THE EFFECT OF MICROBIOLOGICAL INVOLVEMENT ON THE TOPOGRAPHY OF CORRODING MILD STEEL IN COASTAL SEAWATER

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ABSTRACT

Microbiological factors have been implicated in the accelerated corrosion of mild steel in seawater for many years. To date few successful medium-term trials have been carried out to quantify the effect of micro-organisms on the surface of steel exposed to seawater. Presented herein are the results of a two and a half year investigation in which natural seawater was taken from a tributary of Port Stephens, Australia, and split into two streams; natural (raw) seawater and sterile seawater. The waters were pumped directly into tanks in which 25 mm x 25 mm polished steel coupons were suspended. Coupons were removed on an irregular basis and examined. After about a year the coupons in natural water had developed what are commonly referred to as tubercles whereas the oxide build-up on the coupons in sterile seawater was relatively even. There was also a significant difference in size and numbers of pits. Furthermore, the cleaned surfaces of the steel when viewed using a scanning electron microscope (SEM) in all cases showed differences in oxide formation and topography between coupons recovered from natural and sterile water streams. Since the only difference between the two streams of seawater was the removal of microbiological matter, differences in topography and in pitting are most likely the result of bacterial activity.

Keywords: Corrosion, bacteria, pitting, sterile water, raw water

INTRODUCTION

Careful observations of the corrosion of steel surfaces in natural seawater have shown that pitting initiates almost immediately after first immersion and that these pits grow quickly in depth to about 100 microns\(^1\). Usually this is attributed to anode-cathode reactions set up by the small manganese sulfide (MnS) inclusions present in most steels\(^2\). It also has been proposed that the region immediately surrounding the MnS inclusion rather then the inclusion itself may be responsible for the observed pitting\(^3\). Although there is much research still on-going regarding the mechanisms involved in pitting corrosion, the possibility that microbiological activity can have some part in the pitting process for mild steels does not appear to have been given much attention, although the present authors have argued that the changes of the mild steel corroded surfaces with increased exposure time are likely to be the result of microbiological influences\(^4\). They observed that topography of the surface changes considerably with time and includes pit growth both in depth and in area, with subsequent pit coalescence and eventually the initiation of newer pitting.
It is now well-established that upon first immersion of a steel surface in natural seawater it is soon covered by a thin biofilm that harbours micro-organisms\(^5\) that appear to have an interaction with the corrosion process\(^6\). Other evidence points to microorganisms being involved in later corrosion, particularly when anaerobic niches form within the corrosion products, which then provide an appropriate local environment for sulfate reducing bacteria (SRB)\(^7\).

These observations are included in the mathematical models for uniform corrosion developed by the authors\(^8\) and also have been used in models for maximum pit depth\(^9\). Independent confirmation of the early part of the model has been reported\(^10\) and the overall trends are consistent with long-term pit depth trends\(^11\). Although these various observations appeared to provide interesting evidence for the involvement of microorganisms in pitting corrosion of mild steel in seawater, more direct evidence was thought to be desirable. For that reason the project described below was commenced in 2006 and has now reached almost three years of continuous exposure. Two questions were of interest: (a) whether bacteria are involved in very early pitting, that is within a short period of time after a steel surface is first exposed to natural seawater, and (b) whether any such involvement continues with time. The present paper describes the outcome of these investigations.

The next section describes the experimental protocol adopted, the equipment used and the conduct of the experiment. This is followed by a selection of photographic and scanning electron microscope (SEM) images for both the natural and the sterile seawater streams at various exposure periods. Overall the observations indicate very clearly that the topography of the steel surface is very different for natural and for sterile seawater and that this is the case immediately from first exposure. The important practical as well as scientific implications are then discussed briefly.

