BACTERIOLOGICAL INFLUENCE IN THE DEVELOPMENT OF IRON SULPHIDE SPECIES IN MARINE IMMERSION ENVIRONMENTS

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1. INTRODUCTION

The precise role of bacteria and microbiological agents and consortia in immersion corrosion has been the subject of much interest, particularly when anaerobic bacteria control the corrosion process (Videla, 1996). It is known that this may occur quite soon after immersion for tropical waters (Southwell and Alexander, 1970). Conversely, bacteria do not appear to be significantly involved for immersion corrosion at lower water temperatures

The role of anaerobic bacteria in the marine corrosion of steels appears to be particularly important for higher water temperatures and longer durations as anaerobic conditions then govern (Southwell, et al. 1979). There are, of course, other areas for which anaerobic bacteria are of importance, such as their role in the corrosion of stainless steels. Despite their importance it appears that details of their interaction with steel and in particular the iron component in steel is not fully understood and documented (Videla, 1996; Little, et al., 2000).

This work arose out of a project aimed at better understanding the complexities of corrosion under marine immersion conditions. As part of this project a series of exposure trials were conducted at a number of immersion sites in coastal waters on the south-east coast of Australia but principally around Newcastle on the NSW coast and in Lake Macquarie.

2. IRON TO IRON SULPHIDE TRANSFORMATION

The transformation from iron to iron sulphide species through iron oxide is well documented in the literature. It commences with the attachment of bacteria to the metal surface. This appears to occur within hours of immersion of a metal in seawater, with bacterial growth being initially (i.e. within 2-3 days) influenced by surface roughness (e.g. Terry and Edyvean, 1984). The adhesion of the bacteria is a complex phenomenon (Characklis and Marshall, 1990).

Within two or more weeks a bacterial film develops, producing at the liquid-solid interface a number of organic by-products such as organic acids, hydrogen sulphide and 'slime', a protein rich polymeric material (Tiller, 1983).

Bacterial colonization is followed quickly by microbiological consortia that include unicellular diatoms, colonial diatom growths and green and brown algae. Eventually higher order flora and fauna establish themselves (Fletcher and Chamberlain, 1975). It appears however, that bacteriological activity on the metal surface, rather than marine flora and fauna is critical in the control of the rate of oxygen supply to the corroding surface of the metal (and hence of corrosion) (Sanders and Maxwell, 1983).

The transformation from iron to iron sulphide can be represented as a sequence of intermediate transformations. These are shown in Figure 1 (page 4). Anchor points to the images known from the literature are shown also. The figure numbering refers to the figures in the present paper. They all refer to SEM (scanning electron microscope) images.

3. EXPERIMENTAL PROCEDURE

Mild steel coupons (50mm x 100mm x 3.0mm) prepared to ASTM corrosion testing standards were exposed at a number of sites under varying environmental conditions. Corrosion rates were determined using weight-loss measurements. This involved removal of the corrosion product, but rather than discarding it, for some specimens small samples were carefully removed and prepared for SEM examination.

Mild steel coupons 10mm x 10mm x 0.8mm were exposed for periods varying from one hour to 28 days. They were sized specifically to fit easily in the SEM observation chamber without disturbing the corrosion product layer. Typically these coupons exhibited small amounts (<5%) of iron hydroxide after only one hour of exposure. Slime formation was observed after 24 hours of exposure. The coupons were completely covered with corrosion product after one week. After two weeks some colonisation by hydroids was apparent. Macro-biological growth was minimal after 28 days.

The specimens were exposed at three sites, two in

Figure 1:

