



Metal corrosion by aerobic bacteria isolated from stimulated corrosion systems: Effects of additional nitrate sources



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ABSTRACT

Many microorganisms are reported to influence the corrosive behaviour of mild steel and stainless steel in different habitats. In this study, 40 bacterial strains were isolated from corroded mild steel and stainless steel coupons in the nitrate supplemented environments. The corrosion abilities of the isolates against the mild steel and stainless steel coupons were tested with or without additional nitrate sources. The presence of bacterial isolates alone stimulated the corrosion of mild steel coupons. Most of the bio-corrosion processes of mild steel coupons were mitigated by adding nitrate supplement with bacterial isolates. The effects of bacterial isolates and additional nitrogen sources on corrosion of stainless steels were varied. Not all bacterial isolates stimulated the corrosion on stainless steel during the study period. Unlike the effects on mild steel coupons, additional NaNO_3 might stimulate, retard the corrosion rate by the bacterial isolates or have limited effects. Similar results were obtained when NH_4NO_3 was used. Phylogenetic analysis demonstrated that all isolates were closely related. The majority of the bacterial isolates from corroded metal coupons were identified as *Bacillus* species. Others were identified as *Pseudomonas* sp., *Marinobacter* sp., and *Halomonas* species. The results prove that the isolated aerobic microorganisms do play a role in the corrosion process of stainless and mild steel. Adding additional nitrate sources might be a tool to mitigate corrosion of mild steel which was stimulated by the presence of bacteria. However, to prevent the corrosion of stainless steels, it might need a trial and errors approach in each case.

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1. Introduction

Materials such as metals immersed in an aquatic or soil environment adsorb dissolved organic matter onto their surfaces thereby conditioning material's surfaces. Based on the property of the surface, various microorganisms then colonize it and form biofilms (Majumdar et al., 1999). Once a microorganism forms a biofilm on a material's surface, a micro-environment is created that is dramatically different from the bulk surroundings (Lane, 2005; Yuan et al., 2007; Akpabio et al., 2011). In the presence of microbial consortium, many physiological types of bacteria interact in complex ways (Bano and Qazi, 2011). Changes in pH, dissolved oxygen, and organic and inorganic compounds in the micro-environment can lead to electrochemical reactions which increase or slow down corrosion rates (Lane, 2005; Sheng et al., 2008; Akpabio et al., 2011). Microbiological influenced corrosion (MIC) has been defined as an electrochemical process in which the participation of

microorganisms is able to influence the corrosion reaction without changing its electrochemical nature (El-Shamy et al., 2009). Many studies have shown that the chemical modifications in the environments resulted in changing of microbial populations that can be used to attenuate metal corrosion (Zuo et al., 2005; Bano and Qazi, 2011).

Zuo (2007) described that a consortium of aerobic and anaerobic bacteria is usually present and may play a role in the corrosion process of metals. The majority of aerobic bacteria, which is involved in this process, are sulphur oxidizers (SOB) and iron oxidizing bacteria. Corrosion caused by sulphur oxidizers can release aggressive metabolites, such as organic (acetic, succinic, isobutyric, etc.) or inorganic acid (sulphuric). *Thiobacillus* form sulphuric acid, which is a strongly corrosive agent, especially in concrete structures where the steel is corroded and the carbonates solubilized (Coetser and Cloete, 2005). The iron oxidizing bacteria in the genus *Gallionella*, and filamentous bacteria in the genera *Sphaerotilus*, *Crenothrix*, *Leptothrix*, *Clonothrix*, and *Lieskeella* are known to promote corrosion (Coetser and Cloete, 2005). The iron oxidizing bacteria are able to oxidize ferrous iron from solution to ferric hydroxide which is precipitated in sheets surrounding the

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bacteria and result in the formation of tubercles on the steel surface. The tubercles can lead to crevice attack, and can also provide a suitable habitat for the anaerobic bacteria in the region beneath the tubercle. *Pedomicrobium manganicum* oxidizes manganese in drinking water systems, and binds colloidal MnO_2 in extracellular polysaccharides (Coetser and Cloete, 2005). They can even oxidize ferrous iron on stainless steel. These iron bacteria normally contribute to corrosion by excessive slime masses creating differential oxygen concentrations and thus create microenvironments ideal for the growth anaerobic cells (Coetser and Cloete, 2005).

Bacteria in the genus *Pseudomonas* and pseudomonas-like organisms have also been reported in connection with many cases of corrosion (Coetser and Cloete, 2005). *Pseudomonas* is an aerobic slime-former and often forms thin films in combination with corrosion deposits on metal surfaces. Various *Pseudomonas* isolates (Westlake et al., 1986) have been implicated in the reduction of ferric to ferrous iron, exposing steel to further oxidation as ferrous iron compounds are more soluble and the protective ferric iron layer is solubilized by this process. Other aerobic bacteria including *Escherichia*, *Flavobacterium*, *Aerobacter* and *Bacillus* are also included in this group of aerobic bacteria (Coetser and Cloete, 2005). Their growths occur as patchy distributions over the metal surface and exclude oxygen via respiration and play a role in the aggregation of bacteria in flocks on surfaces.

However, recently acquired data indicate the possibility of decreasing corrosion by the formation of biofilms on the surface of steel. The formation of axenic aerobic biofilms by microbes inhibitory to the MIC process on metal surface has been reported (Little and Ray, 2002; Ornek et al., 2002; Zuo et al., 2005). Biofilms produced by gramicidin-producing *Bacillus brevis* have been demonstrated to protect metals from corrosion in the presence of SRB (Nikolaev and Plakunov, 2007). Nitrate or nitrite mediated control of corrosion has also proved successful in combination with a nitrate reducing-sulphur oxidizing (NR-SOB) bacterial culture to inhibit sulphide production in a growing SRB consortium (Hubert et al., 2003; Rempel et al., 2006). Nitrate addition alone did not impose an inhibitory effect but stimulated the NR-SOB metabolic activities leading to sulphide removal (Nemati et al., 2001; Rempel et al., 2006).

