Industrial Biofilms and their Control

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Abstract Biofilms are considered to be ubiquitous in industrial and drinking water distribution systems. Biofilms are a major source of contribution to biofouling in industrial water systems. The problem has wide ranging effects, causing damage to materials, production losses and affecting the quality of the product. The problem of biofouling is operationally defined as biofilm development that exceeds a given threshold of interference. It is for the plant operators to keep biofilm development below the threshold of interference for effective production and to work out values for threshold limits for each of the technical systems. Industrial biofilms are quite diverse and knowledge gained with a certain type of biofilm may not be applicable to others. In recognition of this, the old concept of a universal/effective biocide is a misnomer as physical, chemical and biological parameters of source water vary from site to site and so do the interactions of biocides with these parameters. Control methods have to be tailor-made for a given technical system and cannot be extrapolated. Because of the wide-ranging complexity in industrial technical systems, understanding the biofilm processes, detection, monitoring, control and management is imperative for efficient plant operation. A successful antifouling strategy involves prevention (disinfecting regularly, not allowing a biofilm to develop beyond a given threshold), killing of organisms and cleaning of surfaces. Killing of organisms does not essentially imply cleaning as most industrial systems deploy only biocides for killing, and the cleaning process is not achieved. Cleaning is essential as dead biomass on surfaces provide a suitable surface and nutrient source for subsequent attachment of organisms. A first step in a biofilm control programme is detection and assessment of various biofilm components, like thickness of slime layer, algal and bacterial species involved, extent of extracellular polymeric substances and inorganic components. Prior to adopting a biocidal dose and regime in an industrial system, laboratory testing of biocides using side-stream monitoring devices, under dynamic conditions, should be carried out to check their effectiveness. Online monitoring strategies should be adopted and biocidal

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dosing fine-tuned to keep biofilms under control. Literature on biofilm control strategies in technical systems is rich; however, the choice of the control method often depends on cost, time constraints and the cleanliness (threshold levels) required for a technical process. Currently, there is a trend to use strong oxidizing biocides like chlorine dioxide in cooling systems and ozone in water distribution systems as low levels of chlorine have been found to be ineffective against biofilms. A number of non-oxidizing biocides are available, which are effective but the long-term effects on the environment are still unclear. New techniques for biofilm control like ultrasound, electrical fields, hydrolysis of extracellular polymeric substances and methods altering biofilm adhesion and cohesion are still in their infancy at the laboratory level and are yet to be successfully demonstrated in large industrial systems.

1 Introduction

Water drawn from natural sources is the main industrial coolant for dissipating waste heat from heat exchangers and process systems. Use of "pure" water would not eliminate biofouling problems as pure water systems still contain traces of organic carbon and, thus, also face problems due to biofouling. Apart from this, desalination plants also face biofouling problems related to accumulation of biofilms on pipe and membrane surfaces. The problems due to fouling by biofilms are more pronounced in the open and closed freshwater recirculating systems of power plants and to an extent on desalination membranes, hence problems in these systems are discussed in this chapter. However, with regard to the control of biofilms, experiences in pure water distribution systems are also discussed here as the principles and approaches are similar and they share a common goal, i.e. eliminating biofilms.

The events leading to deterioration of surfaces are:

1. Natural waters contain a large number of macromolecules released by breakdown of dead organisms. These substances adsorb onto submerged surfaces constituting a primary film (Busscher et al. 1995).
2. Initially bacteria are attracted towards this surface and are held to the substratum by weak electrostatic forces, hydrogen bonds and van der Waals interactions (Busscher et al. 1995).
3. As the bacteria grow, extracellular polymeric substances (EPS) are produced and accumulate so that the bacteria are eventually embedded in a highly hydrated matrix (Christensen and Characklis 1990; Flemming 2002). The polymeric material is largely composed of polysaccharides, proteins, nucleic acids and lipids (Flemming and Wingender 2002). It is frequently believed to represent a diffusion barrier; however, this is not the case for small molecules such as biocides as the main component of the EPS matrix is water. Therefore, the diffusion coefficients of such molecules are very close to those in free water (Christensen and Characklis 1990) unless these molecules do interact with matrix components. This effect is called diffusion–reaction limitation(Gilbert et al. 2001). The production of EPS

provides adhesion to the substrate and matrix cohesion and, thus, increases the mechanical stability of biofilms.
4. Subsequently, diatoms and microalgae colonize the substratum and the biofilm grows in thickness, further entrapping nutrients from bulk water (Flemming and Leis 2002).

Control of biofilms in industrial systems is an important component of a successful water treatment programme (Ludensky 2003). Theoretical approaches consider that the primary step in biofilm inhibition is to prevent the initial adhesion of microorganisms (Busscher et al. 1999). However, in practice, this does not work because sooner or later surfaces in technical systems will eventually be colonized. Biofilms serve as a source for production and release of microbial cells, which influences microbial levels in the water column. Codony et al. (2005) reported an interesting observation to this effect: intermittent chlorination resulted in a tenfold increase in the release of microbial cells to the water phase in the absence of biocide. Hence, it becomes more important to control biofilms. Routine monitoring procedures assess the presence of planktonic bacteria, whereas the vast majority of bacteria indigenous to aquatic environments exist attached to solid particles or industrial surfaces and go unnoticed. A particular biocide may inactivate more than one type of microorganism. With our current levels of understanding of the mechanisms of biocidal action and of microbial resistance it is pertinent to consider whether it is possible to explain why some biocides are effective while others are not. The factors that affect antimicrobial activity most are contact time, concentration, temperature, pH, the presence of organic matter and the type of microorganism. Hence, comparative assessments of different biocides are somewhat difficult. For industrial operations, system size, cleanliness, service schedules and monitoring programmes are important factors governing smooth operations.

2 Factors Influencing Biofilm Development in Industrial Systems

Industrial biofilms are quite diverse due to a wide range of contributing factors such as microbial species, temperature, nutrient availability, velocity, substratum physical and chemical characteristics, organic loading, suspended solids and general water chemistry. Therefore it is difficult to generalize about the types of biofilm that form in these systems, let alone about their control methods.

2.1 Temperature

Growth of biofilm and of species colonizing a biofilm is dependent on the operating temperatures of industrial equipment. An increase in temperature is found to favour biofilm growth. Even a small change in temperature (5°C) can cause an increase in

biofilm thickness (Bott and Pinheiro 1977). In heat exchangers, raising the temperature above design value can increase the rate of corrosion, rate of chemical reactions and, for inverse solubility salts, raising the temperature might initiate deposition (Bott 1995). Heat transfer surfaces (titanium, admiralty or aluminium brass, and cupronickel 90:10) in an industrial heat exchanger generally experience temperatures in the range 28–45°C for auxiliary cooling systems and 60–70°C for condenser cooling, where bacterial biofilms have been shown to occur (Nebot et al. 2007).

2.2 Nutrient Availability

The basic mechanism of biofilm development involves the conversion of dissolved nutrients into accumulated biomass. Griebe and Flemming (1998) considered biofouling as a "biofilm reactor in the wrong place" because the same laws apply to both cases. The major factor controlling biofilm growth is nutrient availability. In industrial and drinking water systems, mass transfer of nutrients to the biofilm will tend to increase with flow velocity (Characklis 1990). The rough surfaces of biofilms also aid in increased mass transfer of nutrients by as much as threefold compared to a smooth surface (Characklis and Marshall 1990; Bott and Gunatillaka 1983). Nutrient limitation may be one way to control biofilm development without increasing disinfectant dosing in potable water distribution systems (Griebe and Flemming 1998; Chandy and Angles 2001; Flemming 2002). Adsorption of macromolecular substances increases their availability to bacteria. Industrial cooling systems offer a continuous flow of fresh water bringing in nutrients. A 400% increase in biofilm thickness was observed at a given velocity of 1.2 m s^{-1} for an increase in nutrient level from 4 mg L^{-1} to 10 mg L^{-1} (Melo and Bott 1997). Removal of organic carbon resulted in greater persistence of chlorine (Chandy and Angles, 2001).

Treatment of water to reduce the organic load is a non-viable option for power plants as once-through seawater cooling systems on an average have an intake capacity of 30 m^3 s^{-1} (for 500 MW(e) plants) and freshwater recirculating systems have a circulation rate of 80–120 m^3 h^{-1} with an intake capacity of 10 m^3 h^{-1}. However, this factor is included in this section in order to have a measure of the influence of nutrient concentration on biofilm thickness and density, which have direct implications in biocidal efficacy by reacting with the biocide dosed and neutralizing it. The method of reducing the organic load and, thus, limiting nutrients has been suggested and during the last few years has become more and more accepted in practice as a viable alternative for membrane desalination plants, as the feed water is devoid of biocides to protect the reverse osmosis membranes(Griebe and Flemming 1998; Flemming 2002). The option is viable as these plants require far less quantity of water (intake) and it may prove economical considering the consequences to membranes of biofilms and considering the treatment (organic load removal) required to meet the quality standards of permeate water, involving

infrastructure facilities like coagulation chambers, activated carbon adsorption and cartridge filtration for reducing organic load.

2.3 Flow Velocity

In flowing systems, bacterial populations exist as complex, structurally heterogeneous biofilms attached to surfaces. Residence within these complex matrices provides organisms with a higher localized nutrient concentration than that found in normal waters. In the case of heat exchangers, biofilm growth can be controlled if relatively high velocities are imposed, as shear effects are likely to have an impact on biofilm development. Operating at high velocities to achieve increased shear forces also results in erosion of material surface and hence results in increased damage. An optimum shear force and temperature for minimal adhesion is yet to be worked out specifically for heat exchanger operation. Biofilms have been described as a viscoelastic material with plastic flow properties (Korstgens et al. 2001), based on their response to the modulus of elasticity and yield strength. The viscoelastic property of biofilms makes them mechanically stable and also enables them to resist detachment (Rupp et al. 2005). The EPS functions as a network of temporary junction points and yield points, which above a certain threshold results in failure of the gel system resulting in a highly viscous fluid (Korstgens et al. 2001). Hence it would be of practical importance to obtain data on the flow velocities required to either detach or induce such effects. Flow velocities of water in pure and cooling water systems govern the development of biofilms, their density and have important implications with respect to penetration of biocides.

Studies by Pujo and Bott (1991) have shown that the Reynolds number seems to have a profound effect on biofilm thickness. For a given Reynolds numberof 11,000 and fixed nutrient conditions, a velocity of 0.5 m s^{-1} generated biofilms ten times thicker than at a velocity of 2 m s^{-1} over a period of 15 days. An increase in Reynolds number increased biofilm removal (24%), but total biofilm removal was not found for all conditions (Simoes et al. 2005a) suggesting that biofilms were more mechanically stable to shear forces. Treatment of biofilms with chemicals and surfactants like cetyltrimethyl ammonium bromide (CTAB), ortho-phthalaldehyde (OPA), sodium hydroxide and sodium hypochlorite promoted weakening of biofilm mechanical stability (Simoes et al. 2005a). Similarly, velocity is also known to affect biofilm density. Experiments with unispecies *P. fluorescens* biofilms showed that an increase in velocity from 0.1 to 0.5 m s^{-1} resulted in an increase in density of biofilm from 26 kg m^{-3} to 76 kg m^{-3} (dry mass/wet volume) (Pinheiro et al. 1988). Qualitative analysis of flow effects on biofilms grown from tap water at different velocities showed that under laminar conditions biofilms were patchy and consisted of cell clusters separated by interstitial voids. In contrast, biofilms developed under turbulent flow were found to be filamentous (Stoodley et al. 1999). In flowing systems, bacteria can adapt rapidly to hydrodynamic and chemical stresses (Suci et al. 1998)

and sessile cells are known to undergo complex physiological changes during the process of attachment (Sauer and Camper 2001), which reduce their susceptibility to control measures (Cloete et al. 1997; Gilbert et al. 2002).

Another factor of importance in industrial systems is shear stress on the substratum caused by flowing water. High shear forces at the substratum result in (1) increased flux of nutrients at the surface, (2) increased transport of disinfectants to the surface, (3) a greater shearing of biofilms (Percival et al. 2000) and (4) altered biofilm diversity (Rickard et al. 2004). An increase in flow velocities resulted in re-suspension of biofilms and sediments in water from pipe surfaces (laboratory study), which increased particle and turbidity counts in bulk fluid (Lehtola et al. 2006). The consequences of release of biofilm clumps from surfaces are beneficial in once-through systems where the biofilm load decreases, whereas in recirculatory and drinking water systems they pose problems of bacterial regrowth and suspension of toxic metals from the surface to bulk water. However, recent studies by Tsai (2006) showed that shear stress (0.29 N m^{-1}) and chlorination had no interaction on biofilm formation, reinstating findings of an earlier study by Peyton (1996), who observed no significant effects of flow rate on biofilm thickness. A probable reason for the observed effect in these studies is that the shear stress achieved in these studies was inadequate to remove biofilms.

It is necessary to arrive at shear stress values for biofilm removal on a variety of surfaces. Studies by Cloete et al. (2003) showed that high velocities of 3–4 m s^{-1} were required to detach biofilms from surfaces. Alternatively, fouling deposition was found to occur at a slow rate when a nominal flow velocity of (1.85 m s^{-1}) was maintained in the heat exchanger tubes (Nebot et al. 2007). Increasing the velocity regime may offer some relief from the problem of biofilms in water distribution pipelines but with respect to heat exchangers, increased velocity would increase the overall heat transfer coefficient (Bott 1995). This would mean additional surface area and increased capital costs. Further increase in velocity increases the pressure drop(i.e. pressure drop is the square of the velocity) (Bott 1995). Hence, the use of flow velocity to prevent biofilm formation is not a viable option for heat exchangers and industrial circuits because of technical problems and energy consumption. In addition, the role of velocity effects on biofilm formation is yet to be clearly understood and a clear distinction between the two contrasting schools of thought, viz: shearing effects/biofilm stability, needs to be investigated to improve our understanding of using flow velocity as a biofilm control method.