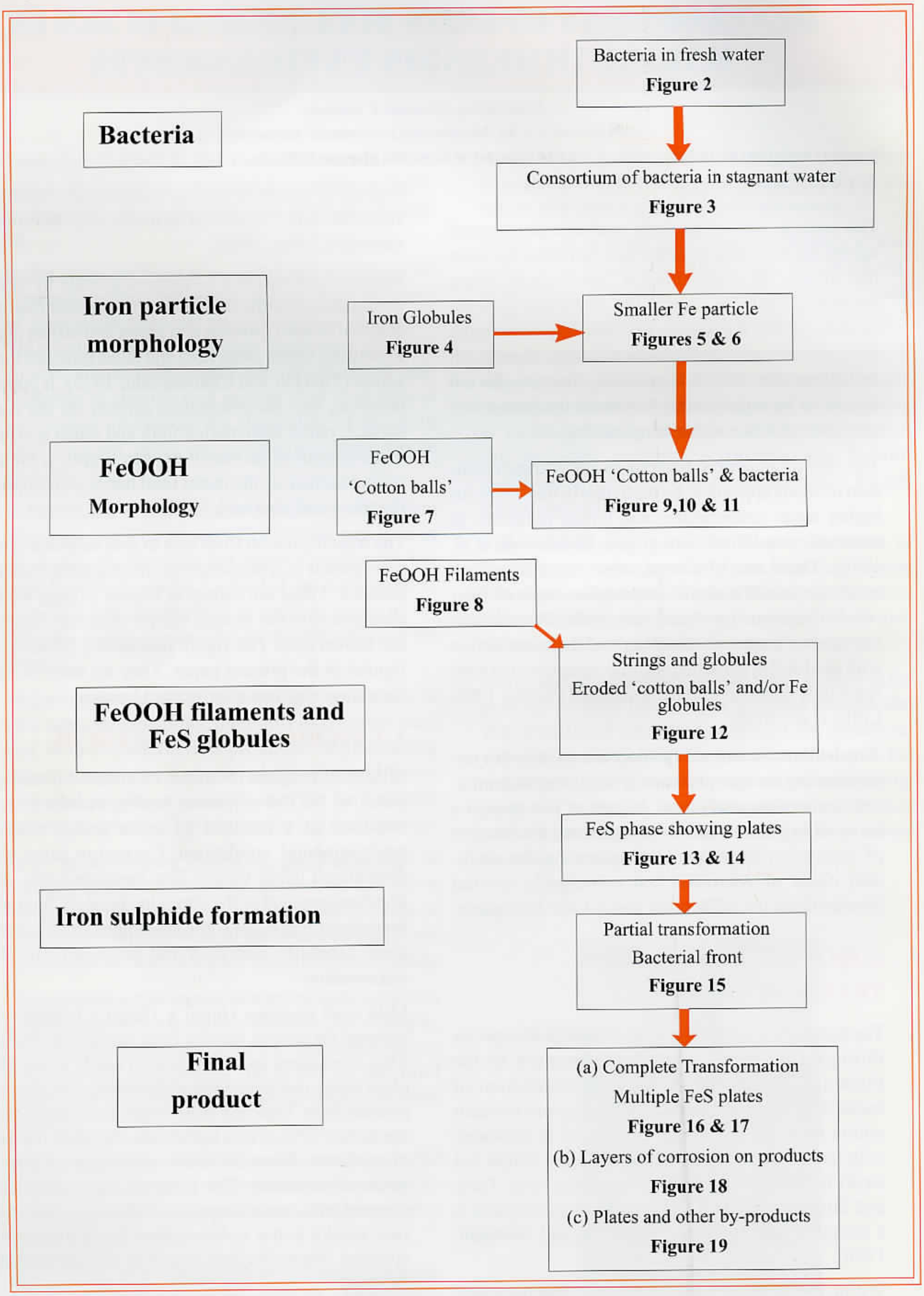

Figure 1 - Iron to iron sulphide transformation with figure numbering for SEM images.

Swansea Channel and one at Taylors Beach, both on the Eastern Australian seaboard. Typical site conditions are shown in Table 1.

Table 1 - Conditions at immersion sites.

Site	Site Conditions	Temp Range (°C)	Max Water Velocity (m/s)	Salinity PPT	Sample Depth m
Swansea Channel	Swift flowing estuary	16-22	1.0	27-34	0.5-1.5m
Taylors	Quiet	12-26	0.15	20-.35	0.5-1.5m

The composition of the steel used for coupons is: 0.17% C, 1.12 % Mn, 0.18% Si 0.022% P, 0.015% S, 0.006% Al, and 0.01% Cu, which complies with AS 3678 grade 250 steel.

The experimental work, performed to ASTM corrosion testing standards, and the operation and analyses using SEM, EDS and XRD equipment involved trained and experienced staff.

4. METAMORPHOSIS OF IRON TO IRON SULPHIDE

4.1 Bacteria in seawater

Because of the central role played by bacteria in the discussion to follow, it is helpful to describe the morphology of bacteria in seawater prior to considering their interaction with corrosion processes. Figure 2 shows an SEM for bacteria cultured from clean seawater. The bacteria shown are relatively small being in the order of 0.5 –1 μm long which is at lower size range the when compared to the typical morphology of bacteria consortia (Prescott et al 1999).

To gain an understanding of the effect of poorer quality seawater on the morphology of bacteria and consortia, corrosion coupons and seawater were recovered from Swansea Channel. They were stored together to maintain the bacterial colonies. After 24

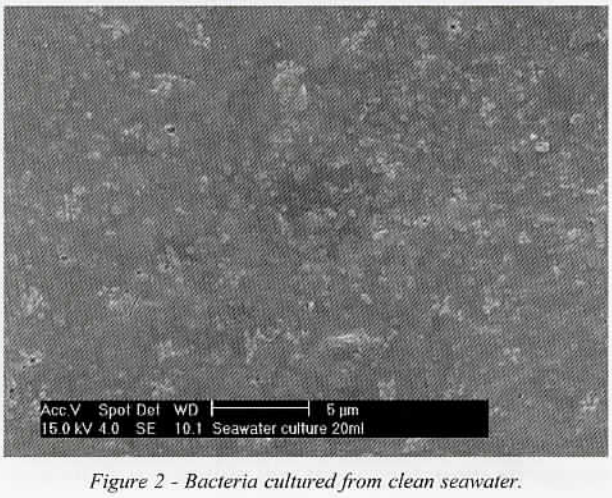

Figure 2 - Bacteria cultured from clean seawater.

hours this produced stagnant conditions. The resultant liquor contained biota from within the general biomass on the coupons. These included amphipods and diatoms as well as bacteria from within the corrosion product. The broth was filtered and the filtrate was cultured for 96 hours. The cultured bacteria were then processed for SEM observations. Some of the unfiltered broth was kept for separate analysis (see section 4.2).

