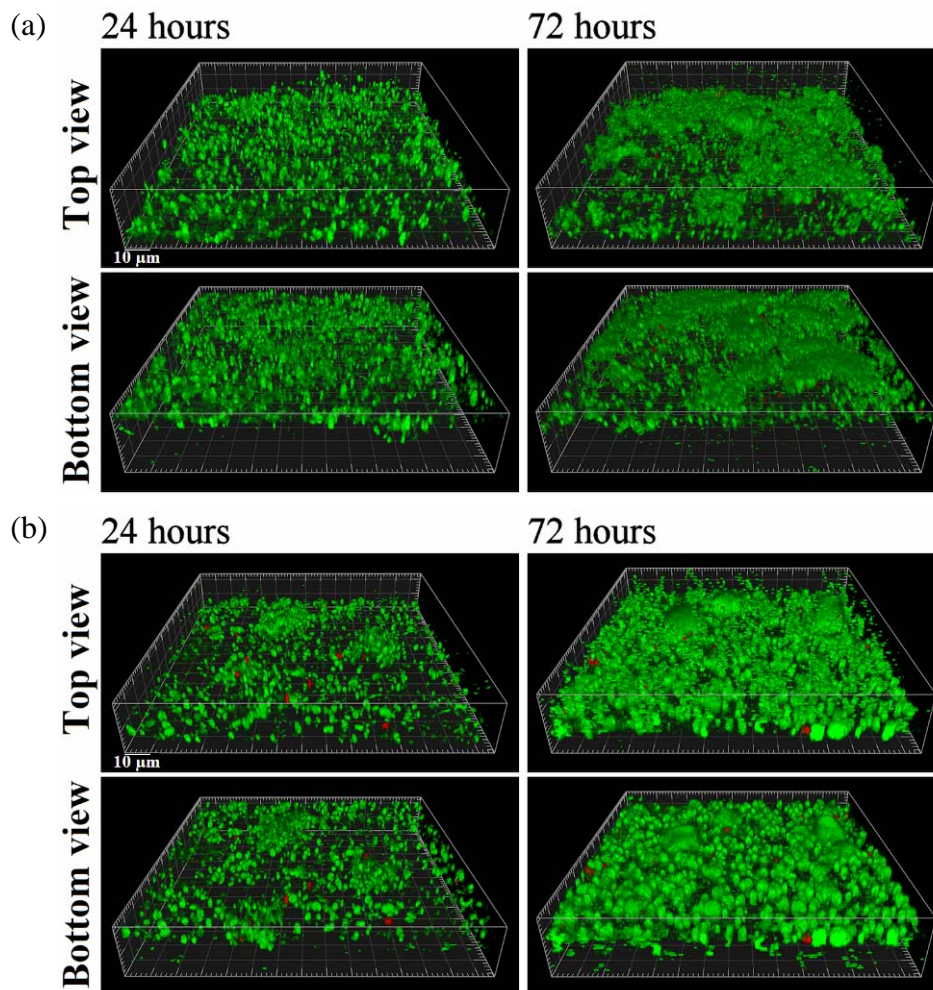


Fig. 2. CLSM micrographs of 24- and 72-hour-old, LIVE/DEAD-stained (a) Ag-sensitive *E. coli* J53, and (b) Ag-resistant *E. coli* J53[pMG101] biofilm on 316 surfaces. The upper panel (top view) shows the biofilm from the growth medium side, whereas the lower panel (bottom view) shows the biofilm from the surface side. The images are representative of three independent experiments. Green fluorescence indicates live cells and red fluorescence indicates dead cells.

