MICROBIOLOGICAL FINGERPRINTING OF ANOLYTE

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INTRODUCTION

Bacterial colonisation of surfaces in an aqueous environments is a basic strategem for survival in nature as nutrients are more available at the solid - liquid interface (Hoppe, 1984; Lawrence, et al., 1987). The resulting aggregates form microcolonies which develop into biofilms (McCoy et al., 1981). These biofilms promote corrosion of metals by creating potential differences across surfaces and by harbouring sulphate-reducing bacteria (Iverson, 1987). They also increase fluid frectional resistance (McCoy et al., 1981) and decrease the rate of heat energy transfer (Characklis and Cooksey, 1983). The above phenomena are termed collectively as microbially induced corrosion (MIC). As the costs attributable to microbially induced corrosion are high, ca. R400 million in 1988 the South African Industry (Von Holy and Cloete, 1988), effective control of bacterial numbers in industrial aqueous environments is essential.

A range of bactericidal substances, commonly termed biocides or microbicides, are available, all of which are claimed by their agents to kill bacteria in aqueous systems quantitatively. However, different bacteria react differently to bactericides, either due to differing cell wall properties (Paulus, 1987), or to other mechanisms of resistance, either inherent or inducible (Heizel, 1988). It mining the percentage kill against a standard laboratory pure culture could lead to misleading results (Allsop and Seal, 1986). Bactericides should be evaluated against the organisms which they are expected to kill, i.e. the dominant ones in the system to be treated. The composition of microbial populations in systems varies with the type of water used (Cloete et al., 1989b). A bacterial population structure of south African water-cooling systems was determined by Cloete et al. (1989a). Eighteen dominant isolates from this study were used to determine the bactericidal fingerprints of 32 commercially available non-oxidising water treatment bactericides.

MIC is caused by the presence of bacteria in water Systems, especially by bacterial biofilms. It is the most obvious conclusion, that such bacterial biofilms must be removed. Five approaches are currently followed: (i) Bacteria are chemically killed by application of bactericidal compounds termed biocides at lethal doses, (ii) biofilms are dispersed by dispersants, (iii) biofilms are removed physically by a variety of processes, (iv) the biofilm structure is weakened by enzymes or chelants and (v) planktonic bacterial numbers are controlled by ultraviolet light.

LITERATURE REVIEW

1. BIOCIDES

Bactericides are antirnicrobial agents employed in various spheres of human activity to prevent, inhibit or eliminate microbial growth. They can be divided into two groups; those occurring naturally and mostly produced by procaryotic organisms (termed antibiotics), and

those not occurring readily in nature (termed antiseptics, disinfectants, biocides, bactericides, sanitisers and preservatives). Members of the second group are classified, depending either on their chemical nature, but more often on their specific field of application.

The use of bactericides to control biofouling in water cooling systems is an accepted practice. A recent market survey indicated that the direct prevention costs in terms of bactericide usage in South Africa was R 19.6 million. Although bactericides are employed to reduce bacterial numbers, mere use of the correct bactericides does not necessarily reduce the fouling rate. It is essential to apply the correct dosage at the correct frequency to maintain antibacterial activity in the water. Incorrect use of biocide gives poor results and is expensive. The building blocks of a successful biocides programme are ideally considered to be:

- knowledge of the organisms to be killed;
- selection of the correct biocide or combinations and their respective concentrations;
- scientific determination of dosage frequency;
- monitoring the control of microorganisms through analysis and data processing;
- monitoring microbial attachment to surfaces.

The modes of action of a plethora of antibiotics have been investigated in detail. Little has been published on the mechanisms of action of most bactericides and antiseptics. Exceptions are quaternary ammonium compounds and biguanides. Generally, bactericides are not as site-specific as are the antibiotics.

