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MONITORING AND CONTROL OF BIOFOULING IN POWER UTILITY OPEN RECIRCULATING COOLING WATER SYSTEMS

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MONITORING AND CONTROL OF BIOFOULING IN POWER UTILITY OPEN RECIRCULATING COOLING WATER SYSTEMS

by

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I certify that the thesis hereby submitted, and the work presented therein, to the University of Pretoria for the degree of M.Sc. has not been previously submitted by myself in respect of a degree at any other University.

Signature .....

Date .....

This thesis is dedicated to my mother, Ruth Poulton

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Department: Microbiology and Plant Pathology
Degree: M.Sc. (Microbiology)

Summary

Surveys of open recirculating cooling water systems at 12 fossil fired power stations and their corresponding raw water supplies were carried out. It was established that all the raw water supplies and recirculating cooling waters contained aerobic and anaerobic bacteria, anaerobic acid producing bacteria, *Thiobacillus*, *Nitrobacter*, sulphate reducing bacteria and algae, with the exception of two potable water supplies. Analysis of the numbers of microorganisms as well as system inspections revealed that each system was unique and no generalisations in terms of presence or activity of microorganisms could be made. Biodispersant/biocide cooling water treatment programmes were monitored at four fossil fired power stations by means of microbiological analysis of Robbins Device biofouling monitors and bulk water. The use of combinations of biodispersants and biocides effectively controlled microbiological growth in all the cooling water systems, but the treatment products produced different effects in different systems. A Robbins Device, modified Robbins Device, a Pedersen Device and the Barry's Device biofouling monitors were evaluated in an open recirculating cooling water system during three different biodispersant dosing regimes. Statistically significant differences were found in the numbers of bacteria recovered from the devices with the exception of the aerobic bacteria when no biodispersant was added and the H_2S producing bacteria when biodispersant was slug dosed. Consistently higher numbers of bacteria were recovered from the modified Robbins Device. A corrosion monitoring device, using the linear polarisation technique was evaluated to determine its suitability as a technique for the monitoring of sessile microorganisms. Bacterial attachment occurred uniformly on the electrodes of the device and on corrosion coupons. The addition of a biocide to the bulk water of two pilot rigs resulted in a statistically significant decrease in corrosion rate in these two rigs when compared to the untreated controls.

MONITERING EN BEHEER VAN BIOBEVUILING IN OOP HERSIRKULERENDE VERKOELINGSWATERSTELSELS VAN KRAGSTASIES

deur

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Opsomming

Opnames van die hersirkulerende verkoelingswaterstelsels en hul ooreenstemmende rouwatertoevoere is by 12 fossielbrandstof kragstasies uitgevoer. Daar is vasgestel dat, met die uitsondering van twee drinkwatertoevoere, al die verkoelingswater en rouwater aërobiese en anaërobiese bakterieë, anaërobiese suur produserende bakterieë, *Thiobacillus, Nitrobacter*, sulfaatreduserende bakterieë en alge bevat het. Ontledings van die hoeveelhede mikroorganismes, asook inspeksies van die verskeie sisteme, het aangedui dat elke sisteem uniek is en dat geen veralgemening gemaak kan word nie. Biodispergeermiddel/biosied behandelings programme is deur middel van mikrobiologiese ontledings van Robbins biobevuilingsmoniteringtoestelle en sirkulerende water by vier fossielbrandstof kragstasies gemonitor. Mikrobiologiese groei in al die verkoelingswaterstelsels is effektief deur die gebruik van kombinasies van biodispergeermiddels en biosiedes beheer. Die behandeling het egter verskillende uitwerkings op die verskeie stelsels gehad. 'n Robbins, gewysigde Robbins, Pedersen en Barry biobe- vuilingsmoniteringstoestelle is tydens drie verskillende doseringsregimes van biodispergeermiddels in 'n oop hersirkulerende verkoelingswaterstelsel geëvalueer. Statisties beduidende verskille is in die hoeveelheid bakterieë wat van die toestelle herwin is, gevind. Die aantal aërobiese bakterieë in die afwesigheid van 'n dispergeermiddel en H_2S produserende bakterieë tydens eenmalige dosering van die dispergeermiddel was egter uitsonderings. Die hoeveelheid bakterieë wat vanaf die gewysigde Robbin toestel herwin is, was deurlopend meer vergeleke met die ander toestelle. 'n Korrosiemoniteringstoestel wat van 'n lineêr polarisasie tegniek gebruik maak, is geëvalueer om die instrument se toepaslikheid vir die bepaling van sessiel mikro-organismes te bepaal. Bakteriologiese aanhegting het egalig op die elektrodes van die toestel en op die korrosie toetsstukke plaasgevind. Die toediening van 'n biosied tot die water van twee loodsaanlegte het tot 'n beduidende afname in die korrosietempo van hierdie aanlegte vergeleke met 'n onbehandelde aanleg gehad.

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Chapter 1

INTRODUCTION

There is an increasing demand for water in South Africa for both domestic and commercial uses, due to the escalation in the population and increased industrialisation (van Leeuwen *et al.*, 1990). South Africa's average rainfall is approximately 497mm per year, in comparison to the world average of 860mm. In addition, severe droughts occur periodically, resulting in further demands on already limited water resources (Anon., 1986). Consequently, industrial installations in South Africa are forced to recycle and reutilise water, particularly during drought periods (Baecker *et al.*, 1988; Bondonno *et al.*, 1989). Thus, the quality of not only cooling water, but also of natural water bodies is rapidly deteriorating due to salination, eutrophication and pollution (Anon., 1986; Nell and Aspden, 1990). This decrease in water quality has increased problems associated with uncontrolled microbiological growth in open recirculating cooling water systems (Honneysett *et al.*, 1985; Poulton and Nixon, 1990).

The types of microbiologically related problems commonly found in open recirculating cooling water systems are generally attributable to sessile microorganisms and include microbiologically influenced corrosion (MIC) and biofouling (Colturi and Kozelski, 1984; Soracco *et al.*, 1988; Cloete *et al.*, 1992). MIC has resulted in severe metal loss in many power utility cooling water systems (Breske, 1990) while biofouling and biofilm formation cause decreases in heat transfer and flow rates (Blenkinsopp and Costerton, 1991).

Many of Eskom's power stations are operated on the principle of zero effluent discharge, thus reducing specific water consumption from 3.2 $l.KWh^{-1}$ to approximately 2.2 $l.KWh^{-1}$ (Nell and Aspden, 1990). In order to be able to achieve this reduction, cooling water systems are operated at high cycles of concentration and water is reused where possible. Due to the resulting poor water quality, the majority of Eskom's power stations experience microbiological problems and associated financial losses (Poulton and Nixon, 1990). It has been estimated that the direct cost of MIC in South Africa is approximately R400 million per annum (von Holy and Cloete, 1988).

To date, the control of sessile microorganisms in cooling water systems has been by means of both oxidising (Connell and Jones, 1991) and non-oxidising biocides (McCoy, 1980). However, it has been reported that bacteria may become resistant to the action of biocides (Russell, 1990), thus prompting investigation into alternate means of controlling microbiological growth. Biodispersants are widely used in industry to disperse sessile bacteria. There is, however, little

published information on their mechanisms of action or case studies on their efficacy in cooling water systems.

The monitoring of biofouling and MIC in South Africa has generally been by means of quantification of the planktonic microorganisms (Cloete *et al.*, 1989) and on occasion by means of the Robbins Device (von Holy and Cloete, 1988). However, the accurate determination of microbiological conditions in cooling water systems remains problematic. To date, no in-depth scientific study has been carried out to determine the most effective techniques for the monitoring of sessile microorganisms in cooling water systems. A need therefore exists for a study of this nature.

The aims of the work outlined in this thesis were therefore as follows:

- to establish the sources and extent of microbiologically related problems in Eskom's open recirculating cooling water systems.

- to investigate the use of biocides and biodispersants as a means of controlling microbiological growth in Eskom's open recirculating cooling water systems.

- to evaluate monitoring techniques for the determination of biofouling and biocorrosion occurring in cooling water systems, as well as for the evaluation of the efficacy of microbiological treatment programmes.

Chapter 2

Literature Review

1. Cooling Water Systems

1.1 Cooling water system design

There are two major types of cooling water systems, namely open and closed cooling water systems. Heat loss in closed cooling systems generally takes place by means of air, or a secondary or auxiliary water cooling system. Thus water loss from the primary closed cooling system is minimal and the cooling water is generally of a high quality. Open cooling systems can be once through systems, i.e. the water is not recirculated, or recirculating cooling systems which operate by means of evaporative cooling via cooling towers. These cooling towers operate as water to air heat exchangers and waste heat is discharged into the atmosphere (Liptak, 1987). Evaporative or open cooling water systems are capable of handling high heat loads with a minimum of water loss (Anon., 1977). The heated water to be cooled is piped to a distribution system and sprayed over a fill or packing. The fill can be composed of splash bars, vertical sheets or honeycomb assemblies that are placed in the cooling tower, to effect heat and mass transfer between the water and the air. The fill aids in increasing the surface area of water in contact with air, either by creating finer droplets, or by causing the water to form a thin film.

Depending on the cooling tower design, the direction of air flow over the water is either counterflow or crossflow relative to the flow of the water (Anon., 1977). The two commonly utilised designs of open cooling towers are natural draft cooling towers and mechanical draft cooling towers (Anon., 1980). Mechanical draft towers operate by means of fans which draw air over falling water droplets. The flow of the air may be counterflow or crossflow to the flow of the water, depending on the design of the cooling tower (Figure 2.1). The temperature change in the water passing through a mechanical draft cooling tower can be controlled by the speed of the fans (Liptak, 1987). Natural draft towers are hyperbolic in shape and a natural upward air flow is created (Figure 2.2). A natural upward movement of air, counterflow to the falling water, is created by the shape of the tower. Variations in the quantity of air that passes through a cooling tower may be caused by the thermal loading conditions, external wind velocity and the temperature and humidity of the outside air (McKelvey and Brooke, 1959).

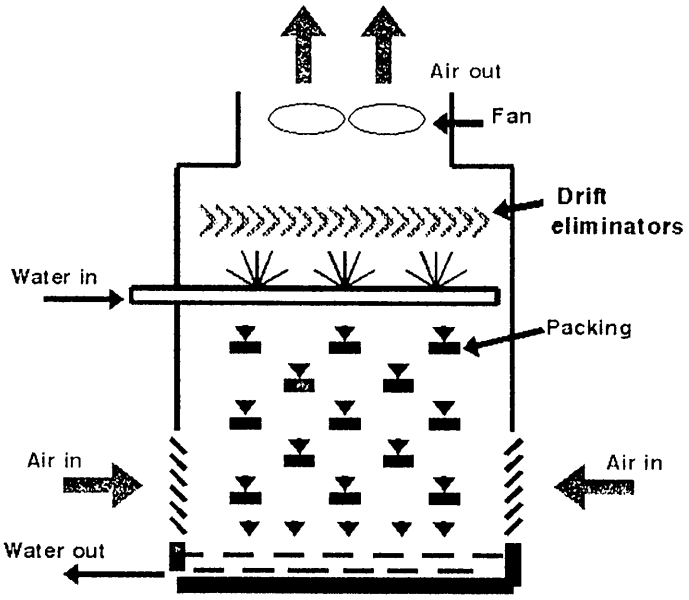


Figure 2.1 : Diagram of a mechanical draft cooling tower.

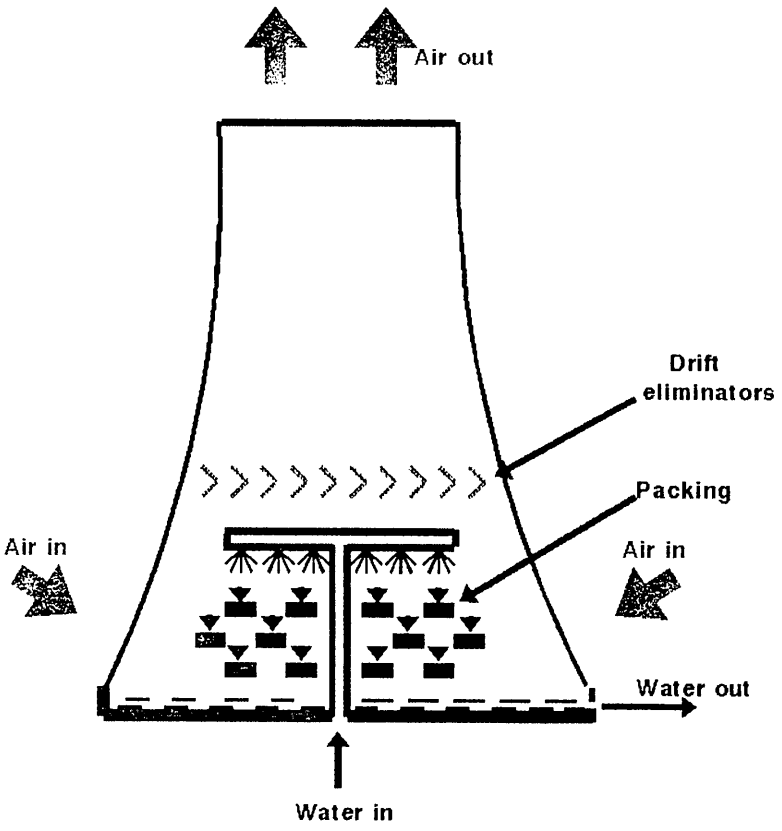


Figure 2.2 : Diagram of a hyperbolic cooling tower.

At power generating plants, open cooling water systems are utilised to cool water that has been heated by passage through a condenser and to cool closed circuits by means of heat exchangers (Schwieger, 1970; Elliott, 1973).

1.2 Cooling water system operation

Due to water shortages in South Africa, cooling water systems are operated at high cycles of concentration in order to reduce the volumes of water used for evaporative cooling. Furthermore, Eskom has implemented various techniques whereby water is reused and many power stations worldwide operate on the principal of "Zero Liquid Discharge" (Freedman, 1984). One of the methods of water conservation is side stream cooling water treatment plants to remove excess salts and reduce the volume of effluent. These side stream plants include softening processes, tubular reverse osmosis and electrodialysis reversal techniques (Murphy and Nel, 1988; Nell and Aspden, 1990). Despite these treatment processes, the majority of the cooling waters in South Africa are of a poor quality due to operation at high cycles of concentration, and are thus able to support the growth of large numbers of microorganisms (Poulton and Nixon, 1990).

Evaporation of water in an open cooling water system results in an increase in the concentration of dissolved and suspended solids in the remaining water. In addition, because air is drawn over the water, airborne debris is entrapped and a further increase in the amounts of dissolved and suspended solids in the recirculating water occurs (Strauss and Puckorius, 1984). This concentration effect is measured in terms of cycles of concentration. The number of cycles of concentration is therefore the ratio of the total dissolved solids (TDS) in the recirculating water and the TDS in the incoming raw water. Alternatively the concentration of a soluble ion such as chloride can be measured (Anon., 1977). Thus cycles of concentration can be calculated as follows:

$$\text{Cycles of concentration} = \frac{\text{Cooling Water Chloride Ions } (mg.l^{-1})}{\text{Raw Water Chloride Ions } (mg.l^{-1})}$$

Operation of a cooling water system at high cycles of concentration can be problematic. In order to prevent the deposition of salts of low solubility (calcium and magnesium), it is necessary to blow down or remove circulating water from the system and replace it with raw or make up water. The raw water is also used to replace water lost by evaporation (Anon., 1988). Thus, the chemistry of the cooling water is controlled to ensure that the water does not have scaling or corrosive tendencies. These tendencies are generally measured by means of

the Langelier and Ryznar Indices. These indices are determined by calculations that take into consideration the temperature, pH, total alkalinity, calcium hardness and total dissolved solid values in the water (McCoy, 1969). Table 2.1 shows the chemical analysis of a typical Eskom water and specifications for various chemical parameters (Anon., 1989).

Table 2.1 : Chemical specificiations and a typical composition of an Eskom cooling water.

	SPECIFICATION	TYPICAL ESKOM WATER
pH at 25°C	8.0 - 8.5	8.5
Conductivity at 25°C ($\mu S\ cm^{-1}$)	<4000	2700
Total alkalinity ($mgl^{-1}\ CaCO_3$)	100 - 130	113
Chloride (mgl^{-1})	not specified	246
Sulphate (mgl^{-1})	variable	905
Total hardness ($mgl^{-1}\ CaCO_3$)	200 - 600	318

2. Microbial ecology of cooling water systems

2.1 Effect of cooling water system operation on microorganisms

The incidence of microbiologically related problems in cooling water systems has increased over the last few years (Pope, 1987). This is considered to be due not only to improved detection techniques (Pope, 1986), but also to the change from low (acidic) to high (alkaline) pH cooling waters. This change results in environments that are more conducive to the growth of microorganisms than the previously used acidic cooling waters (Freedman, 1984). In addition, environmental restrictions on the use of chromate based corrosion inhibitors has limited their use. As these corrosion inhibitors are toxic to many microorganisms, the reduction in their use may also have had an effect on the growth rate and variety of microorganisms present in cooling water (Lutey, 1980).

An open recirculating cooling water system is a favourable environment for the proliferation of certain types of microorganisms (Thierry, 1987). The growth of these microorganisms is encouraged due to the elevated temperatures (ambient to 40°C), an ideal pH (8 - 8.5), the

presence of inorganic and organic nutrients, oxygenated water and in certain areas, sunlight (Zivtins and Casedy, 1980; Strauss and Puckorius, 1984). The major groups of microorganisms found in open recirculating cooling water systems are bacteria, fungi and algae (McCoy, 1980). However, the prevalence of fungi in cooling water systems may diminish in the future, due to the replacement of wooden structures in cooling towers with alternative materials and the increase in the pH of cooling waters (Anon., 1980; Lutey, 1980).

2.2 Planktonic microorganisms

Microorganisms are introduced into a cooling water system by the incoming make up water. Another major source of contamination is the air, as cooling towers can act as scrubbers, by removing airborne microorganisms and introducing them into the circulating water (Strauss and Puckorius, 1984). It has been suggested that the mean wind velocity also plays a role in the degree of microbiological contamination (Bott *et al.*, 1983). Furthermore, planktonic microorganisms in the circulating water may also be released from biofilms in the system, as a result of natural sloughing process or due to shear stresses within the system (Characklis and Marshall, 1990). The microbiological composition of recirculating cooling water will vary depending on the environmental and system conditions (McCoy, 1980). A population structure study of planktonic bacteria in South African cooling water systems showed that the most frequently encountered bacterium was *Pseudomonas (P.) fluorescens* followed by *Chromobacterium violaceum*, *P. picketii*, *P. stutzeri* and *P. putida* (Cloete *et al.*, 1989). Recently, the emphasis has however shifted, from planktonic bacteria, to the role of sessile bacteria in biofilms (Cloete *et al.*, 1992).

2.3 Biofilms

Characklis and Marshall (1990) defined a biofilm as follows:

" A biofilm consists of cells immobilised at a substratum and frequently embedded in an organic polymer matrix of microbial origin."

"Biofilms are distinguished from suspended growth microbial systems by the critical role of transfer and transport processes which generally are rate controlling in biofilm systems."

Bacteria prefer to live in the sessile or attached phase, as they are retained in an ecosystem that is nutritionally favourable and which allows them to trap and use soluble nutrients (Costerton *et al.*, 1985). Biofilms in industrial water systems are composed of consortia of bacteria that

function as a unit (Costerton and Boivin, 1990). Due to the cycling of nutrients within this unit, increased species diversity occurs and the biofilm is more stable and thus resistant to stress (Atlas and Bartha, 1987). In addition, this mode of growth protects microorganisms against potentially harmful substances such as biocides (Ruseska *et al.*, 1982).

2.3.1 Microbial attachment or adhesion in aqueous environments

The mechanism of bacterial attachment varies depending on the surface properties of the bacteria and the surface or substratum to which they are attached (Marshall, 1985). Characklis and Marshall (1990) defined attachment as the capture or entrapment of cells in a biofilm.

There are various schools of thought regarding the attachment of bacteria to surfaces. The interfacial forces approach (Figure 2.3) proposes that bacterial attachment occurs in two phases namely, reversible adhesion and irreversible adhesion (Marshall, 1985). Reversible adhesion is an instantaneous attraction by long range forces and bacteria can still be easily removed from the surface (Marshall *et al.*, 1971). This type of adhesion was first described by Zobell (1943). Irreversible adhesion is a time dependant, firm adhesion. The bacteria no longer exhibit Brownian motion and cannot be removed by washing (Marshall *et al.*, 1971). This type of adhesion occurs when bacteria produce extracellular polymers (Costerton *et al.*, 1978) or pili and fimbriae (Isaacson, 1985). Neu and Marshall (1991) reported on an adhesive polymer which may be synthesised during the early stages of attachment, resulting in irreversible attachment. If bacteria are removed shortly after attachment, microbial "footprints" can be distinguished (Paul and Jeffrey, 1985).

The surface free energy approach postulates that attachment is due to the interaction of the surface free energy of the bacterium and the substratum, and the surface tension of the liquid (Characklis and Marshall, 1990). Baier (1980) predicted that bacterial adhesion should occur within the "minimally bioadhesive" range, in other words, within a critical surface tension range.

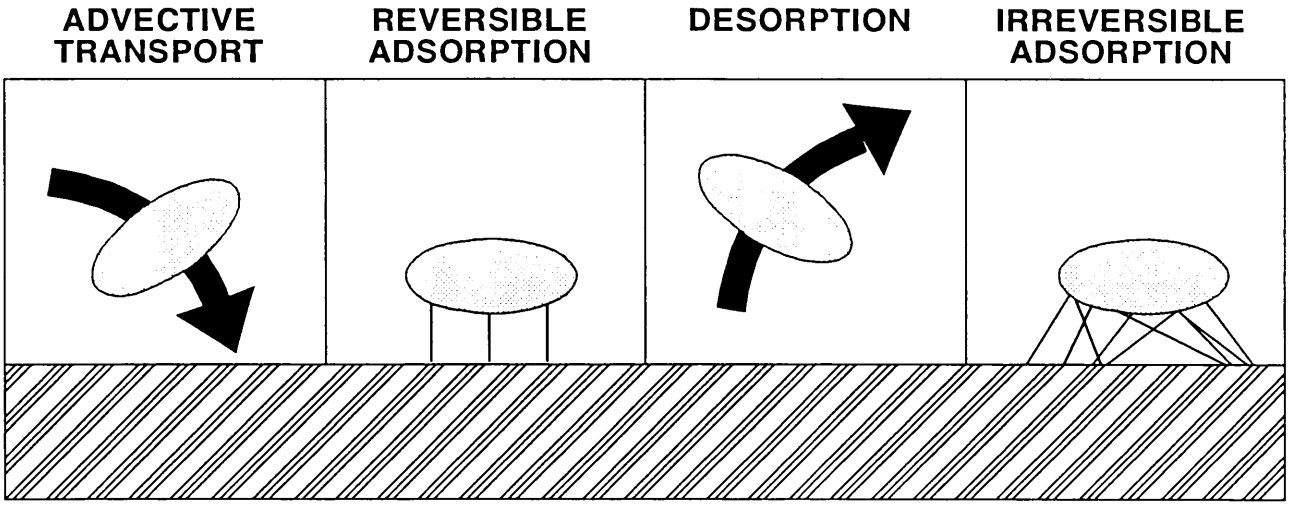


Figure 2.3 : The interfacial forces theory of bacterial attachment (After Characklis and Marshall, 1990).

2.3.2 Factors affecting microbial adhesion

If the theory of surface free energy is correct, then the electrical properties or zeta potential of a surface will affect bacterial adhesion (Rutter and Vincent, 1980). The double layer or DLVO theory of colloid stability allows the calculation of the interaction, at a given distance, between the bacterium and a surface. The interaction depends on the particle radii, surface potentials, the composition of the particles and electrolyte concentration (Loeb, 1985). Bradley and Pritchard (1990) concluded that the formation of sulphate reducing bacteria biofilms was partly as a result of surface charges, as well as sulphide production and iron availability during growth. Soluble molecules may adsorb onto substrata and affect its charge (Fletcher and Marshall, 1982). It has been shown, that adhesion may be enhanced, inhibited or unaffected by the presence of adsorbed molecules or conditioning film on a surface (Marshall, 1985).

The chemical composition of a surface to be colonised may have an effect on microbial attachment. Eaton *et al.* (1980) reported that bacteria attached to copper, produced large amounts of polysaccharides to shield them from the toxic effect of the soluble copper ions. Marsalek *et al.* (1979) observed that brass and copper fouled at a slower rate than glass and stainless steel and that the biofilm was microbiologically less diverse on the metal surfaces.

The roughness of the substratum also plays a role in microbial attachment, but this effect appears to have the greatest impact during initial bacterial attachment (Costerton *et al.*, 1987). However, the surface roughness does not appear to have a substantial effect on the total biofilm thickness (Characklis and Marshall, 1990).

Duddridge and Pritchard (1983) outlined the effects of environmental conditions in the bulk fluid that can also affect adhesion namely, (1) pH, due to changes in electrostatic and ionic charges and changes in exopolysaccharide formation, (2) inorganic ions, as adhesion is influenced by the presence of metal cations, (3) oxygen concentration in the bulk water and (4) temperature, as increases in temperature result in increases in chemical reaction rates and mass transport.

Fluid velocity and turbulence are also important considerations. Shariff and Hassan (1985) reported that biofilm thickness increased with increasing turbulence. An increase in biofouling rate with an increase in water velocity has also been observed by Pedersen (1982) and Wolfaardt and Cloete (1992). However, at high fluid velocities, the shear stress will increase and have a negative effect on the extent of biofilm accumulation (Characklis and Marshall, 1990). Santos *et al.* (1991) reported that *Pseudomonas fluorescens* biofilms were less compact and thicker at flow rates of 0.5 $m.s^{-1}$ than those formed at a water velocity of 2.5 $m.s^{-1}$. In addition, the cells aligned themselves in the direction of flow at the higher flow rate.

Smith and Oliver (1991) presented evidence that under conditions of elevated hydrostatic pressure, bacterial adhesion was affected. At pressures above 405 MBar the initial attachment of *Pseudomonas perfectomarina* was reduced and at 600 MBar, almost completely inhibited. Thus the fouling of surfaces in deep sea environments may be limited.

2.3.3 Structure and functioning of biofilms

Once bacteria are attached to a surface, a biofilm develops that is complex in composition, with many different populations interacting, not only with each other, but also with their environment (Shapiro, 1991). The microbial community will vary with time, as conditions within the biofilm environment change, resulting in changes in the dominant species (McCoy, 1980). Oxygen and chemical gradients will develop within the biofilm. Ledandowski *et al.* (1991) measured changes in oxygen concentration of 8 $mg.l^{-1}$ in the bulk water to 1.2 $mg.l^{-1}$ at the biofilm-water interface to zero at the base of the biofilm. These gradients have an important influence on the species of microorganisms present within the biofilm (Characklis and Marshall, 1990). For example, an anaerobic area at the base of the biofilm provides an environment for the growth of sulphate reducing bacteria, while other bacteria will grow where the conditions best suit their requirements (Costerton *et al.*, 1988). For example, aerobic bacteria will be situated adjacent to the aerated bulk water and are able to utilise organic products, formed by the underlying anaerobic bacteria, as nutrients (Characklis and Marshall, 1990).

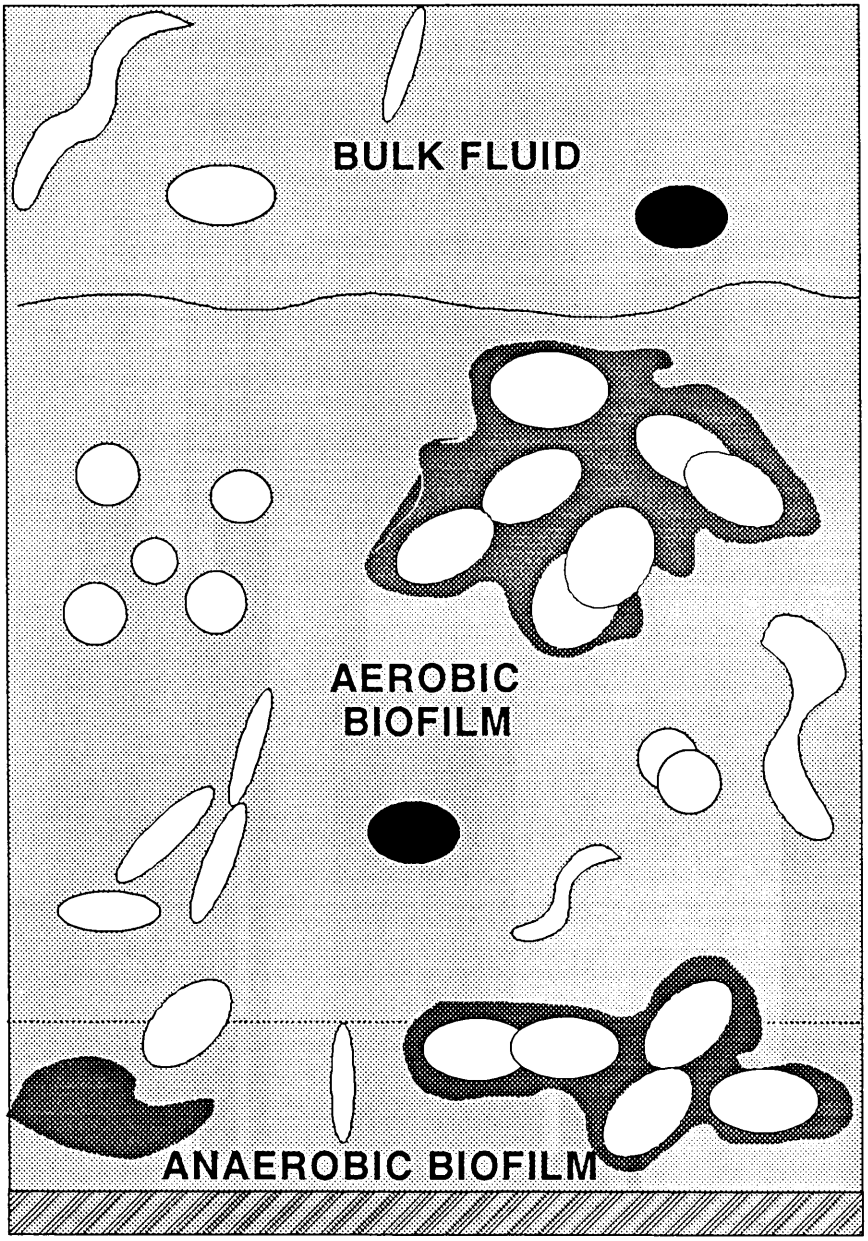


Figure 2.4: Development of an anaerobic region within a biofilm (After Costerton *et al.*, 1988).

As biofilm microbes grow in specialised environments, they differ from their planktonic counterparts both structurally and functionally and thus differ in their response to biocides (Costerton *et al.*, 1987). Bacteria will be released into the bulk water from the biofilm, due to cyclic thickening and sloughing of the biofilm (Characklis and Marshall, 1990).

3. Deleterious effects of microorganisms in cooling water systems

3.1 Effects of biofilms and biofouling deposits

The formation of a biofilm or biofouling deposit can result in a number of deleterious effects in a cooling water system. It is estimated that power industry losses due to condenser fouling in the United States of America, are between one and two billion US dollars per year and that three percent of the total unavailability of power plants was due to condenser fouling (Elmer and Besold, 1988).