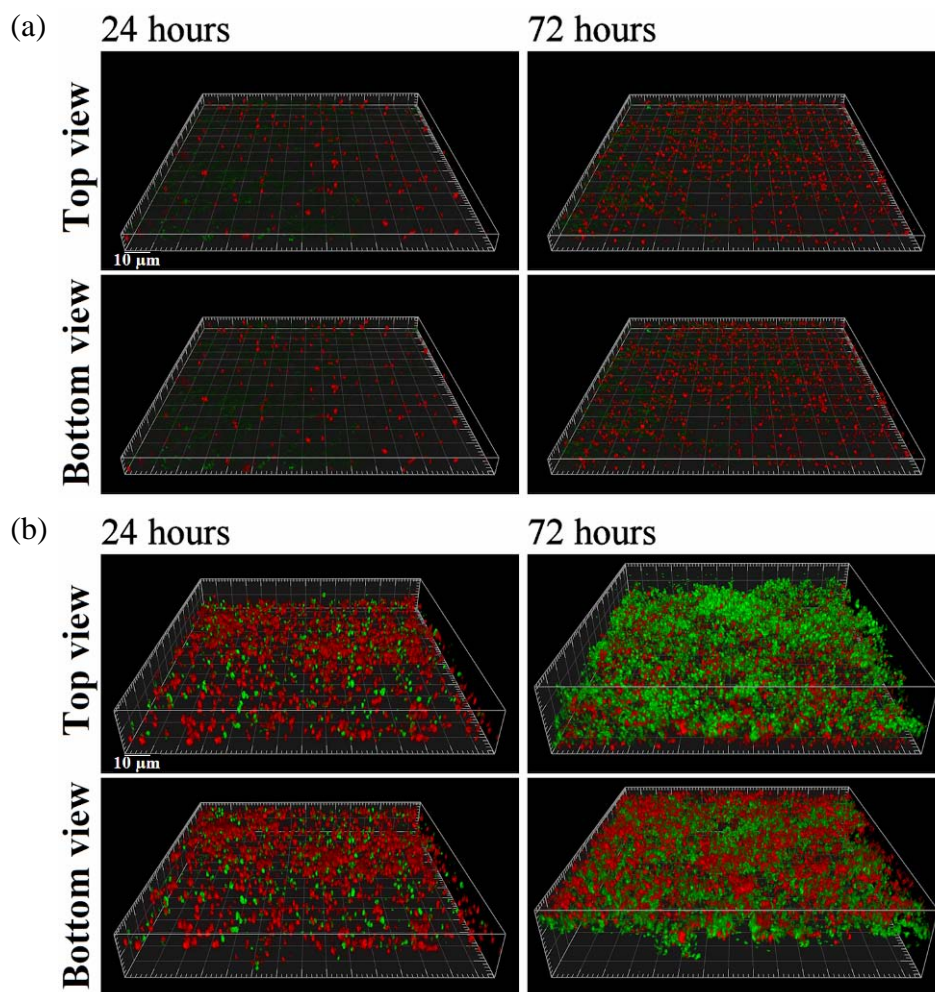


Fig. 3. CLSM micrographs of 24- and 72-hour-old, LIVE/DEAD-stained (a) Ag-sensitive *E. coli* J53, and (b) Ag-resistant *E. coli* J53[pMG101] biofilm on Ag-Pd surfaces. The upper panel (top view) shows the biofilm from the growth medium side, whereas the lower panel (bottom view) shows the biofilm from the surface side. The images are representative of three independent experiments. Green fluorescence indicates live cells and red fluorescence indicates dead cells.

As shown in Fig. 3 (a), unlike biofilm on the 316 surfaces (Fig. 2), in case of the Ag-sensitive bacteria, the Ag-Pd surface killed surface-associated bacteria and prevented biofilm formation, and more dead bacteria were found after 72-hour immersion. In case of the Ag-resistant bacteria (Fig. 3 (b)), the Ag-Pd was covered with a layer of surface-associated dead bacteria close to the surface after 24-hour immersion. When the surface was consistently exposed to solution with a high bacterial load, the Ag-resistant bacteria from their planktonic phase subsequently formed biofilm upon a conditioning layer of dead bacteria that had developed on the surface (Fig. 4). In agreement with our previous studies [10-12], it demonstrated that the Ag-resistant bacteria close to the Ag-Pd surface were killed due to the micro-electric fields/redox processes.

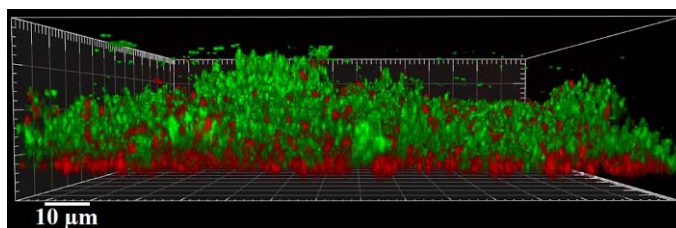


Fig. 4. CLSM micrograph of a side view of 72-hour-old, LIVE/DEAD-stained Ag-resistant *E. coli* J53[pMG101] biofilm on Ag-Pd surfaces. Biofilm formed upon a layer of surface-associated dead bacteria. The images are representative of three independent experiments. Green fluorescence indicates live cells and red fluorescence indicates dead cells.