Bactericides attack functional cell components, placing the bacterium under stress. At low concentrations bactericides often act bacteriostatically, and are only bacteriocidal at higher concentrations. Targets of bactericide action are components of the cytoplasmic membrane or of the cytoplasm. For bactericides to be effective, they must attain a sufficiently high concentration at the target site in order to exert their antibacterial action. In order to reach their target site(s), they must traverse the outer membrane. Therefore different bacteria react differently to bactericides due to differing cell wall properties. Bacteria with effective penetration barriers to bactericides display a higher inherent resistance than those bacteria which are readily penetrated. The rate of penetration is linked to concentration, so that a sufficiently high bactericide concentration will kill bacteria with enhanced penetration barriers.

Water treatment bactericides fall into two categories, oxidising *(eg.* chlorine and hydrogen peroxide) and non-oxidising. Non-oxidising bactericides can be divided into five groups based on their chemical nature or mode of action, and these will be discussed below.

1.1 OXIDISING BIOCIDES

Oxidising biocides are general chemical oxidants. They are not selective for living organisms, but react with any oxidisable matter. However, they are bactericidal because certain bacterial cell components can react readily with them, having a higher oxidation potential than most other chemicals present in water. Three classes of oxidising biocides are available for bactericidal applications; oxidising halogens, peroxides and ozone.

1.1.1 Peroxides

These are unstable oxygen compounds which decompose to form free hydroxyl radicals. These react oxidatively with organic compounds. The peroxides include hydrogen peroxide, peracetic acid, aromatic peroxyacids, persulphates and calcium peroxide.

- **Hydrogen Peroxide**

Hydrogen peroxide is an ideal water treatment bactericide as it is stable if stored correctly, non-corrosive and it is totally miscible with water. It has good antimicrobial properties and decomposes to water and oxygen, leaving no toxic waste. Hydrogen peroxide penetrates cells causing site-directed damage due to metal-dependant OH formation. it causes DNA strand breaks and base hydroxylation. Guanine and thymine are the two main targets of peroxide-generated free radical attack. The resulting 7,8-dihydro-8-oxoguanine mispairs with adenine whereas thymine oxidation products stop DNA polymerase, halting replication. Most bacterial mutants cannot survive due to incoherent metabolism, so that peroxide treatment at low concentration leads to slow death. Hydrogen peroxide also inhibits mitochondrial ADP-phosphorylation.

The development of resistance to oxidising bactericides has not been reported in the biofouling control literature. However, a variety of bacteria, mostly fermentative, exhibit oxidising stress response by producing oxidant-degrading and repair enzymes. These include *Escherichia coli Salmonella typhimurium, Bacillus subtilis* and *Pseudomonas fluorescens.* Stress response means that cells become more resistant to a deleterious factor within hours of exposure to sub-inhibitory quantities of the factor. A variety of defense genes have been characterised in *Escherichia coli,* encoding various superoxide dismutases, catalyses, alkyl hydroperoxide reductases and glutathione reductases, as well as DNA repair enzymes. In addition various regulatory genes have been characterised, including *OxyR, Re~ and SoxR.* These regulators determine intracellular redox potential, and activate stress response when cells are exposed to oxidising agents.

- **Organic peroxides**

Peracetic acid is the best known of the organic peroxides. Like hydrogen peroxide, it forms free hydroxyl radicals which react with various protein structures and DNA. In addition, the dissociation of peracetic acid leads to formation of acetic acid which is mildly antibacterial itself. Application of peracetic acid to systems does not leave any toxic waste behind. The antibacterial activity of peracetic acid is not affected by water hardness or organic contamination, making it suited for application in cooling water.

1.1.2 Oxidising Halogens

Hypochloric and hypobromic acids posess excellent antibacterial activity, although within a defined pH range.

- **Chlorine Compounds**

Hypochloric acid is used in various applications to prevent, control, or decrease bacterial activity. Hypochlorite was first employed as wound disinfectant by Hunter in 1831, and its bactericidal activity was confirmed by Koch in 1881. Hypochlorite is used among others in industrial water systems to control biofouling.