Biofilm accumulation or biofouling in a water system can result in energy losses due to increased fluid frictional resistance, decreases in heat transfer in heat exchange equipment and increased microbiologically influenced corrosion, due to the activity of a variety of microorganisms (Characklis, 1973; Strauss and Puckorius, 1984). The increase in fluid frictional resistance is thought to be due to a number of factors, including an increase in surface roughness and a decrease in the internal diameter of a pipe (Characklis and Marshall, 1990). Siefert and Krueger (1950) reported a reduction of 55% in the flow capacity of a 600mm diameter water supply pipeline, due to the formation of a slimy layer that was 650µm thick.

Characklis *et al.* (1982) demonstrated that conductive heat transfer resistance (transport of heat from a high temperature to a low temperature within a phase such as a fluid), generally increased as a biofilm thickened, due to the insulating layer formed by the biofilm. Advective heat transfer resistance (transport of heat as a result of bulk fluid motion) depends on the roughness of the biofilm. The overall heat transfer resistance increases with an increase in biofilm thickness. However, the advective heat transfer resistance decreases due to an increase in biofilm roughness (Figure 2.5). Ferguson (1981), reported seasonal variations in slime or biofilm thickness and thus heat transfer resistance in power utility condensers.

Uncontrolled biofilms and biofouling can also result in high concentrations of microorganisms, such as *Legionella pneumophila*, that may pose health risks (Characklis and Marshall, 1990).

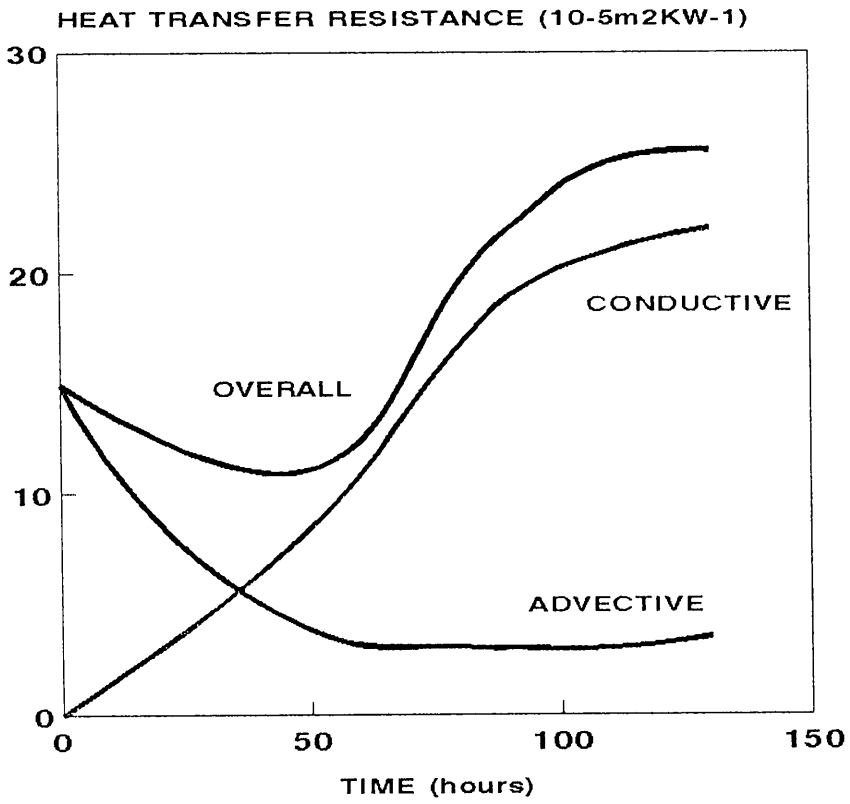


Figure 2.5 : Heat transfer changes due to biofilm formation (After Characklis and Marshall, 1990).

Inorganic materials such as scale or corrosion products trapped in a biofilm, may also influence the rate and extent of heat transfer resistance (Table 2.2). Entrapped scale generally affects the conductive heat transfer resistance as scale has a low relative roughness. However, the thermal conductivity of scale is higher than that of a biofilm, but varies depending on the type and composition of the scale (Characklis and Marshall, 1990).

Table 2.2 : The thermal conductivities of various scales and biofilm (After Characklis and Marshall, 1990).

DEPOSIT	THERMAL CONDUCTIVITY ($Wm^{-1}K^{-1}$)
$CaCO_3$	2.26 - 2.93
$CaSO_4$	2.31
$Ca_3(PO_4)_2$	2.60
$Mg_3(PO_4)_2$	2.16
Fe_2O_3 (magnetic)	2.88
Biofilm	0.63

Algal deposits on cooling tower structures are able to decrease heat transfer by preventing the formation of droplets or films of water, and thus restricting evaporative heat loss (McCoy, 1980). Algae can have an influence on the pH and hardness of water due to their production of carbon dioxide and thus carbonic acid during the night, resulting in a decrease in pH. Algae are also able to increase the quantity of organic matter in water (Palmer, 1962). Large quantities of organic matter may also have an influence on biocide efficacy, particularly chlorine, as the chlorine demand of the water will increase with an increase in organic material (Anon., 1988). In addition, the rate of growth of bacteria will be affected, as organic material may be used as a nutrient source by bacteria (McCoy, 1980).

3.2 Microbiologically influenced corrosion (MIC)

MIC occurs when microorganisms in aqueous solutions significantly increase the corrosion rate of metal and alloys (Buchanan and Stansbury, 1990). MIC can be caused by bacteria, fungi and algae (McCoy, 1980; Iverson, 1987) and has been referred to as the venereal disease of industry (White *et al.*, 1990). The mechanisms by which MIC occurs are varied and include the formation of charged areas or concentration cells on the metal surface, acid and aggressive metabolite production, enzymatic activity, reduction of ferric ion to its more soluble ferrous state and the removal of protective films (Gaylarde, 1990). Since MIC has an economic impact on industry as a whole and affects the performance of safety-related systems, increasing interest is being shown in the mechanisms and microbes involved (Kasahara and Kajiyama, 1990; Witt, 1990).

3.2.1 Sulphate reducing bacteria (SRB)

The sulphate reducing bacteria occur naturally in a wide variety of anaerobic environments and are responsible for the formation of most of the natural sulphur deposits (Postgate, 1988). They demonstrate a remarkable ability to adapt to new environments in terms of temperature, salinity and pressure (Postgate, 1981). They are widely recognised as being economically important due to the fact that they are the major contributors to MIC (von Holy, 1988; Hamilton, 1990). These bacteria have had deleterious effects on the pulp and paper industry (Bennet, 1988), the power industry (Fellers, 1990), the oil and gas industry (Postgate, 1988; Mosley and Holt, 1990) as well as many other industrial or domestic installations.

3.2.1.1 Theories of the mechanism of SRB influenced corrosion

The first mechanism of SRB influenced corrosion was proposed by von Wolzogen Kuhr and van der Vlugt (1934) and was known as the cathode depolarisation theory. This theory postulated that the cathode is depolarised by the action of bacterial hydrogenase enzymes, which remove hydrogen atoms from the cathode, thus accelerating the corrosion reaction (Figure 2.6). In support of this theory, Booth and Tiller (1960) showed that there was a correlation between cathode depolarisation and hydrogenase activity. However, subsequent work revealed that the corrosion rate was independent of enzyme activity as hydrogenase negative organisms were also found to accelerate corrosion rates (Booth *et al.*, 1967).

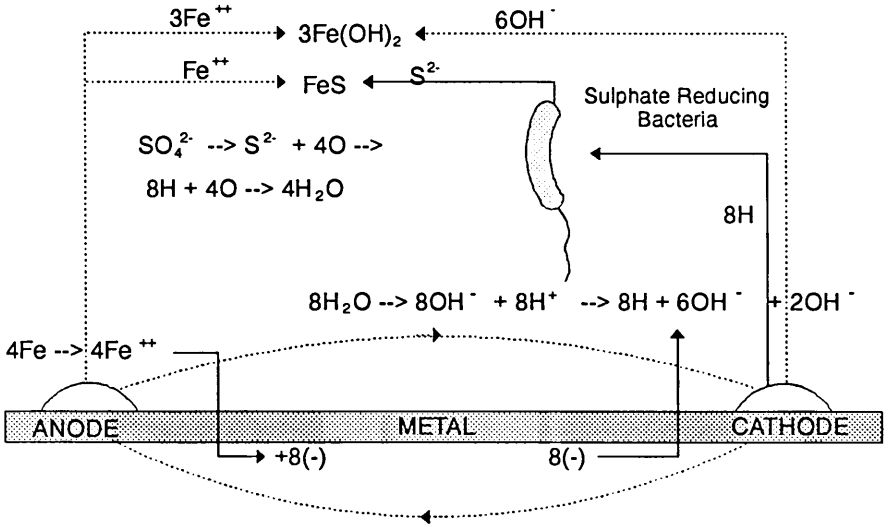


Figure 2.6 : SRB influenced corrosion according to the cathode depolarisation theory (After Lutey, 1980).

Mara and Williams (1972) suggested that the iron sulphide, produced by SRB, absorbed hydrogen atoms, as the hydrogen bonds in between the iron sulphide crystals. This removal of hydrogen would result in depolarisation of the cathode, but would not account for the rate by which the corrosion reaction was accelerated.

King and Miller (1971) proposed that iron sulphide did absorb hydrogen atoms from the cathode, but that it was the action of the bacterial hydrogenase enzymes that removed these atoms from the iron sulphide. Thus, fresh iron sulphide would continuously be brought into contact with the metal surface, and remove more hydrogen atoms. This theory is supported by the fact that when a film of iron sulphide was already present on the metal surface, the corrosion rate for hydrogenase positive organisms was greater than for hydrogenase negative organisms.

Costello (1974) suggested that it is the hydrogen sulphide, produced by the bacteria, that attacks and corrodes the metal surface, by depolarising the cathode by the following reaction:

$$H_2S + e^- \rightarrow HS^- + \tfrac{1}{2} H_2$$

Yet another theory was proposed by Iverson (1984) and is known as the corrosive metabolite theory. Iverson showed that the corrosion by-products associated with SRB growth contained iron phosphide. This by-product could be formed by a volatile phosphorus containing metabolite produced by the bacteria. This metabolite has to be in contact with the bare metal in order to be corrosive. Therefore, if a protective film of iron sulphide is already present on the metal surface, the corrosion process is inhibited until such time as the film is broken down. Thus corrosion may occur immediately, or may be delayed, depending on whether hydrogen sulphide, or the phosphorus containing metabolite reaches the metal surface first.

None of these theories have been proven beyond doubt, and controversy still remains over which theory is correct.

3.2.2 Acid producing bacteria

Many different bacterial species, both aerobic and anaerobic, are able to influence corrosion rates by the production of acids (Tiller, 1983a; Ringas and Robinson, 1987). It has been suggested that acid producing bacteria play a significant, if not primary role in MIC (Soracco *et al.*, 1988).

The sulphur-oxidising bacteria, for example the genus *Thiobacillus*, have been studied extensively regarding their role in MIC, due to their ability to produce sulphuric acid (Cragnolino, 1983). As a result of this acid production, localised, low pH environments are created, which are aggressive to metal or concrete surfaces (Bos and Kuenen, 1983). The reactions catalysed by sulphur oxidising bacteria, are illustrated in Table 2.3.

Table 2.3 : Reactions catalised by the sulphur oxidising bacteria

SULPHUR OXIDISING BACTERIA	REACTION
Aerobic sulphur oxidisers	$H_2S + 2O_2 \rightarrow H_2SO_4$ $2S^o + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$
Nitrate reducing sulphur oxidisers	$5H_2S + 8NO_3^- \rightarrow 4N_2 + 5SO_4^{2-} + 4H_2O + 2H_2$
Photosynthetic sulphur oxidisers	$CO_2 + 2H_2S \rightarrow (CH_2O) + 2S + H_2O$ (cell material) $2CO_2 + H_2S + 2H_2O \rightarrow 2(CH_2O) + H_2SO_4$ (cell material)

Microorganisms that are able to produce organic acids such as acetic, formic and lactic acid, may also play a major role in MIC. Pope *et al.* (1988) demonstrated that *Clostridium* species were able to produce large quantities of organic acids, for example acetic acid, that had damaging effects on carbon steels.

3.2.3 Iron Bacteria

Members of several genera, including *Gallionella*, *Crenothrix* and *Leptothrix* form part of a group of bacteria generally known as the iron bacteria (McCoy, 1980). These iron bacteria aerobically oxidise dissolved ferrous ions to insoluble ferric salts, which may be deposited in a covering sheath, or produce a stalk-like filamentous form (Tatnall, 1981). It has been suggested that these bacteria alter the environment on metal pipe surfaces, encouraging the growth of chemoorganotrophic bacteria. Thus, anaerobic bacteria capable of producing organic acids and hydrogen sulphide, harboured under these deposits, may have further deleterious effects on metal surfaces (Ridgeway *et al.*, 1981). In addition, *Gallionella* can concentrate chlorides, which may result in general corrosion, sub-surface cavitation and stress corrosion cracking, depending on the environmental parameters (Tatnall, 1981). The growth of *Gallionella* has been reported in drinking water systems (Ridgeway and Olson, 1981) and also in cooling water systems (Honneysett *et al.*, 1985).

Obuekwe *et al.* (1981) reported on an Fe(III) reducing *Pseudomonas* species that may prevent the formation of a protective surface coating of insoluble Fe(III). Thus indirectly this bacterium and other iron reducing bacteria may accelerate corrosion by exposure of metal surfaces to the environment.

3.2.4 Algae

Algae are able to cause corrosion of metal components by their ability to produce oxygen. Oxygen concentration cells are thus established and corrosion is initiated (Palmer, 1962). Algae have also been implicated in the degradation or attack of concrete (McCoy, 1980).

3.2.5 The role of biofilms and extracellular polysaccharides in MIC

It has been extensively reported in the literature that the formation of discrete microbial colonies within biofilms can result in the development of concentration cells and localised cathodic and anodic sites on metal surfaces (Obuekwe *et al.*, 1981; Costerton and Boivin, 1990). In addition, the extracellular polymeric substances (EPS) produced by the bacteria have absorptive and ion exchange properties (Hamilton, 1990). Thus, microenvironments are formed within the biofilm, that can further disrupt the metal surface by the formation of local cathodic and anodic sites (Figure 2.7). Characklis and Marshall (1990) outlined the effects of EPS on interfacial processes as: (1) immobilisation of water at the biofilm-substratum interface, (2) entrapment of metal species and corrosion products at the substratum, (3) decrease of the diffusion rates toward and away from the substratum and (4) immobilisation of corrosion inhibitors and biocides. In addition, corrosion rates will be affected due to periodic sloughing of the biofilm. Thus, the substratum will be either exposed or protected from the bulk water resulting in the formation of local cathodic and anodic sites.

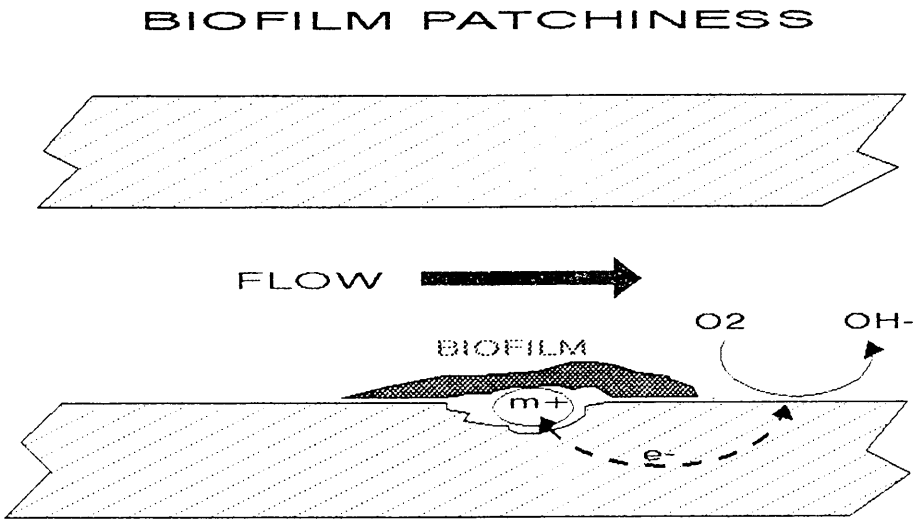


Figure 2.7 : Disruption of a metal surface by a biofilm (After Characklis and Marshall, 1990).

Literature reports on the role of EPS in MIC are conflicting. Beech *et al.* (1990), reported that free extracellular polymeric substances of *Desulfovibrio desulfuricans* are unlikely to have any influence on corrosion rates, but that the biofilm exopolysaccharides may play a role. Hernandez-Duque *et al.* (1990) reported that the attachment of a *Pseudomonas* species and *Serratia marcescens* had a protective effect on mild steel. In direct contrast, Nivens *et al.* (1986) observed an increase in corrosion current density with the production of EPS by a marine *Vibrio natriegens* on stainless steel and Geesey *et al.* (1986) demonstrated that the EPS of an unknown bacterium caused an increase in the deterioration of copper. It would appear that the effects of biofilms and EPS on corrosion rate varies from negligible effects to substantial increases in corrosion rates, depending on the species of microorganisms present and system conditions.

3.2.6 Metals susceptible to MIC

The only materials utilised in industry that appear to be resistant to MIC are the higher nickel-chromium alloys and titanium (Pope *et al.*, 1984). There are case histories detailing the attack of mild steel (Bibb and Hartman, 1984), various grades of stainless steels (Tiller, 1983b; Sinha *et al.*, 1990), copper (Walker *et al.*, 1990), aluminium (Bondonno and Robinson, 1990), nickel alloys (Brennenstuhl *et al.*, 1990), concrete (Kulpa and Baker, 1990) and even metal arsenides (Blake and Bowers-Irons, 1990). Bacteria are known to preferentially attack welds and the area adjacent to the weld (Borenstein, 1988). Buchanan *et al.* (1990) suggested that it is the surface condition of the weld that determines its susceptibility. As the use of materials that are resistant to MIC is cost prohibitive, generally, non-corrosion resistant materials are used in industrial systems. It is therefore essential to take precautionary measures by monitoring and preventing microbiological growth (Pope *et al.*, 1984).

4. Monitoring of microbial populations in cooling water systems

4.1 Monitoring of planktonic microorganisms in cooling water systems

Historically, biofouling has been monitored by the quantification of planktonic microorganisms (Wolfaardt *et al.*, 1991). However, sessile microorganisms are predominant in aqueous environments and more than 10 000 sessile bacteria for each planktonic cell have been reported (Geesey *et al.*, 1978). Traditionally, planktonic bacterial populations have also been quantified for determination of the efficacy of biocide and biodispersant treatment programmes (Cloete *et al.*, 1989; Lutey and Allison, 1991). It is however, generally accepted, that the quantification of planktonic microorganisms is not indicative of the numbers or activity of their sessile counterparts (Costerton and Lashen, 1983).

4.2 Monitoring of sessile microorganisms in cooling water systems

The accurate enumeration of active sessile microorganisms in water systems is problematic (White, 1983). Numerous techniques have, however, been developed, which all have one major disadvantage, namely, that it is impossible to simulate the wide variety of environmental conditions that are found in an operating plant. The majority of these devices therefore give only an indication of microbial activity or numbers.

4.2.1 Indirect techniques for the monitoring of sessile microorganisms

Indirect techniques for the monitoring of sessile microorganisms measure the effects of microbial activity and not microbial numbers. Parameters such as the changes in heat transfer from a metal to a passing water stream or in electrical conductance and the volumetric displacement of a liquid caused by a biofilm have been determined by Characklis *et al.* (1982). Another indirect technique is the measurement of the fluid flow resistance by a differential pressure gauge in the Bio Film Monitor developed by Johnson and Howells (1981).

The increase or influence that microorganisms may have on corrosion rates can also be determined by means of electrochemical techniques. Traditionally, corrosion rates have been determined by means of corrosion coupons. The information obtained is, however, retrospective (Tullmin *et al.*, 1992). The use of electrochemical techniques to monitor MIC and biofilm formation is widely reported in the literature (Pope and Zintel, 1988; Feron, 1990; Salvago *et al.*, 1990; Videla and Characklis, 1992). There are few case studies where these techniques have been utilised to monitor the efficacy of microbiological treatment programmes (Thierry, 1987).

Mansfeld and Little (1990) outlined the electrochemical techniques commonly used to monitor MIC and to determine the mechanisms involved. These techniques are: (1) measurement of the corrosion potential, (2) measurement of the redox potential, (3) measurement of the polarisation resistance, (4) the dual-cell technique, (5) electrical impedance spectroscopy, (6) electrochemical noise analysis and (7) large signal polarisation techniques. It was concluded that a combination of electrochemical, microbiological and surface analytical techniques is a promising approach. The use of corrosion potential measurements is not as widespread as the use of polarisation resistance, and operating variables in a system such as temperature, can limit the application of these electrodes. Polarisation resistance techniques also have a number of limitations, namely, that these systems do not provide information on localised corrosion and that the corroding environment must be one with low resistivity. Electrical impedance tech-

niques are increasingly used, however, more developmental work is required (Scully and Taylor, 1987). Electrochemical noise measurement is the latest development in corrosion monitoring and can be used to monitor the mechanism of corrosion occurring (Tullmin *et al.*, 1992). The major advantages of indirect techniques are that an instantaneous reading is obtained and a trained microbiologist is not required to determine the extent of microbial activity (Johnson and Howells, 1981).

4.2.2 Direct techniques for the monitoring of sessile microorganisms

The use of destructive sampling is generally necessary for monitors where direct monitoring techniques are used. This type of monitor can be placed directly into a water or oil pipe, for example, the CAPROCO monitoring device (Blackburn and Mullin, 1990). This device incorporates a biofilm probe or corrosion coupon and can be sampled without interruption of plant operation (Figure 2.8). Biofouling monitors such as the Robbins Device or the Pedersen Device must be placed into a side-stream line that can be isolated to allow sampling. The Robbins Device (Figure 2.9), is a ported biofilm sampler consisting of removable test surfaces which are exposed to circulating fluids (Costerton and Lashen, 1983). The Pedersen Device consists of test surfaces that can be constructed of glass, mild steel or any other material, which are placed into the device parallel to the direction of flow (Pedersen, 1982). A prescored or presectioned pipe can also be placed on a side stream, and thus entire sections of pipe can be removed and microbiologically analysed, or used for the determination of corrosion rates or biofilm mass.

Algae are often difficult to monitor or control in cooling water systems, as they can grow in spray areas and thus cannot be effectively reached by an algicide in the circulating water. It is common practice to evaluate algal growth in cooling systems by means of visual observations. However, Goysich and McCoy (1989), reported on a quantitive method for determining the efficacy of algicides in industrial cooling towers. This method involves the use of a glass pipe connector which allows the removal of algal deposits from a known area on a cooling tower deck. The algae thus removed can be analysed to determine mass or chlorophyll *a* content.

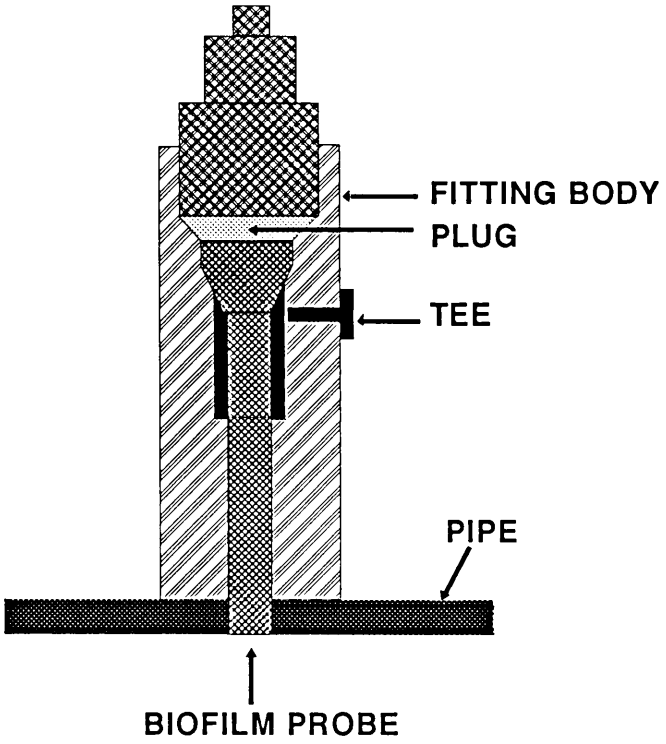


Figure 2.8 : CAPROCO Device biofouling monitor (After Blackburn and Mullin, 1990).

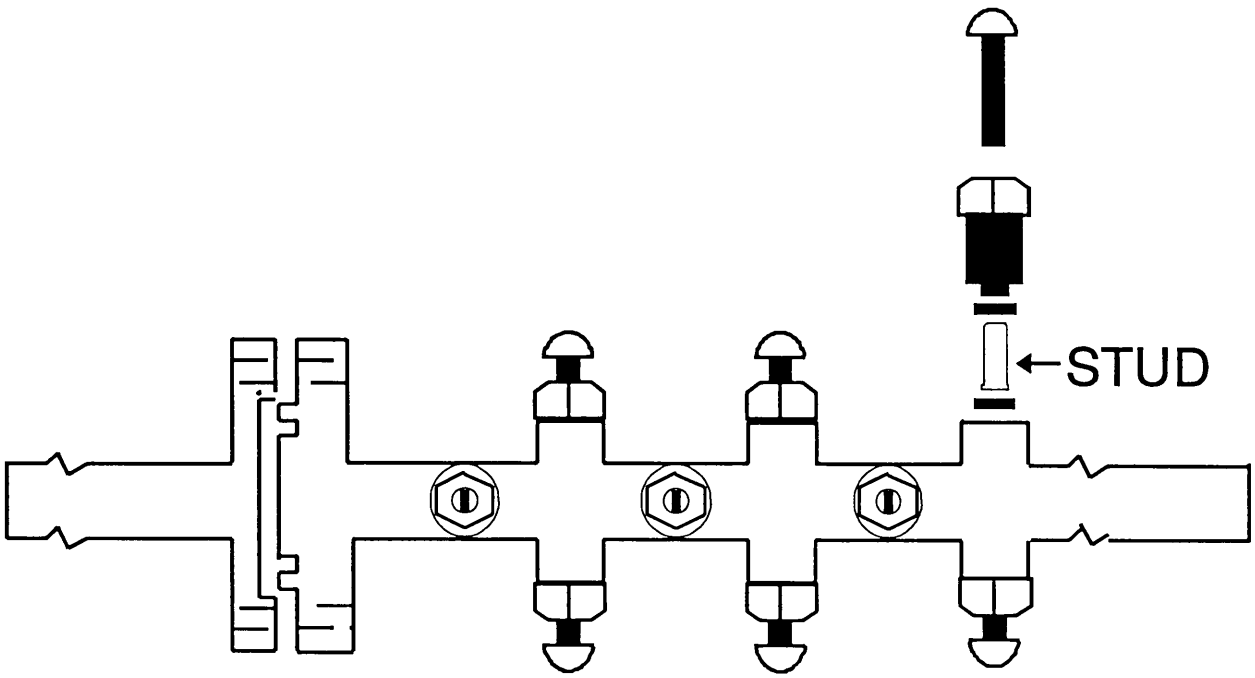


Figure 2.9 : Robbins Device biofouling monitor (After Costerton *et al.*, 1988).

4.3 Determination of microbial numbers and biofilm thickness in industrial water systems

The microbiologist has a number of techniques at his disposal by which to estimate the number or activity of either sessile or planktonic microorganisms in water systems (Table 2.4).

Table 2.4 : Techniques for the determination of microbial numbers or activity in water systems.

PARAMETER MEASURED	ANALYTICAL METHOD	REFERENCE
Biofilm mass	Weight measurements	Trulear, 1980
Biofilm thickness	Optical microscopy	Trulear, 1980
	Transmission electron microscopy	Costerton *et al.*, 1986
	Scanning electron microscopy	Horacek, 1988 Brözel *et al.*, 1990
Biofilm constituents	Polysaccharides, total organic carbon, oxygen demand, protein determinations	Characklis *et al.*, 1982
Bacterial activity or numbers	Viable cell counts	Ruseska *et al.*, 1982 Tatnall *et al.*, 1988
	Adenosine triphosphate (ATP)	Gaylarde, 1990 Challinor, 1991
	Radioisotopic assays	Staley and Konopka, 1985 Maxwell and Hamilton, 1986 Sanders, 1988
	Fluorescein diacetate	Schnurer and Rosswall, 1982
	Fluorescent labelled antibodies	Pope and Zintel, 1988
	Epifluorescence	Wolfaardt *et al.*, 1991
	Enzyme-linked immunosorbent assays (ELISA)	Gaylarde, 1990
	Gene probes	Gaylarde, 1990
	Antibody tests for specific enzymes	Tatnall and Horacek, 1990
	Dye reduction tests	Gaylarde, 1990

The disadvantages of many of the above-mentioned techniques for use in industrial systems, are that they require highly trained personnel for the analyses and in many cases expensive and sophisticated equipment, which is not always avaliable. Typical examples are the use of the scanning electron microscope for the determination of biofilm thickness or maturity (Brözel *et al.*, 1990) and a scintillation counter for radiorespirometric methods (Sanders, 1988). In addition, methods such as the use of fluorescent stains cannot distinguish between living and dead cells (Gaylarde, 1990). Some of the techniques involving the use of antibodies are sensitive, and interferences may occur (Tatnall and Horacek, 1990).

The use of the total viable cell count technique is also questionable, as only a fraction of the microorganisms present in the sample grow on a single culture medium (Quinn, 1984; Karl, 1986). The enumeration of SRB is particularly problematic due to their diverse requirements for both nutrients and environmental conditions (Tatnall *et al.*, 1988; de Bruyn, 1993).

Consequently, the use of rapid techniques that do not require trained personnel is gaining increased popularity. Gaylarde (1990) defined the requirements of rapid techniques for microbiological determinations as: (1) easy to use, (2) highly sensitive, (3) suitable for field use, (4) amenable to automation, (5) economic, (6) specific and (7) adaptable for use with biocide treated samples. However, no single rapid technique has been identified that is able to meet all these requirements. Thus, due to the complexity and diversity of microbiological populations in cooling water systems, it is essential to use combinations of techniques in order to effectively and accurately assess the extent of microbiological growth or activity.

5. Mitigation of microbiological problems in cooling water systems

5.1 Biocides for the mitigation of microbiological problems in cooling water systems

Biocides have been defined as toxicants which are fatal to living organisms. Thus microbicides are chemicals which are toxic to microorganisms (McCoy, 1980). It is, however, common practice to refer to microbicides as biocides. Biocides are extensively utilised throughout the world and the world market for industrial biocides has been estimated at 1.3 billion pounds stirling per annum (Parr, 1990).

Depending on their concentration, biocides have either a biostatic or biocidal effect on microorganisms (Cloete *et al.*, 1992). Denyer (1990) defined the bacteriostatic effect as being metabolic inhibition which is reversed upon the removal of the biocide, whilst the bactericidal effect is irreversible or irreparable damage (Figure 2.10).