2.4 Substratum Physical and Chemical Characteristics

The type of substratum has a pronounced effect on biofilm accumulation. Smooth surfaces accumulate less biofilm mass than rough surfaces. The mechanism behind this is that individual cells are much smaller than crevices (Bott 1999) and an irregular rough surface would offer protection for cells from shear effects. However, such

surface irregularities have a measurable effect only during the initial stages of biofilm development and biofilms are unavoidable in distribution systems (Veeran and Hissett 1999). When biofilm thickness exceeds roughness dimensions, roughness will no longer be of influence for biofilm accumulation; however, it will assist in better anchoring them to surfaces. Vieira et al. (1992) have shown that biofilms of *P. fluorescens* were more pronounced on aluminium plates than on brass and copper. Similarly, more biofilms were observed on polyethylene pipes than on copper pipes (Lehtola et al. 2006). This is commonly attributed to the toxic effects of copper and brass on microorganisms. However, in industrial situations, heat transfer surfaces of copper, brass and cupronickel alloys have all been shown to accumulate biofilms. Titanium heat exchanger tubes were shown to accumulate more fouling than brass tubes (Nebot et al. 2007). From the literature, it is understood that no single surface escapes fouling and that it is impossible to create smooth industrial surfaces as the surface roughness of materials used in industries is dependent on the manufacturing process. Low surface energy coatings, which are characterized by low adhesion forces of the biofilm to the surface (Busscher and van der Mei 1997), could offer some protection for structural materials like pipelines, whereas in heat exchangers chemical control methods are the only alternative.

2.5 Suspended Solids

Industrial cooling water drawn from natural sources (seawater or freshwater) contains common particulate material like sand, silt, clay or quartz and to a certain extent metal oxides resulting from the corrosion of equipment upstream. Although in industrial systems the presence of suspended particles is common, studies on their interaction with biofilms are limited. Deposition of these particles onto surfaces from suspension flows is found to occur in consecutive steps. The presence of particles in suspension influences biofilm growth by: (1) increasing the availability of nutrients to microorganisms, directly influencing their metabolism, (2) the erosion effects of particles, resulting in removal or suppression of biofilm formation and (3) the presence of biofilm enhances the capture of particulate matter from flowing systems, increasing accumulation on surfaces (Bott and Melo 1992). These mechanisms can be observed and are dependent on the shear force and size of the particles. Particulate material in flowing water influences biofilm thickness and growth. If the particle sizes are large, this results in a sloughing effect on the biofilm whereas smaller particles are known to embed within biofilms (Lowe et al. 1988).

In general, to ensure maintaining biofilms within the required threshold limits in industrial circuits, the following are necessary: operating industrial systems at velocities higher than 2–3 m s^{-1}, without additional pumping cost or erosion problems; operating at minimum (ambient) temperatures; avoiding large open sunlit areas; use of appropriate materials and surface coatings with a smooth finish; a proper biocidal and cleaning programme.

3 Problems Associated with Biofilms and Their Control in Industrial Systems

3.1 Heat Exchangers and Cooling Water Systems

In cooling water circuits, the presence of biofilms can restrict flow in pipelines (Bott 1999), decrease heat transfer in heat exchangers, increase pressure drop (Bott 1994; Characklis and Marshall 1990), enhance corrosion (Bott 1995) and alter surface roughness, which in turn can increase fluid frictional resistance resulting in decreased flow and act as a source of contamination (Camper 1993).

Two main problems encountered in heat exchanger systems due to fouling by biofilms are reduction in heat transfer (loss of thermal efficiency) and pressure drop across the heat exchangers due to flow reduction by deposits (Characklis 1990). The restrictions to flow imposed by the presence of biofilm deposits in heat exchanger surfaces increases fluid frictional resistance and, for a given throughput, the velocity will have to increase, which means additional pumping costs. In addition, the presence of biofilms may accelerate corrosion of materials in contact. Other operating costs may accrue from the presence of biofilm deposits, such as increased maintenance requirement and unplanned shutdowns for cleaning. As a result of these factors, the engineering design of heat exchangers usually incorporates allowances for fouling to accommodate a more satisfactory annual cleaning schedule.

Recirculating systems (Fig. 1) are usually located at sites where adequate water is not available for cooling purposes. In open recirculating systems, cooling water drawn from the source (usually a freshwater body) is circulated through a heat exchanger and is conveyed to a cooling tower where evaporation of some of the water results in a cooling effect and lowering of the cooling water temperature for

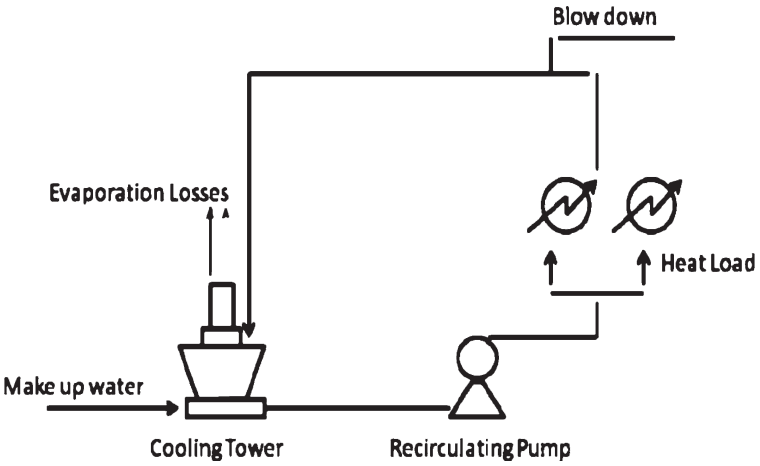

Fig. 1 General schematic of an industrial recirculatory cooling system

further recirculation. After passage through the cooling towers, the water is held in a temporary open reservoir where algal and bacterial growth occurs. In recirculatory systems, both open and closed makeup water is added to compensate for the evaporative losses as well as to maintain the quality of recirculatory water. The conductivity of recirculatory water increases due to concentration of salts on evaporation. This is usually measured as cycles of concentration. Usually, plants operate at two to three cycles of concentration, as an increase of cycles of concentration above four usually results in enhanced scaling and corrosion of equipment.

In open recirculating systems, the problems to be encountered are many as these systems are large (with a resident water of 60–80,000 m^3 for a 1,000 MW(e) power plant). Large open areas and available nutrients in the recirculating water provide adequate conditions for enhanced growth of algal species, resulting in eutrophication. This further leads to organic loading in the system as detrital matter accumulates. Further, the incoming makeup water brings in fresh nutrients that are continuously recycled in the systems.

In closed recirculating systems, the principles are as the name implies, the cooling water is conveyed through pipelines to the heat exchangers and after passage through the cooling tower is recirculated. However, even in these systems it is inevitable to have an open storage point as large volumes of water are involved. Closed recirculatory systems are not preferred as large capital investments have to be made on infrastructure. Recirculating water systems are often designed with an average flow velocity through the condenser tubes in the range of 1.8–2.4 m s^{-1}. Small heat exchangers in the process systems have lower velocities in the range of 0.3–0.6 m s^{-1}, which are prone to fouling. Water filtration devices of various types are always installed in cooling water systems fed by natural waters. These generally consist of a band screen with a coarse grid (about 1–10 cm spacing) where the flow rate is lower than 10 m^3 s^{-1} or drums for higher flow rates. Specific debris filters are also used to protect heat exchangers from clogging. An overview concerning condenser cooling circuits is given in Table 1.

Cooling towers of both open and closed recirculating systems face severe problems due to algal and bacterial growth. Cooling towers represent complex ecological niches and even different towers of identical design on a single site will generally behave quite different microbiologically (Prince et al. 2002). Conventionally, the splash-type cooling tower has been used, in which the heated discharge from the condensers is ejected through fine nozzles from the top of the cooling towers. The discharge trickles down splash bars (either concrete or wood) and collects in the cooling tower basin from where it is pumped for recirculation. The disadvantages of these splash-type towers are their extremely large size and low thermal efficiency. This led to the development of high-performance forced or induced draft cooling towers where the water trickles down through high film fills (polyvinyl chloride) to the cooling tower basin. The high film fills are comprised of corrugated parallel plates with distances of 3–5 mm between the plates. The corrugations or chevron angles result in water being broken up into fine droplets or films by the extended surfaces of the film fills. The corrugation increases the surface area and has resulted in reducing the size of cooling towers. However, these high film fills

Table 1 Biocidal regimes practiced in industrial circuits for condenser cooling

	Concentration (mg L^{-1})	Effect	Reference
Low level targeted Cl_2	> 0.2	Effective if targeted dosing is done at inlet to heat exchangers at EDF power station France	Jenner and Khalanski (1998)
Low level Cl_2	0.2 TRC	Pilot plant device at a 550 MW plant in Spain	Nebot et al. (2007)
Low level Cl_2	0.1 TRC	Effective against planktonic cells of lake water	Nebot et al. (2007)
Discontinuous Cl_2 (30 min every 12 h)	0.5–1.0	Ineffective	Ewans et al. (1992)
Discontinuous Cl_2 (for 1 h every 8 h)	3.0	Effective at EDF Martigues-Ponteau power station on Mediterranean coast	Jenner and Khalanski (1998)
Intermittent Cl_2 (4 h on/4 h off)	0.2–0.3	Effective against biofilms at Maasvlakte power station, Rotterdam	Jenner and Khalanski (1998)
Intermittent Cl_2 (30 min on/1 h off)	1.2	Required for biofilm control on plate heat exchangers	Murthy et al. (2005)
Targeted Cl_2	1.0	Recommended by EPRI for condenser slime control	
Chlorination (30 min day^{-1})	0.5	Effective for fouling control in Netherlands – KEMA	Jenner and Khalanski (1998)
Chlorine dioxide	0.05–0.1	With residual (1 h day^{-1}) or without residuals (10–12 h day^{-1}), effective for sea-water condenser cooling in Mediterranean coast	Petrucci and Rosellini (2005)
Ozone	0.1–0.15	Killing and detaching sessile cells. Followed in Hochst unit, Germany, fed with River Main water	Jenner and Khalanski (1998)

have been prone to both inorganic and biological fouling compared to conventional low fouling, splash bar fills where algal growth is the major problem to be overcome.

In large natural cooling towers, algae tend to develop in the following regions:

- The inner surface of the shell. The wet parts that are exposed to some sunlight become covered with a cyanobacterial and algal layer. Sloughing and detachment of algae during shutdowns leads to a great input of organic matter into the system.
- In the honeycomb-like packing structures of cooling tower fills. Exposure to sunlight and the slow flow of water (0.2 m s^{-1}) are causal factors for growth of filamentous green algae and cyanobacteria where light has access.
- In the cooling tower basins and on concrete walls and pillars of the cooling tower.

Table 2 Biocidal regimes practiced in industrial cooling towers

Regime	Concentration (mg L^{-1})	Effect	References
Discontinuous shock chlorination	2.5	Effective in killing algae; inland power station CEGB, UK	Blank (1984)
Discontinuous mass chlorination	8.0	Exposures of 6 h were effective for killing algae	Lutz and Merle (1983)
Chlorine dioxide	1.5	Elimination of filamentous algae in cooling towers	
	0.3	Requires extended time for achieving similar results	Merle and Montanat (1980)
ACTIV-OX	0.2–0.8	Chlorine dioxide treatment effective against Legionella sp. in cooling towers	Harris (1999)

Generally the walls of the cooling tower basins are not protected. The biocide dosed in the water phase is not effective as the water does not trickle through the wall in forced/induced draft towers with film technology. As a result, thick layers of cyanobacteria develop on the wall and act as source for further contamination. Some of the cooling tower water containing the biocide may come in contact with the walls. This kills the outer layers of the encrusting algae, turning the filaments white, but does not penetrate into the deep layers of horizontal filaments adhering to the walls. When the dead filaments have been washed off, the horizontal filament system is once again exposed to the flow of cooling water and growth begins again. It is important that the walls of the cooling tower basins be treated with a suitable antifouling coating or foul release coating and are subjected to periodical cleaning by high-pressure water jet and disposal of the algal debris. This will ensure smooth operation of the towers.

Chlorine has been the most common biocide used in cooling towers. Biocidal regimes practised in cooling towers are listed in Table 2. Chlorine and copper salts have been used as popular methods for controlling bacterial growth in cooling towers (Fliermans et al. 1982). Chlorine (2–4 mg L^{-1}), silver ions (0.02–0.04 mg L^{-1}) and copper ions (0.2–0.4 mg L^{-1}) have been used for treating cooling towers (Chambers et al. 1962; Cassels et al. 1995; Pedahzur et al. 1997; States et al. 1998; Kusnetsov et al. 2001; Kim et al. 2002a, b). However, the use of metal ions for biofouling control should always take into consideration the development of resistant microbial populations (Schulte et al. 2005).

