MICROBIAL CORROSION OF COMMON PIPING MATERIALS IN THE PWV AREA

by

A. Bondonno ● C. Ringas ● J. Ramotlhola ● C. Prinsloo

Advanced Engineering and Testing Services
Division of Materials Science and Technology, CSIR

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EXECUTIVE SUMMARY

Corrosion of potable water distribution pipelines not only affects the integrity of the system but can also influence water quality by the release of corrosion products into the system. Severe corrosion can lead to leaks and bursts with the associated costs of water loss and pipe replacement. In a recent project carried out by the CSIR for the WRC (K5/254 Research on the effects of varying water quality on the corrosion of different pipe materials in the PWVS/Klerksdorp areas), to examine the effects of the chemical composition of potable waters on the corrosion of common piping materials, the possibility of the involvement of bacteria in the corrosion process was reported. In their recommendations, the Steering Committee suggested that microbiologically induced corrosion of common piping materials be investigated more fully.

The objectives of this study were thus to determine to what extent microorganisms are involved in the corrosion of common piping materials in potable waters and how widespread the problem is in the PWV/Klerksdorp area. Furthermore, the stage during biofilm growth at which corrosion is initiated by microorganisms was to be determined. If microbial corrosion did prove to be a problem, remedial and/or preventative measures were to be recommended. A better understanding of the failure mechanisms of potable water pipes in a widespread area would enable guidelines for material selection and water treatment for various areas to be drawn up.

After a total of 20 months exposure of mild steel, 304 stainless steel, 3CR12 corrosion resisting steel, galvanized steel and copper and brass coupons to potable waters at five sites, it was found that biofilm developed on all alloy surfaces in both chlorinated and non-chlorinated waters. Tubercle formation and colonization by sulphate reducing bacteria were only present on mild steel test and control coupons. The free-chlorine residual in the water retarded biofilm and tubercle formation and colonization by sulphate reducing bacteria, but did not eliminate them. Once sulphate reducing bacteria had colonized the mild steel surface, they initiated pitting attack (after 3-6 months) at the edges which eventually led to perforation of the coupons. The Pretoria site was found to have the most aggressive microbiological attack, while the Vereeniging and Klerksdorp sites had the least. At all sites, though, perforation of

the mild steel coupons had occurred by the end of the exposure period. All other alloys remained unaffected.

It was thus concluded that unprotected mild steel was susceptible to microbiologically induced corrosion in potable waters despite chlorination. This problem does not seem to be area specific, but is more severe in areas with waters of low to zero free chlorine residuals.

All the objectives of the project were met since it was determined that:

(i) Microorganisms, in particular the sulphate reducing bacteria, were the major contributors to pitting corrosion of mild steel in potable waters.

(ii) The corrosion problem was encountered at all five sites around the PWV/Klerksdorp area.

(iii) Pitting was initiated 3-6 months after colonization by sulphate reducing bacteria.

(iv) Post-treatment chlorination stations would retard microbial growth and hence corrosion rate somewhat, but never prevent it.

(v) Unprotected mild steel is not suitable as a piping material for potable waters, since corrosion will cause deterioration in water quality and/or perforation of the pipes.

Previous studies have focused on the effects of biofilm on potable water quality, without much regard for the effect on corrosion of the pipework. When factors influencing corrosion are considered the role played by microbiologically induced corrosion is mostly overlooked. This study has shown that sulphate reducing bacteria play a major role in the corrosion of unprotected mild steel in potable waters while water chemistry is less important. Replacement of burst pipe sections with similarly bare mild steel, as is common practice in many areas, would seem to be a waste of time and money as corrosion by sulphate reducing bacteria of the new unprotected section is inevitable. The other alloys studied were resistant to this type of

corrosion for the period of the project and would thus seem more suited for use in potable waters. However, strength and cost considerations for larger diameter water pipelines must be borne in mind, and although the cost for stainless steel piping material is about five times that of mild steel, fittings, installation costs, protection systems, maintenance expenses and life cycle costs must also all be considered.

It was thus recommended that the use of lining systems for mild steel pipelines and non-metallic material alternatives be investigated. This would entail both chemical and microbiological tests to assess the suitability of these alternatives for potable water systems.

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The Steering Committee responsible for this project, consisted of the following persons:

Mr H.C. Chapman	WRC (Chairman)
Mr F.P. Marais	WRC (Committee Secretary)
Dr H.M. Saayman	WRC
Ms M. Krüger	Western Transvaal Water Co.
Mr S.N. Venter	CSIR
Mr D.T. Nel	Johannesburg City Council
Ms C.E. de Wet	Rand Water Board
Ms J.A. Gründelingh	Rand Water Board
Mr C.A. Lüdik	Department of Water Affairs

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LIST OF ABBREVIATIONS

AOC	Assimilable organic carbon
CFU	Colony Forming Units
DZR	Dezincification Resistant
EDS	Energy Dispersive Spectroscopy
FeS	Iron Sulphide
HCl	Hydrochloric Acid
HPC	Heterotrophic plate count
H_2S	Hydrogen Sulphide
MIC	Microbiologically Induced Corrosion
mpy	Mils per year
MS	Mild Steel
PWV	Pretoria Witwatersrand Vereeniging
RWB	Rand Water Board
SEM	Scanning Electron Microscopy
SRB	Sulphate Reducing Bacteria
TDS	Total Dissolved Solids
TOC	Total Organic Carbon
WRC	Water Research Commission
XRD	X-ray diffraction

1. INTRODUCTION

Growth of microorganisms in a potable water distribution system, despite disinfection, has been well documented. Most studies are concerned with the effect of these growths on the water quality in the distribution systems. However, little information is available on the extent to which these growths can influence corrosion of the metal pipelines. MIC of other metal pipelines such as those for gas and oil transport as well as in cooling water systems is well known.

Corrosion of a potable water pipeline not only affects the integrity of the system but can also influence water quality by the release of corrosion products into the system. Severe corrosion can lead to bursts with the associated costs of water loss and pipe replacement. Although corrosion failures of potable water pipelines may be influenced by many factors, internal corrosion due to MIC should not be discounted.

Recently, the effects of the chemical composition of potable water on the corrosion of various common piping materials were examined by the Corrosion Group of the CSIR in a research programme sponsored by the WRC (Project K5/254). The outcome of the study showed that chemical parameters such as the Langelier Index could not be used to measure the corrosivity of the water. One reason may be that MIC played an important part in the corrosion process, since a preliminary study showed that microorganisms had been involved in the corrosion of specimens at several sites. At the Pretoria site, for example, pitting attack was extremely severe with complete perforation of mild steel specimens after two years' exposure. This equates to a corrosion rate of approximately 0,5 mm per year. The problem of the corrosivity of potable water cannot be addressed until a full understanding of all the elements involved in the process is gained. This study was thus initiated at the suggestion of the Steering Committee for Project K5/254 that MIC be investigated more fully.

This study of MIC of piping material in the PWV area has the following objectives:

1. To determine to what extent microorganisms are involved in the corrosion of common piping materials in potable water.

2. To determine the extent of microbially induced corrosion in potable waters in the PWV area.

3. To determine at which stage of biofilm growth corrosion is initiated by microorganisms.

4. To recommend remedial and/or preventative measures if microbiologically induced corrosion proves to be a widespread problem.

2. LITERATURE SURVEY

A detailed literature review was completed and has been included in Appendix 1.

It is clear from the available literature that the problem of biofilm growth in distribution systems is widespread. Numerous studies investigating composition of the biofilm as well as factors which affect its growth and regeneration have been undertaken. These studies, however, are mostly concerned with the effect of the biofilm on water quality (eg. taste, odour and appearance). Few studies have addressed the problem of internal microbiologically induced corrosion of the distribution system (most deal with soil-side corrosion).

It is commonly found that regrowth of bacteria in potable water distribution systems occurs as sessile growth in biofilms. A large variety of bacteria, fungi and actinomycetes are often associated with tubercles. The biofilm mode of growth offers nutrition and protection from disinfection. The biofilm matrix is able to trap nutrients (such as organic carbon) from the passing water and can also prevent the diffusion of residual chlorine. The total organic carbon (TOC) and residual chlorine concentration seem to be the two most important factors governing biofilm growth. Biofilms seem able to grow on a wide range of piping materials. Once an adherent biofilm has been established on a metal surface, anaerobic bacteria, such as the SRB, colonize beneath this biofilm and can bring about pitting corrosion. The extent to which this occurs in potable distribution systems is not very clear.

In order to investigate this aspect more fully, a study was initiated, in which a range of metals and alloys commonly used as piping materials were exposed in a number of areas so as to observe whether there was a difference in the amount of colonization in different waters and whether this would lead to MIC. Since these sites are situated at various distances from the treatment plant, the effect of diminishing residual chlorine concentration could also be observed. It was decided, however to include control tanks, ie. tanks which would receive chlorinated water, in order to study the effect of chlorine on biofilm growth at the different sites.

Due to logistical considerations, it was necessary to limit the detailed study of the biofilm to mild steel coupons as this material is known to be most susceptible to MIC. A time study of biofilm formation involving culturing and identifying biofilm bacteria as well as SEM observation was carried out at the Johannesburg site only over a 2 month period.

Corrosion of the piping materials was evaluated by determining corrosion rates from weight loss coupons and by visual observation using light and electron microscopy. The latter is important in cases of MIC, since pitting attack with little weight loss is a common feature. The visual observation of mild steel coupons was linked to the biofilm time study in order to determine the initiation of the corrosion process.

3. MATERIALS AND METHODS

3.1 Test Sites

The test sites were located in five areas around the PWV viz the CSIR site in Johannesburg, the CSIR site in Pretoria, the Rand Water Board in Vereeniging, the Western Transvaal Regional Water Company near Klerksdorp and at the Vaal Dam wall. The Johannesburg site receives water treated at the RWB in Vereeniging, a distance of approximately 65 km. The Pretoria site receives some water from Rietvlei and the balance from the RWB and is about 130 km away from the latter treatment works. The Vereeniging and Klerksdorp sites are situated at the treatment works, while the Vaal Dam site receives the pre-treatment raw water. A map showing the location of the sites is presented in Figure 1(a).

Each site contained a once-through flow system. Cold potable water was fed through a Feenix (1 bar) pressure regulator and then through a manifold and into polypropylene tanks (volume 50 l) containing immersed specimens. The flow rate of 200 l/hr was regulated by valves. The control tanks (into which a biocide was dosed) operated in the same way but in addition an on-line chlorine dispenser (Klorman) was fitted to the inlet pipe of the tanks at all sites except the Johannesburg site. At this site water was

treated with a bromine based biocide. The residual halogens were maintained at a concentration of between 0,2 - 1 mg/l (free chlorine). A typical set up is shown in Figure 1b.

3.2 Alloys

The alloys exposed in this investigation were mild steel, hot dip galvanised steel, 304 stainless steel, 3CR12 corrosion resisting steel, DZR brass and copper. The latter two alloys were exposed at the Pretoria and Klerksdorp sites only. Tables 1 and 2 list the chemical composition of all the alloys used.

Coupons (25 mm x 100 mm) were cut from plate, stamped for identification, cleaned, degreased in acetone and weighed. The cleaning solutions for each alloy are listed in Table 3. The alloys were placed in racks and immersed in the tanks for a total period of 20 months. Before immersion, coupons to be placed in the control tanks were autoclaved at 121°C for 15 minutes.

3.3 Biofilm Study

3.3.1 Analyses and Identification

The major study was carried out on mild steel coupons since this metal is known to be most susceptible to MIC. At all sites, coupons were removed at three monthly intervals. The biofilm and corrosion product were scraped off one side of the coupon using a sterile scalpel blade and vortexed in 10 ml of a sterile 0,1% peptone solution. A dilution series was prepared using the peptone solution as a diluent and 0,1 ml aliquots were spread in duplicate onto R2A agar plates[1] and incubated at 28°C for 2 weeks. The number of colony forming units (CFU) of culturable aerobes was then determined for each period. The presence/absence of SRB was tested for by obtaining swab samples from beneath the corrosion product and biofilm on the coupons. These were incubated in SRB medium (Table 4) at 30°C for up to 28 days.

At the Johannesburg, Pretoria and Vereeniging sites, the analyses were carried out more frequently. Samples from these sites were removed daily for five days, twice weekly for two weeks, weekly for six weeks and then twice a month for two months. Total culturable aerobes and the presence or absence of SRB were determined as above.

A more detailed study of the biofilm was undertaken on mild steel coupons from the Johannesburg site. This involved isolating and identifying the dominant bacterial species from the culturable aerobes on R2A agar at each sampling interval and determining the presence or absence of SRB. The dominant bacterial species was determined by picking off random colonies from the R2A plates using the Harrigans disk method[2]. Pure isolates were obtained by streaking onto R3A agar. Identification of isolates involved Gram stains and standard biochemical tests (catalase, oxidase, O/F etc). For the Gram negative bacterial identification, the API 20 NE system was used. The total number of slime forming bacteria was determined by plating samples onto citrimide agar. (Oxoid Pseudomonas agar with CFC and CN supplements).

3.3.2 SEM Study

A time study of biofilm formation was carried out at the Johannesburg site. This involved regular removal of mild steel coupons over a 2 month period and observation of the attached biofilm using SEM. The coupons were prepared for microscopy by fixing in a solution of 2% glutaraldehyde in 0,1% peptone at 7°C overnight. The coupons were then dehydrated in a graded ethanol concentration series (30, 50, 70, 90 and 100% x 3), allowed to air dry and then sputter coated with gold. They were viewed in a ISI SX 30 model scanning electron microscope at an accelerating voltage of 15 kV. Selected coupons of other alloys and from other sites were also observed at the mature biofilm stage. Both the surface of the biofilm and the area beneath the corrosion product was examined.

3.4 Analysis of Corrosion Product

A qualitative analysis of corrosion product from selected coupons at various sites was undertaken using the Energy Dispersive Spectroscopy (EDS) facility attached to the SEM. Both the top and bottom surfaces of the corrosion product were analysed.

3.5 Corrosion Rate Determination

Five samples of each alloy were removed after the appropriate exposure times. After the corrosion product had been scraped off, the coupons were cleaned as per Table 3 and the mass loss and corrosion rates determined.

3.6 Examination of Corroded Coupons

The surfaces of the exposed coupons were examined after cleaning for corrosive damage using both light- and scanning-electron microscopy.

3.7 Water Analysis and Chlorine Level Determination

Periodic sampling of the water at the various sites was undertaken.
Parameters tested included: pH, electrical conductivity, total hardness, calcium, magnesium, sulphate chloride, total alkalinity, TDS, total iron and suspended solids. The Langelier Index was also calculated. The free and total chlorine concentrations for both test and control tanks were determined using the Hach DPD Test kit.

3.8 Stagnant Condition Test

At the end of the 20 month exposure period at the Johannesburg, Pretoria and Vereeniging sites, the tanks containing mild steel coupons were filled with water and left to stand for 2 months in order to simulate stagnant conditions such as would be found at dead ends. After this period, the coupons were removed, tested microbiologically as before and the corrosion rates determined.

4. RESULTS

4.1 Biofilm Study

4.1.1 SEM Study

Observations of the surfaces of both test and control mild steel coupons, removed periodically from the Johannesburg site, revealed the presence of microorganisms on both types of coupons.

In the first two weeks, colonization was relatively slow and single cells could be clearly distinguished on the metal surface (Figure 2). As time progressed, an increasing number of cells were observed as discrete colonies on the surface (Figure 3). The colonization pattern was similar for both test and control coupons, but growth was slower to initiate on the latter. The first organisms seemed to be rod- or cocci-shaped bacteria bound together in a glycocalyx matrix (Figure 4). As the biofilm developed and became thicker, it was more difficult to observe the individual cells which were often obscured by increasing amounts of corrosion products and crystals (Figure 5). Within two months the biofilm seemed to be well established on both test and control coupons. The mature biofilm contained a variety of organisms including diatoms, fungi, filamentous bacteria and algae (Figures 6 and 7). Cells beneath the biofilm and corrosion product were also observed . These were anaerobic organisms, most likely SRB (Figure 8).

A mature biofilm was observed on the surfaces of all the other alloys viz. 304, 3CR12, galvanised steel, brass and copper, for both test and control coupons. An example of a biofilm on a copper coupon is shown in Figure 9.

4.1.2 Enumeration and Identification

Figures 10-12 show the numbers of heterotrophic bacteria/mm^2 (as CFU/mm^2 on R2A agar) on mild steel coupons at periodic intervals at the Johannesburg,

Pretoria and Vereeniging sites. In general, there was a relatively rapid build up of cells on the test coupons to a peak number, after which the numbers decreased and then with time increased again. These fluctuations are most likely due to the sloughing off and regeneration of the biofilm over the 20 month exposure period. In general, the initiation of colonization on the control coupons was slower. However, once an adherent biofilm started to form, the numbers increased rapidly and reached more or less the same levels on the test coupons. Fluctuations in number were also noted for these coupons.

