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The chemical control of biofouling in industrial water systems

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Abstract

Oxidising and non-oxidising biocides are commonly used in an attempt to control biofouling in industrial water systems. Many of these programmes, however, fail due to the incorrect selection and application of these chemical compounds. Knowledge of the organisms to be eliminated and system hydraulics are important operational parameters in ensuring the successful application of chemical control programmes. A further complicating factor is the build up of bacterial resistance to many of these compounds. One way of limiting resistance is the alternation of oxidising and non-oxidising biocides at the correct miminum inhibitory concentration and using these in combination with surface active compounds to dislodge any biofilm. A variety of surface monitoring techniques are in use in order to monitor the success of biofouling control programmes. Unfortunately none of these techniques are ideal and results have to be considered very carefully.

Introduction

The main research effort in the field of biofouling has been to develop methods for the prevention of biodeterioration of materials, thereby preserving their value and service life for as long as possible. The levels of assimilable organic carbon in industrial water systems vary widely, depending on operational parameters and the quality of the make-up water, but will always support microbial growth (Cloete et al. 1992). The incoming water contains salts which are concentrated by evaporation. Systems operated in regions suffering water restrictions are operated at up to 16 cycles of concentration so that they contain up to 514 ppm Ca^{2+} and 4120 ppm SO_4 (Brözel 1992). The water is passaged at high rate over large surface areas in pipelines and heat-exchangers, providing favourable conditions for bacterial attachment (Characklis 1990; Savage & Fletcher 1985). These immobilised cells produce extracellular polymers, the chief constituents of which are polysaccharides and proteins in varying ratios of 0 to 10. These form a tangled mass of fibres termed a biofilm (Characklis & Cooksey 1983; Costerton et al. 1987).

Although biofilms are beneficial in certain natural and modulated environments for removing undesirable substances from waters, e.g. in rivers or waste-water treatment systems, they are responsible for a variety of effects commonly termed biofouling in industrial water systems. Biofouling in water cooling systems for example causes acceleration of metallic corrosion; increased resistance to heat energy transfer; and increased fluid frictional resistance when film thickness surpasses the monolayer. The topic has been reviewed extensively (McCoy et al. 1981; Characklis & Cooksey 1983; Hamilton 1985; Iversen 1987; Ford & Mitchell 1990; Lee et al. 1995).

The most widely practised approach to the minimisation of biofilms in industrial water systems is by way of chemical treatment focusing either on the reduction of microbial numbers using biocides, or their removal using either synthetic dispersants or enzymes. In this publication some critical determinants for successful biofouling control programmes will be discussed.

Biocides as antifouling agents

Biocides

Biocides are antimicrobial 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 prokaryotic organisms (termed antibiotics), and those not occurring readily in nature (termed antiseptics, disinfectants, biocides, 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 biocides to control biofouling is an accepted and commonly used practice. Although biocides are used to reduce bacterial numbers, mere use of the correct biocide does not necessarily reduce the fouling rate. It is essential to apply the correct biocide concentration at the correct frequency. Wrong use of biocides gives poor results and is expensive. The application of biocides has developed into a field of expertise in its own right. The building blocks of a successful biocide programme are ideally considered to be:

- knowledge of the organisms to be killed (Allsop & Seal 1986);
- selection of the correct biocide or combinations and their respective concentrations (Allsop & Seal 1986; Brözel & Cloete 1991a; Freedman 1979);
- scientific determination of dosage frequency (Freedman 1979);
- monitoring the control of microorganisms through analysis and data processing (Freedman 1979; Young-Bandala & Boho 1987; Cloete et al. 1992);
- monitoring microbiological attachment to surfaces (Tamachkiarowa & Flemming 1996).

Selection of the correct biocide programme depends mainly on the variety of bacteria encountered and on their respective numbers. Knowledge of the different kinds of microorganisms in these water systems will greatly assist in selecting the correct biocides. The minimum inhibitory concentration (MIC) of different biocides and the contact time of a particular biocide required for a specific kill percentage against a particular organism (biocide fingerprint) can be determined only once the microbial population structure in a system is known (Brözel & Cloete 1991a). These data would pre-determine the minimum contact time required for a biocide to kill bacteria and therefore directly influence the dosage concentration. This is often ignored. Consequently biocide, dosage concentrations and frequency of dosage have often been selected on an arbitrary basis, resulting in ineffective programmes for the control of biofouling.

Biocides attack functional cell components, placing the bacterium under stress (Wainwright 1988) (Figure 1). At low concentrations biocides often act bacteriostatically and are only bactericidal at higher concentrations (Woodcock 1988). Targets of biocide action are essentially components of the cytoplasmic membrane or the cytoplasm. In order to reach their target site, biocides must therefore traverse the outer membrane and attain a minimum active concentration at that site (Brözel & Cloete 1993b).

Detergent type biocides

Three groups of surface-active antimicrobial agents have been documented to date; anionic, cationic, and amphoteric (Wallhäußer 1995). Anionic antimicrobials are only effective at pH < 3.0 and include the aliphatic acids such as Na dodecyl sulphate (Wallhäußer 1995). The cationic antimicrobial agents are the quaternary ammonium compounds which are well documented and widely used. The best known one is benzalkonium chloride which is actually a group of compounds with varying lengths of the aliphatic chain (C_8–C_{18}) (Wallhäußer 1995).