Legionella sp. is an important component in natural and artificial water environments, cooling towers, plumbing systems and evaporators of large air conditioning systems, and remains a health hazard. *Legionella* sp. is known to occur in biofilms in cooling towers, showers, humidifiers (Fields et al. 2002) and hence knowledge about its response to control measures is important. These Gram-negative aerobic rods have been shown to survive at temperatures of 20–50°C and are inactivated at temperatures above 70°C (Kim et al. 2002a) and in a pH range of 5.5–8.1.The organism is known to occur in stagnant warm water bodies (Sanden et al. 1989).

This aspect is important as power plant exhaust plumes are known sources of *Legionella* deposits. *Legionella* resident within biofilms are a severe problem in cooling tower systems using freshwater.

Several disinfection methods have been tried out. In the technical context, the term "disinfection"is usually not used in the proper sense of the definition (inactivation of infecting microorganisms) but rather as getting rid of microbial problems. Chemical treatments using chlorine were the most common and widely used. Free chlorine concentrations of 1 mg L^{-1} were required for killing planktonic cells whereas a fourfold increase in concentration was required to kill sessile cells (Kim et al. 2002a). An adaptive feature exhibited by *Legionella pneumophila* associated with biofilm protozoa showed that cells were found to be less susceptible to chlorine (residual of 0.5 mg L^{-1}) (Donlan et al. 2005). Resistance by *Legionella* biofilms was also observed for the organic compound chloramine T (*N*-chloro-*p*-toluene sulfonamide), obtained by chlorinating benzene sulfonamide or *para*-toluene, on planktonic and sessile cells (Ozlem et al. 2007). In cooling systems of power plants an organic compound 1-bromo-3-chloro-5,5-dimethylhydantoin (BCDMH)containing bromine as an active ingredient has been used to control *Legionella* (Kim et al. 2002a). Effective bromine concentrations were in the range 1.0–1.5 mg L^{-1}. However, a shock dose of 3–5 mg L^{-1} of ClO_2 for a period of 1 h was required to eliminate *Legionella* from dental chair water systems (Walker et al. 1995).

3.2 Case Study: Microbial Fouling of Cooling Tower Fills in a Power Station

The Talcher super thermal power station (TSTPS) located in the Eastern state of Orissa, India has six units each of 500 MW(e) capacities. The plant operates on an open recirculatory mode with a residence volume of 3,600,000 m^3 h^{-1} of cooling water and a makeup water of 10,000 m^3 h^{-1}. Cooling water comes from the perennial rivers Bahmini, Trika and Singaraj, which converge to form the "Triveni Sangam" from which water is drawn and transported through underground pipelines for approximately 10 km before it reaches the recirculation system. Prior to entering the recirculation system of the plant, the water is aerated and biocide (chlorine) is added before the water is softened using alum. The pH drop after the addition of softening agent (alum) is revived by addition of lime (calcium). This results in a pH value of 8.2–8.3 in the cooling water system. The water is then clarified by removing suspended solids and reaches the pump house feeding the condenser. In the post-condenser section, the heated water from the condensers is fed into the cooling towers. The cooling towers are of forced draft type with a counter-flow direction. The water is then ejected through a fine nozzle below the demisters and falls by gravity down over the PVC fills. The water trickles down the PVC film fills through the "chevron" angle (with a flute size of 17 mm and a peak distance of 34 mm) by gravity flow. Empirical velocity across the fills is estimated to be around 0.2 m s^{-1}. The bottom of the cooling tower is of an open type for air ingress. The water is

collected in a basin from where it is directed through an open channel to reach the pump house.

Severe clogging of high efficiency polyvinyl chloride film fills by deposits (Fig. 2a, b) was observed in the cooling towers (3, 4, 5 and 6) of the 4,000 MW(e) TSTPS, resulting in a loss in condenser vacuum of 40 mbar and operation of the cooling towers reaching criticality (Fig. 2c, d). The problem was found to be specific to high efficiency film fills, and was not observed in splash-type cooling towers (1 and 2) receiving the same waters. Further, the cooling towers connected in parallel and receiving the same water had different bacterial genera. Cooling towers 3 and 4 had predominantly heterotrophs and cyanobacteria (Fig. 2e), whereas iron bacteria (Fig. 2f) dominated in cooling towers 5 and 6. The problem occurred within 3 years of operation with an intermittent chlorination regime of 1.0 ± 0.1 ppm residuals for 12 h in place. The severity of the problem is reflected in the quality of the recirculating water. As a result of insufficient cooling, an increase in temperature (Fig. 3a) in the post-condenser section was observed. Reduction in flow and heat load in the condensers resulted in an increase in conductivity levels of recirculating water (Fig. 3b), further increasing the propensity of scaling in the system.

Experimental data and observations revealed the problem to be a microbially associated phenomenon. The sequence of events leading to the clogging of fills is: (i) establishment of bacterial biofilms on PVC fill surfaces due to long layoff chlorination periods and (ii) the anionic nature of the biofilms aids the entrapment of suspended, airborne particulate matter and of dissolved nutrients like the carbon, phosphate, nitrate and silicate essential for microbial growth. Estimation of bacterial loads in the cooling water during biocide dosing did not reveal significant differences between the pre- and post-condenser sections (Fig. 3c).

Chemical analysis of the high film fill deposits by X-ray photon spectroscopic (XPS) analyses showed 30–45% of silica content, which is known to precipitate, coagulate or adsorb at high concentration levels (Table 3). It is well known that naturally occurring silica can polymerize to form amorphous silica or colloidal silica under supersaturation conditions. The anionic nature of the biofilms resulted in entrapment of this compound into the matrix. The situation was noticed by plant operators when operation of the cooling towers became a concern.

The problem seems to have manifested during the layoff of biocidal dosing (during the night) when bacterial numbers multiplied. Mechanical cleaning was not performed because it is too labour-intensive, time-consuming and physically damaging to the system. Further, the towers could not be taken offline for cleaning. Based on the findings, the chlorination regime was switched over to a low-dose continuous mode (0.2 ppm residuals) and with a shock dose of 5 ppm for 15 min once a shift (8 h), coupled with increased blow-down and intake of makeup water. This resulted in slow break-down of biofilms on the fills and helped solve the problem online. Within 3 months the cooling towers had limped back to normality. The cooling towers now have improved heat transfer efficiency and are inching towards normality. Effective testing, good housekeeping during operation, proper maintenance and prompt antifouling treatment can control microbial activity in the system. The

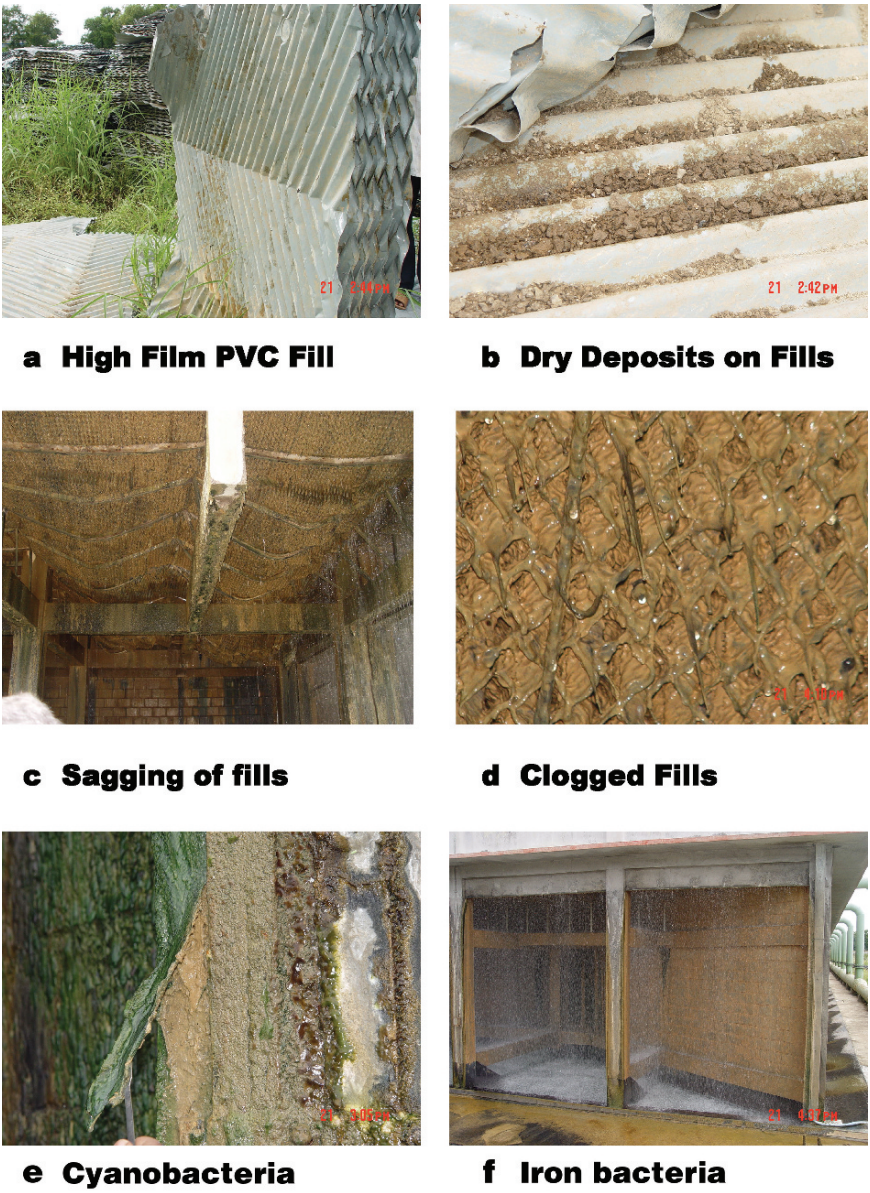

Fig. 2 Biofouling of cooling tower fills of Talcher super thermal power station. a) a high film fill b) dry deposits on fills c) sagging of fills due to fouling load d) closer view of clogging of fills e) Cyanobacteria on cooling tower walls f) Iron bacteria on cooling tower walls

study clearly demonstrated the inefficiency of intermittent chlorination and has also shown that low-level continuous chlorination along with periodical shock chlorination is effective in breaking down biofilms.

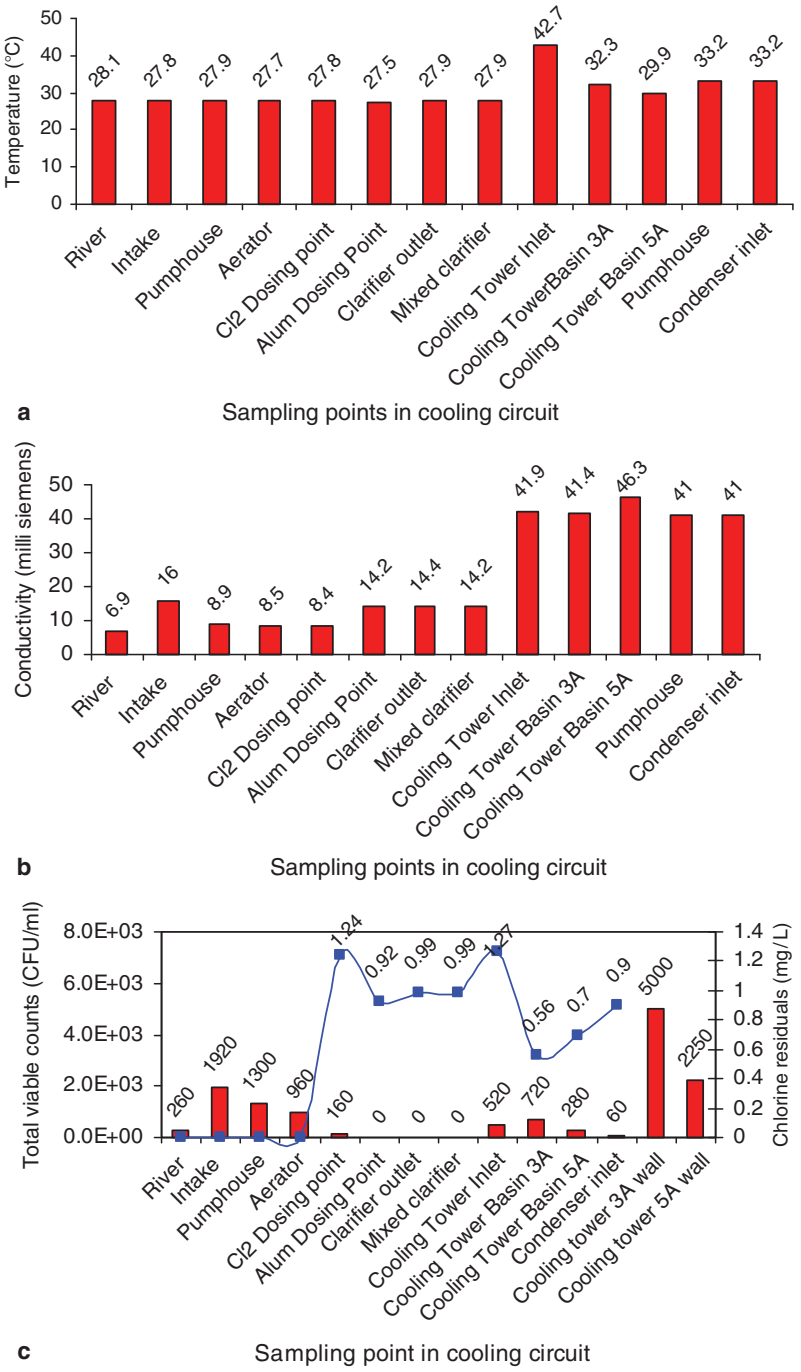

Fig. 3 Distribution of **a** temperature, **b** conductivity, **c** total viable counts and chlorine residuals in the cooling water systems of the 500 × 6 MW Talcher super thermal power station

Table 3 X-ray photon spectroscopy analysis of deposits in cooling tower fills and demisters

	Sample A	Sample B
Element	Deposits on demisters (%)	Deposits on high film fills (%)
Aluminium Al_2O_3	6.90	9.77
Calcium	4.5	3.75
Chlorine	0.0008	0.0008
Iron	–	–
Magnesium	0.63	0.70
Potassium	0.74	1.04
Silicon	31.05	41.39
Sodium	8.46	14.17
Sulfur	0.06	0.06

4 Management of Biofilms in Industrial Systems

Cooling and pure water circuits are typical ecosystems that provide an ideal environment for growth of microorganisms. The steps involved in effective management of industrial systems are: (1) detection of biofilms, (2) biocide dosing, (3) cleaning of surfaces, (4) monitoring of the effectiveness of the management strategy and (5) fine-tuning of biocidal dosing.

