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Effect of sulfate-reducing bacteria on stainless steel: a review

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ABSTRACT

Corrosion-resistant alloys such as stainless steel provide an ideal substrate for microbial colonization due to the absence of corrosion products, similar to inert non-metallic surfaces. Stainless steels are sensitive to pitting and other types of localized corrosion in chloride-containing media such as seawater. Sulfate-reducing bacteria play an essential role in the corrosion of stainless steel in marine and soil environments. Sulfate is utilized by microbes as a terminal electron acceptor as their respiration drives sulfate reduction leading to the formation of H_2S, which can lead to a significant increase in anodic and cathodic processes and corrosion of materials. In reviewing the literature, it was found that most studies on microbially induced corrosion in stainless steels indicate that it is caused by the influence of chlorides and sulfides in the soil resulting from the secretion of sulfate-reducing bacteria. The influence of sulfate-reducing bacteria on stainless steel is described in detail in this review, which can be seen from the following points: general properties of sulfate-reducing bacteria, morphology and chemical composition of biofilm and corrosion products, mechanisms of microbiological corrosion by sulfate-reducing bacteria and electrochemical studies of corrosion rates of stainless steel by sulfate-reducing bacteria under different experimental conditions.

Keywords: *stainless steel, sulfate-reducing bacteria, corrosion*

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Introduction

All definitions of microbiological corrosion (Javaherdashti, 2011; Liu et al., 2018; Pal end Lavanya, 2022) are based on the following statements: microbiological corrosion is an electrochemical process; microorganisms can influence the duration of corrosion, the strength and the direction in which corrosion takes place; and in addition to the presence of microbes, nutrients and water must be present for microbial corrosion to take place. Microbial corrosion accounts for 20% of all corrosion damage (Flemming, 1996). Damage caused by microbial corrosion has always been a significant concern of corrosion researchers. The authors Maxwell et al., (2004) pointed out that corrosion causes losses to the oil industry of around 100 million US dollars annually. It is estimated that 10% of all corrosion damage in the UK is due to microbiological corrosion (De Romero et al., 2000).

Microbes are one of the leading causes of corrosion in underground pipelines (Li et al., 2003), which is related to the influence of different microbes and the physicochemical properties of the soil. It is known that the chloride content in soil and other environments plays a vital role in the corrosion of steel structures (Sun et al., 2011), so the influence of microbiological corrosion on stainless steel is recognized as an essential corrosion phenomenon (Nguyen et al., 2008). Due to the wide application of stainless steel, numerous studies have focused on the microbiological corrosion of these materials (Sheng et al., 2007; Maruthamuthu et al., 2008; Sun et al., 2011; Tang et al., 2021). Stainless steels are alloys with a wide technical-technological application due to their high corrosion resistance; however, the presence and influence of microbes is unavoidable even with these types of steel (Geesey et al., 1996). Due to the wide application of stainless steel in the industry, both in salt and freshwater environments, researchers have focused on studying the influence of sulfate bacteria on these steels. Various analytical techniques have been used to study microbial corrosion, including electrochemical and surface analysis techniques: electrochemical impedance spectroscopy (EIS), atomic force microscopy (MAC) and X-ray photoelectron spectroscopy (XPS) (Sheng et al., 2007; Nguyen et al., 2008; Ramdane et al., 2023).

Work also presents the main aspects of sulfate-reducing bacteria, the morphology and structure of biofilms and corrosion products on stainless steel, and the mechanisms and corrosion processes of microbiological corrosion of sulfate-reducing bacteria on stainless steel. In addition, the role of sulfate-reducing bacteria in the corrosion and fracture of steel was also explained.

General properties of sulfate-reducing bacteria

Sulfate-reducing bacteria is a general term for different types of bacteria that reduce sulfates to hydrogen sulfide or FeS_x. Sulfate-reducing bacteria represent a group of heterotrophic, mixotrophic, mesophilic and halophilic bacteria (Lane, 2005). Sulfate-reducing bacteria have a spindle-shaped, granular or oval form with sessile cell densities of about 107 cells per cm^2 (Duan et al., 2006). The maximum concentrations of sulfides formed by the reduction of sulfates by sulfate-reducing bacteria do not exceed 600 ppm, while sulfide concentrations in sediments hardly exceed 500 ppm. Sulfate-reducing bacteria can tolerate a pressure of up to 506.6 bar (Barton and Tomei, 1995). The optimum temperature for the growth of sulfate-reducing bacteria is 20-30 °C, but they can also survive at 50-60 °C. Sulfate-reducing bacteria survive under anaerobic conditions in soils, seawater, sludge, underground pipelines and oil wells at pH values of 6 to 9 (Yuan and Pehkonen, 2009). Sulfate bacteria can also be found in wastewater (Chen et al., 1998) and the human body, e.g. in the mouth (Maruthamuthu et al., 2008). These organisms exist in a naturally occurring film and form a complex with which they bind to the surface of the metal (Baker et al., 2003).

In their natural habitat, sulfate-reducing bacteria are essential in the biogeochemical sulfur cycle (Yuan and Pehkonen, 2009; Tran et al., 2021). They are a group of classified anaerobic microbes that dissimilate sulfur compounds such as sulfates, sulfites, thiosulfates and elemental sulfur to sulfides (Gibson, 1990). Sulfate-reducing bacteria do not require oxygen but can be found in aerobic environments. They tolerate significant changes in temperature, pH, chloride concentration and pressure fluctuations (Javaherdashti, 2011). Sulfate-reducing bacteria are involved in microbiological corrosion processes affecting various engineering systems and alloys. For example, the sulfate bacteria *Desulfovibrio* can produce insoluble sulfates of Zn, Pb, Ni, Cr, Cd, Cu, Fe, Hg and other elements (Lane, 2005).

The classification of corrosivity, which occurs depending on the number of cells of the sulfate-reducing bacteria, is expressed in units: cell/ml. According to this classification, the environment is considered low corrosive if the number of sulfate-reducing bacteria is 1000 cells/ml or less, while the content of sulfate-reducing bacteria in the solution between 10^3 and 10^5 cells/ml is a medium corrosive environment. If the sulfate-reducing bacteria content is above 10^5 cells/ml, the environment is classified as highly corrosive (Javaherdashti, 2011).