Biguanides

Biguanides are polymer derivatives of the general guanidine structure (Wallhäußer 1995). Two biguanides are currently used as industrial biocides. These are polyhexamethylene biguanide (PHMB) and 1,6-di(4-chlorophenyldiguanido)-hexane, better known as chlorhexidine. Both are not corrosive and are well suited for application in cooling water (Woodcock 1988). Biguanides are bacteriostatic at low concentrations and bactericidal at higher concentrations, and have a wide spectrum of activity, especially against gram negative bacteria (Wallhäußer 1995). They are membrane active agents and attach rapidly to negatively charged cell surfaces (pH neutral or alkaline). Fitzgerald et al. (1992), using ^{14}C-radiolabelled PHMB, showed that it was absorbed into cells of *E. coli* within 20 s after exposure. Bactericidal action did, however, require a few min. Biguanides compete with divalent cations for negative sites at LPS, displacing these. PHMB then interacts by electrostatic interactions with the charged head groups of phosphatidyl glycerol and disphosphatidyl glycerol (negative), but

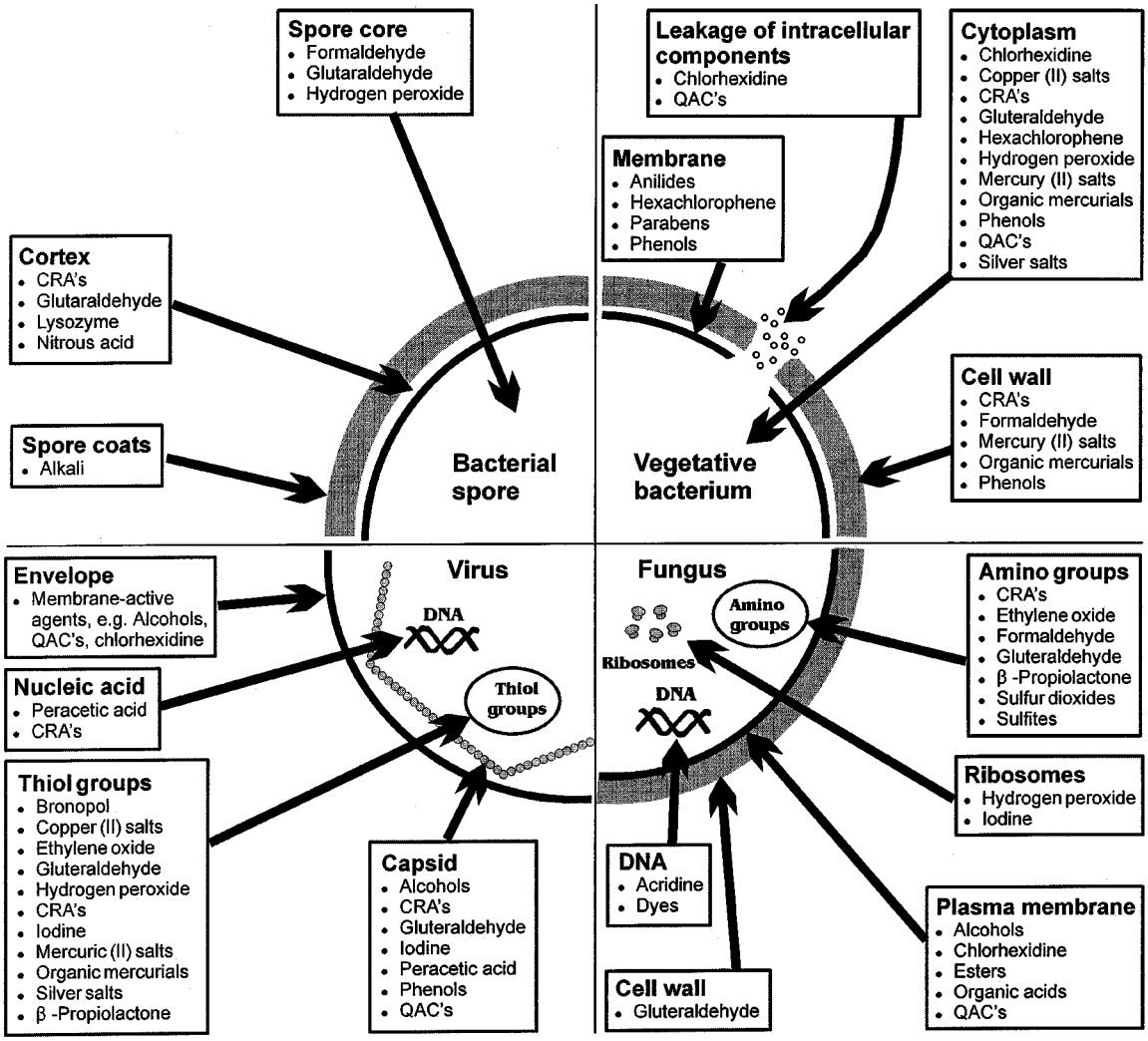

Figure 1. Mechanisms of microorganism inactivation by biocides (after Russel et al. 1997). CRA's = chlorine-releasing agents, QAC's = quaternary ammonium compounds.

not with the neutral phosphatidyl ethanolamine (Broxton et al. 1984). This is supported by TEM studies on *P. cepacia* where both membranes acquired a distinct irregular appearance after treatment with chlorhexidine (Richards & Cavill 1980). Cytoplasmic constituents start leaking out of the cell due to rupturing of the membranes, and the cell loses its viability.

Aldehyde type biocides

Two aldehydes are commonly used as antimicrobial agents, i.e. formaldehyde and gluteraldehyde. Further there is a range of biocides such as the hydroxy ethyl- and ethyl triazine-biocides available which all release formaldehyde (Sondossi et al. 1986). Formaldehyde has a high polarity and high nucleophilic reactivity, so that it reacts primarily with free primary amino groups, but also with amines, amides, sulfides, purines and pyrimidines (Rossmore & Sondossi 1988). In water it hydrates to methylene glycol. Reaction with primary amino groups leads to the information of methylo amines which react further with cellular components (Rossmore & Sondossi 1988). Formaldehyde damages the transport properties of membrane porins, decreasing the rate of proline uptake and of enzyme synthesis (Barnes & Eagon 1986). It is active over a wide pH spectrum (3.0–10.0), and is sporicidal (Wallhäußer 1995).