At the Pretoria and Vereeniging sites, the counts on the test and control coupons were almost identical. At the Johannesburg site, however, there was a comparatively larger difference in bacterial numbers, for the first two months, between the test and control coupons. This could be attributed to the different biocide used in the control tank at this site. The bromine-based biocide seemed to be more effective initially than the chlorine used at the other sites. However, by the end of the exposure period, little difference was noted between counts on control coupons.

A comparison of bacterial numbers on mild steel test coupons at all five sites over the entire test period are shown in Figures 13 and 14. Figure 13 shows the counts for the Johannesburg and Pretoria sites compared to the raw water count at the Vaal site. The former two sites are at some distance from the treatment plant. Although there were a number of fluctuations during the exposure period, in general, bacterial counts were highest at the Pretoria site. The sudden drop in bacterial numbers between 500 and 600 days could be attributed to some disturbance in the system leading to increased sloughing off of the biofilm.

Figure 14 shows the counts for the Vereeniging and Klerksdorp sites, both situated at the treatment works. Although there were fluctuations in numbers at these sites as well, over the entire exposure period, the Klerksdorp site seemed to support more growth. In both cases, the Vaal site counts fell between those of the other two sites. Of all the sites, Pretoria had the highest

counts overall.

Figures 15-19 show the number of heterotrophic bacteria/mm^2 on each alloy after 20 month's exposure at the five sites. Although fluctuations in numbers over the entire exposure period are expected (especially for the mild steel coupons, due to sloughing off of biofilm), in general the mild steel coupons showed the highest colonization and the stainless and corrosion resisting steels the least. The copper and brass samples were fairly well colonized at the Pretoria site. Lower numbers were found at the Klerksdorp site. In general, counts on the control coupons were equal to, or slightly higher than those on test coupons.

Identification of the heterotrophic bacteria from R2A plate counts from mild steel was carried out for 2 months at the Johannesburg site. For the first three weeks, Pseudomonas spp. were predominant on the test coupons while Bacillus spp. predominated on the control coupons. The Pseudomonads were mainly P.paucimobilis and P.luteola. Other species isolated less frequently included P.fluorescens, P.vesicularis, P.diminuta and P.aeruginosa. With time, the Bacillus population increased on the test coupons as did bacteria of the family Micrococcaceae. On the control coupons, Pseudomonas spp. started to colonize after two weeks exposure as did bacteria of the family Micrococcoceae. The mature biofilm on both test and control coupons contained a variety of other bacterial genera including Flavobacterium, Achromobacter, Alcaligenes, Acinetobacter and Moraxella.

The presence of SRB beneath the biofilm on mild steel coupons was determined periodically at the Johannesburg, Pretoria and Vereeniging sites. The first site where SRB colonization initiated was in Pretoria with both test and control coupons positive after seven day's exposure. At the Vereeniging site, SRB were first isolated from test coupons after 15 days and from control coupons after 84 days. For the Johannesburg site the SRB were isolated after 17 days for test coupons and 90 days for control coupons. At the Klerksdorp and Vaal sites, the first tests were carried out at 90 days at which time both test and

control coupons at both sites were positive for SRB. The only other coupons which showed SRB colonization were the 304 and 3CR12 coupons at the Vaal site.

4.1.3 Influence of water composition and chlorine

The maximum, minimum and average values of the various chemical parameters measured periodically at the different sites are shown in Table 5. The Klerksdorp site seems to have the most corrosive water when considering chemical parameters such as chlorides and sulphates (aggressive ions) and conductivity and TDS. All these values were highest at this site. At all sites, the negative Langelier Indices suggested that the waters were non-scaling.

Table 6 gives the values of the total and free chlorine concentrations for the five sites. As expected, there were low to zero concentrations of chlorides at the Johannesburg and Pretoria sites situated at some distance from the chlorination point. The Vereeniging and Klerksdorp sites still had relatively high chlorine levels.

4.2 **Corrosion Study**

4.2.1 Corrosion Product

Both test and control mild steel coupons were covered by large orange/brown tubercles after the 20 month exposure period. These tubercles started to form at the edges of the coupons and gradually built up towards the centre. An example of a rack of coupons covered with tubercles is shown in Figure 20. On removal of the tubercles, a black corrosion product was present underneath. On acidification with HCl, this product released hydrogen sulphide. The metal beneath was shiny and pitted. EDS analyses of the corrosion product revealed that the black product contained high levels of iron (Fe) and sulphur (S), which probably existed as FeS. High sulphur peaks were found for both test and control coupons. Figures 21 and 22 are typical EDS traces. In general, the test

coupons had a higher sulphur peak than the control coupons. Figure 23 shows a typical EDS trace for an analysis of the surface of the corrosion product. The presence of the normal chemical constituents of water are shown.

Coupons of all other alloys were covered by a thin scale layer and biofilm, with the absence of tubercles.

4.2.2 Corrosion Rates

Figures 24-28 show the corrosion rates of mild steel exposed over a 20 month period at the various sites. In general, the corrosion rates were initially high for both test and control coupons, but rapidly fell to more or less stable rates of between 5 and 10 mpy. After about 3 months there was little difference between test and control coupon corrosion rates. In some cases the initially higher corrosion rates for the control coupons could be ascribed to the action of the chlorine.

Corrosion rates for the 304 and 3CR12 specimens were negligible. Rates for the other alloys were also very low at all sites.

4.2.3 Metal Damage

Figures 29 and 30 show the condition of the mild steel test and control coupons after 20 months exposure. The most severely corroded coupons were those at the Pretoria site. At this site, shallow pitting at the edge of the coupons was initiated after 2 months exposure in the test tanks. On the control coupons, pitting was first observed after 3 months. The pitting worsened with exposure time, the pits becoming deeper and wider. Penetration at the edges occurred after 9 months on both test and control coupons (Figure 31). The pitted area mostly coincided with the areas where tubercles had formed (Figure 32). Pitting of the surface metal was observed after 6 months as groups of shallow pits sometimes connected together (Figure 33). On both test and control coupons, these pits deepened at a slower rate than the edge pits, with

penetration occurring after 20 months. The area of the coupon which slotted into the plastic exposure rack, underwent severe crevice corrosion.

At the other sites, the same pattern of attack was observed i.e. pitting corrosion initiating on the edges and then moving to the centre. At the Vaal and Johannesburg sites, edge attack was initiated after 6 months with penetration occurring after 12 months at the Vaal site and 15 months at the Johannesburg site. In the case of the Vereeniging and Klerksdorp sites, edge attack at the former site initiated after 9 months and at the latter after 15 months. Both were penetrated at the 20 month removal period. In general, the control coupons were slightly more severely attacked than the test coupons.

The other alloys suffered minimal damage. The 304 stainless steel coupons were unaffected at all sites and no edge or crevice corrosion was observed after the 20 month exposure period. The 3CR12 corrosion resisting steel specimens suffered surface discolouration at all sites. The only form of corrosion observed was some slight crevice corrosion where the coupons slotted into the exposure rack, at the Vereeniging and Vaal sites only.

The copper and brass coupons at the Pretoria and Klerksdorp sites were also discoloured after 20 months, however, no pitting attack was observed at either site.

The galvanized mild steel coupons remained protected by the zinc coating. No penetration through to the substrate had occurred after 20 months at any site.

4.3 Stagnant Condition Test

The numbers of heterotrophic bacteria /mm^2 on the mild steel coupons, at the Johannesburg, Pretoria and Vereeniging sites, were not significantly different to numbers observed during the flow period. SRB were present on both test and control coupons at all sites.

Corrosion rates were also similar to those of coupons under flow conditions. Pitting attack of the coupons had continued, but was difficult to quantify.

5. DISCUSSION

Biofilm growth in potable water distribution systems is a universal phenomenon[3] and occurs on a variety of piping materials. Large and diverse microbial communities have been found in such systems, despite low-level chlorination of the system[4]. In this study, a range of metals and alloys commonly used as piping materials were exposed to potable water (both chlorinated and non-chlorinated) at a number of sites in the PWV/Klerksdorp area. As many other researchers have reported [8,9,15], it was found that biofilm formation occurred on all materials exposed to both chlorinated and non-chlorinated water.

From investigations of microbial succession, it appears that the first bacteria to colonize a metal surface are invariably those that produce sticky, extracellular slime covers[5,6]. In this investigation, colonization of mild steel coupons was initiated by Pseudomonas spp. which are slime-producing organisms. The presence of Bacillus spp., which predominated afterwards, was also reported by Augostinos et al[7] and Emde et al[9]. The former, in a local study of biofilm composition in potable water distribution systems in the Pretoria area, found that 95% of the organisms isolated belonged to the genus Bacillus. These are endospore-forming bacteria and as such are able to resist treatment processes and multiply.

The eventual production of a continuous biofilm is a function of cell division within micro-colonies and new recruitment of bacteria from the planktonic phase. The mature biofilm thus contains a diverse population of microorganisms. These were viewed on mild steel coupons using SEM and cultured on R2A agar. As reported by other researchers [8,9,10], colonization occurred mainly at or near the surface of tubercles, but bacterial cells were also observed beneath tubercles. These were most likely SRB (which were isolated in culture) and other anaerobic organisms. SRB have been isolated by others from within corrosion tubercles from iron pipes [9,8,11,12]. The surface

organisms viewed included rods, cocci, filamentous organisms, diatoms and algae. This diversity of organisms was also reported by Ridgeway and Olson[4] and Allen[8] in distribution systems.

Enumeration on R2A agar of the biofilm cells on mild steel coupons showed an initially high colonization rate and growth of cells to a more or less stable value. After this period, there were fluctuations in bacterial numbers, as would be expected if sloughing off and regeneration of the biofilm occurred. Enumeration of bacteria in distribution biofilms has demonstrated large variations in the number of bacteria present. Bacterial levels usually fall in the 10^{0} - 10^{6} CFU/cm^{2} range on potable water pipe walls[6]. Cell counts on coupons of all alloys from this study fell within this range. O'Conner and Banerji[14] also found the accumulation of cells on copper, PVC and iron surfaces. Schoenen[13] published data extracted from a number of studies which provides information on the bacterial species which have been isolated from potable water pipe surfaces. (The Table is reproduced in Section 2.3 of the Literature Review). Many of the reported genera were isolated from the coupon surfaces in this study.

There is widespread agreement that free residual chlorine is a critical rate - limiting factor for biofilm growth[6]. In this study, biofilm growth occurred on coupons which had been exposed to chlorinated water, and although initial growth was slower than in the system without chlorine, at the end of the exposure period, the heterotrophic cell levels were the same for both, and SRB had accumulated under tubercles on both test and control coupons. This can perhaps be explained by results obtained by van der Wende et al[16] in a study of bacterial growth in a pilot reactor system designed to model flow in a distribution system. They found that chlorine affected the accumulation and spatial distribution of the biofilm. They postulated that biofilm cell growth and detachment may be even more dominant in water distribution systems with low chlorine concentrations than in chlorine-free systems. The biofilm environment protects cells against the activity of chlorine and may allow for selective accumulation of bacterial species less susceptible to chlorine. Species of the genus Pseudomonas are know to be highly chlorine tolerant, as are Bacillus spp. due to endospore formation. During the exposure period in these tests, there were fluctuations in chlorine levels which could have allowed for selection of chlorine tolerant species at lower chlorine

concentrations. In addition, Berg et al[16] have shown that bacteria grown under low nutrient conditions (such as would be expected in potable waters) are more resistant to disinfectant action than bacteria grown under high nutrient conditions. Slimy biofilm growth and tubercle formation would have protected bacteria such as the SRB. Bacterial levels in raw dam waters were lower than expected but this could be explained by the possibility of inhibition of bacterial species by other organisms such as fungi or algae, in the case of non-chlorinated water and by the high nutrient theory of Berg, explained above, for chlorinated water.

The presence of SRB in anaerobic regions beneath biofilms and within tubercles, the black corrosion product rich in sulphur (as FeS and H_2S) and the pitting nature of attack have all been associated with MIC by SRB[17,18,19]. In this study, both test and control mild steel coupons at all sites had been colonized by SRB within 3 months of exposure. These bacteria were isolated from beneath tubercles which consisted mainly of iron oxide and hardness deposits on the outer surface and iron sulphide and H_2S within. Tubercle formation initiated at the edges of the coupons and it was here too that pitting attack was first noted. On removal of the black corrosion product, the pitted metal surface was shiny, denoting active pits.

Corrosion rates for the mild steel coupons were initially very high for both test and control coupons, but rapidly dropped to more or less stable rates of between 5 and 10 mpy. This can be accounted for by the initial occurrence of rapid general corrosion with an associated high corrosion rate. As the coupons corroded, the iron oxide corrosion product built up on the surface and stifled the cathodic reaction, thus decreasing the corrosion rate. The pitting type corrosion by SRB typically shows far less metal loss as compared to general corrosion. However, it is a more serious type of corrosion as it can lead to penetration. The danger of calculating corrosion rates, using formulae for uniform corrosion, when localized corrosion mechanisms such as pitting are predominant, is thus obvious. Penetration of the coupons at the Pretoria site occurred within 9 months. This translates to a penetration rate of 1,3 mm/yr. In decreasing order of penetration rate the sites ranked as follows: Pretoria (1,3 mm/yr), Vaal (1 mm/yr), Johannesburg (0,8 mm/yr), Vereeniging and Klerksdorp (0,6 mm/yr), which are far higher than the general corrosion rates calculated for each site.

Mild steel coupons at the Pretoria site were the first to support SRB growth (after 7 days exposure). This would be expected as the site is at some distance from the treatment plant and as the water is not re-chlorinated along the way, there are no free chlorine residuals. This site also contained the most severely corroded coupons. The next site to show SRB colonization was Vereeniging after 15 days. This was for mild steel coupons in the test tank. Since this site is at the RWB premises, the water in the test tank always contained a fair level of free chlorine, yet the SRB still colonized as did the aerobic species. The SRB activity was, however, retarded as pitting only initiated much later. The control coupons in tanks with higher chlorine levels were colonized later, but within 3 months SRB had made their appearance at all sites. It thus seems that a free chlorine residual will retard but not inhibit SRB growth and activity. Pitting seems to initiate 3-6 months after SRB colonization, depending on the free chlorine residual and hence SRB numbers. Pitting attack of mild steel coupons at the Vereeniging and Klerksdorp sites, which had the highest free chlorine levels, initiated after 9 and 15 months respectively. Once the attack starts, it seems to progress rapidly leading to penetration within a few months.

SRB were isolated only on 3CR12 and 304 coupons from the Vaal Dam site. It is presumed that they were not isolated from other alloys or other sites as an adequate anaerobic region had not yet developed. At the Vaal site slime and silt on the coupons would have provided such an environment. Pitting attack of the surfaces had not yet occurred.

The flow rate of the water could also play a role in the corrosion rate. In a previous WRC project (K5/254) to study the effects of the chemical composition of potable water on common piping material, it was found that mild steel coupons had suffered MIC. At the Pretoria site, penetration had occurred earlier than was observed in these tests. A major difference between the two project parameters was the flow rate which was almost doubled for this project. It is well known that increased bacterial activity is found in slow flowing or stagnant areas[6:20]. The stagnant condition test carried out for a two month period at the Johannesburg, Pretoria and Vereeniging sites, did not show significant differences in surface colonization or corrosion rate compared to the flow tests. The degree of pitting attack was, however, difficult to quantify and rather

subjective. It is felt that the exposure period was too short to obtain meaningful results and it is suggested that the tests be repeated for a longer period.

Heynike[21], in a desk study of the economic effects of increasing mineralization in the Vaal river barrage, also considered the effects on the corrosivity of the water. He presumed that an increase in the TDS of the water would lead to increased sulphate and chloride levels. It is generally accepted that oxygen, sulphates and chlorides are responsible for the corrosive tendencies of water and the ratio of chloride and sulphate ions to alkalinity is often used as a corrosivity index. Heynike thus postulated that increased corrosion rates would result from increased levels of chloride and sulphate ions.

The Langelier Index is also often used as a corrosivity index in which a negative value is indicative of a non-scaling, aggressive water. It has been found[22], however, that the maintenance of a positive index does not necessarily reduce the corrosivity of the water towards metals.

