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The mutual co-regulation of extracellular polymeric substances and iron ions in biocorrosion of cast iron pipes

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HIGHLIGHTS

- A moderate concentration of iron ions (0.06 mg/L) promoted the production of EPS.
- EPS accelerated corrosion at the initial stage, but inhibited corrosion at the later stage.
- Functional groups in the EPS acted as electron shuttles to bind iron ions.
- The corrosion inhibition of EPS was correlated to phosphorus and corrosion products.

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ABSTRACT

New insights into the biocorrosion process may be gained through understanding of the interaction between extracellular polymeric substances (EPS) and iron. Herein, the effect of iron ions on the formation of biofilms and production of EPS was investigated. Additionally, the impact of EPS on the corrosion of cast iron coupons was explored. The results showed that a moderate concentration of iron ions (0.06 mg/L) promoted both biofilm formation and EPS production. The presence of EPS accelerated corrosion during the initial stage, while inhibited corrosion at the later stage. The functional groups of EPS acted as electron shuttles to enable the binding of iron ions. Binding of iron ions with EPS led to anodic dissolution and promoted corrosion, while corrosion was later inhibited through oxygen reduction and availability of phosphorus from EPS. The presence of EPS also led to changes in crystalline phases of corrosion products.

1. Introduction

Many older pipes in drinking water distribution systems are made of materials such as unlined steel, cast iron, or ductile iron, which are subject to corrosion. Pipe corrosion can result in adverse effects such as pipe clogging, decreases in the water supply, bacterial regrowth, and "red water" or "black water" (Sarin et al., 2004). As a result, high-quality water that originates from drinking water treatment plants can suffer from secondary pollution.

Pipe corrosion in drinking water distribution systems is correlated to biofilm formation, also referred to as biocorrosion. Biocorrosion has been the subject of extensive studies because of its economic and environmental impact, and several models have been proposed to explain its underlying mechanisms (Vu et al., 2009). According to these models, biocorrosion is a synergistic interaction between a metal surface, abiotic corrosion products, and bacterial metabolites. However, these models do not consider the role of extracellular polymeric substances (EPS) in the electron transfer process (Busalmen et al., 2002), despite the fact that metal ions bound by EPS can catalyze cathodic reactions (Jin et al., 2014). EPS play a key role in facilitating cell attachment to cast iron surfaces and in biofilm development. EPS are believed to be polymeric conglomerations consisting mainly of proteins, polysaccharides, and lipids. They play an important role in biocorrosion of cast iron because of their biosorption ability to iron ions (Li et al., 2012). Iron binding by EPS involves interactions between iron ions and the anionic functional groups of the carbohydrate and protein components in EPS. Anionic functional groups in polysaccharides include the carboxyl groups of uronic acids and non-carbohydrate substituents, such as phosphate, sulfate, glycerate, pyruvate, and succinate (Huo et al., 2014). Proteins rich in acidic amino acids, including aspartic and glutamic acid, contain carboxyl groups that also contribute to

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the anionic properties of EPS (Zhou et al., 2013). Some controversy exists about the role of EPS in biocorrosion process. In general, EPS contain functional groups that can bind metals easily. Metal ions concentrated from drinking water or the surface of the metal pipe into the biofilm increase corrosion rates through the enhancement of cathodic reactions. The weak electrostatic interactions between EPS and metal ions have been shown to accelerate metal corrosion (Busalmen et al., 2002). Several reports have described the inhibition of corrosion by EPS. The EPS of *Desulfovibrio vulgaris* was found to promote the corrosion of mild steel, while those of *Desulfovibrio alaskensis* inhibited corrosion due to the absence of oxygen in the biofilm (Stadler et al., 2008). Finkenstadt et al. (2011) showed that purified *Leuconostoc mesenteroides* EPS inhibited the corrosion of low-carbon steel. Dong et al. (2011) investigated the effect of EPS isolated from thermophilic sulfate-reducing bacteria on carbon steel corrosion and found that the adsorbed EPS layers hindered the reduction of oxygen, thereby slowing corrosion, but simultaneously stimulated the anodic dissolution of the underlying steel through chelation of Fe^{2+} ions.

Concentration of iron in the biocorrosion environment affect the formation of the biofilm and the composition of EPS. Iron plays an important role in the formation of the biofilm through the quorum sensing system. Low iron concentrations favor DNA release and biofilm formation. Increased iron concentrations result in suppression of both DNA release and the development of a structural biofilm (Yang et al., 2007). Iron may reduce the production of extracellular polysaccharides by interfering with sucrose metabolism. Moreover, iron ions and their corresponding hydrolysis products can adsorb onto EPS, which not only promotes the formation of EPS, but also changes the isoelectric point of proteins (Baldi et al., 2009).