Gluteraldehyde also reacts with amino and sulfhydryl groups (Russel & Chopra 1990). It is stable in acid solution but is only active at pH 7.5–8.5, so it must be alkalinified before application (Wallhäußer 1995). A 2% solution at the correct pH is ten times more bactericidal than a 4% solution of formaldehyde (Wallhäußer 1995). 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 (Russel & Chopra 1990). In gram negative bacteria it reacts primarily with lipoproteins of the outer membrane, preventing the release of membrane-bound enzymes (Russel & Chopra 1990).

Phenol derivatives

Phenol was the antimicrobial agent which revolutionised invasive surgery, and was pioneered by Lister in 1870 (Franklin & Snow 1981). It enters the cell by dissolving in the membrane, and upon entry into the cytoplasm, precipitates proteins (Wallhäußer 1995). It is, however, harmful 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 (Wallhäußer 1995). 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. Hexachlorophene and 2,2'-methylenebis (4-chlorophenol) (dichlorophen) fall into this group (Wallhäußer 1995). Growth of *P. aeruginosa* is inhibited by 36 μg.ml^{-1} of the bisphenol dichlorophen whereas it is only inhibited by 1000 μg.ml^{-1} of the diphenyl o-phenylphenol (Brözel & Cloete 1993a, Wallhäußer 1995).

Phenol derivatives are membrane active agents. They penetrate into the lipid phase of the cytoplasmic membrane, inducing leakage of cytoplasmic constituents (Russel & Chopra 1990). 3- and 4-chlorophenol uncouple oxidative phosphorylation from respiration by increasing the permeability of the cytoplasmic membrane to protons (Gilbert & Brown 1978).

Thiol oxidising biocides

Thiols on amino acids such as cysteine are important groups which influence the tertiary structure of proteins by forming disulphide bridges (Stryer 1981). Three groups of antimicrobial agents, isothiazolones, Bronopol (2-bromo-2-nitropropane-1,3-diol) and mercury and other heavy-metal compounds, react with accessible thiols, altering the three dimensional structure of enzymes and structural proteins (Collier et al. 1991; Russel & Chopra 1990). Mercury interacts with sulfhydryl groups by complexing with sulphur (Russel & Chopra 1990; Wallhäußer 1995). Bronopol oxidises thiols to disulphides, reacting especially with the active center of hydrogenase enzymes (Wallhäußer 1995).

Three water soluble isothiazolones possess antibacterial activity; 5-chloro-N-methylisothiazolone (CMIT), N-methylisothiazolone (MIT) and benzisothiazolone (BIT) (Collier et al. 1990a; Wallhäußer 1995). Isothiazolones react oxidatively with accessible thiols such as cysteine and glutathione (Collier et al. 1990b). These thiols are reduced to their disulphide adjuncts which, in the case of cysteine, leads to an alteration of protein conformation and functionality. Isothiazolone is hereby oxidised to mercaptoacrylamide, which in the case of CMIT tautomerises to thioacyl chloride, the latter reacting with amines such as histidine and valine (Collier et al. 1991). Isothiazolones are primarily bacteriostatic, and are only bactericidal at high concentrations (Collier et al. 1991).

Chlorination

Chlorination is the addition of chlorine directly to the water being disinfected. The procedure of killing germs via chlorination depends very much on the pH value, and within the permissible pH range of 6.5–9.5 the disinfection effect of chlorine decreases as the pH increases. Chlorine in the form of hypochlorous acid (HOCI) is an effective disinfectant against both bacteria and viruses. Ammonia and particularly organic nitrogen compounds weaken the effect of chlorine as they bind with free chlorine to form inorganic and organic chloramines.

Although chlorine disinfection has been extensively studied, data on the mechanisms whereby chlorine deactivates bacteria and viruses, is sparse. Hypochlorous acid is generally considered to be a destructive, non-selective oxidant which reacts with all biological molecules. However it became clear that HOCI has a specific target site of destruction, which is in conflict with the selective theory. Research has shown that

the first site of interaction of hypochlorous acid with *Escherichia coli* is the cell membrane leading to physical and chemical changes resulting in permeability changes and leakage of macromolecules. Albrich et al. (1981), demonstrated that lethal action occurs before HOCI reaches the cytoplasm, e.g. at the membrane although disruption of the cellular envelope is unlikely. Membrane permeability changes are possible, which might allow leakage of small molecule i.e. electrolytes and metabolites. Studies with *E. coli* revealed that the action site of HOCI involves electron-rich or functional groups located within the cellular envelope. Molecules that possess highly nucleophilic sites are the primary target and these include porphyrins and hemes, ferredoxin-like iron-sulfur centers, purine and pyrimidine bases, conjugated polyene amines and sulfhydryl groups.

In *E. coli*, HOCI terminates adenosine-triphosphate (ATP) production by inhibiting transport of the fermentative substrate, glucose and respiratory succinate, while simultaneously inactivating the membrane-localised proton-translocating ATP-synthetase, which is of utmost importance during respiration. The factors contributing to the widespread use of chlorine include its relatively low cost, ease of application, proven reliability, and the familiarity with its use. The main advantage of disinfecting with chlorine is the development of an easily measured residual of Chlorine. Chlorine can react with organic matter to form organohalogenated compounds. Chloroform and certain other trihalomethanes are formed principally during chlorination. It is also reported that the addition of chlorine at a sufficiently high concentration will kill most bacteria but the chlorine residual soon disappears in a distribution system with a high chlorine demand, allowing bacterial regrowth.

Disinfection of biofilm organisms by chlorine (or chloramine) is very difficult. As high as 1.5 $mg.L^{-1}$ free chlorine residuals may not penetrate biofilms and inactivate bacteria.

Ozonation

Ozone has long been a proven technology for water disinfection and is particularly well established in Europe, Canada, and the Soviet Union. Although many water treatment plants throughout the world still utilize ozone primarily for disinfection, most modern plants rely on ozone to perform one or more oxidation functions. Applications for ozonation now include oxidation of inorganic and organic materials, flocculation and microflocculation for removal of turbidity or suspended solids, and recently promotion of aerobic biological processes conducted in filter and adsorption media. The only method of commercial importance used for ozone generation in water treatment and disinfection is the process using a strong alternating current electric field to produce corona discharge in the feed gas, which is usually air that has been filtered and dried.

