Surface physical roughness correlating to biofilm attachment on galvanized aluminum surfaces by bacteria

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Abstract: Microbial biofilm formation on surfaces of materials is important information to better understand the adhesion mechanisms and to prevent bacterial colonization. Atomic force microscopy is a useful tool for examining bacterial biofilms formed on metal surfaces. The objectives of the present study were to evaluate the metal surface properties including roughness for attachment of the bacterium Janthinobacterium lividum isolated from drinking-water cartridge and to establish the relationship between surface modification through galvanization and susceptibility to biofilm formation. Four metal coupons used in this study were Al Galvanic 0.3%, 5%, 55% and a pure zinc plate. The results showed that several roughness parameters including autocovariance, Z-range, mean roughness, and maximum height increased with bacterial attachment on the selective metal type surfaces. There was a strong positive correlation between different roughness parameters and the number of bacteria attached on the specific metal types. The highest population number of bacteria was observed on Al Galvanized 55% coupon, which was also the roughest surface among the test coupons with different galvanization treatments. Our data suggest that prevention of bacterial attachment on metal surfaces can be achieved by surface treatment to obtain better morphological characteristics.

Keywords: atomic force microscopy; biocorrosion; biofilm; surface characteristics; Zinc; galvanization; surface treatment

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1 Introduction

Microorganisms were first implicated in corrosion of metallic materials by von Volzogen Kuhr and van der Vlught (1934). Microbiological corrosion, also called the microbial influenced/induced corrosion (MIC), affects a wide range of industries, including oil fields, offshore industry, pipelines, pulp and paper, armaments, nuclear and fossil fuel power plants, and petroleum reservoirs (Kobrin, 1993; Gu et al., 2011a, b; Li et al., 2017), aviation and space (Gu, 2003; 2007; 2012). The cost due to failure and prevention was estimated to be 70% of corrosion in gas transmission. American refinery industries lose approximately $1.4 billion a year (see references in review by Dowling and Guezennec (1997). Microorganisms including the aerobic bacterial species and anaerobic sulfate-reducing bacteria (SRBs) as well as methanogens (Daniels et al., 1987), were implicated in the processes of corrosion (Gu, 2012; Gu et al., 2011a, b; Gu and Mitchell, 2013). In addition to SRBs, exopolymer (slime)-producing bacteria were also found to participate in the corrosion by a mechanism in which metal ions are complexed with functional groups of the exopolysaccharides, resulting in release of metallic species (Chen et al., 1995; 1997; Clayton et al., 1992). Similarly, fungi were involved in corroding aluminum and its alloys by a process of organic acids production (Videla et al., 1986), and they were causal microorganisms in materials including concrete (Gu et al., 1996; 1998), stone of cultural heritage (Hu et al., 2013; Meng et al., 2016, 2017), and polymers (Gu et al., 1996; 1998; Gu, 2003).

The biofilm formation on surfaces of materials is a prerequisite for the subsequently observed biofouling and biocorrosion. Once the bacterial cells become attached on a submerged surface, they will proliferate and develop complex architecture of biofilm. In fact a number of factors affect bacterial attachment such as the physicochemical properties of substratum (Azeredo et al., 1999; Clint and Wicks, 2001; Liang et al., 2000) and the bacterial surface. Microbial adhesion and establishment of a complex community of microorganisms (microfouling) are prerequisites for substantial degradation of the underlying materials (Walch, 1992; Gu and Mitchell, 2013). Since all surfaces may act as substrata for formation of these biofilms (Costerton et al., 1995; Geesey and White, 1990; Marshall, 1980), subsequent attack of materials by microorganisms can take place either directly or indirectly, depending on a combination of factors (Gu et al., 1998; Gu et al., 2011a, b). In addition, other factors affecting the physical
environment also influence the extent of bacterial adhesion on surfaces, including ionic strength of the solution, type of cation, hydrodynamic force, and surface properties, e.g., hydrophobicity or hydrophilicity. Corrosion of metals is closely associated with the formation of complex microbial biofilms on surfaces. The microbial communities induce the formation of differential aeration cells under aerobic conditions because dissolved oxygen is consumed within microbial colonies (Gu and Mitchell, 2013). The decrease in oxygen levels provides an opportunity for anaerobic microorganisms to become established within biofilms, where oxygen concentration is low or not available.

