Chapter 16
Effect of Metallurgical Factors on Microbial Adhesion and Microbiologically Influenced Corrosion (MIC)

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16.1 Microbial Adhesion and MIC

Colonization of wet surfaces by bacteria generally leads to the formation of biofilms. However, the microbial adhesion on metal surfaces could sometimes lead to serious problems known as microbiologically influenced corrosion (MIC). MIC causes problems in various industries such as chemical plants, power generation facilities, water and oil pipelines, water tanks, and ship hulls (Borenstein 1994; Licina 1998; Kikuchi and Sreekumari 2002; Miyano and Kikuchi 2008). MIC on metal surfaces once initiated can proceed at a rate of several mm/year; MIC can even occur on corrosion-resistant materials, such as stainless steel.

Stainless steel is one of the major structural materials which have superior corrosion resistance, but there have been many reports on severe local corrosion associated with microbial activity, i.e., MIC. Many corrosion case analyses for stainless steel in which MIC is suspected tend to show the welds or heat-affected zone (HAZ) gets higher bacterial adhesion leading to higher corrosion risk (Enos and Taylor 1996; Borenstein and Lindsay 1987; Borenstein 1991). For this reason, the effect of metallurgical factors, such as changes in metallographic microstructure due to welding heat cycle and manufacturing specifications, has been studied on microbial adhesion (Obukwe et al. 1981; Walsh et al. 1994; Little et al. 1996). Although stainless steel is originally a material with excellent corrosion resistance, if exposed to thermal cycles during welding process, its microscopic properties such as the

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metallic structure or the presence of alloying elements can easily be altered. Although solidification segregation is just an example of such a thermal impact that leads to deterioration of corrosion resistance, heterogeneity of alloying elements like this is suspected to be involved in inducing microbial attachment. The idea that such changes in metallurgical properties not only may contribute to the deterioration of corrosion resistance of materials but also increase the susceptibility to MIC is currently gaining researchers' attention.

In the following section, the authors refer to some cases of MIC of stainless steel and outline the correlation between MIC and microbial adhesion.

16.2   MIC in Stainless Steel Welds

MIC has been reported as the cause for various structural damages in industries. This unexpected corrosion failure and rapid increase in corrosion rate in stainless steel structures exceed the material risk assessment, and this makes MIC a matter of considerable concern in material engineering. To make matters worse, corrosion damage is concentrated in the vicinity of stainless steel welds in many cases. Although the reason has not been elucidated until now, it is known that both bacterial adhesion at the welded portion and the incidences of MIC in and around welds are high.

Figure 16.1 shows a SUS304 steel joint (weld) used in a wastewater treatment plant (Kikuchi and Sreekumari 2002). Large corroded area is confirmed around the weld area. This corrosion incidence has been reported within a few months after being put into use. Although the corrosion resistance of the material to the environment was considered to be sufficient at the design phase and construction stages, both the corrosion dimension and rate were extremely larger than corrosion engineering assumptions. It was confirmed that the possibility of poor welding was infinitely zero during the failure analysis. This was one of the major factors for suspecting the possibility of MIC occurrence.
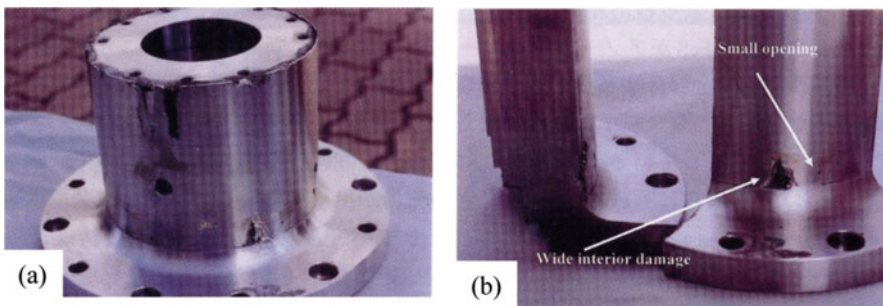