The authors Cheng and Enhou, (2005) showed that the corrosion of stainless steel increases with the decrease in sulfate-reducing bacteria. Wei et al., (2010) demonstrated a positive correlation between sulfate-reducing bacteria and the corrosion process of stainless steel. However, the authors Ilhan-Sungur et al., (2007) showed that there is no evidence of a correlation between the corrosion rate and the number of bacterial cells in the tested environment. Kuang et al., (2007) even showed that instead of the number of sulfate-reducing bacteria, the metabolic products of the bacteria may be of greater importance when testing metal corrosion.

Formation and structure of the biofilm of sulfate-reducing bacteria and their corrosion behavior

According to the general definition, a biofilm's gradual formation can change the metal surface's chemical composition. The physical meaning of the biofilm indicates a passivity that restricts oxygen diffusion to the metal surface. Therefore, the active metabolism of the microorganisms consumes oxygen and produces metabolites. The result of biofilm formation is reflected in the creation of a concentration gradient of chemical species along the entire length of the biofilm, usually between 10 and 400 μm (Xu et al., 1998).

In order to understand the role of sulfate-reducing bacteria in the corrosion of stainless steel, it is necessary to analyse and define the effects of the biofilm on the metal. The heterogeneity of the biofilm is vital for triggering localized corrosion by increasing the corrosion rate, as it can contribute to significant differences in metabolites, pH or soluble oxygen (differential aeration cell), leading to the formation of an active electrochemical corrosion cell (Yuan and Pekhonen, 2007). The resulting biofilm behaves like the growth of a colony of sulfate-reducing bacteria on the metal surface, leading to localized corrosion (Javaherdashti, 2011; Anusha and Mulky, 2023). Microorganisms easily attach to the steel surface. When the metal is immersed in seawater, bacteria excrete organic polymers and adhere to the steel surface, forming a thin film that changes the properties of the metal surface, significantly static charge and wettability. This leads to the adhesion of bacteria to the steel surface and the growth of a bacterial colony (Zhang et al., 2011). In biofilm formation, the initial attachment of bacteria to the steel surface is the most crucial step, which is achieved by electrostatic attraction and other forces, e.g. van der Waals forces and hydrophobic interactions (Ong et al., 1999; Narayana and Srihari, 2019).

Ong et al., (1999) showed that the adhesion force in cell-cell interaction is greater than the force with which *D. disulfuricans* cells adhere to the steel surface. Most researchers (Beech, 2002 and Xu, 2002) indicated stronger adhesion at the cell-cell surface related to the accumulation of exopolymeric substances at the cell periphery, accelerating the mutual binding of the bacteria to the substrate and the steel surface. These results suggest that the sticky exopolymeric substance accumulating on the cell surface increases cell aggregation, leading to the expansion of the biofilm over the surface of the tested steel (Yuan and Pehkonen, 2009). The exopolymeric substance is essential for cell attachment to the biofilm structure. A class of N-acyl-homoserine lactone signalling molecules released by cells into the local environment has also been shown to interact with neighbouring cells.

Studies (İlhan Sungur et al., 2010; Javaherdashti, 2011; Sun et al., 2011; Elmouaden et al., 2016) have shown that on metal surfaces such as stainless steel, galvanized steel and copper, the number of sulfate-reducing bacteria on the examined surface increases over time, indicating that a biofilm has formed. The heterogeneity of the steel structure and the uneven oxygen concentration at the interface between the biofilm and steel can also contribute to forming an electrochemical cell, such as a differential aeration cell, which accelerates corrosion (Vujičić, 2002). The metal at the edge of the biofilm is in contact with the substrate solution, which is relatively richer in oxygen. Due to the decrease in oxygen in the biofilm's centre, differential aeration occurs, manifesting in the anodic dissolution of metals in the form of Fe^{2+} ions. The transport of these ions by diffusion, convection and ion migration towards the cathodic periphery of the biofilm leads to the deposition of hydrated Fe(III) hydroxide as the end product of corrosion (Vujičić, 2002). Due to the different cation concentrations, it is also more likely that a particular site on the steel will become a local cathode, while the opposite site with lower cation concentrations will form a local anode and thus contribute to the occurrence of pitting (Javaherdashti, 2011). Therefore, local anodes become the sites near which the electrolyte contains a lower concentration of passivating substances or a higher concentration of activating substances. Corrosion activators include chloride ions, hydroxyl and ammonia ions, which make the potential of the metal more negative and make it impossible to reach the passive state of the steel (Vujičić, 2002).

However, the biofilm of anaerobic sulfate-reducing bacteria has other properties: a thick biofilm layer impedes oxygen diffusion. The consequences of such processes are anaerobic conditions that lead to better conditions for the growth and reproduction of bacteria (Javaherdashti, 2011). By

reducing the oxygen concentration on the surfaces where the corrosion product is deposited, these surfaces change their polarity, which means that the anode surface area increases at the expense of the cathode surface area (Vujičić, 2002). As a result of chemical reactions promoted by sulfate-reducing bacteria, a film of iron sulfide (FeS_x) was formed. The film forms a corrosion product on the steel, which is very brittle and leads to pits and cracks. A differentiated aeration cell forms above the cracks. Such processes further accelerate corrosion. As the biofilm develops, it begins to change the electrochemical and physical properties of the metal directly on the surface of the metal (Pal and Lavanya, 2022).

Another consequence of biofilm formation can be the saturation of the biofilm, which increases the pitting potential and thus contributes to the occurrence of localized corrosion and premature failure of the steel (Dexter and Chandrasekaran, 2000). The chemical composition of the biofilm boundary layer may also differ from the bulk of the solution, causing the aerated solution to form oxygen pockets in the biofilm. This biofilm structure can be a barrier to chemicals such as biocides or toxic copper ions (Javaherdashti, 2011).

Looking at the chemical composition of biofilms and corrosion products, the results of Duan et al., (2006) show that the inorganic sulfide species include sulfides of Fe, Cr and Mo, as well as organic sulfide species such as sulfur-containing proteins, amino acids and organic molybdenum sulfide. The resulting biofilm and corrosion products can appear on the surface of stainless steel exposed to sulfate-reducing bacteria for seven days. The reaction of the formed biofilm with the passive film and the substrate Fe produces metal oxide Fe_2O_3, which can be reductively dissolved by biogenic hydrogen sulfide, resulting in iron sulfide. Cytochrome and hydrogenase can participate in the reductive dissolution of iron oxide (Amirbachman et al., 1997). Independent of the local corrosion of the thin passive layer, bacterial cells can act directly on elemental Fe by reduction on the cell surface, e.g. by hydrogenase electron transfer, and thus play an essential role in corrosion processes and biomineralization (Da Silva et al., 2004).

