Influence of Cr^{3+} on microbial cluster formation in biofilm and on steel corrosion

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Abstract

Pit corrosion of mild steel in seawater increased with Cr^{3+} concentration. SEM observations showed that increasing Cr^{3+} concentration caused microbes in biofilm on the steel surface to aggregate forming clusters. AFM images suggested that pit corrosion occurred largely on the mild steel surface between clusters, and only little corrosion on the surface covered by microbes.

Introduction

In aquatic environment, microorganisms tend to colonize on solid surface to form biofilm. This could cause biofouling in heat exchanger, ship hull, dental, etc. (Characklis & Marshall 1990). The biofilm can also cause deterioration for the solid surface, be it metal, polymer or concrete. However, little is known about the mechanism of biofilm formation and its controlling factors. Some suggested that biofilm formation could be determined by the metallurgical characteristics, such as surface free energy, surface roughness, hydrophobicity (Muller *et al.* 1992) and grain boundary (Geesey *et al.* 1996). On the other hand, it can also be dependent on the surface characteristics of the microbes and the extracellular polymeric substances (EPS) excreted by the microbes. The EPS, consisting primarily of polysaccharides and proteins, contain functional groups that can bind metal ions (Geesey *et al.* 1988, Paradies 1995), and can also affect the electrochemical character of metals and play an important role in the biocorrosion of metals (Roe *et al.* 1996).

In an anaerobic sulphate-rich environment, such as in the sea-bed, sulphate-reducing bacteria (SRB) capable of using sulphate as electron acceptor tend to out-compete other anaerobes. However, SRB also cause corrosion. Sulphide produced by SRB causes cathodic hydrogen depolarization and may also corrode the metal surface by accelerating anodic interaction (Chen & Clayton 1997, 1998). Corrosion caused by SRB is the most well-known microbial-induced corrosion, which could account for 20% of all the corrosion (Flemming 1996). This study was conducted to examine the influence of Cr^{3+} on the microbial colonies of biofilm on mild steel in the anaerobic marine environment. Chromium is a heavy metal commonly found in the polluted marine environment. Its concentration in the marine sediment from Hong Kong's coastal waters is in the range of 25–75 mg Cr kg^{-1} (Blackmore 1998). Chromium is known to be toxic to microbial growth (Lin 1992). It also affects the SRB growth and the EPS production (Bremer & Loutit 1986, Paradies 1995). But little is known about the influence of chromium on the microbial colonies in biofilm and the resulting corrosion of steels.

Materials and methods

Mild steel coupons

Mild steel coupons ($10 \times 10 \times 1.5$ mm) were used as the substratum for biofilm growth. Its compositions (by wt%) as analysed by inductively coupled plasma optical emission spectrometry and spark optical emission spectrometry include: Fe: 98.48%; C: 0.06%; Si: 0.13%; P: 0.03%; S: 0.045%; Mn: 0.51%; Cr: 0.10%; Mo: 0.02%; Ni: 0.14%; and Cu: 0.49%. The coupons were wet polished with a series of grit SiC papers (grades 320, 400, 600, 800) followed by a further polish using 0.3 μm alumina particles.

SRB culture enrichment

The SRB seed was isolated from marine sediment and cultured in a complete-mix reactor using the modified Postgate's marine medium C (Videla 1996) at room temperature for over six months. The medium was prepared by adding to each litre of seawater 0.5 g of KH_2PO_4, 1 g of NH_4Cl, 0.06 g of $CaCl_2 \cdot 6H_2O$, 0.06 g of $MgSO_4 \cdot 7H_2O$, 6 ml of sodium lactate (70%), 1 g of yeast extract, 0.004 g of $FeSO_4 \cdot 7H_2O$, and 0.3 g of sodium citrate. The medium was adjusted to pH 7.2 ± 0.1 using 1 M NaOH solution. Seawater was taken from the Victoria Harbour, Hong Kong, and was sterilized by filtering through a 0.45 μm membrane before use.