**EXPERIMENTAL DESIGN AND PROCEDURES**

It was considered that the most appropriate approach to examine the questions raised above would be simply a comparison of the surface topography resulting from exposure to natural seawater and that resulting from exposure to seawater for which bacterial activity was in some way controlled. While in principle this could be achieved by control over the rate of metabolism through control over nutrients essential for bacterial activity and survival, a more practical approach, consistent with earlier work, was considered to be the use of seawater from which all bacterial activity had been removed, preferably by killing all bacteria. Chemical or autoclaving have been used for this in the past but were discounted as they could alter the normal chemical and physical characteristics of the seawater. Some studies have used ultra-fine filtering to produce abiotic seawater for use in laboratory experiments (e.g.\(^12\)), but the volumes of water produced were very small. For the present work it was considered highly desirable to replicate field conditions as closely as possible, both for the natural exposures and for the exposures with controlled bacterial activity, and this precluded small test vessels and short-term tests. It is now clear that laboratory experiments that do not closely replicate field conditions may produce very misleading results\(^13\).

Closely realistic natural exposure conditions were achieved by using two parallel streams of low velocity seawater, one natural and one natural without bacterial activity, both sourced continuously from the one body of natural coastal seawater. The removal of bacterial activity in the second stream was achieved through the use of filtration and ultraviolet radiation to produce ‘sterile’ seawater. The latter was monitored continuously using periodic frequent testing for SRB and iron related bacteria (IRB).

Although a variety of techniques exist to study bacteria in biofilms\(^14\) these were considered to have the potential to interfere with the very processes being studied, that is, those within the biofilm and within the corrosion products. Moreover direct observation of bacterial processes within these environments was considered impractical for actual metal surfaces exposed to seawater. Similarly, it was recognized that detailed observation of individual pit development under natural conditions would be difficult experimentally, in part also because it is well-known that the location of individual pits cannot be
predicted with any degree of accuracy, even for small coupons or isolated surface areas. As a result of these limitations, a compromise experimental procedure was adopted, as described below.

The test rig, shown schematically in Figure 1, was established in a field laboratory within the Port Stephens Fisheries Centre site at Taylors Beach. This site is located on a tributary of Port Stephens, a coastal water body larger than Sydney Harbour and situated approximately 150 kilometers north of Sydney. The seawater at this site is generally similar to that of Pacific Ocean water in the adjacent coastal regions (Table 1). The Fisheries Centre site is used for fish breeding and fisheries research and has a large pumped fresh seawater supply taken directly from the local tributary. The seawater for the experiment was taken-off directly from this source.

![FIGURE 1 - Schematic Flowchart For Natural And Sterilized Seawater Trial At Taylors Beach.](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>Ammonia mg/l</th>
<th>Nitrate mg/l</th>
<th>Nitrite mg/l</th>
<th>Total P mg/l</th>
<th>Sulfate mg/l</th>
<th>Salinity ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.05 – 0.080</td>
<td>0.017 – 0.034</td>
<td>&lt; 0.003- 0.011</td>
<td>&lt; 003 – 0.07</td>
<td>1600 - 2750</td>
<td>25.7 – 31.3</td>
</tr>
</tbody>
</table>

The incoming seawater was split into two streams, one of which went directly into the ‘natural seawater’ exposure tank. After passing (slowly) through this tank the seawater was returned directly to the estuary. The second seawater stream was passed through a series of filters and sterilized using ultra-violet (UV) lamps prior to entering a similar but sealed tank, itself fitted with UV sterilization lamps. This was the ‘sterilized seawater’ tank. Again the water passed slowly through this tank and was then returned to the estuary. The filtration and sterilization process consisted of settling tanks, primary and secondary filters, a battery of 20, 50 and 5 micron filter bags and finally through two ultra-violet light sterilizing units. For both streams the water velocity was kept very low (< 1mm/sec) to ensure there were no velocity effects that would interfere with the observations.

The exposure tanks were 200 liter fiber-glass rectangular units. To attempt to ensure uniform flow conditions across the tanks, they have uniformly decreasing depth in the flow direction and water was discharged over a spillway to the drain. An underflow weir was used to minimize ‘dead spots’ within the water body. The sterilized tank had an airtight lid (Figure 2) and the raw water unit had a similar but not airtight fitted cover.
The area available on the stage of the SEM determined coupon size. The coupons were 25 mm x 25 mm x 1.3 mm thick mild steel plates that had been mirror polished on both sides prior to laser cutting. The coupons were stored in a desiccator until needed. They were sterilized with alcohol immediately prior to exposure.