In this study, the chemical composition of the water did not seem to have a major effect on the corrosion rate. Similar results were obtained in Project K5/234 in that no correlation could be found between commonly used corrosion indices (e.g. Langelier Index and chloride and sulphate to alkalinity ratio) and corrosion rate. The Klerksdorp site, for example, in both projects had by far the highest chloride, sulphate and TDS values but the lowest overall corrosion rates and amount of pitting attack of all sites. This would seem to point to the influence of some other factor in the corrosion process. It is felt that the important role played by SRB in the corrosion process has been disregarded in many other studies. The effects that SRB can have on industrial water systems has long been recognized and acknowledged, but their presence in potable water systems is mostly overlooked. This project has demonstrated that SRB can and do exist in potable water despite chlorination and are responsible for a large part of the corrosion of mild steel in these waters. Compared to industrial or raw waters, potable waters have relatively low levels of aggressive ions and the corrosivity of these waters thus seems to be less dependant on chemical parameters than on the presence of SRB.

6. CONCLUSIONS

The following conclusions can be drawn from this study.

- Despite chlorination, biofilm developed on all alloys at all sites.
- Tubercle formation and SRB colonization took place only on mild steel coupons (both test and control) at all sites.
- At sites where the free chlorine residual was high (ie those at the treatment works and in the control tanks), SRB colonization and activity was delayed but not prevented.
- Pitting attack of mild steel coupons beneath tubercles was due to MIC by SRB and initiated 3-6 months after SRB colonization.
- Pitting attack on mild steel coupons resulted in perforation of the coupons at the edges.
- Penetration rates were highest at the Pretoria site and lowest at the Klerksdorp and Vereeniging sites.
- Penetration rates were far higher than general corrosion rates thus emphasising the danger of using mass loss coupons to calculate corrosion rates when pitting is involved.
- The influence of the chemical parameters of the water on corrosion was minor compared to the role played by SRB.
- Unprotected mild steel in potable waters is susceptible to MIC by SRB.

7. RECOMMENDATIONS

- Maintenance of a free chlorine residual of at least 0,2 mg/l throughout the distribution system should delay SRB activity. This may necessitate post-treatment chlorination stations.
- Since SRB colonization of bare mild steel is inevitable, despite chlorination, alternatives such as lining should be considered.
- Replacement of mild steel pipes with non-metallic alternatives should also be considered.

8. RECOMMENDATIONS FOR FURTHER RESEARCH

- It is recommended that the susceptibility of lining systems and non-metallic pipes to MIC be investigated.

- The stagnant condition tests should be repeated for a period of up to one year.

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APPENDIX 1

TABLES AND FIGURES

TABLE 1: **Chemical composition of alloys**

ELEMENT	MILD STEEL	3CR12	304	BRASS
Carbon	0,042	0,028	0,045	
Manganese	0,35	1,05	1,51	≤ 0,01
Sulphur	0,009	≤ 0,01	≤ 0,01	
Phosphorus	0,005	0,016	0,017	
Silicon	≤ 0,01	0,36	0,46	≤ 0,01
Chromium	≤ 0,01	12,1	18,4	
Molybdenum	≤ 0,01	≤ 0,01	≤ 0,01	
Nickel	≤ 0,01	0,20	8,3	0,04
Copper	≤ 0,01	0,13	0,18	59,6
Aluminium	≤ 0,01	≤ 0,01	≤ 0,01	≤ 0,01
Vanadium	≤ 0,005	0,04	0,04	
Niobium	≤ 0,005	≤ 0,01	≤ 0,01	
Boron	≤0,0005	0,02		
Iron	Matrix	Matrix	Matrix	0,081
Titanium			0,03	
Tin				0,12
Zinc				Remainder
Lead				1,95

TABLE 2: Chemical composition of copper alloy

ELEMENT	COMPOSITION ppm
Copper	Balance
Tin	≤ 5
Zinc	≤ 5
Lead	≤ 5
Nickel	≤ 5
Iron	9
Aluminium	≤ 5
Manganese	≤ 5
Silicon	≤ 5
Antimony	≤ 5
Oxygen	153
Arsenic	≤ 5
Phosphorus	≤ 5
Cadmium	≤ 5
Bismuth	≤ 5
Tellurium	≤ 5
Silver	≤ 5
Selenium	≤ 5
Sulphur	≤ 5
Cobalt	≤ 5
Chromium	≤ 5

TABLE 3: **Cleaning procedures for alloys**

MATERIAL	SOLUTION	TIME	TEMPERATURE	ADDITIONAL
Mild Steel	Hibitol (inhibited HCl)	1-3 mins	20-25°C	Specimens brushed
Stainless Steel	Hibitol	30 sec	20-25°C	Acetone used for specimens with little corrosion product
Copper, Brass	500 ml HCl, reagent water to make 1000 ml	1-3 mins	20-25°C	Deaeration of solution with nitrogen
Galvanized Steel	100g ammonium persulphate, reagent water to make 1000 ml	5 mins	20-25°C	

TABLE 4: SRB Medium

Sodium lactate	7,0 g
Beef extract	1,0 g
Peptone	2,0 g
$MgSO_4.7H_2O$	2,0 g
Na_2SO_4	1,5 g
K_2HPO_4	0,5 g
$CaCl_2$	0,1 g
Distilled Water	1 l
Prepare medium and adjust pH to 7,5. Autoclave at 121 °C for 15 mins.	
Prepare separate solutions of ferrous ammonium sulphate (78,4 g/l) and sodium ascorbate (20 g/l) and filter sterilize. Add 5 ml/l of each solution to the medium before use.	

TABLE 5: Summary of the chemical parameters of the water samples from the various sites.

CHEMICAL PARAMETERS		JOHANNESBURG	PRETORIA	VEREENIGING	KLERKSDORP	VAAL
Electrical	max	24,2	35,8	33,6	85	16,4
conductivity	min	9,2	27,6	20,7	44	9,9
(mS/m)	ave	17,4	31,95	25,9	65	13,2
Total Hardness	max	96	103	98	266	46
(mg/l)	min	52	82	51	139	32
	ave	73	89,8	75	133	39
Calcium	max	38	31	35	70	12
(mg/l)	min	13	21	12	40	9
	ave	25	25	24	53	10,5
Magnesium	max	4	9	4	22	4
(mg/l)	min	2	5	2	9	3
	ave	3	6,7	2,7	16	3,5
Sulphate	max	25	41	47	187	28
(mg/l)	min	13	24	13	58	26
	ave	19	31,7	24,7	119,6	27
Chloride	max	18	20	19	68	11
(mg/l)	min	5	11	6	39	5
	ave	10	17,3	9,8	51	8
Total alkalinity	max	98	95	102	98	77
(mg/l)	min	52	65	40	62	51
	ave	68	86,7	65,5	75,6	64
TDS	max	155	205	165	528	157
(mg/l)	min	107	169	109	289	120
	ave	127	186,7	137	408	139
Total iron	max	0,03	0,92	0,03	2,7	17,2
(mg/l)	min	0,19	0,03	0,21	0,03	6,16
	ave	0,08	0,52	0,09	0,92	11,7
Suspended solids	max	15	25,	27	25	120
(mg/l)	min	1	1	1	1	25
	ave	6,2	10,8	6,3	13,6	92,7
pH	max	8,1	8,0	8,2	8,4	7,8
	min	6,9	6,9	6,9	6,7	7,3
	ave	7,6	7,6	7,73	7,6	7,6
Langelier Index	max	-0,1	-1,1	0	0,4	-1,1
	min	-1,1	-0,1	-1,4	-1,3	-0,9
	ave	-0,35	-0,45	-0,47	-0,5	-1,0

TABLE 6: Summary of total and free chlorine values for test and control waters

		JOHANNESBURG	PRETORIA	VEREENIGING	KLERKSDORP	VAAL
Total Chlorine						
Test	max	0,4	0	0,8	0,9	0
	min	0,1	0	0,4	0,5	0
	ave	0,25	0	0,6	0,6	0
Control	max	0,8	0,8	0,8	0,9	0,8
	min	0,4	0,5	0,65	0,6	0,4
	ave	0,6	0,6	0,75	0,8	0,7
Free Chlorine						
Test	max	0,09	0	0,6	0,65	0
	min	0,05	0	0,2	0,4	0
	ave	0,06	0	0,38	0,5	0
Control	max	0,7	0,7	0,7	0,8	0,7
	min	0,35	0,4	0,34	0,4	0,3
	ave	0,5	0,5	0,65	0,6	0,45

Values in **mg/l.**

Figure 1(a): Location of exposure sites.

Figure 1(b): Test site in Johannesburg.

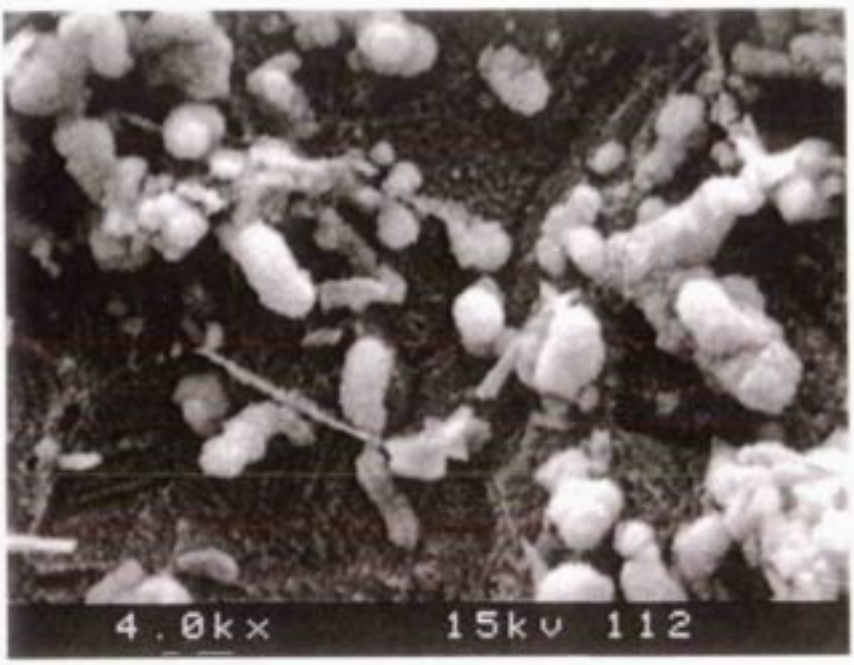

Figure 2: MS coupon after 10 day's exposure. Note the single cells.

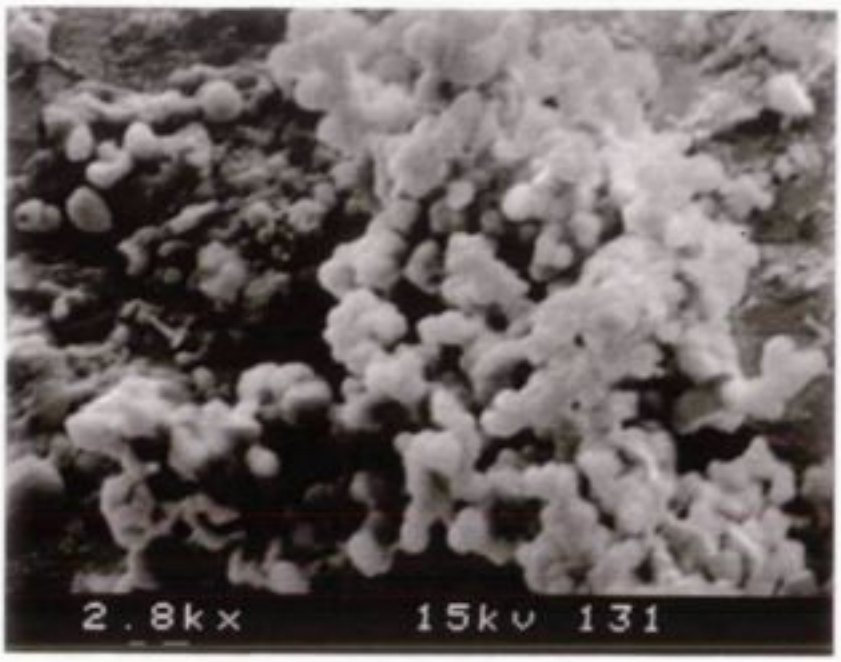

Figure 3: A colony of cells on the surface of a MS control coupon.

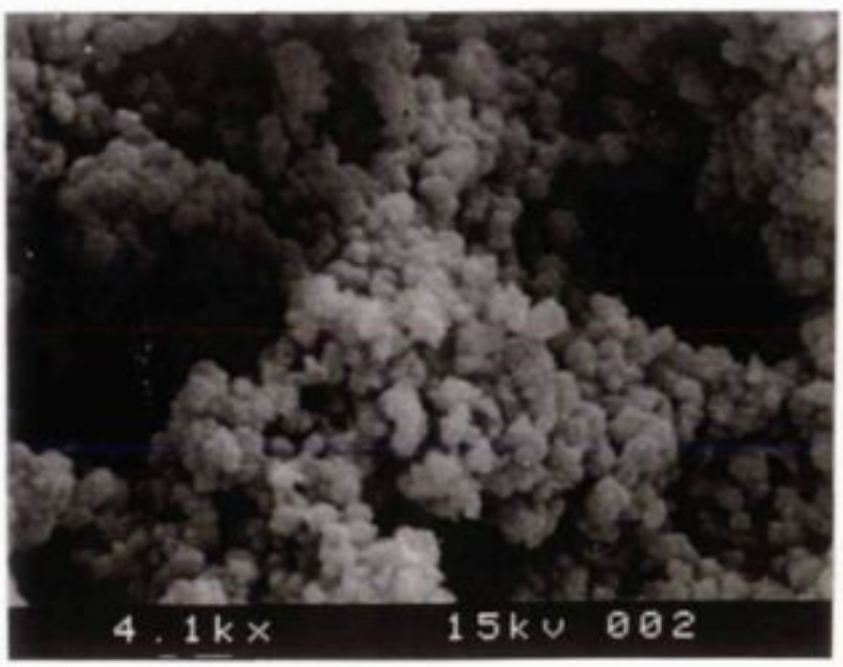

Figure 4: Cells bound in a glycocalyx matrix.

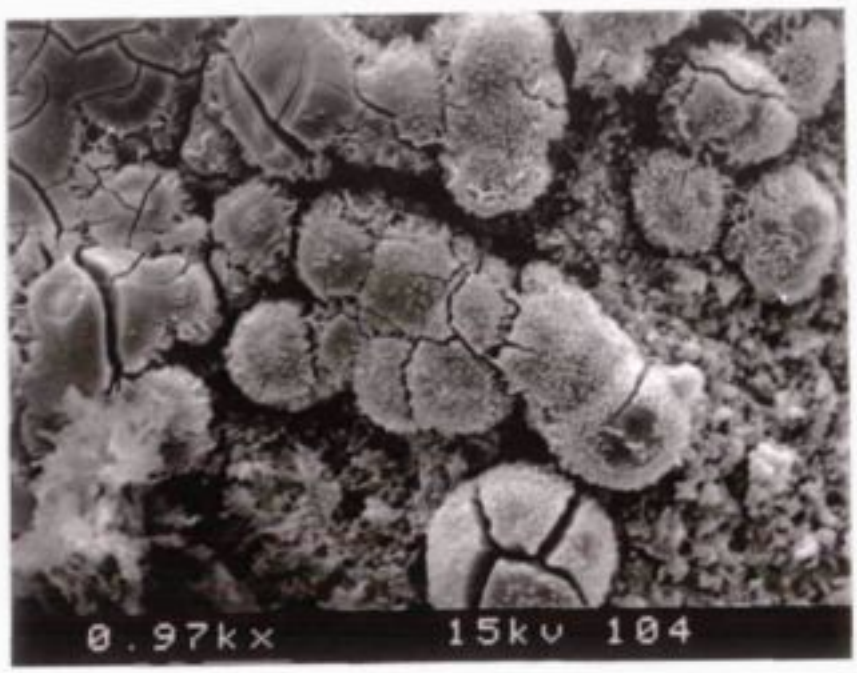

Figure 5: Corrosion product and crystals on the MS coupon surface.

