Development of a laboratory test for microbial involvement in accelerated low water corrosion

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Microbially influenced corrosion (MIC) is a general term for when microbes affect material corrosion processes. The rapid corrosion that can occur due to MIC can cause significant dangers and costs for owners of relevant assets in relation to predicting structural safety, design of new structures and maintenance. Verification and/or prediction that a structure may be subject to MIC is not straightforward and, when metal surfaces are involved, it requires a series of metallurgical, microbiological and chemical tests. A useful part of this testing can be laboratory-based studies of microbial consortium samples from the environment of interest. However, there are no standard guidelines for how to perform such tests. Here we report the results of a preliminary study of laboratory corrosion simulations with biomass from a marine metallic corrosion event and show that simple changes in the test conditions can alter the rate of corrosion and the composition of microbial consortia during the test.

Accelerated low water corrosion (ALWC) is increasingly being recognised as a form of MIC, which occurs on metal structures in the marine environment[1–3] (Figure 1*a*). ALWC occurs in ports and harbours worldwide and is often associated with orange patches on steel surfaces at the low-tide water level (see Figure 1*a*, *b*). The orange patches are a combination of iron oxide-rich corrosion products and microbial biomass. The exact details of the corrosion mechanisms and microbial processes involved in ALWC are still not well understood but are believed to be mediated by a combination of microbial sulfate reduction and sulfur oxidation and there is good evidence that electrochemistry is centrally involved. Currently, there is limited guidance available for those wanting to determine if microbially-induced ALWC is present at a particular site. Some of the suggested tests include visual surveys for orange patches and holes in sheet piling, ultrasonic thickness measurements and tests for the presence of sulfate reducing bacteria (SRB) and/or their by-products such as iron sulfide. A possible addition to these tests could be to use microbial samples from field corrosion studies, for corrosion simulations in the laboratory or at some other suitable test location. These ALWC tests should be able to be carried out with relevant microbial communities, basic equipment and initiated in the field by technical staff.

Laboratory-scale corrosion testing to ascertain MIC/ALWC requires standard test procedures (including sample collection and storage) and operational conditions, since they are critical for generating comparative test outcomes and they do affect microbial community composition, as has previously been shown[4–10]. Some important test factors include the simulation medium composition, including nutrients and dissolved oxygen supply, physicochemical aspects of redox potential and pH, and the biomass used as inoculum. Thus, with a view to widespread application in the corrosion industry, we used samples of naturally occurring ALWC orange patches as an inoculum and a relatively simple laboratory set-up to study the effect of the selected microbes and the impact of several environmental conditions on corrosion of metal coupons.

Samples of an orange patch were taken from a steel sheet pile wall suffering ALWC located in a port in southeastern Australia. The test set-up involved solutions of seawater and nutrients in 500 mL Schott bottles incorporating suspended marine grade steel coupons, and the homogenised orange patch as inoculum (see Figure 1*c*, *d*). The range of experimental variables included oxygen availability, nutrient addition and filtering of the seawater. After

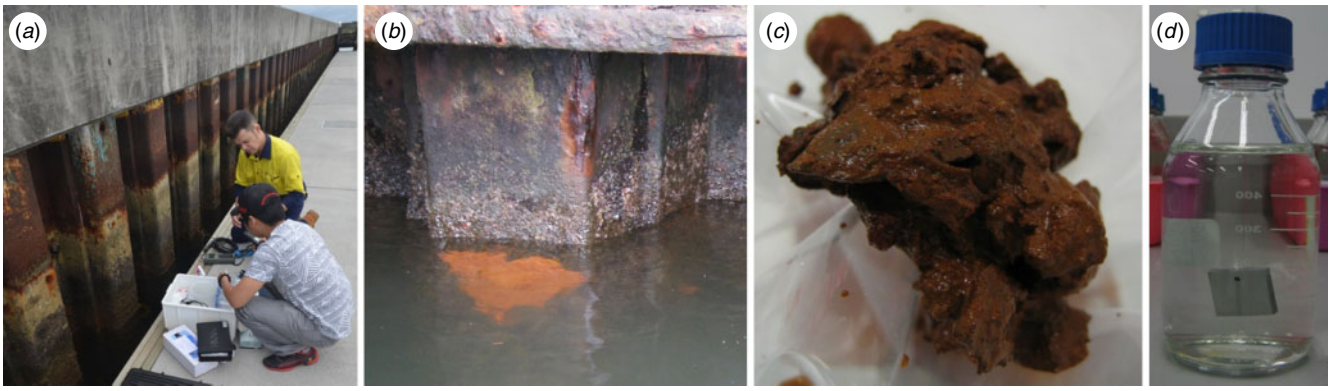

Figure 1. (*a*) Photo of steel sheet piling, the type of which can be subject to ALWC, (*b*) example of steel sheet piling with patch of orange bloom, (*c*) collected orange patch material (= corrosion products and microbial biomass), and (*d*) test set-up prior to inoculation, including suspended marine grade steel coupon.