The antibacterial mechanism of action of hypochlorite is not clear to date although much work on the mechanism of action in eukaryotic cells has been done. HOCl does not enter freely into eukaryotic cells but attacks surface and plasma membrane proteins, impairing transport of solutes and the salt balance. It oxidises sulfhydral groups and inhibits plasma membrane ATPases. It appears to halt protein synthesis in cells at low concentrations for ca. 2 hours following exposure. It does, however, not cause any damage to eukaryotic genomic material.

The stability and antimicrobial activity of hypochloric acid is dependant on pH. It dissociates at pH greater than 7, and the undissociated moiety is the antibacterial one. Above pH 7,5 it looses its antibacterial activity. It is excellent for biofouling control as it weakens the extracellular polysaccharide (EPS) structure, leading to sloughing and removal of sections of the biofilm.

- **Bromine Compounds**

Hypobromous acid works similarly to hypochloric acid. It is, however, stable at a pH up to pH *8.5.* This makes it more suited for application in cooling waters which are often maintained at a slightly alkaline pH. Certain organic compounds release hypobromic and hypochloric acid slowly when in solution. An example is 3-bromo -1-chloro -5,5 - dimethylhydantoin. Such compounds maintain a longer antibacterial level of hypohalous acid in the system treated.

1.1.3 Ozone

Ozone is a strong oxidising material capable of killing bacteria and algae and of inactivating viruses. It is an unstable gas with a pungent odour. It further degrades the EPS holding biofilms together, so that treatment results in loosening of the biofilm. This leads to loosening of scale from the surface. Ozone has a very short half-life and therefore has to be generated on site. In distilled water its half-life at 20 °C is 25 minutes. Its solubility in water is 13 times that of oxygen. Upon reaction with organic material it decomposes to oxygen. It does, however, react with several cations and anions such as Fe^{2}+, Mn^{2}+, MnO_4, $N0^{2-}$, and CN. Ozone is toxic to humans, and detectors should be installed together with ozone generators. However treated water is perfectly safe as ozone degenerates to oxygen.

1.2 NON-OXIDISING BIOCIDES

These include a variety of organic chemical compounds which have antimicrobial activity. Their modes of action differ vastly, and their only common denominator is that they are non-oxidising organic molecules. They can be grouped into five distinct categories.

1.2.1 Detergent-type Biocides

Three groups of surface-active antimicrobial agents have been documented to date; anionic, cationic, and amphoteric. Anionic antimicrobials are only effective at pH < 3.0 and include the aliphatic acids such as sodium dodecyl sulphate. The cationic antimicrobial agents are the quaternary ammonium compounds which are well documented and widely used. The best known is benzalkonium chloride.

Benzalkonium chloride adsorbs to the cell surface of negatively charged cells (pH > 7.0) in an irreversible way. The pH minimum for antimicrobial activity is 3.0. It is membrane active and induces leakage of cytoplasmic constituents. Upon exposure to benzalkonium chloride, membranes of *P. cepacia* appeared irregular, indicating membrane damage. At 37 °C it is twice as active as at 20 °C. It is active against gram positive and also against gram negative cells, but not against spores. Cations such as Ca^{2}+ and Fe^{3}+ decrease its activity, as does NaCI.

- **Biguanides**

Biguanides are polymer derivatives of a general guanidine structure. Two biguanides are currently used as industrial bactericides. These are polyhexamethylene biguanide (PHMB) and 1,6-di-(4-chlorophenyldiguanido)-hexane, better known as chlorhexidine. Both are not corrosive and all are well suited for application in cooling water. Biguanides are bacteriostatic at low concentrations and bactericidal at higher concentrations, and have a wide spectrum of activity, especially against gram negative bacteria. They are membrane active agents and attach rapidly to negatively charged cell surfaces (pH neutral or alkaline). By making use of ^{14}C-radiolabelled PHMB, it has been shown that PHMB 15 absorbed into cells of *E. coli* within 20 S after exposure. Bactericidal action, however, requires a few minutes. Biguanides compete with divalent cations for negative sites at LPS, displacing these. PHMB then interacts by elecrostatic interactions with the charged headgroups of phosphatidylglycerol and diphosphatidylglycerol (negative), but not with the neutral phosphatidylethanolamine. By binding to phospholipids of the inner leaflet of the outer membrane and of the outer leaflet of the inner membrane, the two membranes attain net positive charges and are repelled from each other, causing membrane damage by distortion. This is supported by TEM studies on *P. cepacia* where both membranes acquired a distinct irregular appearance after treatment with chlorhexidine. Cytoplasmic constituents start leaking out of the cell due to rupturing of the membranes, and the cell loses its viability.