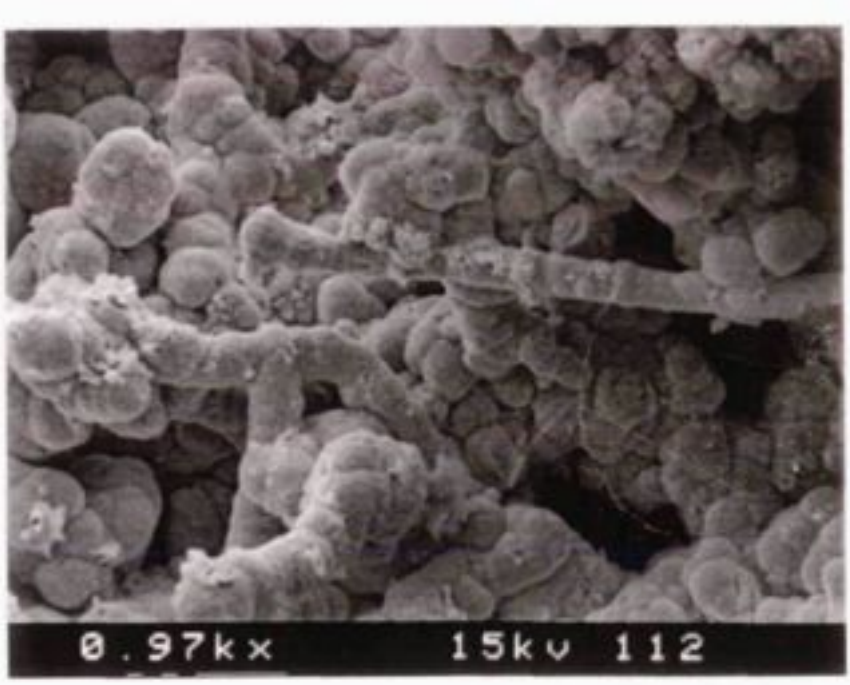

Figure 6: Mature biofilm on a MS test coupon showing fungal hyphae and cocci-shaped bacteria.

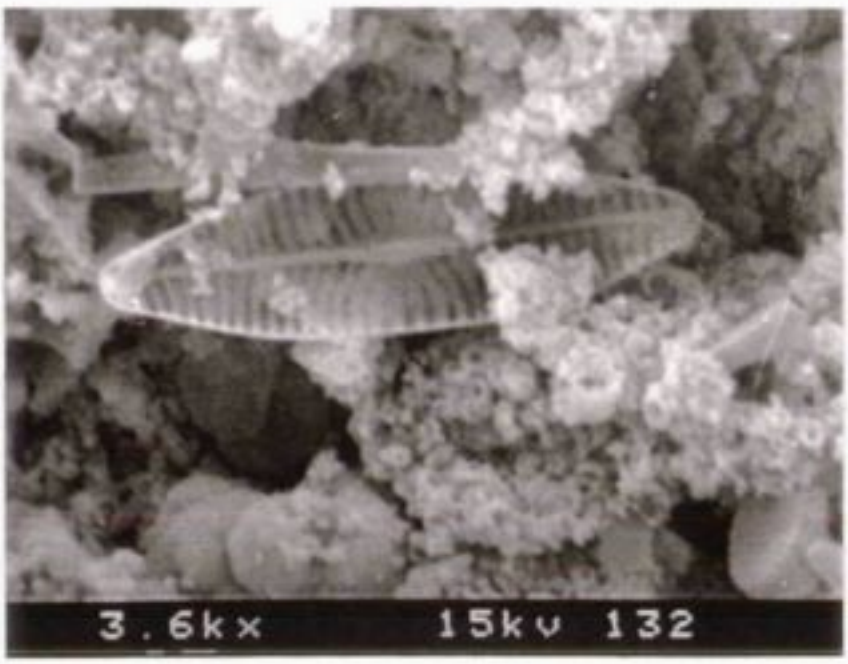

Figure 7: Diatom and filamentous bacterium in a mature biofilm.

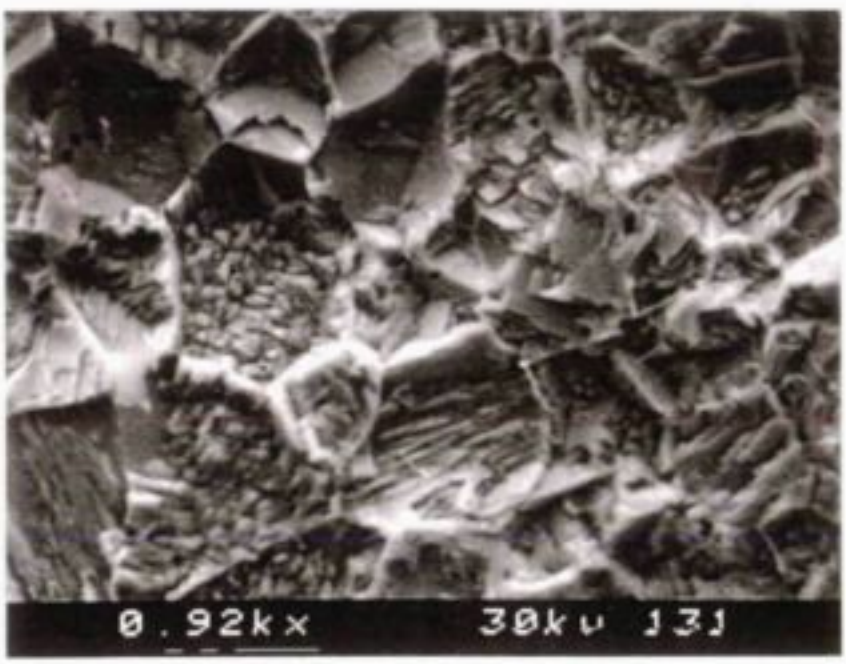

Figure 8: Bacterial cells on the metal surface beneath the corrosion product. Note the individual grains.

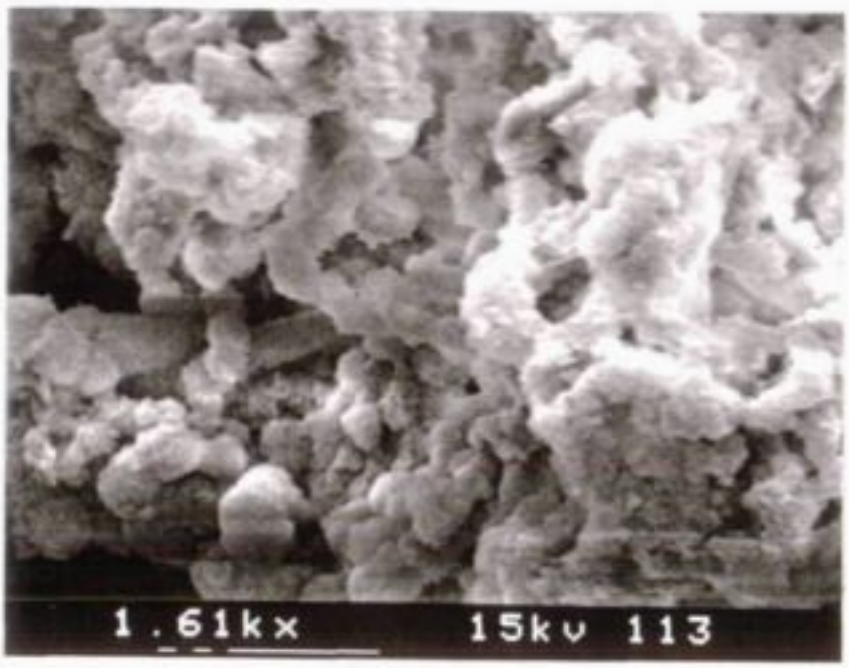

Figure 9: Mature biofilm on the surface of a copper coupon.

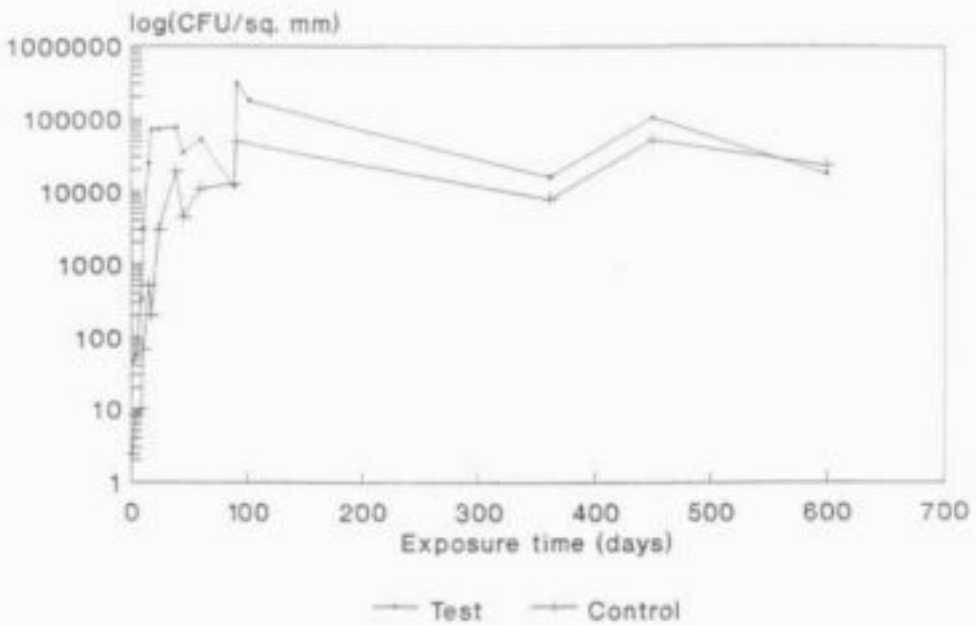

Figure 10: Number of heterotrophic bacteria/mm^2 with time at Johannesburg site.

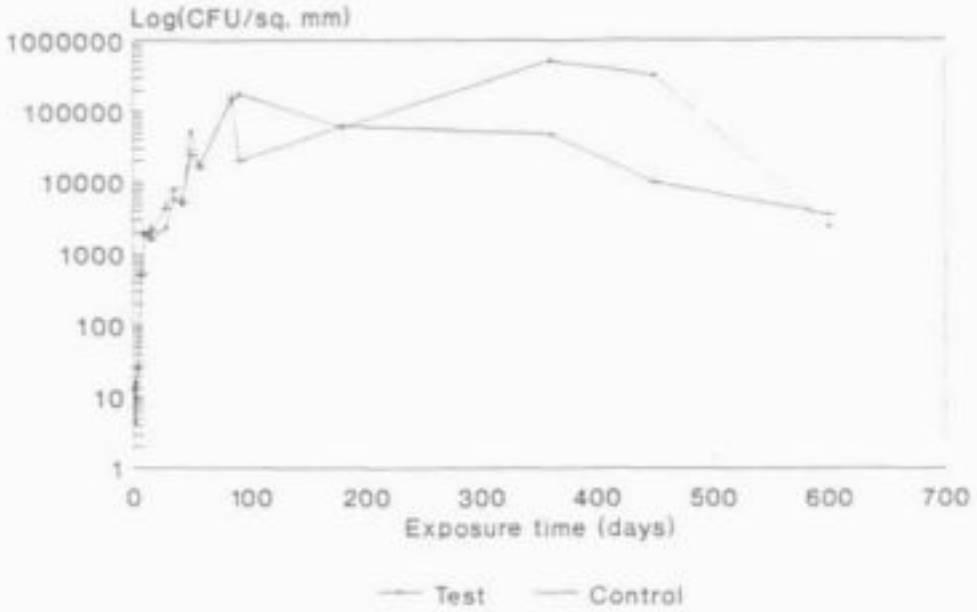

Figure 11: Number of heterotrophic bacteria/mm^2 with time at Pretoria site.

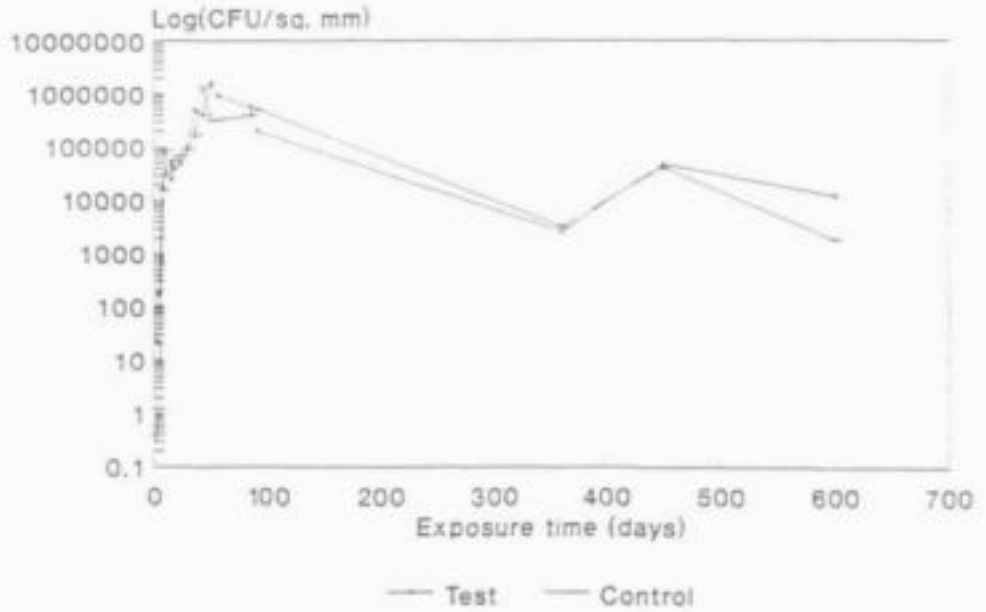

Figure 12: Number of heterotrophic bacteria/mm^2 with time at Vereeniging site.

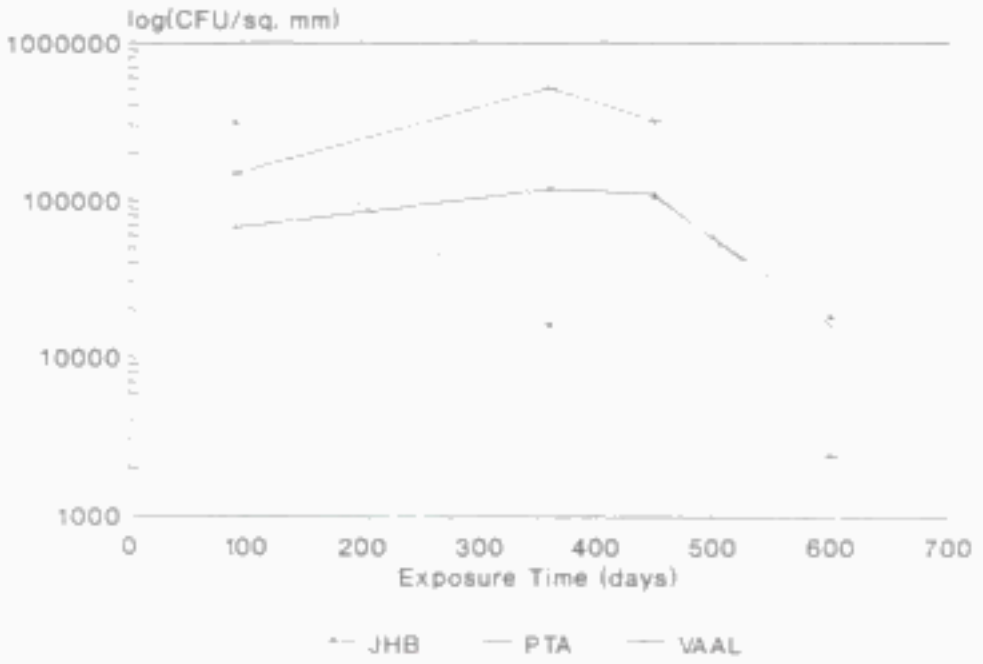

Figure 13: Heterotrophic bacterial counts on MS coupons for the Johannesburg, Pretoria and Vaal Dam sites.

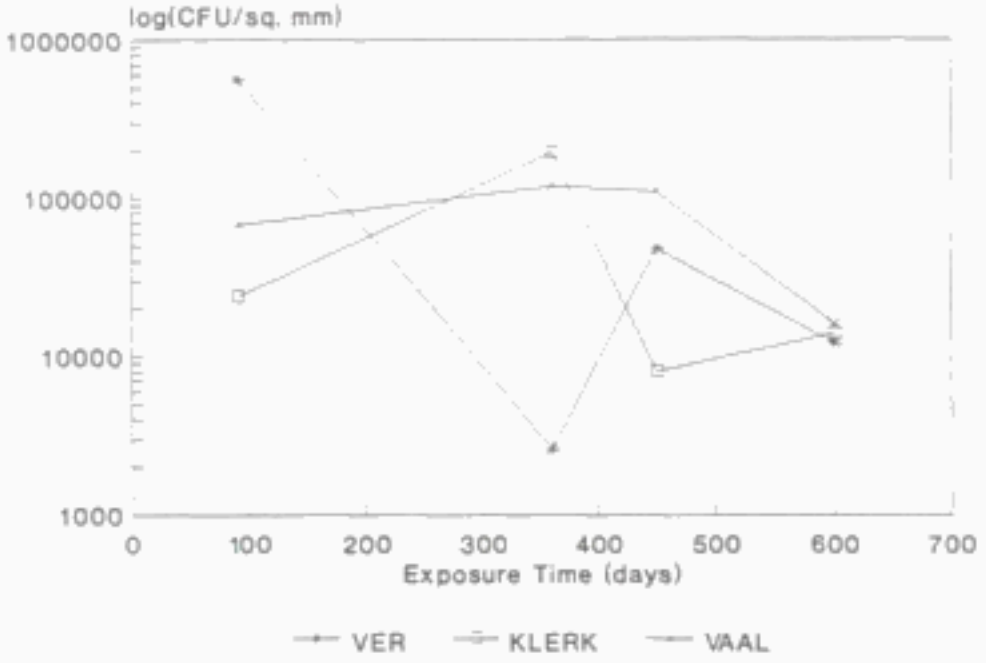

Figure 14: Heterotrophic bacterial counts on MS coupons for the Vereeniging, Klerksdorp and Vaal Dam sites.