Fig. 16.1   Figure showing suspected MIC in a SUS304 piping fitting units used in a wastewater treatment plant. (**a**) Appearance of piping fitting units. (**b**) Enlarged image of the corrosion site
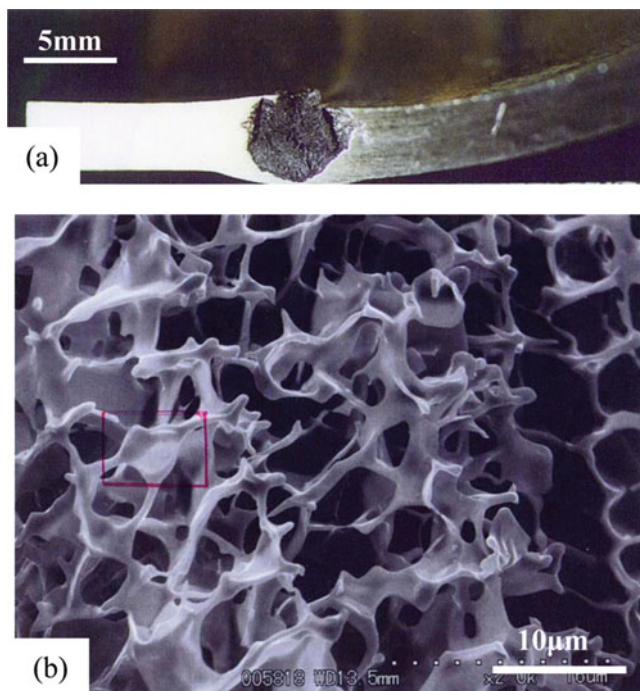
Fig. 16.2 A suspected MIC case occurred on a SUS316 cooling water piping in a power plant. (**a**) Cross-sectional image of corrosion part around welds. (**b**) SEM micrograph of corrosion site. (The tendency of selective corrosion of austenitic phase was confirmed by EDX)

Another failure case analysis involved SUS316L from a cooling water piping system in an energy plant. Figure 16.2 shows a cross-sectional image of corrosion failure part in the welds perpendicular to the welding direction (Miyano and Kikuchi 2008). In visual observation, significant corrosion damage was confirmed at the inner surface contacting the cooling water around weld and the HAZ regions. Observation of these parts under a scanning electron microscope (SEM) showed selective corrosion dissolution of the austenitic phase. Since the cooling water flowing through the pipe was neutral and at room temperature, the corrosion risk was considered to be low from the corrosion engineering viewpoint. However, a water leak was discovered within a few months after construction of the plant and starting its operations. This is the reason why mechanical welding defects were initially suspected during the initial inspection, but a detailed re-examination concluded that the possibility of construction failure was extremely low.

The samples from the above incidence were brought back to the laboratory, and by using simulation experiments, the risk posed by MIC was examined (Miyano et al. 2004). As shown in Fig. 16.3a, the SEM images showed remarkable bacterial attachment and biofilm formation on the SUS316 weld test pieces exposed to the bacterial culture for 56 days. Figure 16.3b shows the results of SEM observation
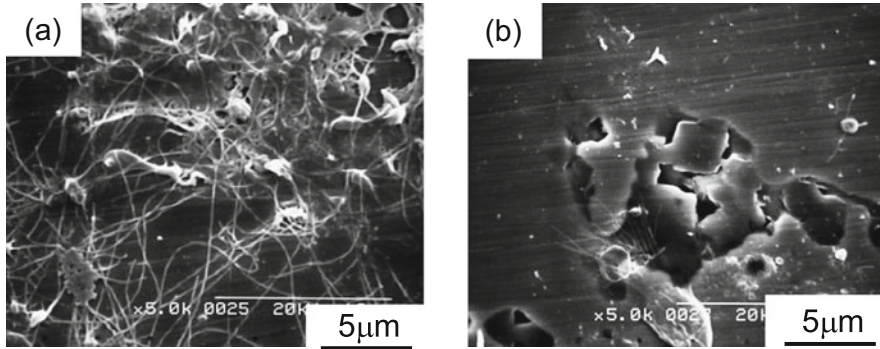
Fig. 16.3 Figures showing MIC in laboratory simulation tests. (**a**) Biofilms formed on the surface of a SUS316 weld specimen by exposure test in a flask. (**b**) Pitting corrosion confirmed in the same field of view as (**a**) after the removal of biofilms