Figure 3 shows a consortium of cultured bacteria. For the following discussion the small light coloured triangular species are of particular interest. In an attempt to identify these bacteria DNA sequencing was carried out on bacteria cultured from coupons recovered that had been exposed under similar conditions. Although a number of candidate species were identified, no specific conclusion could be drawn. Nevertheless, the triangular species appear to play an important role, as will be discussed in section 4.2 below. An important feature is that the triangular shape is relatively uncommon in bacterial morphology (Prescott, et al, 1999).

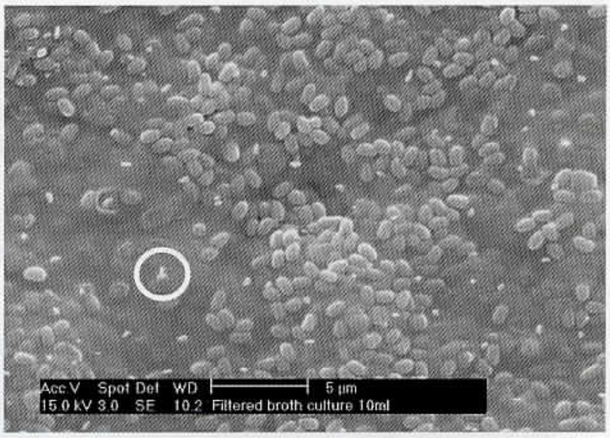

Figure 3 - Consortium of bacteria cultured from stagnant seawater.

4.2 Iron morphology and bacteria

SEM images were prepared from a number of corrosion product samples recovered from the unfiltered broth described in Section 4.1. Figure 4 shows a typical example. Most of the image shows iron hydroxide (the identification of which is discussed in Section 4.3). Also shown are what appear to be globules of metallic iron. These are interspersed throughout the background material. The three smooth, roughly rectangular objects are identifiable as diatoms (Miller, 1971, Borenstein, 1994).

Of particular interest in Figure 4 is the metallic iron globule near the centre of the image. It can be distinguished from the surrounding background of iron hydroxide by its flaking surface morphology. An EDS analysis of the globule would show a strong presence of Fe, indicating that the globule is composed almost entirely of iron. This is in contrast to the surrounding material. Indications are that some traces of chloride, calcium and other minor elements also are present.

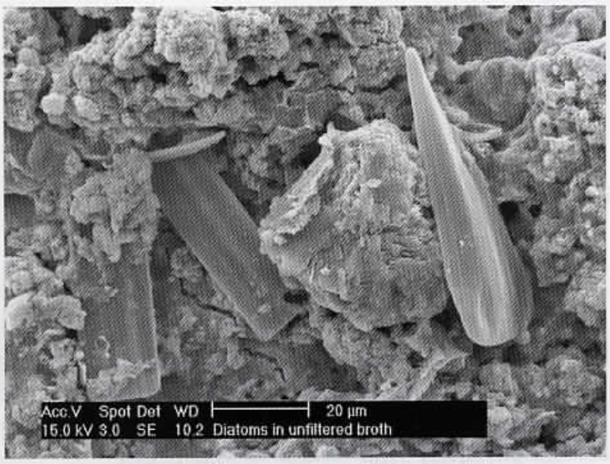

Figure 4 - Diatoms and metallic iron globule

Figure 5 shows a diatom against a background of iron oxide species and a small iron particle at the bottom right of the diatom. The iron particle is covered with white triangular particles. A more detailed view of this area is shown in Figure 6.

Visual examination of the white triangular particles in Figure 6 suggests that they are similar in morphology to those shown in Figure 3. Since they are from the same broth it is inferred that they are the same bacteria. In contrast, the diatom shown in Figure 5 appears to be composed mainly of silicon with some background iron.

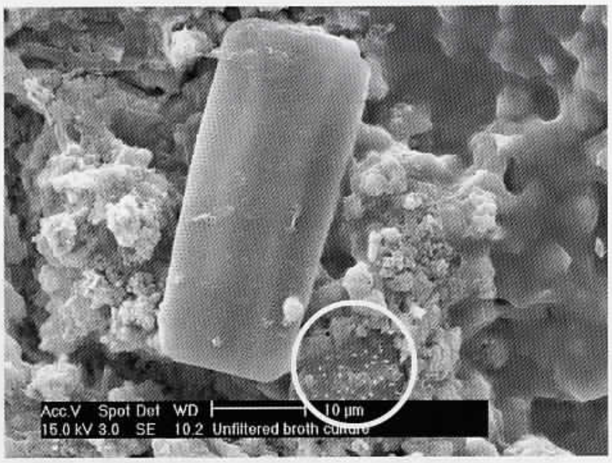

Figure 5- A diatom against a background of iron hydroxide and a small iron globule at the bottom right of the diatom.