1.1.4 Aldehyde-type Biocides

Two aldehydes are commonly used as antimicrobial agents, ie. formaldehyde and gluteraldehyde. Further there is a range of bactericides such as the hydroxyethyl- and ethyltriazine- bactericides available which all release formaldehyde. Formaldehyde has a high polarity and high nucleophilic reactivity, so that it reacts primarily with free primary amino groups, but also with amines, amides, suffides, purines and pyrimidines. In water it hydrates to methylene glycol. Reaction with primary amino groups leads to the formation of methyloamines which react further with cellular components. Formaldehyde damages the transport properties of membrane porins, decreasing the rate of proline uptake and of enzyme synthesis. It is active over a wide pH spectrum (3.0-10.0), and is sporicidal.

Gluteraldehyde also reacts with amino and sulfhydryl groups. It is stable in acid solution but is only active at pH 7.5 - 8.5, so it must be alkalinified before application. A 2 % solution at the correct pH is ten times more bactericidal than a 4 % solution of formaldehyde. Its reactivity is related to temperature; a 2 % solution kills spores of *Bacillus anthracis* in 15 min at 20 °C, whereas it requires only 2 min at 40 °C. In gram positive bacteria it reacts with, and binds to, peptidoglycan and teichoic acid, and is also sporicidal. In gram negative bacteria it reacts primarily with lipoproteins of the outer membrane, preventing the release of membrane-bound enzymes.

Phenol Derivatives

Phenol was the antimicrobial agent which revolutionised invasive surgery, and was pioneered by Lister in 1870. It enters the cell by dissolving in the membrane, and upon entry into the cytoplasm, precipitates proteins. It is, however, harmfull to humans, and its antibacterial activity is not very high. A range of halogenated phenols, cresols, diphenyls and bisphenols have been developed from phenol, and have excellent antimicrobial activity, many being applied in the preservation of pharmaceutical products. Halogenation increases the antimicrobial activity of phenol, as does the addition of aliphatic and aromatic groups. Bisphenols have the highest antimicrobial activity of the phenol derivatives, especially halogen substituted ones. Hexachlorophen and 2,2'-methylenebis(4-chlorophenol) (dichlorophen) fall into this group.

Phenol derivatives are membrane active agents. They penetrate into the lipid phase of the cytoplasmic membrane, inducing leakage of cytoplasmic constituents. 3- and 4-chlorophenol uncouple oxidative phosphorylation from respiration by increasing the permeability of the cytoplasmic membrane to protons.

1.1.5 Thiol-oxidising Biocides

Thiols on amino acids such as cysteine are important groups which influence the tertiary structure of proteins by the forming disulphide bridges. Three groups of antimicrobial agents, isothiazolones, Bronopol (2-bromo-2-nitropropane-1,3-diol), mercury and other heavy-metal compounds, react with accessible thiols, altering the three dimensional structure of enzymes and structural proteins. Mercury interacts with sulfhydryl groups by complexing with sulphur. Bronopol oxidises thiols to disulphides, reacting especially with the active center of hydrogenase enzymes.

Three isothiazolones possess antibacterial activity; 5-chloro-N-methylisothiazolone (CMIT), N-methylisothiazolone (MIT) and benzisothiazolone (BIT). Isothiazolones react oxidatively with accessible thiols such as cysteine and glutathione. These thiols are oxidised to their disulphide adjuncts which, in the case of cysteine, leads to an alteration of protein conformation and functionality. Isothiazolone is hereby reduced to mercaptoacrylamide, which in the case of CMIT tautomerises to thioacyl chloride, the latter reacting with amines such as histidine and valine. Isothiazolones are primarily bacteriostatic, and are only bactericidal at high concentrations.