Biofilm formation and corrosion

All experiments were carried out in four enclosed one-litre glass reactors filled with the modified Postgate's marine medium C. The medium was further sterilised by filtering through a 0.22 μm membrane, and then flushed with nitrogen to remove dissolved oxygen. The enriched SRB culture was used as the microbial seed at the initial concentration of 2×10^6 cell ml^{-1} in the medium. $CrCl_3$ was added to three reactors to the targeted concentrations of 10, 50 and 100 mg Cr^{3+} l^{-1}, respectively. $CrCl_3$ was not added to the fourth reactor, which served as control. Mild steel coupons hung on the Nylon string were then immersed. The initial sulphate concentration in all the seawater medium was 2090 mg l^{-1}. The activity of SRB in each reactor was monitored by analyzing the decrease of sulphate concentration over time using ion chromatography. Since over 80% of sulphate in the control became depleted after 9 days, half of the medium in each reactor

was replaced on day 10 with the fresh modified Postgate's medium C. After 20 days, the steel coupons were removed for microscopic observations and for surface corrosion analysis.

SEM and AFM analyses

The biofilm-coated coupons were fixed for 8 h in 2.5% glutaraldehyde, followed by dehydrating in a graded series of ethanol (Lee & Characklis 1993). The coupons were then dried in a critical-point dryer and coated with carbon for SEM (scanning electron microscopy) examination. The metal surface was analysed for pit corrosion, after removing the biofilm, by AFM (atomic force microscopy). A Nanoscope IIIA (Digital Instruments, USA) was used in tapping mode with the standard etched silicon probes. The detailed procedures for sample preparation and AFM operation were described in a previous study (Xu *et al.* 1999).

Results and discussion

Influence of Cr^{3+} on SRB activity

Within 5 days, formation of biofilm on the steel coupon surface became visually noticeable in all the reactors. The bioactivity of SRB in each reactor was monitored daily by analysing the sulphate concentration. Results clearly indicated a toxic effect of Cr^{3+} on SRB. After about two days of acclimation, the sulphate concentration began to decrease in all reactors; but the rate decreased with the increase of Cr^{3+} concentration. After nine days, sulphate in the control reactor was lowered by 85%, from the initial 2090 mg l^{-1} to 305 mg l^{-1}. The reduction of sulphate was, on the other hand, only 61% in the reactor with 10 mg Cr^{3+} l^{-1}, 38% in 50 mg Cr^{3+} l^{-1} and 27% in 100 mg Cr^{3+} l^{-1}.