performed on the same field of view but after removing the biofilm. Corrosion pits generated below biofilms were clearly confirmed. The extend of bacterial attachment was observed to be more in stainless steel welds where MIC incidence was remarkably higher than in the base metal.

Similar rate of microbial adhesion was observed on polished surfaces of stainless steel in the areas of welds. There are many reports showing that a large amount of microorganisms adheres to the stainless steel welds even after polishing to a smooth surface finish. This shows stainless steel welds can easily attract microorganisms. However, there is not much data available as to why the microbial adherence is predominant on stainless steel welds. In the following section, the authors would like to mention the available information on this issue from a material engineering viewpoint.

16.3 Lab Study on Microbial Adhesion on Stainless Steel Welds

As previously mentioned, there is a correlation between bacterial adhesion and occurrence of MIC. The process begins by the adhesion of bacteria on the wet material surface leading to biofilm formation and initiation of MIC. Metal surfaces to which microbes are adhered have different properties than those of glass, resin, etc. The metal microstructure includes grain boundaries and precipitates, i.e., a disorder of the atomic arrangement or a nonuniform part. These metallurgical factors characterize the physical and chemical properties of metal surfaces.

The following results show the effect of macroscopic morphologies of metal surface on the bacterial adhesion. Figure 16.4 shows results of numerical analysis at a two-dimensional laminar flow field that is showing macroscopic (mm-order scale)

Fig. 16.4 Results of the numerical analysis at a two-dimensional laminar flow field around the inner side of welded pipes
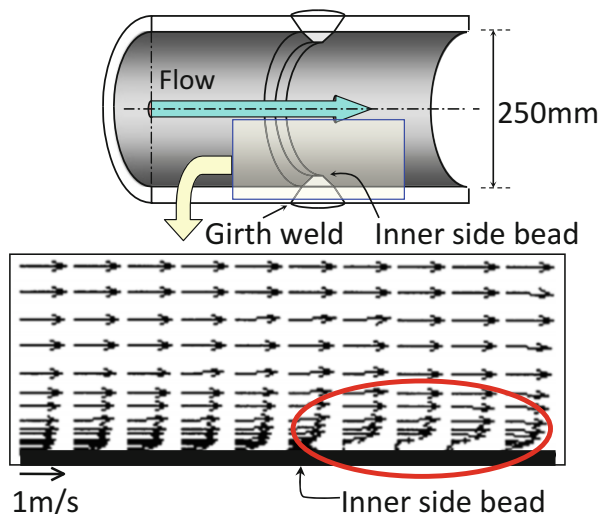
geometry near stainless steel (SUS304) welds that assist microbial adhesion and induce corrosion (Amaya et al. 2001). When a weld shape is present in a flow field such as a cooling water pipe, there may be flow velocity variation around the weld part compared to the non-weld (base metal) part. The concept is that this slowing down of flow near the weld surface assists the adhesion of microbes and, as a result, MIC initiation. As shown in Fig. 16.5, bacterial adhesion is significantly higher near the weld area where the flow velocity is slow. From the uniaxial flow field reproduced by the numerical calculation of the flow field, the tendency of increased microbial adhesion around the convex part of the weld (the toe (Toe) and the heat-affected zone (HAZ)) has been confirmed.

