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).

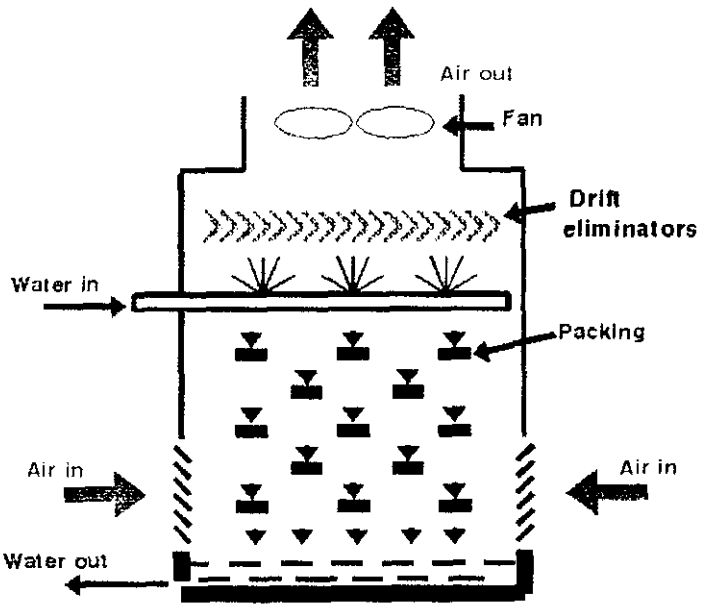

Figure 2.1 : Diagram of a mechanical draft cooling tower.

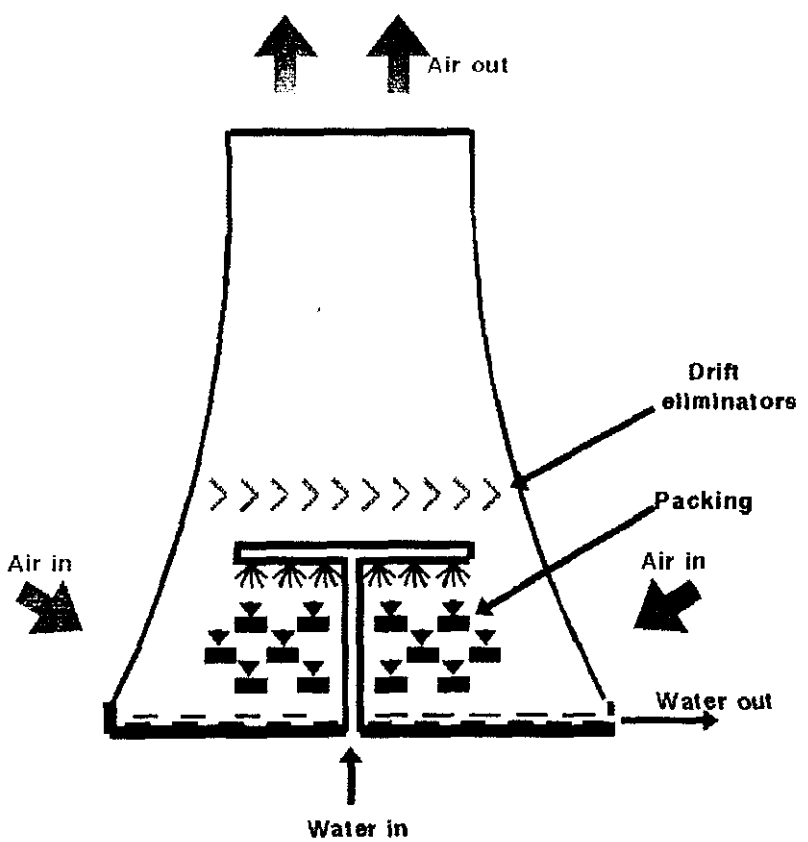

Figure 2.2 : Diagram of a hyperbolic cooling tower.

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.