Antony et al., (2007) found that the site of attack occurs mainly at locations with low Cr and Mo content in the steel, as biogenic sulfides trigger the attack on duplex steel. The initial systolic state of the bacteria tends towards metal corrosion, as it depends on the chemical elements of the steel alloy under investigation. Thus, the high content of Ni and N in the structure of stainless steel increases the binding of bacteria to the metal surface (Flint et al., 2000; Lopes et al., 2005). Direct dissolution of Cr_2O_3 with biogenic H_2S forms chromium sulfide, which indicates a possible

sulfidation of chromium oxide in the passive layer through contact with the substrate of sulfate-reducing bacteria. Duan et al., (2006) demonstrated the described phenomenon of passivity reduction on stainless steel 317L exposed to sulfate bacteria of the species Desulfovibrio desulfuricans.

On the other hand, mackinawite ($Fe_{1+x}S$) is generally regarded as the first FeS_x layer on the surface of stainless steel. It is formed by the activity of sulfate-reducing bacteria in water. It has also been hypothesized that the microstructure of pyrrhotite on the surface of stainless steel is due to the action of sulfate-reducing bacteria. Mackinawite is less stable than pyrrhotite under slightly acidic conditions, as pyrrhotite (Fe_7S_8) is stable in slightly acidic, neutral and alkaline environments ($5.3 < pH < 13.3$) (Jeffrey and Melchers, 2003). In contrast, pyrite is not a typical corrosion product for such experimental conditions. Although sulfate-reducing bacteria can produce pyrite from mechinavite in contact with sulfur or polysulfides, no pyrite is formed under the given experimental conditions (120 °C, pH=7.2-8.0, sulfate-reducing bacterial substrate) (Ilkin and Barner, 1996).

Morphology and chemical composition of biofilm and corrosion products

The surface morphology of stainless steel samples buried in soil for 136 days was studied with and without sulfate-reducing bacteria and chloride ion substrate (0.5%). The scanning electron microscopy (SEM) investigations show rough and fine-grained corrosion products on the surface of the tested steel. In some places, pitting was observed to tend to spread over the entire surface. It was also shown that the sulfur content as a corrosion product was much higher in the soil with sulfate-reducing bacteria than in the soil without sulfate-reducing bacteria. This indicates that FeS is present in the corrosion product, which increases the tendency to pit the steel. A high Mn and Si content and a low S and Fe content were also found in the corrosion products, indicating the onset of pitting near the site containing these elements. Stainless steel samples were placed in a soil substrate containing 3.0% Cl^- and sulfate-reducing bacteria, and after 136 days the concentration of sulfate-reducing bacteria was no longer detectable (Sun et al., 2011).

On the other hand, Antony et al., (2008) showed by microscopic examination that the entire surface was covered with a biofilm on the base metal after 40 days of exposure to sulfate-reducing bacteria. Loosely associated extracellular polymeric substances, cracks and pits were seen in biofilm and

stainless steel imaging. Microholes are determined in the ferrite phase, in the heat-affected zone. Microscopy shows a welded spot on steel with a fusion zone, i.e., the base metal zone, under the influence of heat. The microstructure consists of a solidified ferrite zone and transformed austenite, as dendrites, in the fusion zone. In the area affected by heat, the ferrite fraction increased significantly. It was also observed that the welding wire, with a higher Ni content, helped to achieve a balanced microstructure on the duplex surface in the fusion zone. At higher saturations of the solution with sulfate-reducing bacteria, the tested austenite and intragranular austenite growth was observed within the heat-affected zone. The ferrite phase is characterized by the appearance of chromium nitride (Cr_2N) (Antony et al., 2008).

Even though duplex steels are known for their corrosion resistance in a chloride environment, the influence mentioned above of the presence and activity of sulfate-reducing bacteria contributes to the wear of the passive layer (Song et al., 2018). Therefore, this work showed that the welded spot and sulfate-reducing bacteria could contribute to the depassivization of steel via the formation of the inevitable microstructure on the steel surface by controlling these properties. Microstructural studies revealed that the weld spot can cause an imbalance in the heating zone with higher fractions of ferrite than the base metal. Also, the metal welding wire has a high Ni content, which increases appearance of austenite fraction in the fusion zone. Due to the solidification of the welded area in the ferritic form, the cooling rate is higher because there is little time for the transformation to the austenite phase. This leads to the trapping of interstitial nitrogen in the ferrite phase and the formation of Cr_2N (Muthupandi et al., 2003; Chen and Yang, 2002). On the other hand, the transformation process from ferrite to austenite begins in connection with the saturation of Ni in the melting zone at high temperatures. It helps to achieve phase equilibrium and a microstructure free from precipitates in the fusion zone.

The initial concentration of bacteria attached to steel depends on the properties of the metal microstructure. The population of bacteria is higher in the fusion zone than the number of bacteria on the base (parent) metal. The formation of clusters of bacterial cells in the heating zone indicates that the bacteria tend to attach in certain places, as in this case, at the heat-affected zone/fusion zone phase boundary (Antony et al., 2008).

The austenitic phase of the base metal only corrodes in a medium with sulfate-reducing bacteria (Antony et al., 2007). The reformed austenite in the ferritic matrix remained intact at high Cr and Mo content compared to the austenite of the base metal. This can be explained by the low diffusion

of substitutional elements of the alloy during the transformation to the solid state (Antony et al., 2008). The authors pointed out that duplex steel with a higher Cr and Mo content and a defective microstructure may have better passivity in the environment with sulfate-reducing bacteria (Wan et al., 2023). Also, the appropriate choice of molten weld and welding parameters affects the distribution of alloys and the more difficult formation of Cr_2N, which may contribute to the passivity of the welded area (Antony et al., 2008). Chen and Yang, (2002) showed through experiments that the addition of nitrogen from the shielding gas can contribute to the reformed austenite of the heating zone by diffusion of nitrogen at the surface of the fusion zone/heat-affected zone interface, thereby avoiding the formation of Cr_2N in the ferrite phase.

According to Sreekumara et al., (2005), the combination of physical and chemical changes caused by stainless steel welding favors the accumulation of organic matter on the surface and the gradual binding of microorganisms to the metal. The morphological appearance of the corrosion products is masked by a biofilm, which, according to energy dispersive X-ray spectroscopy (EDS) analysis, consists of C, Ca, O, Si and P (Beech and Sunner, 2004). A cross-section of the sample shows further damage where the stainless steel is skeletonized.