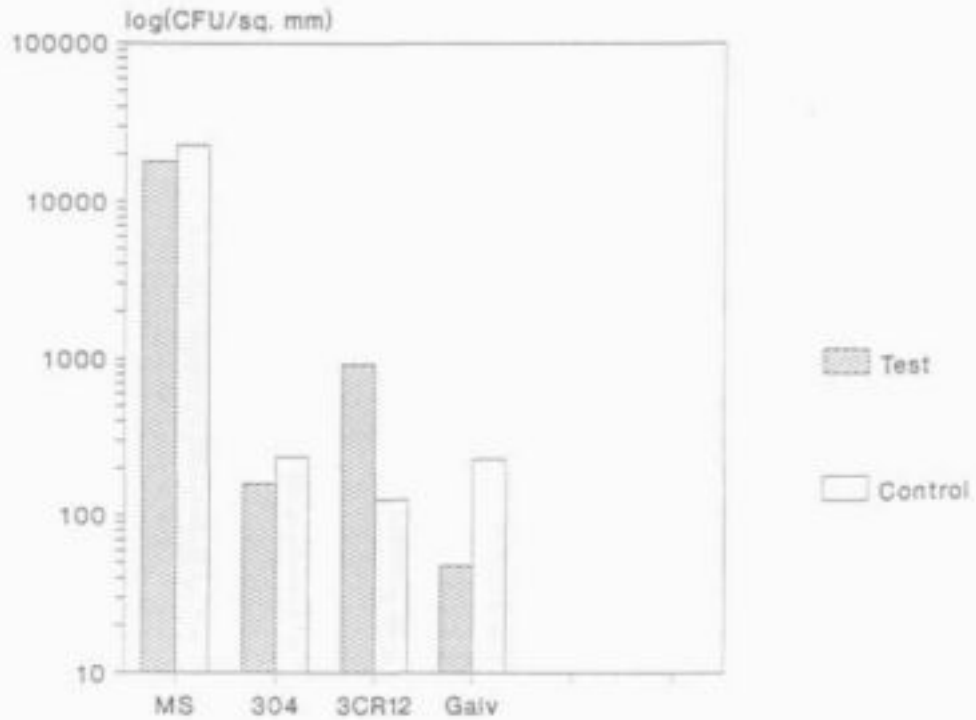

Figure 15: Heterotrophic bacterial counts after 20 months for the Johannesburg site.

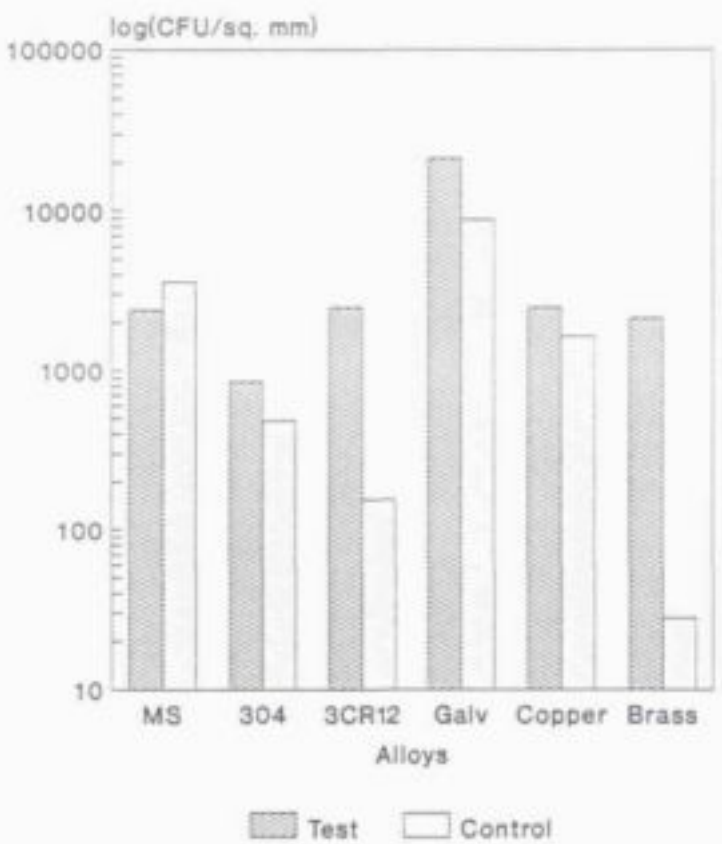

Figure 16: Heterotrophic bacterial counts after 20 months for the Pretoria site.

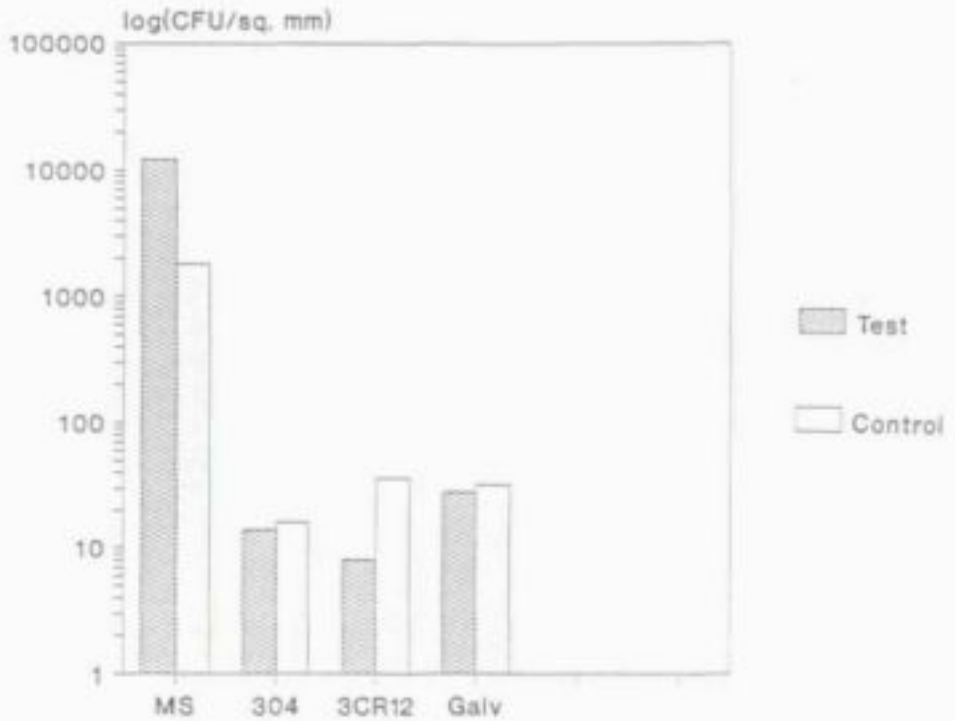

Figure 17: Heterotrophic bacterial counts after 20 months for the Vereeniging site.

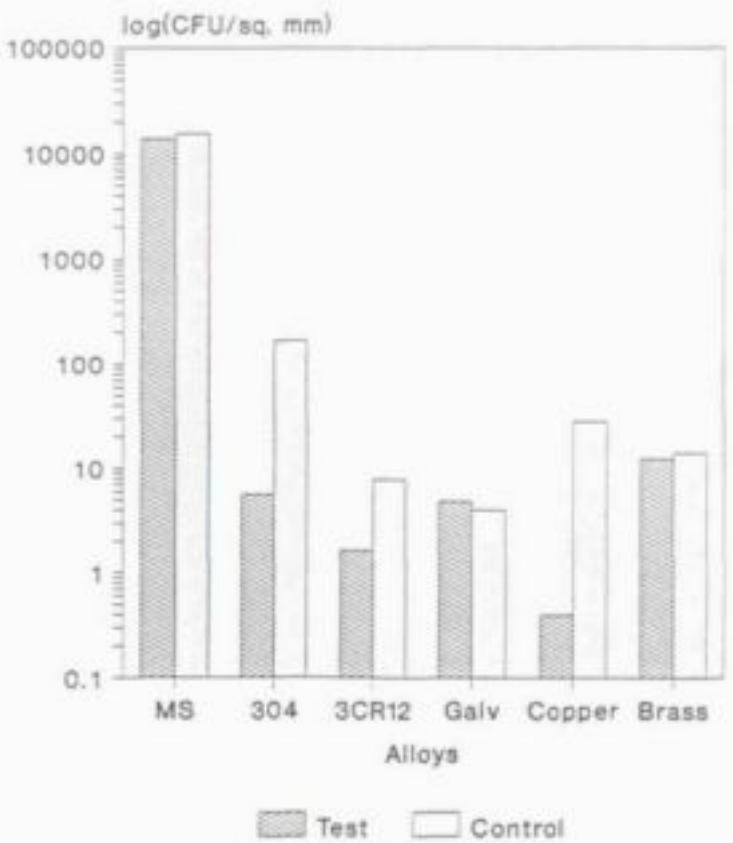

Figure 18: Heterotrophic bacterial counts after 20 months for the Klerksdorp site.

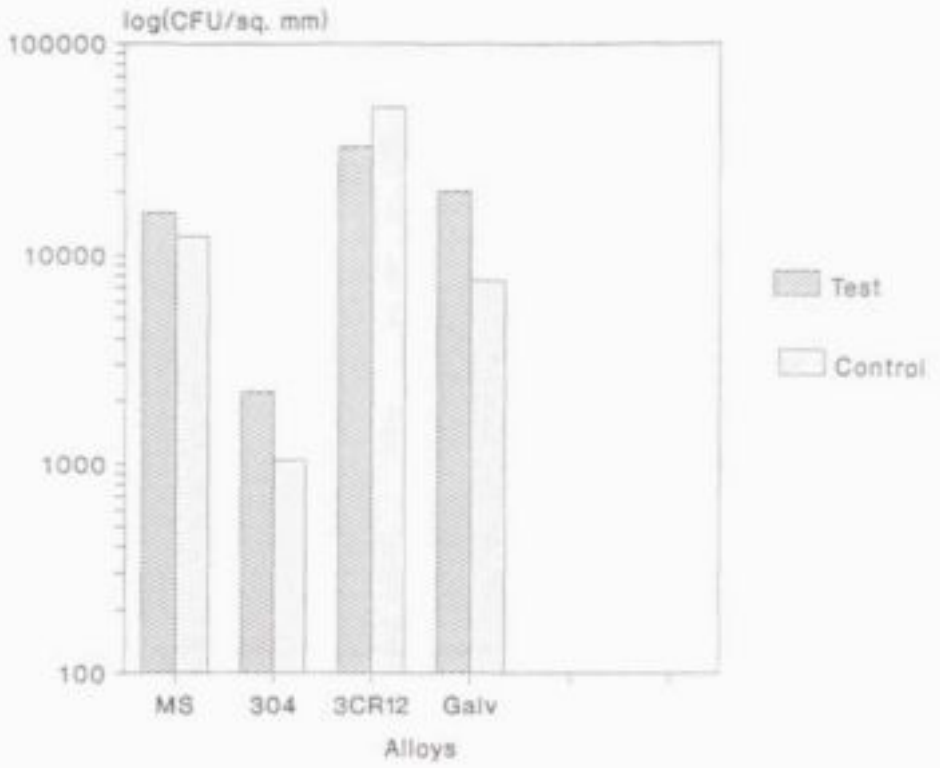

Figure 19: Heterotrophic bacterial counts after 20 months for the Vaal Dam site.

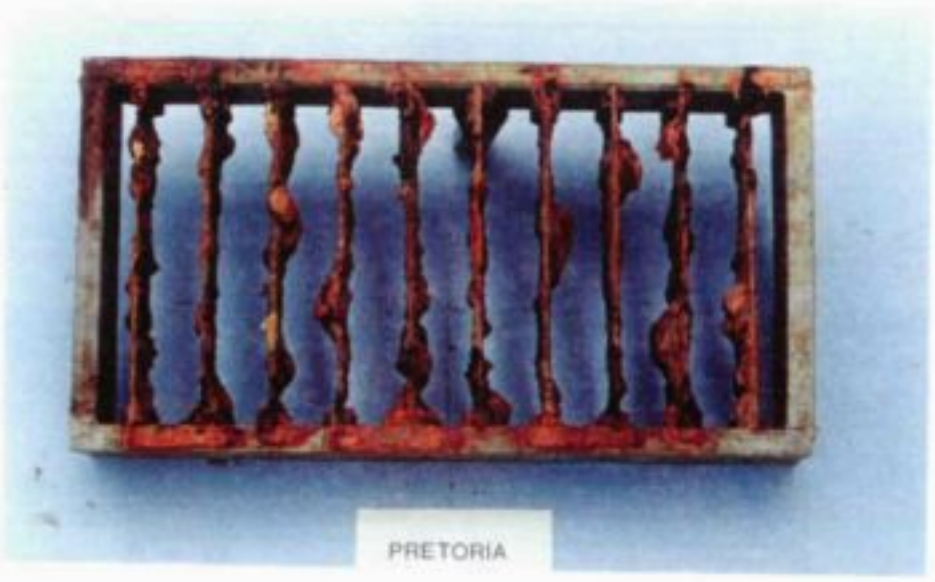

Figure 20: Tubercles on MS test coupons after 20 months exposure.

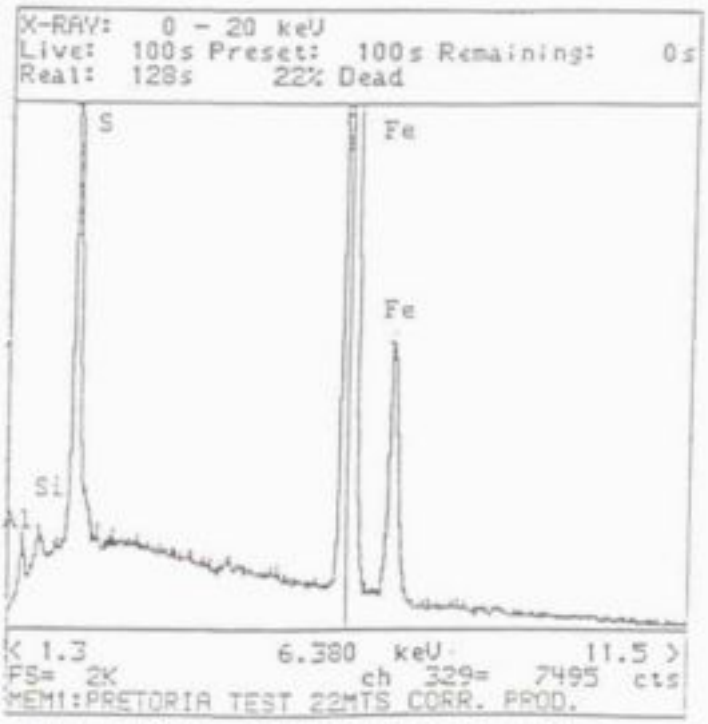

Figure 21: EDS trace of black corrosion product on a MS coupon after 20 months exposure at the Pretoria site (test).

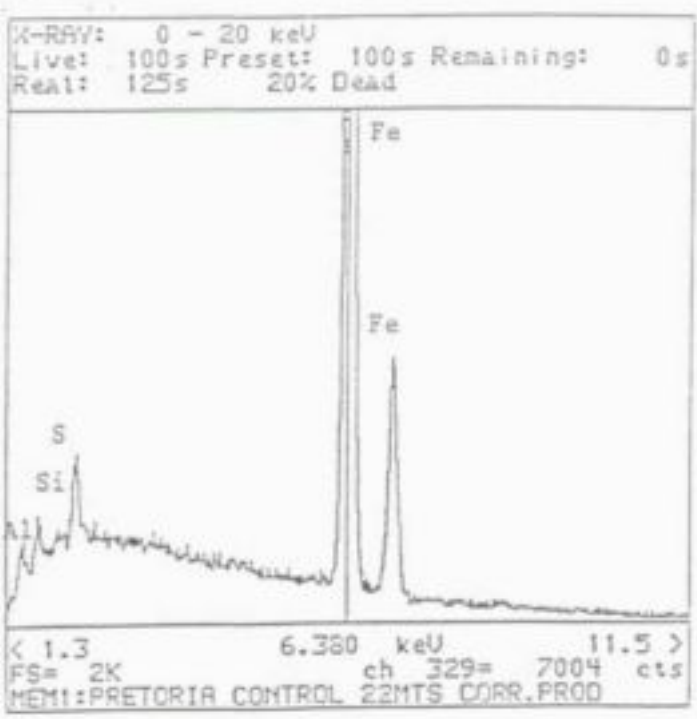

Figure 22: EDS trace of black corrosion product on a MS coupon after 20 months exposure at the Pretoria site (control).