1.1.6 Miscellaneous Biocides

The mechanisms of action of various antimicrobial agents, employed to control bacterial growth in cooling water systems, have not been formally published to date. These include phosphonium chloride (tetra-alkyl phosphonium chloride), thiocarbamates (Na diethyldithiocarbamate) and MBT (methylene bis-thiocyanate). Phosphonium chloride probably has surfactant properties, damaging the bacterial cell envelope. The mechanism of antimicrobial action of MBT is not known to date. Thiocarbamates are used as agents for the extraction of trace metals such as Fe, Cd, Co, Cu, Mn, Ni, Pb and Zn. This would imply that it chelates iron, a vital trace element of most bacteria. The nucleophilic sulphur atom indicates potential reactivity with accesible thiols. Thiocarbamates do react with accessible thiols and amines. Therefore their antibacterial mechanism of action would rest partially on denaturation of surface proteins. We have found that the antibacterial mechanism of action depends on the

alkyl chain length of the thiocarbamate. Sodium diethyldithiocarbamate is inactivated by free ferrous iron, indicating that it removes iron (a growth factor) from the cell. Sodium dimethyl dithiocarbamate is not inactivated to the same degree, showing that its antibacterial activity does not rest as much on iron removal.

- **ECA technology**

Water of varying mineralisation is passed through an electrochemical cell, the specific design of which, permits the harnessing of two distinct and electrically opposite streams of activated water. Aside from its distinctive attributes, the negatively charged anti-oxidant solution **(Catholyte)** can also be channeled back into the anode chamber, thereby modulating the quality of the positively charged oxidant solution **(Anolyte)** that is produced. Depending on the specifications of the required application, variations in the design of the hydraulic systems can be effected to meet the requisite objectives.

The design of the cell is such, as to ensure a uniformly high voltage electrical field through which each micro-volume of water must pass. This unipolar electrochemical activation created by potential gradients of millions of volts per cm^2 between the anode and cathode terminals, results in the creation of solutions whose pH, Oxidation Reduction Potentials (ORP) and other physico-chemical properties, lie outside of the range which can be achieved by conventional chemical means.

Properties of Activated water

The properties of the activated solutions are dependent upon a number of factors. These comprise the solution flow rate through the reactor cell, the current being applied, temperature, the degree of feedback of catholyte into the anolyte chamber and the degree of mineralisation of the water.

During electrochemical activation, three categories of products within the solution are generated. They comprise:

1. Stable products which include acids and bases which influence the pH of the solutions,
2. Highly active unstable products including free radicals and electrolytic gases in the form of micro-bubbles which influence the ORP of the solutions, and
3. Quasi-stable products comprising complexes of hydrated membranes which form clusters of water molecules which impart the catalytic activity of the solutions.

Without maintenance of the activated state, these diverse products degrade to the relaxed state of benign water and the anomalous attributes of the activated solutions such as altered conductivity and surface tension similarly revert to pre-activation status.

It is important to note that the level of mineralisation of input water required to generate optimally metastable solutions is insignificantly different from the composition of benign potable water. However, the heightened electrical activity and altered physico-chemical attributes of the solutions differ significantly from the benign state, but yet remain non-toxic to mammalian tissue and the environment.

Biocidal properties of Anolyte

Earlier technologies that have employed electrochemical activation to generate biocidal solutions have not been capable of separating the output the Anolyte and catholyte solutions. In these cases the two opposing solutions have neutralised each other with regard to potential electrical activity.

