Chapter 6
A LINEAR POLARISATION TECHNIQUE FOR IN-SITU BIOCIDE EFFICACY MONITORING

ABSTRACT

Sessile bacteria can cause deleterious effects in cooling water systems. It is therefore essential to monitor the activities of these bacteria and determine the efficacy of biocide treatment programmes on their populations. A corrosion monitoring device, using the linear polarisation technique, was evaluated to determine its suitability as a technique for the monitoring of sessile microorganisms. Initially, the device was evaluated in jar tests to determine whether bacterial attachment was occurring on the electrodes of the device. Uniform bacterial attachment occurred on all of the electrodes and on corrosion coupons. Four pilot rigs were constructed, filled with cooling water, containing no biofouling control chemicals and the corrosion rates monitored over a three month period by means of the corrosion monitoring device. A gradual increase in corrosion rate from approximately 0.3 mm.year\(^{-1}\) to 0.6 mm.year\(^{-1}\) was observed. It was concluded that this increase in corrosion rate was due to the formation of biofilms. Planktonic bacteria numbers were also determined weekly. A net increase in aerobic bacteria (1.0x10\(^4\) CFU.ml\(^{-1}\) to 1.0x10\(^7\) CFU.ml\(^{-1}\)) and anaerobic bacteria (1.0x10\(^3\) CFU.ml\(^{-1}\) to 1.0x10\(^5\) CFU.ml\(^{-1}\)) occurred in the bulk water, but no sulphate reducing bacteria were detected. The biocide isothiazolin was then added to two of the rigs to give a final concentration of 5 ppm, while the remaining two rigs were retained as untreated controls. A significant decrease in corrosion rate was observed in the two rigs to which biocide was added. The corrosion rates in the two rigs to which biocide was added decreased by 0.2 mm.year\(^{-1}\) and 0.3 mm.year\(^{-1}\). It was concluded that this type of corrosion monitoring device could be utilised as an on-line monitor of sessile microbiological activity and biocide efficacy.
INTRODUCTION

In flowing, aqueous environments, microorganisms occur in two phases, the planktonic (or free floating) phase and the sessile (or attached) phase (Geesey et al., 1978; Costerton et al., 1987). Although no correlation between the numbers of microorganisms in the sessile and planktonic phases has been observed to date, the sessile bacteria outnumber their planktonic counterparts, especially in nutrient poor environments (Blenkinsopp and Costerton, 1991).

Sessile microorganisms can have deleterious effects on the operation and integrity of cooling water systems (Lutey, 1980; Ferguson, 1981; Bott et al., 1983). These adverse effects can cause significant financial losses, and it is therefore essential to monitor and control the activities of these sessile microbial populations (Cloete et al., 1992). Sessile bacteria are more resistant to biocides than their planktonic counterparts (Russell, 1990; Blenkinsopp and Costerton, 1991). Thus, when evaluating biocide efficacy, their effect on sessile microorganisms must be determined.

The majority of monitors utilised to directly determine sessile microbiological growth and MIC in aqueous systems require the removal of sampling surfaces (Blackburn and Mullin, 1990; Nivens et al., 1990). As a result, damage of the biofilm may occur. In addition, the techniques for removal of biofilms from the sampling surfaces and subsequent quantification of the microorganisms are often inadequate (Tatnall and Horacek, 1990; Clancy and Cimini, 1991). Thus, many indirect, non-destructive techniques for the determination of biofouling have been developed. Some of the indirect methods of determining biofilm thickness are the tubular reactor system which determines the volumetric displacement of a biofilm (Characklis et al., 1982), the Bio Film Monitor which measures fluid-flow resistance (Johnson and Howells, 1981) and fouling monitors that measure decreases in heat transfer (Matson and Characklis, 1982). However, these monitors are not able to assess corrosion induced or influenced by sessile microorganisms.