Figure 16.6 shows the correlation between the initial microbial adhesion (within 2 h from the start) and the microstructure, i.e., grain boundary or phase interface, of a SUS304 base metal and welds (Sreekumari et al. 2001). The images were obtained by a superimposing method using two types of images. One type is fluorescence micrographs from microbial adhesion tests on mirror-polished test specimens (tested bacteria are appearing as orange spots); another is an optical microscopy image acquired after crystal grain boundaries are revealed by etching (same field of view as fluorescence microscope observation for bacterial adhesion). The tendency for preferential bacterial attachment to grain boundaries or phase interfaces is clearly confirmed. More interestingly, although the specimens were mirror finished to eliminate the effect of the surface morphology on bacterial adhesion, the weld specimen shows larger amount of adhesion and proliferation of tested bacteria than the base metal. To summarize the observation here, in the case of stainless steel, the amount of bacterial adhesion increases as the microstructure becomes finer and the microbial adhesion to the weld is larger than that of the base metal possibly because of more grain boundaries and inclusions. However, the reason has not been clearly identified yet.

Fig. 16.5 Comparison of
adhesion area of bacteria on
as-weld specimen in
laboratory simulation tests
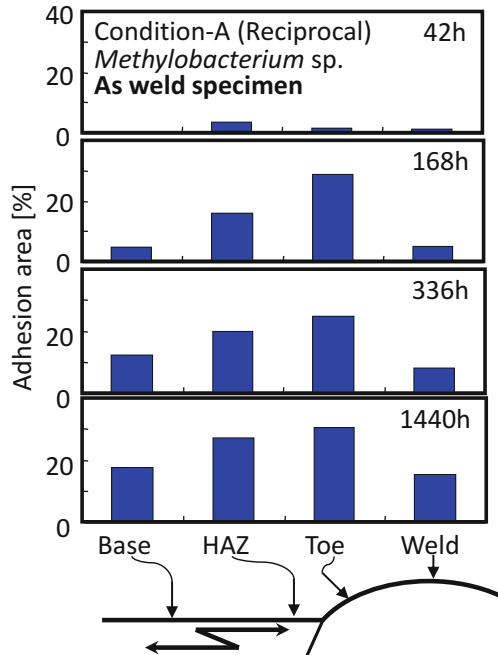
Certainly, the microstructure of stainless steel welds forms fine solidification structure with fine phase interface at high density due to quick heating and quenching during welding process. Generation of grain boundary segregation and sensitized structure also occur in this process. In previous studies on the correlation between microstructure and bacterial adhesion, the possibility that the localization of surface energy, the precipitation including elements with high affinity for bacteria, and the grain boundary energy inducing the bacterial adhesion are discussed (Sreekumari et al. 2001). In the field of material science, how the metallurgical factors such as the ones mentioned above affect the bacterial adhesion has become a challenging research theme and is attracting attention. From this point of view, the authors would like to introduce the results of investigation on the effects of alloying elements on bacterial repellency, i.e., alloying elements which have antibacterial properties, on the attachment of microorganisms.

## 16.4 Bacterial Adhesion on Antibacterial Stainless Steel

It is well known that metals such as Cu or Ag suppress the growth of microorganisms. Although not originally developed for suppressing MIC, materials that are classified as antibacterial stainless steels are manufactured by adding Cu or Ag as alloying elements. Here, the authors would like to introduce some results of