A black-colored substance visible on the surface of a stainless steel sample (AISI 304) formed after an incubation period of 7 weeks in seawater containing the substrate *Desulfovibrio desulfuricans*. XRD analysis was used to determine the binding energies and composition of the biofilm on the surface of the investigated steel. The chemical composition of the *Desulfovibrio desulfuricans* biofilm was as follows: monosulfide, iron sulfide, chromium sulfide and molybdenum sulfide, then disulfide, polysulfide and organosulfide-like proteins, which can be formed by bacterial excretion during the experiment with a binding energy of 163 to 168 eV. Organic molybdenum sulfide (Mo(V)-S (cysteine)) was also detected in the passive layer during the described experiment (Nguyen et al., 2008).

The surface saturated with Mo was also considered when the stainless steel was exposed to an abiotic and biotic corrosion environment with a certain chlorine content (Bastidas et al., 2002). Molybdenum is usually found in the +4 and +6 oxidation states in the form of molybdenum chloride salts and MoO_4^{2-}, which can improve the pitting resistance of steel. The study indicated the presence of inorganic MoS in the passive film, which is more insoluble than the molybdenum chloride salt and can, therefore, be converted to molybdenum sulfide by reaction with biotic H_2S. Thus, organic sulfides, including organic molybdenum sulfide, also occur in the passive film. The

extracellular polymeric substance and the bacterial cells adhering to the stainless steel surface are the source of the organic sulfides. The Mo(V) complex can be formed in interaction with a hydrogenase (FeS_x protein) or a cysteine residue formed in the biofilm (Duan et al., 2006).

Based on the experimental data, the XPS results of Nguyen et al., (2008) on the influence of sisal bacteria on corroding steel are presented. The change in the chemical composition of the typical elements on the surface of the SS 304 steel under consideration (Fe and Ni) was determined. The biofilm on stainless steel samples formed under the action of sulfate-reducing bacteria shows the presence of elements such as Na, K, Cl and S. In this way, it was revealed that the biofilm can retain soluble and insoluble particles and metabolic products of sulfate-reducing bacteria.

By analyzing and recording C 1s spectra, three types of carbon bonds were identified: C-C, C-O or C-N, and C=O or C=N. The presence of the analyzed layer is also explained by the CHn hydrocarbon layer of 0.5 to 1 nm on the surface of the metal (Vinnichenko et al., 2002). The presence of the analyzed layer was attributed to production processes, preparation of the metal surface or contact with the atmosphere. The presence of C-O and C=O bonds is explained by polishing paste on the analyzed surface. C-N and O=C-N bonds were explained by proteins from the solution or bacterial cells (Nguyen et al., 2008). Also, in the presence of sulfate-reducing bacteria adsorbed on the metal surface, the presence of the O=C-N bond is explained by the presence of peptide chains of the protein components of the bacteria (Johansson and Saastamoinen, 1999), while the C-C bond indicates the accumulation of long aliphatic chains from bacterial cells on the stainless steel surface. The C=O bond indicates intracellular lipids, while the C-O bond may originate from extracellular polymers (Kusy et al., 2002).

The primary spectrum of O 1s includes the following compounds: metal oxides and hydroxides. A decrease in the oxide layer was observed with increasing concentration of sulfate-reducing bacteria. In addition, O-C=O and N-O-S bonds were detected on the stainless steel surface of the analyzed samples exposed to sulfate bacteria.

In the continuation of the experiment, analyzes of S 2p spectra were performed, and based on the results, a conclusion was drawn about the low sulfur content on polished and bacteria-free steel surfaces, in contrast to surfaces exposed to a solution of sulfate-reducing bacteria (Nguyen et al., 2008). Sulfates are formed from dissolved sulfate salts adsorbed on the metal surface, while sulfites are formed as intermediates during sulfate reduction. Precipitated FeS and FeS_2 are

corrosion products of iron substrates formed in artificially saturated salt water (Keresztes et al., 2001).

Organic sulfides are also present in biofilm as an integral part of the exopolymer. In sulfate-reducing bacteria, FeS_x proteins, such as the hydrogenases of *D. desulfuricans*, play an essential role in biological electron transfer processes and many enzymatic reactions (Keresztes et al., 2001). For this reason, the presence of organic sulfides in the biofilm of sulfate-reducing bacteria suggests that these bacteria play an essential role in biocorrosion by accelerating the cathodic reaction. There are specific changes in the chemical composition of the biolayer in the spectra of C 1s, O 1s and S 2p for the different samples.

The XPS spectra showed changes in stainless steel under the influence of sulfate-reducing bacteria for three elements: Fe, Cr and Ni. The outer oxide layer on the surface of SS 304 steel contains lower amounts of Ni, and Ni is obtained by dissolving the lower oxide layers, which are then exposed to the outer layer of the biofilm. On the other hand, the Fe-2p spectrum indicates higher concentrations of iron on the corroded surface under the influence of sulfate bacteria, suggesting that the passive film becomes thinner due to the dissolution of metal oxides in the studied electrolyte (Nguyen et al., 2008).

The curve corresponding to the S 2p spectrum shows the following compounds: monosulfide S^{2-}, disulfide S_2^{2-} and polysulfide (S_n^{2-}) as well as S^0, SO_3^{2-}, organic sulfide / SO_4^{2-}, and $S_2O_3^{2-}$. A passive film of FeO, Fe_2O_3, Cr_2O_3, $Cr(OH)_3$ and FeOOH formed on the steel surface exposed to sterile seawater and aerobic conditions (Yuan and Pehkonen, 2007). However, sulfidation of the passive layer by biogenic sulfide ions leads to a gradual loss of passivity of the steel.

Therefore, the studies above show that the corrosivity of steel samples with sulfate bacteria is higher than that of samples exposed to *Pseudomonas* bacteria, mainly related to biogenic sulfide anions (Yuan and Pehkonen, 2009).

Mechanisms of microbiological corrosion by sulfate-reducing bacteria

Due to the significant role of sulfate-reducing bacteria in microbial corrosion, many researchers are focusing on determining the corrosion mechanisms of steel by sulfate-reducing bacteria (Abdullah et al., 2014; Liu et al., 2019; Lv et al., 2019). To date, different versions of the

corrosion mechanisms of stainless steel by sulfate-reducing bacteria have been developed, specifically cathodic and anodic depolarization (Nguyen et al., 2008).