4.3 Iron hydroxide morphology

In order to discuss the transformation of iron to its corrosion products it is necessary at this point to introduce their morphologies. The SEM of a typical mild steel coupon recovered within one hour of immersion in free flowing clean seawater is shown in

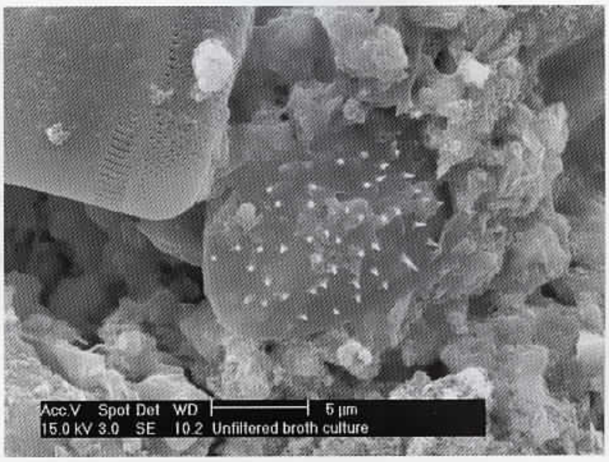

Figure 6 - Magnification of iron globule showing it is covered with white triangular particles.

Figure 7. XRD analysis of coupons with this corrosion product confirms hydrated ferric oxide in the form of g-FeOOH as the primary phase after one day's exposure. XRD analyses of coupons recovered over the course of the present investigation indicate iron oxide hydrate ($Fe_2O_3.H_2O$) and the g-FeOOH form of hydrated ferric oxide as the primary forms of iron corrosion product. For the purpose of this paper any combination of these will all be referred to as iron oxide.

The fine needle-like structures in Figure 7 should be noted. Their EDS indicates high counts of sulphur. This confirms the presence of sulphur in the corrosion product even at this early stage (one hour) of immersion.

Other images, obtained from a specimen after 10 hours exposure, are generally similar to Figure 7. One of them exhibited the formation shown in Figure 8, observed around the attachment hole. It depicts strings of iron oxide similar to those shown by Videla (1996) and others. On the basis of the observations by Videla (1996) it is proposed that the shape of the corrosion product has been influenced by the bacteria species

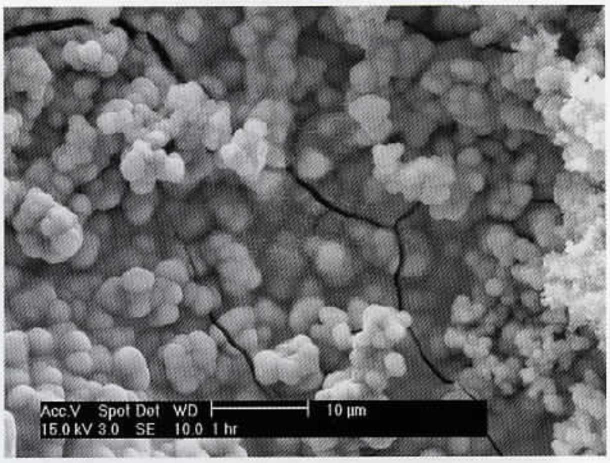

Figure 7 – Morphology of hydrated ferric oxide species after one hour of immersion

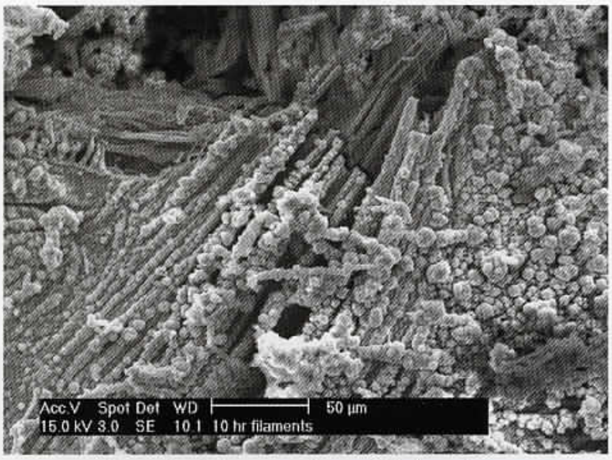

Figure 8 - Filamentous iron oxide formations.

Gallionella ferruginea. If this is the case it means that significant bacterial activity has occurred within 10 hours of exposure. The remainder of the coupon consisted of a morphology similar to Figure 7.

The observations shown in Figure 7 were also made in other cases. A typical image of bacterial activity after 14 days exposure is shown in Figure 9. It shows that the general surface condition consists of a relatively hard crust with typical globular iron oxide morphology underneath.

Higher magnification of the central globular region in Figure 9 revealed bacterial activity.