One coupon was recovered from each of the natural seawater and the sterile seawater tanks after 1, 4, 7, 50, 100, 135, 190, 280, 360, 554, 726 and 943 days. All coupons were examined externally immediately upon recovery and the nature of the rust formation noted. They were then cleaned; typically a strong stream of water was sufficient to remove the rust layer back to bright steel. In cases where the rust layer was adhesive coupons were cleaned with dilute inhibited hydrochloric acid then rinsed in water and then alcohol before drying. All coupons were gold coated prior to being examined in the SEM.

The efficacy of the sterilization system was monitored at regular intervals by taking samples from the water stream immediately after various filtration and ultra-violet units. Samples were taken also from the sterile water tank. These were tested for sulfate reducing bacteria (SRB) and iron related bacteria (IRB) using commercial test kits. Over the whole test period no evidence of SRB or IRB was found in the sterile seawater tank. However, bacterial activity was found regularly in the natural seawater tank. Water temperature in both tanks was monitored over a one-year period, as summarized in Table 2. The temperature in the sterile tank was on average 2°C warmer than in the raw water tank. This was attributed to the water passing over ultra-violet lights as well as the UV light in the roof of the tank.

### Table 2

<table>
<thead>
<tr>
<th>Water temperature °C</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
<th>Typical diurnal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile seawater tank</td>
<td>11.3</td>
<td>33.6</td>
<td>21.5</td>
<td>1.0-2.5</td>
</tr>
<tr>
<td>Natural seawater tank</td>
<td>10.3</td>
<td>31.3</td>
<td>19.5</td>
<td>1.0-2.0</td>
</tr>
</tbody>
</table>

### EXPERIMENTAL RESULTS AND DISCUSSION

#### Overall Observations

Coupons recovered during the first three months had generally similar rust layers, irrespective of the type of seawater. This gradually changed with increased exposure period. After about 3 months (135 days) some differences were noted. The coupons from the sterilized seawater tank showed a much more regular rust build-up than did that the coupons from the natural seawater tank (Figures 3 and 4).
The rust layer on the natural seawater coupons typically showed definite signs of biological influence with the presence of pustules and slime (Figure 3). In contrast, for the coupons from the sterilized water the rust layer appeared very uniform, and showed what appeared to be lines of oxide (Figure 4). Again, it is reasonable to suppose that the difference is the result of the presence of microbiological components in the case of the rusts shown in Figure 3.

The nature of the corrosion products did not appear to change much with longer periods of exposure, although there was a clear increase in volume. After about 2.6 years there was a clear difference between the rusts on the coupons from the sterilized seawater tank and those coupons from the natural seawater tank (Figures 5 and 6). Extended exposure produced prolific rust on both sets of steel coupons. The rusts on the coupon exposed to natural seawater for 943 days showed a large orange tubercle (Figure 5). When this was broken open it contained a black oxide that is often found in similar tubercles. The black oxide layer was readily washed off to reveal a bright steel surface. In contrast, for the sterilized seawater the recovered coupon showed an oxide layer that was less regular than that observed at, say 135 days exposure (Figure 3). There also was no evidence of tubercles. Removal of the outer orange crust revealed a black oxide underneath that readily washed off to expose a bright steel surface.
Differences in surface topography also were clearly seen after coupons were cleaned and before more detailed examination. For example, the coupon exposed to natural seawater for 100 days (Figure 7) showed a surface that was more uneven in surface texture and also was more heavily pitted than the coupon exposed to sterilized seawater for the same time period (Figure 8). In contrast, the only pitting on the coupon from this tank was around the top attachment hole. This probably is a result of differential aeration. Otherwise the surface was relatively flat and free of pitting.

Generally similar observations were made for the surfaces of coupons exposed for 554 days with the coupon exposed to natural seawater showing more and deeper pitting (Figure 9) than the coupon exposed to sterile seawater (Figure 10).
SEM Observations

Selected detailed observations made using the SEM are given below, in sequence, to illustrate some of the features observed. It should be noted that the SEM observations showed considerable differences on each surface, depending on where attention was focused. Herein most attention is given to pitting effects rather than other features.