Licina et al., (1990) described the general requirements for an on-line monitoring device for the determination of microbiologically influenced corrosion (MIC) as simple to use, simple to interpret, rugged, accurate, cost effective and sensitive. If all these requirements were met, it would ensure that results could be rapidly interpreted by operating personnel. Preventative action could therefore be taken to circumvent deleterious effects, such as metal loss as a result of MIC. The added advantage of this type of monitoring device would be that a qualified microbiologist would not be required for the operation and interpretation of results.
Thus, indirect techniques such as electrochemical monitoring have been extensively utilised to monitor sessile microbiological activity, particularly MIC (Feron, 1990; Kasahara and Kaji- yama, 1990; Mansfeld and Little, 1990; Salvago et al., 1990; Tullmin et al., 1992). There are, however, few reports on corrosion monitoring devices being utilised to evaluate the efficacy of biocides (Tatnall, 1981). Linear polarisation resistance techniques provide instantaneous and continuous readings of corrosion rates. Three electrodes are used, the test, the reference and the auxiliary electrode. The test electrode should be constructed from the same metal as found in the system to be monitored. The potential of the test electrode is measured against the reference (non-polarised) electrode. The current resulting from a 10mV shift in the potential of the test electrode is measured by the auxiliary electrode. A direct relationship exists between the resulting current and corrosion rate. The major disadvantage of linear polarisation techniques is that they do not provide information on localised corrosion (Scully and Taylor, 1987).

The aim of this work was therefore to evaluate a linear polarisation technique to not only monitor the corrosive effects of sessile microorganisms, but also to evaluate biocide treatment programmes. The hypothesis was that biocide addition will result in a lowering of the corrosion rate and that the corrosion rate is indicative of sessile microorganisms.

MATERIALS AND METHODS

Corrosion monitoring device

Four Petrolite corrosion rate instruments (Model M-3011) were used. These instruments measure corrosion rates by means of a polarisation admittance instantaneous rate, or linear polarisation technique. Each instrument consists of three electrodes, the test, reference and auxiliary electrodes which are mounted on a support probe which is immersed in the water to be tested (Figure 6.1).

The electrodes used in these tests were constructed from mild steel. The surfaces of the electrodes were abraded with 200 grit sandpaper before being fixed onto the support probe, to remove any existing corrosion products (C. Gross pers. comm.)*. The corrosion monitors were linked to a data logger (Squirrel 1200, Grant, USA), that recorded the corrosion rates in milliamperes.

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Figure 6.1: Petrolite corrosion rate instrument probe.

Jar tests to determine microbial attachment and MIC on electrodes of the corrosion monitor and corrosion coupons

Linear polarisation corrosion monitoring device utilisation for determination of the activity of microorganisms, requires the microorganisms to attach to the electrodes of the device. To establish whether this was occurring, three corrosion monitors were each suspended above two litre jars containing water from the main, open recirculating, cooling water system at Duvha Power Station (see Table 6.2 for the chemical composition). This cooling water was used since it contained no biofouling treatment chemicals. The corrosion monitors were positioned such that their electrodes were totally immersed in the water and the jars were placed on magnetic stirrers (approximately 100rpm) to circulate the water. A mild steel corrosion coupon was placed in each of the jars so that corrosion rates could also be determined by the standard mass loss technique (Dean and Sprowls, 1987).
Table 6.1: Chemical composition of the cooling water obtained from the main, open recirculating cooling water system, Duvha Power Station.

<p>| | |</p>
<table>
<thead>
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<tbody>
<tr>
<td>pH at 25°C</td>
<td>8.5</td>
</tr>
<tr>
<td>Conductivity at 25°C</td>
<td>2700 μS.cm⁻¹</td>
</tr>
<tr>
<td>Total alkalinity</td>
<td>113 mg.l⁻¹ CaCO₃</td>
</tr>
<tr>
<td>Chloride</td>
<td>246 mg.l⁻¹</td>
</tr>
<tr>
<td>Sulphate</td>
<td>905 mg.l⁻¹</td>
</tr>
<tr>
<td>Total hardness</td>
<td>318 mg.l⁻¹ CaCO₃</td>
</tr>
</tbody>
</table>

After one week the electrodes and corrosion coupons were removed from the holders and the numbers of sessile microorganisms determined as described in Table 6.2.

New electrodes were then placed on each of the corrosion monitors which were immersed in Duvha cooling water, for one week, as described above. The electrodes and corrosion coupons were removed and processed for examination by scanning electron microscopy, as described below.

**Pilot rig tests for evaluating the use of a linear polarisation technique to determine the control of MIC using a biocide**

Four identical pilot rigs, each consisting of a pump, a reservoir tank and a pipe into which a corrosion coupon and the corrosion monitor probes could be inserted, were constructed. The system volume of each rig was 190 litres. Stainless steel cooling coils were placed inside the reservoir tanks to provide cooling (Figure 6.2).