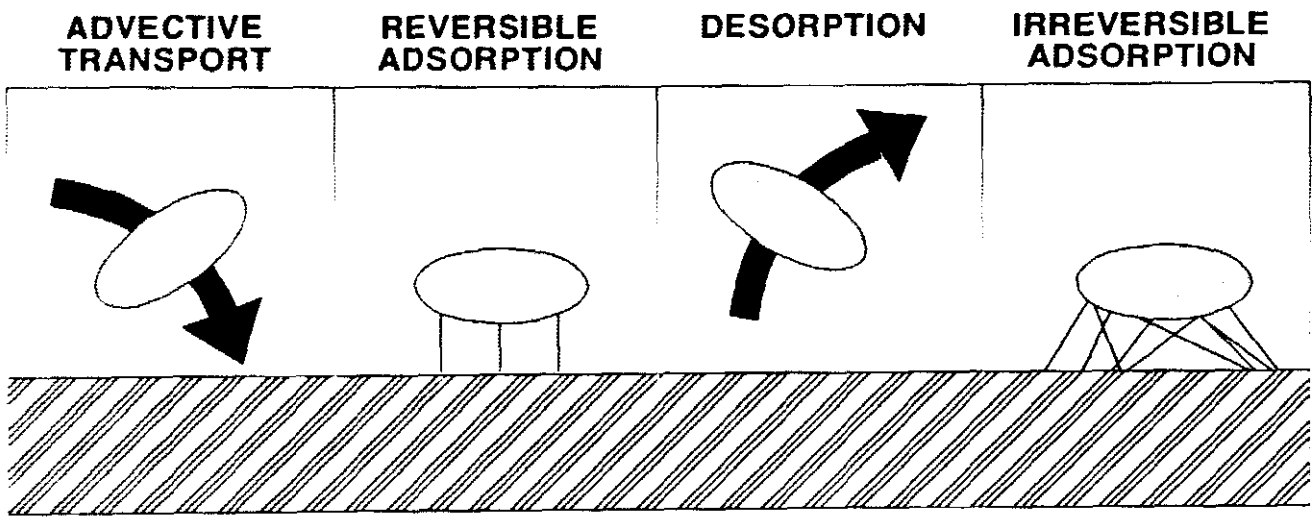

Figure 2.3 : The interfacial forces theory of bacterial attachment (After Characklis and Marshall, 1990).

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).