A proper understanding of the identity and role of microbial populations in the specific environment of metal surface may be exploited to induce corrosion inhibition by bacteria as a useful tool to prevent MIC effects in practice. This paper reports the identifications of bacterial species isolated from corroded mild steel and stainless steel coupons during stimulated corrosion processes. The effects of these microbial isolates with nitrate supplements on the corrosion of mild steel and stainless steel coupons were also investigated.

2. Materials and methods

2.1. Bacterial isolation from the corroded metal coupons

Seawater and sea sand were collected at the water/sediment interface of Durban beachfront, KwaZulu-Natal. Loam soil was collected in Shallcross, KwaZulu-Natal and air-dried for one week. The soil/sand was then passed through a 7.5 mm (porous aperture) Madison Test Sieve before use. Metal coupons (mild steel and Type 304 stainless steel with dimensions of $25 \times 25 \times 1.2$ mm and a 2 mm drilled mounting hole) were obtained from the Academic Instrumentation Unit (University of KwaZulu-Natal, Westville). Experimental set-up was constructed according to Orfei et al. (2006). The coupons were degreased with acetone, polished with fine polishing paper, rinsed with distilled water and finally sterilized by autoclaving at 120°C for 20 min. Prior to experiments, the

weight of the pre-treated coupons was determined using an analytical balance (Sartorius Basic).

In order to study the impact of adding nitrate sources on the metal corrosion under different soil environments, the following experiments were initiated. For the seawater/sediment system, four 3 L catering jars were filled with 1 L of seawater and 2 Kg of sea sand and subjected to different conditions (autoclaved sea water and sea sand, non-autoclaved sea water and sea sand, additional $5\text{ mM} \cdot \text{NaNO}_3$ or NH_4NO_3) in an attempt to inhibit or reduce the rates of steel corrosion. In a separate set of experiment with a similar set-up, 2 kg of air-dried, sieved loam soils was used in each of 3 L catering jars. All jars were maintained at room temperature in the laboratory for the study period. Pre-treated mild steel or pre-treated stainless coupons were suspended in a vertical position into each prepared jar to initiate the metal corrosion.

Two of the exposed metal coupons were removed from each jar at different sampling times over a 20 week period to determine the weight loss (Zuo et al., 2004; Ryhl-Svendsen, 2008). The weight loss of mild steel coupons was ranged from 7.09 mg g^{-1} – 22.49 mg g^{-1} in the sea sand environment and from 2.90 mg g^{-1} – 20.74 mg g^{-1} in the loam soil. The weight loss of stainless steel coupons was ranged from 0.075 mg g^{-1} to 0.12 mg g^{-1} in the sea sand environment and from 0.065 mg g^{-1} to 0.13 mg g^{-1} in the loam soil (Pillay, 2013). Additional 2 coupons were removed at different sampling times and swabbed with the cotton swabs (25 mm Gamma sterilized), spread plated onto nutrient agar (5 g/L peptone, 2 g/L yeast extract, 1 g/L meat extract, 8 g/L NaCl and 15 g/L agar from Merck) plates. The agar plates were incubated at 30°C overnight or until growth was observed. Pure cultures were obtained using four way streaks on nutrient agar plates. Once pure isolates were obtained, these were subjected to Gram staining and spore staining. The presence or absence of sulphate-reducing bacteria was determined using the 3-tube Most Probable Number technique using the Most Probable Number Calculator[®] Version 4.04 (Klee, 1996). The cotton swabs (25 mm Gamma sterilized) were immersed into Postgate C solutions and vortexed to disperse the cells from the cotton swabs. One millilitre of serial diluted samples was inoculated into Postgate B medium (Postgate, 1984) for 7–10 days. The tubes exhibiting a black precipitate were scored positive for sulfate-reducing bacterial growth Lopez et al. (2006). No sulphate reducing bacteria were detected.

2.2. Corrosion experiments with bacterial isolates

Each bacterial culture was grown in 30 ml nutrient broth (5 g/L peptone, 2 g/L yeast extract, 1 g/L meat extract and 8 g/L NaCl from Merck) at 37°C on a rotary shaker at a speed of 150 rpm for 24 h. One-day culture was harvested using the Beckman Coulter, Avanti, J-26 XPI centrifuge at 4000 rpm for 20 min. The subsequent pellet was washed three times with 0.85% (w/v) saline solution. The inoculum was standardized to a final absorbance of 1.5 at 600 nm. Two metal coupons (mild steel or stainless steel) were immersed in 250 ml flasks containing 100 ml of deionized water (Millipore Elix purification system, 17 m Ω), 5 mM NaNO_3 and 5 mM NH_4NO_3 solution, respectively. One ml of standardized bacterial culture was introduced into each solution and one ml of deionized solution was used as the control. The experiments were performed in duplicate. The flasks were then incubated at 30°C and at 120 rpm for 2 weeks and 3 months in the case of mild steel and stainless steel coupons respectively. At the end of the experiment, weight losses of the metal coupons were measured as described previously.

2.3. Bacterial identification

The bacterial isolates were identified by 16S rRNA sequencing (Marchesi et al. 1998) followed by NCBI Blast comparison software

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