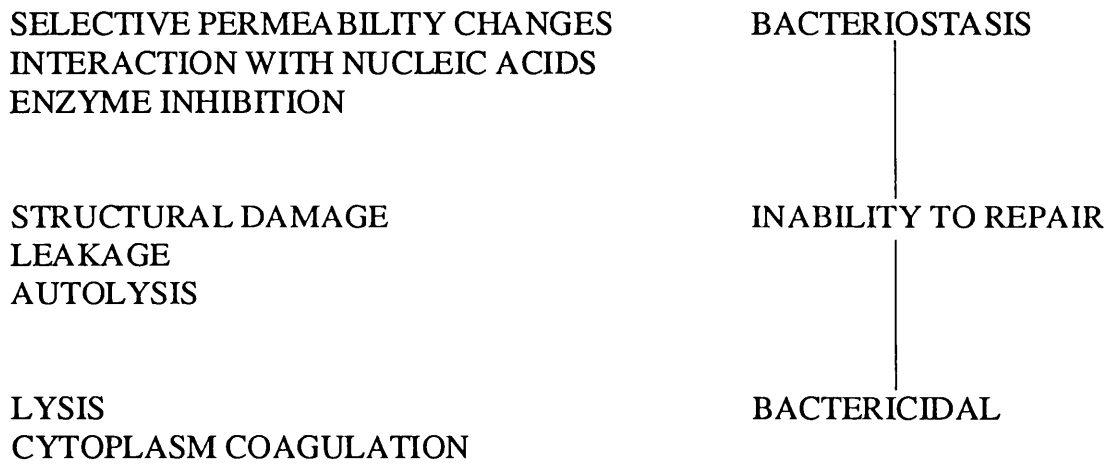


Figure 2.10 :The consequence of biocide-induced damage of bacterial cells (After Denyer, 1990).

Bernarde *et al.* (1967) identified three factors that can influence the biocidal effect on bacteria, namely (1) the mass transfer of toxicant to the bacteria/water interface (2) the chemisorption of toxicant at active centres on the cell wall (3) diffusion and chemical attack on extracellular or intracellular structures.

The efficacy of a biocide is dependant on the susceptibility of the target microorganisms and the compatibility of the biocide with the environmental parameters of the system. For example, Bessems (1983), illustrated that SRB vary in their susceptibilities to different quaternary ammonium compounds and chlorine is not an effective biocide at highly alkaline pH values (Anon., 1988). Thus each system must be individually evaluated before a biocide is added to the bulk water. Gaylarde and Johnston (1983) demonstrated that factors such as the bacterial species present in the test suspension, growth conditions of the bacteria prior to testing, oxygen concentration, pH and the presence of iron coupons can have profound effects on biocide efficacy against SRB, under laboratory conditions. The efficacy of individual biocides against the microbes to be controlled and the compatibilty of that biocide with the water to be treated, must therefore be evaluated.

5.1.1 Mechanisms of action of biocides

Biocides can be generally classified as either oxidising or non-oxidising. Traditionally, chlorine products have been utilised for biofouling control in cooling water systems (Al-Hoti, 1989). However, due to environmental controls and the move towards alkaline cooling waters, other oxidising biocides such as ozone and bromine based compounds are gaining increasing popularity (Puckorius, 1991).

The three target regions for biocides are the cell wall, the cytoplasmic membrane and the cytoplasm (Hugo 1967; Denyer, 1990). A correlation between the chemical structure of a biocide and its toxicity has been identified (Hugo 1967; McCoy, 1980). In addition, the microbial species to be controlled, plays an important role in determining the efficacy of a biocide (Albert, 1963). Thus a biocide may be effective against bacteria, but ineffective against fungi. Table 2.5 lists the major groups of both oxidising and non-oxidising biocides utilised in cooling water treatment and details their mechanisms of action.

Table 2.5 : Cooling water biocides and their mechanisms of action.

BIOCIDE	TARGET AREA	MECHANISM OF ACTION	REFERENCE
OXIDISING BIOCIDES			
Sodium hypochlorite	Cell wall	Lysis	Denyer, 1990
Chlorine dioxide	Cytoplasm	Protein synthesis	McCoy, 1980
Ozone	Cytoplasm	Lysis	Anon., 1977
NON-OXIDISING BIOCIDES			
Quaternary ammonium compounds	Cell membrane	Leakage of cell material	Denyer, 1990
Methylene bisthiocyanate	Cytoplasm	Enzyme poison	McCoy, 1980
Heavy metal salts	Cytoplasm	Coagulation of colloids	McCoy, 1980
Dodecylguanidine hydrochloride	Cytoplasm	Enzyme poison	McCoy, 1980
Dithiocarbamates	Cytoplasm	Enzyme poison	McCoy, 1980
Chlorophenols	Cell wall/ cytoplasm	Disruption of cell walls and proteins	McCoy, 1980
Isothiazolones	Cytoplasm	Inhibition of macromolecular synthesis	McCoy, 1980
Acrolein	Cytoplasm	Enzyme poison	Anon., 1977

5.1.2 Resistance of water microorganisms to biocides

The phenomenon of microbial resistance to biocides, is well documented in the literature (Chaplin, 1952; Neu, 1984; Russell, 1990; Brözel and Cloete, 1991). Russell (1990) defined two types of microbial resistance to biocides (1) intrinsic or a natural chromosally-controlled property of an organism and (2) acquired resistance resulting from genetic changes in a cell such as mutation of existing cell material, or the acquisition of a plasmid. In addition, changes in cell structure, for example sporulation, can also impart biocide resistance (Russell, 1982).

The sensitivity of a particular microorganism to a biocide is related to its morphology (Albert, 1963). For example, the differences in membrane and cell wall characteristics between gram positive and gram negative bacteria play an important role in determining biocide sensivity (Russell, 1990). Gram positive bacteria are more sensitive to biocides than are gram negative bacteria, and gram negative bacteria are thus intrinsically more resistant (Russell and Gould, 1988). The production of extracellular polysaccharides or a glycocalyx also enhances biocide resistance. Thus sessile bacteria present in a biofilm, will have an intrinsic resistance to biocides when compared to their planktonic counterparts (Costerton and Lashen, 1983; Blenkinsopp and Costerton, 1991). The production of large quantities of polysaccharides by bacteria attached to copper surfaces, to shield them from the effect of soluble copper ions, is a well known phenomenon (Eaton *et al.*, 1980). It has also been suggested that the age of a biofilm plays a role in determining biocide resistance. Young biofilms have been found to be more susceptible to antibacterial agents than ageing biofilms (Anwar and Strap, 1992). A microorganism may also have the ability to detoxify a biocide and thus shield other sensitive microorganisms from the effect of the biocide. For example, certain bacteria can detoxify formaldehyde based biocides by means of the formaldehyde dehydrogenase enzyme system (Sondossi *et al.*, 1989).

Microorganisms can acquire biocide resistance either by exposure to increasing concentrations of a biocide, or by plasmid mediated resistance (Russell, 1990). Development of resistance to a particular biocide may also induce cross resistance to other biocides, although the exact mechanisms by which this cross resistance occurs are not clear (Sondossi *et al.*, 1989; Brözel and Cloete, 1991). Mutagenic activity of biocides in microorganisms is also an important consideration, and this has been reported in cooling tower water after the addition of 5-chloro-2-methyl-4-isothiazolin-3-one (Woodall *et al.*, 1987).

5.1.3 Environmental concerns regarding the use of biocides in industrial water systems

Increasing environmental restrictions and legislative pressure for safer and environmentally friendly biocides will intensify the need for new biocide formulations (Halleux, 1990; Parr, 1990). In addition, the trend towards the use of biodispersants, which are unlikely to induce mutations in microorganisms, may accelerate in the future.

5.2 Mitigation of microbiological problems in cooling water systems by biodispersants

Although they are widely used in industry, little information is available on the use of biodispersants in cooling water systems. The use of biodispersants is anticipated to increase the efficacy of biocides by reducing the protection offered by the glycocalyx produced by sessile microorganisms (McCoy, 1980). In addition biodispersants can increase the penetration of biocides into biofilms and inorganic deposits (Lutey and Allison, 1991). Biodispersants or surfactants cause fouling material to remain in suspension by imparting a charge to the material, resulting in a charge replusion between the foulant and the surface (Anon., 1977). Further research into mechanisms of action of biodispersants and factors affecting their efficacy in cooling water systems is required.

5.3 Coatings for the protection of cooling water systems

Coatings are widely utilised as protection systems against corrosion, including MIC (Jones and Walch, 1990). These coatings may be required to protect the inner pipe surfaces or exterior surfaces in the case of buried pipelines (Jack *et al.*, 1990). These coatings can consist of a wide variety of materials, but those most commonly used are epoxy-based (Jones and Walch, 1990). The major disadvantage of this type of coating, is that inadequate or incorrect application results in pinholing of the coating, allowing microorganisms access to metal surfaces (Severyn, 1990). In addition, it has been reported that epoxy coatings can be degraded by bacterial activity and extensive surface preparation is required before application (Jones and Walch 1990; Spires, 1990).

5.4 Mitigation of microbiological problems in cooling water systems by cathodic protection

Cathodic protection incorporates the use of impressed current or sacrificial anodes as a mechanism of protection for buried or immersed metallic structures and components against

corrosion and has been successfully used since 1944 (Kaiser, 1984; Guezennec, 1991). Impressed current cathodic protection is the process by which a current is applied to a metal to neutralise or overcome any currents which are attempting to flow from the metal (Edyvean *et al.*, 1992). This current has to be of sufficient magnitude to prevent any base metal dissolution (Berkley, 1968). More specifically, cathodic protection is an electrochemical means of corrosion control in which the oxidation reaction in a galvanic cell is concentrated at the anode and suppresses corrosion of the cathode in the same cell (Heidersbach, 1987). Sacrificial anodes consisting of metals that will corrode preferentially to the metal to be protected can also be utilised. It has been reported that zinc or magnesium are the most effective materials for the sacrifical anode protection of mild steel (Moosavi *et al.*, 1990).

It has been suggested that the application of a cathode potential may inhibit not only MIC, but also biofilm development (Maxwell, 1986). There are numerous reports of the use of cathodic protection for use against MIC (Fischer, 1983; Guezennec *et al.*, 1990; Nekoksa and Gutherman, 1990). However, the results are conflicting and it appears that each case must be investigated separately as environmental factors play an important role (Fischer, 1983). Mollica (1992) suggested that a combination of cathodic protection and biocide addition was the most effective technique for the prevention of MIC in seawater systems.

5.5 Mitigation of microbiological problems in cooling water systems by pigging

Pigs are bullet nosed devices that are propelled through pipes containing biofilms or biofouling deposits attached to their interior surfaces (Smart and Smith, 1992). Many different types and designs of pigs are commercially available. Due to their design, pigs aid in the removal of deposits from the interior wall of the pipe through which they are propelled. Biofilms and inorganic deposits are therefore disturbed and the number of microorganisms in the bulk water increases (Allison, 1990). Pigs are often utilised in conjunction with treatment chemicals, such as biocides, to enhance their performance (Smart and Smith, 1992). Planktonic bacteria are more susceptible to the action of biocides (Costerton and Lashen, 1983). The addition of a biocide immediately after pigging thus results in the more efficient mitigation of MIC and removal of biofilms than treatment with a biocide alone (Blenkinsopp and Costerton, 1991; Lutey and Allison, 1991).

South African industry faces many unique problems in terms of water quality due to the increasing demand for water and recurring droughts. Thus, microbiological problems in open recirculating cooling water systems can be expected to increase in the future. Although extensive research has been carried out worldwide on the monitoring and control of biofouling and biocorrosion, there are still many unanswered questions. In particular, little information is available on the use of biodispersants for microbiological control in cooling water systems. Furthermore, the industrial microbiologist still does not have accurate tools for the monitoring of sessile microorganisms in cooling water. Although many biofouling monitors have been developed and tested, few studies have been carried out where the accuracy and practicality of different monitors have been compared on an industrial site. The majority of the literature available on biofouling and biocorrosion reports on laboratory studies. A need therefore exists for research to investigate practical methods for the monitoring and control of microorganisms in industrial cooling water systems.

Chapter 3

MICROBIOLOGICAL SURVEY OF OPEN RECIRCULATING COOLING WATER SYSTEMS AT TWELVE FOSSIL FIRED POWER STATIONS AND THEIR RAW WATER SUPPLIES

ABSTRACT

Raw water supplies utilised at 12 fossil fired power stations, as well as the corresponding open recirculating cooling water systems were surveyed. Visual inspections were carried out and total aerobic and anaerobic bacteria, anaerobic acid producing bacteria, *Thiobacillus*, *Nitrobacter*, sulphate reducing bacteria (SRB) and algae were quantified. All raw water supplies and recirculating cooling waters contained all of the above groups of microorganisms, with the exception of the two potable raw water supplies. In 75% of the systems, the numbers of SRB in the recirculating cooling waters were higher than in the corresponding raw water supplies and in 92% of the systems, the numbers of total aerobic bacteria were higher in the recirculating cooling waters than in the raw water supplies. However, no relationship between the sulphate levels in the recirculating cooling waters and the numbers of SRB could be distinguished, or between the percentage increase in the numbers of total aerobic bacteria and the cycles of concentration at which the system was operated. The frequency polygons of the occurrences of total aerobic and anaerobic bacteria in the raw water supplies and recirculating cooling waters did not follow normal distribution patterns. Visible biofouling deposits were observed at six of the power stations surveyed and the predominant algal group was the blue green algae. However, in the raw water supplies, the predominant algal groups were green algae and diatoms. Microbiologically influenced corrosion was identified in all five of the condensers inspected. Each system was found to be unique and no generalisations in terms of presence or activity of microorganisms could be made. Thus the need to evaluate and monitor microorganism presence and activity in each individual system, was highlighted.

INTRODUCTION

The Department of Water Affairs of South Africa has requested that dry-cooled power stations be constructed preferentially, as this type of power station utilises only 22% of the volume of water required by a wet-cooled station. Higher capital expenditure is, however, required for the construction of dry-cooled stations and operating costs are also elevated when compared with wet-cooled power stations. It is therefore evident, that wet-cooled power stations will be constructed in the future. It is estimated that by the year 2010, a total of 900 million m^3 per annum of water will be required for power generation, as compared with the 282 million m^3 of water consumed during 1980 (Anon., 1986). Thus the need for water conservation and reuse will be of extreme importance in the future.

The awareness of microbiologically related problems in open recirculating cooling water systems has increased over the last few years and has been extensively reviewed (Soracco *et al.*, 1988; Cloete *et al.*, 1992). It has been widely reported that in aqueous systems, microorganisms attach themselves to available surfaces by means of extracellular polysaccharide polymers, forming biofilms or biofouling deposits (Duddridge and Pritchard, 1983; Characklis *et al.*, 1990). The attachment of microorganisms to surfaces enables them to function as a multicellular tissue (Costerton *et al.*, 1987). The physical presence of such deposits in cooling water systems can result in decreased heat transfer and increased frictional resistance (Characklis, 1973; Ferguson, 1981). In addition, discrete microbial colonies within biofilms or biofouling deposits on metal or concrete structures, can give rise to microbiologically influenced corrosion (MIC). The major groups of microorganisms responsible for this phenomenon are the sulphate reducing bacteria (SRB), aerobic acid producing bacteria such as *Thiobacillus* and *Nitrobacter*, anaerobic acid producing bacteria such as *Clostridium* and iron bacteria such as *Gallionella* (Tatnall, 1981; Cragnolino, 1983; Pope *et al.*, 1988). Algae are also responsible for numerous problems in cooling water systems, for example, reduction in heat transfer across cooling towers (McCoy, 1980).

Due to the intensified demand on limited water resources for a wide variety of industrial and domestic uses, the quality of water supply in South Africa is degenerating (Anon., 1986). As a result, the incidence of MIC and biofouling in industrial water systems in South Africa is increasing, resulting in costly control programmes and down-time (Poulton and Nixon, 1990). Iverson (1987), estimated that MIC constituted 10% of all metallic corrosion while the estimated direct cost of MIC in South Africa is approximately R400 million (von Holy and Cloete, 1988). As the presence of potentially troublesome groups of microorganisms can have profound effects

on cooling water system operation and integrity, it is essential to determine their presence, activity and source.

The aim of this work was therefore to survey the open recirculating cooling water systems at 12 fossil fired power stations and their corresponding raw water make up supplies. It was anticipated that this survey would not only reveal the extent of microbiological contamination in these systems, but also indicate which make up supplies contained undesirable microorganisms.

MATERIALS AND METHODS

Description of power stations surveyed

The 12 power stations surveyed and their operational parameters are shown in Table 3.1 and their locations in Figure 3.1.

Details of the chemical composition of the recirculating cooling waters are shown in Table 3.2.

Table 3.1 : Power stations where microbiological surveys of the recirculating cooling water and raw water supply were carried out and their corresponding operational parameters.

POWER STATION	RAW WATER SUPPLY	SYSTEM VOLUME (Megalitres)	CYCLES OF CONCENTRATION
1. Arnot	Nooitgedacht	50	12 - 15
2. Camden	Jerico Dam	50	10
3. Duvha	Komati/Naauwpoort	128	10
4. Grootvlei	Vaal Dam	36	10
5. Hendrina	Vygeboom/Komatipoort	60	7 - 10
6. Kendal	Potable water	0.7	11
7. Komati	Komati River	40	7
8. Kriel	Usutu scheme	128	15 - 20
9. Lethabo	Vaal River	128	15 - 20
10. Matimba	Potable water	0.7	5
11. Matla	Grootdraai Dam	128	10
12. Tutuka	Grootdraai Dam	128	20

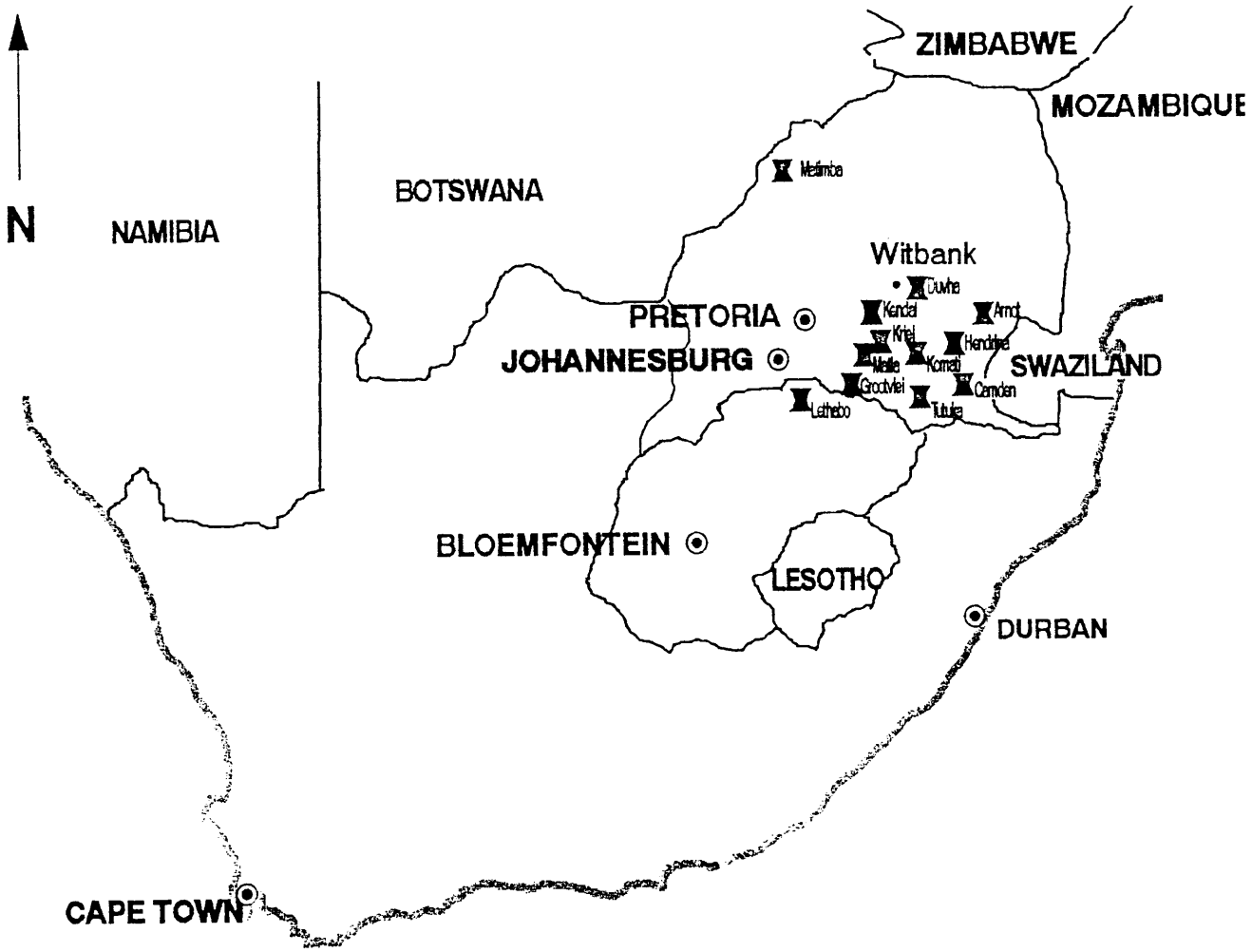


Figure 3.1 : Locations of 12 power stations where microbiological surveys of the recirculating cooling water and raw water supply were carried out.

Table 3.2 : Chemical analysis of the recirculating cooling waters at the 12 power stations where microbiological surveys were carried out.

POWER STATION	pH AT 25°C	CONDUCTIVITY AT 25°C ($\mu S\ cm^{-1}$)	TOTAL HARDNESS ($mg.l^{-1}\ CaCO_3$)	SULPHATE ($mg.l^{-1}$)
1.[a]	8.4	1 200	350	350
2.	8.4	1 040	56	21
3.	8.3	2 100	350	830
4.	8.3	1 310	206	470
5.	8.3	1 490	400	330
6.	8.6	450	120	50
7.	8.5	960	350	300
8.	8.5	1 500	500	400
9.	8.5	2 700	318	900
10.	8.6	800	200	20
11.	8.6	2 350	550	500
12.	8.6	4 700	428	1 300

[a] for key to figures see Table 3.1

Sampling points

At each power station, recirculating cooling water was sampled from the cooling tower sump and raw water from the inlet pipe into the cooling tower sump. Visual inspections were carried out on the cooling towers, clariflocculators and condensers, circumstances permitting. Samples of visible deposits on the cooling tower or areas of the clariflocculators, together with visible deposits or nodules in the condensers, were collected.

Sampling procedure

The 12 power stations were surveyed over a three month period during summer. One power station was surveyed per week. The same sampling procedure was followed for both the raw water supplies and recirculating cooling waters. Four 550 ml aliquots of each water type were sampled in sterile Whirl Pak bags (Nasco, USA). One of the aliquots was purged with nitrogen before transportation and was utilised for the quantification of anaerobic microorganisms. Air

was trapped in the remaining Whirl Pak bags which were utilised for the quantification of the aerobic microorganisms and algae.

Deposits were removed by sterile forceps and immediately placed into Whirl Pak bags. Whirl Pak bags containing deposits that were thought to contain SRB were purged with nitrogen before transportation. All samples were kept at 4°C during transportation to the laboratory and analysed within six h of sampling.

Quantification of microorganisms in water samples

The samples were diluted in sterile, quarter strength Ringer's solution and subjected to duplicate plate counts. All incubation was at 37°C. Anaerobic incubation took place in an anaerobic incubator (Forma Scientific Anaerobic System, Labotec, S.A.), where the atmosphere consisted of 5% hydrogen, 15% carbon dioxide and 80% nitrogen. The techniques used to quantify the bacteria in the raw water supplies and recirculating cooling waters are detailed in Table 3.3. Plates containing between 30 and 300 colonies were counted.

Table 3.3 : Techniques used to quantify bacteria in the recirculating cooling waters and raw water supplies.

MICROBIAL TYPE	TECHNIQUE	INCUBATION TIME (d)	ATMOSPHERE	GROWTH MEDIUM
Total Aerobic Bacteria	Pour plate	2	aerobic	Nutrient Agar (Biolab)
Total Anaerobic Bacteria	Pour plate	3	anaerobic	Nutrient Agar (Biolab)
Anaerobic Acid Producing Bacteria	Spread plate	3	anaerobic	Dextrose Tryptone Agar (Oxoid)
*Thiobacillus**	Spread plate	7	aerobic	Clesceri *et al.*, 1989
*Nitrobacter**	Spread plate	7	aerobic	Martin *et al.*, 1988
SRB	Agar tubes	14	anaerobic	SABS method 1497-1989, 1989

* Bacterial morphology observed and Gram stain carried out

The presence of algae in the recirculating and raw waters was determined by analysis of chlorophyll *a* according to the method of Sartory (1982). For each analysis, 500ml of water was filtered.

Calculations

The following calculation was used to determine the percentage increase in the numbers of total aerobic bacteria (TAB) in the recirculating cooling water as compared to the raw water supply:

$$\frac{\text{(number of TAB in recirculating water - number of TAB in raw water)}}{\text{number of TAB in raw water}} \times 100$$

Microscope examination of deposits and water

All deposits were examined under a light microscope at 500 x magnification. If the deposits contained mostly algae, they were classified into unicellular or filamentous green algae, blue green algae or diatoms (Palmer, 1962). If the deposit appeared to be primarily inorganic in composition, it was treated with dilute (0.1N) hydrochloric acid and examined under a light microscope at 500 x magnification for the presence of *Gallionella* (ASTM D932-72, 1972).

One hundred ml of each of the raw water supply and recirculating cooling water samples were filtered through a 0.45μm pore size cellulose acetate filter (Millipore) and the filters were air dried. A drop of immersion oil was placed onto the filter to clear it and the surface examined under a light microscope at 500 x magnification, for the presence of *Gallionella* and planktonic algae.

Condenser inspections

When a system was off line, condensers were visually examined. The bare metal or coated metal surfaces in the condensers were examined for signs of MIC e.g. nodules of iron oxides or blisters in the coating, with underlying shiny metal pits, filled with a black liquid (McCoy, 1980). Inspections were carried out within 12 h after the system had been drained, to ensure that the surfaces were still moist. Deposits were examined under a light microscope at 500 x magnification for the presence of *Gallionella* and cultured to determine the presence of SRB as described in Table 3.3.

RESULTS AND DISCUSSION

Quantification of microorganisms in water samples

Microbial numbers quantified in the raw waters are detailed in Table 3.4.

Table 3.4 : Microbiological analysis of the 12 raw water supplies used in the open recirculating cooling water systems.

Power Station	Total Aerobic Bacteria	Total Anaerobic Bacteria	Anaerobic Acid Producing Bacteria	*Thio-bacillus*	*Nitro-bacter*	SRB	Algae
1.[a]	$2.9x10^3$	$4.0x10^2$	Positive	$1.0x10^1$	$2.9x10^1$	$2.0x10^1$	3.7
2.	$2.0x10^2$	$6.0x10^1$	Positive	$1.0x10^1$	$2.9x10^1$	$1.0x10^0$	8.6
3.	$5.1x10^2$	$1.5x10^2$	Positive	$1.2x10^2$	$3.7x10^1$	$9.0x10^0$	12.3
4.	$7.3x10^2$	$3.5x10^2$	Positive	$4.1x10^2$	$3.0x10^0$	$5.0x10^0$	2.7
5.	$4.8x10^2$	$2.0x10^2$	Positive	$1.6x10^1$	$4.0x10^0$	$1.8x10^1$	11.4
6.	$5.0x10^0$	$<1.0x10^0$	$<1.0x10^0$	$1.3x10^1$	$1.0x10^0$	$<1.0x10^0$	<1.0
7.	$4.2x10^2$	$3.4x10^2$	Positive	$7.0x10^1$	$1.0x10^1$	$5.0x10^0$	2.7
8.	$1.0x10^2$	$2.0x10^1$	Positive	$6.0x10^0$	$<1.0x10^0$	$3.2x10^1$	9.3
9.	$7.0x10^3$	$8.8x10^2$	Positive	$1.3x10^2$	$3.3x10^1$	$3.0x10^0$	4.3
10.	$1.0x10^0$	$1.0x10^0$	$<1.0 x10^0$	$<1.0x10^0$	$<1.0x10^0$	$<1.0x10^0$	<1.0
11.	$1.0x10^3$	$2.1x10^3$	Positive	$7.1x10^1$	$9.0x10^1$	$1.5x10^1$	14.8
12.	$1.4x10^3$	$2.4x10^2$	Positive	$1.3x10^1$	$2.2x10^1$	$9.0x10^0$	1.6

All bacterial counts are reported as colony forming units (CFU) ml^{-1}
Algae are reported as $\mu g.l^{-1}$ chlorophyll *a*
[a] for key to figures see Table 3.1

The raw water supplies contained all groups of microorganisms, with the exception of the potable waters at Kendal and Matimba Power Stations (Table 3.4). As these potable waters are chlorinated, low numbers of microorganisms were expected. Although Matla and Tutuka Power Stations obtained their raw water from the same source, variations in the numbers of microorganisms were noted. For example, the raw water used by Matla contained $2.1x10^3$ CFU.ml^{-1} anaerobic bacteria, whereas the water used by Tutuka contained $2.4x10^2$ CFU.ml^{-1} (Table 3.4). The raw water used by Matla also contained 14.8 $\mu g.l^{-1}$ of chlorophyll *a*, considerably more than the 1.6 $\mu g.l^{-1}$ recorded at Tutuka (Table 3.4). In addition,

higher numbers of *Thiobacillus*, *Nitrobacter* and SRB were noted. The number of aerobic bacteria in the raw water used by Matla was lower than the number recorded in the Tutuka water, $1.1x10^3$ CFU.ml^{-1} as compared with $1.4x10^3$ CFU.ml^{-1} (Table 3.4). These variations could be due to the fact that these power stations are not equidistant from the shared water supply and changes in the water may occur during transport to the power stations. Alternatively, the variations may be due to the samples not being taken on the same day. Figure 3.2 shows the frequency polygons of the aerobic and anaerobic bacteria in the raw water supplies.

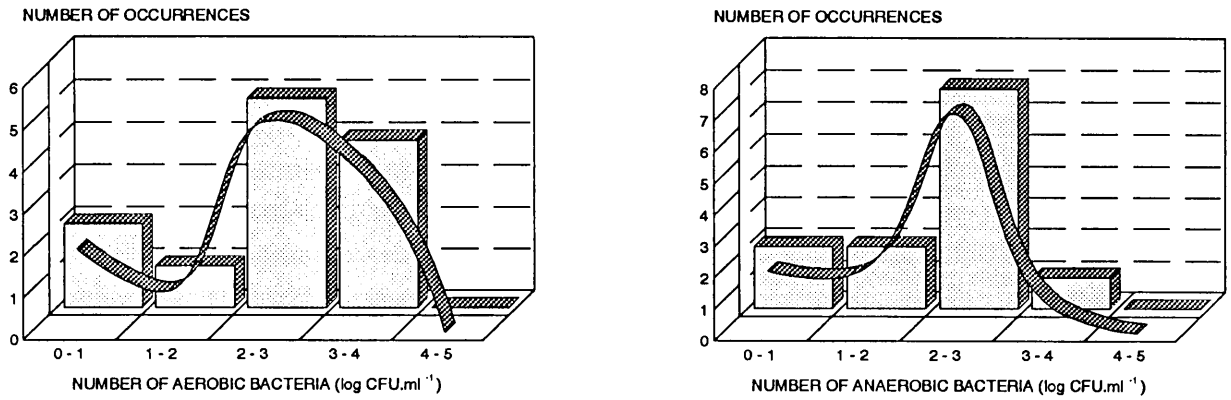


Figure 3.2 : Frequency polygons of aerobic and anaerobic planktonic bacteria counts in the raw water supplies for the 12 power stations surveyed.