Comparing Fig. 3 (a) and 3 (b), the apparent difference is that less dead Ag-sensitive bacteria were present close to the surface in the tests of 24-hour immersion, and no obvious biofilm formed in the tests of 72-hour immersion. Taken together with the analyses of Ag concentration as shown in Fig. 1, this can be explained by the ability of Ag-resistant bacteria to tolerate higher Ag concentration than Ag-sensitive bacteria [16, 17]. When chemicals aggressive to Ag-Pd surfaces were present in solution, an increased Ag dissolution occurred and dissolved toxic levels of Ag into the surrounding solution. This killed planktonic Ag-sensitive bacteria in solution in addition to the killing effects of the surface, where low numbers of Ag-sensitive bacteria could not initiate biofilm formation. Therefore less dead Ag-sensitive bacteria were present on the Ag-Pd surface.

Ag dissolution mechanism

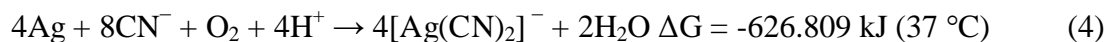
As shown in Fig. 1 (b) and Fig. 3, these observations suggested that surface-associated bacteria increased Ag dissolution from the surfaces, and the amount of surface-associated bacteria improved this effect. The tests with Ag-resistant bacteria showed more surface-associated dead bacteria and had an even higher Ag concentration than those of Ag-sensitive bacteria.

The influence of surface-associated bacteria on the increased rate of Ag dissolution can be explained by the interactions between cell components and Ag-Pd surface. It is well-known that Ag can react with amino acids (H_2NCHR_1COOH , where R is an organic substituent) or amino groups ($-NH_2$) of membranes or enzymes inside bacteria, [24-26]. When surface-associated bacteria were present, these reactions can lead to increased rate of Ag dissolution from the surface by Ag complex formations. Furthermore the silver resistant strain utilises effects like silver binding proteins and efflux pumps, which may possibly affect the silver dissolution rate [16, 17].

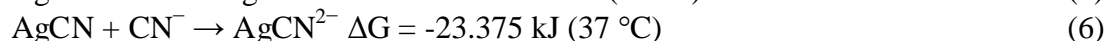
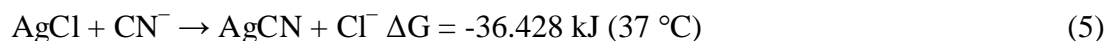
In Fig. 1 (b), the rate of Ag dissolution was observed not to increase proportionally with increasing incubation time from 24 to 72 hours. This can be explained by a steady state of the reaction because of mass-transfer control.

In natural media different from this ABTG designed for optimal biofilm formation in tests, we might find other effects. In some specific environments (not above-mentioned test conditions), a number of bacterial species, e.g. *E. coli*, can perform respiratory reduction of nitrate (NO_3^-) to nitrite (NO_2^-) and of nitrite to ammonia (NH_3) under anaerobic condition [27, 28]. If Ag-Pd surfaces are applied in these environments, these NH_3 or NH_4^+ ions can cause Ag and AgCl to form Ag complexes (Eq. 2 and Eq. 3). It also has been reported that microbial cyanide biosynthesis, so called microbial cyanogenesis, can occur in some species

of bacteria, e.g. *Pseudomonas aeruginosa* (*P. aeruginosa*) [29, 30]. Cyanide produced by bacteria or already presented in an environment can cause Ag and AgCl to form Ag complexes (dicyanoargentate ion). For Ag dissolution, the calculated reaction is as follows [31, 32]:



For AgCl dissolution, a series of reactions can happen, and these calculated reactions are as follows [23, 32]:



Since the Gibbs free energy (ΔG) for these reactions is negative, these calculated reactions are thermodynamically favorable.

Conclusion

This study indicated that it is important to select applicative environments to avoid the degradation of Ag-Pd surfaces. In some specific environments, an undesired increased Ag dissolution can occur and add to the bacteria inhibiting effect when chemicals aggressive to Ag and AgCl, such as NH_4^+ ions, are present. Surface-associated bacteria can increase Ag dissolution from Ag-Pd surfaces due to the interactions between cell components and the surfaces, and the amount of surface-associated bacteria can improve this effect. This could indicate that in a natural environment Ag-dissolution may be low unless bacteria or activating ions are present. Furthermore silver resistant strains seem to enhance this microbiologically influenced corrosion as compared to silver sensitive strains.

Biofilm formation evidently can occur if the Ag-Pd surface becomes covered with a conditioning layer of dead bacteria. Highest bacteria inhibiting efficiency of an Ag-Pd surface may therefore be achieved under conditions where appropriate cleaning processes can be applied.

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