In addition, when ozone decomposes, it generates radical intermediates that have much greater oxidizing power than ozone itself does. Ozone suffers from two major limitations as an alternative to chlorine. Firstly, it is unstable in water. For example, at pH 8, its half-life is less than one hour, too short to ensure that a residual disinfectant capacity will remain. Secondly, ozone reacts with natural organic substances to produce low-molecular-weight oxygenated by-products that generally are more biodegradable than their precursors. These substances will promote biological growth and further limit the disinfection efficacy of ozone. An other disadvantage of ozone is the inability of on-site storage.

Ultraviolet irradiation

Ultraviolet light is a non-chemical form of disinfection. As research has developed cheaper, more efficient and more reliable UV light sources, interest in the disinfection of drinking water by UV rays has been growing in recent years. The reduction of bacteria through UV-irradiation is achieved by photons with a wavelength in the UV-C band or region, i.e. 200 nm to 280 nm. These high energy photons, most effective at a wavelength of 253.7 nm, destroy RNA or DNA molecules, thereby killing bacteria without the addition of chemicals to the drinking water.

The efficiency of UV-irradiation also depends on the quality of the water, especially in relation on the turbidity of the water, agglomeration of microorganisms and organic and inorganic dissolved substances. Suspended particles in the water absorb UV-rays, and thus the effective dosage is reduced. Most organic components absorb UV-irradiation, as do certain inorganic salts. The more substances there are present in the water the lower is the transmittance of the water for UV irradiation. The rapid diffusion of UV has always been hampered by the lack of residual. The main advantages of UV-irradiation are related to the no introduction of foreign substances to the water and no energy input and the high operational security.

Hydrogen peroxide

Hydrogen peroxide is a disinfectant with bactericidal and sporicidal properties. Hydrogen peroxide is effective against a broad range of bacteria, including many which have become resistant to chlorine-based chemicals, such as *Pseudomonas aeruginosa*. However, hydrogen peroxide is used on a large scale in conjunction with other disinfectants such as ultraviolet-irradiation and ozone, particularly the latter. In this case, it is known as the peroxone process which is becoming popular in many parts of the world.

Hydrogen peroxide itself is not reactive and therefore not bactericidal. It has to be converted to radicals such as the hydroxyl radical (·OH) which react with cell components such as nucleic acids, proteins and lipids. Hydrogen peroxide can act as a weak oxidising agent and will attack thiol groups of proteins or reduced glutathione. It can also react directly with some keto acids. The antimicrobial action of hydrogen peroxide may involve impingement of surface membranes through ·OH formation, oxidation of protein sulphydryl groups and double bonds. Also having a sporicidal effect, hydrogen peroxide causes lysis of the spores which include damage to the spore coat, oxidative cortex hydrolysis or germination like changes due to activation of cortex lytic enzymes.

Knowledge of the organisms to be killed

Bacterial colonization of surfaces in aqueous environment is a basic stratagem for survival in nature as nutrients are more available at the solid-liquid interface (Hoppe 1984; Lawrence et al. 1989). The resulting aggregates form micro colonies 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 frictional resistance (McCoy et al. 1981) and decrease the rate of heat energy transfer (Characklis & Cooksey 1983). The costs attributable to the above phenomena are high. In South Africa this cost is estimated at $ 100 million per annum. The effective control of bacterial numbers in industrial aqueous environments is therefore essential.

Most non-oxidizing biocides will not kill all the different types of bacteria which are found in water cooling systems (Heinzel 1988). The application of only one such non-oxidizing bactericide will result in the selection of unaffected bacteria (Cloete et al. 1992). On the other hand oxidizing biocides are non-selective in their action. Biocides attack targets of cell function, placing the bacterium under stress (Wainwright 1988). It is well recognized that communities under stress have a lower species diversity and select for fitter species (Atlas & Bartha 1987). Where a bactericide is the stress factor, fitter species would be those resistant to or more tolerant of the specific bactericide. As diversity is inversely proportional to productivity, it would influence the corrosive nature of the biofilm.

As cells in biofilms and planktonic communities are in continuous exchange, death of cells in the planktonic phase would influence the equilibrium and shifts would occur in both the planktonic and the sessile populations (Costerton et al. 1987). Few studies have been reported regarding the effect of stress on species diversity in water cooling systems (Cloete et al. 1989b; Cloete et al. 1992). Although information on the selective activity (bactericide fingerprints) of a range of biocides has been published (Brözel & Cloete 1991a) the result of *in situ* application warrants further investigation (Cloete et al. 1989b, Brözel & Cloete 1992b).

The problem of bacterial resistance to biocides

Biocides have often been classified as non-specific protoplasmic poisons. This broad generalisation is, however, not acceptable. Instead, it is convenient to consider the modes of action of biocides in terms of their targets within the bacterial cell. The targets are the cell wall, the cytoplasmic membrane, the membrane-bound enzymes, the cytoplasm, the genome, thiol groups and amino groups (Gilbert & Wright 1987; Russel & Chopra 1990).

Few biocides are equally active against all bacteria (Brözel & Cloete 1991a; Cloete et al. 1992). Three types of resistance have been documented: inherent resistance, also termed natural or intrinsic; acquired resistance due to mutation, and adapted resistance (Brözel & Cloete 1993b; Heinzel 1988). Acquired resistance is usually plasmid mediated. An example is QAC (quaternary ammonium compound) resistance in *Staphylococcus aureus* encoded by a transposable element (Sasatsu et al. 1992). Adair et al. (1971) reported a *Pseudomonas aeruginosa* strain growing on commercial benzalkonium chloride and chlorhexidine as nitrogen source. Adair et al. (1971) found that cells resistant to QAC had an increased lipid content in the cell envelope compared to the wild strains. QAC resistant cells appear to contain more unsaturated fatty acids compared to wild strains (Jones et al. 1989). Brözel &

Cloete (1991b) found that biocides induce cross resistance to other biocides. The cross-resistance by induction indicates that a bacterial community can become more resistant to any given biocide after treatment with any other (Brözel & Cloete 1991b).