The frequency polygons for both the aerobic and anaerobic bacteria did not follow normal distribution patterns. However, in both cases, bacteria numbers most frequently fell into the range $1.0x10^2$ - $1.0x10^3$ CFU.ml^{-1} (Figure 3.2). As these raw waters were taken from a number of different sources, a normal distribution pattern would not be expected. In addition, the potable water supplies had been treated, thus changing the original microbiological composition.

Table 3.5 : Microbiological analysis of the 12 cooling waters from the open recirculating cooling water systems.

Power Station	Total Aerobic Bacteria	Total Anaerobic Bacteria	Anaerobic Acid Producing Bacteria	*Thio-bacillus*	*Nitro-bacter*	SRB	Algae
1.[a]	$3.0x10^3$	$8.0x10^2$	Positive	$5.0x10^1$	$6.9x10^1$	$1.0x10^0$	6.9
2.	$5.9x10^4$	$2.3x10^2$	Positive	$7.1x10^3$	$6.9x10^1$	$5.2x10^1$	32.6
3.	$2.2x10^3$	$3.2x10^3$	Positive	$9.5x10^2$	$1.2x10^2$	$3.1x10^1$	26.6
4.	$3.2x10^3$	$1.5x10^3$	Positive	$1.0x10^3$	$7.9x10^1$	$5.0x10^0$	16.0
5.	$4.0x10^2$	$4.1x10^2$	Positive	$3.5x10^1$	$1.4x10^1$	$2.0x10^1$	21.3
6.	$3.0x10^2$	$4.4x10^1$	Positive	$1.6x10^1$	$6.0x10^0$	$4.0x10^0$	17.3
7.	$1.8x10^3$	$1.3x10^3$	Positive	$7.0x10^1$	$1.9x10^1$	$2.0x10^0$	2.1
8.	$2.9x10^4$	$2.1x10^3$	Positive	$7.2x10^2$	$1.1x10^2$	$1.5x10^2$	29.8
9.	$3.0x10^5$	$3.6x10^3$	Positive	$2.2x10^3$	$2.2x10^3$	$1.6x10^1$	14.5
10.	$4.4x10^2$	$2.4x10^2$	Positive	$3.0x10^1$	$1.3x10^1$	$5.0x10^0$	1.5
11.	$2.2x10^3$	$1.1x10^3$	Positive	$7.4x10^1$	$1.1x10^2$	$1.1x10^1$	10.4
12.	$8.5x10^4$	$7.5x10^2$	Positive	$1.4x10^2$	$9.5x10^1$	$1.1x10^2$	6.2

All bacterial results are reported as $CFU.ml^{-1}$
Algae are reported as μgl^{-1} chlorophyll *a*
[a] for key to figures see Table 3.1

All groups of microorganisms were present in all the recirculating cooling waters. Again, differences in the microbial numbers quantified at Matla and Tutuka Power Stations were noted. The number of aerobic bacteria quantified in the recirculating cooling water at Tutuka was $8.5x10^4$ $CFU.ml^{-1}$, while in the Matla recirculating cooling water only $2.2x10^3$ $CFU.ml^{-1}$ were recorded (Table 3.5). Whereas in the Matla raw water the numbers of *Thiobacillus*, *Nitrobacter* and SRB were higher than in the Tutuka water, these bacteria were present in higher numbers in the Tutuka recirculating water when compared to the Matla recirculating water. A possible explanation for this could be that the Tutuka cooling water system was operated at 20 cycles of concentration and the Matla system at 10 cycles (Table 3.1). Thus, not only microorganisms, but also nutrients would be more concentrated, in the Tutuka water, due to system operation.

The frequency polygons for aerobic and anaerobic bacteria in the recirculating cooling waters are shown in Figure 3.3.

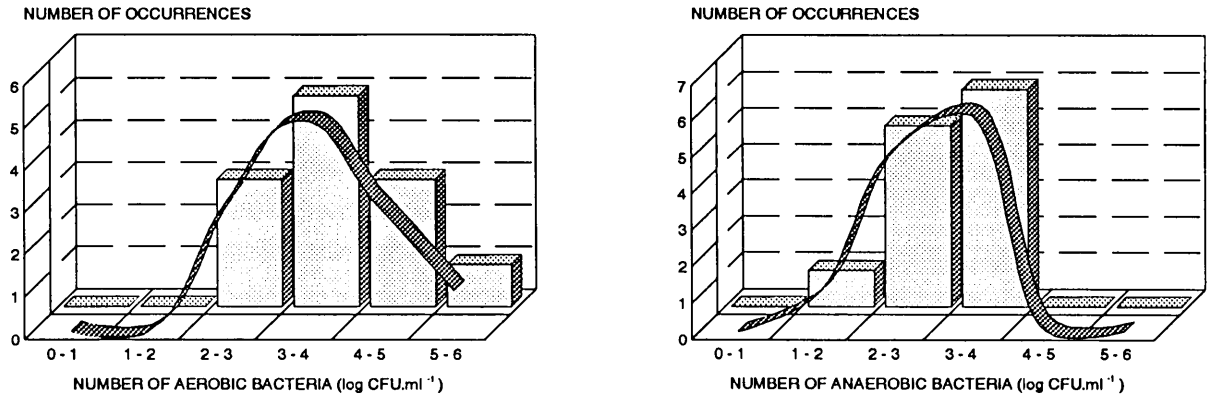


Figure 3.3 : Frequency polygons of planktonic aerobic and anaerobic bacteria counts in the recirculating cooling waters at the 12 power stations surveyed.

As with the frequency polygons for the raw water supplies, the distribution of the aerobic and anaerobic bacteria in the recirculating cooling waters did not follow normal distribution patterns. However, the highest number of occurrences shifted to the range $1.0x10^3$ - $1.0x10^4$ CFU.ml^{-1} as compared with $1.0x10^2$ - $1.0x10^3$ CFU.ml^{-1} in the raw water supplies (Figures 3.2 and 3.3). This shift can be explained by the fact that conditions are more favourable for microbiological growth in open recirculating cooling water systems when compared to raw water supplies (Strauss and Puckorius, 1984; Thierry, 1987).

In 75% of the systems, the numbers of SRB in the recirculating cooling waters were higher than in the raw waters. However, a number of disparities were noted. For example, the numbers of SRB in the raw water supply to Camden Power Station were $1.0x10^0$ CFU.ml^{-1} and $5.2x10^1$ CFU.ml^{-1} in the recirculating water. In direct contrast, $2.0x10^1$ CFU.ml^{-1} SRB were quantified in the raw water supply to Arnot Power Station and only $1.0x10^0$ CFU.ml^{-1} in the corresponding recirculating cooling water (Tables 3.4 and 3.5). Numbers of SRB in the recirculating cooling waters versus the corresponding sulphate concentration are illustrated in Figure 3.4.

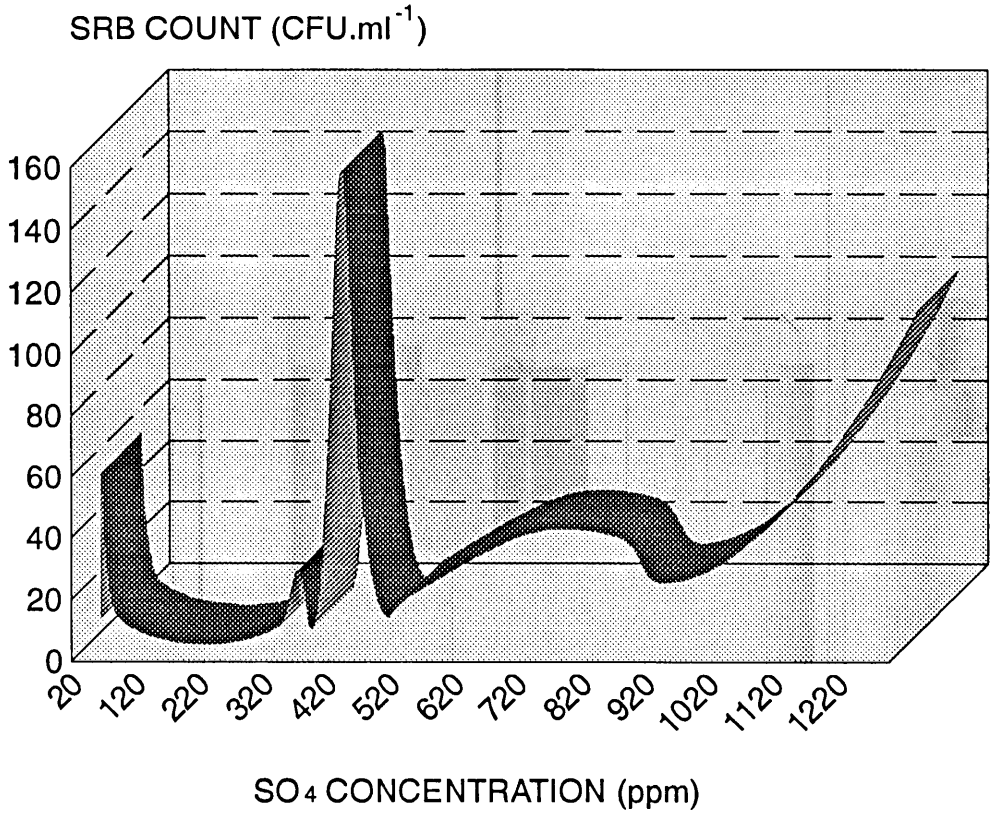


Figure 3.4 : Numbers of SRB compared to corresponding sulphate concentrations in the recirculating cooling waters at the 12 power stations surveyed.

Numbers of SRB did not increase with increasing concentrations of sulphate. Thus it appeared that sulphate concentration is not the only factor that determines SRB activity (Postgate, 1981). In addition, as SRB are likely to preferentially migrate to anaerobic areas in the system, the quantification of their numbers in the bulk water cannot be used to accurately predict their activity (Postgate, 1981).

Other inconsistencies included minor changes between the numbers of aerobic bacteria in the raw and recirculating waters at Arnot and Hendrina Power Stations. Numbers of aerobic bacteria in the recirculating cooling water at Arnot Power Station had increased by only 3% when compared to the raw water supply. At Hendrina Power Station, numbers of aerobic bacteria in the recirculating water were lower than those in the corresponding raw water supply. In addition, the number of anaerobic bacteria in the recirculating cooling water at Matla Power Station was 53% of the number quantified in the raw water supply (Tables 3.4 and 3.5). In 92% of the cases, however, numbers of aerobic bacteria in the recirculating cooling waters were

higher than those in the raw water supplies. Percentage increases in the numbers of aerobic bacteria between the raw water supplies and recirculating cooling waters were thus compared to the cycles of concentrations at which the corresponding power stations operated (Figure 3.5).

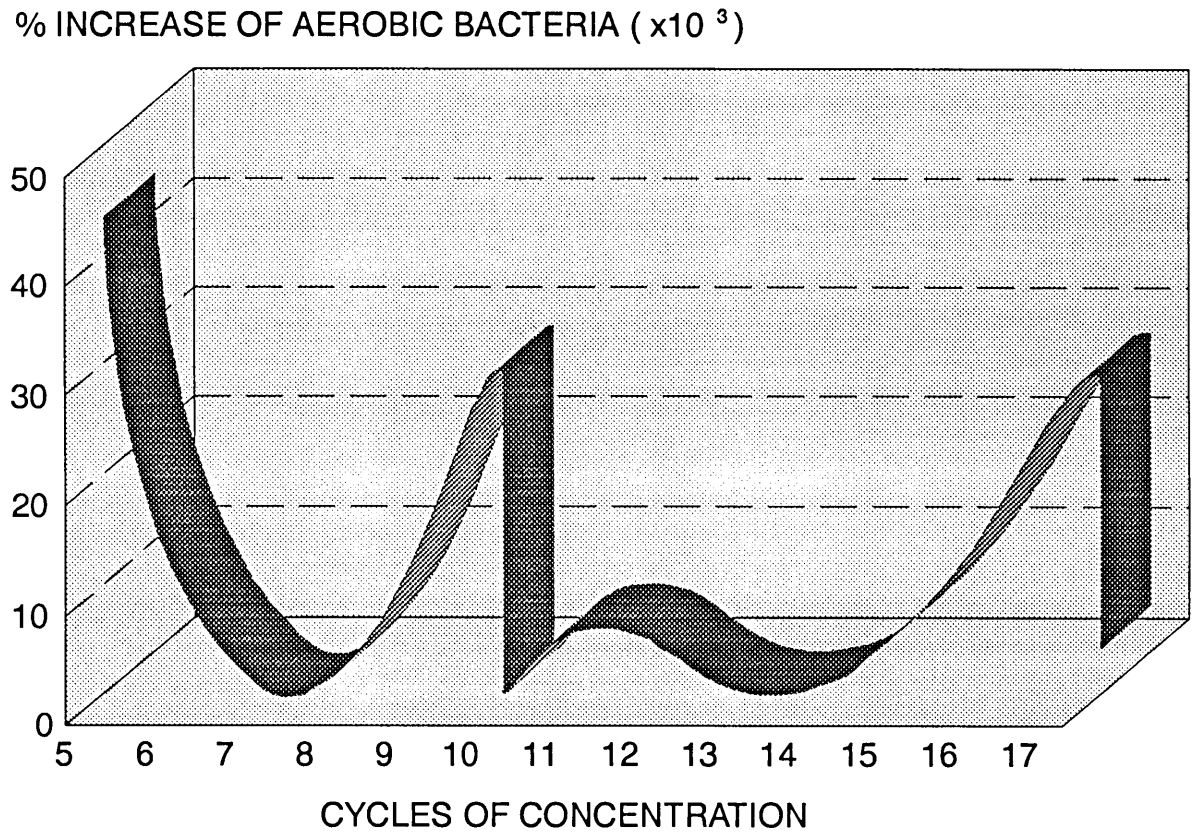


Figure 3.5 : Effect of cycles of concentration on the percentage increase in the numbers of aerobic bacteria in the raw water supplies and corresponding recirculating cooling waters.

No obvious relationship between the percentage increase in aerobic bacteria and cycles of concentration could be distinguished. At five cycles of concentration, the aerobic bacteria at Matimba Power Station increased by 43400%, whereas at Arnot Power Station, which is operated at 12 - 15 cycles, a 3% increase occurred (Figure 3.5).

Microscope examination of biofouling deposits and filtered water samples

Biofouling deposits were examined under the light microscope and predominant algal groups identified (Table 3.6).

Table 3.6 : Microscope examination of biofouling deposits removed from the open recirculating cooling water systems surveyed.

POWER STATION	LOCATION OF DEPOSIT	PREDOMINANT ALGAL GROUPS (Palmer, 1962)
3.[a]	Clariflocculator outlet	Filamentous blue green algae
4.	Clariflocculator outlet Cooling tower	Filamentous blue green algae Filamentous green and blue green algae Filamentous and unicellular diatoms Unicellular green algae
5.	Cooling tower	Filamentous blue green algae Unicellular diatoms
7.	Cooling tower	Filamentous blue green and green algae Unicellular diatoms
9.	Clariflocculator	Filamentous blue green algae unicellular diatoms and green algae
11.	Clariflocculator	Filamentous blue green algae

[a] for key to figures see Table 3.1

In all biofouling deposits examined, the predominant algal group was filamentous blue green algae. Unicellular and filamentous diatoms and green algae were also observed, at irregular intervals.

Microscope examination of filters from recirculating cooling waters and raw water supplies, revealed that the predominant algal groups in the waters were unicellular green algae and filamentous and unicellular diatoms. All recirculating cooling waters contained filamentous blue green algae. The raw water from the Naauwpoort system into Duvha Power Station and both the recirculating cooling water and the raw water supply at Matla Power Station contained filamentous green algae. The predominant algal group in the raw water supplies were green algae and diatoms. In direct contrast, in the recirculating cooling waters, the predominant algal group, both in the recirculating water and in the deposits, were blue green algae and diatoms. *Gallionella* was not detected in any of the samples.

When the results of the chlorophyll *a* content of the waters were examined, in relation to the presence of biofouling deposits, again no correlation could be found. The raw water supplies

for Duvha and Matla Power Stations contained the highest chlorophyll *a* content, (12.3 and 14.8 $\mu g.l^{-1}$ respectively) and biofouling deposits were present in these systems (Table 3.4). Although biofouling deposits were observed at Grootvlei and Komati Power Stations, the chlorophyll *a* content of both the raw water supplies for those power stations was only 2.7 $\mu g.l^{-1}$ (Table 3.4). Evidence of sessile algal growth was observed in 50% of the cooling towers and clariflocculators (Table 3.6). The microscope examination of these algal deposits and the recirculating waters determined that they consisted predominantly of filamentous blue green algae. The predominant algal groups in the raw waters were, however, green algae and diatoms. It has been reported that blue green algae are the most prominent group of algae found in cooling systems, as was found during this study (McCoy, 1980). However, no explanation is given for this phenomenon.

Condenser Inspections

Condenser inspections were carried out at all power stations where general maintenance outages were in progress, thus allowing entrance into the cooling water systems (Table 3.7). SRB were identified as detailed in Table 3.3 and *Gallionella* as per ASTM D932-72 (1972).

Table 3.7 : Visual inspections of condensers at five power stations.

POWER STATION	INSPECTION POINT	OBSERVATIONS
3.[a]	Cross-over loop of condenser 3.	Blisters in epoxy coating, shallow pitting of mild steel, SRB present.
4.	Cooling water strainer boxes.	Numerous nodules overlying shallow pits in the mild steel, SRB present.
5.	Cross-over loops of condensers 1, 3 and 5.	Numerous nodules overlying shallow pits in the mild steel, SRB present. *Gallionella* in 20% of the 10 nodules sampled.
8.	Cross-over loop of condenser 6.	Blisters in epoxy coating, shallow pitting of mild steel, SRB present.
9.	Cross-over loop of condenser 3.	Blisters in epoxy coating, shallow pitting of mild steel, SRB present.

[a] for key to figures see Table 3.1

All of the condenser inspections revealed evidence of MIC. Evidence of MIC was shown at Grootvlei Power Station even though only 5 $SRB.ml^{-1}$ were enumerated in the recirculating cooling water (Table 3.5). SRB were isolated from condenser pipework which had been protected with an epoxy coating. Epoxy coatings do offer a measure of protection against MIC. However, where there are defects in the coating, MIC attack of the underlying mild steel can still occur (Severyn, 1990).

The reasons for the inconsistencies in the results and observations made during this study could encompass a number of environmental and physiological changes that occur when raw water enters a cooling water system. The temperature of the water in certain areas of a cooling system is 10 - 20°C higher than it would be under ambient conditions, thus increasing the growth rate of microorganisms (McCoy, 1980). Due to cycling of the water, nutrients are more freely available and the pH of the water is increased. Oxygen is also freely available as oxygenation

of the water also occurs as a result of turbulence, and the mechanism by which heat exchange is achieved in the cooling tower (Strauss and Puckorius, 1984). These changes in environmental conditions are expected to result in changes in the numbers of microorganisms (McCoy, 1980). However, sessile microbiological populations are predominant in aqueous environments (Costerton *et al.*, 1985). More than $1.0x10^4$ sessile bacteria have been quantified for each planktonic cell (Geesey *et al.*, 1978). Furthermore, sessile microorganisms form highly organised microbial communities, in which nutrients may be cycled (Costerton *et al.*, 1985). This phenomenon could explain why no direct correlation could be found between sulphate levels in the recirculating cooling water and SRB numbers, and between total aerobic bacteria and cycles of concentration. The recirculating cooling water can also be further contaminated by windborne microorganisms (Bott *et al.*, 1983). Evidence of this type of contamination, is the number of microorganisms isolated from the recirculating cooling waters at Kendal and Matimba Power Stations. The recirculating waters contained all groups of microorganisms, whereas the raw water supply to both these systems was potable water which had been chlorinated and thus contained low numbers of all the groups of microorganisms. These microorganisms could therefore have been introduced into the recirculating cooling water from the surrounding environment.

Changes in the environmental conditions that microorganisms are exposed to on entering a cooling water system, could also explain the shifts in the frequency polygons for aerobic and anaerobic bacteria between the raw water supplies and the recirculating cooling waters. Higher numbers of bacteria were enumerated in the recirculating cooling waters. In addition, the environmental conditions in each system are unique. Thus a normal distribution pattern would not be expected due to variations in operating temperatures and chemical parameters.

CONCLUSIONS

The quality of the raw waters may contribute to the microbiological composition of the recirculating cooling water. However, other prevailing environmental and system conditions such as temperature and pH appeared to have a greater influence on the extent of microbiological contamination in any given system. This study emphasised the fact that each system was unique and thus no generalisations could be made in terms of the presence or activity of microorganisms. The need to monitor and evaluate the microbiology of each individual system was highlighted.

ACKNOWLEDGEMENTS

The staff of all power stations surveyed are gratefully acknowledged for their assistance.

Chapter 4

BIOFOULING CONTROL BY BIOCIDE AND BIODISPERSANT TREATMENT IN POWER STATION OPEN RECIRCULATING COOLING WATER SYSTEMS

ABSTRACT

Cooling water treatment programmes were monitored in the open recirculating cooling water systems at four fossil fired power stations. Combinations of three biodispersants and four biocides were evaluated. The bulk water and a Robbins Device biofouling monitor were analysed microbiologically for total aerobic and anaerobic bacteria as well as sulphate reducing bacteria. Visual system inspections were carried out where possible. All of the biodispersants resulted in increases in the numbers of planktonic bacteria, ranging from 22592% to 654%. Biocides resulted in percentage kills of planktonic bacteria of between 83.2% and 100%, with only one exception, where no change in the numbers of anaerobic bacteria occurred. The biodispersants resulted in decreases in sessile aerobic bacteria numbers in 80% of the cases and the biocides resulted in decreases in all of the systems evaluated. Inspections at Grootvlei Power Station revealed that the biodispersant/biocide combination removed sessile microbiological deposits and aided in the penetration of the biocides into inorganic deposits by softening the overlying nodules. The use of combinations of biodispersants and biocides effectively controlled microbiological growth in all the cooling water systems. However, the treatment products did not produce the same effects in different systems. Thus, biodispersants and biocides have varied effects and treatment products have to therefore be evaluated for each individual system.

INTRODUCTION

To conserve water, modern power stations are operated on the policy of zero liquid discharge. This policy ensures that no water is discharged back into public water sources, but is treated and reutilised in the power station (Nell and Aspden, 1990). In an attempt to further conserve water, open recirculating cooling water systems are operated at increased cycles of concentration (8 - 20 cycles). Due to this cycling of water, nutrients and microorganisms are concentrated in the water and consequently, problems associated with unchecked microbiological growth occur (McCoy, 1980). These problems include biofouling and microbiologically influenced corrosion (MIC), particularly that caused by the influence of the sulphate reducing bacteria (SRB) (Pope, 1987). These types of problems are commonly experienced in power utility cooling water systems (Fellers, 1990; Puckorius, 1991).

One of the methods of controlling microbiological growth is the use of biocides (Matson and Characklis, 1982). Oxidising and non-oxidising biocides have been tested in cooling water systems and it has been found that planktonic microbiological populations quickly recover from the effect of these biocides (Cloete *et al.*, 1989). In addition, it has been widely reported that planktonic bacteria are more susceptible to the action of biocides than are their sessile counterparts (Le Chevallier *et al.*, 1988; Blenkinsopp and Costerton, 1991). It is, however, still common practice to add biocides to cooling water systems to control sessile bacteria and thus alternative treatments need to be evaluated.

One of the alternate treatments for the control of microbiological growth in open recirculating cooling water systems, is the use of biodispersants (Poulton and Nixon, 1990). An effective biodispersant should disperse sessile microbial populations into the bulk water, rendering them more susceptible to the action of biocides (Strauss and Puckorius, 1984). Furthermore, biodispersants should aid in the penetration of biocides into inorganic deposits, thus assisting in the destruction of SRB growing in anaerobic areas. These deposits are at the same time softened, allowing their removal by the turbulence of the circulating water (Hart *et al.*, 1990; Lutey and Allison, 1991). Laboratory and field studies carried out by Lutey *et al.* (1989), showed that a biodispersant was effective in removing established biofilms and resulted in an increase in the number of planktonic bacteria. In addition, the biodispersant was shown to restrict the formation of biofilms on clean surfaces, was able to mitigate MIC and had no effect on biocide efficacy.

Thus, if bacteria could be dispersed into the bulk water prior to the addition of a biocide, the biocide would be used more cost effectively. Biodispersants are generally less costly than biocides and can be used at lower concentrations. It is unlikely that biodispersants will have any mutagenic effects on bacteria, or that microorganisms would be able to become resistant to the action of biodispersants, as can be the case with biocides (Russell, 1990; Brözel and Cloete, 1991).

No correlation has been established between the numbers of sessile and planktonic bacteria in cooling water systems (Cloete *et al.*, 1989). It is therefore essential, to monitor both the planktonic and sessile microbiological phases, in order to establish the efficacy of a microbiological treatment programme (Wolfaardt *et al.*, 1991). One of the techniques commonly used to monitor sessile bacteria is the Robbins Device biofouling monitor (Blenkinsopp and Costerton, 1991). This device has been in use in South Africa for a number of years and is reported to be an effective method of monitoring sessile bacteria (Costerton *et al.*, 1986).

The objectives of this study were to evaluate the effect of combinations of biocides and biodispersants on both planktonic and sessile microorganisms, in the open recirculating cooling water systems at four fossil fired power stations.

MATERIALS AND METHODS

Biocide and biodispersant treatment programmes were monitored in the open recirculating cooling water systems at four different fossil fired power stations.

System parameters

The system parameters of the four power stations where treatment programmes were monitored are detailed in Table 4.1. As Tutuka Power Station has two separate cooling water systems, two different biodispersant/biocide treatment programmes were monitored simultaneously.

Table 4.1 : System parameters of four open recirculating cooling water systems where treatment programmes were monitored to determine their efficacy in controlling microbiological growth.

POWER STATION	SYSTEM VOLUME (MEGALITRES)	CYCLES OF CONCENTRATION
1. Grootvlei	36	10
2. Lethabo (west)	64	15
3. Matimba	0.7	5
4. Tutuka (west)	64	20
5. Tutuka (east)	64	20

Treatment chemicals

Three biodispersants and four biocides were tested at concentrations recommended by the suppliers (Table 4.2).

Table 4.2 : Biodispersants and biocides utilised for the treatment of the open recirculating cooling water systems at four power stations.

PRODUCT	DESCRIPTION
BIODISPERSANT	
A	Liquid non-ionic penetrant/surfactant
B	Liquid non-ionic biodispersant
C	Liquid anionic biodispersant
BIOCIDE	
A	Dithiocarbamate
B	Organosulphur
C	Bromine containing oxidising biocide
D	Isothiazolin

The biodispersant/biocide combinations used at the various power stations, the dosing method, time of addition and concentrations used are detailed in Table 4.3.

Table 4.3 : Biodispersants and biocides programmes used to treat the open recirculating cooling water systems at four power stations.

POWER STATION	BIODISPERSANT				BIOCIDE			
	PRODUCT	DOSING METHOD	TIME[a] (h)	CONC (ppm)	PRODUCT	DOSING METHOD	TIME[a] (h)	CONC (ppm)
1.[b]	A[c]	continuous	2.0	12.0	A[c]	slug	46.5	12.0
					B	slug	46.5	12.0
2.	A	continuous	2.0	7.0	C	slug	95.0	8.0
3.	A	slug	2.0	21.0	A	slug	42.0	49.0
		slug	5.0	14.0	B	slug	42.0	84.0
		slug	17.0	14.0				
		slug	53.5	14.0				
4.	B	slug	2.0	9.0	D	slug	24.0	21.0
5.	A	continuous	2.0	8.0	A	slug	68.0	11.0

[a] time in hours after the start of each evaluation
[b] For key to figures see Table 4.1
[c] For key to letters see Table 4.2

Robbins Device biofouling monitors

Robbins Device biofouling monitors were installed on by-pass lines, on the "hot" side of the cooling water systems where the temperature (37°C), was considered to be favorable for microbial growth. Robbins Devices were installed two to four weeks before the start of the evaluation, to allow a biofilm to develop on the sample stud surfaces. Robbins Devices were constructed of mild steel, with nylon studs in brass holders. A water velocity of between 1 and 1.2 $m.s^{-1}$ was maintained through the devices.

Sampling times for microbiological analysis

The sampling times varied for each evaluation, depending on the individual system parameters and conditions. The sampling times, in hours after the start of each evaluation, are detailed in Table 4.4.

Table 4.4 : Sampling times for microbiological analysis at four power stations where biodispersant/biocide treatment programmes were monitored.

	POWER STATION									
	1[a]		2		3		4		5	
	BW[b]	RD[c]	BW	RD	BW	RD	BW	RD	BW	RD
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2.5	23.0	2.0	30.0	5.0	3.0	3.0	21.0	3.0	21.0
	4.5	53.0	5.0	50.0	17.0	41.0	9.0	45.0	21.0	31.5
S	7.0	72.0	7.0	122.0	21.0	49.0	21.0	67.5	25.5	45.0
A	11.0		10.0	223.0	25.0	65.5	27.5	76.0	29.5	67.5
M	15.0		13.0		41.0	113.0	31.5	92.0	31.5	92.0
P	19.0		16.0		45.0		39.0		39.0	
L	23.0		19.0		49.0		45.0		45.0	
I	26.5		23.0		59.0		57.0		67.5	
N	32.0		27.0		65.5		53.0		92.0	
G	36.0		31.0		89.5		67.5		116.0	
	45.0		35.0		113.5		69.0		140.0	
T	46.5		40.0				70.0		164.0	
I	47.5		45.0				72.0			
M	49.5		50.0				74.0			
E	53.0		59.0				75.0			
S	58.0		69.0				76.0			
	63.0		75.0				80.0			
	68.0		98.0				84.0			
(h)	72.0		117.0				88.0			
			122.0				92.0			
			146.0				116.0			
			223.0				140.0			
							164.0			

[a] For key to numbers see Table 4.1
[b] BW = bulk water
[c] RD = Robbins Device

Sampling procedure

One 500 ml sample was taken from the recirculating cooling water of all systems for each analysis, in a sterile Whirl Pak bag (Nasco, USA). Recirculating cooling water samples were taken from sample points on the "hot" side of the system.

Two studs were removed from the Robbins Device, for each sampling time using sterile forceps. Studs were placed into 10ml sterile, quarter strength Ringer's solution, immediately after removal from the Robbins Device. Prior to analysis, the studs were agitated on a vortex mixer for 2 min, to disperse the sessile bacteria on the stud surfaces. The resultant suspension was then analysed as described in Table 4.5. All samples were analysed within 12 h of sampling and retained at 4°C until they were analysed.