Figure 10 shows iron oxide spheres with clear small surface indentations. Precisely what these might be

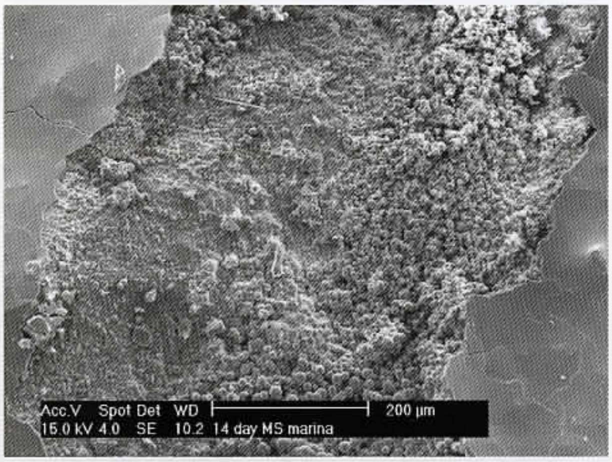

Figure 9 - Morphology of corrosion product after 14 days exposure.

is not immediately obvious but reference might be made to imprints, somewhat similar in shape and size, to those observed in iron sulphide deposits recovered from deep-sea hydrothermal 'black-smokers' (Vetari et al., 1999). These authors suggest that the imprints they observed were the cavities created by microbes that have bioleached tunnels through the iron sulphide deposit. It is possible that the indentations shown on the iron oxide spheres in Figure 10 are of a similar origin. In this case the imprints can be interpreted as the start or part of the decomposition of iron oxide.

In the same Figure (10) the relatively small white particles are similar in shape and size to those in Figures 1, 2 and 5 and which were earlier considered to be bacteria. This supposition is reinforced by micrographs which show bacteria with copper–sulphur corrosion products (e.g. Little 2000).

A clearer view of the process appears to be shown in Figure 11, with nearly perfect iron oxide spheres at

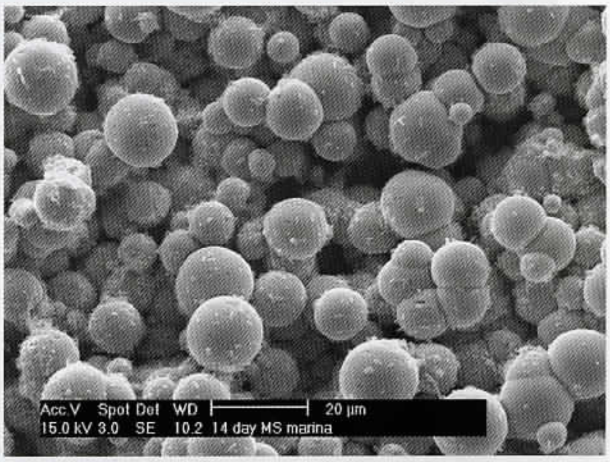

Figure 10 - Bacterial activity on iron oxide spheres.

left of the micrograph and what are assumed to be deteriorated spheres at mid-right.

Further support for the above supposition is provided in Figure 12. It shows iron oxides from a sample recovered after 7 months exposure. Two different shapes of the species can be seen, namely globules (see also Figure 7) and strings (see also Figure 8). It is considered that the globules in Figure 12 are those of Figure 11 but exhibiting greater bacterial induced degradation as shown by their 'spongy' nature.

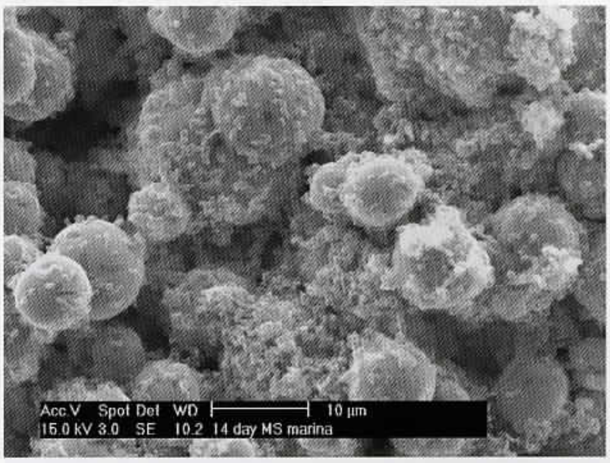

Figure 11 - Deterioration of iron oxide species due to bacterial activity.

The conclusion from these observations is that the iron oxide globules in Figures 10 and 11 have transformed to the globules shown in Figure 12 and that these are iron-sulphides.