4.1 Detection of Biofilms

In industrial situations biofilms are visible to the naked eye as copious slime layers on surfaces. Biofilms in industrial systems are detected indirectly by symptoms noticed in the operational parameters (Flemming 2002) or failure to meet the required standards in desalination and potable water systems. The first step in detection of biofilms is sampling on surfaces, which can be a real challenge. However, water samples reveal neither the site nor extent of biofouling layers (Flemming 2002), as also demonstrated by Goysich and McCoy (1989) for cooling towers. The type of sampling method used is critical for the data to be obtained. Various methods have been used for collecting biofilm samples like sterile nylon brushes, utility knife, swabbing and stomaching for removing them from surfaces. Among the various methods, use of the stomaching procedure was found to be efficient to culture biofilm cells (Gagnon et al. 1999).

Laboratory analysis of samples involves culturing of microorganisms in biofilms and estimating the number of colony forming units. However, most of the bacteria occurring in industrial circuits cannot be cultured by standard plate methods. An European task force, with scientists from 18 different participating laboratories under the French Association des Hygienistes et Technicians Municipaux (AGHTM)

Table 4 Methods for estimating components of biofilms

Biofilm parameters	Method	References
Direct cell counting	Epifluorescence microscopy	Daley and Hobbie (1975)
Biofilm thickness	Light microscopy	Blakke and Olson (1986)
Colony forming units	Standard methods	APHA (1995)
Total living biomass	Adenosine triphosphate	Chalut et al. (1995)
	Fluorescein diacetate estimation	Rosa et al. (1998)
Total biomass	Total organic carbon	
Dry weight	Biofilm total suspended solids	APHA (1995)
Algal biomass	Chlorophyll and phaeophytin estimation	APHA (1995)
Total proteins	Protein determination	Bradford (1976)
Total sugars	Carbohydrate determination	Dubois et al. (1956)
Lipids	GC-MS	Geesey and White (1990)
Uronic acids	Uronic acid determination	Mojica et al. (2007)
Respiratory activity	CTC staining method	Schaule et al. (1993)

during the period 1996–1997, validated methods for evaluation of aqueous biofilms and recommended the use of glass beads or slides and plate counts of cells for quantifying biofilms (Keevil et al. 1999). Advances in microscopy, microfiltration membranes (nucleopore or polycarbonate) and molecular staining techniques like the Live/Dead BacLight assays are now available, which minimizes the errors in estimating viable and dead bacterial cells. A comparison of microscopic methods for biofilm examination has been reviewed by Surman et al. (1996). The use of redox dyes like CTC, which forms fluorescent and insoluble crystals after reduction, also provide a more accurate quantification of microbial numbers and activity in biofilms (Schaule et al. 1993). Several other biofilm measurement techniques or methods have been listed by Donlan (2000) and Flemming and Schaule (1996); however, in practice, results from the methods listed in Table 4 were found to be more realistic in gaining an insight into the nature and extent of the deposits.

4.2 Biocide Dosing

Biocide addition in industrial systems (Table 5) is the main method of controlling problems associated with microbial biofilm formation (Chen and Stewart 2000). The use of biocides is a common response to biofouling problems, resulting from a “medical paradigm” that implies that biofouling can be considered as a “technical disease” and “cured” by substances that kill the causing bacteria. However, it always should be kept in mind that killing of bacteria is not equivalent to cleaning. The complexity involved in combating biofilms in industrial systems is wide ranging, elements of which have been discussed by Flemming (2002), who has formulated

Table 5 Biocides used in industrial circuits

Oxidizing	Non-oxidizing
Bromine Chlorine Chlorine dioxide Ozone Hydrogen peroxide Para-acetic acid Bromine chloride 1-Bromo-3-chloro-5, 5-dimethylhydantoin (BCDMH)	Clamtrol: alkyl dimethyl benzyl ammonium chloride (ADBAC); Bulab 6002: poly[oxyethylene (dimethyliminio) ethylene-(dimethyliminio) ethylene dichloride]; biguanides; β-bromo-β-nitrostyrene; 2-bromo-2-nitropropano-1,3-diol (BNPD); chlorophenols; H-130; dodecyl dimethyl ammonium chloride (DDAC); 2,2-dibromo-3-nitrilo-propionamide (DBNPA); 2-dithiobisbenzamide; glutraldehyde; isothiazolone; kathion; methylenebisthiocyanate; organic sulfur and sulfones; phosphonium biocides; 2-(thiocyano-methylithio)-benzothiazole (TCMTB); thiocarbomate

a toolbox for an integrated antifouling strategy. Various devices (Robbins device, annular reactors, continuous stirred batch reactors, flow cells, mixed consortia reactors) and processes (cooling water systems, drinking water systems, model cooling towers, synthetic mediums containing high and low nutrients) have been used for assessing biocide efficacies, and have been listed extensively by Donlan (2000). Each of these systems and processes is unique and hence comparisons or extrapolation of data to other systems is very difficult. Furthermore, knowledge from these studies on the response of microorganisms to different biocides and processes is difficult to utilize in choosing a biocide type, dosing or regime. For a given industrial system and process, preliminary studies have to be carried out to arrive at the biocidal dose and concentration with respect to the environmental and hydro-biological conditions on site.

As indicated above, elimination of biofilms is an important task, and mechanical cleaning has been found to be the most satisfactory method for removing biofilms (Walker and Percival 2000) because the problems caused by biofilms in heat exchanger systems are due to their physical presence and properties. However, in most industrial systems, design and construction of equipment does not facilitate mechanical cleaning, except for tubular heat exchangers where online and offline cleaning techniques have been used. Several complexities are involved in the action of biocides in controlling biofilms, which are discussed in this section.

Good housekeeping practices (cleaning regularly) along with appropriate biocidal and surfactant or biodispersant dosings are required to keep biofilms under the threshold of interference. Biocides aid only in killing of cells, and dead biomass often accelerates the attachment process by offering a rough surface. Further, biocides increase the biodegradable organic matter(BDOC) in treated water. Instead of cleaning the system they actually increase the amount of nutrients available for growth. In general, the cleanliness and the effectiveness of the microbial control agent used should be periodically monitored using a combination of visual inspection and monitoring of differential bacterial counts like total autotrophs, heterotrophs, iron oxidizers, iron reducers, sulfate reducers, slime formers and pathogens

such as *Legionella pneumophila* in both bulk water and on surfaces in order to determine the efficacy of the biocidal programme in practice.

4.2.1 Role and Action of Biocides on Microorganisms

The ideal biocide for a particular system would meet each of the following requirements: (1) active at a low concentration against a wide range of microorganisms, (2) a low order of toxicity to humans and non-target aquatic life, (3) biodegradable, (4) active in hard and soft water, (5) non-corrosive and (6) not readily inactivated in the presence of a wide range of soils.

The essential duty of the microbiocide is both to prevent primary biofilm formation and to prevent excessive growth of microorganisms, which can either induce corrosion (e.g. sulfate-reducing bacteria) or cause degradation of chemical additives (e.g. nitrifying bacteria).

4.2.2 Factors Influencing Efficacy of Biocides in Industrial Cooling Systems

In practice, the effectiveness of a biocidal programme is assessed by recovery of process parameters in industrial systems (Flemming and Schaule 1996). In turn, efficacy of biocides is determined by the Chick and Watson law (Chick et al. 1908; Watson et al. 1908):

$$\ln(N/N_o) = -kC^n t$$

where N/N_o is the ratio of surviving organisms at time t, C is the disinfectant concentration, and k and n are empirical constants (n is referred to the coefficient of dilution).

The Chick and Watson law, with its concentration C multiplied by the contact time t (Ct) factor, has been the basis for all subsequent models (LeChevallier et al. 1988). Further, the efficacy of a disinfectant programme can be assessed by the recovery of process parameters (Flemming and Griebe 2000). In an industrial cooling system or water distribution system, dosing of biocides is done to prevent bacterial growth and colonization. However, experience over the years has shown that maintaining a biocide residual alone could not result in preventing microbial growth and biofilms in industrial systems. From a better understanding of the principles of microbial adhesion, the action of biocides and the quality of abstracted water, it is now becoming obvious that living with biofilms is imperative (Flemming and Griebe 2000). Biofilms are ubiquitous and cannot be totally eradicated even at a very high cost factor and for environmental safety. All systems in contact with water carry biofilms, but not all have biofouling problems. It is now being increasingly recognized that to control biofouling means to maintain biofilm development below the threshold limits so that operations are not affected (Flemming 2002).

The *Ct* values for all biocides and disinfectants are affected by a number of parameters including temperature, pH value and biocide demand as commonly caused by organic matter and protective cell aggregations (Walker and Percival 2000). Temperature and pH effects on oxidizing biocides have been well documented (refer to White 1999), whereas the most important parameter responsible for determining biocidal availability for killing is the organic content of water, which is a site- and season-specific dynamic parameter for which no specific value could be assigned. In this context the influence of organic matter on the efficacy of biocides is of utmost practical importance. The presence of even small quantities of organic matter reduces the efficiency of oxidizing biocides to varying degrees. The types of action that may occur are as follows:

- The biocide may react chemically with the organic material, giving rise to a complex that is in many instances non-biocidal, or it may form an insoluble compound with the organic matter, thus rendering it inactive
- Particulate and colloidal matter in suspension may absorb biocides so that it is subsequently, if not totally, removed from solution
- Naturally occurring fats, phospholipids etc. may dissolve or absorb biocides preferentially, rendering them inactive
- Organic and suspended particulate matter may form a coating on the surface that may render the fluid in the immediate vicinity rather more viscous, and so tend to prevent the ready access or penetration of biocides to the cell before any biocidal activity can occur

Antifouling efficacy on mixed population biofilms in low nutrient environments revealed a relationship between the nature of organic matter and disinfection efficiency. Chlorine was effective in removing natural biofilms with low organic carbon content, whereas it was ineffective with biofilms grown using amino acids and carbohydrates as the nutrient source (Butterfield et al. 2002). Organic load requires additional dosing of biocides to compensate for the demand in the system and to make available the biocide for reaction with biofilms. The price will be an increased concentration of chlorination by-products. Compared to chlorine, monochloramine-was found to be stable and is used in many recirculating and drinking water systems and is effective against biofilms (Murthy et al. 2008). Biofilm bacteria challenged with monochloramine retained significant respiratory activity even though they could not be cultured (Huang et al. 1995).

Application of biocides to industrial cooling water systems is done either on a continuous or on an intermittent basis. It is important when applying biocides to a cooling water circuit that the concentration developed within the system exceeds the minimal inhibitory concentration for the microbiological contaminants present and that it also has a sufficient contact time to exert its activity. Unless the system has a low retention time, there will be little difference between the inhibitory concentrations, whether dosing is continuous or intermittent. Conventionally, before the advent of surfactants, intermittent dosing along with an increase in velocity was practised in industrial cooling water systems where fouling caused by biofilms was found to be a problem to be overcome. This is dependent upon the generation of a

relatively high concentration of microbiocide within the system at regular intervals of time and the use of high velocities intermittently to slough off biofilm layers.

Due to a wide diversity and varying population of microorganisms that can be present in any cooling system, it is impossible to establish definitive dosage figures that will have universal application. In general, however, high dosages are necessary in the case of severe microbial fouling. In effect, dosages are frequently applied in a two-phased manner. The initial dosage is usually high and aims at disrupting and dispersing any biomass present in the system, in addition to reducing the microorganisms to an acceptable level. Once the load is within the threshold limit then a lower concentration of biocides will inhibit further growth. In this context, cooling systems operate on a continuous low dose biocidal treatment with an intermittent shock dosing.

4.2.3 Efficacy of Biocides in Drinking Water Systems

Experience from drinking water systems can be adopted at least partially to biofouling control of heat exchanger circuits. However, drinking water disinfection has a different goal (i.e. the control of hygienically relevant microorganisms) while antifouling measures in heat exchanger systems do not have to meet such high hygienic standards but rather focus on limitation of microbial growth. Therefore, the term "disinfection" has a strictly hygienic connotation in drinking water, while in heat exchanger systems it refers in a more loose sense to partially inactivating the overall microbial biofilm population, while cells in suspension usually do not represent the dominant problem.

In drinking water distribution systems, growth of biofilms generally exceeds the growth of their planktonic counterparts (Camper 1996). Biofilms in drinking water systems are thin and patchy (Characklis 1988; Wingender and Flemming 2004). Control of biofilms in potable water systems is straightforward and usually achieved by establishing stable water through control of biologically degradable organic carbon (BDOC). This keeps the naturally occurring microbial population in drinking water in an oligotrophic situation. Furthermore, the drinking water industry is continually seeking novel disinfection strategies to control biofouling in distribution systems where nutrient limitation cannot be secured.