Fig. 16.6 Correlation between initial bacterial adhesion and stainless steel microstructure, i.e., grain boundary in the base metal or phase interface formed by ferrite and austenite phases in welding materials. (**a**) Base metal surface. (**b**) Weld metal surface
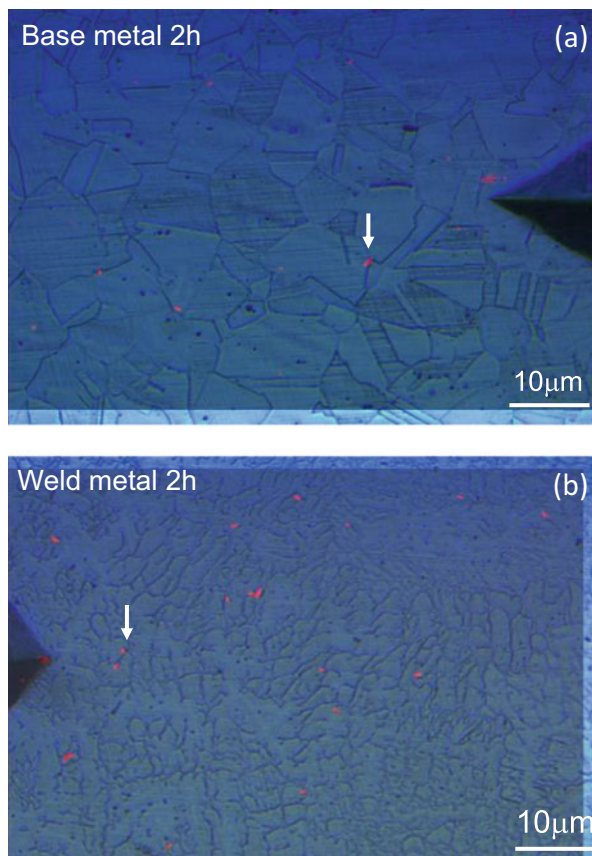
interaction of microorganisms and antibacterial materials. Figure 16.7 shows the results of bacterial adhesion on copper-containing antibacterial stainless steel and general stainless steel SUS304 (Sreekumari et al. 2005). The orange color spots indicate bacteria attached on the material surface. It can be seen that the bacterial adhesion on antibacterial stainless steel is significantly lower compared to the general stainless steel. Figure 16.8 shows the result of a similar study performed on silver-containing antibacterial stainless steel and general stainless steel SUS304 (Kikuchi and Sreekumari 2002). Similar to the previous study, the bacterial adhesion was significantly lower in the silver-containing antibacterial stainless steel. These studies were conducted in the laboratory using isolated pure cultures of corrosive bacteria. Further studies were carried out to evaluate the performance of silver-containing antibacterial stainless steel exposed to natural microbial flora in a fresh-water pond. Although the evaluation period was only 30 days, as shown in Fig. 16.9, the antibacterial stainless steel significantly reduced the natural biofilm formation (Kikuchi and Sreekumari 2002).

Fig. 16.7 Images of copper-containing antibacterial stainless steel in a laboratory study. (**a**) General stainless steel. (**b**) Copper-containing antibacterial stainless steel
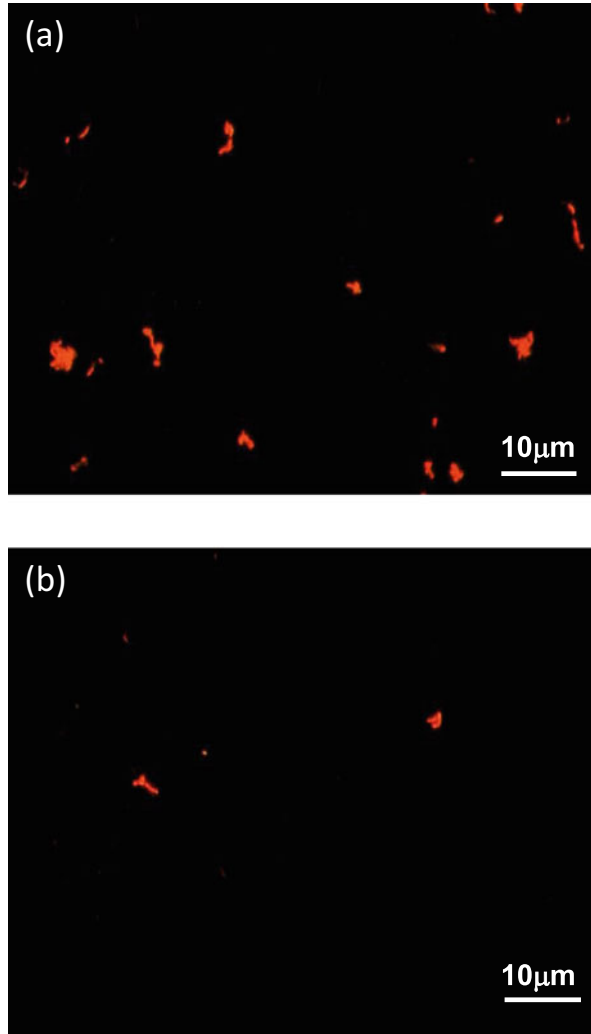
Before these materials can be used commercially, we need more intense studies to understand the effectiveness of these materials to prevent biofilm formation on a longer period. However, biofilm formation or biofouling prevention function of these materials proposes new possibilities for industrial application. Recently, one of the practical strategies for using antibacterial materials to control MIC, the choice of copper as over pack barrier materials for geological treatment of radioactive waste (METI Database 2018), attracted attention.