Microbiological analysis

Samples were diluted in sterile, quarter strength Ringer's solution and subjected to duplicate plate counts. All incubation approximated the temperature of the systems from which the samples were taken, i.e. 37°C. Anaerobic incubation was carried out in an anaerobic jar, filled with nitrogen. The techniques used for microbiological analyses are detailed in Table 4.5. Plates containing between 30 and 300 colonies were counted.

Table 4.5 : Techniques used to quantify planktonic and sessile bacteria in four open recirculating cooling water systems where biodispersant/biocide treatment programmes were monitored.

MICROBIAL TYPE	TECHNIQUE	INCUBATION TIME (d)	ATMOSPHERE	GROWTH MEDIUM
Total aerobic bacteria	Pour plate	2	aerobic	Nutrient Agar (Biolab)
Total anaerobic bacteria	Pour plate	3	anaerobic	Nutrient Agar (Biolab)
SRB	Agar tubes	14	anaerobic	SABS method 1497-1989

Calculations to determine changes in numbers of planktonic and sessile bacteria

Percentage increases in planktonic bacteria were calculated as follows :

$$\% \text{ increase} = \frac{\text{Increase in number of bacteria after treatment}}{\text{original number before treatment}} \times 100$$

Percentage kills or decreases in planktonic and sessile bacteria were calculated as follows :

$$\% \text{ kill or decrease} = 100 - \left(\frac{\text{Number of survivors}}{\text{original number}} \times 100\right)$$

Visual inspections

Visual inspections were carried out at Grootvlei Power Station before the initiation of treatment, and then after three weeks and after nine months of treatment. It was possible to inspect this system as the cooling water strainer boxes, located at the condenser inlets, could be isolated and inspected while the system was in operation. Deposits observed in the strainer boxes were removed using sterile forceps and tested for the presence of SRB (Table 4.5). The cooling towers were also visually inspected for signs of algal growth.

RESULTS AND DISCUSSION

Microbiological analyses

For practical reasons, at some of the power stations, it was not possible to analyse the anaerobic bacteria for the duration of the evaluation. In addition, all the systems could not be monitored for the same length of time.

Numbers of both planktonic and sessile bacteria quantified in the bulk water and by means of the Robbins Device, are shown in Figures 4.1 - 4.5.

Changes in the numbers of planktonic and sessile bacteria, after biodispersant and biocide treatment, are detailed in Table 4.6.

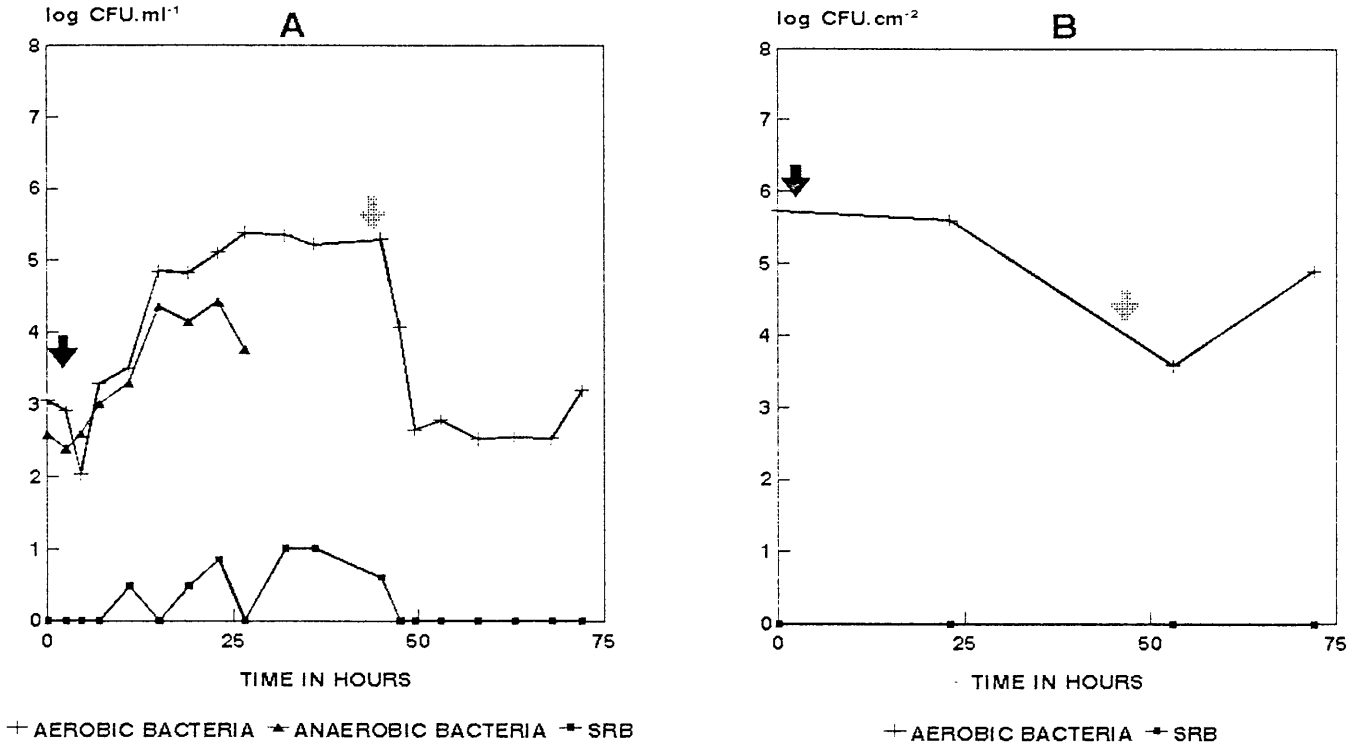


Figure 4.1 : Microbiological analysis of bulk water (A) and Robbins Device studs (B) at Grootvlei Power Station. ⬇ indicates biodispersant addition and ✣ biocide addition.

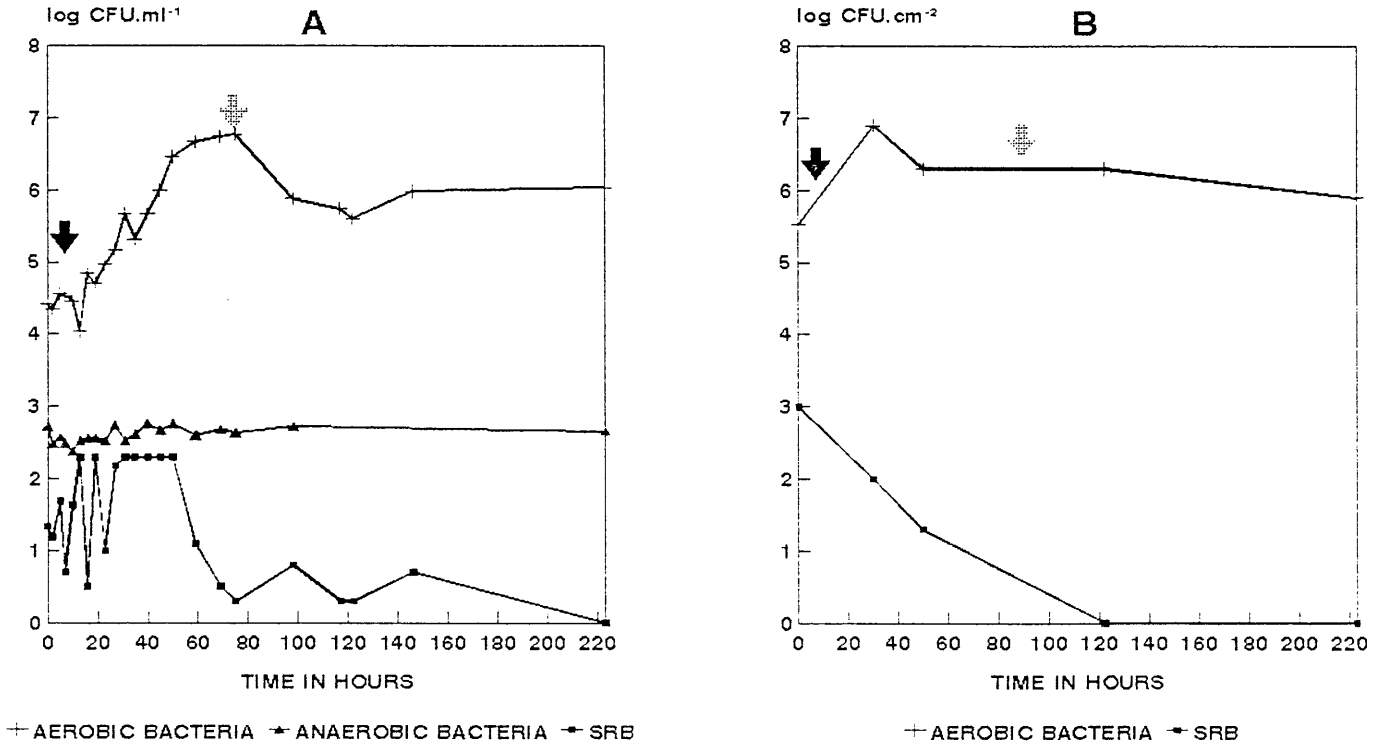


Figure 4.2 : Microbiological analysis of bulk water (A) and Robbins Device studs (B) at Lethabo Power Station. ⬇ indicates biodispersant addition and ✣ biocide addition.

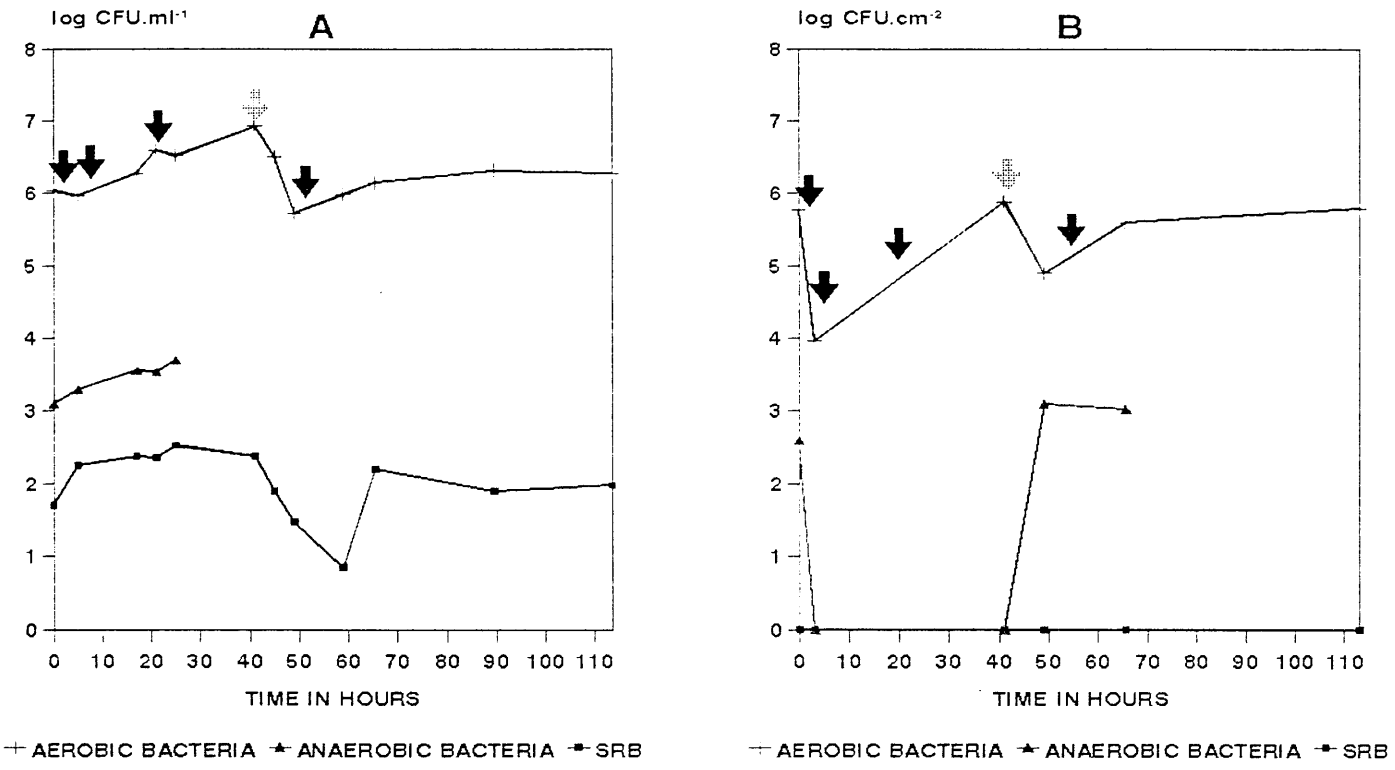


Figure 4.3 : Microbiological analysis of bulk water (A) and Robbins Device studs (B) at Matimba Power Station. ↓ indicates biodispersant addition and ✣ biocide addition.

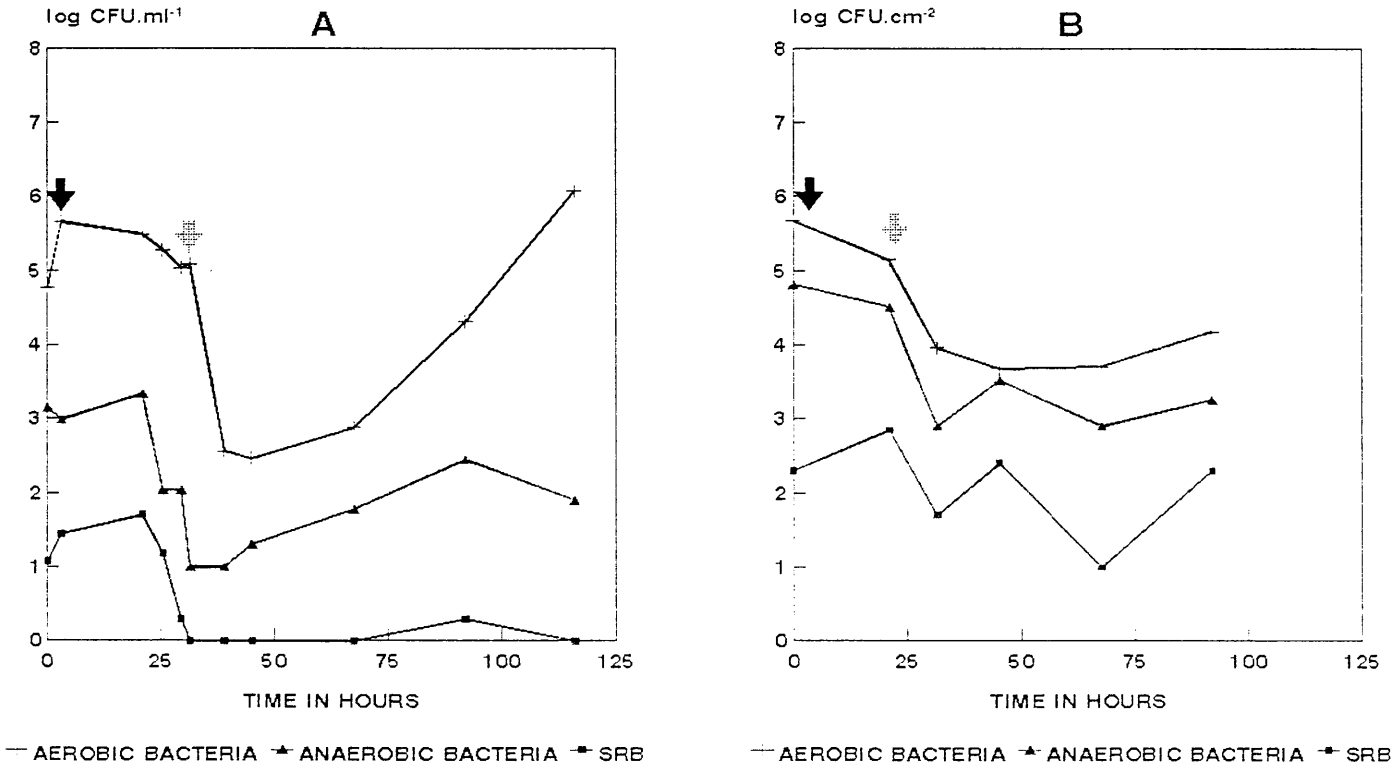


Figure 4.4 : Microbiological analysis of bulk water (A) and Robbins Device studs (B) at Tutuka (W) Power Station. ↓ indicates biodispersant addition and ✣ biocide addition.

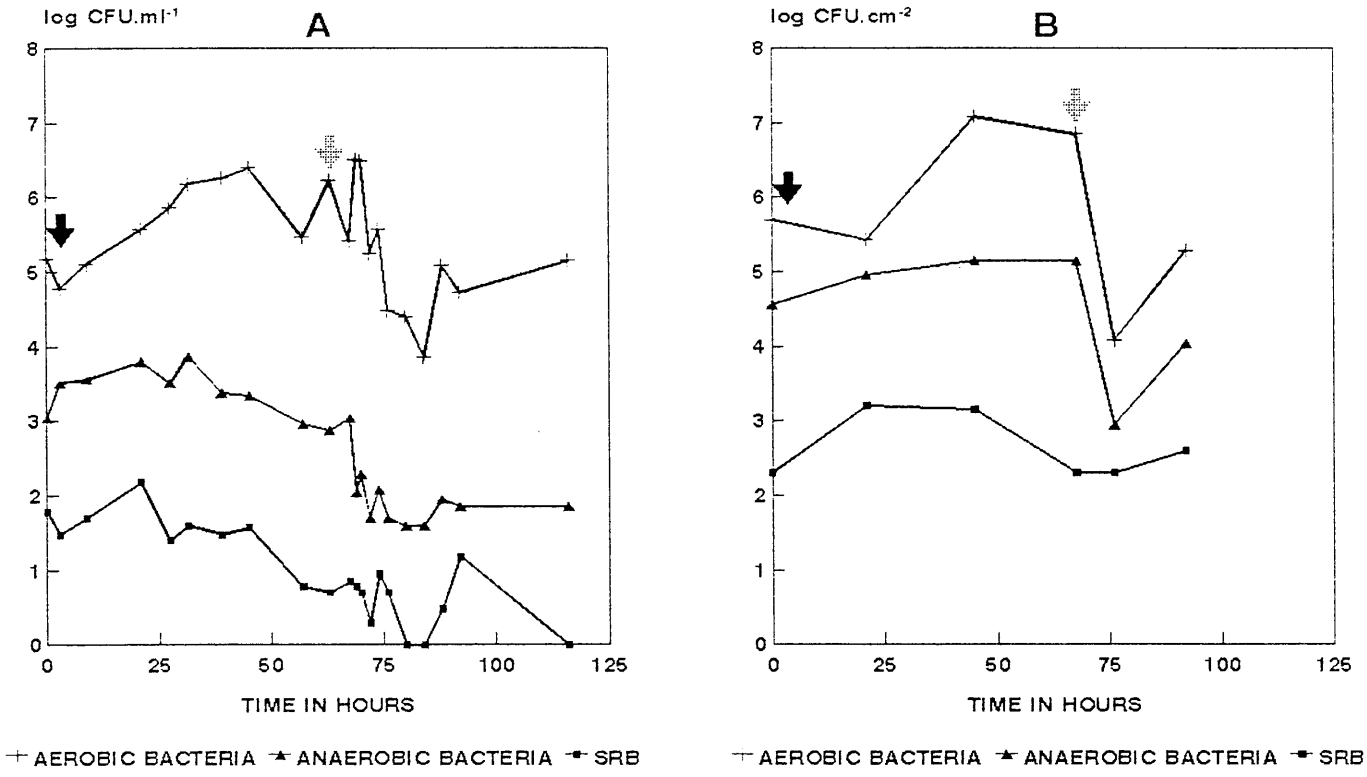


Figure 4.5 : Microbiological analysis of bulk water (A) and Robbins Device studs (B) at Tutuka (E) Power Station. ⬇ indicates biodispersant addition and ✣ biocide addition.

Table 4.6 : Planktonic and sessile bacteria numbers in the four open recirculating cooling water systems treated with biodispersant/biocide combinations.

		BIODISPERSANT (% increase, planktonic) (% decrease, sessile)			BIOCIDE (% kill)		
	POWER STATION	Aerobic Bacteria	Anaerobic Bacteria	SRB	Aerobic Bacteria	Anaerobic Bacteria	SRB
PLANKTONIC	1[a]	21718.0	1000.0	7005.0	99.8	NM[b]	100.0
	2	22592.0	650.0	90.0	92.0	NC[c]	100.0
	3	654.0	287.0	576.0	83.2	NM	91.0
	4	667.0	57.0	316.0	97.2	99.5	100.0
	5	1033.0	573.0	2400.0	99.9	96.4	100.0
SESSILE	1	26.0	NM	ND[d]	98.0	NM	ND
	2	NC	NM	98.0	60.0	NM	100.0
	3	98.3	100.0	ND	89.9	0.0	ND
	4	70.2	49.0	NC	96.6	97.6	92.9
	5	46.0	NC	NC	99.8	99.4	NC

[a] For key to figures see Table 4.1
NM[b] = not monitored
NC[c] = no change or increase
ND[d] = not detected

Changes in planktonic bacteria numbers with the addition of a biodispersant to the bulk water

Little published information is available on the effects of biodispersants on sessile microorganisms in cooling water systems. However, in all the systems monitored, the addition of a biodispersant when slug or continuously dosed, resulted in appreciable increases in the numbers of planktonic microorganisms. These increases varied from 22592% for total aerobic bacterial counts at Lethabo Power Station, to 654% at Matimba Power Station (Table 4.6). The lowest percentage increase in total aerobic bacterial counts (654%), occurred at Matimba Power Station, over a 42 h period (Table 4.6). The dominant bacterial genus in South African cooling water systems is reported to be *Pseudomonas* (Cloete *et al.*, 1989). The minimum recorded generation time for *P. putida*, under ideal conditions, is 45 minutes (Stanier *et al.*, 1986). As a cooling water system is a low nutrient environment, it is unlikely that bacterial growth would be occurring at the maximum division rate (McCoy, 1980). Thus, the increases in the numbers of planktonic bacteria in the bulk water may be attributed to the action of the biodispersant. This assumption is supported by the fact that increases in the numbers of planktonic, total anaerobic bacteria and SRB were also recorded. As it is unlikely that anaerobic bacteria would multiply in the aerobic bulk water, these bacteria must have been released from biofilms. This indicated that the biodispersants were able to penetrate not only the upper aerobic regions of a biofilm, but also the underlying anaerobic areas (Lutey and Allison, 1991).

Although increases in planktonic bacteria numbers were recorded each time a particular biodispersant was added to the bulk water, these increases were not comparable to each other. For example, the same biodispersant (A), was added continuously to the cooling water systems at Lethabo, Grootvlei and Tutuka (East) Power Stations at 7, 12 and 8ppm respectively and slug dosed at Matimba Power Station at 21ppm (Table 4.3). The corresponding increases in planktonic aerobic bacteria numbers observed at these power stations were 22592%, 21718%, 1033% and 654%, respectively (Table 4.6). However, parameters such as the chemical composition of the water, ambient conditions, extent of initial fouling and raw water source varied from one system to another. These differences in system conditions may account for the variations in the effects of a particular biodispersant.

Changes in the planktonic bacteria numbers with the addition of a biocide to the bulk water

Percentage kills of the planktonic bacteria of between 83 - 100% were achieved by all of the biocides evaluated (Table 4.6). As for the biodispersants, the same biocide did not always achieve the same effect in different systems. A combination of biocides A and B was added to the cooling water at Grootvlei Power Station at concentrations of 12ppm of each product and

at Matimba Power Station at concentrations of 49ppm of biocide A and 84ppm of biocide B (Table 4.3). However, a percentage kill of 99,8% of the planktonic, aerobic bacteria was recorded at Grootvlei Power Station and only 83,2% at Matimba Power Station (Table 4.6). At Tutuka Power Station (east) where only biocide A was added, a percentage kill of 99.9% of aerobic bacteria was achieved (Table 4.6). The variations in the numbers of planktonic microorganisms as a result of biocide addition, was again attributed not only to the different products and dosage regimes, but also to the extent of fouling already established in the systems. It was noted that after the initial decrease in planktonic bacterial numbers, following the addition of a biocide, a subsequent increase was recorded. This phenomenon has been reported by other researchers and may be due to the recovery of resistant bacteria, or a as a result of the low retention time of a slug dose of biocide in a cooling water system (Cloete *et al.*, 1989). In those systems where a biodispersant was continuously added, this increase may also have been due to the continuing action of the biodispersant.

Changes in the sessile bacteria numbers with the addition of biodispersants or biocides to the bulk water

As with the planktonic bacteria, considerable variations in the effect of a particular biodispersant or biocide on sessile bacteria, were noted. Biodispersant A achieved a 26% decrease in attached bacterial numbers at Grootvlei (12ppm), a 98.3 % decrease at Matimba (21 and 14ppm) while an increase in sessile bacteria was recorded at Lethabo Power Station (7ppm) (Tables 4.3 and 4.6). The combination of biocides A and B achieved percentage kills of sessile aerobic bacteria of 98% at Grootvlei Power Station and 89.9% at Matimba Power Station (Table 4.6). Biocide A achieved a 99.8% kill of sessile aerobic bacteria at Tutuka Power Station (Table 4.6). These variations could be due to the differences in system conditions or to the inaccuracy of the monitoring device. For example, SRB were not detected on the studs removed from the Robbins Device at two of the power stations. This could be due to the fact that tearing of the biofilm may occur when studs are removed from the Robbins Device. Furthermore, it has been shown that biofilms may be unevenly distributed over metal surfaces in recirculating cooling water systems (Characklis *et al.*, 1990; Donlan *et al.*, 1990). This uneven distribution could affect the detection of not only SRB, but the total number of sessile bacteria detected by biofouling monitors with a small sample surface area, such as the Robbins Device. The accuracy of the Robbins Device will be addressed in a later chapter.

Visual Inspections

The results of the visual inspections are detailed in Table 4.7. and in Figures 4.6 - 4.10.

Table 4.7 : Visual inspections of the open recirculating cooling water system at Grootvlei Power Station before and during the implementation of a biodispersant/biocide treatment programme.

TIME OF INSPECTION	LOCATION	OBSERVATIONS
Before initiation of treatment	Cooling towers	Biofouling deposits consisting primarily of blue green algae attached to the support columns and hanging in strands from the packing (Figure 4.6).
After three weeks	"	Virtually all biofouling deposits removed or lost green colouration (Figure 4.6).
After nine months	"	Slight regrowth of algae in spray area. Packing and support columns free of algae (Figure 4.7).
Before initiation of treatment	Strainer boxes	Hard nodules on mild steel, covering shallow pits filled with a black liquid. All of the 10 nodules sampled contained SRB (Figure 4.8).
After three weeks	"	Nodules softened, could be brushed off the surface by hand. Many of the pits had lost the black colouration. Half of the 10 nodules sampled contained SRB (Figure 4.9).
After nine months	"	The majority of the nodules removed from metal surface. Only nodules in low flow areas contained SRB (Figure 4.10).

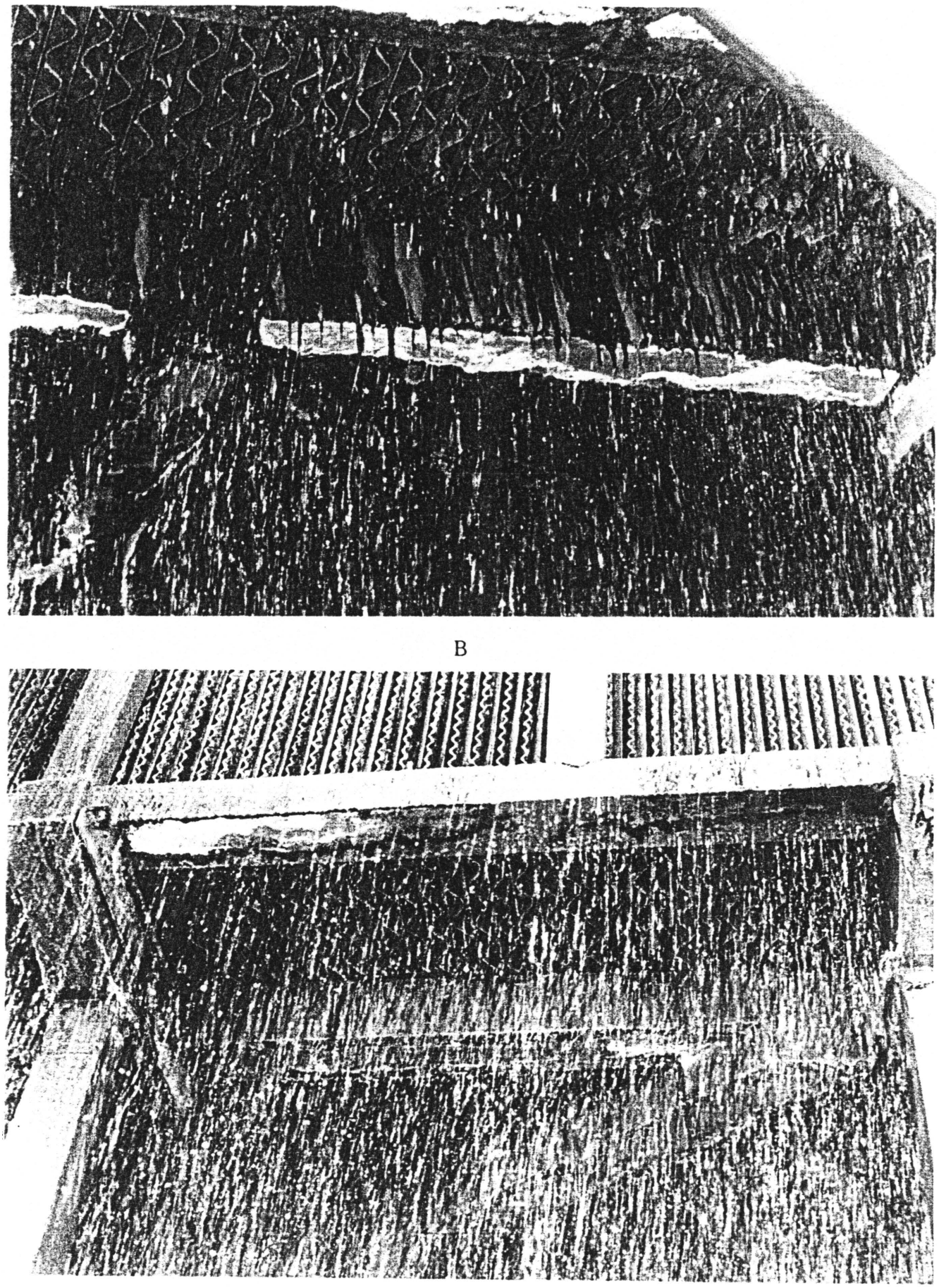


Figure 4.6: Biofouling deposits at Grootvlei Power Station on cooling tower packing before biodispersant/biocide treatment (A) and packing clear of biofouling deposits after three weeks of treatment (B).