Conventionally, chlorine and chloramines are used as disinfectants in potable water distribution systems (US Environmental Protection Agency (US EPA) 1992). The efficacy of different biocides on test organisms is listed in Table 6. The problem in drinking water distribution systems is similar to cooling circuits with respect to the development of multi-species biofilms. Studies by Williams et al. (2005) have shown that biofilm communities in distribution systems are capable of changing in response to disinfection practices. Comparing two different treatments using monochloramine and chlorine it was found that after 2 weeks, increased dosing was required to maintain monochloramine levels in the system. In monochloramine-treated systems *Mycobacterium* and *Dechloromonas* were dominant whereas in chlorine-treated systems proteobacteria were dominant. Hence, it is advisable to use a combination of biocides or to alternate between biocides in distribution systems in order to prevent microorganisms from developing resistance.

Table 6 Biocides used for disinfecting planktonic and sessile cells in drinking water systems

Biocide	Test system and organism	Concentration (mg L^{-1})	Effect
Planktonic cells			
Cl_2	*E. coli*	0.2	Bacterial survival even after 2 weeks of continuous exposure (Williams et al. 2003)
	Legionella pneumophila	4 Monochloramine	
	δ- and β-Proteobacteria		
Ozone	*P. fluorescence* Laboratory cultures	0.1 and 0.3	Effective at 10–3 min (Viera et al. 1999)
Biofilms			
Cl_2 and $NHCl_2$	*K. pneumoniae* *P. aeruginosa* Steel surfaces Natural biofilms Pipe surfaces	2	Respiratory activity observed deep in biofilm with CTC stain (Huang et al. 1995)
Chloramine T	*L. pneumophila*	0.1 – 0.3%	Reduction in planktonic cells only (Ozlem et al. 2007)
Oxsil 320 N	*P. aeruginosa*	3	Wood et al. (1996)
Potassium mono persulfate	*P. aeruginosa*	20	Eliminated total viable counts (Wood et al. 1996)
Oxsil 320N			A tenfold increase in concentration required to eliminate sessile cells (Surdeau et al. 2006)
Chlorine dioxide	Diverse microbes in a Chemostat	0.25	Percentage kill of 73.8%
		1.0	Percentage kill of 88.4% (Walker and Morales 1997)
		1.5	Percentage kill of 99.3%
	Heterotrophic Biofilms	0.25 low 0.5 high	Disinfection (Gagnon et al. 2005)
Chlorite ion	Heterotrophic Biofilms	0.1 low 0.25 high	Disinfection (Gagnon et al. 2005)
Ozone	Laboratory biofilms	0.15	Diminish sessile cell population by three orders of magnitude (Viera et al. 1999)

Another important observation is that discontinuous or intermittent addition of biocides increased the release of cells from the biofilm to bulk water. A tenfold increase in microbial cells in the water phase was observed in the absence of chlorine dosing (Codony et al. 2005). Intermittent dosing of biocides resulted in planktonic cells developing resistance, corresponding to the number of times layoff periods occurred. Results indicated that intermittent biocidal dosings may accelerate the development of microbial communities with reduced susceptibility to disinfection in drinking water systems (Codony et al. 2005).

Maintenance of a chlorine residual level does not inactivate all bacteria in a water distribution system (Momba et al. 1998). Biofilm formation was observed at residuals of 16.5 mg L^{-1} hydrogen peroxide, 1 mg L^{-1} monochloramine and 0.2 mg

L^{-1} free chlorine (Momba et al. 1998). Studies with chlorine have shown that 3–5 mg L^{-1} (Nagy et al. 1982) and 10 mg L^{-1} (Exner et al. 1987) of free chlorine eliminates biofilms in pure water systems.

Chlorine dioxide is another option for disinfection in distribution systems. Chlorite ion, a by-product generated in systems dosed with chlorine dioxide, was found to be less effective at concentrations between 0.20 and 0.34 mg L^{-1} in eliminating heterotrophic bacteria (Gagnon et al. 2005). Field trials at the East Bay Municipal Utility District (EBMUD) in California comparing the efficiency of UV/ClO_2, ClO_2, UV/Cl_2 and Cl_2 for biofilm control showed that UV/ClO_2 was most effective against suspended and sessile heterotrophic bacteria. ClO_2 was more effective than Cl_2 against suspended and sessile bacteria, and that UV treatment alone was not as efficient as ClO_2 and Cl_2 treatments (Rand et al. 2007). On the other hand, ozone has been a very effective agent for disinfecting potable water systems. The formation of by-products like iodate and bromate has been observed with ozonated waters. A low drinking water standard of 10 mg L^{-1} has been set for drinking water, and hence disinfection strategies should be designed to operate at these ranges (Gunten 2003). It is generally believed that increasing the concentration of a disinfectant should control regrowth but many instances exist where the opposite is seen (LeChevallier et al. 1987; Martin et al. 1982; Reilly and Kippen 1984; Oliveri et al. 1985).

4.2.4 Efficacy of Biocides and Resistance of Biofilm Organisms

It is well known that biofilm organisms display a resistance to biocides. For their inactivation, sometimes more than two orders of magnitude higher concentrations are required than for planktonic cells (for review see Schulte et al. 2005). The reasons for this phenomenon are under research and not fully elucidated. Among the mechanisms discussed in terms of increased resistance are:

- Influence of abiotic factors such as limited access of biocides to biofilms in crevices or in dead legs of water systems, and attachment to particles
- Diffusion–reaction limitation, due to the reaction of oxidizing biocides with EPS components (main inactivation factor for chlorine)
- Slow growth rate, which protects dormant organisms from biocides interfering with physiological processes
- Biofilm-specific phenotypes that express, e.g., copious amounts of EPS in response to biocides or enzymes such as catalase that inactivate hydrogen peroxide
- Persister cells, which is the term for the small number of organisms in a population that survive even the most extreme concentrations by mechanisms still unknown

Ranking of halogen biocides against biofilms of *Pseudomonas fluorescens* (a contaminant in cooling water circuits), *Pseudomonas aeruginosa* (a contaminant in potable water distribution systems) and *Klebsiella pneumoniae* (a contaminant in potable water distribution and hygiene systems) showed stronger resistance of biofilms than of planktonic cells (Tachikawa et al. 2005). Results of this study showed that efficacy

of different biocides varied with respect to the microorganism. In the case of *P. fluorescens* biofilms exposed to various biocides, survival increased as follows:

$$NH_4Br > NH_2Cl > HOCl > STARBEX^{®} > Br_2Cl$$

with *K. pneumoniae* biofilms, percentage survival increased as follows:

$$Br_2Cl > HOCl > NH_2Cl > STARBEX^{®} > NH_4Br$$ (Tachikawa et al. 2005).

STARBEX is a stable liquid bromine-based antimicrobial compound marketed by NALCO (Naperville, IL). It is imperative from the results to ascertain the dominant microorganisms present in an industrial system before a biocidal regime can be put into place. Further, bacterial species having a high inherent susceptibility to water-treatment biocides become dominant in systems in the presence of biocides. This has been attributed to the formation of resistant cells. The effect was demonstrated by Brözel et al. (1995) with *P. aeruginosa*, *Pseudomonas stutzeri* and *Bacillus cereus* sub-cultured repeatedly in the presence of sub-inhibitory concentrations of biocides, and thus adapted to grow in the presence of increasing concentrations. Hence, in industrial water systems it is advisable to alternate between biocides to maintain biofilms within the threshold levels.

Ozone was found to be effective at concentrations between 0.1 and 0.3 ppm at eliminating planktonic cells of *Pseudomonas fluorescens* (a contaminant in industrial systems) (10^7–10^8 cells mL^{-1}) within a contact period of 10–30 min, whereas ozone at a concentration of 0.15 ppm was only able to diminish cells by two to three orders of magnitude (Viera et al. 1999). Biofilms have also been reported to develop resistance to quaternamonium compounds like benzalkonium chloride as a result of an increase in hydrophilicity of the bacterial cell surface by the production of exopolysacchrides in *P. aeruginosa* CIP A22. However, this change in hydrophobicity was intermediate as the cells returned to normalcy after washing (Campanac et al. 2002). This study shows that bacteria have similar mechanisms of resistance for oxidizing and non-oxidizing compounds, i.e. development of EPS. Quaternary ammonia compounds dosed along with a domestic detergent did not induce microbial resistance in long-term exposures (McBain et al. 2004).

Due to the enhanced resistance exhibited by biofilms towards biocides, novel approaches like dosing a combination of biocides are currently under investigation. A laboratory study by Son et al. 2005 using a mixture of biocides showed that combinations of Cl_2/O_3, Cl_2/ClO_2 and Cl_2/ClO_2 showed enhanced efficiency (52%) compared to a single biocide (Cl_2) in killing *Bacillus subtilis* spores. In comparison, a combination of Cl_2/H_2O_2 was not found to be as effective. This approach of a combination of biocides could be tried out in heat exchangers (targeted biocide addition) where improvement in threshold levels of biofilm would amount to significant savings. Another study supporting the concept of application of dual biocides was by Rand et al. (2007), who tested a combination of UV/ClO_2, UV/Cl_2, ClO_2 and Cl_2 and showed that the combination of UV/ClO_2 was the most effective against suspended (3.93 log reduction) and attached (2.05 log reduction) heterotrophic bacteria. In contrast, UV light alone was not effective in disinfecting

suspended or sessile bacteria compared to both ClO_2 and Cl_2. Pretreatment with UV aided in increased disinfection efficiencies with both the biocides ClO_2 and Cl_2.

The approach of using a combination of biocideshas also been tested in pure water systems. Comparison of the disinfection efficiency of chlorine and chlorine dioxide against microbial cells revealed chlorine dioxide to be effective over a wide range of pH (Junli et al. 1997a). Further, disinfection efficiency of ClO_2 on algae (*Ulothrix* Cl_2 94.2%, ClO_2 100%; *Chlamydomonas* Cl_2 92.9%, ClO_2 75%; *Microphorimidum* Cl_2 81.3%, ClO_2 100%) was found to be the same or slightly better than liquid chlorine. Enhanced disinfection was observed with ClO_2 against virusesand zooplankton (Junli et al. 1997b). Chlorine dioxide inactivation of *Bacillus subtilis* spores in natural waters and spiked ultrapure waters were far more effective than chlorine (Barbeau et al. 2005). Intermittent application of chlorine dioxidewas found to be ineffective in disinfecting bacteria in dental unit water lines (Smith et al. 2001). Comparison of efficacies of non-oxidizing biocides, e.g. Macrotrol MT200, Microtreat AQZ2010 and Microbiocide 2594, assayed against 23 groups of bacteria showed susceptibility of Gram-positive (MIC <4 mg L^{-1}) and Gram-negative bacteria (MIC <16 mg L^{-1}) that were in ranges far lower than those for alkylated naphthoquinonederivate molecules (MIC 1–64 mg L^{-1}) (Chelossi 2005).

Another example of a multiple biocide strategy is using oxidizing biocides like hydrogen peroxide and potassium monopersulfate and a surface-active agent (copper and cobalt phthalocyanine) incorporated in the surface matrix, which reduced the quantity of the biocide (potassium monopersulfate) required (Wood et al. 1996). This successful approach, demonstrated for surfaces of medical importance, could be tried in industrial systems where a multiple strategy of using biocide and a low surface energy antifouling coating in tandem would reduce the amount of biocide to be dosed.

4.3 Cleaning of Surfaces: Role of Surfactants or Surface-Active Agents

Biocides have been used for killing both planktonic as well as sessile cells (Chen and Stewart 2000) but killing alone is not enough, as explained earlier. In addition to the killing action, oxidizing biocides like chlorine, ozone, hydrogen peroxide and peracetic acid are known to weaken the biofilm matrix (Flemming 2002). The basic concept is to apply shear forces to a weakened biofilm matrix for removal. This can be achieved by the use of mechanical forcessuch as an increase in water flow velocity or flushing with air or steam. Basically, biofilms are kept together by weak physicochemical interactions (see above). Understanding the requirement, industry has adopted the use of surfactants or surface-active agents (the majority of which are biodegradable and less toxic) addressing van der Waals interactions, as well as complex-forming substances such as citric acid in order to overcome electrostatic interactions (Flemming et al. 1999).

Quaternary ammonium compounds(QACs) are amphoteric surfactants that are widely used for the control of bacterial growth in clinical and industrial environments (Brannon 1997). These are known to have broad-spectrum antimicrobial and surfactant properties, which have made QACs such as benzalkonium chloride the favoured agents (Shimizu et al. 2002). QACs are known to act on the cell membrane and rupture cells (Simoes et al. 2005a). Cetyltrimethylammonium bromide(CTAB), a cationic QAC is known to act on the lipid component of the membrane causing cell lysis as secondary effect (Gilbert et al. 2002). These are usually applied to open or closed recirculating systems and are non-toxic for short-term applications, against non-target organisms. QACs are dosed in small closed recirculating cooling systems where the water is inaccessible for potable and domestic purposes, as the effects of these compounds on the biota are yet to be worked out completely. These biocides are required at milligram levels and are dosed periodically, once a day or once in 8 h and are effective for short-term periods like 12 or 48 h after dosing.