The mechanism that explains the metal corrosion caused by sulfate-reducing bacteria is the consumption of hydrogen produced at the cathode by the enzyme hydrogenase. Stott, (1988) pointed out that the main effect of sulfate-reducing bacteria is the elimination of hydrogen from the corroding metal, which refers to the hydrogenase reversibly catalyzing the activation of hydrogen. The classical theory of cathodic depolarization is represented by reactions occurring in three domains: metal, solution and microorganisms, which is given by equations 1, 2, and 3.

In the absence of oxygen, the cathode surface of the metal is rapidly polarized by atomic hydrogen. Under anaerobic conditions, an alternative cathodic reaction, such as oxidation by gaseous or free oxygen atoms, is not possible. Under such conditions, water dissociation occurs, so this reaction, together with the cathodic reaction of the adsorbed hydrogen ions produced, is the most crucial cathodic reaction. The products of the cathodic reactions are then adsorbed on the metal surface (polarization) and consumed by the hydrogenase. The classical theory of microbiological corrosion of metals by sulfate-reducing bacteria is shown schematically in Fig. 1. The bacterial cells are shown separately; in reality, they live directly on the surface of the iron. The reduced hydrogen is transported from the iron to the bacteria at the cathode and used to reduce sulfate to sulfide. Only a quarter of the dissolved iron(II) ions react stoichiometrically with H2S to form FeS at the anode. In the presence of CO_2 and bicarbonate, the Fe^{2+} residue transforms into $FeCO_3$; without bicarbonate, $Fe(OH)_2$ precipitates (Song et al., 2018; Victoria et al., 2021).

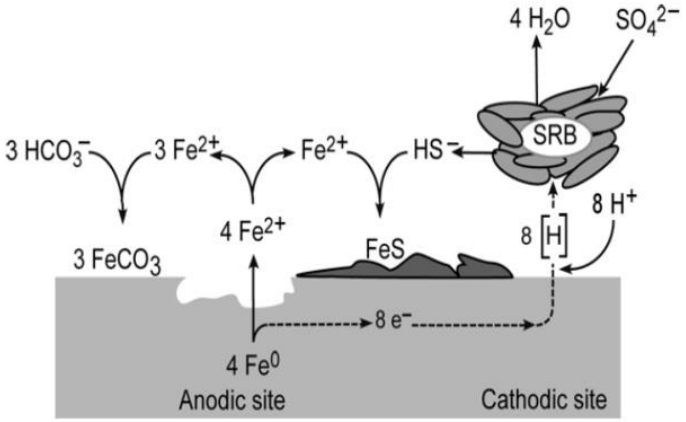

Figure 1. Schematic representation of the classical theory of cathodic depolarization by SRB activity (Hang, 2003).

The most critical corrosion mechanisms of sulfate-reducing bacteria are:

1. Cathodic depolarization mechanism: sulfate-reducing bacteria produce sulfates that oxidize adsorbed H^+ under anaerobic conditions to accelerate corrosion and release hydrogen. The reactions proceed as follows (Javaherdashti, 2011):

$$Anode: 4Fe^0 \rightarrow 4Fe^{2+} + 8e^- \quad (1)$$

$$Cathode: 8H^+ + 8e^- \rightarrow 8H_{ad} \quad (2)$$

$$Cathodic\ depolarization: SO_4^{2-} + 8\,H_{ad} \rightarrow S^{2-} + 4H_2O \quad (3)$$

$$Ionization\ of\ water: 8H_2O \rightarrow 8H^+ + 8OH^- \quad (4)$$

$$Corrosion\ products: Fe^{2+} + S^{2-} \rightarrow FeS \qquad 3Fe^{2+} + 6OH^- \rightarrow 3Fe(OH)_2 \quad (5)$$

$$Overall\ reaction: 4Fe^0 + SO_4^{2-} + 4H_2O \rightarrow FeS + 3Fe(OH)_2 + 2OH^- \quad (6)$$

According to Kuhr's theory, hydrogenase and hydration of the bacterial cell can promote depolarization and accelerate corrosion.

2. The concentration cell mechanism: This mechanism shows that a concentration cell forms when corrosion products, such as iron hydrate, form on the metal surface. In most cases, this type of corrosion would form an area of hypoxia near the metal surface under anaerobic conditions and create suitable conditions for the growth of sulfate-reducing bacteria that accelerate corrosion.

3. Mechanism of metabolites: Anaerobic corrosion of sulfate-reducing bacteria results from phosphide, which is highly active and volatile and is produced by the metabolism of sulfate-reducing bacteria. Phosphides can react with iron compounds or H_2S to form iron phosphide, which enhances corrosion.

4. Other mechanisms (Zhang et al., 2011): (a) extracellular polymers of sulfate-reducing bacteria react with the metal to form Fe^{2+}, which oxidizes to Fe^{3+}, accelerating corrosion; (b) under anaerobic conditions, the accumulation and release of H_2O_2 in the biofilm participate in the electrode reaction of steel, causing an increase in electrode potential and leading to localized corrosion; (c) sulfate-reducing bacteria attack the metal by attacking the grain boundary and selectively releasing austenite; d) the oxygen present reacts with sulfur and leads to the formation of intermediates, accelerating the corrosion process; e) sulfide ions (S^{2-}) react with iron to form FeS, which is the cathode, while Fe is the anode; the cathodic depolarization reaction, which releases hydrogen, can corrode the metal surface.

Based on the proposed mechanisms, there are no unified views, so further investigation of possible mechanisms of microbiological corrosion of sulfate-reducing bacteria on stainless steel should be continued, mainly focusing on the most promising mechanism of cathodic depolarization.

The authors Videla and Herrera, (2005) summarized a new picture of the microbiological mechanisms of corrosion by sulfate-reducing bacteria, which includes the following phases:

- In a saline environment, steel dissolves at high Fe^{2+} concentrations and forms a film of iron hydroxide whose thickness and protective properties depend on the solution's pH.
- Anion adsorption processes, which take place in the boundary layer between the metal and the solution, have a further influence on the acceleration or retardation of corrosion.
- The physicochemical properties of the iron sulfide film can control the effect of sulfide on steel dissolution, which depends on the iron/sulfide anion ratio, the presence of sulfate-reducing bacteria and the coverage of the metal surface with biofilm.