immersion for 60 days at laboratory temperature, the metal coupons were removed and the extent and nature of corrosion was determined by surface contour changes (Bruker Contour GT-K1 3-D optical profilometer), scanning electron microscopy (Zeiss SUPRA 40VP-25-38) and mass loss (Mettler Toledo MS205DU mass balance). Samples of the planktonic suspensions representing the different test conditions were evaluated for microbial communities by Illumina MiSeq metabarcoding of the V6-V8 part of the 16S rRNA genes.

The corrosion rates of the coupons in the tests containing microbial inocula and additional nutrients (two test set-ups) were 100–125 µm yr^{-1}, which are about 3 times that of coupons from the solutions lacking inoculum. We concluded that the microbes present in the orange patch have the ability to increase corrosion rates. While the greatest corrosion rates were observed when nutrients were added to the test solutions (glucose and yeast extract, after 15 and 44 days), teasing out the exact reason for this is not straightforward. These nutrients can act as a corrosion inhibitor[7] and also cause changes in microbial community composition and dominant microbial metabolisms, which also can affect corrosion. Oxygen availability was also found to affect corrosion, where the corrosion rates of nominally aerobic tests (bottles with ~100 mL air headspace, with holes drilled in lids and weekly manual agitation) were about 3 times greater than for the nominally anaerobic tests (the liquid completely filled the tightly sealed, non-agitated bottles).

A total of 100 different bacterial genera were identified in all the test setups with the number of unique bacterial taxa found in each of the test solutions varying from 15 to 37. Overall the phylum *Proteobacteria* (73.9%) made up the majority of the bacteria identified, with phylum *Bacteroidetes* (13.2%) the second most dominant bacterial phylum. The two test set-ups with the highest corrosion rates contained seawater, nutrients and inoculum, and they selected for unique upper taxonomic level microbial populations comprising the following groups in the Bacteria:

- *Proteobacteria* (63%)
  - Class Deltaproteobacteria: 24% (comprised of different Families)
  - Class Gammaproteobacteria: 35%
- *Bacteroidetes* (17%)
  - All Class Bacteroidia
- *Tenericutes* (7.5%)
  - All Class Mollicutes
- *Firmicutes* (8%)
  - All Class Clostridia
- *Lentisphaerae* (5%)
  - All Class Lentisphaeria.

In the Archaea, one high corrosion test set-up contained Candidate Division WCHD3-30 from Phylum *Parvarchaeota* (48.1%), but very little is known of this group.

Phylum *Tenericutes* were present only in the two tests with the highest corrosion rates, implying a key role in ALWC. High abundances of Class *Deltaproteobacteria* were also found in the two highest corrosion rate tests. Class *Deltaproteobacteria* contains many SRB, which have a noted role in MIC and ALWC. The SRBs that we identified were from several different families, largely *Desulfobulbaceae*. There is plenty of scope for multivariate analyses of the microbial community (diversity and evenness) with phenotypic aspects including corrosion rate, oxygen and nutrient availability and these studies will statistically tease out linkages between specific microbes and function.

We showed that a simple, laboratory test set-up can potentially be used to predict and identify ALWC. In addition, the results obtained demonstrate how changing the test conditions can affect both the magnitude of corrosion that takes place and the microbial community that develops. The test arrangements used in this work were deliberately chosen to be rudimentary so as to mimic a practical application. Some of the areas of further study include oxygen effects, changes in the community with time, sessile versus

planktonic communities and the timing/types/levels of nutrient addition. Reducing the test volumes (from 500 mL), increasing the throughput, and making the test available in an interpretable form to the industry are future practical directions. From a fundamental perspective, we are delving into the functional aspects of the microbial groups that were specifically selected in the high corrosion tests and exploring the microbes that grew on the coupons.

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Biographies

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