Ortho-phthalaldehyde(OPA), an aromatic compound with two aldehyde groups (McDonnell and Russell 1999) having excellent microbiocidal and sporicidal activity (McDonnell and Russell 1999; Rutala and Weber 2001) has received clearance by the FDA (US Food and Drug Administration in 1999) and is currently being tested with different biofilm models (Simoes et al. 2003, 2007, 2008). Some commercial products based on quaternamonium compounds are also available (NALCO, Naperville, IL; GE-Betz Dearborn, Decatur, IL) for treating cooling water systems. Several detergents are available for disinfecting medical equipment for hygienic purposes, whereas their use in potable water distribution systems is non-existent. Investigations on the mode of action of these surface-active agents on biofilm components, their interaction with water systems and their degradation and by-product formation all need to be carried out before these can be recommended in real-time systems. The cleaning efficacyof QACs, however, is very limited.

Surfactants to a certain extent are also known to inactivate microbial cells, apart from removing them from the surface. The efficacy of CTAB (cetyltrimethyl ammonium bromide), a cationic surfactant, on *Pseudomonas fluorescens* biofilms (Simoes et al. 2005a; 2006a) grown under laminar and turbulent conditions revealed biofilms generated under laminar conditions to be more susceptible to CTAB than biofilms generated under turbulent conditions. Total inactivation of cells was not achieved for either flow condition. In comparison, an anionic surfactant sodium dodecyl sulfate(SDS) was effective in inactivation at higher concentrations, but neither CTAB nor SDS promoted detachment of biofilms from surfaces (Simoes et al. 2006b). These results indicate that surfactants alone are not sufficient to remove biofilms. Furthermore, post-surfactant treatments resulted in biofilms recovering respiritory activity to levels found in untreated controls. Subsequent studies demonstrated resistance of *P. fluorescens* cells attached to glass surfaces on treatment with CTAB and the aldehyde OPA (Simoes et al. 2008). The low cell detachment observed with CTAB treatments has been attributed to a change in the bacterial cell surface charge (it acquired a positive charge) and increased electrostatic interaction of the microbe to the surface (Azeredo et al. 2003). In comparison, the combined exposure to CTAB application and increased shear stresses promoted

increased biofilm removal, demonstrating physical and chemical forces to be effective in removing biofilms (Simoes et al. 2005a). Alternatively, the response of biofilms to combined exposure to oxidizing biocides and surface-active agents needs to be evaluated to improve our understanding of their efficacies. Screening for biofilm detachment using other surface-active agents needs to be carried out and their mechanism of action with respect to their molecular and antimicrobial properties needs to be studied.

5 Monitoring the Effectiveness of Biocidal Dosings

Monitoring is of particular importance when water treatment is the primary approach to prevention of biofouling in industrial systems (Bruijs et al. 2001; Flemming 2002). Microbial growth can be prevented by a good biocidal (Maukonen et al. 2003; Simoes et al. 2005b; Meyer 2006) with a biofilm monitoring programme in place (Flemming 2003). Control or prevention of microbial attachment may form the basis of a successful treatment programme (Meyer 2003). Various monitoring techniques are available of which the following would be of practical use in industrial systems: (1) in-situ analysis where fouling deposits are collected and analyzed, (2) online monitoring devices and (3) side-stream monitoring devices.

In-situ analysis is a labour-intensive job requiring special laboratory skills for estimation of various physical, chemical and biological parameters. On the other hand, online monitoring techniques are found to offer an indication of surface deterioration to plant operators to review their dosing strategy (e.g., Flemming 2003; Jahnknecht and Melo 2003). Characklis proposed as early as 1990 an online biofouling monitoring system from which the data collected is relayed to a central processor system. This would allow for early warning, effective countermeasures and efficacy determination.

The requirements for online monitoring are very demanding: it should give the information online, in real time, non-destructively, automatically and possibly remotely sensed. In general, only physical methods can meet these requirements. The problem is that they usually detect a deposit but not its nature. Therefore, they will respond to abiotic fouling as well as to biofouling. This requires experience and advanced application research, which is not often performed.

Different types of online monitoring systems are available and it is up to the operator to choose between them. They have been systematically considered by Flemming (2003). These involve in-place monitors like test substrates (Yohe et al. 1986; Donlan et al. 1994); retractable bioprobes (Jones et al. 1993); an optical fouling monitor (Wetegrove et al. 1997; Tamachkiarow and Flemming 2003); the BioGeorge electrochemical biofilm activity monitoring system (Bruijs et al. 2001); and the Bridger Scientific Fouling Monitor described by Bloch and DiFranco (1995). Flemming et al. (1998) have described the design features and functioning of some of the online industrial fouling monitoring devices (fibre optical sensor FOS; differential turbidity measurement device DTM; Fourier transformation infrared spectroscopy flow cell).

An important aspect brought out by Donlan (2000) on the use of online fouling monitors in operational industrial units is that these devices may throw light on the extent of deterioration occurring in the system with respect to the current levels of biocidal dosing but may not mimic exactly the system condition (pipe or electrically conductive surface) where biofilms have been accumulating for years. Each of these online monitoring methods has its own strengths and weaknesses and the type of monitor should be carefully chosen for a particular application. As pointed out by Donlan (2000), online sensors and detection devices are indicative of surface deterioration rather than the nature of fouling (biological, inorganic fouling), which is essential in determining the biocidal action. These devices measure total fouling,which includes clay/silt, corrosion and scale deposits, and biofilms. Addressing this aspect the electrochemical sensor BioGeorge has been developed, which measures the change in electrochemical reactions produced by biofilms on stainless steel electrodes (Bruijs et al. 2001).

In spite of the large number of online devices available, the concept of online monitoring has not been widely adopted by the industry, partly because there is no real consensus on accepted biofilm monitoring techniques and the paucity of information regarding the concentration of biocides required to control biofilms in industrial systems (as opposed to laboratory data) (Donlan 2000). To resolve the concern an "expert system approach" has been proposed by Donlan (2000) that involves studies comparing biofilm levels using different techniques. In other words, it is the threshold levels of interference for a particular technical system that is the scope of an industrial operator and not the online monitoring equipment. Hence, the expert system approach should involve a study of threshold levels of interference on a site- and season-specific basis and the results extrapolated to the online monitoring device for its effective usage.

Compared to online monitoring devices, side-stream monitoring devices are more practical and offer data of real-time value to operators. Several types of side-stream monitoring devices are available: Robbins device (McCoy and Costerton 1982), annular reactors (Chexal et al. 1997) and parallel plate flow-through systems (Pedersen et al. 1982). Measuring devices (pressure gauges) for D*p* would also offer an indication of the effectiveness of the control measure in practice. However, this method is more appropriate for macrofouling organisms. The use of different methods to evaluate biocide efficacy can lead to different conclusions about the effects caused by the biocide (Simoes et al. 2005b). Simple flow-through systems housing the material of interest and connected to the main system would be the best method of understanding fouling development.

A regular monitoring programmeshould be a part of an antifouling programme. Since fouling follows an asymptotic pattern in industrial systems, this curve should be established for a given system to arrive at the sampling strategy. The next step is the sampling strategy, where three types of coupons needs to be introduced. For the time being, the most common method is to expose a short-term coupon (exposed for a period of 15–20 days in a system, retrieved and quantified). Later, a long-term coupon is exposed for a period of 30–40 days, retrieved and quantified. The time intervals cited are arbitrary and need to be standardized for a given geographical

location based on the asymptotic fouling curve. Short-term exposure refers to the log phase of the curve (15–20 days) and long-term exposure refers to the plateau phase where deposition levels off (30–40 days). The third is a permanent coupon (for visual observation, which is to be observed by the naked eye to note seasonal changes). This is a less expensive and effective method compared to a simulated side-stream monitoring device where these sampling procedures can be overcome.

In power stations, when a more precise control over the process parameters is required, side-stream monitors incorporating both D*p* and D*t* measuring devices to determine the thermal resistance of fouling deposits are a more precise and accurate method of evaluating the effectiveness of the biocide. Data from such monitors could be logged and available online through a computer for operators to fine-tune their biocidal programmes. However, monitoring remains a highly neglected field in improvement of antifouling measurements and early warning systems, as well in minimizing the environmental burden of biocides. Still, preventive overdosing of biocides is very common, causing considerable damage to the environment due to interference with biological treatment of waste water and to excessive formation of by-products.

6 Concluding Remarks

Every industrial cooling water system is unique with respect to biological, chemical and process parameters. As pointed out in this chapter, cooling water treatment programmes have to meet a compromise between cost, cleanliness and environmental requirements, wherein the threshold factor is of importance. Biofilms are ubiquitous in industrial systems and have been demonstrated to be mechanically stable. Elimination of biofilms in industrial systems is not necessary. However, it is vital to learn how to live with biofilms and how to prevent their excessive development. For this point, the threshold of interference due to biofilms becomes important and has to be ascertained in order to evolve suitable control measures. This level is up to the subjective tolerance of the operator and is only operationally, not scientifically, based.

Increasing the biocide dose to combat biofilms is neither a sufficient nor a completely acceptable option as biofilms in industrial cooling circuits have been shown to develop resistance to biocides in the long run. Alternating between biocides would help solve the problem to a certain extent; however, it is not viable in huge industrial circuits where capital investments are involved. Although several mechanisms of resistance have been put forward, biofilm resistance observed in industrial systems is mainly due to failure of the most popular biocide, i.e. chlorine, to penetrate into deep biofilm layers before being consumed by EPS components and interaction with the process fluid. Quite often, cells deep in the biofilm are unaffected and multiply to reach levels expected in untreated systems. To tackle this problem, more persistant biocides are used, like monochloramine or bromine chloride, or a stronger oxidant like chlorine dioxide for power plants and ozone for potable water distribution systems, along with a surface-active agent to remove the biomass from surfaces.

Increased biocidal doses would initiate other problems like corrosion and by-product accumulation. Instead, fine-tuning of the biocide dose and regime based on continuous monitoring or surveillance should be adopted to keep biofilms under the threshold level. In addition, it must be kept in mind that killing is not cleaning and that it is imperative to use surfactants in a fouling control programme.

Biomass offers copious nutrients for increased colonization and regrowth of bacteria. Hence, cleaning of surfaces is an important aspect of an antifouling programme. As a consequence, heat exchanger systems should be designed to be cleaning-friendly, with surfaces easily accessible (e.g. for pigging) and with low adhesion forces of biofilms. Cleaning is more important than killing the organisms and leaving them in place. Nutrients are potential biomass but are not addressed by biocides – some biocides make nutrients even more bioavailable (e.g., by chlorination of humic substances). Treating of water for removal of nutrients is a non-viable option for power plants, whereas this can be used as a limiting factor for biofilm prevention in desalination membranes. Suitable devices for removal of organic load need to be developed for industrial applications. The concept of living with biofilms is a reality to be accepted, and it can be achieved by understanding the laws of biofilm development.

Currently, more is known about the action of oxidizing biocides like chlorine, chlorine dioxide and ozone than about the organic and synthetic biocides that are now flooding the market. Even though these organic biocides are toxic at low concentrations, long-term environmental effects on the receiving water bodies need to be assessed. This leaves us with chlorine dioxide and ozone as the potential biocides to replace chlorine because of increasing legislations on the upper discharge limits of chlorine. Comparatively little literature is available on the type, action and efficiencies of surfactants, the main reason being insufficient success. Under these conditions, chlorine dioxide promises an interesting alternative due to it high oxidizing nature and low by-product formation.

From earlier studies it is clear that biocides alone are not sufficient to control fouling. For efficient industrial operations, an integrated antifouling programme involving a practical and reliable monitoring programme, biocide dosing, biodispersant dosing, online cleaning and, eventually, off-line cleaning has to be put into practice. Online mechanical cleaning methods assist biocides in combating fouling. Offline cleaning methods should be included in the design of industrial systems. The frequency of offline cleaning is again dependent on the required threshold levels of interference. Side-stream monitoring devices simulating D*p* and D*t* with online data recording are a convenient method of fine-tuning biocide dosing with respect to spikes in biofilm formation. Comparatively, the use of coupons for periodic monitoring would offer a better understanding of the diversity and density of organisms at surfaces.

Basically, the most elegant way to prevent biofouling is always nutrient limitation. Considering biofouling as a “biofilm reactor in the wrong place”, it can be put in the “right” place by using a biological filter ahead of the system to be protected. The biofilm develops here, “in the right place”, where it does not disturb the process and can be handled easily (Flemming 2002). Of course, this cannot be achieved in

all situations but certainly much more often than it is done now. It requires nothing but a little shift of perspective.