Biofilm bacteria are more resistant to biocides than the corresponding planktonic cells. Herewith a number of possible reasons why biofilm bacteria could be more resistant to biocides:

- Biofilms contain large amounts of EPS, which may protect bacteria from biocides (Christensen & Characklis 1990),
- Attached bacteria have a higher ratio of unsaturated to saturated fatty acids (Sakagami et al. 1989a, b),
- The microorganisms can produce an enzyme that destroys the biocide (Heinzel 1988),
- The permeability of the cytoplasmic membrane may be altered to prevent the entry of the biocide (Brözel & Cloete 1993a; Russel & Chopra 1990),
- A change may take place in the composition of the cell wall (Brözel & Cloete 1991b; Gilbert & Brown 1978),
- A spontaneous mutation may occur on the chromosome or in a plasmid (Summers 1986; Hall 1990).

Bacteria respond to changes in their environment by profound phenotypic variations in enzymatic activity, cell wall composition and cell surface structure (Anwar et al. 1985). These phenotypic changes often involve the target molecules that control the access of biocides (Brözel & Cloete 1993b). The susceptibility to antibacterial agents is dictated by the structure of biofilms. Biofilms can increase the concentration of a soluble antibacterial agent in the cellular environment by trapping and concentrating its molecules, as it traps and concentrates nutrients (Costerton et al. 1987). This would make the bacteria more vulnerable to biocide attack. However these effects are not yet well understood and warrant further investigation.

Pietersen et al. (1995) indicated that bacteria do not become resistant to oxidizing biocides, like for example chlorine. In order to prevent microbial resistance build-up, an oxidizing biocide should always (where possible) be part of a biocide programme for the control of biofouling.

Factors affecting bactericide effectivity

The antibacterial activity of biocides is determined by their chemical reactivity with certain organic groups. Biocides do not select between free and cell-bound groups. Therefore oxidising biocides react with any readily oxidisable organic compound, and not only with live cells. Bactericide activity is influenced by the chemistry of the surroundings where it is employed (Wallhäußer 1995).

Factors effecting 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 affect different biocides to different degrees.

Some biocides 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 (Wallhäußer 1995). Isothiazolones are unstable at temperatures above 40 °C and chlorhexidine is unstable above 70 °C (Wallhäußer 1995). 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 (Cloete et al. 1992).

Monitoring biocide concentration

A certain minimum inhibitory concentration of a biocide is required to maintain control over the microbial community in a system. Monitoring the concentration of a biocide after application to a system is therefore a critical determinant in making a biocide programme work. If the minimum inhibitory concentration were known, it may be more important to ensure that this concentration is maintained, rather than to monitor microbial attachment. In practice most biocides, especially oxidizing agents, react with substances contained in the water, decreasing the available concentration. Furthermore, even if the residual concentration could be determined accurately, it would not reflect the antimicrobial activity of the product. Techniques for the determination of antimicrobial activity of biocides are available (Cloete et al. 1993; Hill et al. 1989; Payne 1988).

In this regard, at least one bioindicator which is commercially available has been developed (Hill et al. 1989). Bioindicators are considered to be biological preparations that usually contain spores of a single bacterial strain with a known susceptibility towards an antimicrobial agent (Cloete et al. 1993). Sterikon^R

is used as a bioindicator in heat sterilisation. It is a glass vial containing spores of the *Bacillus stearothermophilus* ATCC 7953 suspended in a broth containing glucose and a pH indicator. After heat exposure the vial is incubated at 45 °C and viable spores germinate, produce acid and render the indicator yellow. Cloete et al. (1993) adapted this procedure for determining biocide concentrations.

Environmental considerations

Sooner or later biocides used to treat industrial water will be released into the environment. Ideally a biocide should affect only the target microorganisms against which its use was intended. All chemicals, however, have some effect at a greater or lesser concentration on plant and animal life. It is always assumed that dilution and natural degradation will inactivate any biocide and laboratory investigation has indicated that the commercially available biocides can be biodegraded. Such findings do not necessarily imply that biodegradation will take place equally readily in the environment.

It must be clear from the foregoing that, if industry is to continue to use biocides for the control of biofouling, questions of '*in situ*' biocide effectiveness, resistance, biodegradability and environmental impact will have to be answered. These answers will only be found by co-operation between biocide manufacturers and producers, operators, chemists, biochemists, microbiologists, marine biologists and legislative authorities (Parr 1990).

Biodispersants as antifouling agents

Biodispersant is a term normally used to described dispersants produced by micro-organisms. However, this term as used in biofouling control is actually a misnomer, since it refers to synthetic surfactants and not a product produced by micro-organisms. One of the alternate treatments for the control of microbiological growth in open recirculating cooling water systems, is the use of biodispersants (Poulton & Nixon 1990). An effective biodispersant should disperse sessile microbial populations into the bulk water, rendering them more susceptible to the action of biocides (Strauss & 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). Laboratory and field studies carried out by Poulton (1993), 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 were dispersed into the bulk water prior to the addition of a biocide, the biocide could be used more cost effectively. Biodispersants are generally less costly than biocides and can be used at lower concentrations (Poulton 1993). 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 (Russel 1990).

Surfactants and emulsifiers are integral components to many industrial, agricultural and food processes. Most of the compounds are chemically synthesized. Their surfactant and emulsification properties result from the presence of both hydrophilic and hydrophobic regions on the same molecules (Fiechter 1992). Surfactants are also an important constituent of biocides (Cloete et al. 1992). They are employed to achieve both uniform wetting of the surface to be treated and have an additional cleaning effect (Cloete et al. 1992).