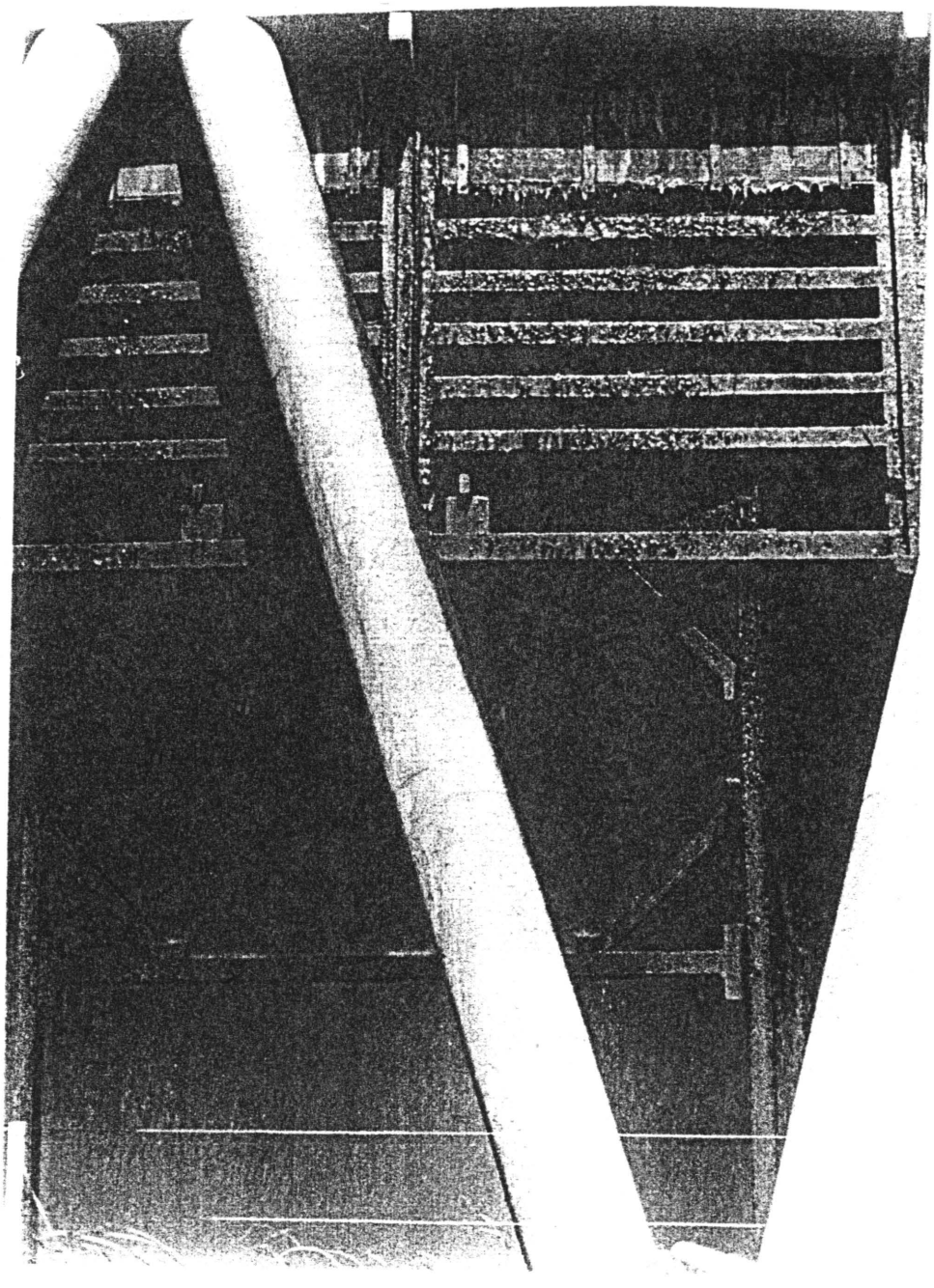


Figure 4.7: Slight algal regrowth in the spray area of the cooling tower at Grootvlei Power Station, after nine months of biodispersant/biocide treatment.

A

B

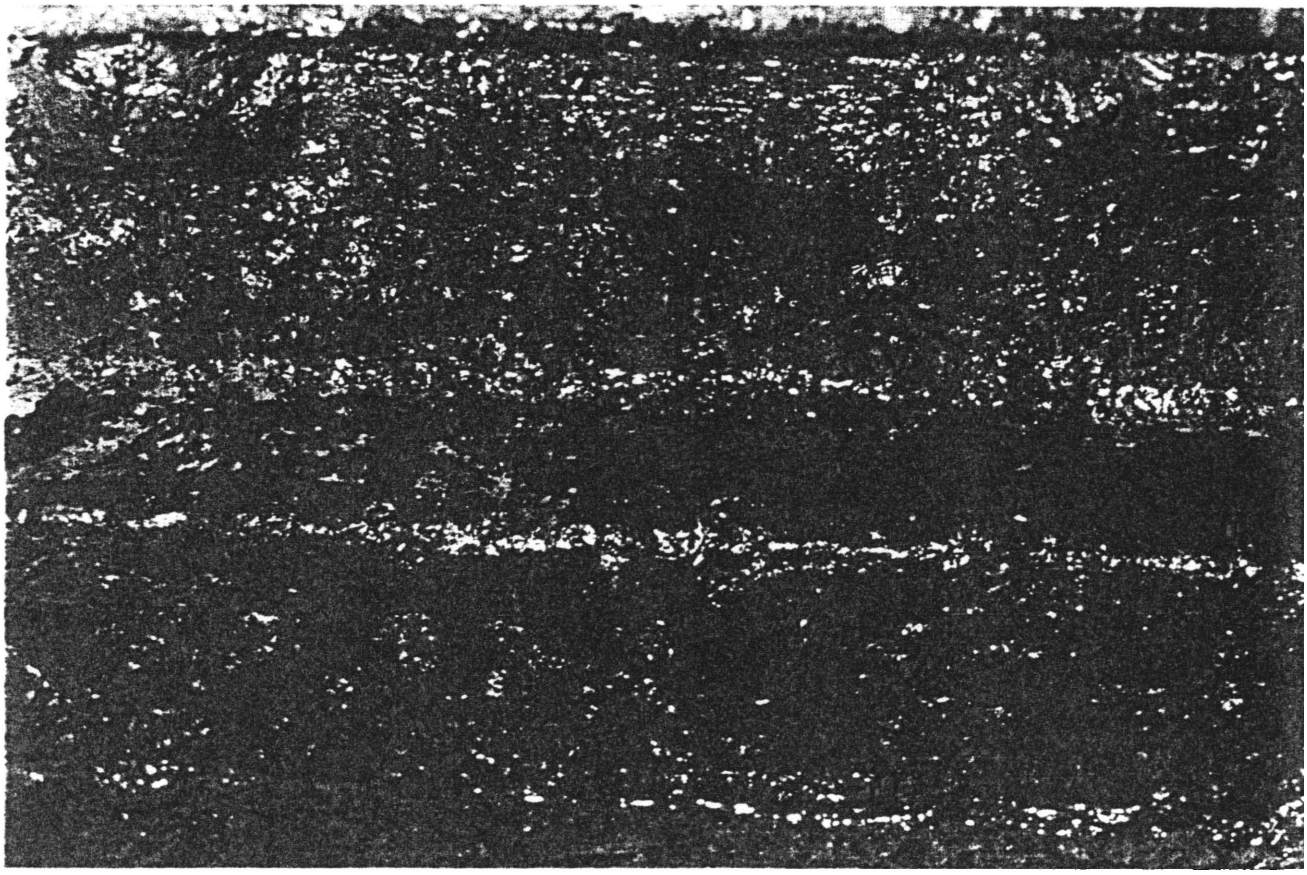
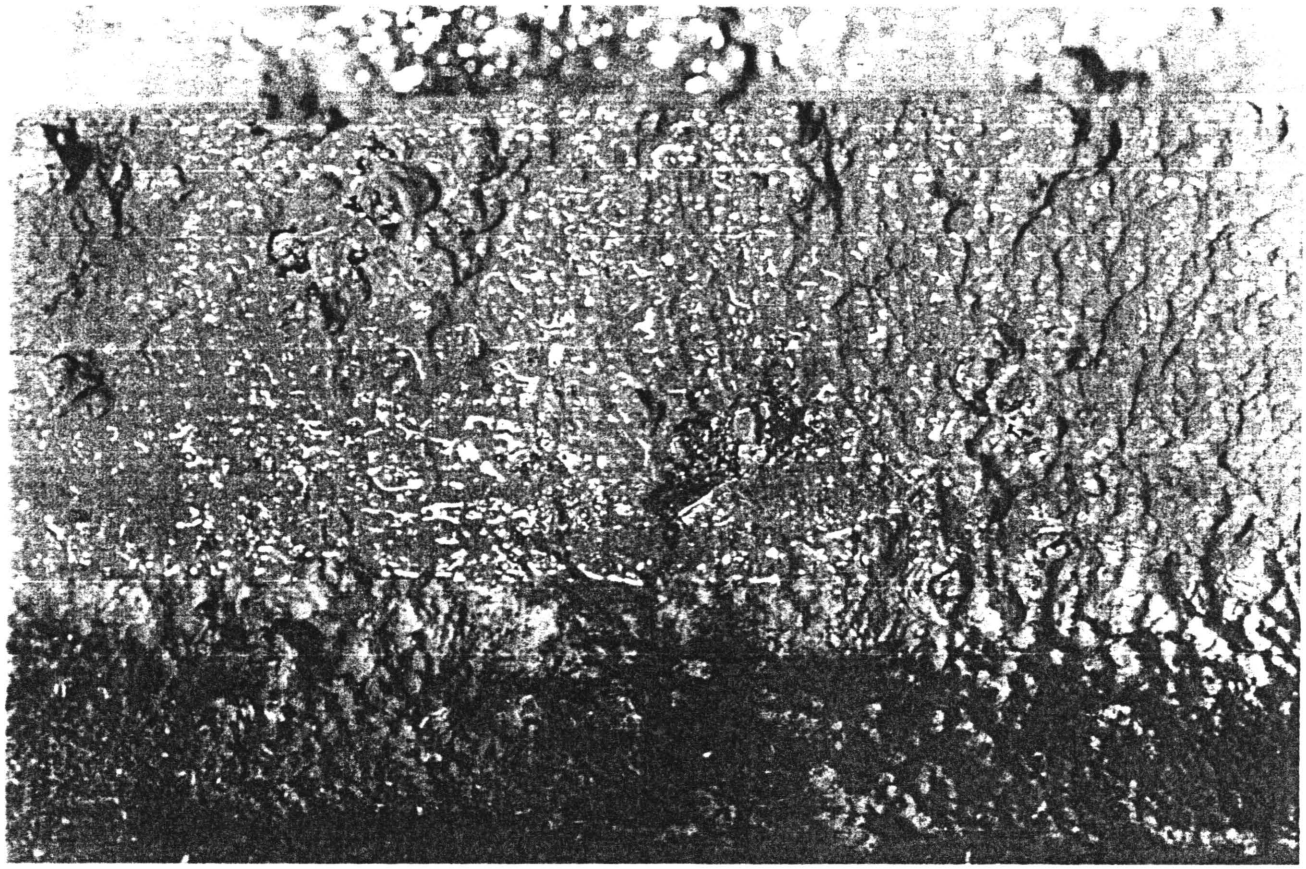


Figure 4.8 : Strainer box at Grootvlei Power Station before the initiation of biodispersant/biocide treatment showing hard nodules on surface (A) and underlying pits filled with black liquid (B).

A



B

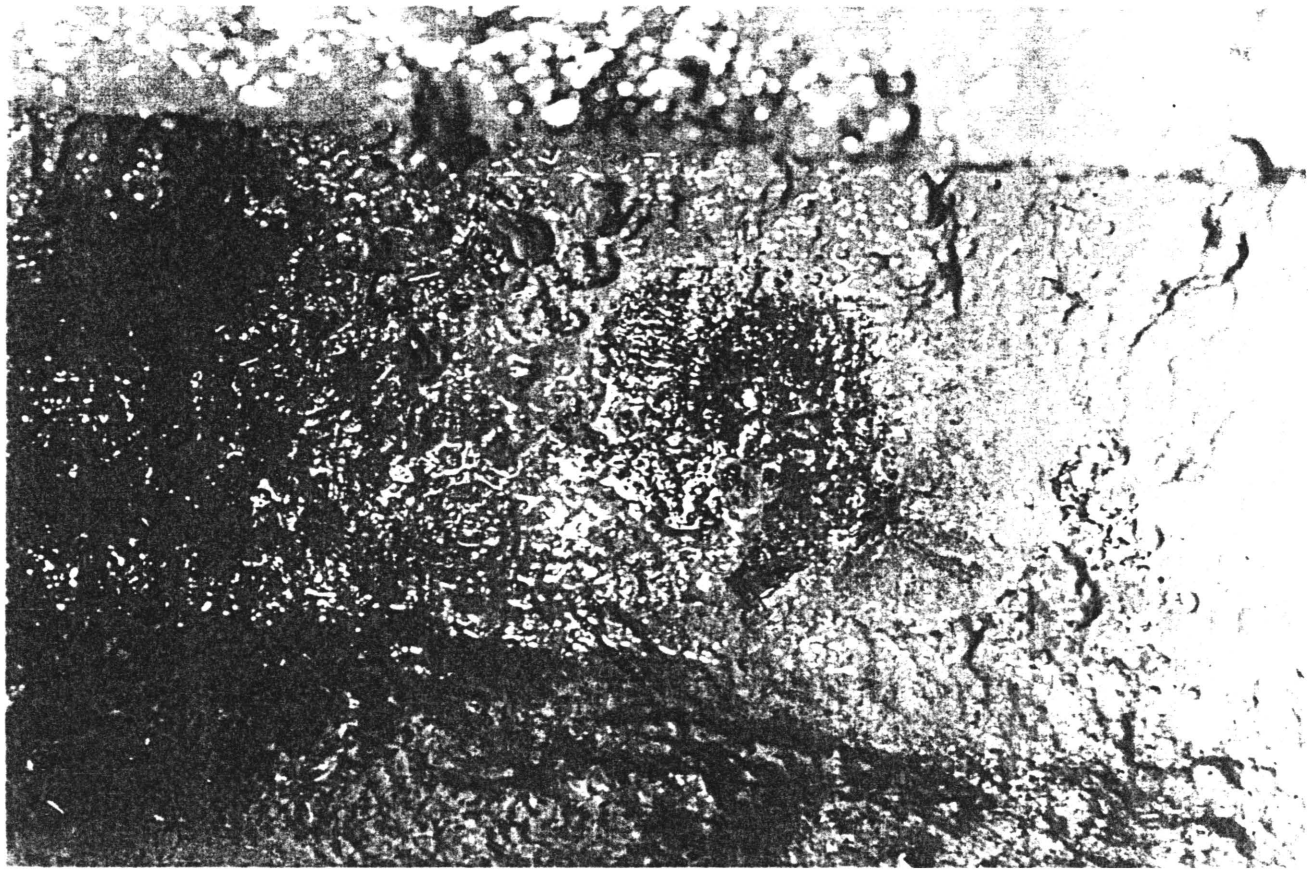


Figure 4.9 : Strainer box at Grootvlei Power Station after three weeks of biodispersant/biocide treatment showing softened nodules on surface (A) and loss of black colouration in underlying pits (B).

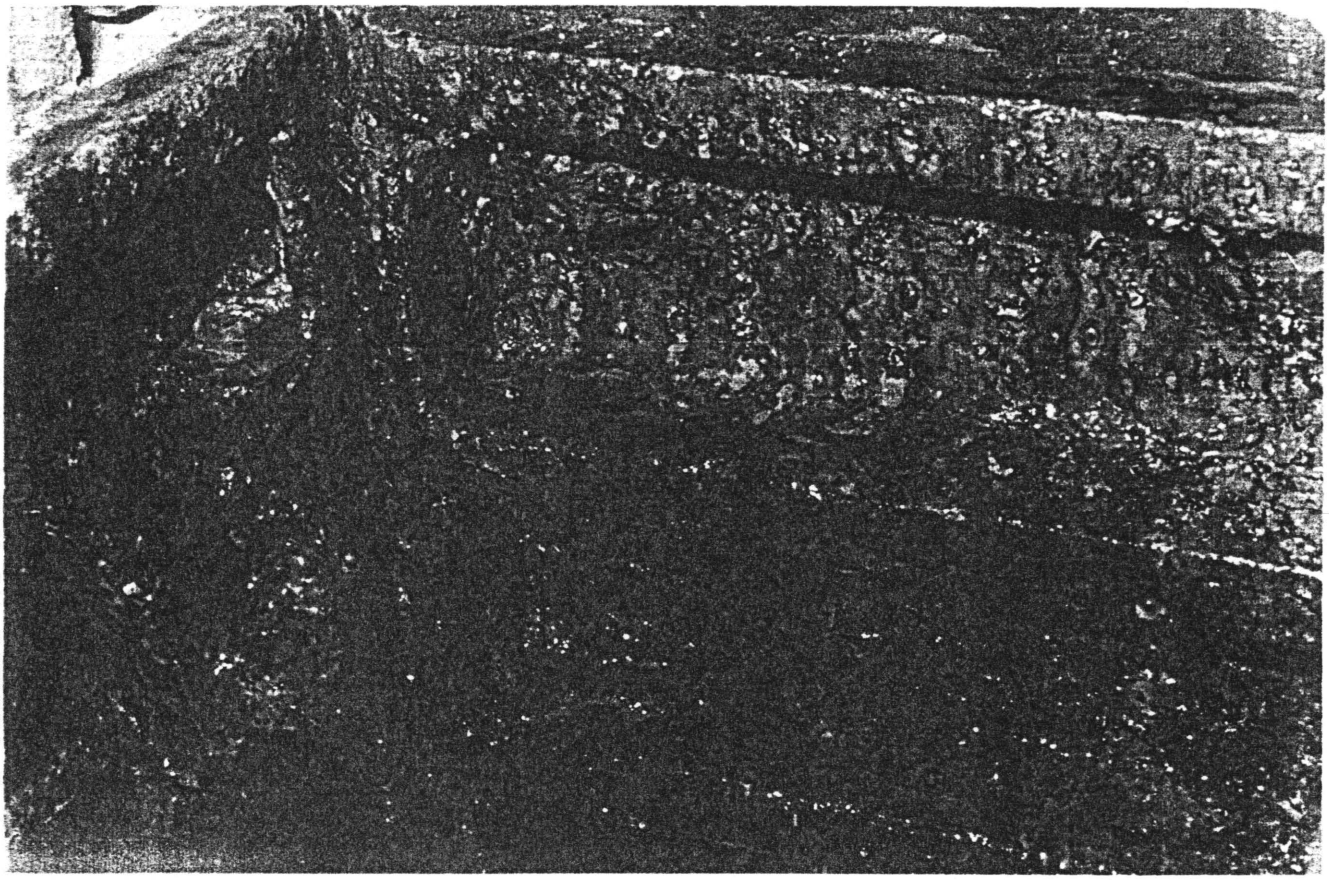


Figure 4.10 : Strainer box at Grootvlei Power Station after nine months of biodispersant/biocide treatment showing almost complete removal of nodules.

The visual inspections confirmed that a combination of a biodispersant and biocides was effective in removing biofilms, biofouling deposits and nodules of iron oxide, thus assisting in the mitigation of MIC (Table 4.7). After three weeks, the biofouling deposits on the cooling tower packing and clarifiers were either removed or had lost their green colouration, indicating cell death. In addition, little algal regrowth occurred over the nine month observation period. Thus the biodispersant/biocide programme was effective not only in the removal of biofouling deposits, but also in preventing any reattachment. The softening and removal of the nodules in the strainer boxes occurred over a longer time period. After three weeks of treatment, the nodules had been softened and only half of them contained SRB. However, it was only after nine months that almost complete removal of the nodules and thus mitigation of MIC occurred. These results were similar to those reported by Lutey *et al.* (1989), where a biodispersant was evaluated. This indicates that the biodispersant was the primary cause of biofouling deposit removal and mitigation of MIC, and not the biocide. However, the use of biocides, particularly in the "clean-up" phase of a treatment can assist with the control of those microorganisms dispersed into the bulk water, where they are more susceptible to the action of biocides (Blenkinsopp and Costerton, 1991).

CONCLUSIONS

The use of biodispersants in conjunction with biocides to control biofouling and MIC in cooling water systems operating at high cycles of concentration, was shown to be more effective than the use of biocides alone. The addition of a biodispersant aided in the dispersion of sessile bacteria into the bulk water where they are susceptible to the action of biocides. The accurate prediction of the effect of a particular biodispersant or biocide on the microbial populations in a cooling water system is not possible. In addition, the relative efficacy of different treatment products could not be determined. Therefore, biodispersants and biocides must be evaluated for each individual system.

ACKNOWLEDGEMENTS

The assistance of the power station personnel where treatment programmes were evaluated is gratefully acknowledged. The staff of Buckman Laboratories and Chemserve Systems are also acknowledged for their assistance and co-operation.

Chapter 5

BIOFOULING MONITORING DEVICE COMPARISON IN A COOLING WATER SYSTEM

ABSTRACT

Although different biofouling monitors are currently in use in South Africa, few studies, to determine which devices most accurately reflect biofouling conditions, have been conducted under standard operating conditions. A study was initiated to compare different biofouling monitoring devices under the same system parameters. Four monitoring devices, a Robbins Device, a modified Robbins Device, a Pedersen Device and a Barry's Device were evaluated in the main, open recirculating cooling water system at a power station. The devices were evaluated for four week periods, under three different dosing regimes, namely when no biofouling control chemicals were added to the system, when a biodispersant was slug dosed weekly at 15 ppm and when the biodispersant was continuously dosed at 10 ppm. Total aerobic, anaerobic, and hydrogen sulphide producing bacteria were quantified on the monitoring devices and in the bulk water. Scanning electron microscopy was carried out on the sampling surfaces of the monitoring devices. Experiments using the Pedersen Device were discontinued after one week, as actual system water velocities could not be achieved through the device. No correlation between the numbers of planktonic and sessile bacteria was found during any of the three dosing regimes. No statistically significant differences were found between the numbers of total aerobic bacteria recovered from the remaining three devices, when no biodispersant was added to the system and in the H_2S producing bacteria when biodispersant was slug dosed. Statistically significant differences were found between the numbers of all the other bacteria during the three dosing regimes. However, numbers of all bacteria recovered from the modified Robbins Device were consistently higher than those from the other devices. No visible differences in the biofilms on the sampling surfaces of the different biofouling monitors, could be distinguished by scanning electron microscopy. No statistically significant differences, in all the numbers of sessile bacteria recovered from the monitoring devices, were found between the three dosing regimes. The modified Robbins Device and the Barry's Device were found to be the most suitable biofouling monitors.

INTRODUCTION

It has been common practice to monitor the extent of microbiological contamination in industrial water systems by the enumeration of microorganisms in the bulk water (Wolfaardt *et al.*, 1991). However, microorganisms in aqueous environments exist in both sessile and planktonic phases (Costerton *et al.*, 1981; Characklis *et al.*, 1982). Microorganisms in the sessile phase form biofilms that cause deleterious effects in cooling water systems, such as decreased heat transfer, decreased flow rates and microbiologically influenced corrosion (MIC) and should therefore be monitored (Colturi and Kozelski, 1984).

Techniques that have been developed to monitor sessile microbial populations in water systems can be indirect, where the effects of these microbial populations are determined, or direct, where microbial numbers, or biomass, are quantified (Characklis *et al.*, 1982; Costerton and Lashen, 1983). Corrosion monitors or devices that measure decreases in heat transfer as a result of microbial activity, are classified as indirect techniques (Characklis *et al.*, 1982). Indirect techniques do not require trained personnel to carry out microbiological analyses and an instantaneous reading can usually be obtained (Mansfeld and Little, 1990; Tullmin *et al.*, 1992). The Robbins Device is commonly utilised as a direct technique to monitor biofilm development in water systems worldwide (Bondonno *et al.*, 1989). Other direct techniques commonly used in South Africa are the Pedersen Device (Pedersen, 1982) and the Barry's Device. The latter is a jointed pipe, 25mm in diameter, constructed from an inert material such as polyvinyl chloride (B. Luddick pers. comm.)*. The major advantages of direct techniques are that more comprehensive information on the microbiological and chemical composition of a biofilm can be obtained. The numbers of sessile bacteria per square centimetre, or the bacterial species present can for example be determined (Pedersen, 1982). Although there are many devices for the monitoring of biofilms in water systems, limited information is available on the comparison of these devices.

If direct monitoring techniques are used to quantify sessile microorganisms, it is essential to be able to enumerate, as accurately as possible, the microorganisms removed from the sampling surface of the monitoring device. Commonly, bacteria removed from such sampling surfaces have been quantified on standard nutrient media, such as Nutrient Agar. However, Brözel (1990) demonstrated that low nutrient media yielded the highest number of planktonic bacteria isolated from cooling water systems. In keeping with these results, half strength

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Nutrient Agar was found to be the most appropriate for the analysis of cooling water from Eskom power stations (M. Santa, pers. comm.)*. Historically, sulphate reducing bacteria have been identified as the major contributors to MIC in cooling water systems (Poulton and Nixon, 1990). It has however been suggested, that H_2S may play a role in anaerobic corrosion by directly attacking metal surfaces (McCoy, 1980). Numerous researchers have implicated H_2S producing bacteria in contributing to MIC (Pope *et al.*, 1982; Puckorius, 1983; Stoecker, 1984; Pope and Zintel, 1988). Mara and Williams (1970) demonstrated that Iron Sulphite Agar (ISA) could be utilised to quantify sulphate reducing bacteria (SRB), as well as other H_2S producing bacteria. Bacteria removed from the surfaces of biofouling monitoring devices in cooling water systems should therefore be quantified on low nutrient media and H_2S producing bacteria should be quantified and not only SRB.

The aims of this study were therefore, to evaluate a number of direct technique biofouling monitors, under standard operating conditions and to identify suitable monitors for use in South African cooling water systems. The devices were also evaluated when a biodispersant was added to the circulating water to determine their suitability for the assessment of the efficacy of biodispersants as biofouling treatment programmes.

MATERIALS AND METHODS

Experimental cooling water system parameters

Four biofouling monitoring devices were installed at Lethabo Power Station. This power station is operated at elevated cycles of concentration with make up water of poor microbiological and chemical quality. Visual inspections of the cooling water system were carried out over the last five years. Biofouling and MIC were identified in the system, even in those areas where the mild steel pipework was protected with an epoxy based coating (Figure 5.1). To date, the microbiological condition of the system was determined by microbiological analysis of the bulk water, in conjunction with a Robbins Device biofouling monitor. This type of on-line monitor was considered suitable for this particular cooling water system, as a resident microbiologist was available to sample and analyse for sessile bacteria. System parameters and chemical composition of the cooling water at Lethabo Power Station are detailed in Table 5.1.

*M. Santa, ESKOM, TRI, Private Bag 40175, Cleveland, 2022.

Table 5.1 : System parameters and chemical composition of the open recirculating cooling water at Lethabo Power Station.

System volume	128 megalitres
Cycles of concentration	15 - 20 cycles
pH at 25°C	8.5
Conductivity at 25°C	2700 $\mu S.cm^{-1}$
Total alkalinity	113 $mg.l^{-1}$ $CaCO_3$
Chloride	246 $mg.l^{-1}$
Sulphate	905 $mg.l^{-1}$
Total hardness	318 $mg.l^{-1}$ $CaCO_3$

Biodispersant dosing regimes

Biofouling monitors were evaluated under three different dosing regimes, each of four weeks duration, namely :

no biofouling control chemicals utilised.

slug dosing of an anionic biodispersant once a week at 15 ppm.

continuous dosing of an anionic biodispersant at 10 ppm.

Biofouling monitors

Four biofouling monitors were compared, the Robbins Device, a modified version of the Robbins Device, the Pedersen Device and the Barry's Device (Table 5.2). The modifications to the Robbins Device involved an increased stud surface area (factor of 10) and the counter sinking of the stud into the holder (Figure 5.2). These modifications were intended to minimise errors incurred during sampling, since tearing of the biofilm would occur on the holder and not on the stud surface. The modified stud could therefore be removed without disturbing the biofilm. In addition, more representative samples of patchy or uneven biofilms would be obtained due to the larger surface area.

The four biofouling monitors were installed in series, on a by-pass line on the "hot" side of the main cooling water system, i.e. after passage of the cooling water through the condensers (Figure 5.3). The devices were exposed to the circulating water for three weeks before the commencement of each dosing regime. During these three weeks, no biofouling control chemicals were added to the system, to allow a biofilm to develop. A water velocity of 1.2 $m.s^{-1}$ was maintained through all the devices with the exception of the Pedersen Device where the maximum velocity was limited to 0.2 $m.s^{-1}$, due to the design.

Table 5.2 : Biofouling monitors evaluated in the open recirculating cooling water system at Lethabo Power Station.

BIOFOULING MONITOR	MATERIALS OF CONSTRUCTION		SAMPLING SURFACE AREA (cm^2)	SUPPLIER
	SAMPLING SURFACE	HOUSING		
Robbins Device	Nylon	Mild steel	0.5	Hydralube, P.O.Box 8725, Edenglen, South Africa
Modified Robbins Device	Nylon	PVC[a]	4.9	Eskom, TRI, Private Bag 40175, Cleveland, South Africa
Pedersen Device	Nylon	PVC	41.6	Chemserve Systems, P.O. Box 12055, Chloorkop, South Africa
Barry's Device	PVC	-	34.7	Iscor, P.O. Box 2, Vanderbijlpark, South Africa

[a] PVC = polyvinyl chloride

The sampling surfaces of the four monitoring devices are illustrated in Figure 5.4.

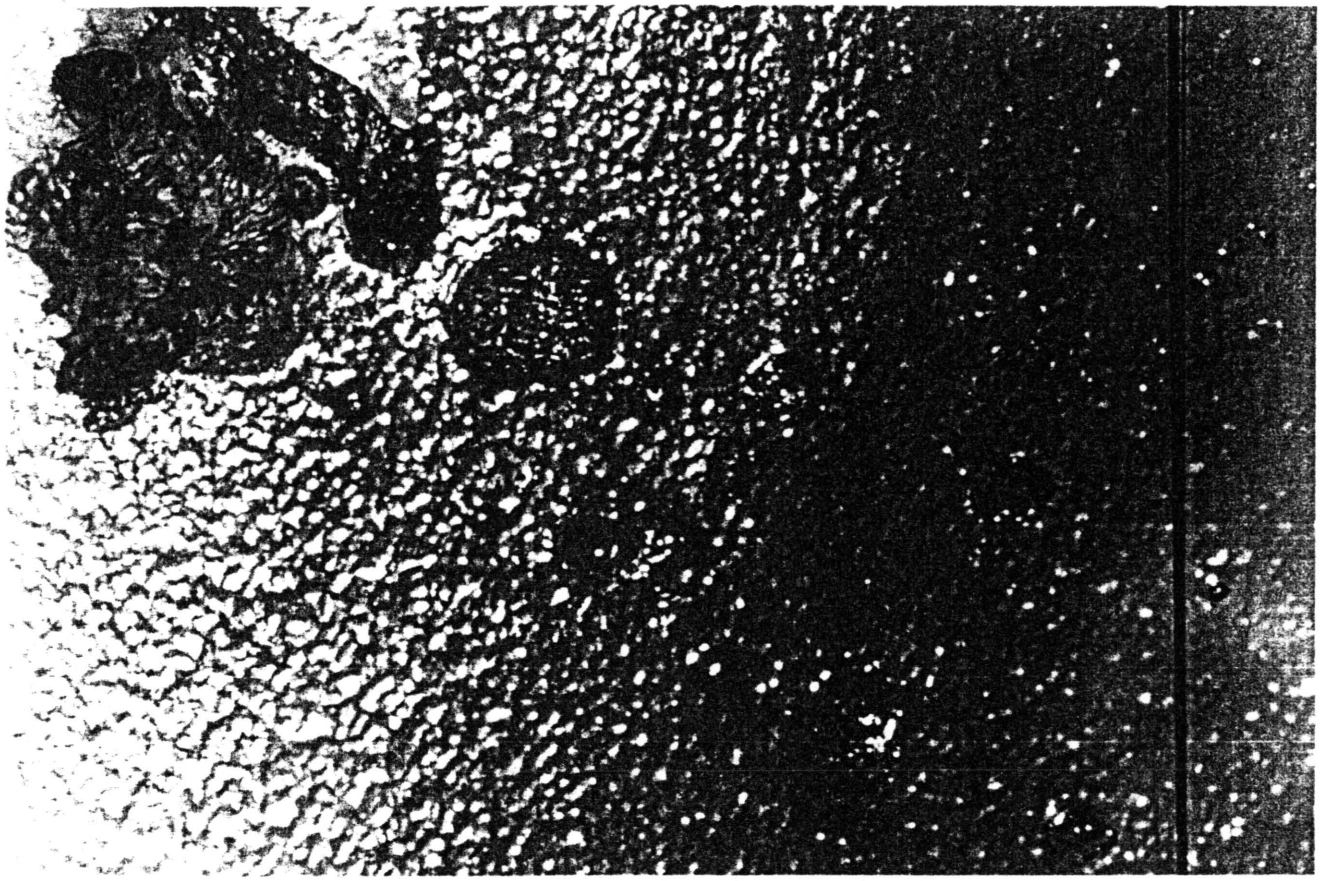

Figure 5.1 : Blistering of the epoxy coating and subsequent MIC in a condenser at Lethabo Power Station.