References

APHA (1995) Standard methods for the examination of water and wastewater 19th edn. American Public Health Association/American Water Works Association/Water Environment Federation, APHA Washington DC, 9:34–9.41

Azeredo J, Pacheco AP, Lopes I, Oliveira R, Vieira MJ (2003) Monitoring cell detachment by surfactants in a parallel plate flow chamber. In: Proceedings of the 2002 international specialized conference on biofilm monitoring. Water Sci Technol 47:77–82

Barbeau B, Desjardin R, Mysore C, Prevost M (2005) Impacts of water quality on chlorine and chlorine dioxide efficacy in natural waters. Water Res 39:2024–2033

Bloch KP, DiFranco P (1995) Preventing MIC through experimental on-line fouling monitoring. National Association of Corrosion Engineers annual conference; paper no 257

Blank LW (1984) Control of algal biofouling at High Marnham; 1981–83. CERL note no TPRD/L/2649/N84, Central Electricity Research Laboratories, Leatherhead

Blakke R, Olsson PQ (1986) Biofilm thickness measurements by light-microscopy. J Microbiol Methods 5:93–98

Bott TR, Pinheiro MMVPS (1977) Biological fouling – velocity and temperature effects. Can J Chem Eng 55:473

Bott TR, Gunatillaka M (1983) Nutrient composition and biofilm thickness In: Bryers RW, Cole SS (eds.) Fouling of heat exchanger surfaces. United Engineering Trustees New York, pp. 727–734

Bott TR, Melo LF (1992) Particle–bacteria interactions in biofilms In: Melo LF, Bott TR, Fletcher M, Capdeville B (eds.) Biofilms – science and technology. NATO ASI Series Kluwer Academic Netherlands pp. 199–206

Bott TR (1994) The control of biofilms in industrial cooling water systems In: Wimpenny W, Nichols D, Sticker, Lappin-Scott H (eds.) Bacterial biofilms and their control in medicine and industry Bioline, Cardiff

Bott TR (1995) Fouling of heat exchangers Elsevier, Amsterdam p. 524

Bott TR (1999) Biofilms in process and industrial waters: the biofilm ecology of microbial biofouling, biocide resistance and corrosion. In: Keevil CW, Godfree A, Holt D, Dow C (eds.) Biofilms in the aquatic environment. Royal Society of Chemistry, London pp. 80–92

Brannon DK (1997) Cosmetic microbiology: a practical handbook. CRC, Boca Raton

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72:248–254

Bruijs MCM, Venhuis LP, Jenner HA, Licina GJ, Daniels D (2001) Biocide optimization using an on-line biofilm monitor. Power Plant Chem 3(7):400–405

Brözel VS, Pietersen B, Cloete TE (1995) Resistance of bacterial cultures to non-oxidizing water treatment bactericides by adaptation. Water Sci Technol 31(5–6):169–175

Busscher HJ, Handley PS, Bos R, van der Mei HC (1999) Physico-chemistry of microbial adhesion from an overall approach to the limits. In: Baszkin A, Norde W (eds.) Physical chemistry of biological interfaces Marcel Dekker, New York pp. 431–458

Busscher HJ, Bos HC, van der Mei H (1995) Initial microbial adhesion is a determinant for the strength of biofilm adhesion. FEMS Microbiol Let 128:229–234

Busscher HJ, van der Mei H (1997) Physico-chemical interactions in microbial adhesion and relevance for biofilm formation. Adv Dent Res 11:24–32

Butterfield PW, Camper AK, Ellis BD, Jones WL (2002) Chlorination of model drinking water biofilm: implications for growth and organic carbon removal. Water Res 36:4391–4405

Campanac C, Pineau L, Payard A, Baziar-Mouysset G, Roques C (2002) Interactions between biocide cationic agents and bacterial biofilms. Antimicrob Agents Chemother 46:1469–1474

Camper AK (1993) Coliform regrowth and biofilm accumulation in drinking water systems: a review In: Geesey GG, Lewandowski Z, Flemming HC (eds.) Biofouling/biocorrosion in industrial systems Lewis Chelsea, MI

Camper AK (1996) Factors limiting microbial growth in distribution systems: laboratory and pilot-scale experiments. AWWA Research Foundation, Denver

Cassels JM, Yahya MT, Gerba CP, Rose JB (1995) Efficacy of a combined system of copper and silver and free chlorine for inactivation of *Naegleria fowleri* amoebas in water. Water Sci Technol 31:119–122

Chambers CW, Protor CM, Kabler PW (1962) Bactericidal effect of low concentrations of silver. J Am Water Works Assoc 54:208–216

Chalut J, D'Arise K, Bodkin PM, Stodolka C (1995) Identification of cooling water biofilm using a novel ATP monitoring technique and their control with the use of biodispersants. In: Corrosion/88. NACE International Houston, paper no 211

Chandy JP, Angles ML (2001) Determination of nutrients limiting biofilm formation and the subsequent impact of disinfectant decay. Water Res 35:2677–2682

Characklis WG (1988) Bacterial regrowth in distribution systems. AWWA Research Foundation, Denver, p. 332

Characklis WG (1990): Microbial fouling. In: W.G. Characklis, K.C. Marshall (eds.) Biofilms. Wiley, New York, 523–584

Characklis WG, Marshall K (1990) Biofilms: a basis for an interdisciplinary approach. Wiley New York, pp. 3–15

Chen X, Stewart PS (2000) Biofilm removal caused by chemical treatments. Water Res 34:4229–4233

Chelossi E, Faimali M (2005) Comparative assessment of antimicrobial efficacy of new potential biocides for treatment of cooling and ballast waters Sci Total Environ

Chexal B, Horowitz J, Munson D, Spalaris C, Angell P, Gendron T, Ruscak M (1997) Biofilm monitoring in power plant waters for use in prediction and control of MIC EPRI. Presented at tenth service water reliability improvement seminar, Denver, CO

Chick H (1908) An investigation of the laws of disinfection. J Hygiene 8:92–158

Christensen BE, Characklis WG (1990) Physical and chemical properties of biofilms. In: Characklis WG, Marshall KC (eds.) Biofilms. Wiley, New York, pp. 93–130

Cloete TE, Jacobs L, Brozel VS (1997) The chemical control of biofouling in industrial water systems. Biodegradation 9:23–37

Cloete TE, Westaard D, van Vuuren SJ (2003) Dynamic response of biofilm to pipe surface and fluid velocity. Water Sci Technol 47(5):57–59

Codony F, Morato J, Mas J (2005) Role of discontinuous chlorination on microbial production by drinking water biofilms. Water Res 39:1896–1906

Daley RJ, Hobbie JE (1975) Direct counts of aquatic bacteria by a modified Epifluorescence technique. Limnology and Oceanography 20:875–883

Donlan RM (2000) Biofilm control in industrial water systems: Approaching an old problem in new ways In: Evans LV (ed.) Biofilms: recent advances in their study and control Harwood Academic Netherlands, pp. 333–360

Donlan RM, Pipes WO, Yohe TL (1994) Biofilm formation on cast iron substrata in water distribution systems. Water Res 28:1497–1503

Donlan RM, Forster T, Murga R, Brown E, Lucas C, Carpenter J, Fields B (2005) Legionella pneumophila associated with the protozoan *Hartmannella vermiformis* in a model multi-species biofilm has reduced susceptibility to disinfectants. Biofouling 21(1):1–7

Dubois M, Gilles KA, Hamilton JK, Rebers A, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Ewans DW, Griffiths JS, Koopmans R (1992) Options for controlling zebra mussels. Ontario Hydro Res Rev 7:1–25

Exner M, Tuschewitski GJ, Scharnagel J (1987) Influence of biofilms by chemical disinfection and mechanical cleaning. Zentrablatt Bakteriologie Mikrobiologie Hygeine B. 183:549–563

Fields BS, Benson RF, Besser RE (2002) Legionella and Legionnaire disease: 25 years of investigation. Clin Microbiol Rev 15:506–526
Flemming H-C (2002) Biofouling in water systems-cases causes and countermeasures. Appl Microbiol Biotechnol 59(6):629–640
Flemming H-C (2003) Role and levels of real time monitoring for successful anti-fouling strategies. Water Sci Technol 47 (5):1–8
Flemming H-C, Griebe T (2000) Control of biofilms in industrial waters and processes In: Walker J, Surman S, Jass J (eds.) Industrial biofouling. Wiley, New York, pp. 125–141
Flemming H-C, Leis A (2002) Sorption properties of biofilms. In: Bitton G (ed.) Encyclopedia of environmental microbiology, vol 5. Wiley, New York, pp. 2958–2967
Flemming H-C, Schaule G (1996) Biofouling. In: Heitz E, Sand W, Flemming HC (eds.) Microbially influenced corrosion of materials-scientific and technological aspects. Springer, Heidelberg Berlin New York, pp. 39–54
Flemming H-C, Wingender J (2002): Extracellular polymeric substances: structure, ecological functions, technical relevance. In: Bitton G (ed.) Encyclopedia of environmental microbiology, vol 3. Wiley, New York, pp. 1223–1231
Flemming H-C, Griebe T, Schaule G (1996) Antifouling strategies in technical systems: a short review. Water Sci Technol 34(5–6):517–524
Flemming H-C, Tamachkiarowa A, Klahre J, Schmitt J (1998) Monitoring of fouling and biofouling in technical systems. Water Sci Technol 38(8–9):291–298
Flemming H-C, Wingender J, Moritz R, Mayer C (1999): The forces that keep biofilms together. In: Keevil W, Godfree AF, Holt DM, Dow CS (eds.) Biofilms in aquatic systems. Royal Society of Chemistry, Cambridge, pp. 1–12
Fliermans CB, Bettinger GE, Fynsk AW (1982) Treatment of cooling systems containing high levels of Legionella pneumophila. Water Res 16(6):903–909
Gagnon GA, Stawson RM (1999) An efficient biofilm removal method for bacterial cells exposed to drinking water. J Microbiol Methods 34:203–214
Gagnon GA, Rand JL, O'Leary KC, Rygel AC, Chauret C, Andrews RC (2005) Disinfectant efficacy of chlorite and chlorine dioxide in drinking water biofilms. Water Res 39:1809–1817
Geesey GG, White GC (1990) Determination of bacterial growth and activity at solid–liquid interfaces. Ann Rev Microbiol 44:579–602
Gilbert P, Das JR, Jones MB, Allison D (2001) Assessment of resistance towards biocides following the attachment of micro-organisms to, and growth on, surfaces. J Appl Microbiol 91(2):248–254
Gilbert P, Allison DG, McBain AJ (2002) Biofilms in vitro and in vivo: do singular mechanisms influx cross-resistance? J Appl Microbiol 92:98S–110S
Goysich MJ, McCoy WF (1989) A quantitative method for determining the efficacy of algicides in industrial cooling towers. J Ind Microbiol 4:429–434
Griebe T, Flemming HC (1998) Biocide-free antifouling strategy to protect RO membranes from biofouling. Desalination 118:153–156
Gunten Urs von (2003) Ozonation of drinking water: Part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine. Water Res 37:1469–1487
Harris A (1999) Problems associated with biofilms in cooling tower systems. In: Keevil CV, Godfree A, Holt D, Dow C (eds.) Biofilms in the aquatic environment. Springer, Berlin Heidelberg New York, pp. 139–144
Huang CT, Yu FP, McFeters GA, Stewart PS (1995) Non-uniform spatial patterns of respiratory activity with biofilms during disinfection. Appl Environ Microbiol 61:2252–2256
Jahnknecht P, Melo L (2003) Online biofilm monitoring. Rev Environ Sci Biotechnol. 2:269–283
Jenner HA, Whitehouse JW, Taylor CJL, Khalanski M (1998) Cooling water management in European power stations: biology and control of fouling. Hydroecologie Appliquee 10(1–2)225:

Jones DS, O'Rourke PC, Caine CS (1993) Detection and control of microbiologically influenced corrosion in a Rocky Mountain oil production system-a case history National Association of Corrosion Engineers annual conference, paper no 311

Junli H, Li W, Nanqi R, Fang MA, Juli (1997a) Disinfection effect of chlorine dioxide on bacteria in water. Water Res 31(3):607–613

Junli H, Li W, Nenqi R, Li LX, Fun SR, Guanle Y (1997b) Disinfection effect of chlorine dioxide on viruses, algae and animal planktons in water. Water Res 31(3):455–460

Keevil CW, Mackerness CW, Colbourne JS (1990) Biocide treatment of biofilms. Int Biodeter Biodeg 26:169–179

Keevil CW, Walker JT, Maule A, James BW (1999) Persistence and physiology of Escherichia coli O157:H7 in the environment. In: Duffy G, Garvey P, Coia J, Wasteson W, McDowell D(eds.) Verocytotoxigenic *E. coli* in Europe: survival and growth. Teagasc, Dublin, pp. 42–52

Kim BR, Anderson JE, Mueller SA, Gaines WA, Kendall AM (2002a) Literature review-efficacy of various disinfectants against *Legionella* in water systems. Water Res 36:4433–4444

Kim J, Cho M, Chung WK, Kang SJ, Yoon JY (2002b) Biogrowth control in cooling tower with EEKO-BALL. Fall conference of Korean Society of Water and Wastewater, abstract A-47

Korstgens V, Flemming HC, Wingender J, Borchard W (2001) Uniaxial compression measurement device for investigation of the mechanical stability of biofilms. J Microb Method 46:9–17

Kusnetsov J, Livanainem E, Elomaa N, Zacheus O, Martikainen PJ (2001) Copper and silver ions more effective against *Legionella* than against mycobacteria in a hospital warm water system. Water Res 35:4217–4225

LeChevallier MW, Babcock TM, Lee RG (1987) Examination and characterization of distribution system biofilms. Appl Environ Microbiol 53:2714–2724

LeChevallier MW, Cawthon CD, Lee RG (1988) Inactivation of biofilm bacteria. Appl Environ Microbiol 54:2492–2499

Lehtola JM, Laxander M, Miettinen TI, Hirvonen A, Vartiainen T, Martikainen PJ (2006) The effects of changing water flow velocity on the formation of biofilms and water quality in pilot distribution system consisting of copper or polyethylene pipes. Water Res 40:2151–2160

Lowe MJ (1988) The effect of inorganic particulate materials on the development of biological films. PhD thesis, University of Brimingham

Ludensky M (2003) Control and monitoring of biofilms in industrial applications. Int Biodeter Biodeg 51:255–263.