Turning now to the strings in Figure 12, an analysis by EDS shows a very strong presence of iron. There

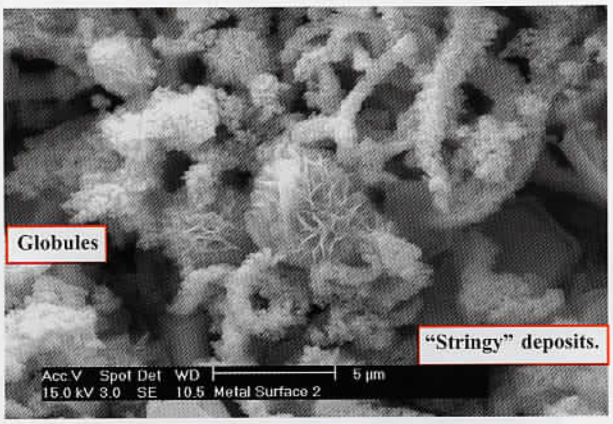

Figure 12 - Image of morphology of iron oxide region of sample recovered after 7 months exposure. Two different shapes of the species can be noted, namely strings and globules.

is only a trace of sulphur. This indicates that the strings are a form of iron oxide or hydroxide. It is postulated that the strings in Figure 12 probably are the result of the action of the iron oxidising bacteria. It is known that the bacteria have the common feature of oxidising Fe^{+2} to Fe^{+3}, which generally precipitates as iron hydroxide. Frequent corrosion-causing agents are bacteria from the family *Caulobacteriaceae* in general with the genera *Gallionella* and *Siderophacus* playing particularly prominent roles (Vidella 1996). The strings appear to be irrelevant to the iron sulphide development process as they were found not to take a further part in the transformation.

4.4 Iron sulphide species morphology

It is known that iron sulphides can exist as six recognised species and the existence of a strictly amorphous iron sulphide is highly unlikely (Rickard, 1969; Miller and King, 1975). The species can change from one form to another either biogenically or abiogenically. In what follows, images are presented which appear to show a transformation to iron sulphide.

For purposes of identification, the structure of the various forms of iron sulphide is of interest. From mineralogical observations it is known that greigite has a cubic form and the polymorph smythite is rhombohedral with a structure consisting of slabs of pyrrhotite (FeS) stacked on each other to give a sheet structure (Wedepohl, 1978). It is known that pyrrhotite has a hexagonal form, as noted, for example, by Neal et al. (2001) through XRD identification.

In the observations of corrosion product for the samples in the present study, a tabular hexagonal morphology was observed in the corrosion product of many specimens (Figure 13). However, an interesting observation is the presence in Figure 13 of 'spongy' globules similar to those of Figure 12.

Figure 13 - Corrosion product showing tabular hexagonal crystals and 'spongy' globules.

A closer view of these globules and their relationship to the tabular hexagonal plates is shown in Figure 14. Analyses of the plates and of the globules makes it is clear that the spectra are almost identical. This suggests that the plates and the 'spongy' globules are different morphologies of the same chemical species. Moreover, the presence of bacteria in the 'spongy' globules suggests that the bacteria are instrumental in the transformation process. As far as can be ascertained, a photograph similar to Figure 14 has not appeared previously in the literature.

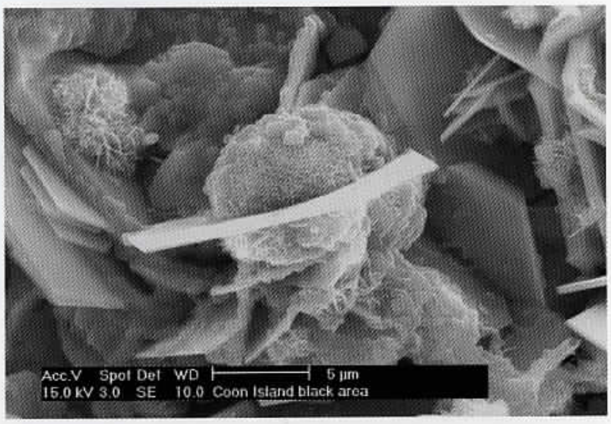

Figure 14 - Closer view of iron sulphur crystal forming from a globular mass.

A view of the likely progression of the above transformation can be seen in Figure 15, obtained from a specimen exposed for 2 months. It shows a number of morphological states. Under the outer oxide crust there are distinct areas of both the globular and tabular plate formations of the iron-sulphur phases. It appears that a "front" of bacteria consortia has moved from left to right through the corrosion product and has produced the plate-phase iron sulphide product (as shown on the left).

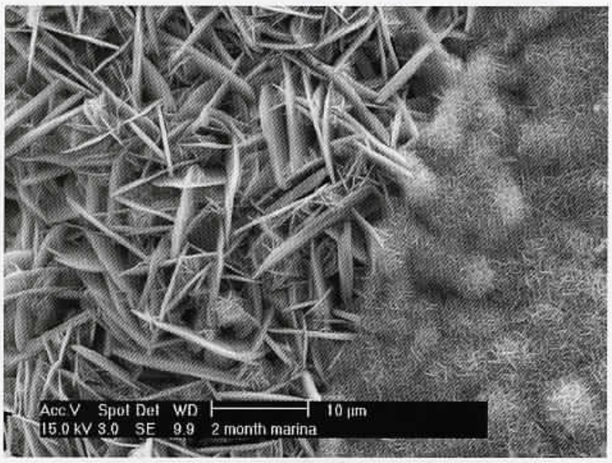

Figure 15 - Trailing edge of bacterial consortium transforming iron-sulfur globules to plates (final product on the left).