Dispersants function by breaking up foulants into smaller particles and keeping them suspended in cooling water (Strauss & Puckorius 1984). This prevents deposit formation and enables foulant removal from the system via blowdown or filtration. Natural dispersants such as lignins and tannins provide good results, but must be applied continuously and at relatively high dosages (50 to 200 ppm of active chemical) (Strauss & Puckorius 1984). Unfortunately, these dispersants are derived from natural products, making them excellent food for organisms which may stimulate growth and result in the loss of fouling control.

Synthetic water-soluble polymers are the most common dispersant chemicals currently used. Synthetic dispersants represent a major improvement over natural dispersants for several reasons; they can be made to any specific molecular weight; they are not easily degraded by biological organisms; they do not react with chlorine or iron salts; and, most importantly, they are more cost effective (Strauss & Puckorius 1984).

Surfactants are also commonly used to mobilise oily or gelatinous foulants. They are low foaming, nonionic surfactants that are added at dosages of 10–20 ppm. Surfactants damage the cell by reducing its permeability; they disrupt the normal flow of nutrients

into the cell and the discharge of wastes, causing the organism's death (Cloete et al. 1992). Anionic surfactants reduce cell permeability and eventually dissolve the entire membrane (Strauss & Puckorius 1984).

Anionic surfactants

The hydrophilic group of an anionic surfactant is an anion, of which the most common are sulfonates and sulfates (Attwood & Florence 1983). Alkyl aryl sulfonates are the most widely used surfactants, since they have excellent detergent power, are made of easily available materials which make them low in price and their formulations have attractive properties. They consist of alkyl chains, with a mixture of 10–15 C atoms, but principally C_{11} and C_{12} are attached to the benzene ring in the para position of the sulfonate group (Attwood & Florence 1983). These surfactants can be used as the sodium salt as the sole surfactant or in conjuction with other anionic, non-ionic or cationic surfactants (Karsa 1992).

At low concentrations (1 mgL^{-1}) surfactants normally dissolve in water and each individual molecule or ion is present as a separate entity. As more is added, a concentration is reached (100–1000 mgL^{-1}), depending on the salt content of the solution, the temperature and the chemical nature and structure of the surfactant, at which micelles are formed. This concentration is called the critical micelle concentration (CMC). Micelles contain molecules which are orientated with their hydrophobic groups clustered together and the hydrophilic ends extending outwards. Beyond the CMC much more surfactant can be dissolved and the micelles increase in number. Micelles play a part in the cleaning action of surfactant (Attwood & Florence 1983).

Non-ionic surfactants

Surface active agents that have no electronic charge are referred to as non-ionic surfactants (Karsa 1992). Non-ionic surfactants have the advantage over ionic surfactants in that they are compatible with all other types of surfactant and their properties are generally not affected by pH (Attwood & Florence 1983). The non polar regions are mainly derived from hydrocarbon, alcohol or fatty acid sources with carbon skeletons in the range of C_8–C_{20} (Attwood & Florence 1983). The polar region is usually provided by a poly oxyethylene glycol. Examples of non-ionic surfactants are: n-octyl glucoside, Triton and polyethylene oxide (10) cetyl ether.

Cationic surfactants

Quaternary ammonium compounds (QAC) represent the cationic surfactant group. They adsorb onto the cell membrane and chemically react with the negative charge associated with the cell wall (Cloete et al. 1992). These cationic surface-active chemicals are organically substituted nitrogen compounds that are generally more effective against algae and bacteria in alkaline pH ranges (Strauss & Puckorius 1984). Their action is attributed to their positive charge, which forms an electrostatic bond with negatively charged sites on microorganism cell walls (Strauss & Puckorius 1984). The electrostatic bonds create stresses in the wall, leading to cell lysis and death. The QAC's also cause cell death by protein denaturation; cell-wall permeability is distorted, reducing the normal intake of life-sustaining nutrients to the cell (Strauss & Puckorius 1984). Problems associated with the use of these compounds are that they lose their activity in heavily fouled systems and also react with divalent cations. Because of their surface activity, they tend to emulsify oils (Strauss & Puckorius 1984). These compounds become less effective as the temperature increases. Anionic surfactants promote the inactivation of QAC's (Cloete et al. 1992).

Poulton (1993) and Jacobs (1996) both illustrated that a combination of biocide and biodispersants was more effective for biofouling control than any of these on their own. Individual programmes have however to be worked out for each system. Poulton (1993) indicated that there was no universal programme which would work in all systems.

Monitoring of biofouling

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. 1987; 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 & Kozelski 1984). Attached bacterial numbers

can exceed planktonic ones by 3 to 4 logarithm units in water systems (Costerton et al. 1987). Yet the common practice of monitoring industrial water systems still frequently involves the determination of planktonic bacterial numbers with the consequent underestimation of numbers and types of bacteria present in the biofilm. The full extent of bacterial numbers can only be determined by investigating microbial populations on the surfaces within the systems. Methods for monitoring and studying of biofilms will now be discussed briefly.

In-situ *deposit development probes*

Test surfaces can be exposed *in situ* to pressurised injection water systems, using existing technology for access into the process pipework. Test surfaces are incorporated into holding assemblies compatible with the high pressure fittings so that sampling can be performed without partial shutdown and depressurisation of the system. The Petrolite and Caproco bioprobes are the most common devices of this kind (Cloete et al. 1992).

Side-stream deposit developing monitoring devices

Process water is taken from some point in the water injection system and pumped into a side-stream experimental rig incorporating the exposure surfaces, monitoring and control equipment (Cloete et al. 1992).

Tubular geometry biofilm monitoring devices

The tubular section most closely mimics the flow conditions encountered in water injection system pipeworks. The test liquid is pumped through the centre of the tube and the fouling deposit forms on the inside surfaces. The three types of devices used to monitor biofilms in this way, are:

- *Ported tubes*. This type of device consists of small diameter pipes fitted with a series of sampling ports containing removable studs that incorporate the test surface for exposure (Cloete et al. 1992).
- *Sectioned tubes*. The tubes may be pre- sectioned and held within another tube assembly, or broken off as required or sectioned using a pipe cutter.
- *Monitored tubes*. This equipment measures the rate and extent of deposit growth by monitoring the effect of the accumulation on the heat transfer resistance (HTR) and fluid frictional resistance (FFR) (Cloete et al. 1992).