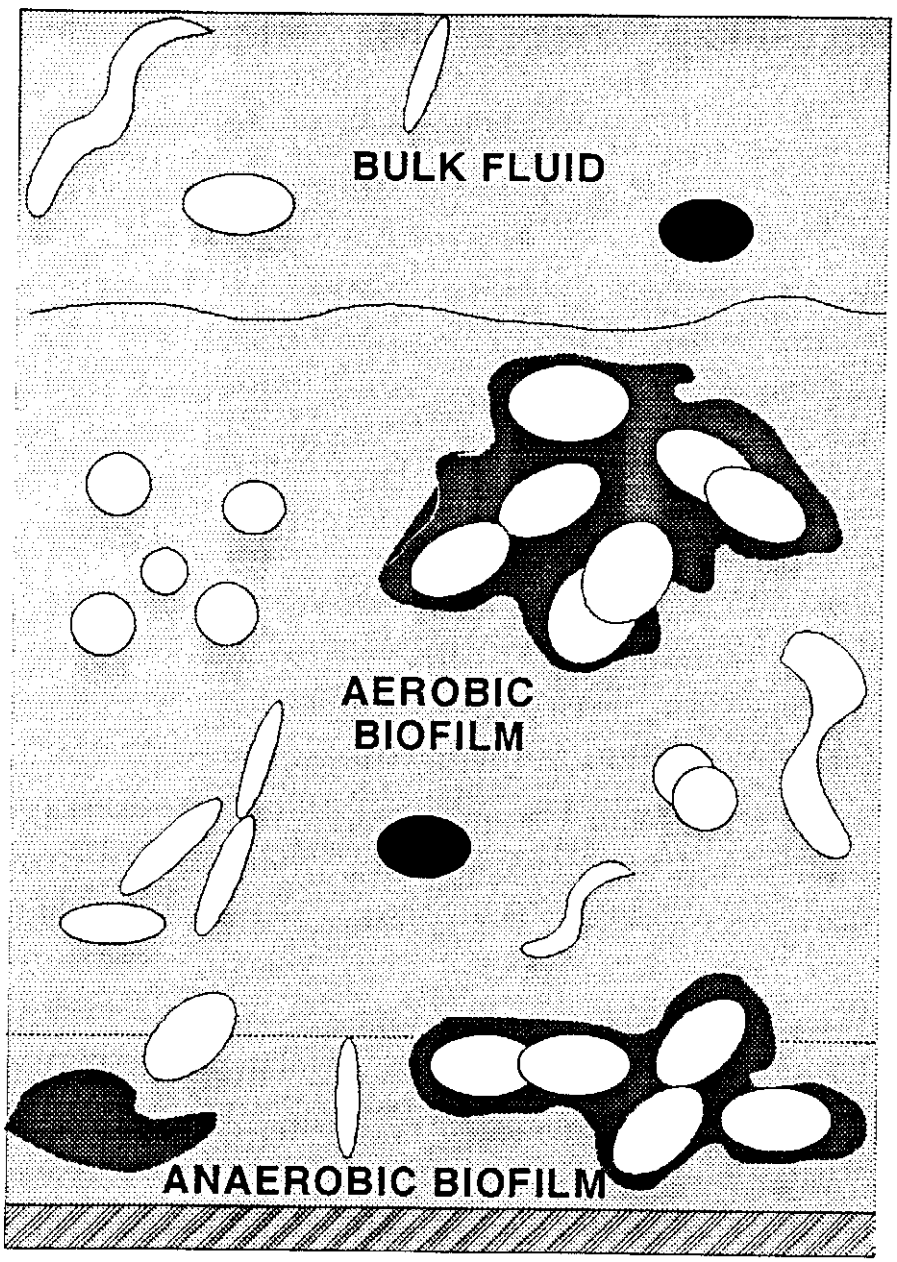

Figure 2.4: Development of an anaerobic region within a biofilm (After Costerton *et al.*, 1988).

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).

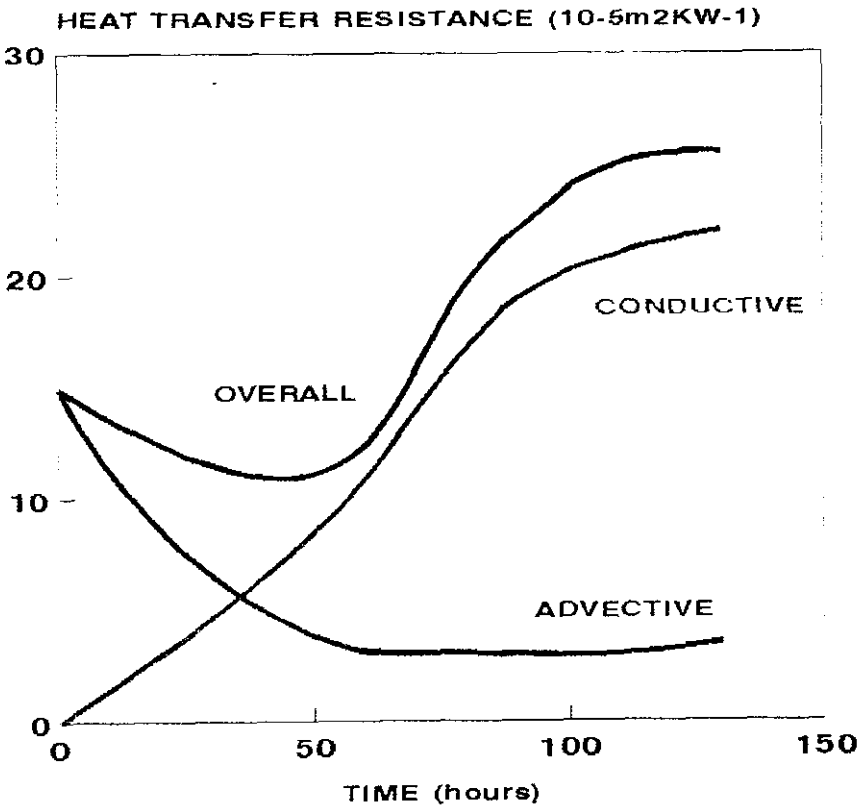

Figure 2.5 : Heat transfer changes due to biofilm formation (After Characklis and Marshall, 1990).

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).

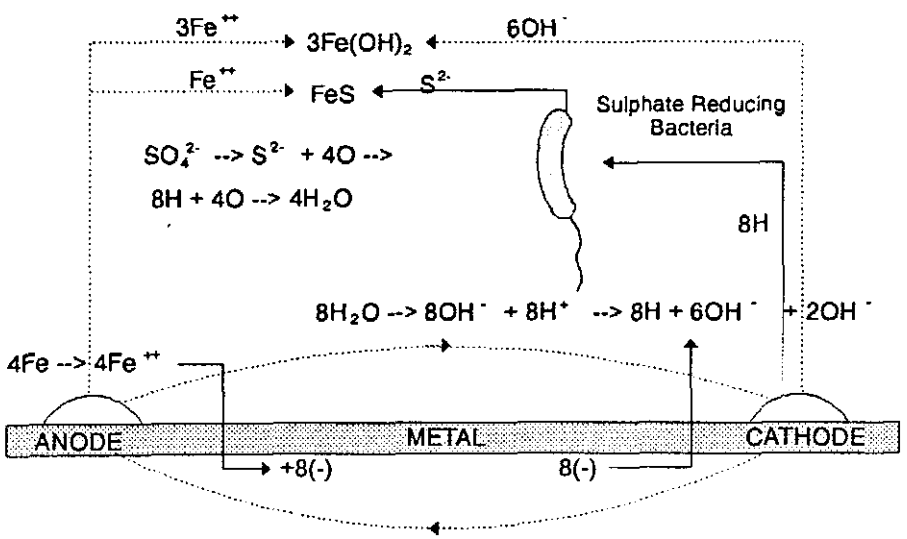

Figure 2.6 : SRB influenced corrosion according to the cathode depolarisation theory (After Lutey, 1980).