Influence of Cr^{3+} on formation of microbial clusters

Mild steel coupons were removed from the reactors on day 20 for biofilm observations by SEM. Figure 1 illustrates the SEM images of the biofilms on steel surface in reactors of (a) the Cr^{3+}-free control, (b) 10 mg Cr^{3+} l^{-1}, (c) 50 mg Cr^{3+} l^{-1}, and (d) 100 mg Cr^{3+} l^{-1}. Figures 1a and 1b illustrate that microbes are uniformly scattered over the biofilm in Cr^{3+}-free and dilute (10 mg l^{-1}) Cr^{3+} seawaters. However, Figures 1c and 1d illustrate that microbes in biofilm tended to
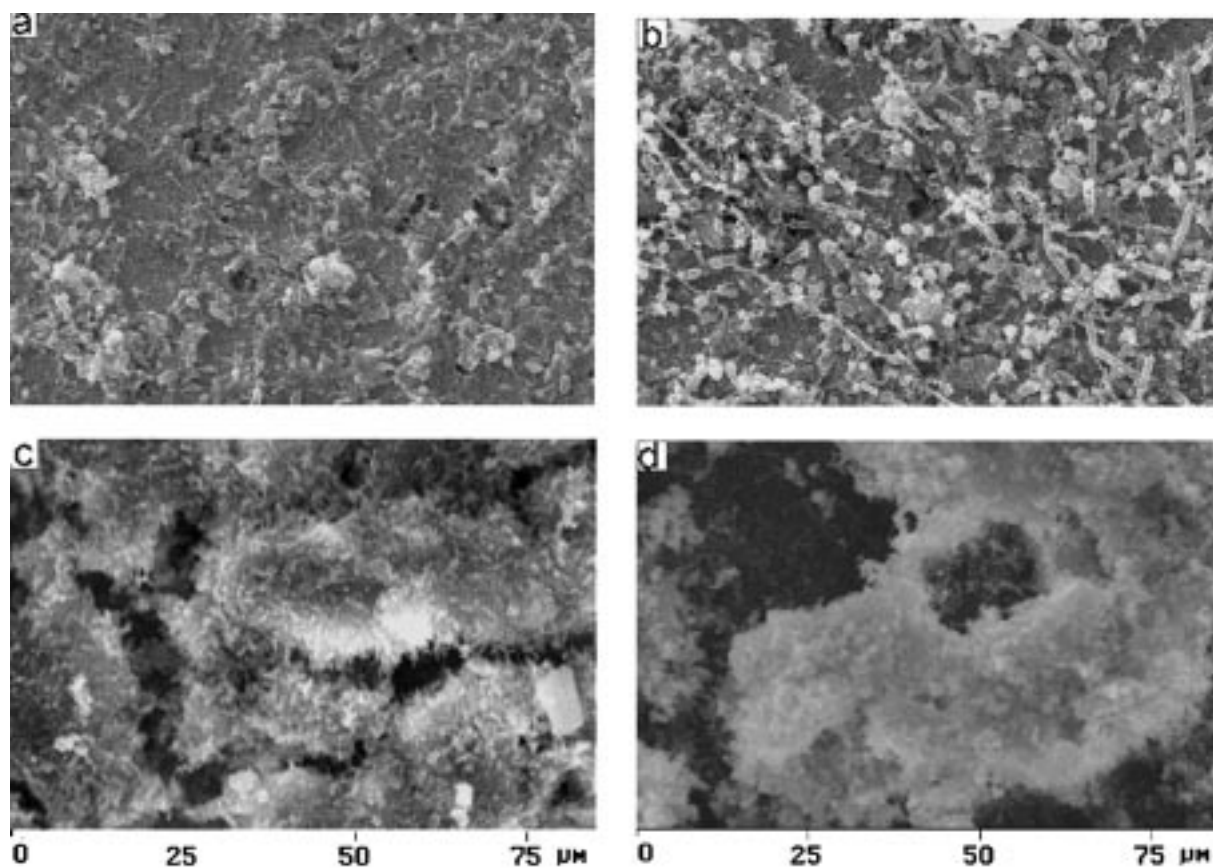
Fig. 1. SEM images of biofilm exposed to media of (a) control, (b) 10 mg Cr^{3+} l^{-1}, (c) 50 mg Cr^{3+} l^{-1}, and (d) 100 mg Cr^{3+} l^{-1} showing increased tendency of microbial cluster formation with Cr^{3+} concentration.

aggregate forming clusters as Cr^{3+} concentration in seawater increased; regions between microbial clusters were devoid of cells. The tendency increased with Cr^{3+} concentration, as the microbial clusters and the regions in between were noticeably larger in biofilm exposed to 100 mg Cr^{3+} l^{-1} (Figure 1d) than those exposed to 50 mg Cr^{3+} l^{-1} (Figure 1c). This is most likely due to the increased toxicity of Cr^{3+} in the medium. As Cr^{3+} concentration increased, microbes tended to cluster so as to minimise the area (Costerton *et al.* 1987) exposed to the toxic Cr^{3+}.

Influence of Cr^{3+} on corrosion

After removing the coated biofilm, surfaces of mild steel were analysed by AFM. The corroded sites are illustrated in grey; the darker the site the deeper the corrosion. Figure 2a illustrates that there was little corrosion of the steel exposed to Cr^{3+}-free medium after 20 days. But the degree of corrosion increased with the Cr^{3+} concentration, from 10 mg l^{-1} in Figure 2b

to 50 mg l^{-1} in Figure 2c, and to 100 mg l^{-1} in Figure 2d, even though Cr^{3+} was toxic to SRB. Based on the AFM scanning, the surface roughness of the original mild steel coupon was 5.8 nm. After 20 days in the seawater medium, the roughness of the control coupon increased to 30.1 nm, as compared to 38.1 nm for that exposed to 10 mg Cr^{3+} l^{-1}, 57.4 nm for 50 mg Cr^{3+} l^{-1} and 150.3 nm for 100 mg Cr^{3+} l^{-1}. AFM scanning also showed that, for each 100 μm × 100 μm scanned area, 69.5 μm^3 of steel was corroded in the control medium, as compared to 75.3 μm^3 for that exposed to 10 mg Cr^{3+} l^{-1}, 251.9 μm^3 for 50 mg Cr^{3+} l^{-1} and 347.5 μm^3 for 100 mg Cr^{3+} l^{-1}.