Iron is typically not available in the ecosystem because it forms oxy-hydroxides. As a result, iron acquisition is difficult for many aerobic organisms found in drinking water. Indeed, the redox chemistry of iron plays an important role in its speciation. In oxic freshwaters, oxidized iron is thermodynamically-stable (Perdue et al., 1976). The concentration of both free and complexed dissolved ferric iron tends to be very low in the majority of surface waters (pH ~6–8), as extensive hydrolysis leads to the precipitation of insoluble (particulate/colloidal) oxides and hydroxides. While dissolved iron oxides may sometimes be detected in drinking water, this may be attributed to the presence of organic complexes of ferric iron or small hydrous ferric oxide particles coated with natural organic matters. However, there are environments in which iron is abundant due to the presence of a large amount of ferric hydroxides, which produce a rusty color. One such environment is the biocorroded surface of a cast iron pipe. In biocorrosive ecosystems, iron affects EPS production, while EPS play a significant role in iron release and biocorrosion. Understanding this interaction of EPS and iron may contribute to a better understanding of the complexity of the biocorrosion micro-interface.

In this study, the interaction of EPS and iron ions was investigated. The effect of iron ions on heterotrophs and EPS production within biofilms was explored. In addition, the effect of EPS on the release of iron and the corrosion of cast iron was examined. EPS were extracted from the biofilm at different stages of incubation to determine the effect of EPS composition on the corrosion of cast iron. Based on observations of the interaction between EPS and iron ions, a hypothesis was proposed to explain the pathway of biocorrosion.

2. Methods

2.1. Bacterial culture and biofilm growth

Biofilm samples were taken from cast iron pipes in a drinking water distribution system (Shenzhen, China) with a sterile brush and suspended in 10 mL 0.85% NaCl. The suspension was used as the inoculum and was cultured in four 1 L beaker reactors with polyethylene (PE) coupons (length of 15 cm and width of 1.5 cm) placed vertically along the wall of the beaker. PE, rather than cast iron, coupons were used to eliminate the presence of extrinsic iron ions from the corrosion of cast iron. Ferric ions were dosed into the beakers at concentrations of 0.03 mg/L (reactor A), 0.06 mg/L (reactor B), and 0.1 mg/L (reactor C). The final reactor was not dosed with ferric ions and was used as the control (reactor D). Sodium acetate was added as the carbon source to produce an assimilable organic carbon (AOC) level of 0.5 mg/L. The growth of biofilm has been shown to be sensitive to the AOC levels, with maximum growth occurring at an AOC level of 0.5 mg/L, and the quantity of biofilm was slightly affected when the AOC level was greater than 0.5 mg/L. (Tsai et al., 2004). Phosphorus buffer solution (5 μmol/L NaH_2PO_4 and 45 μmol/L Na_2HPO_4) was used as the phosphorus source and to achieve a pH of 8.0. The phosphorus level was maintained at 10 μg/L because levels of more than 5 μg/L favor rapid biofilm formation in drinking water as the content of ATP is increased at these concentrations (Lehtola et al., 2002). Salinity is another important factor for the growth of biofilm; the concentrations of chlorine and sulfate ions may not be higher than 250 mg/L according to the drinking water standard of China. An appropriately-simulated salinity is important to obtaining accurate results in corrosion experiments. Our previous studies have shown that chlorine and sulfate ions have particularly serious impacts on the corrosion of cast iron (Jin et al., 2013). In addition, increasing the ionic strength of the solution enhances bacterial adhesion to metal surfaces due to the strengthening of attractive electrostatic forces (Magalhaes et al., 2005). Thus, it was necessary to control the salinity in this study. A final concentration of 500 mg/L NaCl was chosen to simulate the salinity and the total ionic strength of drinking water. All solutions were autoclaved at 121 °C for 20 min before use. The water in the reactors was displaced with new simulated water every 2 days. Beakers were sealed with sterilized gauze and placed in a clean room to maintain sterility.

Beakers were stirred magnetically at a constant speed (800 rpm) and were covered with a black cloth to protect them from light exposure. PE coupons were removed from the beakers on days 7, 15 and 30. Biofilm on the surface of the PE coupons was removed with a small sterile brush and suspended in 2 mL deionized water for heterotrophic plate counts (HPCs) and EPS extraction. The bulk water in the reactors was also sampled (100 μL) for HPCs measurement on days 7, 15, and 30. The sampling process was performed in an aseptic work station (at UVC/T-M-AR, Beijing Funuo, China) in order to maintain a strict aseptic environment so as to avoid the introduction of bacteria to the solutions.

Biofilm samples were decimally diluted to 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} gradients. The diluted biofilm was shaken by hand for 5 min, and 100 μL of solution was spread on R_2A agar for HPCs testing. Colonies were enumerated after 7 days of incubation at 20 °C. The average value of three parallel samples was reported. For the bulk water of the reactor, 100 μL of water was spread directly on R_2A agar without dilution. The biofilm solutions were heated in a 60 °C water bath for 40 min and then centrifuged at 12,000*g* at 4 °C for 20 min to release EPS (Mishra and Jha, 2009). The supernatant was concentrated to 100 mL in an 80 °C water bath. Dissolved EPS were purified by dialysis for 1 day against distilled water using a 10 kDa molecular weight cutoff dialysis membrane (Sangon Biotech, China), and the purified EPS was lyophilised at −60 °C for 24 h. Polysaccharide and protein content of EPS were analyzed. Each sample was measured in duplicate, and concentrations are presented as $\mu g/cm^2$. The purified EPS were analyzed by Fourier transform infrared (FTIR) spectroscopy and transmission electron microscopy (TEM).