Research by Hang, (2003) has also shown that different types of bacteria play a significant role in corrosion processes. A solution of sulfate-reducing bacteria was tested, enriched directly with iron and sulfate and a CO_2/bicarbonate buffer. The bacterial strains used iron, lactate and pyruvate for sulfate reduction. In the presence of iron, the strains of *Desulfobacterium corrodes* reduce sulfate much faster than *Desulfovibrio*. In contrast, sulfate reduction slows down in the presence of hydrogen and lactate compared to *Desulfovibrio* species. This work indicated a new species of *Desulfovibrio ferrophilus*, which can reduce sulfates faster than other Desulfovibrio species in the presence of iron but slower than *D. corrodens*.

The complete genome of *Desulfovibrio vulgaris* encodes oxidase, oxidoreductase, plasmid-encoded catalase and superoxide dismutase genes. Analysis of the incomplete genome sequences of gram-positive *Desulfitobacterium hafniense* reveals genes encoding three catalyzes, one of which is extracellular. These catalyzes are homologs of hydroperoxidase I and II. The genome of *Desulfovibrio vulgaris* encodes several proteins that are similar to the chemerythrin domain and contain Fe or O. Overall, the genome sequence of *Desulfovibrio vulgaris* indicates the presence of 27 methyl-accepting chemotaxis proteins, which include the oxygen- or redox-potential-responsive proteins DcrA and DcrH (Beech and Sunner, 2004). These proteins may be necessary for sulfate-reducing bacteria on oxygenated or non-oxygenated surfaces.

The development of biofilms is promoted by producing an extracellular polymeric substance containing macromolecules such as proteins, polysaccharides, nucleic acids and lipids. The ability of the exopolymeric substances to bind metal ions depends on the type of bacteria and metal ions (Rohwerder et al., 2003). The binding between the exopolymeric substance and the metal occurs via the bond between metal ions and anionic functional groups (carboxyl, phosphate, sulfate, glycerol, pyruvate and succinate groups) common to the proteins and carbohydrate compounds of the exopolymer. The affinity of anionic ligands can be vital for multivalent ions such as Ca^{2+}, Cu^{2+}, Mg^{2+} and Fe^{3+}. The presence and affinity for metal ions with different oxidation numbers in the biofilm can constantly change the standard reduction potential. For example, the electrode potential of Fe (III/II) changes with the change of ligands (from +1.2 V to -0.4 V). Extracellular polymeric substances bound to metal ions can act as electron acceptors and thus contribute to redox reactions in the biofilm matrix, e.g. by direct electron transfer from metals (e.g. Fe) or biominerals (e.g. FeS). In the presence of a specific acceptor (e.g. O_2 under aerobic conditions or nitrate under anaerobic conditions), the redox pathway would lead to a depolarization of the cathode and thus intensify the corrosion. A schematic model of the corrosion reactions of ferrous metals, which includes the binding of exopolymeric substances to metal ions in an oxidized biofilm, can be found in the work of Beech and Sunner, (2004).

Studies of the Fe-hydroxide biofilm layer have shown that bacterial exopolymers and acidic polysaccharides can occur in acagenite (b-FeOOH) (Chan et al., 2003). It has been demonstrated that polymer production aims to localize the precipitation of iron oxyhydroxide immediately outside the cell to increase the energy of cell metabolism by accelerating proton movement. In the presence of iron, the Fe-oxyhydroxide surface is associated with biofilm polymers to adsorb Fe(II) ions, leading to their oxidation and contributing to the cathodic reaction (Beech and Sunner, 2004). It is well known that catalysis of the cathodic proton/water reaction occurs mainly on an iron alloy under anaerobic conditions (Stott, 1993):

$$2H^+ + 2e^- \rightarrow H_2 \text{ or } 2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (7)$$

The production of metabolites of sulfide ions activates sulfate-reducing bacteria:

$$SO_4^{2-} + 4H_2O + 8e^- \rightarrow S^{2-} + 8OH^- \quad (8)$$

This forms a FeS deposit that catalyzes the metal surface's cathodic reaction between protons and water. However, the mechanism of anaerobic biocorrosion is more complex than can be seen in the work of Beech and Sunner, (2004). Hydrogen consumption by sulfate-reducing bacteria cannot

have a direct effect on the corrosion rate because reaction 8 can be decomposed into a Vollmer reaction (Lee et al., 1995):

$M + H_2O + e^- \leftrightarrow M\text{-}H_{ads} + OH^-$ (9)

which is followed by the Tafel reaction:

$2M\text{-}H_{ads} \leftrightarrow 2M + H_2$ (10)

or Hierovski's reaction:

$M\text{-}H_{ads} + H_2O + e^- \leftrightarrow M + H_2 + OH^-$ (11)

Tafel and Hierowski reactions are limiting reactions on the surface of the iron alloy. The consumption of generated hydrogen cannot accelerate them. Sulfate-reducing bacteria have an advantage in hydrogen production during corrosion processes (reaction 7) and use it as an electron donor that supports sulfide production (Mehanna et al., 2009). Hydrogenase enzymes produced by sulfate-reducing bacteria can be adsorbed on the steel surface, catalyzing the reduction of protons (Da Silva et al., 2002), and the presence of phosphate buffers can cause an additional cathodic reaction.

On the other hand, EDS spectra analysis shows the entry of active chloride ions (Cl^-) under the biofilm and the lowering of the pH value in the anode area when $FeCl_3$ is formed. This favored initiating and propagating pitting corrosion (Yuan and Pehkonen, 2007). The anodic reaction describes the effect of chloride ions on the passive layer of stainless steel (Yuan and Pehkonen, 2009):

$Fe^0 \rightarrow Fe^{2+} + 2e^-$ (12)

$Fe^{2+} + 2H_2O + Cl^- \rightarrow Fe(OH)_2 + 2HCl$ (13)

$Fe(OH)_2 + 3Cl^- \rightarrow FeCl_3 + 2OH^- + e^-$ (14)

$FeCl_3 + 3H_2O \rightarrow Fe(OH)_3 + 3HCl$ (15)

Chloride ions react with the oxyhydroxide layer and displace OH^- ions in the oxide layer until the soluble product $FeCl_3$ is formed. The intermediate product $FeCl_3$ is hydrolyzed to the porous precipitate $Fe(OH)_3$ under decreasing pH at the original corrosion site. Such reactions result in a self-reinforcing or autocatalytic mechanism of hole growth.