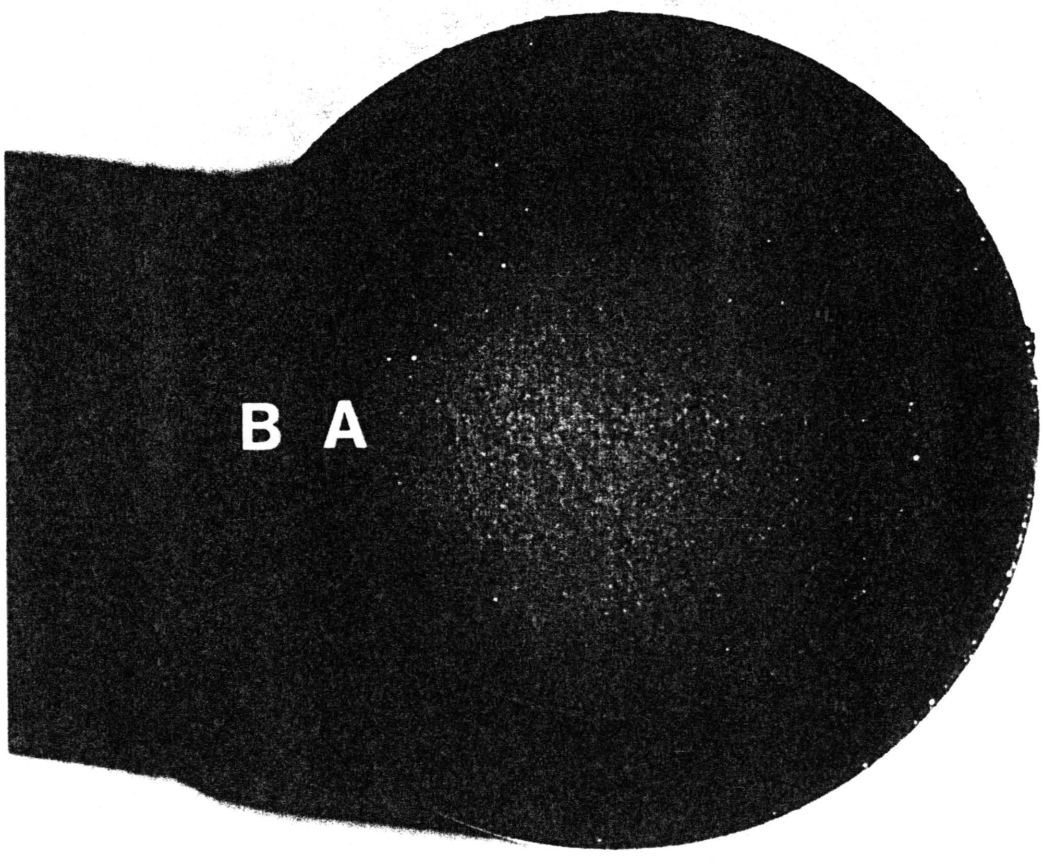

Figure 5.2 : Stud from a modified Robbins Device illustrating larger surface area of 4.9 cm^2 (A) countersunk into the stud holder (B).

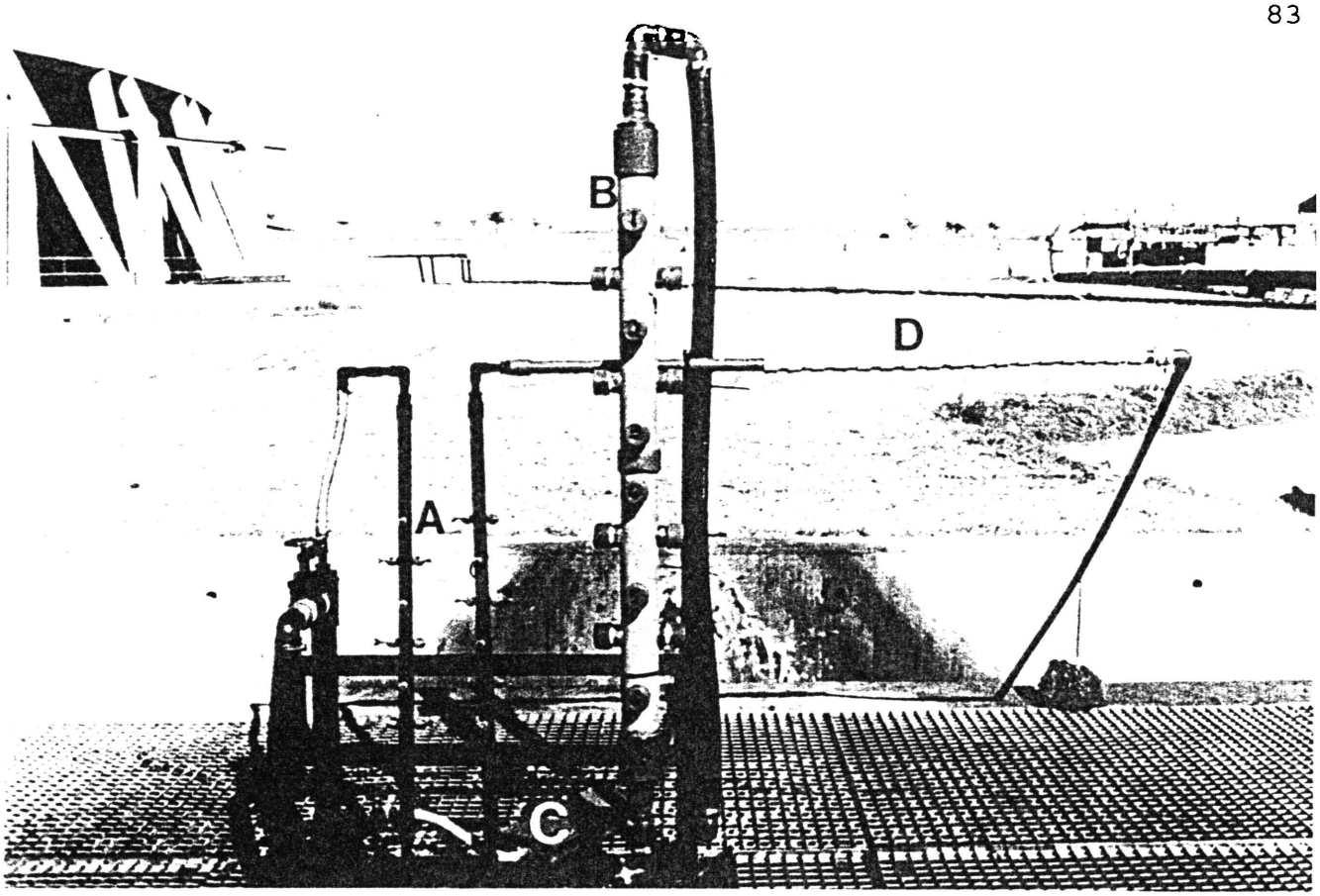

Figure 5.3 : Robbins Device (A), modified Robbins Device (B), Pedersen Device (C) and Barry's Device (D) evaluated in the open recirculating cooling water system, Lethabo Power Station.

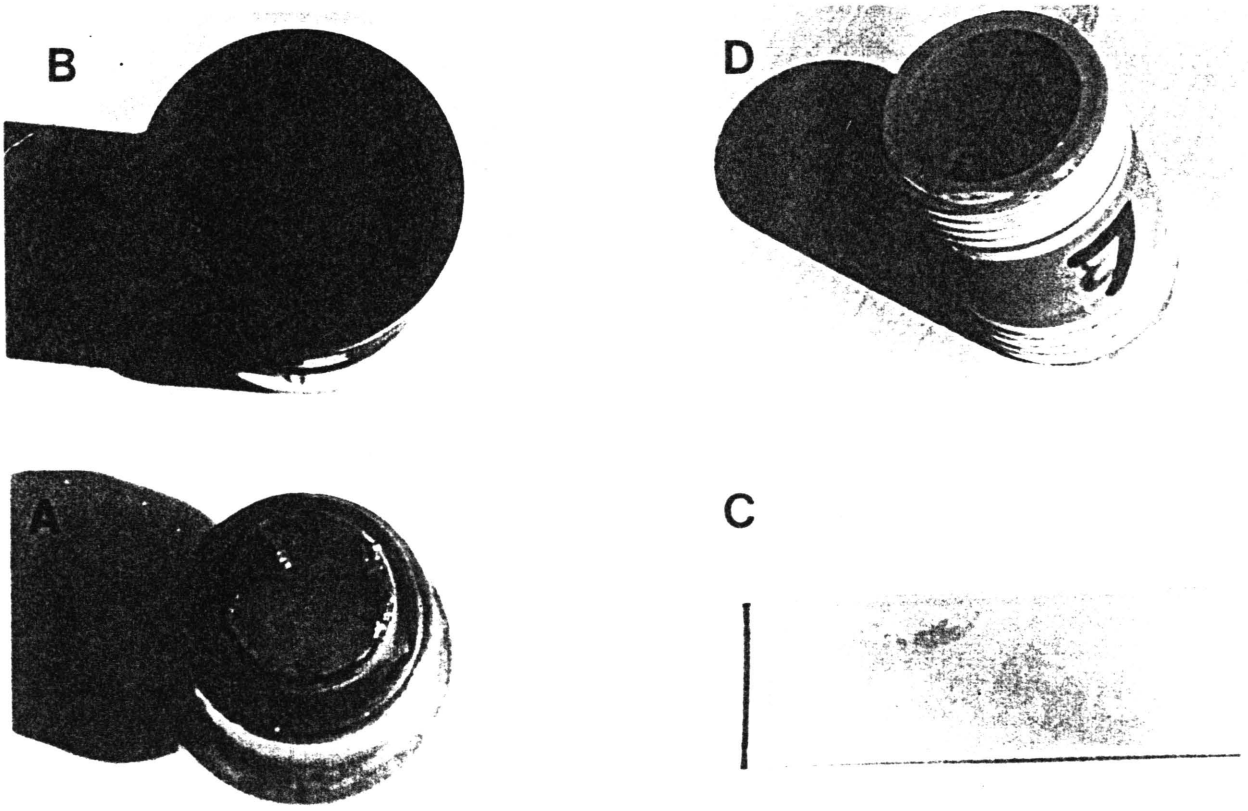

Figure 5.4 : Sampling surfaces of Robbins Device (A), modified Robbins Device (B), Pedersen Device (C) and Barry's Device (D).

Sampling procedure

Numbers of sessile bacteria were not determined during the three week period when no treatment chemicals were added. This allowed the development of a biofilm. Sampling surfaces were, however, removed from each of the devices before the start of each evaluation and thereafter, twice a week for four weeks. Two samples were removed from each device, at each sampling interval.

Sampling surfaces were removed from each device using sterile forceps and rinsed in sterile, quarter strength Ringer's solution. A sterile scalpel was run around the outer edge of the sampling surface in the modified Robbins Device, before it was removed from the holder. The sampling surfaces were placed in bottles containing 100ml of sterile, quarter strength Ringer's solution, 20 ppm of a proprietary biodispersant and glass beads, to maximise biofilm removal. The one end of the section of PVC pipe removed from the Barry's device was sealed using a sterile end cap. The section of pipe was then filled with the beads, biodispersant and Ringer's solution and another end cap used to seal the remaining open section of pipe (Figure 5.5). The bottles and sealed sections of pipe were agitated on a shaker for a period of 30 min, after which the resultant bacterial suspensions were analysed (Table 5.3).

A single 500ml bulk water sample for microbiological analysis, was collected in a sterile Whirl Pak bag (Nasco, USA) from the by-pass line, each time sampling surfaces were removed from the biofouling monitors.

Figure 5.5 : Sampling procedure used for the Robbins Device, modified Robbins Device and Pedersen Device (A) and for the Barry's Device (B).

Microbiological analysis

Samples were diluted in sterile, quarter strength Ringer's solution and subjected to duplicate plate counts. All incubation was at 37°C, as this temperature was the approximate system temperature. All the samples were analysed within two h of sampling and stored at 4°C until they were analysed. Anaerobic incubation took place in a nitrogen atmosphere, in an anaerobic jar.

Half strength Nutrient Agar was prepared by using 50% of the amount of Nutrient Agar specified by the manufacturers and adding purified Agar (Biolab) to yield the specified amount of agar per litre as in full-strength Nutrient Agar. The techniques used for bacteriological analysis are detailed in Table 5.3. Plates containing between 30 and 300 colonies were counted.

Table 5.3 : Techniques used to quantify bacteria detached from the sampling surfaces of four biofouling monitors and in the bulk water of the open recirculating cooling water system at Lethabo Power Station.

BACTERIAL TYPE	TECHNIQUE	INCUBATION TIME (d)	ATMOSPHERE	GROWTH MEDIUM
Total aerobic bacteria	Pour plate	2	Aerobic	Half strength Nutrient Agar (Biolab)
Total anaerobic bacteria	Pour plate	3	Anaerobic	Half strength Nutrient Agar (Biolab)
H_2S producing bacteria	Agar tubes	5	Anaerobic	Iron Sulphite Agar (Oxoid)

Scanning electron microscopy

Sampling surfaces removed from each of the biofouling monitors were viewed using a scanning electron microscope (SEM) before the start and at the end of each of the four week dosing regimes.

Surfaces removed from the biofouling monitors were fixed in 2.5% gluteraldehyde for four 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

Numbers of sessile microorganisms on the sampling surfaces of the four monitoring devices were analysed using the f-test procedure for the analysis of variance at a 95% confidence interval. Variances between the four monitoring devices and the three treatments were determined (Brown and Hollander, 1977).

RESULTS AND DISCUSSION

Suitability and visual observations of the four biofouling monitors

The experiments involving the Pedersen Device were discontinued one week after the start of the evaluation, due to the fact that a maximum water velocity of only 0.2 $m.s^{-1}$ was achieved. Sludge had deposited on the base of the device (Figure 5.6) and as this device did not simulate actual water velocities occurring in the system, it was decided to discontinue its use.

A visible biofilm was observed on the sampling surfaces of the other three biofouling monitors, particularly on the exposed surfaces of the Barry's Device. Visual examination of the nylon studs in the Robbins Device, after exposure to the recirculating cooling water for four weeks, revealed deposits of what appeared to be corrosion products (Figure 5.7).

Microbiological analysis of bulk water and biofouling monitor sampling surfaces

Numbers of attached bacteria recovered from the sampling surfaces of the Robbins Device, Modified Robbins Device and Barry's Device are shown in Figures 5.8 - 5.10. Numbers of planktonic bacteria in the bulk water are plotted on the same graphs. Since all the monitors were evaluated under the same operating conditions, it was suggested that the monitor from which the highest number of sessile bacteria were recovered, represented the most accurate simulation of system conditions. It was hypothesised that there would be significant differences between the numbers of sessile bacteria on the sampling surfaces of the different monitoring devices.

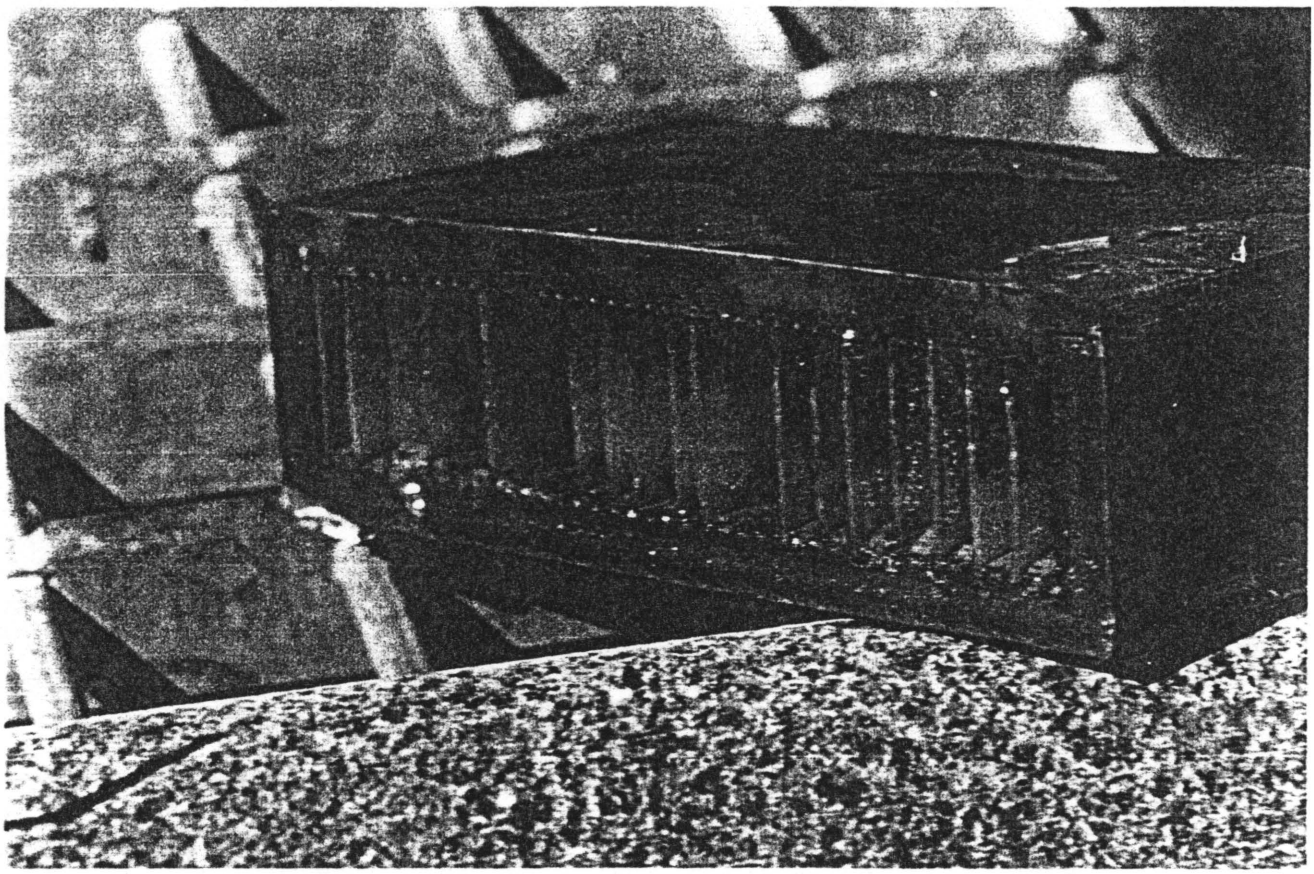

Figure 5.6 : Sludge deposition in the Pedersen Device one week after installation in the open recirculating cooling water system at Lethabo Power Station.

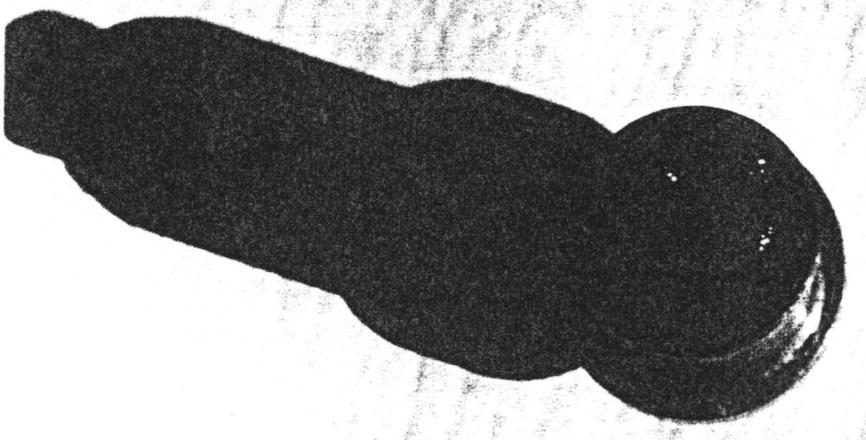

Figure 5.7 : Deposition of corrosion products on the surface of a Robbins Device stud after four weeks exposure to the recirculating cooling water at Lethabo Power Station.

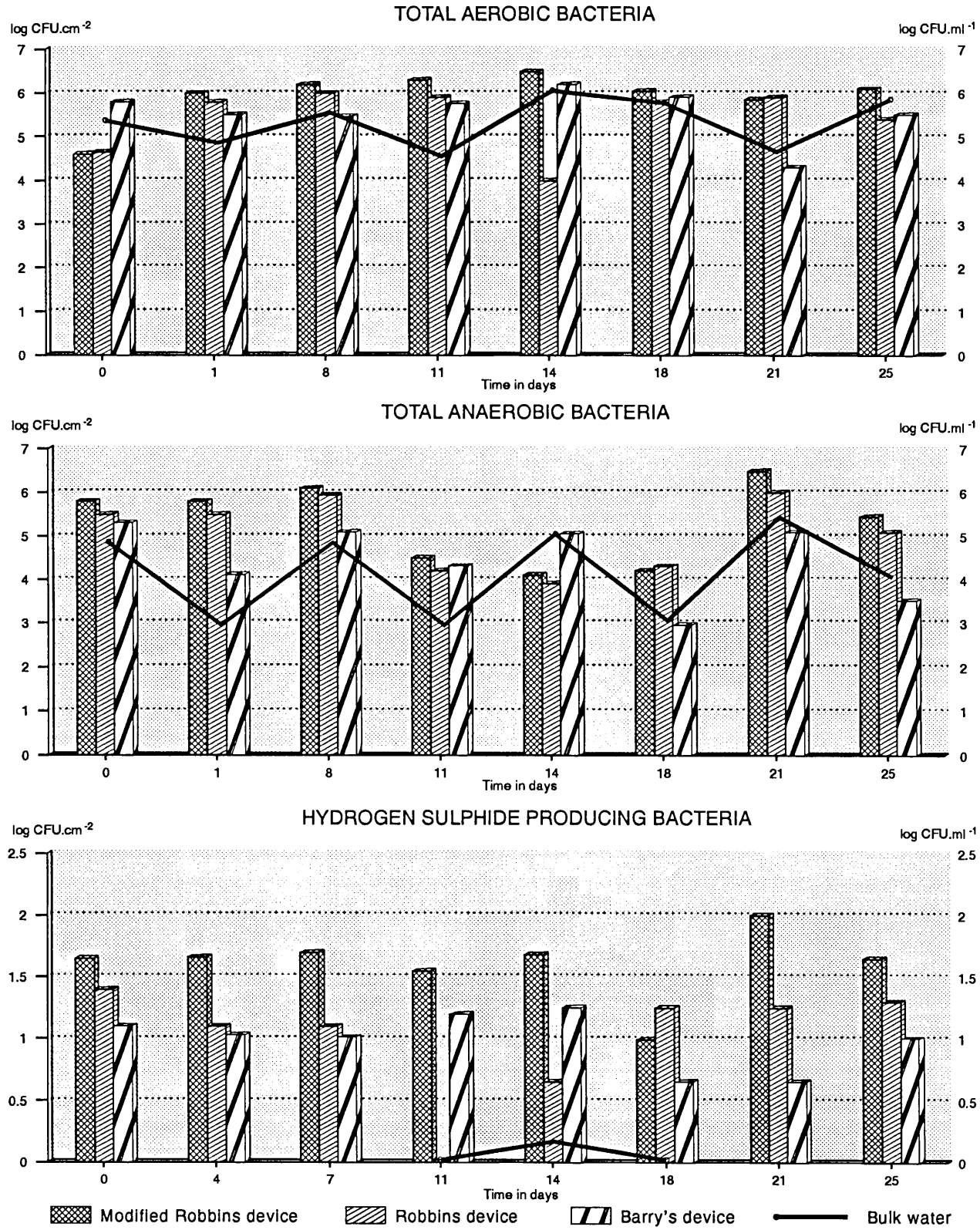

FIGURE 5.8 Comparison of attached bacteria on modified Robbins, Robbins and Barry's device biofouling monitors where no specific treatment chemicals were used.

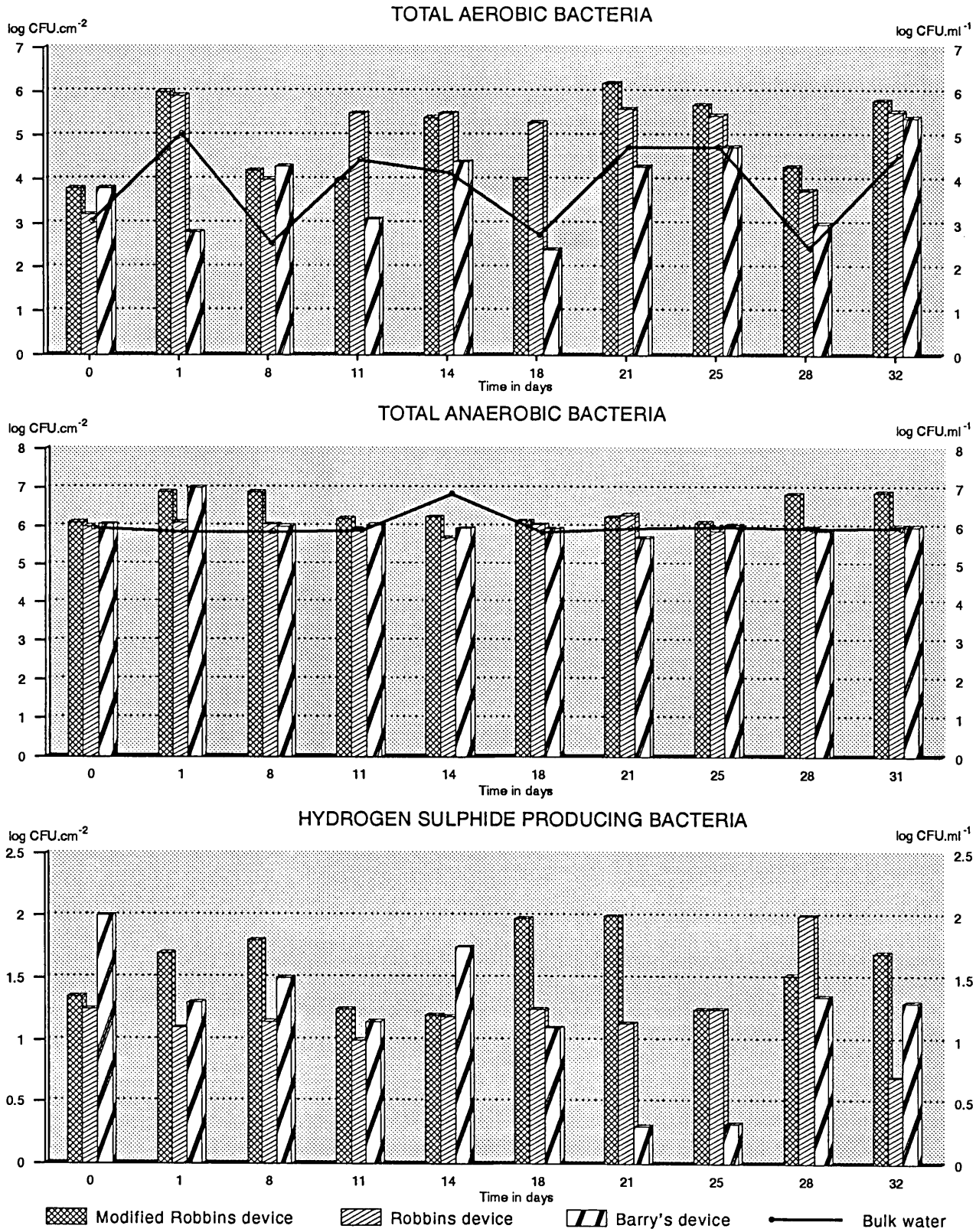

FIGURE 5.9 Comparison of attached bacteria on modified Robbins, Robbins and Barry's devicebiofouling monitors with continuous dosing of biodispersant at 10ppm.