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.

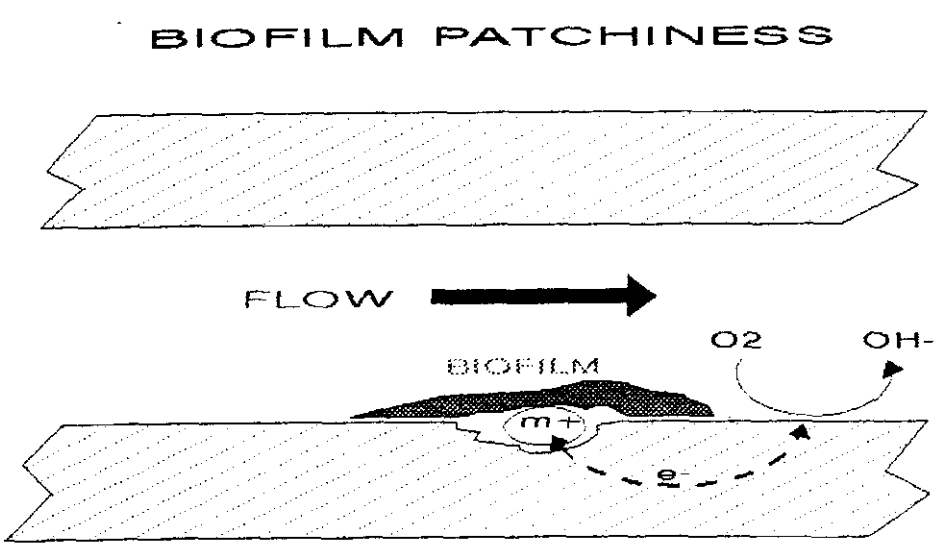

Figure 2.7 : Disruption of a metal surface by a biofilm (After Characklis and Marshall, 1990).

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.

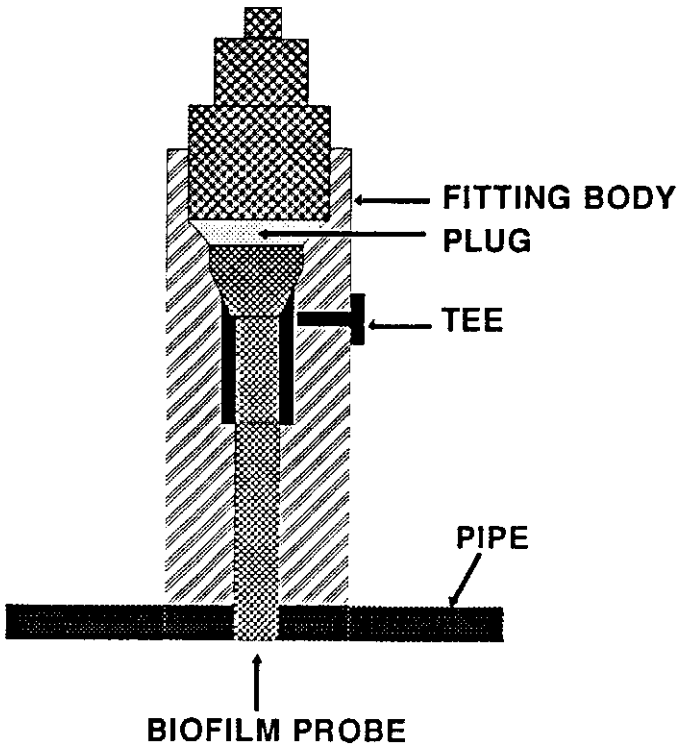

Figure 2.8 : CAPROCO Device biofouling monitor (After Blackburn and Mullin, 1990).