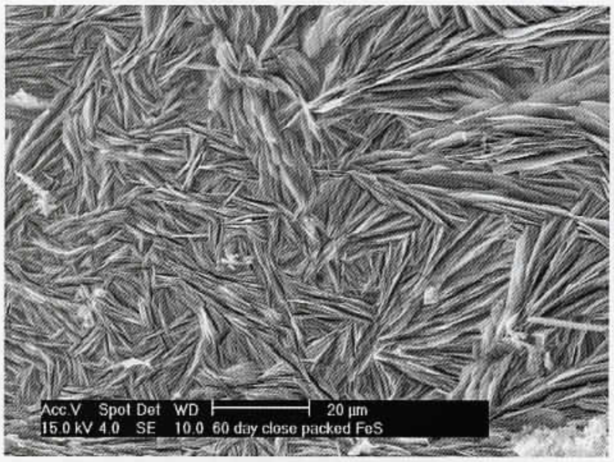

Figure 16 - Image of closely packed tabular plates.

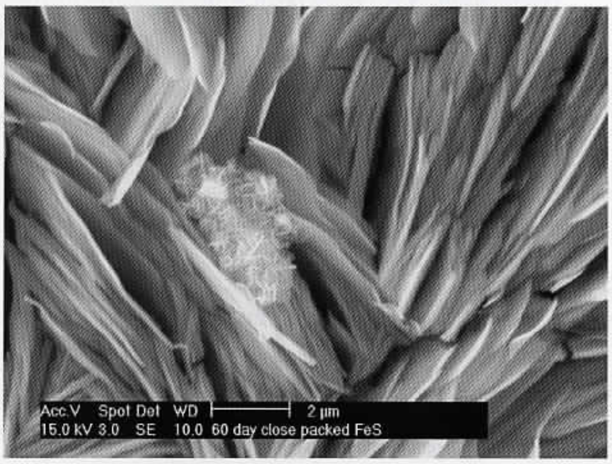

Figure 17 - Magnification of part of Figure 16.

The final iron-sulphide species are shown in Figures 16-19 for specimens recovered after two months exposure. They exhibit a considerably denser form of the tabular plates than seen on the left of Figure 15, suggesting an on-going process of plate development. This is suggested also by the presence of the small white iron-sulphide crystals in the centre of Figure 17. These should be compared to the small white crystals apparent in Figure 7. They were found to have a similar EDS spectrum. It was suggested earlier that these are involved in the transformation process. Figure 17 indicates that they continue to be involved.

4.5 Overview

A specimen recovered after 5 months exposure can be used to illustrate the complete sequence (Figure 18). Three discrete layers of corrosion product could be identified by visual observation - two layers of black and one overlying brown oxide layer. These conform to the observations reported in the literature (e.g. Hamilton, 1994), and also the transformations described above.

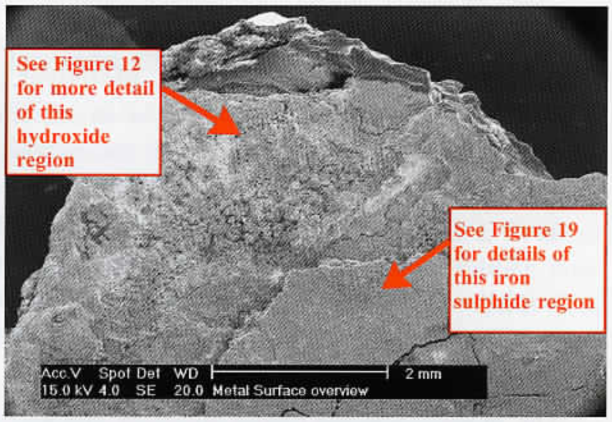

Figure 18 - Image showing distinct regions or phases of corrosion product.

In SEM observations the layers are seen as light, dark and grey respectively. The light and grey areas consist of iron oxide species, whereas the dark regions indicate iron sulphur species in a metamorphic state. Each of these corrosion product layers was examined. The image shown in Figure 18 is from the underside

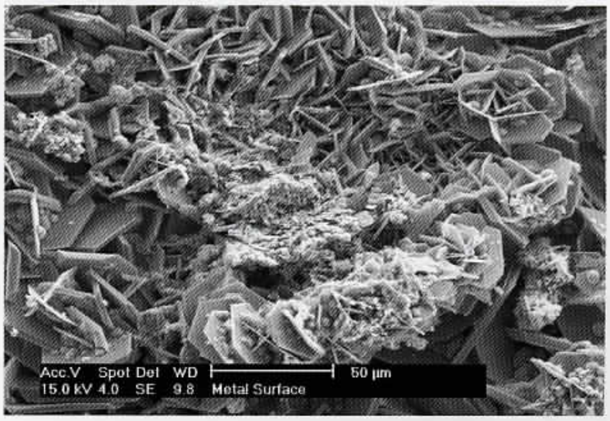

Figure 19 - Higher magnification of Figure 18

of a section of corrosion product removed from a coupon that had been exposed for 7 months. A closer view of the outer orange oxide layer is given in Figure 12 (Section 4.3).