The pilot rigs were filled with cooling water (Table 6.1). Once a week, 20 litres of water was removed from each pilot rig and replaced with fresh cooling water. After the addition of the fresh cooling water, Nutrient Broth (Oxoid) was added to the water in each pilot rig to give a final concentration of 1.5 g.l⁻¹. Nutrient Broth was added to the pilot rigs to accelerate microbiological growth as cooling water is a low nutrient medium (McCoy, 1980). A flow rate of 1.3 m.s⁻¹ was maintained through the pipes containing the coupons and probes. The temperature of the water was maintained at 36°C.
The pilot rigs were operated for three months and corrosion rates were recorded every eight h. A pure culture of a sulphate reducing bacterium (SRB), *Desulphovibrio desulphuricans* (obtained from E. de Bruyn)* was added to the water at four weeks and six weeks after initiation of the experiment. This ensured that bacteria previously implicated in MIC, were present in the water (Lutey, 1980). The calculated number of *D. desulphuricans* in the water was 25 CFU.ml−1. The numbers of total aerobic and anaerobic bacteria and *D. desulphuricans* in the bulk water were quantified weekly as described in Table 6.2.

After three months, Kathon WT, a water treatment microicide (Rohm and Haas, South Africa) was added to two of the pilot rigs, to give a final concentration of 5 mg.l−1 of the active ingredient, a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one. The other two rigs were monitored as untreated controls. Corrosion rates were monitored every four h, for a further forty eight h.

**Sampling procedure**

All water samples were taken in 500ml aliquots in sterile Whirl Pak bags (Nasco, USA). Corrosion monitor probes and corrosion coupons were removed from their holders using sterile forceps and each placed in bottles containing 100ml of sterile, quarter strength Ringer’s solution and 20 ppm of a biodispersant. These bottles were then agitated on a vortex mixer for a period of two min, to dislodge the sessile bacteria into the Ringer’s solution. Samples were retained at 4°C and analysed within two h.

**Microbiological analysis**

Samples of bulk water and suspensions of microorganisms recovered from the electrodes or corrosion coupons, were serially diluted in sterile, quarter strength Ringer’s solution and subjected to duplicate plate counts. Samples were retained at 4°C and analysed within two h. Anaerobic incubation took place in an anaerobic incubator (Forma Scientific Anaerobic System, Labotec, S.A.), in an atmosphere of 5% hydrogen, 15% carbon dioxide and 80% nitrogen. All incubation was at 37°C. Plates containing between 30 and 300 colonies were counted.

The techniques used to quantify bacteria in the bulk water and recovered from the electrodes or corrosion coupons are detailed in Table 6.2.

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Table 6.2: Techniques used for the quantification of bacteria in the bulk water from the pilot rigs and recovered from the electrodes of the corrosion monitoring device and corrosion coupons.

<table>
<thead>
<tr>
<th>MICROBIAL TYPE</th>
<th>TECHNIQUE</th>
<th>INCUBATION TIME (d)</th>
<th>ATMOSPHERE</th>
<th>GROWTH MEDIUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Aerobic bacteria</td>
<td>Pour plate</td>
<td>2</td>
<td>Aerobic</td>
<td>Half strength Nutrient Agar (Biolab)</td>
</tr>
<tr>
<td>Total anaerobic bacteria</td>
<td>Pour plate</td>
<td>3</td>
<td>Anaerobic</td>
<td>Half strength Nutrient Agar (Biolab)</td>
</tr>
<tr>
<td>H₂S producing bacteria</td>
<td>Agar tubes</td>
<td>5</td>
<td>Anaerobic</td>
<td>Iron Sulphite Agar (Oxoid)</td>
</tr>
</tbody>
</table>

Scanning electron microscopy

Electrodes and corrosion coupons were fixed in 2.5% gluteraldehyde for 4 h at room temperature, dehydrated for 10 min in each of a series of 10, 20, 30, 40, 50, 60, 70, 80, 90, 95 and 100% ethanol, critical point dried, sputter coated with gold using an Edwards 150B sputter coater and viewed using a Phillips 5020 scanning electron microscope.