Fig. 16.8 Images of silver-containing antibacterial stainless steel in a laboratory study. (**a**) General stainless steel. (**b**) Silver-containing antibacterial stainless steel
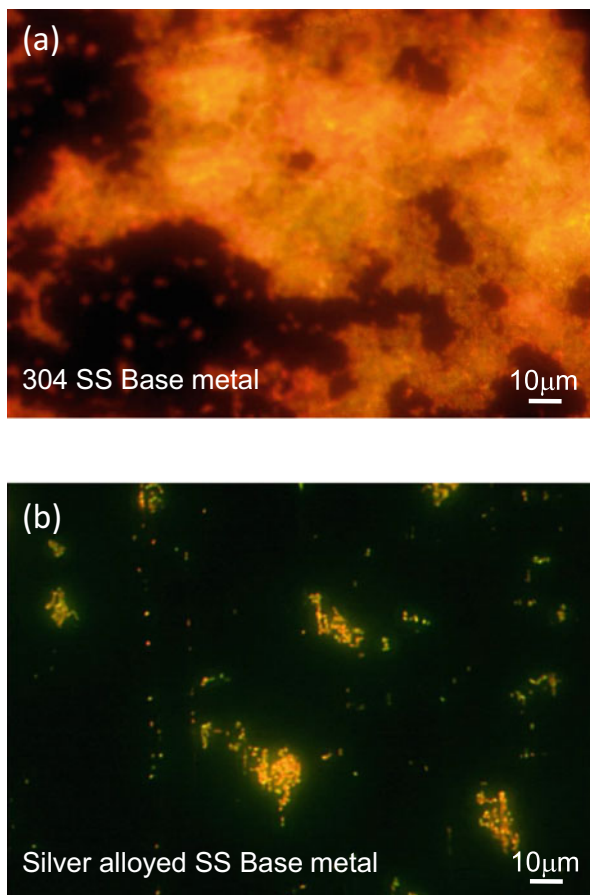

16.5 Research Prospects for Material Microbe Interactions

The authors have reviewed the relationship between metal surfaces and adhesion of microorganisms. However, it is clear that various metallurgical factors that can affect the adhesion of microorganisms have been unexplored. It is very difficult to study the factors influencing the material microbe interactions in a comprehensive way as it needs both metallurgy and microbiology expertise. This is thought to be a major factor hindering the progress of research in this field. Here, the authors would like to introduce a pioneering result of in situ observation of MIC initiation process on stainless steel welds by COCRM (Miyano et al. 2015). Figure 16.10 shows the results of continuous observation at a fixed point on SUS303 steel weld surface exposed to natural seawater for 15 h. Figure 16.10a shows a mirror-finished initial surface by buff polishing using diamond paste after wet polishing with emery paper. Although there are some fine damages due to embedding of abrasive particles, a