On the other hand, the EDS spectrum indicates a synergistic interaction of active biogenic sulfide and chloride anions that are responsible for the initiation of grey spot corrosion on the steel surface exposed to sulfate bacteria. It is generally concluded that chloride ions can catalyze sulfide dissolution, which causes metastable pitting corrosion in stainless steel. The anodic dissolution of

sulfide ions (S^{2-}) in the marine environment is represented by the following reactions (Yuan and Pehkonen, 2009):

$$Fe^0 + H_2S \rightarrow FeSH^-_{ads} + H^+ \quad (16)$$

$$FeSH^-_{ads} \rightarrow FeSH^+_{ads} + 2e^- \quad (17)$$

$$FeSH^+_{ads} \rightarrow FeS_{1-x} + xHS^- + (1-x)H^+ \quad (18)$$

$$Fe^0 + 2Cl^- + H_2O \rightarrow [Fe(OH)^+ + Cl^-] + HCl + 2e^- \quad (19)$$

$$Fe(OH)^+ + HS^- \rightarrow FeS + 2H_2O \quad (20)$$

Electrochemical investigations of the corrosion rates of stainless steel by sulfate-reducing bacteria under different experimental conditions

Javed et al., (2022) investigated the corrosion effect of sulfate-reducing bacteria on stainless steel samples, UNS30400, exposed to sulfate-reducing bacteria and conditions where no sulfate-reducing bacteria existed. The samples that exhibited pitting corrosion were exposed to an environment containing iron ions and an environment containing chloride ions for 32 days under anaerobic conditions and at a temperature of 30 °C. The electrochemical tests show that the difference between the passivity breakdown potential, E_b and E_{corr} for the 304 stainless steel was smallest for the samples exposed to sulfate-reducing bacteria and a solution containing chloride ions. Based on the criteria of Pardo et al., (2000), the authors concluded that pitting corrosion occurs on UNS30400 stainless steel specimens due to the interaction of sulfate-reducing bacteria and a relatively high salt concentration in the tested medium.

The influence of sulfate-reducing bacteria on the corrosion behavior of the X80 steel pipeline in acidic soil was investigated using electrochemical impedance spectroscopy (EIS). The formed biofilm of sulfate-reducing bacteria developed over the entire surface of the tested steel in a thin layer of 20μm, and after 14 days, the number of living sessile cells was more significant than the number of dead cells of the sulfate-reducing bacteria. Under the abiotic conditions of the formed biofilm, an EIS analysis was performed, according to which the size of the semicircle of the Nyquist plot decreases with time, indicating an acceleration of the corrosion processes over time. However, under the conditions of the inoculated soil solutions, the activity of the sulfate-reducing bacteria and their metabolic products inhibit the corrosion of the steel during the first four days of the experiment and then accelerate the corrosion until the end of the experiment (Chen et al., 2021).

The authors Sun et al., (2011) investigated the corrosion effect of sulfate-reducing bacteria (23000-35000 cells/g soil) on stainless steel samples, 1Cr18Ni9Ti, buried for 136 days and the addition of a particular concentration of chloride ions to the soil. The work showed that the electrode potential (Eh) decreases with increasing chloride ion concentration, which is explained by the acceleration of the activity of sulfate-reducing bacteria in the soil. These results also indicate that the dissolved oxygen in the soil decreases with increasing Cl^- concentration, which is suitable for the respiration of the sulfate-reducing bacteria.

In addition, the authors presented the relationship between the corrosion potential (mixing potential) and the concentration of chloride ions in the soil with and without sulfate-reducing bacteria injection. In both cases, the corrosion potential of stainless steel decreases with increasing chloride ion concentration. However, the potential of steel in soils with sulfate-reducing bacteria is more negative than the potential of steel samples in soils without sulfate-reducing bacteria, suggesting that the presence of sulfate-reducing bacteria increases the tendency of steel corrosion. The same authors showed changes in corrosion rates and maximum pitting depth of stainless steel as a function of Cl^- concentration, with and without injection of sulfate-reducing bacteria into the soil. It was found that higher corrosion rates occur at lower Cl^- concentrations (1.0%) in the presence of sulfate-reducing bacteria when hydrogenase is also observed. Hydrogenase from sulfate-reducing bacteria consumes hydrogen atoms on the steel surface and thus accelerates the cathodic depolarization reaction (Guo et al., 1992). However, when the Cl^- concentration increases to 2.0%, a constant corrosion rate of the steel in the soil occurs.

Sun et al.'s, (2011) experimental results show that the maximum depth of pits formed on steel with sulfate-reducing bacteria is more remarkable than without sulfate-reducing bacteria. This indicates that sulfate-reducing bacteria increase the pitting tendency. It has also been shown that stainless steel corrosion does not occur when the Cl^- concentration is 0.05% in soils with sulfate-reducing bacteria.

Considering that we are dealing with soil that contains pores, it is clear that the contact area between the steel and the soil particles has a low oxygen content, while the area of the boundary layer between the steel and the pores is in an area with a high oxygen concentration. The metal corrosion that occurs under the influence of unequal oxygen concentrations is called corrosion by differential aeration (Vujičić, 2002). The sites mentioned above may be available for the growth of sulfate-reducing bacteria. As is known, sulfate-reducing bacteria reduce sulfates to sulfide

(FeS), sulfates being electron acceptors under anaerobic conditions. Sites on the surface of stainless steel where FeS accumulates act as cathodes, forming a new electrochemical cell (Iv et al., 1984). Therefore, the passive layer is no longer stable due to increased chloride ion concentration.

EIS results showed that the corrosion processes of stainless steel in the presence of sulfate-reducing bacteria are controlled by concentration polarization (Sheng et al., 2007). Based on the impedance values of the film resistance (R_f), they concluded that the number was higher in soil samples with sulfate-reducing bacteria than in other samples without sulfate-reducing bacteria. On the steel surface occured complex film of a corrosion products and bacterial biofilm, and the compactness of the film thus formed is better than the compactness of the single-layer film.

Effective prevention of surface reactions by the film formation was also found. Feng et al., (2008) showed that the film formed on steel in soil with sulfate-reducing bacteria is less stable than the film formed on steel without sulfate-reducing bacteria. This indicates that the complex film gradually degrades when the Cl^- concentration exceeds 1.0%. Thus, sulfate-reducing bacteria increase the pitting tendency of stainless steel, 1Cr18Ni9Ti. The author concludes that pitting corrosion is due to the influence of chloride and sulfide ions in the soil, which are metabolic products excreted by sulfate-reducing bacteria. In other words, sulfate-reducing bacteria increase the susceptibility of steel to pitting corrosion in Cl^- containing soils.