Statistical analysis

The corrosion rates obtained from rigs to which biocide was added, were statistically compared with those from untreated controls, by means of the Kolmogrov-Smirnov two sample test and the two sample analysis (Steyn et al., 1989).
Figure 6.2: Pilot rigs used for the evaluation of a linear polarisation corrosion monitoring device.
RESULTS AND DISCUSSION

Jar tests to determine microbial attachment and MIC on electrodes of the corrosion monitor and corrosion coupons

The corrosion rates, as determined from the corrosion coupons are shown in Table 6.3 and those determined by the linear polarisation technique are shown in Figure 6.3.

Table 6.3: Corrosion rates measured on corrosion coupons in the jar tests to determine microbial attachment and MIC.

<table>
<thead>
<tr>
<th>JAR</th>
<th>CORROSION RATE (mm.year⁻¹)</th>
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<tr>
<td>1</td>
<td>0.316</td>
</tr>
<tr>
<td>2</td>
<td>0.300</td>
</tr>
<tr>
<td>3</td>
<td>0.305</td>
</tr>
</tbody>
</table>

Figure 6.3: Corrosion rates as measured by the linear polarisation technique in the jar tests to determine microbial attachment and MIC.
Numbers of sessile aerobic and anaerobic bacteria per cm², on the electrodes and corrosion coupons are shown in Figure 6.4.

The electrodes and corrosion coupons were also examined by SEM, but the extent of bacterial attachment could not be estimated due to the abundance of corrosion products, which appeared to screen the attached bacteria.

Figure 6.4: Sessile aerobic and anaerobic bacteria enumerated on three corrosion monitor electrodes and corrosion coupons in jar tests to determine microbial attachment and MIC.
The results of the jar tests indicated that aerobic and anaerobic bacteria did attach to the probes of this particular corrosion monitoring device to form a biofilm. Although there were variations in the numbers of attached bacteria per cm² on the electrodes and the corrosion coupon, all the results corresponded within half a log number. This indicated that the bacteria did not preferentially attach to any particular electrode or to the corrosion coupon. Confirmation of the formation of a biofilm on the electrodes of the corrosion monitor was important, as in the pilot rig tests this could not be confirmed. The ratio of sessile aerobic bacteria to anaerobic bacteria was consistent with the ratios found in cooling water systems (Poulton and Nixon, 1990). Although the corrosion rates in all the jars were not the same, they followed the same general trend (an increase in corrosion rate from approximately 0.25 mm.year⁻¹ to 0.35 mm.year⁻¹). The corrosion rates measured on the corrosion coupons were all approximately 0.3 mm.year⁻¹, which correlated with the corrosion rates as measured by the corrosion monitors. Historically, corrosion coupons have been utilised to monitor both MIC and chemical corrosion (Colturi and Kozelski, 1984; Blackburn and Mullin, 1990). However, the information obtained is retrospective (Tullmin et al., 1992). In this study, approximately the same corrosion rates were obtained by both techniques, the linear polarisation technique, however, has the added advantage that instantaneous corrosion rate results can be obtained.

**Pilot rig tests for evaluating the use of a linear polarisation technique to determine the control of MIC using a biocide**

The corrosion rates as measured by the corrosion monitoring device in four pilot rigs over a three month period with no biocide addition, are shown in Figure 6.5.

Numbers of planktonic aerobic and anaerobic bacteria enumerated in the bulk water are shown in Figure 6.6.
Figure 6.5: Corrosion rates measured by the linear polarisation technique in four pilot rigs during a three month period when no biocide was added.

Figure 6.6: Numbers of aerobic and anaerobic planktonic bacteria in the bulk water of four pilot rigs during a three month period when no biocide was added.
Although exactly the same corrosion rates were not recorded for all pilot rigs, similar trends were observed (Figure 6.5). Fluctuations in corrosion rates occurred in all four rigs. These variations were likely to be due to environmental changes such as temperature fluctuations and not due to unique changes in any individual rig. However, a gradual increase in corrosion rates from approximately 0.3 mm.year\(^{-1}\) to 0.6 mm.year\(^{-1}\), over the three months when no biocide was added to the rigs, was observed. It is possible that this increase was due to the development of biofilms on the electrodes, since it could not be attributed to the chemical composition of the water, which remained constant. The sharp increase and subsequent decrease in the corrosion rate in rig three between 22 d and 26 d cannot be explained, but did not affect the overall increase in corrosion rate (Figure 6.5).