Fig. 16.9 Images of silver-containing antibacterial stainless steel exposed in a freshwater pond. (**a**) General stainless steel. (**b**) Silver-containing antibacterial stainless steel
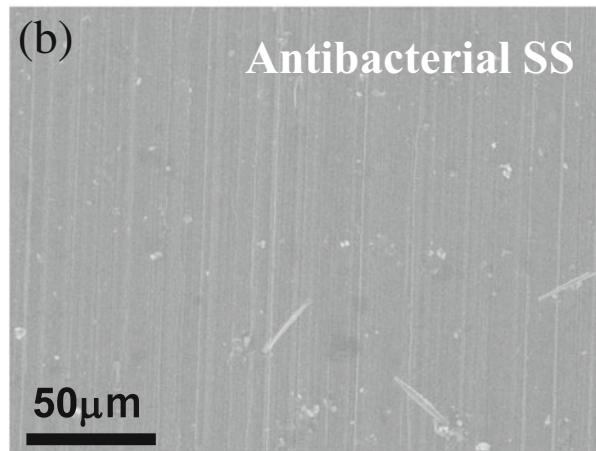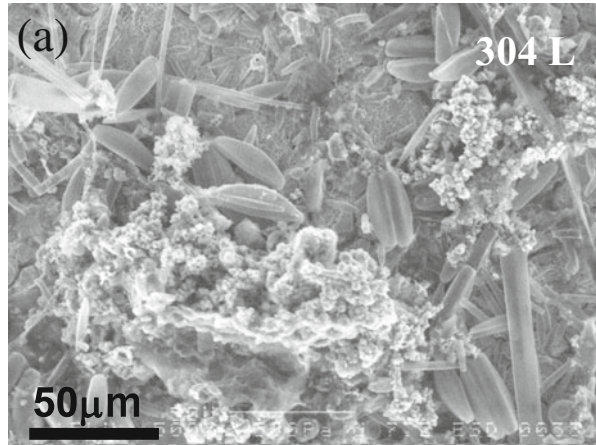

sound initial surface without substantial irregular morphology can be clearly con-firmed. Figure 16.10a–e are time lapse images of the surface of the test specimen during 15-h exposure test. This results show the beginning of bacterial attachment leading to the formation of biofilm and its impact on the changes of surface morphology of the test specimen. Figure 16.10f is a synthesized image of the fluorescence microscope image and the COCRM one acquired immediately after the image acquisition of Fig. 16.10e. The dye staining operation was performed during microscopic observation. The localization of the microbial cells identified by the green color is almost corresponding to the state of the test material shown in Fig. 16.10e. Note the fact that the microbial adhesion is localized, even though the initial surface was almost evenly smooth. This result suggests that the attachment of the microorganism to the metal and its subsequent growth are affected by
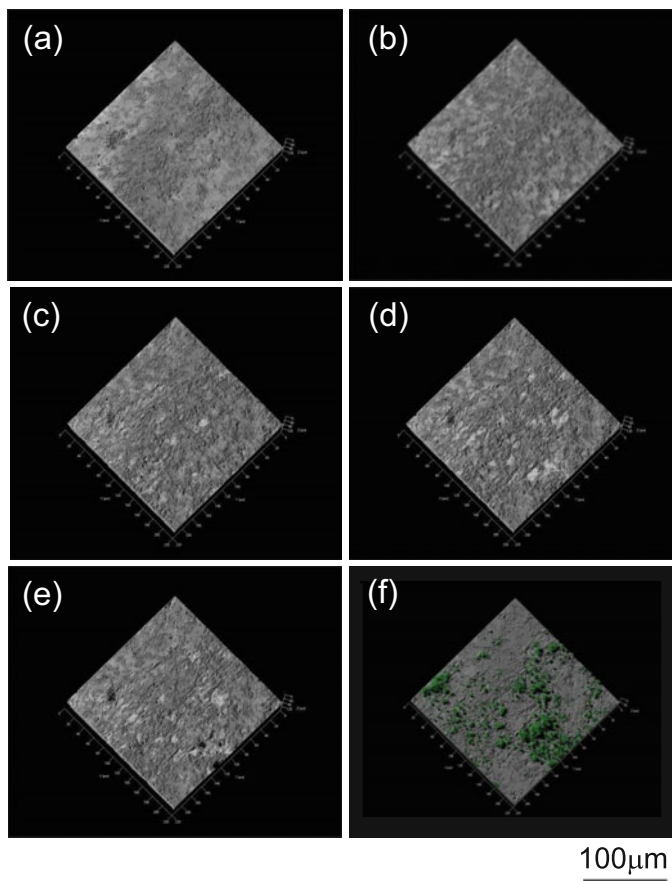
Fig. 16.10 15-hours fixed-point continuous observation results for in situ metal/microbial simultaneous observation designed to analyze biofilm formation and corrosion initiation process. The observation surface is mirror-finished SUS303 welds exposed in fresh marine water. (**a**) 0 h (immediately after exposure) and (**b**) 4 h, (**c**) 8 h, (**d**) 12 h, and (**e**) 15 h (immediately before the end of exposure test) and (**f**) a synthesized image of the fluorescence observation image and the COCRM one acquired immediately after the image acquisition of (**e**)