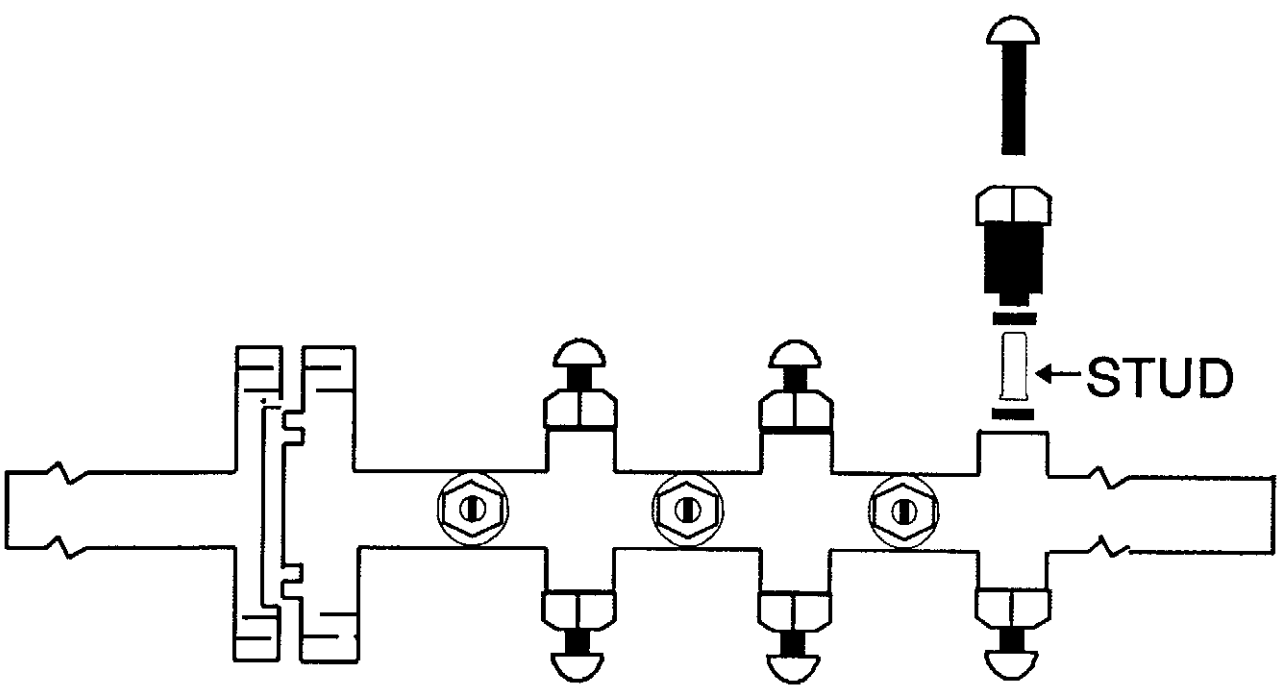

Figure 2.9 : Robbins Device biofouling monitor (After Costerton *et al.*, 1988).

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).

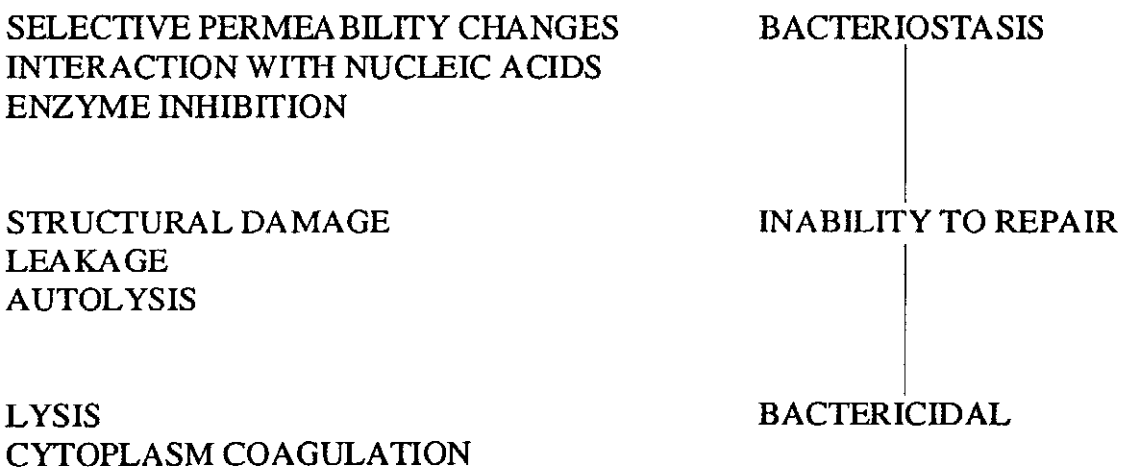

Figure 2.10 :The consequence of biocide-induced damage of bacterial cells (After Denyer, 1990).

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.