SEM images of the typical surface of coupons recovered after 24 hours of immersion are shown in Figures 11 and 12. Already after one day exposure the cleaned surfaces of the coupons showed distinct anode-cathode regions as indicated by some regions of the surface having evidence of oxidation whilst other regions appeared to be unaffected. Similar behavior has been noted previously\(^8\). There was some light-colored, adherent rust that could be removed with a very short (< 1 min) soak in dilute acid. Visible pitting was found on both coupons, entirely consistent with much earlier observations by Butler et al. \(^1\) who found pits of depth approaching 100 microns within days of exposure to seawater. Although Figures 11 and 12 show only a small part of the overall coupon surface, it was very clear that there were a greater number of pits for the natural seawater coupon. Figure 12 appears to show some steps or “benches” within some pits but this is not evident in Figure 11.

![FIGURE 11 - Mild Steel Surface Of After 24 Hours Exposure In Natural Seawater Showing A Multitude Of Pits Approaching 100 Microns In Diameter. Bar = 100 μm.](image1)

![FIGURE 12 - Mild Steel Surface Of After 24 Hours Exposure In Sterile Seawater Showing A Small Number Of Pits But One Pit Already 100 Microns In Diameter. Bar = 100 μm.](image2)

The surface topography changes little in the next few days, as seen after 7 days, shown in Figures 13 and 14. There is now more evidence of ‘steps’ or ‘benches’ for the surface of the natural seawater coupon (Figure 13). It has been proposed previously that such topography is the result of bacterial activity\(^4\). This suggests that the bacteria present in the natural seawater, as evident from the water quality, influenced the pitting corrosion process within the first week of immersion. The surface exposed to sterile seawater appears to be less pitted with larger sized pits (Figure 14).

Although Figures 13 and 14 show quite regular surface topography, the coupon surface topography overall varied considerably. The surface of the coupon exposed to natural seawater (Figure 15) appears in places to be covered by a cracked film of material, which also appears to cover what seem like pits under the film. This could be consistent with metal deposition around the edges of pits\(^15\). A different form of somewhat unusual surface topography is shown in Figure 16 for the natural seawater coupons. As with all samples, these coupons were acid cleaned, so the surface topographical features are not calcareous or biological in nature. EDS analysis confirmed they have a high iron content.
FIGURE 13 - Pitting Of Steel Surface After 7 Days In Natural Seawater. Bar = 100 μm.

FIGURE 14 - Pitting Of Steel Surface After 7 Days In Sterilized Seawater. Bar = 100 μm.

Both Figures 15 and 16 show inchoate dough-nut-like forms on some of the regions without pitting, and, again, this occurs for both natural and sterile seawater, suggesting it is not a function of bacteria activity. These surface patterns also are unlikely to be oxides as the samples had been acid cleaned immediately prior to SEM preparation. Energy dispersive spectrographic (EDS) analysis of the formations gave identical traces to that of the original base steel. Similar images have been reported previously by the authors on coupons in seawater immersion conditions⁴ and are also seen in images reported recently by Caceres et al.¹⁶

FIGURE 15 - Steel Surface After 7 Days In Natural Seawater Showing What Appear To Be A Pits With (Cracked) Metal Deposits Over. Bar = 100 μm.

FIGURE 16 - Steel Surface After 7 Days In Sterilized Sea Water Showing Unusual Surface Features That EDS Analysis Showed Are High In Iron Content. Bar = 50 μm.

Although several other observations were made after 7 days, the surface topography did not change significantly over time, as illustrated by Figures 17 and 18 for coupons obtained after 50 days continuous exposure. Although the photographs appear to show that pitting was more severe for the sterile seawater exposure, this was not generally the case. Figures 19 and 20 show some typical samples of surface topography after 135 days exposure.
FIGURE 17 - Pitting Of Steel Surface After 50 Days In Natural Seawater. Bar = 200 μm.

FIGURE 18 - Pitting Of Steel Surface After 50 Days In Sterilized Sea Water. Bar = 200 μm.

FIGURE 19 - Typical Pitting On Steel Surface After 135 Days In Natural Seawater. Bar = 50 μm.

FIGURE 20 - Typical Pitting On Steel Surface After 135 Days In Sterilized Seawater. Bar = 100 μm.

Figures 21 and 22 show the surfaces after 360 days (one year) of exposure. The natural seawater coupon showed an irregularly pitted surface and the sterilized water coupon showed more and larger crystalline and irregular features.