The Pedersen device

The Pedersen device carries replaceable sampling surfaces that allow more detailed observation of relatively undisturbed portions of biofilm. Wolfaardt et al. (1991) used metal slides from a Pedersen device to follow biofilm development by staining attached cells with DAPI (4',6-Diamidine-2'-phenylindole dihydrochloride) and counting these cells by epifluorescence microscopy.

The Robbins device

The Robbins device is a ported biofilm sampler consisting of removable test surfaces which are exposed to circulating fluids (McCoy et al. 1981). The Robbins device provides quantifiable samples of biofilms growing on submerged surfaces in aqueous systems. The stud surface of the device, which is exposed to the flowing bulk fluid, can be aseptically removed from the system and sampled for biofilm bacteria. The device can also be used to determine the concentration of biocides and antibiotics that kill planktonic bacteria in bulk fluids (Costerton & Lappin-Scott 1989).

Scanning confocal laser microscopy

SCLM (Scanning Confocal Laser Microscopy) allows direct, nondestructive observation of biological materials (Wolfaardt et al. 1994). The laser produces a high-intensity illumination, and since the returning signal is processed point-by-point, even low levels of fluorescence can be imaged with a sensitive photomultiplier (Wolfaardt et al. 1994). This high sensitivity, and the capability to observe samples *in situ*, render SCLM suitable to demonstrate the presence and distribution of fluorescent molecules in biological material such as biofilms (Wolfaardt et al. 1994).

Monitoring of biofilm thickness

Biofouling can also be studied with scanning electron microscopy techniques (Jacobs 1996), as well as Image analysis and Confocal Laser Microscopy, that give 3-D images of biofilms (Caldwell & Lawrence 1989). These highly sophisticated methods are not suitable for routine monitoring of biofilms due to the high cost and expertise required for operation. In addition, methods such as the use of fluorescent stains cannot distinguish between living and dead cells (Gaylarde 1990). Tech-

niques involving the use of antibodies are sensitive and interferences may occur (Tatnall & Horacek 1990).

Future monitoring technologies

- *Spectrophotometry*. Jacobs (1996) developed a spectrophotometric method for monitoring microbial attachment and indicated the viability of this technique for evaluating biodispersant and biocide efficacy under laboratory conditions. This technique showed a lot of promise for on-line biofouling monitoring.
- *Reflectance*. A very new development in biofouling monitoring is the use of fibre optic probes (Tamachkiarowa & Flemming 1996).

Microbiological analysis techniques

Monitoring microbial activity is an integral part of biofouling control and also an area where the greatest need exists for development. The lack of suitable techniques often leads to the misinterpretation of results and consequent biofouling.

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 & 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 & Little 1990; Tullmin et al. 1992). The Robbins device is commonly utilised, as a direct technique to monitor biofilm development in water systems worldwide (Poulton 1993). 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 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 Plate Count Agar. The use of the total viable cell count technique is questionable, as only a fraction of the microorganisms present in the sample grow on a single medium. However, Brözel & Cloete (1992a) demonstrated that low nutrient media yielded the highest number of planktonic bacteria isolated from cooling water systems. Historically, sulphate reducing bacteria have been identified as the major contributors to MIC in cooling water systems (Ford & Mitchell 1990; Iversen 1987; Poulton & Nixon 1990). The enumeration of SRB (sulphate reducing bacteria) is particularly problematic due to their diverse requirements for both nutrients and environmental conditions (De Bruyn 1992). It has, however, been suggested, that H_2S may play a role in anaerobic corrosion by directly attacking metal surfaces (Hamilton 1985). Numerous researchers have implicated H_2S producing bacteria in contributing to MIC (Iverson 1987). De Bruyn (1992) postulated focussing on all sulphide producing bacteria rather than the strictly anaerobic SRB only. She clearly showed that various agar media for the enumeration of SRB supported the growth of facultative sulphide producers. These include *Shewanella putrefaciens* and *Aeromonas veronii* biotype *sobria* (Dawood & Brözel 1997). Dawood & Brözel (1997) have subsequently shown that *S. putrefaciens* contributes significantly towards MIC of ferrous metals. Bacteria removed from the surfaces of biofouling monitoring devices in cooling water systems should therefore be quantified on low nutrient media and all H_2S producing bacteria should be quantified and not only the true SRB.

Thus, due to the complexity and diversity of microbiological populations in cooling water systems, it is essential to use combinations in order to effectively and accurately assess the extent of microbiological growth or activity (Poulton 1993). A variety of techniques may be used to estimate microbial activity in and on the different monitoring systems. These include, light microscopy, epifluorescence microscopy, fluorescent antibody staining techniques, electron microscopy, biochemical assays using enzyme markers or measuring adenosine triphosphate (ATP) concentrations, measurement of cell wall components, chlorophyll measurements for algae, respiration rate measurements and also more recently a variety of molecular techniques have been used. The most common molecular techniques include PCR (polymerase chain reaction), 16s RNA oligonucleotide probes and confocal laser microscopy in combination with marker genes.

Critical operational determinants for making biofouling control programmes work

Control of water chemistry in an open recirculating cooling system is an exercise in establishing and maintaining an acceptable water quality. Once target concentrations of selected chemicals are attained, the effort reverts to regular analytical checks, corrosion-rate monitoring and an occasional economic review.

Since biocides and biodispersants, like all other water treatment chemicals, are subjected to system flow dynamics, it is important to know the basics of system hydrodynamics in order to ensure that the correct biocide concentration is maintained in a system.