The advantages of the current ECA technology has been confirmed, wherein the biocidal activity of hypochlorous acid generated by the current ECA technology is 300 times more active than the Sodium hypochlorite generated by earlier systems. Additionally comparison of neutral Anolyte (pH=7), with alkaline Gluteraldehyde (pH=8.5), showed that the latter required a concentration of 2% versus 0.05% of the former, in order to achieve the same biocidal efficacy. Similarly, it has been shown that a 5% solution of sodium hypochlorite (Jik) can only be used for purposes of disinfection whilst a 0.03% solution of neutral Anolyte, has both disinfectant and sterilising properties. In general, the biocidal activity of non-activated neutral Anolyte (only stable products and no electrical charge) is 80 times the potential activity of the hypochlorite solution, but still exhibits only one third of the full biocidal potential of the optimally activated ECA solution.

Thus, activated solutions have been conclusively shown to exceed chemically derived equivalents both in low dosage effectiveness as well as physico-chemical purity. This heightened biocidal capacity relative to traditional chemical solutions, permits the incorporation of ECA solutions at substantially lower dose rates, therein obviating the risk of intoxication, adverse environmental impact, while providing cost effective resolutions

1.3 FACTORS AFFECTING THE EFFICACY OF BIOCIDE TREATMENT PROGRAMMES

The antibacterial activity of bactericides is determined by their chemical reactivity with certain organic groups. Bactericides do not select between free and cell-bound groups. Therefore oxidising bactericides react with any readily oxidisable organic compound, and not only with live cells. Bactericide activity is influenced by the chemistry of the surrounding where it is employed. Factors affecting bactericide effectivity are the following:

- pH
- water hardness
- organic compounds such as proteins or saccharides
- additives such as antiscaling agents or corrosion inhibitors

These factors effect different bactericides to different degrees. Some bactericides are not very stable in concentrated form and undergo changes. Formaldehyde polymerises when exposed to polar compounds (acids or alkalis) or high temperature and oxidises to formic acid when exposed to air. Isothiazolones are unstable at temperatures above 40 °C and chlorhexidine is unstable above 70 °C. A decrease in the efficacy of a bactericide treatment programme can be due to a decrease in bactericide activity, or due to inactivation by adverse conditions, and does not always indicate bacterial resistance.

Water is the essence of life and as a chemical compound, its composition and characteristics have no analogue in nature.

The molecular composition of water comprises two hydrogen and one oxygen atom, and the

opposite polarity of the charges between the molecules, results in a process of "hydrogen bonding". It is the very nature of this polarity of charge that imparts the capacity of water to be activated into metastable states with distinctive and unique attributes.

ECA technology is based upon the generation, by means of specialised electrochemical systems, of metastable or activated solutions being derivatives of basic water molecules which display abnormal physico-chemical and catalytic activity. The source components of these activated water solutions are simply potable water and a small quantity (0.2-5g/l) of salt.

During the period of increased activity, these meta-stable solutions have been shown to have both real and potential applications in a diverse array of technological processes, often as a substitute for traditional chemical agents. Water has been described as the universal solvent, and hence the variety of applications for the use of the activated water solutions are potentially beyond constraint.

Irrespective of the characteristics of the specific solution, where activation status may extend from hours to days, the resultant product following decay of the state of activation remains benign water.

The ability to consistently produce a solution of a specific quality, possessed of unique and proven attributes, on a demand driven basis, with no adverse environmental consequences, significantly differentiates ECA technology from current customary interventions in water treatment and its allied applications.

OBJECTIVE

The objective of this study was to determine the minimum inhibitory concentration of anolyte using 15 reference bacterial strains.