After 943 days (Figures 23 and 24) the coupons showed extensive corrosion plateaus, similar to those reported earlier. The large pitted regions in Figure 23 also appear to show smaller pitting within these large pits, a phenomenon previously associated with bacterial activity.
Corrosion Products

At 943 days exposure the corrosion products were analyzed by XRD techniques and then by a Rietveld analysis. Figures 25 and 26 show the XRD traces for rusts from natural and sterile seawaters respectively. Table 3 shows the estimated percentage of each phase detected by XRD in the rust samples. These results were obtained using a standard analysis package that employs a least squares technique to estimate theoretical patterns for each of the phases present and then fits these against sample patterns. As a result the estimated relative compositions are not necessarily very accurate and should be considered indicative only.

Nevertheless, both the XRD traces in Figures 25 and 26 and the XRD results given in Table 3 show very considerable differences in rust composition between the rust obtained in natural seawater and that obtained in sterile seawater. The sample of rust from the natural seawater exposure shows a much greater presence of magnetite and hematite while that obtained in natural seawater has a much higher presence of goethite, lepidocrocite and aragonite.
### TABLE 3
**COMPARATIVE OCCURRENCE OF RUST COMPONENTS**

<table>
<thead>
<tr>
<th>Component</th>
<th>Natural seawater %</th>
<th>Sterile seawater %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetite</td>
<td>Fe$_3$O$_4$</td>
<td>38</td>
</tr>
<tr>
<td>Goethite</td>
<td>$\alpha$-FeOOH</td>
<td>24</td>
</tr>
<tr>
<td>Lepidocrocite</td>
<td>$\gamma$-FeOOH</td>
<td>27</td>
</tr>
<tr>
<td>Aragonite</td>
<td>CaCO$_3$</td>
<td>7</td>
</tr>
<tr>
<td>Quartz</td>
<td>SiO$_2$</td>
<td>2</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>NaCl</td>
<td>1</td>
</tr>
<tr>
<td>Green Rust 2</td>
<td></td>
<td>trace</td>
</tr>
<tr>
<td>Akageneite</td>
<td>$\beta$-FeOOH</td>
<td>-</td>
</tr>
<tr>
<td>Hematite</td>
<td>Fe$_2$O$_3$</td>
<td>-</td>
</tr>
<tr>
<td>Ferric oxychloride</td>
<td>FeOCl</td>
<td>-</td>
</tr>
</tbody>
</table>

**FIGURE 25** - Annotated XRD Trace Of Rust Sample From Natural Seawater Exposure After 943 Days.
FIGURE 26 - Annotated XRD Trace Of Rust Sample From Sterile Seawater Exposure After 943 Days.

The above observations of the forms of the rusts and the topography of the cleaned surfaces of the steel coupons show that a clear difference exists at all times between those surfaces exposed to natural seawater and those exposed to sterilized seawater. The only known or detected difference between the two seawater streams is their living microbiological content (apart from a very small difference in average water temperature resulting from the UV lamps). Various previous investigations have indicated that bacterial activity can influence the corrosion of steel in seawater both in the period soon after immersion (e.g.\textsuperscript{10,17}) and subsequently\textsuperscript{18} and the present observations are generally consistent with these earlier findings. Although microbiological influence has previously been associated with pitting\textsuperscript{19}, and more particularly so for stainless steels\textsuperscript{12}, direct, clear evidence has been lacking. There also has been no direct, clear evidence to date that the overall corrosion products can be significantly different between seawater with and without living microbiological content, such as shown herein.

The bacteria involved in the natural seawater stream have not, in the present project, been specifically characterized although it is known from earlier work that for immersion conditions the SRB are likely to be the most influential\textsuperscript{17}. The water quality testing used in the project for checking the effectiveness of the sterilization system was based on verifying the presence or absence of SRB and IRB using commercial test kits.

CONCLUSION

The images presented herein show that both the topography of the cleaned steel surfaces and the composition of rust products are considerably different for those coupons exposed to natural seawater and those exposed to sterilized seawater. It is noted that the only significant difference between the exposure conditions was the removal of living microbiological (and other living) material. On this basis it
is concluded that microbiological factors are responsible, directly or indirectly, for the more severe pitting observed on the natural seawater coupons.

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REFERENCES