microstructure such as uneven distribution of elements on the metal surface. This research achievement has been evaluated as a breakthrough result of simultaneous in situ observation for microbial behavior and metal surface changes.

Similar studies on microbial attachment on metal surfaces using COCRM have been conducted on stainless steel base metals and its two welds types (Miyano et al. 2015). Figure 16.11 shows the difference of bacterial adhesion in each of these samples. Although the surface of the test specimen was smooth-finished by polishing, in SUS303 weld which has welding solidification structure and contains higher P and S content compared with SUS304, a large amount of bacterial adhesion
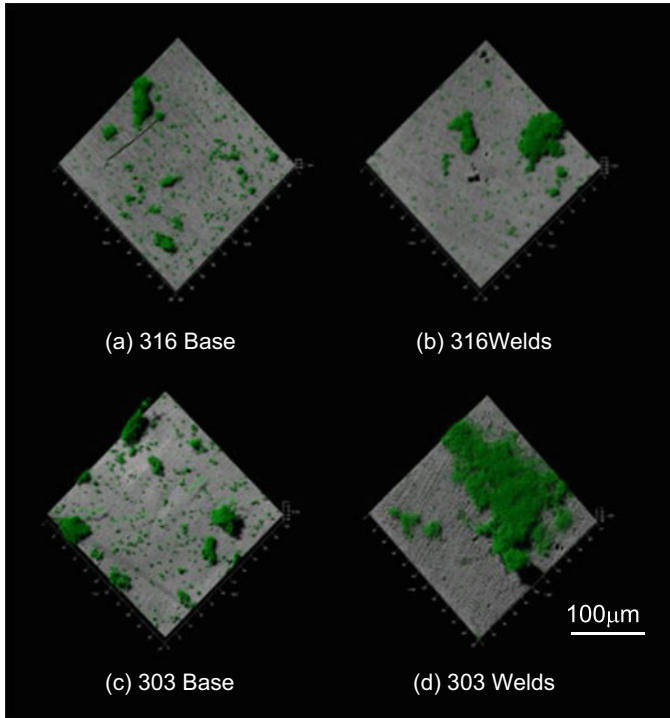
Fig. 16.11 Synthesized image of the fluorescence micrograph and the in situ metal/microbial simultaneous observation after 12-h exposure test in fresh marine water. (**a**) SUS316 steel base metal, (**b**) SUS316 steel weld metal, (**c**) SUS303 steel base material, (**d**) SUS316 steel weld metal. The surface of each specimen was mirror finished with diamond paste

was confirmed. Previous studies have shown that microbial adhesion to welds tends to be significantly greater than the base metal and that the reason for this is considered to be the effect of alloying elements to which microbes have some affinity. The results presented here indicate that the alloying element concentration influences the initial bacterial adhesion and thereby the biofilm formation. These results indicate the potential for acquiring new knowledge based on dynamic analysis of bacterial behavior to metals by applying new technologies such as COCRM. The authors hope that research related to the interaction between micro-organisms and metals will be further expanded, starting from the analysis of MIC, and may result in the prevention of MIC.

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