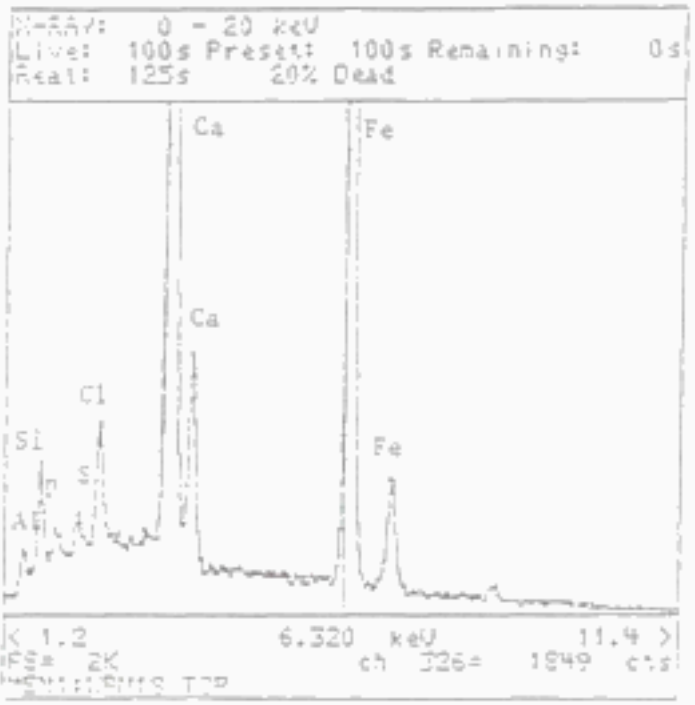

Figure 23: EDS trace of surface of corrosion product on a MS coupon from the Pretoria site (control).

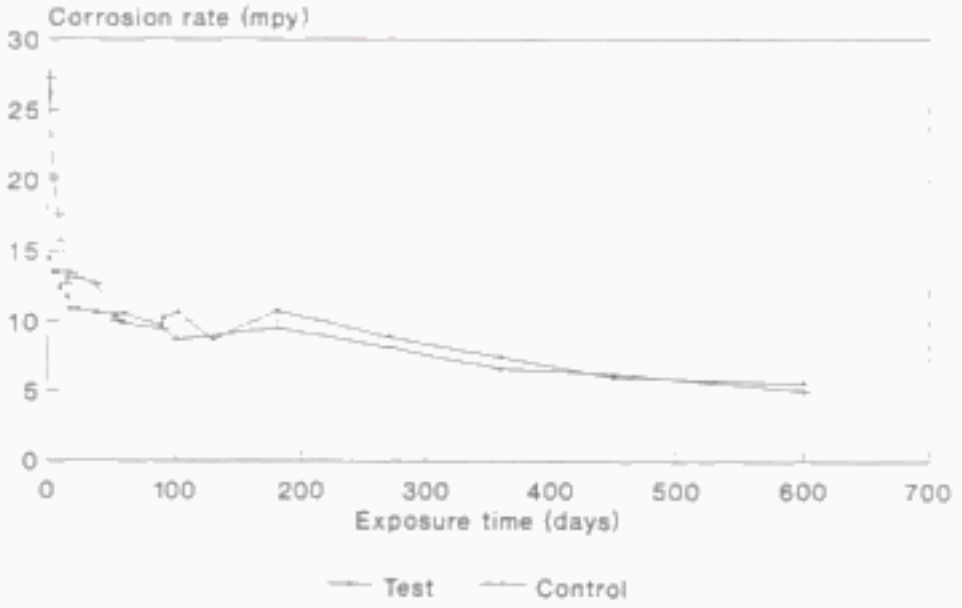

Figure 24: Corrosion rate of MS at the Johannesburg site.

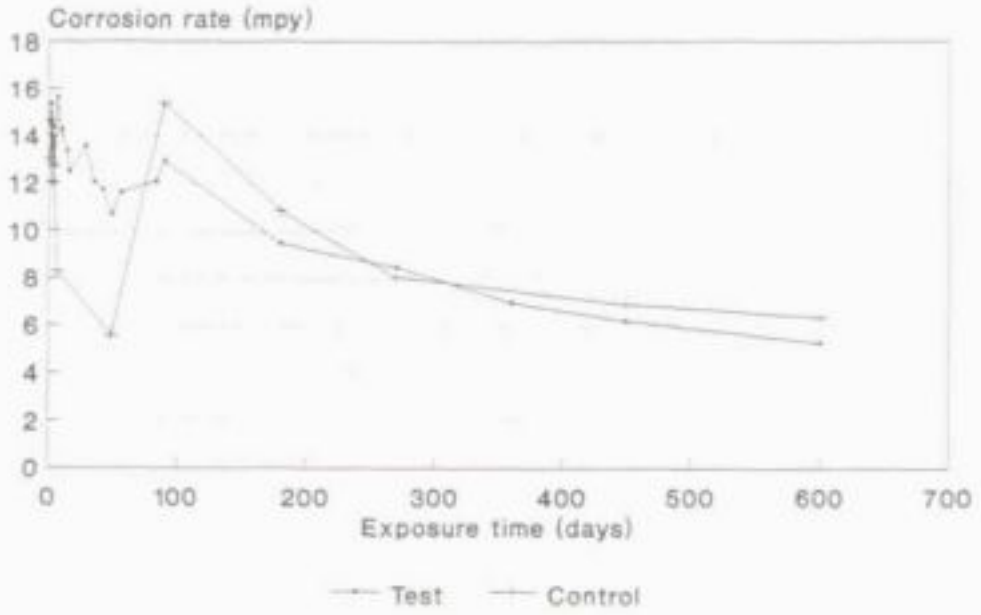

Figure 25: Corrosion rate of MS at the Pretoria site.

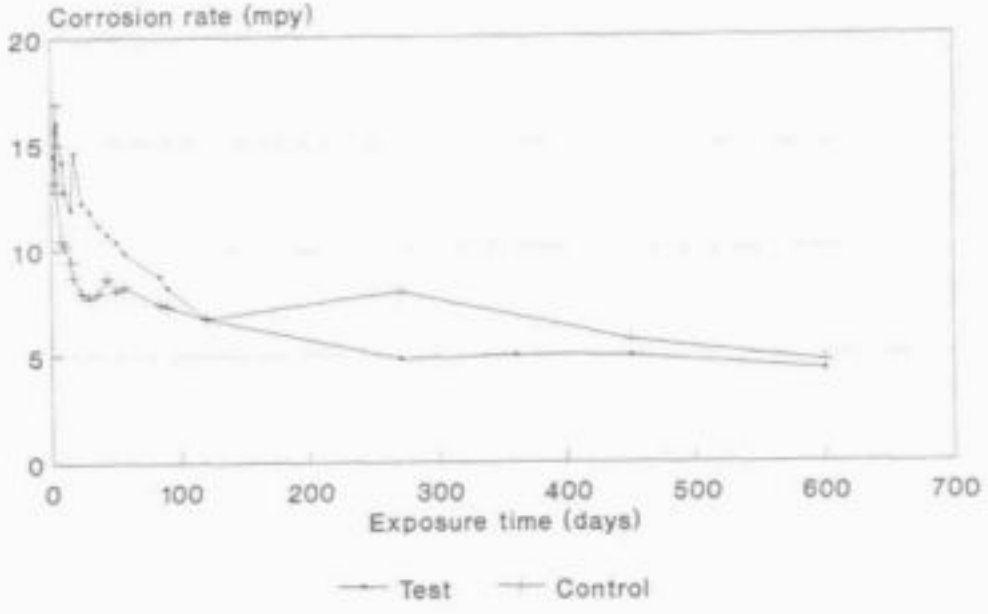

Figure 26: Corrosion rate of MS at the Vereeniging site.

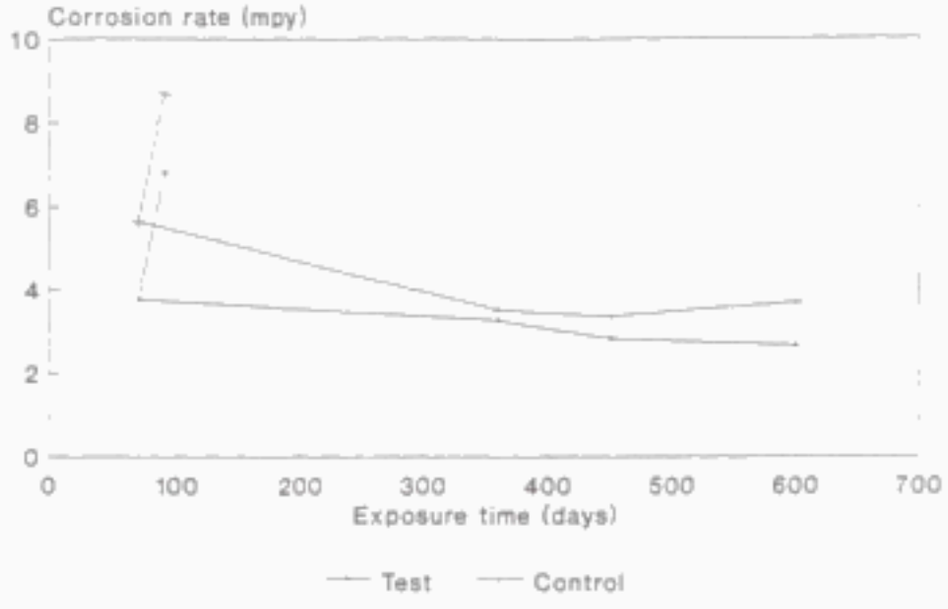

Figure 27: Corrosion rate of MS at the Klerksdorp site.

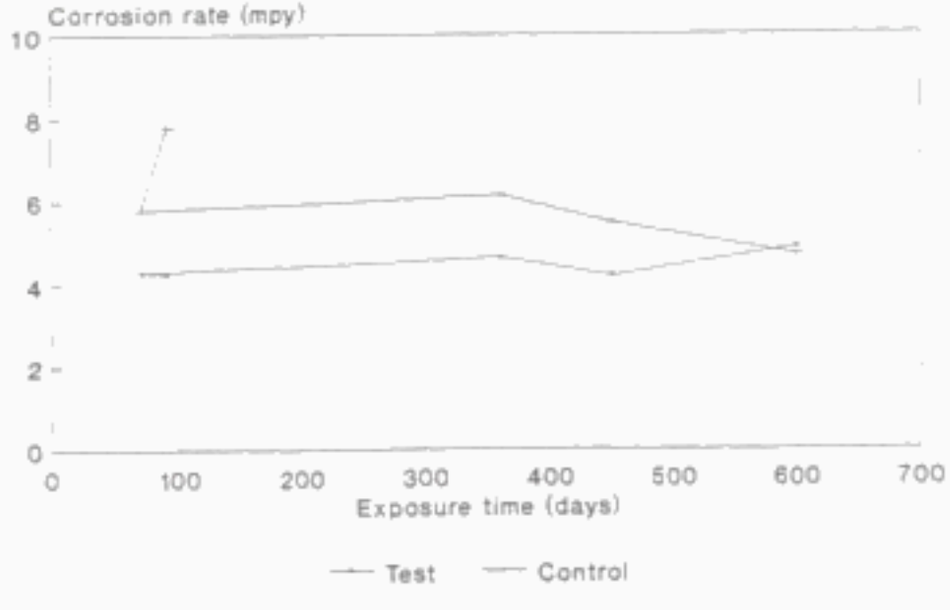

Figure 28: Corrosion rate of MS at the Vaal Dam site.

Figure 29: Condition of MS test coupons after 20 months exposure.

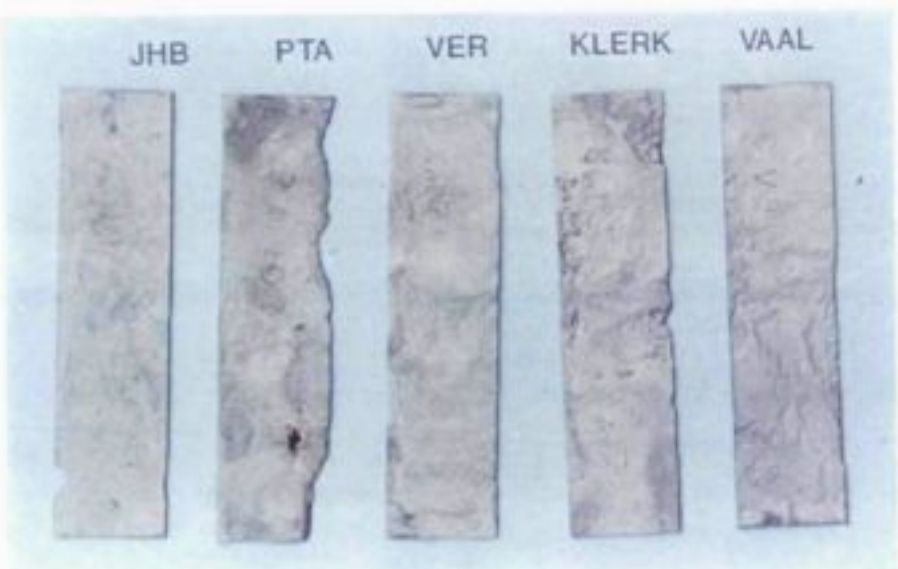

Figure 30: Condition of MS control coupons after 20 months exposure.

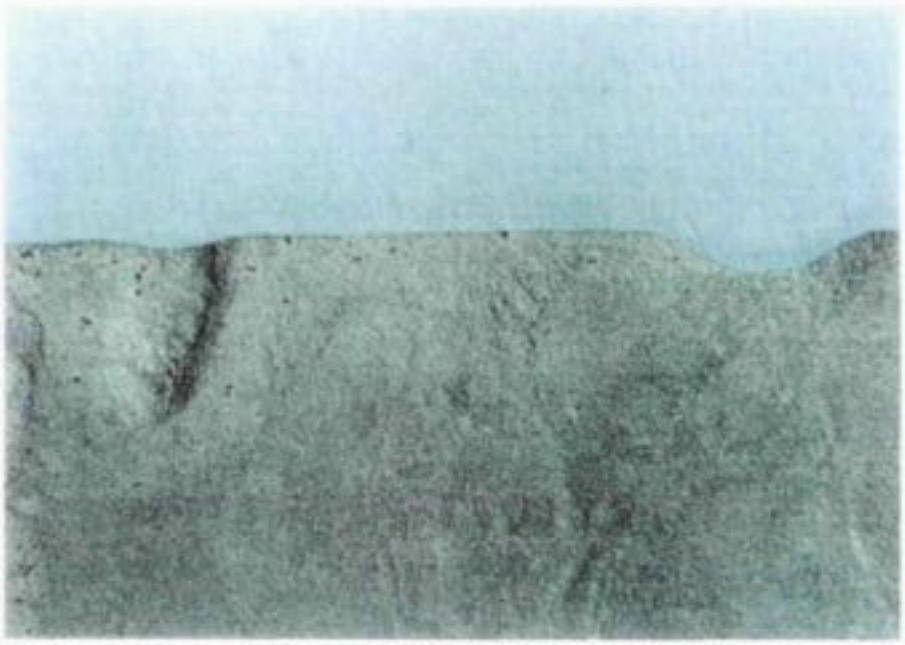

Figure 31: Penetration of a pit at the edge of a MS coupon.