Figure 19 shows more detail of the black iron-sulphide region. It was taken from the area that was closest to the metal surface and which appeared as a black deposit. As confirmed by the EDS in Figure 20, the plates are a form of iron sulphide.

5. DISCUSSION

The images and analysis presented above were part of the outcomes of a series of experimental trials conducted primarily for the elucidation of particular influences on corrosion. This work allowed examination in detail of the corrosion products obtained. The transformation pattern described herein gradually became apparent as new observations were made.

It is recognised that iron sulphide can exist in a wide range of forms -crystalline and amorphous - each with its characteristic iron/sulphur stoichiometry (Rickard 1969, Hamilton 1994). Moreover, the corrosion products are known to be an extremely complex mixture of sulphides, oxides and carbonates. This was also found in the present study with magnetite, schwertmannite, akaganeite, nahcolite, northuptite, mountkeithite, brugnatellite, iron sulphate hydroxide, iron chloride, and, green rust (iron carbonate hydroxide) being some of the most common species identified by XRD analysis.

The images reported herein and their arrangement represent an effort to reconstruct the transformation processes involved in the marine immersion corrosion of steel. Each image is only a 'snap-shot' of the physical conditions at a discrete point in time. Naturally the processes themselves cannot be captured in this way, although reasonable inferences can be made with the aid of the observations and conclusions of previous investigators, both in the marine corrosion literature and elsewhere.

It should be noted that all the observations were taken in coastal and seawater estuary conditions in the Newcastle, Australia, region at average water temperatures of about 20°C, ranging from 11°C in winter up to 23° in summer.

Finally, it is acknowledged that the observations made herein represent only a proportion of all the processes involved in marine corrosion and marine product formation.

6. CONCLUSION

Scanning electron microscope (SEM) images were obtained for steel specimens exposed to actual corrosion conditions in a temperate seawater climate. Energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD) were used to analyse the corrosion products.

In general the observations tend to confirm that bacteria and microbiological consortia play an important role in the corrosion of steel in marine environments. A sequence of SEM images was presented for the metamorphosis of iron to forms of iron oxide and then to iron-sulphur compounds for steel under marine corrosion conditions. The images show what appear to be new details of the involvement of bacteria in the transformations.

7. ACKNOWLEDGEMENTS.

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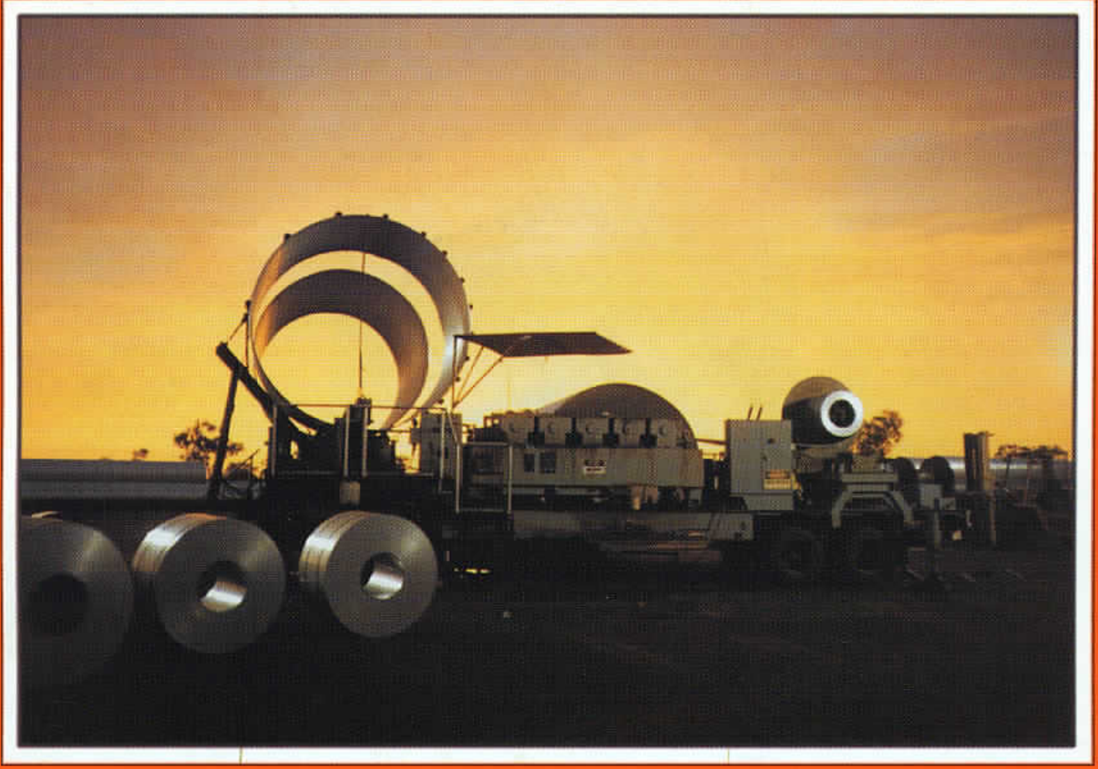

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