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Microorganisms associated with corrosion of structural steel in diverse atmospheres

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ABSTRACT

The influence of atmospheric conditions on the corrosion of steel and its associated microbial community was studied. Surface analysis revealed greater localized corrosion in steel exposed to near-ocean atmospheres with high chloride deposition compared to inland and subalpine sites. High-throughput sequencing analysis of corrosion products showed that dissimilar microbial communities and dominant species were deposited on steel in the different atmospheres. Close to the ocean, *Brevundimonas diminuta* were predominant whereas *Clostridium* and *Pseudomonas* species dominated for inland sites with agricultural or forestry activities. *Bacillus* and *Enterococcus* were dominant for sites close to a fertilizer plant and a sewage treatment plant, respectively. *Actinobacteria* species dominated at sub-alpine conditions. Results from this study indicate that microbial communities on corroding steel exposed to atmospheric conditions are the result of deposition of locally-generated aerosols. Acid-producing activity and exopolysaccharide (EPS) production was widespread and rapidly detected in microbial cultures from all the exposure sites. Sulphate-reducing bacteria were not detected in this study. These results suggest that acid production and EPS synthesis can be important mechanisms for microbial corrosion of steel under atmospheric conditions.

1. Introduction

The atmospheric corrosion of structural steels continues to be a topic of interest because of the potential implications for the safety, performance and economic viability of critical infrastructure. Corrosion of steel and other surfaces in the atmosphere involves the availability of moisture either as rain, condensation through relative humidity mechanisms or deposition of sea or other water spray. Such corrosion is also influenced by atmospheric pollutants such as chlorides, NOx and SOx and their role on both short and long term corrosion has received much research interest for many years. Likewise, low levels of atmospheric corrosion have been associated with the lack of deposition of airborne pollutants (Evans, 1960) (de la Fuente et al., 2011).

Aerosols have been shown to play a major role in the biogeochemical processes at the air-water interface of oceans (Prospero, 2002) and are known to play a similar role in air-land surface interfaces (Foissner, 2006; Morris and Nicot, 2014). Aerosols can transport a wide variety of inorganics, particulates of iron and other metals, and microorganisms including algae, bacteria and archaea. Typically, they are generated and transported by local wind action, thermal gradients, evaporation and dust (Prospero, 2002). Deposition to substrates such as vegetation, animals and inanimate surfaces including stones, rock and steel is commonly observed. Similarly, winds, rain, snow and mechanical abrasion can remove all or part of such deposition. In a study by Bovallius et al. (1976), the numbers of airborne bacteria and deposited bacteria were compared at four locations in Sweden. The authors found a degree of correlation between airborne and deposited bacteria with an elevated number of deposited bacteria in summer and autumn seasons. Rain and high humidity decreased the counts of deposited bacteria whereas high winds tended to increase their numbers. The authors did not investigate the microbiological species involved.

There is significant evidence that the corrosion of steels can be influenced by microbial activity, a phenomenon known as microbiologically-induced corrosion (MIC) (Melchers and Jeffrey, 2008), (Machuca et al., 2014). The main species involved in MIC have been identified as sulphate reducing bacteria (SRB) and iron-

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oxidizing bacteria (Little and Lee, 2007; Chang et al., 2015) but causative microbes can vary depending on location, environment and materials. Likewise, the extent of their influence on the corrosion processes appears to be dependent on the availability of critical nutrients (Melchers, 2007, 2014; Little and Lee, 2007; Melchers, 2014). However, there is only limited evidence that MIC may be involved in atmospheric corrosion.

Santana Rodriquez et al. (2002) reported SRB on plastic test strips exposed for one month to three different sites in the coastal regions of Gran Canaria Island. The authors concluded that SRB were transported and deposited on the test strips via seawater aerosols. Santana Rodriquez and Gonzalez Gonzalez (2006) reported SRB in Green rusts corrosion products and concluded that SRB were responsible for the aggressive atmospheric corrosion observed on steel. Similarly, De Belie et al (De Belie et al., 2000). reported aggressive corrosion associated with the presence of SRB in local areas of intensive animal husbandry. In a study on atmospheric corrosion of inland Indian railways (Maruthamuthu et al., 2011) corrosion of the steel rails was attributed to toilet droppings. Using DNA analysis of corrosion products the authors identified the microbial species involved and concluded that corrosion was caused by ureolytic bacteria that produce carbonic acid and by iron/manganese-oxidizing microbes. These observations highlight the potential role of microorganisms in atmospheric corrosion.