A fundamental understanding of bacterial adhesion on morphologically different surfaces of materials, and their impact on the substratum materials requires integration of information from several disciplines, including materials science and engineering, microbial ecology, and physiology. Basic microbiological information is required for a better understanding of the role of microorganisms in corrosion and deterioration. Traditionally, microscopic techniques were used to evaluate the bacterial attachment on the substratum (Caldwell et al. 1997). Scanning electron microscopy (SEM) was also commonly used for this purpose. However, this technique is comparatively time-consuming and does not provide detailed information about the morphologies of the substratum nor in situ bacterial biofilms. In contrast, atomic force microscopy (AFM) has a unique advantage in offering information about the biofilm/substratum interactions in situ. In addition, it can obtain topographical details of the cells under fully hydrated and realistic state, and measure the roughness of the substratum quantitatively. The objectives of this study were to assess metal surfaces physical properties using AFM and elucidate the relationship between surface morphologies and bacterial attachment.

2 Materials and Methods

2.1 Preparation of Metal Coupons

Four types of metals with different surface treatments were used in the present study and they were Al Galvanized 0.3%, 5%, 55%, and pure Zinc plate (99.8% purity). The coupons (10 mm × 10 mm) were degreased by immersing in acetone over night, dried in fume hood and stored in a desicicator prior to AFM study.

2.2 Microorganism and Culturing Conditions

A bacterium, initially isolated from a drinking water filter, was described by Gu and Cheung (2001) and was then identified as Janthinobacterium lividum with 99.9% similarity using 16S rRNA sequences. At the starting of the experiment, each type of the coupons was introduced into an individual Erlenmeyer flask containing sterile 1% Nutrient Broth medium (Difco Lab., Detroit, Michigan, USA) and 1.0 ml of fresh culture of J. lividum. All flasks were incubated at 25°C in the darkness on a shaker. During the incubation period, one set of coupons was removed from each flask for examination using SEM and the sample preparation procedures are described below. At the same time, another set of coupons was removed for AFM.

2.3 Preparation for Scanning Electron Microscopy

The coupons after removal from the flasks were first treated with 3% glutaralddehyde buffer in 0.2 M Na cacodylate overnight in glass vials as described before (Gu et al., 1998). The samples were then washed with 0.2 M Na cacodylate three times, further fixed in 1% OsO4 in 0.1 M Na cacodylate for at least 1 h, and rinsed with Na cacodylate and deionized water, respectively. Dehydration of the samples was achieved in an ethanol-distilled water series of 40 to 80% ethanol with a 10% increment and then 90 to 100% with a 5% increment. Samples were kept in 100% ethanol and sealed in glass vials until they were critical point dried in liquid CO2. After drying, samples were coated immediately with Palladium-Gold and observed on a Leica Cambridge Scan 440 Scanning Electron Microscopy.

2.4 Preparation for Atomic Force Microscopy

The morphologies of the four types of metal coupons were analyzed before the experimental exposure in order to characterize surface physical properties including roughness. A Nanoscope IIIA AFM (Digital instruments, Santa Barbara, CA, USA) operating in the tapping mode was used to view the morphologies of the metal coupons. The AFM probe had a cantilever length 125 µm and the tip radius of the curvature was 5-10 nm. The Digital Nanoscope software (version 4.23) was used to process the data of the roughness before the experimental exposure in order to characterize surface physical properties including roughness. Representative AFM micrographs of the four metal coupons are shown in Figure 1. Variability of metal surface physical properties was due to galvanization process upon surface treatment to variable degrees. Using these several roughness parameters mentioned above, surface roughness could be ranked in the following order: Al Galvanized 55% > Al Galvanized 5% > Zn > Al Galvanized 0.3%. For example, the depth of the valley on surface of Al galvanized 55% was 2.72 µm while it was 399 nm for Al galvanized 3% by the surface treatment process (Table 1).