Lutz P, Merle G (1983) Discontinuous mass chlorination of natural draft cooling towers. Water Sci Tech15:197–213

Martin RS, Gates WH, Tobin RS, Sumarah R, White P, Forestall P (1982) Factors affecting coliform bacterial growth in distribution systems. J Am Water Works Assoc 74:34–37

Maukonen J, Matto J, Wirtanen G, Raaska L, Matila-Sandholm T, Saarela M (2003) Methodologies for the characterization of microbes in industrial environments: a review. J Ind Microbiol Biotech 30:327–356

McBain AJ, Ledder RG, Moore LE, Catrenich CE, Gilbert P (2004) Effect of quaternary ammonium based formulations on bacterial community dynamics and antimicrobial susceptibility. Appl Environ Microbiol 3449–3456

McCoy WF, Costerton JW (1982) Fouling biofilm development in tubular flow systems. Dev Ind Microbiol 23:551–558

McDonnell G, Russell AD (1999) Antiseptics and disinfectants: activity action and resistance. Clin Microbial Rev 12:147–179

Merle G, Montanat M (1980) Essai d'utilisation du dioxyde de chlore dans la boucle TERA installee a Montereau: resultats des tests d'efficacite EDF DER report HE/31–80.045

Melo LF, Bott TR (1997) Biofouling in water systems. Exp Therm Fluid Sci 14:375–381

Meyer B (2003) Approaches to the prevention, removal and killing of biofilms. Int Biodeter Biodeg 51:249–253

Meyer B (2006) Does microbial resistance to biocides create a hazard to food hygiene? Int J Food Microbiol 112:275–279

Mojica K, Elsey D, Cooney MJ (2007) Quantitative analysis of biofilm EPS uronic acid content. J Microb Methods 71(1):61–65

Momba MNB, Cloete TE, Venter SN, Kfir R (1998) Evaluation of the impact of disinfection processes on the formation of biofilms in potable surface water distribution systems. Water Sci Technol 38(8–9):283–289

Murphy HM, Payne SJ, Gagnon GA (2008) Sequential UV- and chlorine-based disinfection to mitigate *Escherichia coli* in drinking water biofilms. Water Res 42(8–9):2083–2092 doi:10.1016/j.watres.2007.12.020

Murthy PS, Venkatesan R, Nair KVK Inbakandan D, Syed Jehan S, Magesh Peter D, Ravindran M (2005) Evaluation of sodium hypochlorite for fouling control in plate heat exchanger for seawater application. Int Biodeter Biodeg 55:161–170

Nagy LA, Kelly AJ, Thun MA, Olson BH (1982) Biofilm composition formation and control in the Los Angeles aqueduct system. In: Proceedings of the American Water Works Association water quality technology conference. American Water Works Association, Denver, pp. 141–160

Nebot E, Casanueva JF, Casanueva T, Sales D (2007) Model for fouling deposition on power plant steam condensers cooled with seawater: Effect of water velocity and tube material. Int J Heat Mass Transfer 50:3351–3358

Oliveri VP, Bakalian AE, Bossung KW, Lowther ED (1985) Recurrent coliforms in water distribution systems and the presence of free residual chlorine. In: Jolley RL, Bull RJ, Davis WP, Katz S, Roberts MH, Jacobs VA (eds.) Water chlorination chemistry environmental impact and health effects Lewis Boca Raton

Ozlem N, Sanli-Yurudu, Ayten Kimiran-Erdem, Aysin C (2007) Studies on the efficacy of chloramine T trihydrate (*N*-chloro-*p*-toluene sulfonamide) against planktonic and sessile populations of different *Legionella pneumophila* strains. Int J Hyg Environ-Health 210:147–153

Pedahzur R, Shoval HI, Ulitzur S (1997) Silver and hydrogen peroxide as potential drinking water disinfectants their bactericidal effects and possible modes of action. Water Sci Technol 35:87–93

Pedersen K (1982) Factors regulating microbial biofilm development in a system with slowly flowing seawater. Appl Environ Microbiol 44:1196–1204

Percival SL (2000) Detection of biofilms in industrial waters and processes. In: Walker J, Surman S, Jass J (eds.) Industrial biofouling detection prevention and control. Wiley, Toronto, pp. 103–124

Petrucci G, Rosellini X (2005) Chlorine dioxide in seawater for fouling control and ost disinfection in potable waterworks. Desalination 182:283–291

Peyton BM (1996) Effects of shear stress and substrate loading rate on *Pseudomonas aeruginosa* biofilm thickness and density. Water Res 30(1):29–36

Pinheiro MM, Melo LF, Bott TR, Pinheiro JD, Leitao L (1988) Surface phenomena and hydrodynamic effects on deposition of *Pseudomonas fluorescens*. Can J Chem Eng 66:63–67

Prince EL, Muir AVG, Thomas WM, Stollard RJ, Sampson M, Lewis JA (2002) An evaluation of the efficacy of Aqualox for microbiological control of industrial cooling tower systems. J Hosp Infect 52:243–249

Pujo M, Bott TR (1991) Effects of fluid velocities and Reynolds numbers on biofilm development in water systems. In: Keffer JF, Shah RK, Ganie EN (eds.) Experimental heat transfer, fluid mechanics and thermodynamics. Elsevier, New York pp. 1358–1362

Rand JL, Hofmann R, Alam MZB, Chauret C, Cantwell R, Andrews RC, Gagnon GA (2007) A field study evaluation for mitigating biofouling with chlorine dioxide or chlorine integrated with UV disinfection. Water Res 41:1939–1948

Reilly KJ, Kippen JS (1984) Relationship of bacterial counts with turbidity and free chlorine in two distribution systems. J Am Water Works Assoc 75:309–314

Rickard HA, McBain AJ, Stead AT, Gilbert P (2004) Shear rate moderates community diversity in freshwater biofilms. Appl Environ Microbiol 70:7426–7435

Rosa DES, Sconza F, Volterra L (1998) Biofilm amount estimation by fluorescein diacetate. Water Res 32(9):2621–2626

Rupp CJ, Fux CA, Stoodley P (2005) Viscoelasticity of *Staphylococcus aureus* biofilms in response to fluid shear allows resistance to detachment and facilitates rolling migration. Appl Environ Microbiol 71(5):2175–2178

RutalaWA, Weber DJ (2001) New disinfection and sterilization methods. Emerging Infect Dis 7(2):348–353

Sanden G, Fields BS, Barbaree JM, Feeley JC (1989) Viability of *Legionella pneumophila* in chlorine free waters at elevated temperatures. Curr Microbiol 18:61–65

Sauer K, Camper AK (2001) Characterization of phenotypic changes in *Pseudomonas putida* in response to surface associated growth. J Bacteriol 183:6579–6589

Schaule G, Flemming HC, Ridgway HF (1993) Use of 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) for quantifying planktonic and sessile respiring bacteria in drinking water. Appl Environ Microbiol 59:3850–3857

Schulte S, Wingender J, Flemming HC (2005) Efficacy of biocides against biofilms. In: Paulus W (ed.) Directory of microbicides for the protection of materials and processes. Kluwer Academic, Doordrecht, pp. 90–120

Shimizu MK, Okuzumi A, Yoneyama T, Kunisada M, Araake H, Ogawa, Kimura S (2002) In vitro antiseptic susceptibility of clinical isolates from nosocomial infections. Dermatology 204(Suppl 1):21–27

Simoes M, Carvalho H, Pereira MO, Vieira MJ (2003) Studies on the behaviour of *Pseudomonas fluorescens* biofilms after ortho-phthalaldehyde treatment. Biofouling 19:151–157

Simoes M, Pereira MO, Vieira MJ (2005a) Action of a cationic surfactant on the activity and removal of bacterial biofilms formed under different flow regimes. Water Res 39:478–486

Simoes M, Pereira MO, Vieira MJ (2005b) Validation of respirometry as a short term method to assess the toxic effect of a biocide. Biofouling 47:217–223

Simoes M, Pereira MO, Machado I, Simoes LC, Vieira MJ (2006a) Comparitive antibacterial potential of selected aldehyde based biocides and surfactants against planktonic *Pseudomonas fluorescens*. J Ind Microbiol Biotech 33:741–749

Simoes M, Simoes LC, Machado I, Pereira MO, Vieira MJ (2006b) Control of flow generated biofilms with surfactants - Evidence of resistance and recovery. Inst Chem Eng 84(c4):338–345

Simoes LC, Simoes M, Oliveira R, Vieira M (2007) Potential of the adhesion of bacteria isolated from drinking water to materials. J Basic Microbiol 47:174–183

Simoes M, Simoes LC, Cleto S, Pereira MO, Vieira MJ (2008) The effects of a biocide and a surfactant on the detachment of *Pseudomonas fluorescens* from glass surfaces. Int J Food Microbiol 121:335–341

Smith AJ, Bagg J, Hood J (2001) Use of chlorine dioxide to disinfect dental unit waterlines. J Hosp Infect 49:285–288

Son H, Cho M, Kim J, Oh B, Chung H, Yoon J (2005) Enhanced disinfection efficiency of mechanically mixed oxidants with free chlorine. Water Res 39:721–727

States S, Kuchta J, Young W, Conley L, Ge J, Costello M, Dowling J, Wadowsky R (1998) Controlling *Legionella* using copper-silver ionization. J Am Water Works Assoc 90:122–129

Stoodley P, Boyle J, Cunningham AB, Dodds I, Lappin-Scott HM, Lewandowski Z (1999) Biofilm structure and influence on biofouling under laminar and turbulent flows In: Keevil CW, Godfree A, Holt D, Dow C (eds.) Biofilms in the aquatic environment. Royal Society of Chemistry Cambridge pp. 13–24

Surdeau N, Laurent-Maquin X, Bouthors S, Gelle MP (2006) Sensitivity of bacterial biofilms and planktonic cells to a new antimicrobial agent, Oxsil 320 N. J Hosp Infect 62:487–493

Suci PA, Vrany JD, Mittelman MW (1998) Investigation of interactions between antimicrobial agents and bacterial biofilms attenuated total reflection Fourier transform infrared spectroscopy. Biomaterials 19:327–339

Surman SB, Walker JT, Goddard DT, Morton LHG, Keevil CW, Weaver W, Skinner A, Hanson K, Caldwell D, Kurtze J (1996) Comparison of microscope techniques for the examination of biofilms. J Microbiol Method 2(5):57–70

Tamachkiarow A, Flemming H-C (2003) On-line monitoring of biofilm formation in a brewery water pipeline system with a fibre optical device (FOS). Water Sci Technol 47(5):19–24
Tachikawa M, Tezuka M, Morita M, Isogai K, Okada S (2005) Evaluation of some halogen biocides using a microbial biofilm systems. Water Res 39:4126–4132
Tsai YP (2006) Interaction of chlorine concentration and shear stress on chlorine consumption, biofilm growth rate and particle number. Bioresource Technol 97:1912–1919
US Environmental Protection Agency (US EPA) (1992) Control of biofilm growth in drinking water distribution systems. EPA/625/R-92/001. USEPA, Washington, DC
Verran J, Hissett T (1999) The effect of substratum surface defects upon retention of and biofilm formation by micro-organisms from potable water. In: Keevil CW, Godfree A, Holt D, Dow C (eds.) Biofilms in the aquatic environment. Royal Society of Chemistry Cambridge, pp. 25–33
Vieira MJ, Oliveira R, Melo LF, Pinheiro MM, van der Mei HC (1992) Biocolloids and biosurfaces. J Dispersion Sci Tech 13(4):437–445
Viera MR, Guiamet PS, de Mele MFL, Videla HA (1999) Use of dissolved ozone for controlling planktonic and sessile bacteria in industrial cooling systems. Int Biodeter Biodeg 44:201–207
Walker JT, Mackerness CW, Rogers J, Keevil CW (1995) Biofilm – a haven for waterborne pathogens. In: Lappin-Scott HM, Costerton JW (eds.) Microbial biofilms. Cambridge University Press, London, pp. 196–204
Walker JT, Morales M (1997) Evaluation of chlorine dioxide (ClO2) for the control of biofilms. Water Sci Technol 35 (11–12):319–323
Walker JT, Percival SL (2000) Control of biofouling in drinking water systems. In: Walker J, Surman S, Jass J (eds.) Industrial biofouling. Wiley, New York, pp. 55–76
Watson HE (1908) A note on the variation of the rate of disinfection with change in the concentration of the disinfectant. J Hygiene 8:536
Williams MM, Braun Howland EB (2003) Growth of *Escherichia coli* in model distribution system biofilm exposed to hypochlorous acid or monochloramine. Appl Environ Microbiol 5463–5271
Williams MM, Jorge W, Domingo S, Meckes MC (2005) Population diversity in model potable water biofilms receiving chlorine or chloramines residual. Biofouling 219 (5/6):279–288
Wetegrove RL, Banks RH, Hermiller MR (1997) Optical monitor for improved fouling control in cooling systems. J Cooling Tower Inst 18:52–59
White GC (1999) Handbook of chlorination and alternative disinfectants, 4th edn. Wiley, New York, p. 1568
Wood P, Jones M, Bhakoo M, Gilbert P (1996) A novel strategy for control of microbial biofilms through generation of biocide at the biofilm surface interface. Appl Environ Microbiol 2598–2602
Wingender J, Flemming HC (2004): Contamination potential of drinking water distribution network biofilms. Water Sci Technol 49: 277–285
Yohe TL, Donlan RM, Kyriss KK (1986) Sampling device for determining conditions on the interior surface of a water main. US patent no 4,631,961