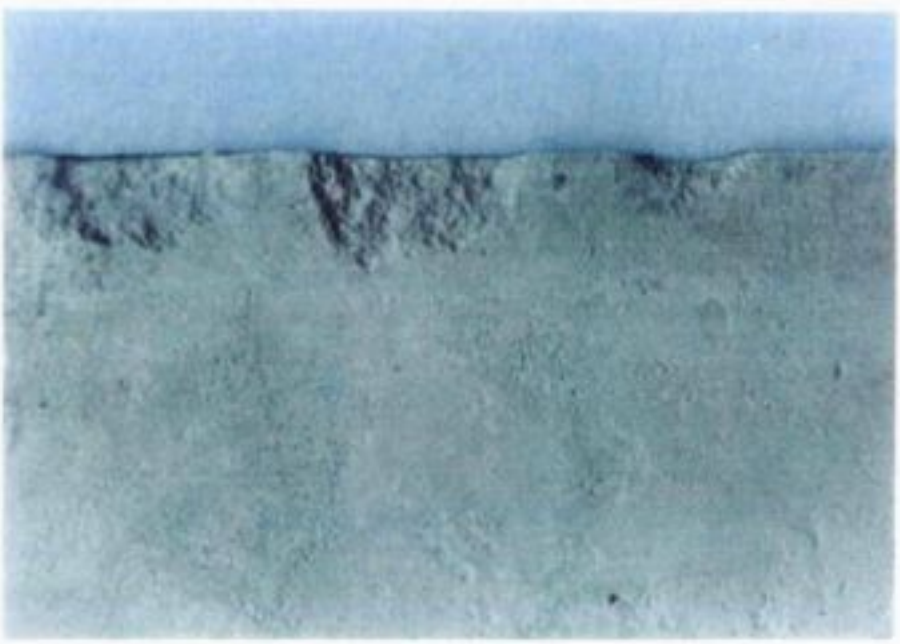

Figure 32: Pitting attack at the edge of a MS coupon after removal of the tubercles.

Figure 33: Shallow pitting attack on the surface of a MS coupon.

APPENDIX 1

LITERATURE REVIEW

1. INTRODUCTION

In the last two decades, microbiologically induced corrosion (MIC) has been recognised as a serious problem in most industries. Almost all metals and alloys are affected and the process occurs in most aqueous systems. The potable water industry has not escaped this problem either, as a review of recent literature has shown.

Distribution of drinking water through a complex network of pipes, reservoirs and household plumbing systems, inevitably results in changes of the water quality as attained at the treatment plant[1]. The nature and extent of the quality changes may depend on the composition and temperature of the produced water, the structure and dimensions of the distribution network and the materials of construction.

Water quality degradation during distribution may have aesthetic, hygienic and technical consequences. Water quality and hygienic factors are covered widely in the literature and have recently been studied in a WRC project[2]. This literature survey will deal mainly with internal MIC of potable water pipes and the consequences thereof.

The impact of corrosion of a distribution system can range from significant physical damage to pipes, service connections or fittings, to water quality changes due to the addition or removal of chemicals and microorganisms[3]. The water quality may change due to the removal of chemicals such as dissolved oxygen or chlorine from the water or by the addition of chemicals such as iron, lead, zinc and manganese due to corrosion of the pipe materials. The water quality may also be affected by the sloughing off of attached microorganisms or their byproducts from the pipe wall as corrosion advances. Some of these microorganisms may be opportunistic pathogens which may have been protected from disinfection by the corrosion product. In all, these factors may not only result in the deterioration of water distribution infrastructure, but also in deterioration of water quality.

2. BIOFILMS

2.1 Formation Process

A solid object such as a pipe surface in contact with flowing water will undergo a series of physical-chemical and biological changes at the solid-liquid interface[4]. These changes consist of four main stages: (1) absorption of organic molecules onto the solid surface; (2) adhesion of microorganisms to this "conditioned" surface; (3) multiplication of microorganisms to form microcolonies and eventually a continuous biofilm; (4) dynamic equilibrium of the biofilm with film growth and film destruction in relative balance.

Due to chemotactic responses, bacteria are able to seek out higher concentrations of nutrient sources and are thus attracted to the organic molecules on the pipe surface. From investigations of microbial successions at solid-liquid interfaces it appears that the primary colonizers are invariably bacteria that produce sticky extracelluar slime covers, generally composed of polysaccharides and water [4,5]. Once slime forming bacteria colonize a pipe surface, inorganic and organic particles as well as other microorganisms can become entrapped in the matrix. The eventual production of a continuous biofilm on the colonized surface is a function of cell division within micro-colonies and new recruitment of bacteria from the planktonic phase. Such structured consortia allow for nutrient trapping which occurs when organic nutrients are bound to the biofilm matrix and readily dissociated for use by the component organisms. Nutrients produced by component organisms also enter the biofilm and micro-colonies capable of primary production of nutrients are often surrounded by heterotrophic organisms that are stimulated by the exudates to grow and produce adjacent micro-colonies. The death and cell lysis of primary producers often radically stimulates biofilm growth since biofilms tend to trap and recycle cellular components. Because of the matrix enclosed mode of growth of biofilm bacteria, a substantial ion exchange matrix arises between the component cells and the liquid phase of their environment. Additionally, the gell-like state of the predominantly polysaccharide biofilm matrix limits the access of antibacterial agents to its component bacteria. Therefore biofilm bacteria are substantially protected from surfactants and

biocides[6].

Biofilms sometimes form continuous, evenly distributed layers but are often quite patchy in appearance. Biofilms in water distribution systems are thin but contain more than one distinct micro-environment eg. they have aerobic and anaerobic strata[7]. Inorganic debris entrapped in the biofilm may result from the adsorption of silt, precipitation of inorganic salts, or corrosion products.

2.2 Studies of Biofilm Accumulation in Distribution Systems

The study of biofilm accumulation and composition throughout a water distribution system is very difficult due to limited access to these systems. Most observations consist of analysis of samples obtained during flushing or pigging of the water mains. These samples consist of biofilm, sediment and corrosion product and thus bacterial enumeration procedures carried out on these samples cannot be accurately related to biofilm surface area. In addition, the extent of biofilm removal from the pipe wall is unknown. Direct cell counts on samples are complicated by the presence of corrosion products. Electron micrographs of biofilm of a single pipe surface sample are fragmentary and neglect the spatial heterogeneity of the biofilm[7]. All these factors are limiting in water distribution system research.

The condition of the distribution system strongly influences changes in the microbiological quality of the water. These system-mediated changes, which affect corrosion and sediment deposition, appear to be related to the ability of microorganisms to colonize within encrustations (tubercles) on the interior surfaces of the distribution pipes[8].

Allen[8] examined the occurrence of microorganisms on water main tubercles by electron microscopy. He found that on most tubercles, microorganisms were mainly observed at or near the surface. Active bacterial colonization was, however, also detected beneath the surface veneer, while some cells were found embedded in the granular matrix deep in the tubercle. It was also found that bacterial enumeration, by scraping of the tubercle surface followed by viable cell

counts, greatly underestimated the true populations present.

Emde[3] investigated MIC in a low temperature potable water distribution system and found that the highest populations of microorganisms were found in corrosion tubercles.

Ridgeway and Olson[10] used scanning electron microscopy to observe the bacterial colonization of a distribution main consisting of galvanised iron pipe with a cement lining. They found a diverse population of microorganisms able to colonize the walls of the mains as randomly distributed microcolonies, mainly in crevices in the mineral layer.

Victoreen[11] also found evidence of bacterial association with tubercles in experiments conducted with sections of tuberculated cast-iron main which were treated with different antibiotics. He concluded that the production of rust and turbidity can be reduced by reducing the bacterial population.

Studies carried out in a pilot reactor system to determine the relative contribution of biofilm accumulation to the bacterial populations in distribution mains, indicated that relatively large biofilm populations can accumulate in the system[12]. Another pilot plant experiment[13] to study biofilm growth in a drinking water network also found biofilm accumulation occurred in the system. Fixed biomass was approximately 10^7 bacteria /cm^2 and decreased constantly with the distance from the feedpoint of the network.

2.3 Bacterial and Chemical Analyses of Tubercles

Populations of bacteria found in both chlorinated and unchlorinated distribution systems have been shown to fluctuate seasonally and the microbial community that can colonize pipe surfaces and suspended particles is large and diverse[14]. The ability of various microorganisms to grow and survive on the surface of drinking water pipes was relatively well established by the turn of the century[4]. During the next 60-70 years, the ability of pipe surface microorganisms to influence water quality was most clearly demonstrated by iron and manganese

bacteria, resulting in considerable corrosion and water discolouration. As a result of increased availability of research tools and a wider range of media, there was a resurgence of activity in drinking water microbiology in the 1970's[4]. It was then thought that after-growth in distribution systems was largely as a result of microbial growth on pipe surfaces in the distribution system and its subsequent re-entry into the passing water. Greater emphasis was placed on understanding the microbial ecology of the distribution system leading to a number of investigations using a variety of microbiological media as well as scanning electron microscopy[4].

Tuovinen[15] examined tubercles from iron pipes. Using selective media, he isolated a variety of bacterial groups including sulphate reducers, nitrate reducers, nitrite oxidizers, ammonia oxidizers, sulphur oxidizers and various unidentified heterotrophic organisms. Redox potential measurements throughout the tubercles indicated that bacterial niches were available for aerobic, facultative anaerobic and anaerobic bacteria. The presence of large amounts of reduced iron in the tubercles was thought to contribute to the chlorine demand of the pipe wall and therefore hinder efforts to control bacteria.

Investigation of MIC in a low temperature distribution system (year-round water temperature was near 0 °C) revealed a diverse population of microorganisms, both in the water and in corrosion tubercles, which could potentially induce corrosion[3]. The groups isolated included sulphite reducers, sulphate reducers, iron reducers, sulphur oxidizers, iron precipitators, sulphate reducing actinomycetes and iron reducing fungi. The highest populations of microorganisms were found in the pipe corrosion tubercles and included both pathogenic bacteria and those associated with corrosion. Iron reducing bacteria were the predominant group isolated from tubercles, with Bacillus sp. and Pseudomonas sp. being the most common. Extensive tuberculation was attributed to the presence of iron oxidizing bacteria. Although the study indicated that a diverse group of microorganisms are associated with corrosion deposits, the authors felt that further studies were needed to elucidate the exact contribution of each organism to the nett corrosion process and the effect on electrochemical corrosion processes.

Iron oxidizing bacteria, such as Gallionella sp., Lepothrix sp. and Sphaerotilus sp., seem to play an important role in tubercle formation. These bacteria metabolize ferrous ions already present in the water and deposit large masses of ferric oxide in sheaths around their cells, thus producing voluminous tubercles[16]. They were indicated as being the predominant bacteria associated with tubercle formation in studies of steel and/or cast iron pipes [17;9;10;7].

SRB are the organisms most commonly associated with MIC in industry. Their presence in potable water systems is no exception. These bacteria have been isolated from within corrosion tubercles from iron pipes [16;15;7;3]. Their presence in the Johannesburg distribution system was first demonstrated by their isolation from the water itself and from within tubercles during the drought period in the early 1980's [18]. Bitumen lined steel mains were also found to contain SRB beneath defects in the lining[9]. Most studies seem to concentrate on bacteria found on or near the surfaces of tubercles and which would be involved with the quality and health aspects of potable water. Few studies are concerned with the anaerobic regions.

Aerobic bacteria on the surfaces of tubercles may, however, play an important role in the corrosion process either directly by oxidation or reduction activities or indirectly by their formation of slimy biofilm which then harbours SRB. Surface examination of tubercles using SEM[10] revealed a diverse population of microorganisms. Many observed cells had a coccoid shape and were sometimes linked together in chains. Filamentous bacteria were the most frequently observed, while iron-oxidizing bacteria of the genus Gallionella were also often seen. Rod-shaped bacteria were also observed. Allen[8] also using SEM showed high population of bacteria in mains tubercles. Diatoms, algae and filamentous and rod-shaped bacteria were commonly encountered. SEM showed that 17% of the 10- to 50- μm sized particles were colonized with 10 to 100 bacteria per particle[10].

Culture examination of distribution system biofilms has demonstrated large variations in the number of bacteria present. Variations in the number of heterotrophic plate count (HPC) bacteria range from $10^9/cm^2$ for Acinetobacter

on a mortar lined pipe surface to 10^8 bacteria/g of tubercle from water distribution pipelines[19]. It is difficult to compare bacterial numbers on surfaces of pipes due to the different criteria used. However, when calculated in terms of surface area, bacterial levels usually fall in the 10^0 - 10^6 colony forming units (CFU)/cm^2 range on potable water pipe walls[4]. Costerton and Geesey[20] found that the number of organisms/ml of flowing water was approximately 200 times less than the number of organisms/cm^2 of attached growth. It has been reported[4] that bacteria isolated from distribution pipe walls are basically the same as those isolated from water. Bacterial genera associated with distribution system biofilms include Arthrobacter, Flavobacterium, Moraxella, Acinetobacter, Bacillus, Pseudomonas, Alcaligenes and Achromobacter [4;12;19]. Schoenen[21] published data extracted from a number of studies which provides information on the species that have been isolated from drinking water surfaces. (The Table is reproduced below). Most of the bacterial isolates examined were gram negative rods and were identified as members of the genera Pseudomonas, Flavobacter, and Acinetobacter. Yeasts and filamentous fungi have also been found associated with pipe wall biofilms, while high levels of coliforms have been detected in tubercles [19;11].

The chemical composition of tubercles from potable water distribution systems has also been widely studied since the chemistry of the pipe surface may strongly influence the ability of certain microorganisms to attach and multiply. Iron seems to be the most common element present. Le Chevalier[19] found that dislodged tubercles consisted of 98,7% iron, as did Tuovinen[15], who reported large amounts of reduced iron (Fe^{2+}/Fe^{3+} ratio of between 1,2 and 2,0). Badan[17] further characterized the type of iron species present on steel and cast iron pipes using chemical analysis, SEM, XRD and Mössbauer Spectroscopy. In all samples the following iron compounds were found: Goethite (d-FeOOH), Lepidocrocite (y-FeOOH), non-stoichiometric Fe_3O_4 and colloidal hydrate oxide ($Fe(OH_3).0,9\ H_20$). Ridgeway[10] detected the presence of five major elements from X-ray energy scans of tubercles from a distribution system. These were Si, P, S, Ca and Fe with smaller quantities of the elements Zn, Mg, Al, K and Mn. Rothwell[16] reported that chlorides and sulphates are found to concentrate within the tubercle.

TABLE 1: **Microorganisms found in surface growth on different materials (after Schoenen[21]).**

GENUS/SPECIES/ OTHER GROUPS	FREQUENCY	GENUS/SPECIES/ OTHER GROUPS	FREQUENCY
Gram Negative Bacteria	+ + + + +	Moraxella-like bacteria	+ + +
		Pasteurella sp.	+
Pseudomonas fluorescens	+ + + +		
P. putida	+ + + +	Citrobacter sp.	+
P. aeruginosa	+	Klebsiella sp.	+
P. cepacia	+ + +	Enterobacter agglomerans	+ +
P. diminuta	+ + +	Vibrio sp.	+ +
P. acidovorans	+ +	Spirillum sp.	+ +
P. putrefaciens	+	Zoogloca sp.	+ +
P. alcaligenes	+ +	Acetobacter sp.	+
P. pseudoalcaligenes	+ +	Aeromonas sp.	+ +
Pseudomonas sp.	+ + + + +	Unidentified Gram negative rods	+ + + + +
Plesiomonas sp.	+	Gram Positive Bacteria	+ +
Comamonas terrigena	+		
		Bacillus sp.	+
Flavobacterium devorans	+ +	Micrococcus sp.	+
F. breve	+ + +	Planococcus sp.	+
F. aquatile	+ + +	Arthrobacter sp.	+ +
F. rigense	+ +	Streptomyces sp.	+
Flavobacterium sp	+ + + + +	Actinoplanes sp.	+
CDC-Gr. Vel/Vell/llk	+ + +	Nocardia sp.	+
Cytophaga sp.	+ +	Pasteurella sp.	+
Caulobacter sp.	+ +	Occurrence	
Hyphomicrobium sp.	+ +	+ + + + + always	
Sheathed bacteria	+ + +	+ + + + most frequent	
		+ + + frequent	
Chromobacterium sp.	+	+ + occasional	
Acinetobacter calcoaceticus	+ + + +	+ sporadic	
Alcaligenes sp.	+ + +	CDC Center of Disease	
Achromobacter sp.	+ +	Control (Atlanta)	

2.4 Factors Affecting Biofilm Growth

Distribution of potable water through a complex network of pipes, reservoirs and household plumbing systems, inevitably results in changes of the water quality as attained at the treatment plant[1]. The biological quality is altered as living organisms and nutrients enter the distribution system in one or more of the following ways: recontamination events (back siphonage, cross-connections, line breaks and repairs); inadequate or incomplete treatment, including disinfection; break through of particle-associated bacteria; and survival and repair of chlorine-injured organisms[14]. It is generally accepted that bacterial after-growth occurs on surfaces[4]. This biofilm mode of growth within an exopolysaccharide matrix ensures that the organisms are afforded some degree of protection from adverse conditions.

Biofilm growth on distribution system pipe surfaces is a universal phenomenon[14]. However, the extent of biofilm growth depends on a number of factors which have been the subject of numerous studies[8]. The parameters most often cited include: chemical-biological quality, residual disinfection, physical integrity of the distribution system, temperature, residence time, pH, concentration of assimilable nutrients, flow rate and redox potential [4,8,14]. Some of these parameters will be considered in more detail below.