A net increase in the numbers of planktonic aerobic and anaerobic bacteria in the circulating water was observed during the three month period when no biocide was added to the rigs (Figure 6.6). However, no SRB or H\(_2\)S producing bacteria were detected, even after the addition of *D. desulphuricans* to the circulating water. Numbers of planktonic aerobic bacteria in the bulk water increased from approximately 1.0\times10^4 CFU.ml\(^{-1}\) to 1.0\times10^7 CFU.ml\(^{-1}\) (Figure 6.6). The planktonic anaerobic bacteria in the bulk water increased from approximately 1.0\times10^3 CFU.ml\(^{-1}\) to 1.0\times10^5 CFU.ml\(^{-1}\) (Figure 6.6). As Nutrient Broth was periodically added to the rigs to encourage microbiological growth, this gradual increase in bacterial numbers was expected. The ratios of aerobic bacteria to anaerobic bacteria in the bulk water were consistent with the ratios found in cooling water systems (Poulton and Nixon, 1990). However, as the numbers of anaerobic bacteria gradually increased in the oxygenated bulk water, this indicated that they may have been released into the bulk water due to the periodic sloughing of biofilms (Characklis *et al.*, 1990).

Changes in corrosion rates as measured by the corrosion monitoring device after addition of the biocide to two of the rigs are shown in Figure 6.7.
Figure 6.7: Corrosion rates measured by the linear polarisation technique in two pilot rigs treated with biocide and two untreated controls (arrow indicates time of biocide addition).
After addition of the biocide, a statistically significant decrease in the corrosion rate was detected by the corrosion monitoring devices, when compared to the untreated controls (Figure 6.7). The variances in corrosion rates, obtained in those rigs where biocide was added, were found to be significantly different from the corrosion rates in the control rigs at the 95% confidence level (Steyn et al., 1989). The corrosion rate in the rig to which biocide was added in experiment one, dropped from 0.6 mm.year\(^{-1}\) to 0.4 mm.year\(^{-1}\) and in experiment two from 0.8 mm.year\(^{-1}\) to 0.5 mm.year\(^{-1}\) (Figure 6.7). The addition of a biocide did produce a significant reduction in corrosion rate. This indicated that microbiological activity contributed to corrosion rates. No SRB or H\(_2\)S producing bacteria were, however, detected in the weekly bulk water analyses. Although these bacteria may have been present in the biofilm, it is possible that the presence of other bacteria may have had an influence on the corrosion rate. It has been reported in the literature, that the formation of discrete colonies within biofilms can result in the development of concentration cells and localised cathodic and anodic sites on metal surfaces which affect the corrosion rate (Obuekwe et al., 1981; Costerton and Boivin, 1990). In addition, the extracellular polymeric substances produced by biofilm bound bacteria have absorptive and ion exchange properties (Hamilton, 1990). Microenvironments are thus formed within the biofilm, which can further disrupt the metal surface with a subsequent increase in corrosion rate. The presence of a biofilm that does not contain SRB or H\(_2\)S producing bacteria may therefore be able to influence corrosion rates.

Electrodes and corrosion coupons removed from the two rigs to which biocide had been added and from the two untreated controls, were examined by SEM after the 48 h experimental period. The abundance of corrosion products again masked the bacteria. No visible difference could be distinguished between the biocide treated electrodes and coupons and the untreated controls.

CONCLUSIONS

The hypothesis that biocide addition will result in a lowering of the corrosion rate, which is in part indicative of sessile microorganisms, was confirmed. The advantages of utilising an on-line monitor such as the one described in this study, are that it can monitor the corrosive activities of sessile microorganisms, as well as be utilised to determine the efficacy of biocide treatment programmes. This type of monitor is also non-destructive and thus immediate remedial action, such as the addition of a biocide can be taken if any increases in corrosion rate are detected.
A corrosion monitoring device, using the linear polarisation technique can be used to monitor the efficacy of a biocide programme. However, further work on the on-site practical application of this technique needs to be carried out.

ACKNOWLEDGEMENTS

Mr J. Pressly of Anglo American is gratefully acknowledged for providing the corrosion monitoring device and for his technical input as well as Mr G. Lok, Mr A. Kimpton, Mr G. Gericke, Mr K. McNaughton and Mr A. Bokhorst for their technical advice and assistance.