The following sections describe a study on the corrosion of steel and its associated microbial community composition after 3 months exposure to 7 different atmospheric sites including near-ocean, industrial, farming and subalpine locations. The identification of microbial communities on corroding steel has been widely applied to help understand the role of microorganisms in corrosion (Machuca et al., 2011; Li et al., 2014; Liengen et al., 2014) Ithough most attention has been given to immersed conditions. Measuring the exact contribution of microorganisms to atmospheric corrosion is very challenging because of the logistical difficulty of setting up abiotic (sterile) controls in field studies and as such it was not the purpose of this work. The aim of the study was to gain insight into the possible mechanisms by which microorganisms can accelerate atmospheric corrosion through the identification of the microbial communities deposited on steel at the different locations. High-throughput 16SrRNA gene sequencing was used to identify the microbial communities. Microbial deposition was assessed via fluorescence microscopy and culture-dependent method was used to assess the activity of major metabolic groups associated with corrosion. Corrosion was evaluated using 3D surface profilometry.

2. Materials and methods

2.1. Test specimens and exposure sites

Mild steel coupons of 50 × 50 × 3 mm were used in this study, all cut from the same sheet of cold-rolled steel. The coupons were used in the 'as supplied' state, without polishing, to reflect normal industrial applications. Random samples were examined under an optical microscope to confirm that the surfaces were essentially identical. Atomic emission spectrometry was used to determine their composition (C 0.16, Mn 0.32, P 0.015, S 0.019, Si 0.08. Ni 0.02, Cr 0.02, Mo 0.00, V 0.003, Al 0.041, Cu 0.01, bal. Fe %wt). Prior to exposure, coupons were acid cleaned and rinsed in deionised water, alcohol and acetone, consecutively. Coupons were air dried and sterilized by spraying 70% ethanol (v/v) over the surface before deployment. In all cases the coupons were suspended vertically from a support frame using nylon cable ties that permitted free orientation. They were spaced very close together (suspended no more than 150 mm apart) on the assumption that this would have a very high likelihood that the microbial communities deposited would be similar for each coupon.

Five coupon replicates were exposed at different test sites located in New South Wales, Australia, for three months. The sites have different environmental characteristics (Table 1). All coupons were exposed primarily during the Autumn season. After exposure, coupons were removed from the test rack using sterile gloves and placed in individual sterile plastic bags. From the five coupon replicates at each site, two coupons were processed for DNA extraction followed by 3D surface profilometry, one coupon was used for direct DAPI staining and fluorescence microscopy, one coupon used for cultivation, and one coupon for chloride deposition measurement. The average chloride deposition rates for the sites was measured using a standard technique (ASTM, 2002).

2.2. Corrosion analysis

Previous corrosion studies conducted on the same test sites have shown that corrosion mass losses (general corrosion) are not significant in these conditions and corrosion is best evaluated using surface analysis of pits. For this reason, corrosion evaluation was limited to surface examination. Coupons were cleaned in accordance with standard procedures (ASTM, 2003). Corrosion was evaluated using an optical 3D surface profilometer (IFM G4g system, Alicona Imaging) that allows measurements of pit density and pit depth.

2.3. Direct assessment of bacteria deposition on steel by DAPI

For direct evaluation of bacteria deposited onto steel, coupons from each site were stained with 4,6-diamidino-z-phenylidole (DAPI), a fluorescent nucleic acid dye, as described previously (Machuca et al., 2013). Briefly, the coupon was rinsed with sterile phosphate buffered saline (PBS) solution to remove unattached contaminants and stained with DAPI (2 μg/mL; Sigma). Coupons covered with DAPI were incubated in the dark at room temperature for 15 min. DAPI-stained coupons were examined using a BX-51 Olympus epifluorescence microscope.

2.4. Bacteria abundance and DNA extraction from coupons

Coupon replicates from each site were suspended in sterile phosphate buffer saline (PBS) containing 0.1% (w/v) Tween 20. Two coupon replicates were used for DNA extraction and 16SrRNA gene sequencing (Section 2.5) and one coupon was processed for cultivation (Section 2.6). For microbial cell detachment, vortex mixing at maximum speed was used for 2 min (Vortex Genie®, MO BIO Laboratories, Inc.) followed by sonication in an ultrasonic bath (60 s-sonication steps). The PBS/Tween solution was replaced between sonication steps so as to avoid prolonged exposure of dislodged bacteria to sonication. Bacterial cells in suspension were harvested by microfiltration (0.2 μm pore size polycarbonate membrane filters). The retained cells were washed from the filter with phosphate buffered saline (PBS) and further processed to separate iron oxides (centrifugation 1 min, 700 × g, 4 °C). Microbial cells in the supernatant were counted using a Petroff-Hausser counting chamber and phase-contrast microscopy. Cell abundance is given as cells/cm^2 surface area. One-way analysis of variance (ANOVA) was conducted to examine the significance of variations in bacterial numbers as a function of exposure site at a significance level of 0.05 (calculated as statistical p values).

For DNA extraction, bacterial cells in supernatant from each coupon replicate (as above) were mixed and harvested by high speed centrifugation (20 min, 13,000g, 4 °C) and the pellet used for DNA extraction using a DNA extraction kit (PowerSoil™ DNA Isolation Kit, MO BIO Laboratories Inc.) following manufactures'