There is widespread agreement that free residual chlorine is a critical rate-limiting factor for biofilm growth[4]. Van der Wende et al [12] studied bacterial growth in potable water distribution systems in a pilot reactor system designed to model the plug flow characteristics in a water main. They found that chlorine affected the accumulation and spatial distribution of the biofilm. They postulated that biofilm cell growth and detachment may be even more dominant in water distribution systems with low chlorine concentrations than in chlorine-free systems. The biofilm environment is believed to protect cells against the activity of chlorine by diffusional resistance and neutralization of chlorine through the reaction with biofilm and pipe wall materials while planktonic cells do not find such protection in their environment. Thus, microbial growth in the biofilm will be even larger than the growth of planktonic cells which experience a higher

chlorine concentration.

An increase in chlorine concentration has been found to decrease heterotrophic plate counts (HPC) of surfaces sampled from cast iron cylinders exposed inside water mains[22]. Sampling of water from a distribution system in France showed that the zones of highest bacterial concentration correlated with lower levels of chlorine residuals[23]. Le Chevalier[24], studying the regrowth of coliform bacteria in distribution systems predicted from a multiple linear regression model that 1,0 mg of free chlorine residuals per litre would be necessary to limit HPC bacterial levels to less than 500 CFU/ml. To achieve this residual, however, much higher chlorine doses would be required, which would result in unacceptable trihalomethane levels.

Olson and Nagy[4] have formulated a model to conceptualize the relationship between chlorine levels, contact time and bacterial detachment in a drinking water distribution system. This model explains the contradictory results obtained by many researchers and is reproduced below.

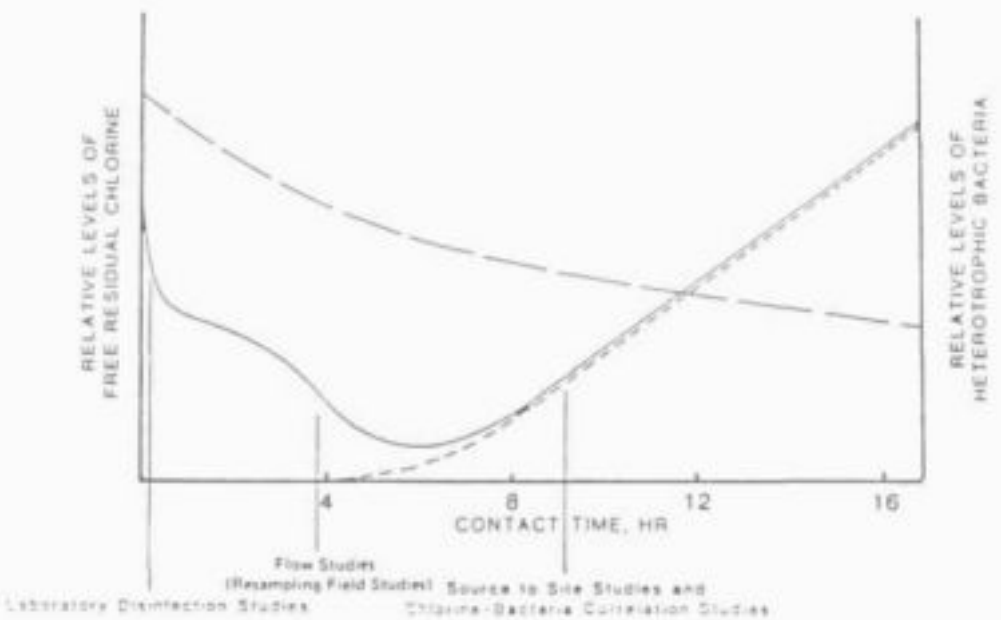

Figure 1: A conceptual model for the relationship between chlorine levels, contact time and bacterial detachment in a drinking water distribution system (after Olson and Nagy[4]).

The model relies on two factors which act as opposite forces on bacterial numbers viz. free chlorine residual (contact time) and detachment of bacteria from pipe surfaces, and includes information from both laboratory and field studies using different contact times and chlorine levels.

Van der Kooij[1] reported that the densities of bacteria on surfaces depend on the concentration of free residual chlorine in the water as well as on the concentration of energy sources. These sources may include both inorganic compounds (eg. NH_3) and organic compounds (eg. carbon). Haudidier[13] concluded, from experiments in a pilot plant using potable water free of residual chlorine, that substrate conversion really controlled biofilm formation. He reported that heterotrophic bacterial growth is supported only by nutrients (organic carbon, nitrogen and phosphates) found in the water. In contrast, Donlan[22] found that the attached population on cast iron pipes were not limited by nutrient concentration, including organic nitrogen, ammonia, nitrite, nitrate, total-and orthophosphate and total organic carbon (TOC).

Organic carbon is utilized by heterotrophic bacteria for production of new cellular material and as an energy source. Most organic carbon in water supplies is natural in origin and is derived from living and decaying vegetation. Assimilable organic carbon (AOC) is the portion of TOC which can be readily utilized by aquatic organisms for growth. Often, the AOC constitutes a fraction (0,1 - 9,0%) of the TOC[24]. In studies of regrowth of coliform bacteria in distribution systems, Le Chevalier[24] reported that growth of these bacteria is influenced by TOC levels greater than 2,4 mg/l and AOC levels greater than 50 µg/l. Olson & Nagy[4], in a review of the microbiology of potable water, reported that together with residual chlorine, it was widely agreed that TOC is a critical rate-limiting factor for biofilm growth. In the case of the presence of SRB, the concentration of sulphates in the water may also be and important nutrient[18].

A great and increasing variety of synthetic materials is being applied to distribution systems (including reservoir and plumbing systems). Materials which have been reported to promote bacterial growth range from lubricants for pipe joints to coatings of reservoirs and rubber gaskets in plumbing systems[1]. The

materials may promote growth by releasing organic constituents which can be utilized by microorganisms. Cases of increased bacterial growth due to utilization of hydrocarbons from bitumen lining of pipes and reservoirs have been reported [9;21]. In a study of microbial growth due to materials used in drinking water systems, Schoenen[21] concluded that materials of purely inorganic composition and also metals do not in themselves promote microbial growth. However, materials of an organic nature, such as coatings, or those with organic additives, such as plastic-containing cement-mortar, may promote the growth of microorganisms.

Water temperature seems to be another important rate-controlling factor in bacterial growth with greater growth at warmer temperatures. Le Chevalier[9] found a significant increase in coliform bacterial counts in potable water systems at temperatures above 15 °C. A strong positive relationship between water temperature and HPC of biofilm-associated bacteria was found to exist[22]. Maul[23] reported increased bacterial growth in summer months, as did Olson & Nagy[4]. Reasons given for this [4;22;24] are that (i) growth rates of microorganisms are linearly related to temperature within the organisms' temperature range, and (ii) increase in temperature can increase chlorine demand reactions resulting in loss of chlorine residuals in dead end sections.

The structure and dimensions of the distribution system may also affect biofilm growth. Stagnant areas such as found in dead ends and household plumbing systems may allow for increased bacterial growth[1]. Increased coliform counts have been reported from reservoir surfaces and from pipe surfaces from dead ends[4]. This may also relate to retention/residence time within the system. Maul[23] noted that zones of highest bacterial concentrations were attributed to prolonged retention time of the water in the network, especially in the storage units, before reaching the various distribution areas.

Flow velocity may affect the biofilm bacteria both positively and negatively. Increasing velocities will allow for a higher flux of disinfectant and produce a greater shear of attached microbial growths. At the same time the higher velocities will flux a greater loading of nutrients and cells to the surface growth[22].

The correlation between flow rates and bacterial levels indicates that the microbiology of distribution systems is highly dynamic[4].

Finally, dissolved oxygen in the water distribution system generally decreases as organic content and bacterial numbers increase. It appears that bacterial numbers have a greater effect on reducing oxygen than does TOC. A decrease in dissolved oxygen also leads to an increase in chlorine demand making it more difficult to maintain a chlorine residual.

3. CONSEQUENCES OF MICROBIAL ACTIVITY

3.1 Corrosion

Potable water pipelines provide an ideal habitat for microorganisms: (i) the pipe walls supply a surface for attachment; (ii) they have a relatively sheltered environment in which the biofilm mode of growth ensures a nutrient supply and protection from undesired outside factors; (iii) the water flowing through the pipeline provides a constant supply of nutrients which are trapped by the polysaccharide slime of the biofilm; (iv) flow quickly and widely disperses cells so that they can establish themselves in other parts of the system.

It is now widely accepted that a large number of diverse microorganisms are able to colonize potable water pipe surfaces and are most frequently found on or within tubercles and encrustations. These microorganisms may affect the corrosion of the pipeline either directly or indirectly.

3.1.1 Mechanisms of MIC in Potable Water Distribution Systems

(i) Concentration Cells

Aerobic microorganisms have been thought to participate principally through the formation of oxygen concentration cells on the metal surface. As microorganisms attach to the metal surface,

the biofilm grows in density and thickness. Oxygen will be depleted more quickly in heavily populated areas relative to thinner sections of the biofilm. This results in the area of oxygen depletion becoming more anodic relative to surrounding areas, with metal dissolution occurring at the anode [3,5]. The formation of tubercles by aerobic iron-precipitating bacteria such as Gallionella and Sphaerotilus may also create oxygen concentration cells [17,25]. Microbial colonization may also enhance the formation of ionic concentration cells increasing the net corrosion rate[3]. Chlorides and sulphates have been found to concentrate within the tubercle[16].

(ii) **Products of Microbial Metabolism**

Microbial metabolic by-products such as inorganic and organic acids, hydrogen sulphide (H_2S) and others enhance metal dissolution and precipitation which, in turn, depolarizes the anode and furthers the corrosion process [3,25]. The most important inorganic acid producing organisms are species of Thiobacillus and Ferrobacillus which are both capable of producing sulphuric acid through the oxidation of sulphur species[26]. Sulphuric acid up to the level of 15-20% has been found on many occasions beneath these organic deposits leading to very rapid corrosion of the metal surface[5]. Organic acid production is often associated with the growth of fungi and moulds [26,5]. Certain microorganisms can influence corrosion by metabolism of corrosion inhibitors or degradation of protective coatings.

(iii) **Anaerobic Corrosion by SRB**

Most microbiologically induced corrosion studies have focused on the SRB (such as Desulfovibrio and Desulfotomaculum) as they are known to be potent contributors to metal corrosion. Being

anaerobic organisms, they are found in areas beneath biofilms or within tubercles where they are protected from oxygen. They grow well at ambient and slightly elevated temperatures and in the neutral pH range. They require an organic source of carbon and derive their energy for growth by the reduction of sulphate to sulphide with the eventual production of hydrogen sulphide. This is highly corrosive and will attack most metals. More detailed discussions of the mechanisms of SRB corrosion have been extensively covered [26,27,28].

3.1.2 Identification of MIC in Potable Water Systems

Nearly all confirmed cases of MIC have been accompanied by characteristic deposits. These are usually discrete mounds of corrosion product. Bacterial deposits usually have a slimy feeling when fresh and wet, and are generally soft and easily deformed[29].

On mild steel, corrosion mediated by SRB has several characteristic features. Usually the metal surface is distinctly nodular, the nodules consisting mainly of iron and sulphur species. On removal, the nodules display a hard outer crust and a relatively soft accumulation near the metal, often containing hydrogen sulphide. Beneath the nodule, the metal is clearly pitted in a localized fashion and the metal within active pits when cleaned is usually shiny and bright[16]. The pitting is often described as "gouging" of the metal[33].

Tubercles formed by iron-depositing bacteria, may or may not contain SRB in their interior. They are usually an orange-brown colour. The metal beneath the tubercles normally has hemi-spherical pitting attack in the absence of SRB. In a study of cast iron and steel potable water pipes, it was found that tubercles present on pipe surfaces were formed due to the action of iron depositing bacteria. The tubercles consisted mainly of iron species with significant amounts of organic matter also present. No significant amounts of sulphur compounds were detected. Corrosion

beneath the tubercles was in the form of shallow saucer-shaped pits. The metal surface was extremely corrugated[17].

As corrosion proceeds, the pipe wall thickness is reduced and the water main may be prone to fracture from internal and external loads[25].

3.1.3 Potable Water Piping Metals and Alloys affected by MIC

All piping materials used to convey potable water have been affected by MIC. This type of attack is most common in steel and cast iron pipes. Attack of the former results in pitting [34;25;3;18] while that of the latter in graphitization [16;35]. Graphitization occurs when the iron matrix at anodic areas dissolves, leaving a soft, porous mass of corrosion product bound together by a network of graphite flakes and phosphide residues. Copper pipes have also suffered pitting and some general corrosion due to the action of microorganisms [30;31;32]. An austenitic stainless steel pipeline which had been hydrotested with low chloride potable water was reported to have failed by through wall penetration 18 months after testing. This was found to be due to the action of SRB and Gallionella. Galvanized steel is also prone to pitting attack by SRB[34].

3.2 Quality Problems

Briefly, the corrosion of distribution systems can have a number of implications for a treated water quality. Microbial after-growth and/or regrowth in distribution systems has been linked to sloughing of the pipe biofilm into the passing water. Bacteria of public health concern have been isolated from well-functioning distribution systems.

Distribution system biofilms have also been shown to support growth of actinomycetes and certain filamentous fungi, which have been linked to taste and odour complaints about potable water[3].

4. REMEDIAL MEASURES

Rehabilitation of distribution systems affected by MIC includes several methods varying from flushing and chemical treatment to replacement. It is essential, however, to determine the cause and extent of corrosion before a choice between different rehabilitation methods can be made. It is also necessary to obtain information on the condition of the pipeline, such as thickness of the corrosion layer and amount and composition of deposits[36].

4.1 Disinfection

Water utilities usually apply one or more disinfectants during or at the end of the water treatment process. The most often applied is chlorine. In some cases chloramines, chlorine dioxide or ozone may be used. After a disinfectant has been applied to the water, its residual concentration begins to decline due to the reaction with various dissolved and suspended compounds in the water phase and with biofilm and pipe wall materials. The disinfectants are reactive compounds which oxidize substances like Fe^{2+}, Mn^{2+}, sulphide and organic compounds. Disinfectant booster stations throughout the distribution system are sometimes used to maintain appropriate disinfectant residuals in the extremities of the system[7]. The presence of chlorine has been shown to reduce the accumulation of biofilm and influence its distribution in a pilot reactor system[12]. Problems may arise, however, in old or heavily encrusted water mains since the disinfectant may have limited penetration into the biofilm and/or tubercles. Le Chevalier et al[19] reported that mono-chloramine was more successful in penetrating the biofilm than free chlorine. Researches have, however, determined that chlorine reacts much faster and with a larger variety of biological organic compounds[7]. The range of disinfectants which can be used in a potable water system is very limited due to their toxic properties.

4.2 Physical Cleaning

Flushing has a short term effect on the improvement of water quality and unfortunately, has hardly any effect on tubercles and on pipelines > 200 mm in

diameter. Air scouring is a discontinuous addition of compressed air to the water. High turbulence removes all loose material but not the tubercles. Swabbing with soft foam pigs removes only loose sediment. In order to remove adherent tubercles, pigs with a coating, brushes or scrapers are used. The pipes remain clean for a long period but where the corrosion layer is removed completely, corrosion is accelerated. High pressure water jet cleaning will almost completely remove the internal corrosion layer, however, as before the corrosion is increased[36]. In a study of the occurrence of coliform bacteria within tubercles of a water mains system, Le Chevalier et al[19] reported that flushing and pigging of the study area had not been an effective control for coliform occurrences in that section. They suggested that to make flushing and pigging effective, the entire distribution system, even short segments, would have to be treated.

4.3 Relining

Cement-mortar lining is an expensive but permanent method. The pipe has to be cleaned with scrapers before the lining is applied. It is important, however, that the pipe material has enough strength and there is not too much corrosion on the outside of the pipe. In-situ relining using epoxy resins is becoming more popular [37,36]. Relining results in a small decrease in diameter of the pipe, while the strength of the pipe increases. The resin must be approved as acceptable for use in pipelines transporting drinking water.

4.4 Replacement

Replacing an old pipeline is an expensive solution. Replacement of steel mains with the same material in an uncoated condition could lead to re-occurrence of the problem as the new surface is colonized by biofilm from older parts of the system. Replacement with plastics can be effective but the type of plastic chosen must be resistant to biodegradation and chemical attack by the specific metabolic products of microbial activity, principally H_2S and organic acids.

4.5 Design Considerations

The engineering design of a system should be streamlined to eliminate areas of low flow or total stagnancy, since it is in these areas that biofilm growth is most prolific.

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