It is interesting to note that the corrosion pattern in Figures 2c and 2d matches the pattern of microbial clusters in Figures 1c and 1d respectively. Corrosion appeared to occur mostly in the regions of biofilm devoid of microbial cells, instead of underneath the microbial clusters.
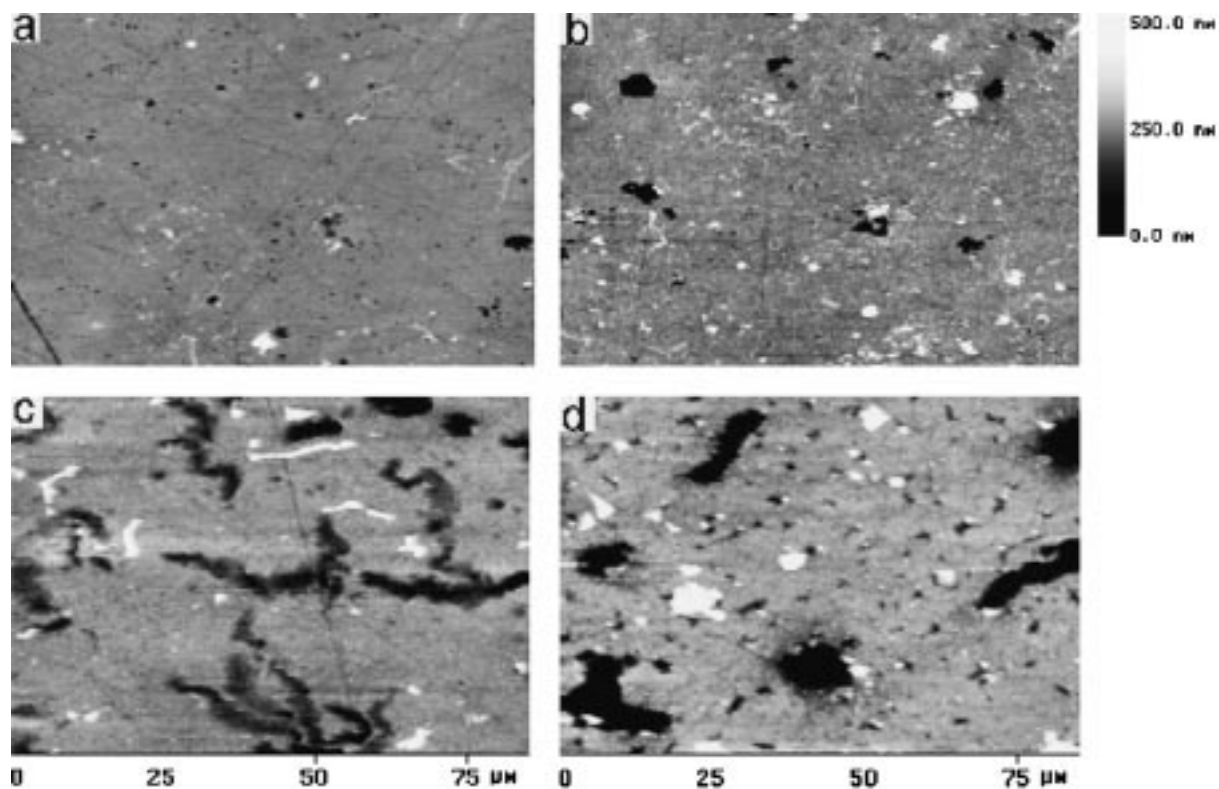
Fig. 2. AFM images of mild steel surface exposed to media of (a) control, (b) 10 mg Cr^{3+} l^{-1}, (c) 50 mg Cr^{3+} l^{-1}, and (d) 100 mg Cr^{3+} l^{-1} showing corrosion patterns resembling those of microbial clusters in Figure 1. (The depth of each corroded pit is indicated by the degree of darkness.)

Acknowledgement

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