Recirculating water systems repeat the process of re-use of water, taking in sufficient fresh water makeup to balance the water evaporated and blow down from the system, so as to control the chemical characteristics (quality) of the recirculating water.

A critical factor in any biofouling control program is hence system hydrodynamics. The following section will give a brief overview of the water balance equations to be considered when designing an antifouling strategy.

The basic equation

The basic equation is a variation of 'what goes in, must come out (sooner or later)'. Make-up rate (M) is just that; water fed to make up for the water removed by evaporation (E) and blowdown (B).

$$M = E + B$$

While makeup keeps the water volume constant, it is blowdown that controls the solids content, and cycles of concentration is their report card. Since pure water vapour is discharged by evaporation in the cooling tower, the dissolved and suspended solids left behind are concentrated. If there were no water loss other than evaporation, these solids would concentrate to brine, causing massive scale and corrosion problems. These scale deposits will also provide conditions where microorganisms could proliferate. This would result in an increased organic loading by microbiological slime, which will then again increase the solids content in the recirculating water and foul the system.

In calculating the water balance the following operating parameters have to be estimated:

- Drift loss: Drift loss is usually in the range of 0,05–0,2% loss based on recirculation rate.
- System loss: System loss is the water lost in the plant through pump or valve leaks, compressor jackets or bearings or draw-off.
- Evaporation (E): Evaporation is the water lost to the atmosphere in the cooling process. The evaporation rates are dependent on the amount of water being cooled and the temperature differential. As a rule of thumb, for each 6 °C temperature decrease across the cooling process, 1% of the recirculation rate is evaporated. Therefore a 11 °C decrease across a cooling tower produces an evaporation loss of 2% of the recirculation rate. The amount of evaporation that can take place over a given tower is limited by the relative humidity.

System volume (V)

In order to determine the biocide dosage concentration to maintain a minimum inhibitory concentration in a system, the total system volume is important.

The total volume of water contained in an industrial water system is approximately constant. An estimate of the volume of water contained is done in a number of ways. Some approaches are:

- Volume = 2 × cooling water tower basin volume (very inaccurate)
- Volume = 10 × recirculation rate in 1/min (inaccurate)
- 'Salt test' (exact).

The 'Salt test' gives an excellent estimate of system volume and can be conducted as follows:

- A rough estimate of the system volume is made and the blowdown valve is closed.
- A known concentration of a new ion, for example, chromate is added to give a measurable concentration based on the system-volume estimate.
- The concentration of this ion is then determined, hereafter windage and leaks will begin to deplete it.
- The time to reach peak and the duration of the peak is not important; only the value of peak concentration is required.
- If the peak value (ppm) of the ion is more than the estimated value, based on the estimated volume, the real volume is less than estimated.
- If the peak value (ppm) of the ion is less than the estimated peak value, based on the estimated volume, the real value is more than estimated.

e.g. The estimated cooling tower volume = 100 000 litres and the ion is added to give a concentration

of 60 ppm (i.e. 6 litres) the actual peak value measured = 40 ppm, then the real volume (V) would be: V = 60/40 × 100 000 litres.

Retention time (R) (Turnover)

Note that a system's turnover time by definition is V/B, which is its retention time during steady state operation. Mathematically: R = V/M.

Time cycle (TC)

This is defined as the time it takes the water to make one 'trip' around the system, or mathematically, V/R. The physical picture is one of plug flow around the cooling-heating loop of the cycle rather than displacement to drain. It also ignores mixing and redistribution. Time cycle is useful for estimating the response time of the system to initial contact with a slug-fed chemical such as a biocide. It also indicates the time necessary to begin analytically tracking known concentration changes.

Holding-time index (HTI)

Holding-time index is a measure of the half-life of the chemical in a system. By extending its use, the time when 1/4, 1/8, 1/6, etc. of the original concentration is still present can be predicted.

If the make-up rate (M) and system volume (V) is known: HTI = (0.693) (V/M) = 0.693 Holding-time index is also a measure of the time for makeup solids to double in the absence of blowdown. Cloete et al. (1989b) illustrated the importance of HTI and other system parameters affecting hydrodynamics in biocide programmes. They indicated that as soon as the biocide concentration decreased to below the minimum inhibitory concentration, rapid regrowth occurred.

Summary

The efficacy of biocide programmes for biofouling control in industrial recirculating water systems relies not only on the kill spectrum of the biocide, but also on the concentration of the biocide. In many cases the correct concentration is not achieved due to a lack of knowledge of the size of the system or the difficulty to determine the active concentration of the biocide. In recirculating water systems, biocides are diluted out of the system depending on the retention time of the system. Normal practice would be to top-up the biocide in order to maintain the required concentration. It must be emphasized that the concentration of a biocide is not related linearly to its activity; a concentration exponent is involved in the relationship. In many cases a small decrease in concentration will result in a dramatic decrease in activity. For biocide programmes to be effective one would ideally want enough biocide for long enough. This would prevent the depletion of the biocide to sub-lethal concentrations. In order to prevent this the following should always be considered in designing a biofouling control programme:

- Obtain as much information about the plant as possible, e.g. draw a complete flow diagram of the water system. This diagram should also include a list of additives (corrosion inhibitors, current biocides being used, microbiological sampling points, etc.) and the point of biocide addition to the system.
- Determine the system volume. The system volume is critical, since it will determine the amount of chemical that will have to be added to give a particular concentration of that chemical i.e. a biocide.
- Determine the retention time of the system. Retention time is important because it will determine the time required to remove a specific percentage of a slug fed biocide.
- Determine the time cycle of the system. This will indicate the time in which the water makes one trip around the system. Time cycle is useful for estimating the response time of the system to initial contact with a slug fed biocide.
- Determine the holding time index. This calculation is of paramount importance, since it will indicate the half-life of a slug fed biocide concentration with blowdown.
- The biocide dosage concentration should be known and by making use of the above information the dosage frequency can be calculated.

This will ensure that the biocide and/or biodispersant concentration is maintained at minimum inhibitory concentrations.

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