On the other hand, a thermomechanical process such as welding can form undesirable phases on the steel if not carried out under suitable conditions (Tavares et al., 2007). For example, Antony et al., (2008) investigated the influence of microstructure formation of the weld in tungsten gas on duplex steel (2205) under anaerobic conditions with Cl^- and sulfate-reducing bacteria as substrate. The chemical composition of the welded area consists of Ni and Mo, with a content of more than 2%. The duplex steel samples were exposed to an aqueous medium with a pH of 7.5 and a temperature of 30 °C for 40 days. The shift in corrosion potential, E_{corr}, relative to the active value of the potential, which remained constant until the end of exposure of the steel samples, was indicated. Similar E_{corr} variations were determined for passivated steels in an environment with sulfate-reducing bacteria (Xu et al., 2008). In the work of Antony et al. (2008), it was also shown that the change in sulfide concentration in the given environment does not affect the E_{corr} value. It was revealed that the E_{corr} value reaches a particular active value even at low sulfide concentrations in the reactive environment. In contrast, the E_{corr} value in sterile samples indicates an improvement

in metal passivity compared to samples exposed to an environment with sulfate-reducing bacteria during the 40 days of the experiment (Antony et al., 2008).

The electrochemical data show no significant difference between the base metal and the spot weld. However, the spot has higher anodic currents and a lower E_{corr} value than the base metal. Nevertheless, the destruction of the passive layer of the base metal and the welded spot increased when exposed to sulfate-reducing bacteria compared to a sterile environment.

The current value increases with the potential differences for all samples for the three distinct phases formed on the base metal. The current density of samples previously exposed to sulfate-reducing bacteria for 40 days is twice as high as the current density of samples exposed to a sterile medium during the same period. This indicates a strong influence of sulfate-reducing bacteria on the increase of corrosion of steel 2205. The current density of the samples before exposure was an order of magnitude lower than that of the samples in a sterile environment for the same period. In each of the three different environments (before and after exposure in a sterile environment and in an environment with sulfate-reducing bacteria), the weld exhibited a higher current density at all potentials than the base metal's current density. In the two steel samples previously exposed to an environment with sulfate-reducing bacteria, the current density of the welded area increases faster at higher potentials than the current density of the base metal (Antony et al., 2008).

Continuous exposure of SS 316L samples to sulfate-reducing bacteria and a combination of sulfate-reducing bacteria and ferric bacteria results in a significant decrease in E_{corr} and the steel's polarization resistance, R_p, and corrosion acceleration compared to the observed samples exposed only to ferric bacteria or sterile medium for the same time interval. During the additional exposure to the solution with sulfate-reducing bacteria and iron bacteria, E_{corr} was strongly reduced, and a value of -0.54 V was reached for 24 days. The results thus indicate the metabolic activity of bacteria and its influence on corrosion processes. The presence of sulfate-reducing bacteria led to higher corrosion rates than iron-containing bacteria. Still, it was also shown that the combination of sulfate-reducing and iron-containing bacteria led to higher corrosion rates than the single effect of the same bacteria (Zhang et al., 2007).

Similarly, based on graphical EIS methods, the authors Nguyen et al., (2008) found that the polarization resistance of SS 304 steel is reduced in the presence of sulfate-reducing bacteria. This indicates that the metabolic products of sulfate-reducing bacteria cause changes in the electrochemical properties of steel (Sheng et al., 2007).

In addition, diffusion processes occur within the biofilm, involving bacterial cells and their insoluble products, such as metal sulfides in solution. Impedance parameters indicate the difference between abiotic and biotic corrosion. The first difference between abiotic and biotic corrosion is the charge transfer resistance (R_{ct}), whose value is high at 805.6 Ω in the presence of sulfate-reducing bacteria compared to 600 Ω in the environment without sulfate-reducing bacteria. In addition, the roughness coefficient n for stainless steel in a sterile environment is around 1, indicating a relatively homogeneous passive film. A lower coefficient value of 0.60 for the passive film in the presence of sulfate-reducing bacteria indicates the roughness of the passive film. Also, forming an anaerobic film of stainless steel in a saturated artificial seawater solution with a substrate of sulfate-reducing bacteria leads to a reduction in the resistivity of the passive film, R_{pf}. The decrease in the resistivity and roughness parameters of both types of coatings, the steel and the passive film indicate two critical points. First, sulfate-reducing bacteria and their products can be adsorbed on the metal surface or become part of the passive film, changing the charge of the Me/solution interface and the passive film, which more easily leads to metal surface corrosion. Secondly, the EIS results show that the biofilm plays a vital role in metal corrosion, and this has been confirmed by other authors (Wang and Liang, 2008; Xu et al., 2008).

Conclusion

Based on various tests of stainless steel with sulfate-reducing bacteria, corrosion of the tested metal was demonstrated during a very short period of exposure to these bacteria under anaerobic conditions.

It was shown that sulfate-reducing bacteria lead to more severe pitting corrosion of SS 304 passive steel than *Pseudomonas* bacteria. When investigating metal corrosion, it is not the number of sulfate-reducing bacteria but the metabolic products of the bacteria that may be of greater importance. The exopolymeric substance is also important for binding the cells in the biofilm structure.

The weld on the steel allows access to and binding microorganisms, i.e., grease and oil deposits located on the pipe wall, which could be the primary source of the carbon needed for the bacteria's development and growth. In sulfate-reducing bacteria, FeSx proteins, such as the hydrogenases of

D. desulfuricans, play an essential role in biological electron transfer processes and many enzymatic reactions.

Pitting corrosion is caused by the influence of Cl^- and sulfides in the soil, which are produced by the secretion of sulfate-reducing bacteria. In other words, sulfate-reducing bacteria increase steel's susceptibility to pitting in Cl^- containing soils. Regardless, it was found that the destruction of the passive layer of the base metal and the welded area was greater when exposed to sulfate-reducing bacteria than in a sterile environment.

The sulfate-reducing bacteria resulted in a higher corrosion rate than iron-containing bacteria. Still, it was also shown that the combination of sulfate-reducing and iron-containing bacteria resulted in higher corrosion rates than the single effect of the same bacteria.

Despite the significant research in the field of microbiological corrosion, many questions remain unanswered, such as the influence of biofilms on the electrochemical properties of metals. For this reason, many authors believe that the study of sulfate bacteria on stainless steel should be continued.

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Conflict-of-Interest Statement

Declarations of interest: none

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