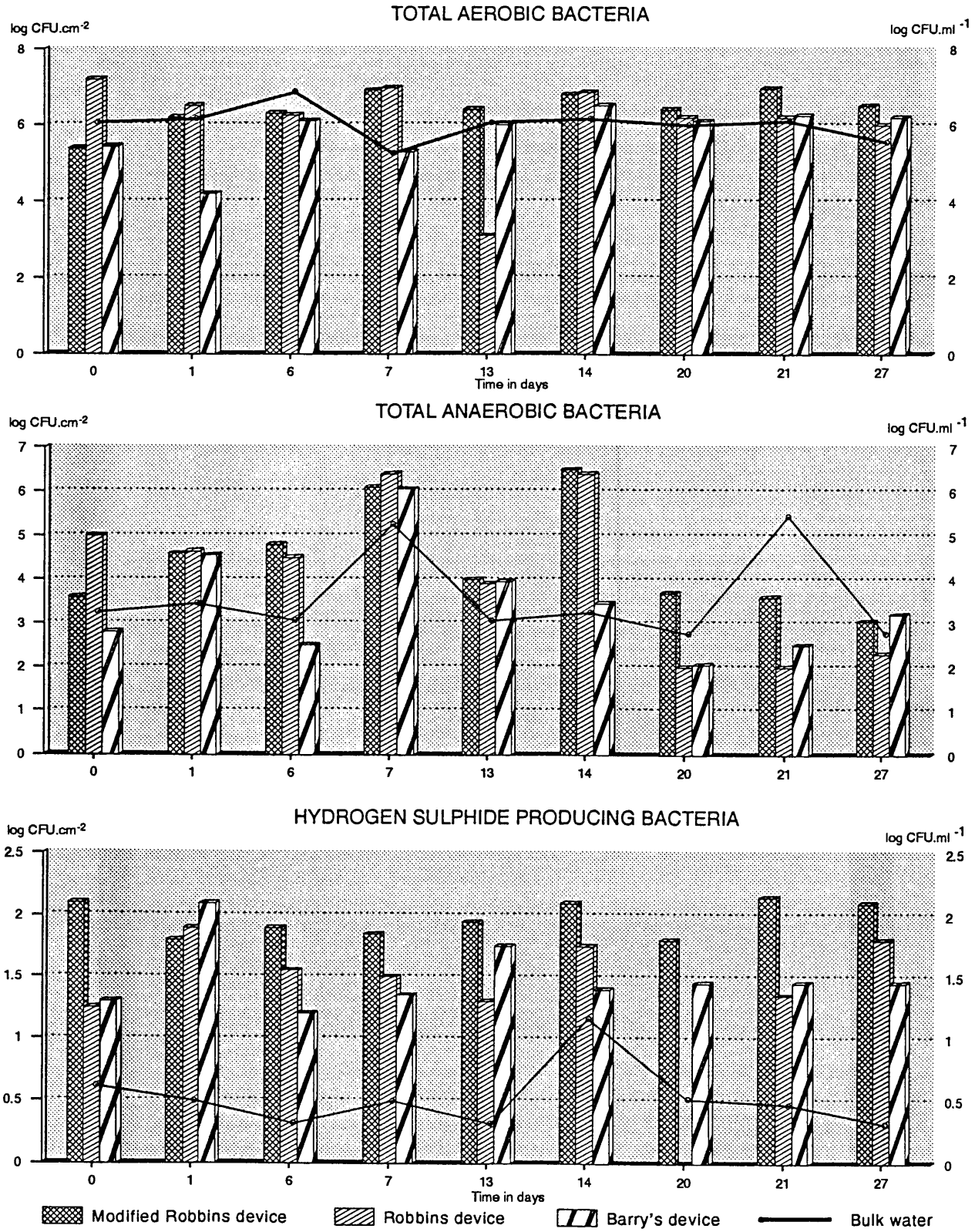

FIGURE 5.10 Comparison of attached bacteria on modified Robbins, Robbins and Barry's device biofouling monitors with slug dosing of biodispersant at 15 ppm once a week.

Changes in numbers of planktonic and sessile bacteria

In all four-week dosing regimes, changes in the numbers of attached bacteria measured by the monitoring devices, did not correspond with changes in the numbers of planktonic bacteria. For example, when no biodispersant was added to the system, the anaerobic bacteria in the bulk water decreased after one day and then increased after eight days, while the sessile anaerobic bacteria recovered from the Modified Robbins and Robbins Devices, gradually increased over the same time period (Figure 5.8). It has, however, been widely reported that no correlation can be made between numbers of planktonic and sessile bacteria, thus, these results were expected (Costerton *et al.*, 1985; Blenkinsopp and Costerton, 1991). A noticeable increase in the numbers of planktonic bacteria, after the biodispersant was added, either continuously or as a slug dose was, however, not recorded (Figures 5.9 and 5.10). Previous studies have shown that the addition of an effective biodispersant, will result in a characteristic increase in the numbers of aerobic and anaerobic planktonic bacteria (Poulton and Nixon, 1990; Lutey and Allison, 1991). These results indicated that the particular biodispersant used in this study, was not effective in dispersing sessile microorganisms.

During the four-week period, when no treatment chemicals were added, no overall increasing or decreasing trends in numbers of sessile or planktonic bacteria were noted on any of the devices. There were, however, variations in the numbers of sessile and planktonic bacteria (Figure 5.8). For example, the total aerobic bacteria recovered from the modified Robbins Device, varied from approximately $3.0x10^4$ CFU.cm^{-2} to $3.0x10^6$ CFU.cm^{-2}, the total anaerobic bacteria from $1.0x10^4$ CFU.cm^{-2} to $3.0x10^6$ CFU.cm^{-2} and the H_2S producing bacteria from $1.0x10^1$ CFU.cm^{-2} to $1.0x10^2$ CFU.cm^{-2} (Figure 5.8). These variations in sessile bacterial numbers could be due to natural phenomena such as periodic sloughing and thickening of biofilms (Characklis and Marshall, 1990). In addition, anaerobic bacteria could be more sensitive to slight changes in biofilm thickness than their aerobic counterparts. Changes in oxygen concentrations within a biofilm may range from supersaturation to total depletion within micrometer distances (Jorgensen and Revsbech, 1983; Ledandowski *et al.*, 1991). Thus, slight changes in biofilm thickness may result in anaerobic bacteria being exposed to lethal levels of oxygen and fluctuations in numbers of anaerobic bacteria.

Comparison of the four biofouling monitors

Numbers of sessile bacteria recovered from the three remaining biofouling monitoring devices were analysed statistically, to determine variances between the devices (Table 5.4).

Table 5.4 : Statistical analysis of variances between numbers of bacteria quantified on the biofouling monitoring devices, for each of the three different biodispersant dosing regimes.

		DEVICES	BACTERIAL TYPE	f VALUE	SIGNIFICANT DIFFERENCE ($p<0.05$)
NOT TREATMENT		MRD[a] : RD[b]	Total aerobic bacteria	3.43	No
		RD : BD[c]		1.02	No
		MRD : BD		3.48	No
		MRD : RD	Total anaerobic bacteria	7.19	Yes
		RD : BD		5.66	Yes
		MRD : BD		40.72	Yes
		MRD : RD	H_2S producing bacteria	8.06	Yes
		RD : BD		2.85	No
		MRD : BD		23.01	Yes
BIODISPERSANT DOSING	SLUG	MRD : RD	Total aerobic bacteria	3.43	No
		RD : BD		25.42	Yes
		MRD : BD		9.00	Yes
		MRD : RD	Total anaerobic bacteria	2.82	No
		RD : BD		10.91	Yes
		MRD : BD		10.34	Yes
		MRD : RD	H_2S producing bacteria	1.06	No
		RD : BD		2.86	No
		MRD : BD		1.68	No
	CONTINUOUS	MRD : RD	Total aerobic bacteria	5.03	Yes
		RD : BD		15.47	Yes
		MRD : BD		77.81	Yes
		MRD : RD	Total anaerobic bacteria	69.91	Yes
		RD : BD		51.07	Yes
		MRD : BD		1.37	No
		MRD : RD	H_2S producing bacteria	9999.99	Yes
		RD : BD		59.31	Yes
		MRD : BD		9999.99	Yes

[a]MRD = Modified Robbins Device [b]RD = Robbins Device
[c]BD = Barry's Device

No statistically significant differences were found amongst the variances of the numbers of total aerobic bacteria recovered from the Robbins, Modified Robbins and Pedersen Devices when no biodispersant was added to the system (Table 5.4). This was also true for the numbers of H_2S producing bacteria when the biodispersant was slug dosed.

Consistently higher f values (1.37 - 9999.99), and thus significant variances between the numbers of sessile bacteria enumerated on the devices, when the biodispersant was continuously added to the system, were noted (Table 5.4).

Variances between the devices were lower (1.02 to 40.72), when no biodispersant was added and when the biodispersant was slug dosed (Table 5.4). However, the numbers of sessile bacteria on the modified Robbins Device were consistently higher for all groups of bacteria tested. Eight sampling surfaces were removed from each monitoring device during the period when no biodispersant was added to the cooling water. During this period, the total aerobic and anaerobic bacteria recovered from the sampling surfaces of the modified Robbins Device, were higher than on the other devices in 75% of the cases, while the H_2S producing bacteria were higher in 88% of the cases (Figure 5.8). This pattern was also observed when the biodispersant was added to the system (Figures 5.9 and 5.10). This may be due to the larger sampling surface area of the modified Robbins Device (4.9 cm^2), as compared to the standard version (0.5cm^2). The larger surface area may have allowed a more uniform biofilm attachment, or increased the sampling accuracy for patchy or unevenly distributed biofilms (Donlan *et al.*, 1990). Another possible explanation is the difference in the materials of construction. The modified Robbins Device was constructed entirely of PVC and nylon, whereas the Robbins Device consisted of nylon studs in brass holders in a mild steel pipe (Table 5.2). It was observed that deposition of corrosion products from the mild steel pipe occurred on the nylon studs of the Robbins Device (Figure 5.7). These corrosion products may have interfered with the quantification of sessile microbiological populations, as they were firmly attached to the stud surface and therefore difficult to disperse. Thus, a reduced number of bacteria may have been recovered from the sampling surface. Another contributory factor may be the changes in the design of the stud holder in the modified Robbins Device, which allowed removal of the studs without tearing the biofilm.

No visible differences on the sampling surfaces of the biofouling monitors could be distinguished when viewed by scanning electron microscopy. It was, however, noted, that large amounts of amorphous material were attached to the surface of the Barry's Device (Figure 5.11). The sampling surface area of the Barry's Device was larger than both the Robbins and modified Robbins Devices (Table 5.2). However, larger numbers of attached bacteria per

cm^2 were not recorded. Visual observations of thicker biofilms on the sampling surfaces of the Barry's Device also indicated that other, non-microbiological material was entrapped in the biofilm. This could be due to the different material of construction of the sampling surface of the Barry's Device which was PVC, as opposed to nylon in the other devices (Duddridge and Pritchard, 1983). Fletcher (1985) reported that the nature of a surface can affect the number and composition of sessile bacterial populations on it, as well as the activity of the attached bacteria. As the PVC surface was rougher than the nylon surfaces, it may have facilitated improved biofilm formation (Characklis and Marshall, 1990).

Figure 5.11 : Scanning electron micrograph of biofilm attached to a sampling surface removed from the Barry's Device after four weeks exposure to the recirculating cooling water at Lethabo Power Station when no biodispersant was added (arrow indicates amorphous material).

Comparison of dosing regimes

Statistical analysis of the variance detailed below, showed no significant differences between the three dosing regimes.

Null hypothesis (H_0) was that the means are equal
$F_{2.231,0.025} = 3.69$
$f = 3.25$
H_0 is accepted if $f \leq F_{v2, vl, \alpha}$
$f \leq 3.69$

Therefore, the null hypothesis that there is no significant difference between the three treatments in terms of bacterial types or monitoring devices was accepted.

These results indicated that slug doses or continuous dosing of the biodispersant were apparently ineffective in removing sessile bacteria. The fact that increases in the planktonic bacteria were not recorded after the addition of the biodispersants, confirmed the ineffectiveness of the biodispersant treatment (Figures 5.9 and 5.10).

CONCLUSIONS

The hypothesis that there would be significant differences between the numbers of sessile bacteria on the sampling surfaces of the different monitoring devices was found to be valid. The modified Robbins Device was found to be the most accurate monitoring device for the direct monitoring of sessile microbiological populations in the open recirculating cooling water system at Lethabo Power Station. It is anticipated that the modified Robbins Device will be utilised to directly quantify sessile microbiological populations, and the Barry's Device for indirect biomass determination. Thus, the realistic assessment of not only biological fouling but also possible organic deposition and will be determined. These two monitors will allow the optimisation and assessment of cooling water treatment programmes with a higher degree of accuracy. The biodispersant used in this study was found to be ineffective in removing sessile bacteria.

ACKNOWLEDGEMENTS

Chemserve Systems and Mr B. Luddick at Iskor are gratefully acknowledged for supplying biofouling monitors and the staff at Lethabo Power Station and TRI, particularly Mr C. Scholtz, for their assistance and co-operation.

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 Kajiyama, 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 auxilliary 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 milliamps.

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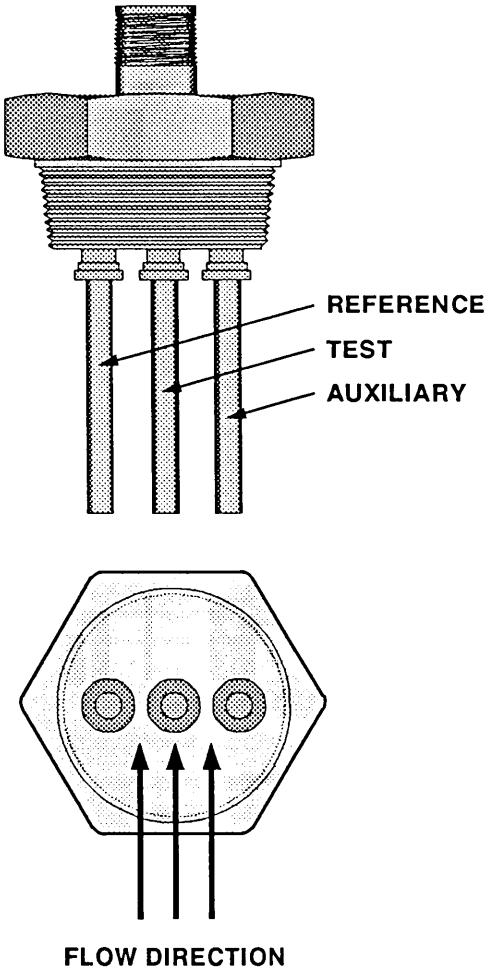

Figure 6.1 : Petrolite corrosion rate instrument probe.

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.

pH at 25°C	8.5
Conductivity at 25°C	2700 $\mu S.cm^{-1}$
Total alkalinity	113 $mg.l^{-1}$ $CaCO_3$
Chloride	246 $mg.l^{-1}$
Sulphate	905 $mg.l^{-1}$
Total hardness	318 $mg.l^{-1}$ $CaCO_3$

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 microbicide (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.

MICROBIALTYPE	TECHNIQUE	INCUBATION TIME (d)	ATMOSPHERE	GROWTH MEDIUM
Total Aerobic bacteria	Pour plate	2	Aerobic	Half strength Nutrient Agar (Biolab)
Total anaerobic bacteria	Pour plate	3	Anaerobic	Half strength Nutrient Agar (Biolab)
H_2S producing bacteria	Agar tubes	5	Anaerobic	Iron Sulphite Agar (Oxoid)
Sulphate reducing bacteria	Agar tubes	14	Anaerobic	SABS method 1497-1989 (1989)

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.

JAR	CORROSION RATE ($mm.year^{-1}$)
1	0.316
2	0.300
3	0.305

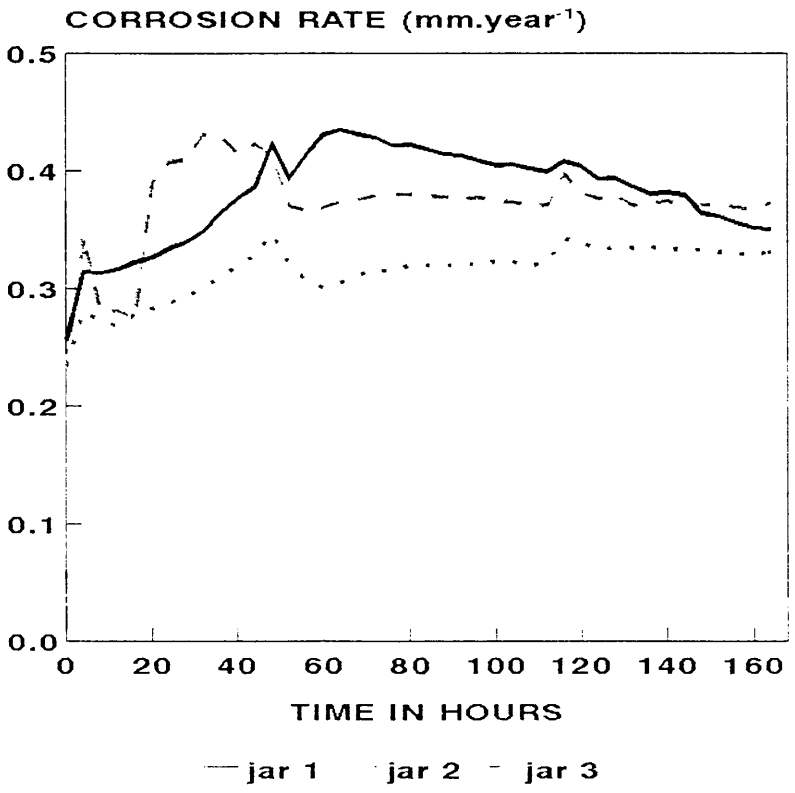


Figure 6.3 : Corrosion rates as measured by the linear polarisation technique in the jar tests to determine microbial attachment and MIC.

Numbers of sessile aerobic and anaerobic bacteria per cm^2, 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.

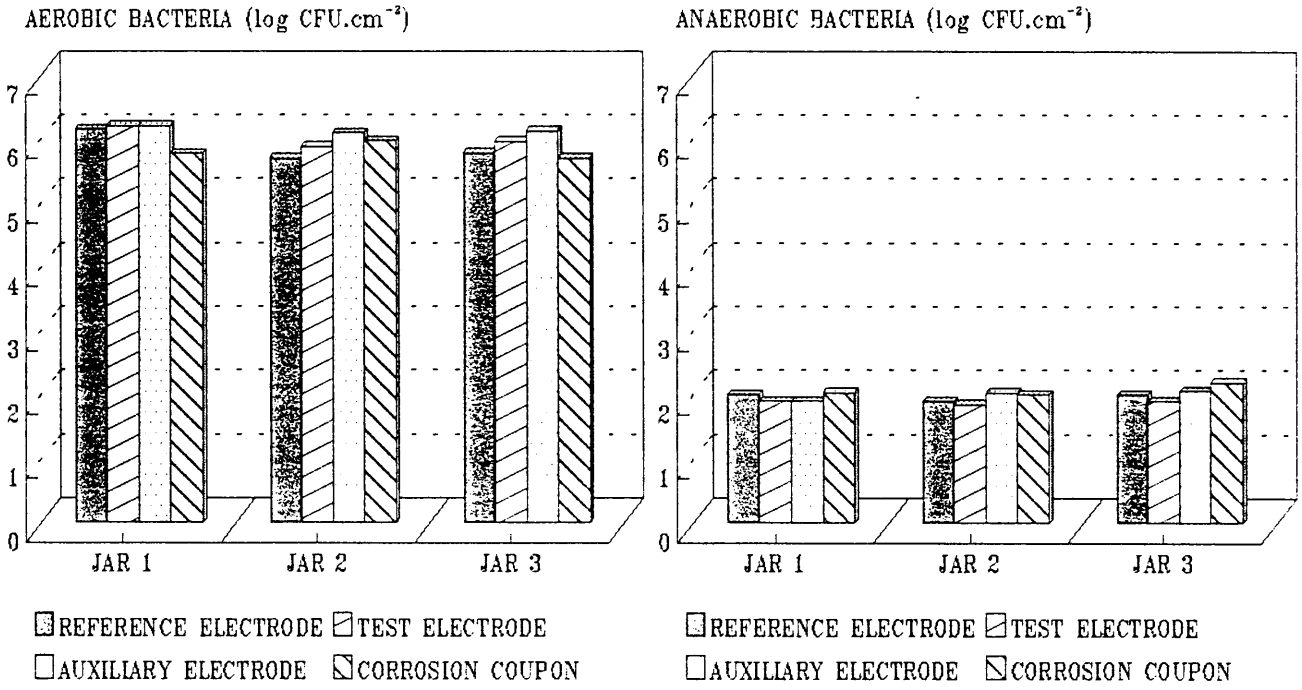


Figure 6.4 : Sessile aerobic and anaerobic bacteria enumerated on three corrosion monitor electrodes and corrosion coupons in jar tests to determine microbial attachment and MIC.

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 cm^2 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 rigs 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.

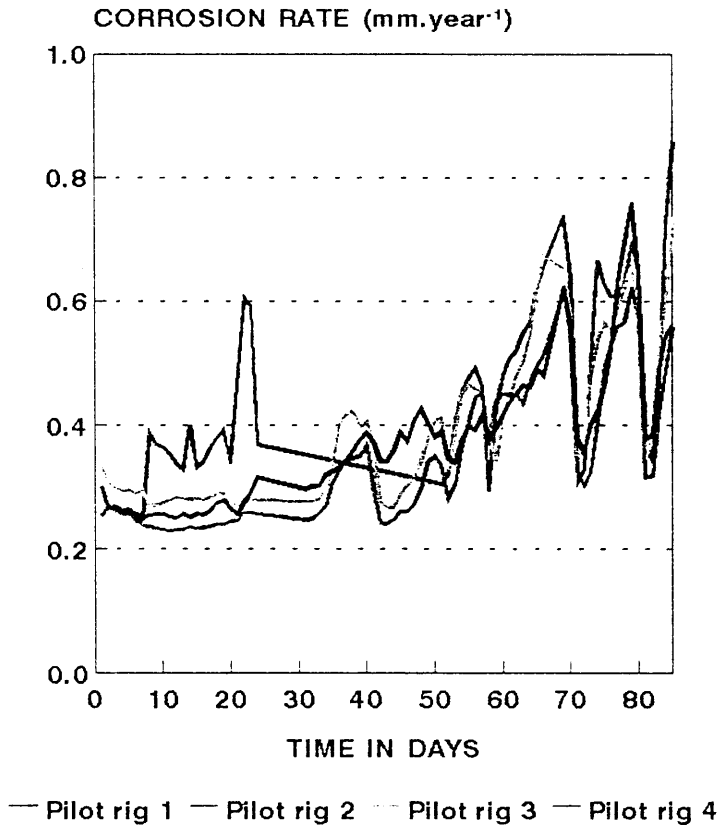


Figure 6.5 : Corrosion rates measured by the linear polarisation technique in four pilot rigs during a three month period when no biocide was added.

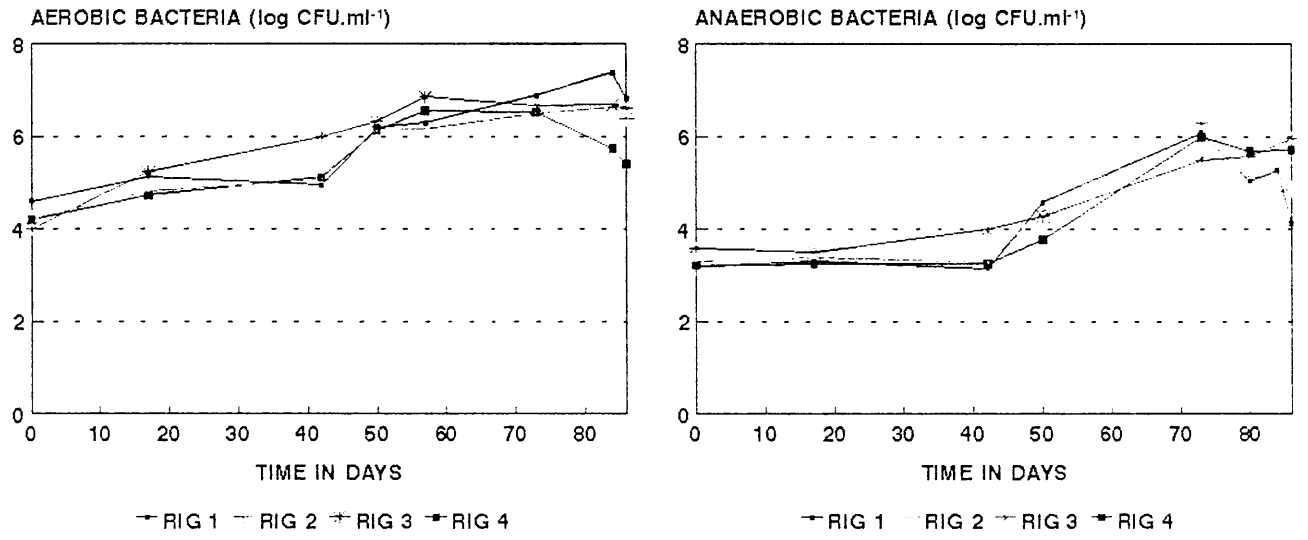
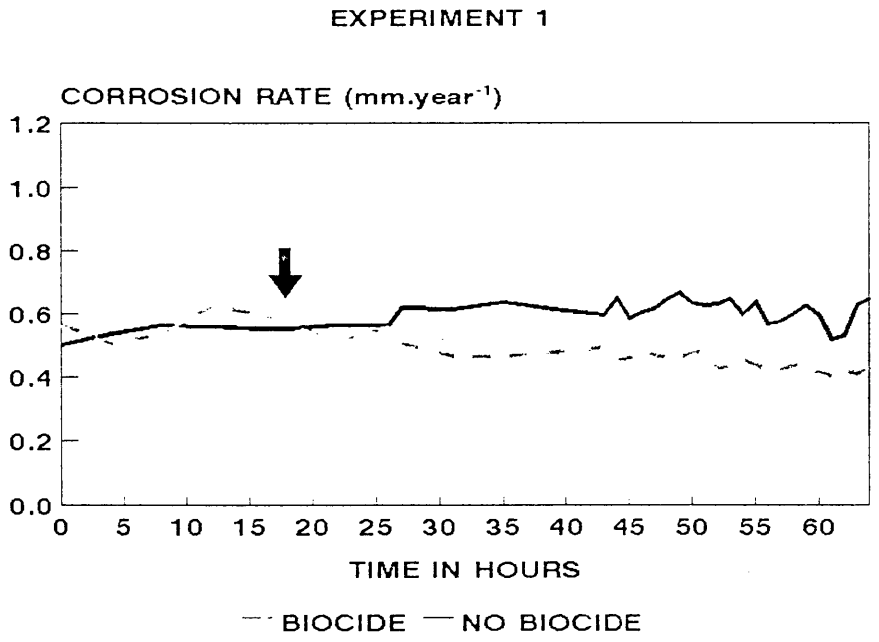


Figure 6.6 : Numbers of aerobic and anaerobic planktonic bacteria in the bulk water of four pilot rigs during a three month period when no biocide was added.

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_2S 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 $1.0x10^4$ $CFU.ml^{-1}$ to $1.0x10^7$ $CFU.ml^{-1}$ (Figure 6.6). The planktonic anaerobic bacteria in the bulk water increased from approximately $1.0x10^3$ $CFU.ml^{-1}$ to $1.0x10^5$ $CFU.ml^{-1}$ (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.



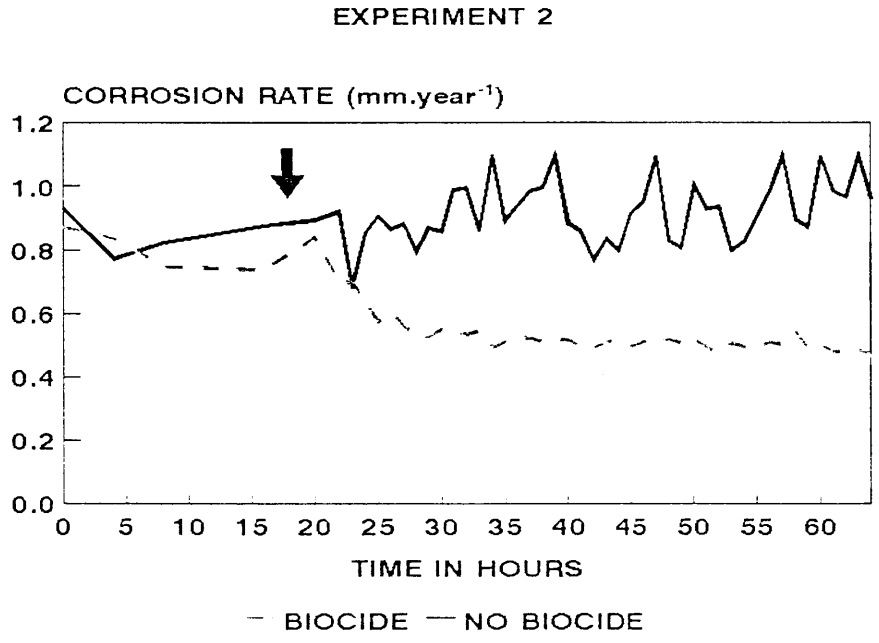


Figure 6.7 : Corrosion rates measured by the linear polarisation technique in two pilot rigs treated with biocide and two untreated controls (arrow indicates time of biocide addition).

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_2S 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_2S 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.

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Chapter 7

CONCLUSIONS

The microbiology of cooling water systems has been extensively researched and thus our understanding of the microorganisms and processess involved has expanded. However, this study clearly showed that there are still numerous problems that face the industrial microbiologist.

Microbiological surveys were carried out on the recirculating cooling waters and corresponding raw water supplies at 12 power stations. Microbiological analyses of the water samples revealed that total aerobic and anaerobic bacteria, anaerobic acid producing bacteria, *Thiobacillus, Nitrobacter*, SRB and algae were present in all the recirculating cooling waters and raw water supplies with the exception of two potable raw water supplies. Although in 75% of the systems the numbers of SRB were higher in the recirculating cooling waters than in the raw water supplies, no correlation between SRB numbers and sulphate concentration could be distinguished. In addition no correlation between the percentage increase in the numbers of total aerobic bacteria and cycles of concentration was evident. The raw water supplies predominantly contained green algae and diatoms whereas in the recirculating cooling water systems algae deposits consisted primarily of blue green algae.

Variations exist between the environmental conditions, water quality and composition in the raw water supply and recirculating cooling water at a power station (Mc Coy, 1980). These changes in environmental and system conditions appeared to have a significant influence on the extent of microbiological contamination in any given system. Thus each system was found to be unique, and the need for the monitoring of each individual system was highlighted.

The individuality of cooling water systems was also highlighted when biodispersant/biocide treatment programmes were monitored at four power stations. Biodispersants resulted in increases in numbers of planktonic bacteria ranging from 22592% to 654%, and biocides in percentage kills of between 83.2 to 100%. Decreases in the numbers of sessile aerobic bacteria always occured when a biodispersant was added to the system and in 80% of the cases when a biocide was added. System inspections revealed that removal of sessile microorganisms and inorganic deposits occured. Although the combinations of biodispersnats and biocides were effective in controlling microbiological growth, they had varied effects in different systems and therefore have to be evaluated for each individual system.

The monitoring of cooling water systems is problematic. Although general trends can be distinguished, the wide variety of environmental conditions in any one cooling water system,

constrain the accuracy of any monitoring device. Monitoring devices using both direct (bacterial numbers) and indirect (effect of bacterial activity or MIC) were evaluated in this study. Four monitoring devices using direct techniques were evaluated at a power station. The devices were the Robbins device, modified Robbins device, Pedersen Device and the Barry's Device. The Pedersen device was discared after one week as it could not simulate system conditions. Statistically significant differences were found between the numbers of bacteria recovered from the sampling surfaces of the remaining three monitoring devices. The numbers of bacteria recovered from the modified Robbin's device were consistently higher than the numbers recovered from the Robbin's device and Barry's device. This was thought to be due to the modification made to the Robbin's device which was to countersink the stud into a stud holder and to increase the surface area by a factor of 10. The indirect technique evaluated was a corrosion monitor using the linear polarisation technique. Bacterial attachment occured uniformly on the electrodes of the device, indicating that this technique can be used to monitor the corrosive effects of sessile bacteria. The corrosion monitor was also evaluated in four pilot rigs. After the addition of a biocide to two of the rigs, a statistically significant decrease occured in the corrosion rate in these two rigs when compared to the untreated control. Thus it was concluded that this technique can be utilised to monitor sessile microbiological activity as well as biocide efficacy.

Due to increasing industrialisation and recurring droughts, the demand for water in South Africa will increase and the quality of water will deteriorate in the future. Thus, there is a need for intensified research into the practical implementation of methods of monitoring and mitigating the effects of microorganisms in cooling water systems.

Chapter 8

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