Materials and Methods:

Test isolates were obtained from the culture collection of the Department of Microbiology and Plant Pathology, University of Pretoria. Bacterial cultures were grown on Nutrient agar for 24 h at 30^0C. Suspensions were diluted to ca. 3×10^8 cfu.ml^{-1} using the McFarland scale (McFarland, 1970). Initial counts of suspensions were determined by spreading 0.1 ml quantities of a serial dilution series onto Nutrient agar. Anolyte was initially added to the culture suspensions to a final concentration of 1:10 (anolyte: water), 1:20 and 100% pure. Suspensions with anolyte were incubated at 25^0C for 6 h. Viable counts were determined as described above. The killing percentage was calculated for each individual culture-anolyte combination using the formula:

% Kill = 100 - (survivor count/initial count) x 100

RESULTS:

Table 1: Percentage kill of bacterial strains at different anolyte concentrations

		Concentrations of anolyte		
Bacterial strain	**Gram stain**	**100%**	**1:10**	**1:20**
Bacillus subtilis	-	100	100	78
Pseudomonas aeruginosa	-	100	100	87
Acinetobacter calcoaceticus	-	100	100	100
Lactobacillus brevis	+	100	100	100
Micrococcus luteus	+	100	100	100
Streptococcus feacalis	+	100	100	31
Pseudomonas flourescens	-	100	100	66
Staphylococcus aureus	+	100	100	100
Pseudomonas alcaligenes	-	100	100	52
Pseudomonas medocina	-	100	100	88
Pseudomonas putida	-	100	100	90
Bacillus cereus	-	100	100	92
Micrococcus roseus	+	100	100	100
Pseudomonas stutzeri	–	100	100	57
Pseudomonas syringae	–	100	100	87
Campylobacter jejuni		100	100	Not determined

Anolyte gave a 100 % kill of all the test isolates at a concentration of 100 % and 10 % (Table 1). At a 1:20 dilution, variable kill percentages were obtained ranging from 100 % - 31 % (Table 1). This indicated variable susceptibility of different bacteria to anolyte. This is not an uncommon phenomenon (Brözel and Cloete, 1991). Many organisms are intrinsically more tolerant of antimicrobial substances, than others. Anolyte was more effective against the Gram positive bacterial strains at a 1:20 dilution giving a 100 % kill against all Gram positive strains excepting *S. faecalis* in contrast with its efficacy against Gram *A calcoaceticus* where anolyte also gave a 100 % kill at a 1:20 dilution.

CONCLUSION

Anolyte had a minimum inhibitory concentration at a 10 % concentration against all test strains.

REFERENCES

ALLSOP, D and SEAL, KJ (1986) *Introduction to biodeterioration.* Edward Arnold, London.

CLOETE, TE, PRESSLY, J and BROZEL, VS (1989a) A bacterial population structure study of water cooling systems in South Africa. *Water SA* 15(1), 37-42.

CLOETE, TE, SMITH, F and STEYN, PL (1989b) The use of planktonic bacterial populations in open and closed recirculating water cooling systems for the evaluation of biocides. *Int. Biodeterioration* 25, 115-122.

HEINZEL, M (1988) The phenomena of resistance to disinfectants and preservatives. In: KR Payne (ed.) *Industrial Biocides.* John Wiley and Sons. 52-67.

HOPPE, HG (1984) Attachment of bacteria: Advantage or disadvantage for survival in the aquatic environment. In: KC Marchall (ed.) *Microbial Adhesion and Aggregation.* Berlin Springer Verlag. 283-301.

IVERSON, WP (1987) Microbial corrosion of metals. *Adv. Appl. Microbiol.* 32, 1-36.

LAWRENCE, J.R., DELAQUIS, PJ., KORBER, DR and CALDWELL, DE (1987) Behaviour of *Pseudomonas fluorescens* within the hydrodynamic boundary layers of surface microenvironments. *Microb. Ecol.* 14 , 1-14.

McCOY, WF., BRYERS, JD., ROBBINS, J and COSTERTON, JW (1981) Observations of fouling biofilm formation. *Can. J. Microbiol.* 27, 910-917.

PAULUS, W. (1987) Development of microbiocides for the protection of materials. In: Dr Houghton, RN Smith and HOW Eggins (eds.) *Biodeterioration.* London. Elsevier Applied Science. 1-19.

VON HOLY, A and CLOETE, TE (1988) Practical aspects of monitoring biofilms and microbiologically induced corrosion in industrial water systems. *S. Afr. J. Science.* 84, 17-19.