3 Results

3.1 Surface Morphologies of Different Metal Coupons

The four different metal coupon surfaces containing variable Zn for surface treatment against corrosion could not be distinguished visually but the differences were clearly revealed using AFM. Information obtained by AFM was expressed in terms of several quantitative parameters including Z-range, mean roughness, maximum height, raw mean, autocovariance, and the surface roughness. Representative AFM micrographs of the four metal coupons are shown in Figure 1. Variability of metal surface physical properties was due to significant differences among the four treatments. After 7 days of incubation,
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Figure 1. The AFM micrographs of four different metal coupons. (A) Galvanized 55% Al; (B) Galvanized 5% Al; (C) Zn; (D) Galvanized 0.3% Al.

the metal coupons were removed from the incubation and the number of bacterial cells attached onto the metal coupons was quantified from SEM images. It was observed that the number of bacterial cells attached to each coupon surface was significantly different. Al Galvanized 55% coupons showed the highest density of the bacterial cells attached than all the other types of coupons. The order of bacterial attachment followed: Al Galvanized 55% > Al Galvanized (Galfan) 5% > Al Galvanized 0.3% > Zn (Figure 2) in agreement of the surface roughness observed (Table 1).

Bacterial adhesion on surfaces was positively correlated to the surface roughness parameters. The correlation coefficient was 0.807, 0.891, 0.894 and 0.900 for autocovariance, $Z$-range, mean roughness and maximum height, respectively (Table 2).

4 Discussion

Microbial involvement in the corrosion of metals has been known for a long time (Gu and Mitchell, 2013; Gu et al., 2011a, b). Large amount of information based on research using Pseudomonas aeruginosa is also readily available. In this study, surface morphological characteristics of roughness of several systematic treated metal types were investigated to determine the relationship between surface roughness and bacterial attachment using AFM and SEM. A previous study showed that as the roughness of the stainless steel surface decreased, fewer bacterial cells attached to the surface using a mixed bacterial culture derived from poultry processing plant (Arnold and Bailey, 2000). Our results are in good agreement with theirs despite of the different materials types and the galvanization of Al used. The difference is that their...
Table 1. Different metal surface roughness parameters as shown by atomic force microscopy

| Measurement          | Metal                  | Galvalume 55% Al | Galfan 5% Al | Galvanized 0.3% Al | Zn |
|----------------------|------------------------|------------------|--------------|-------------------|----|
| Autocovariance       | 246542                 | 25540            | 1131         | 16406             |    |
| Z-range              | 2770                   | 960              | 403          | 744               |    |
| Mean Roughness       | 405                    | 122              | 22.26        | 98.18             |    |
| Raw mean             | 3.2                    | 4.45             | 5.34         | 5.26              |    |
| Maximum height       | 2.72 μm                | 990 nm           | 399 nm       | 775 nm            |    |

1 Autocovariance (nm²) indicates the degree of surface structural or morphological regularity. The regularity is inversely related to the magnitude of the number.
2 The Z-range (nm) controls the vertical range of the image. This is the difference between the highest and lowest points within the given range.
3 The mean roughness (Ra, nm) is the mean value of the surface relative to the center plane.
4 The raw mean (nm) is the SD of the Z-values within the given three-dimensional area.
5 The maximum height (nm) is the difference in height between the highest and lowest points on the surface relative to the mean plane.

Table 2. Correlation between surface roughness of the test materials and the bacteria attached

| Measurement          | R²  |
|----------------------|-----|
| Autocovariance       | 0.8068 |
| Z-range              | 0.891 |
| Mean Roughness       | 0.8937 |
| Maximum height       | 0.8998 |

In our study, galvanized Al 55% coupons had the roughest surface and the most abundant number of attached J. lividum. This suggests that Al Galvanized 55% was favorable for bacterial attachment. However, the susceptibility to biofouling and biocorrosion needs to be further investigated in the future study. It would be interesting to know the settlement of invertebrate larvae on Al Galvanized 55% coupons because bacterial biofilm is thought to play a role in the induction and inhibition of larvae settlement (Holmström and Kjelleberg, 1994). Moreover, Xu et al. (1999) also showed that bacteria may not participate in the corrosion process directly but they can change the nature of the substratum so as to facilitate the corrosion process.
5 Conclusions

Surface treatment of materials can be effective means to prevent microbial deposition and colonization of materials surfaces. The surface roughness of the galvanized coupons increased with an increase of the attached bacteria when incubated under laboratory conditions. The high correlation between surface morphological characteristics of metal coupons and the number of bacteria attachment can offer effective material engineering against bacteria in applications.

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Conflict of Interest

All authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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