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CHAPTER 8

The Control of Microbiological Problems*

Methods for controlling the microbiological problems include control of the contamination sources and control of microbiological populations (Blanco et al., 1996, 1997; Baurich et al., 1998; Bendt, 1971; Bennett, 1985). The treatment of fresh water and additives, control of the residence time in the storage tanks, and control of the stagnant flow areas should be done to control the contamination sources and the microbiological populations. Systematic maintenance and cleaning of the system by using different chemicals or treatments to reduce the formation of deposits and to eliminate the already formed deposits is required. A microbiological control program requires a good knowledge of the system and the main sources of contamination. Once the sources of contamination have been reduced, the paper manufacturer can control the microbiological populations of the system. Good housekeeping and regular inspection of all areas, effective boilouts, and regularly scheduled washup reduces slime development. To reduce the number of maintenance shutdowns in the paper machines, chemical products are introduced into the system to get rid of the microorganisms by preventing their growth or by reducing the harmful effects they produce. The chemical products most commonly used are dispersants and biocides. Other control methods are the enzymatic degradation of microbiological deposits and controlling the population by limiting nutrients (Gould, 1992, 2001; Geller, 1996; Blanco et al., 1997, 2011; Torres et al., 2011, 2012; Kanto Öqvist, 2008; Kiuru, 2011; Ullan, 2011). Joyce et al. (1980) reported that the operation of most paper mills would be virtually impossible without using chemicals to control slime formation. The desirable characteristics of slimicides, which include high toxicity and slow biodegradability, attracted regulatory attention. There is a need for green biocides derived from natural products that would be easily biodegradable. The desire for safe biocides that are green to the environment is a policy concern in the United States, Canada, and Europe. The strict US Environmental Protection Agency (USEPA) regulations may result in the removal of a large number of biocidal products that are incompatible with the environment (Treskonova and Wingenfeld, 2001).

8.1 Good Housekeeping

Good housekeeping is extremely important to deal with microbial problems (Kiuru, 2011). Thorough control of the whole-plant operations, including regular and sufficient cleaning and precise control of the delays in the use of easily spoiled materials, is of great importance

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(Blanco, 2003). Some conceptual tools to make running practices systematic and more controllable include for example good manufacturing practice and hazard analysis critical control points analysis. Basically these systems were developed for other purposes, but their principles and those of other quality-system-based approaches, can contribute to better process hygiene. This is because control of microorganisms is connected to the overall operational practices in the mill. One should also remember that the design of the equipment in mill will have important consequences in terms of microbiology (Blanco et al., 1996). Good housekeeping includes:

- Controlling the contaminants entering the process
- Performing regular boilouts of pipelines and tanks

It is much easier and significantly less expensive to keep a clean system clean than to stop a dirty system from getting dirtier. Even the best biocide program will be stressed to maintain microbial control of the process without the execution of good housekeeping practices. According to Woodward (2009), basic housekeeping starts with the standard "first in, first out" rule. To avoid long storage times, this should be basic rule for all the containers, particularly for the chemicals because they might be stored by the machine for weeks. The hoses should also be washed when filling the tanks and containers. Other good housekeeping practices include: cleaning tanks on a routine basis, sufficient agitation and recirculation, and at least annual system-wide boilouts. If the time and temperature in boilouts are limited, using some special chemicals or enzymes could be taken into consideration (Woodward, 2009). To some extent, good housekeeping can also be automated (Kiuru, 2011). On-line cleaning can be applied to essentially any position in the papermaking process. An electric-driven water pump can be used that uses the cleanest return water available from the process. The commodity items are the spray nozzles and the joint piping connections permitting ease of piping travel through elevation and direction changes to continuously clean, for example, the entire wire channel with a single flow. Parks (2007) has reported that this type of automated mechanical cleaning

- Reduces manual cleaning
- Reduces paper quality issues like spots and tears
- Reduces need for unexplained, unplanned refeed and restart exercises
- Reduces waste amount
- Improves paper quality
- Reduces biocide usage

8.2 Boilouts

Boilouts are widely used in the paper industry, particularly in fine paper mills (Lindvall, 2000; Kiuru, 2011). They are very effective at cleaning the internals, piping and headbox of the short whitewater circuit. Boilout emphasizes the use of high temperatures to clean

papermaking equipment. Steam is mostly used for the heating of solutions used for washups of the short loop of paper machine systems, but temperatures are normally in the range of 60–71 °C (Guillory, 1998; Guillory and Towery, 1998). Boilout involves treatment with an alkaline detergent solution, having a pH in the range of 10–13. But acidic solutions may also be used depending on the nature of the deposited materials. By using enzymes capable of breaking down the binder materials in a variety of deposits, the use of high or low pH solutions can be avoided (Anstey et al., 1998a,b). Paper mills periodically shut down operations and clean the wetted surfaces of a paper machine, usually with an emphasis on the short-loop recirculation system and wet-press section (Glazer, 1991; Guillory, 1998; Guillory and Towery, 1998). Papermaking efficiency and product quality often suffer if too much time elapses between such cleanups (Stitt, 1997). Schwamberger and Wormsbaker (2006) report the importance of well-planned boilouts and the advantages they offer. The boilout should include careful washing of the whole system and removal of any deposits or biofilms. The chemistry should be optimized during the boilout for optimal washing and successful startup. Careful washing without considering the chemical interactions might cause many serious problems, such as excessive foaming in startup and wrong pH levels. Boilouts can significantly increase the runnability and also improve the product quality (Schwamberger and Wormsbaker, 2006).

8.3 Biocides

Biocides are products used to control the growth of microbes and have become a necessary part of paper production as the modern process and the water supply conditions in most plants greatly promote the growth of microorganisms. Various biocides are used in the paper industry (Kanto Öqvist, 2008; Kiuru, 2011; Ullan, 2011; Blanco et al., 1997, 2002; Brattka, 1992; Bunnage and Schenker, 1995; Camp, 1989; Carvalho, 1978; Cloete and Brozel, 1991; Cloete and Gray, 1985; Eriksson et al., 1995; Farkas, 1990; Giatti, 1993; Goldstein, 1983; Haack et al., 1997; Himpler, 2001; Hootmann, 2002; Lindvall, 1998b; Lustenberger and Deuber, 1991; Mobius et al., 1986; Nelson, 1982; Paulus, 1993; Purvis and Tomlin, 1991; Rantakokko et al., 1994; Scharschmied, 1975; Schirch et al., 1993; Stomps, 1995; Van Haute, 2000; Weir et al., 1981; Kanto Öqvist, 2008; Kiuru, 2011; Kulkarni et al., 2003; Ullan, 2011; Lazonby, 1997; Barnes, 1984; Bigotte, 1979; Blanchard et al., 1987; Burka, 1993). Biocides act either by killing the microorganisms, which are known as a biocidal effect, or by inhibiting the growth of microorganisms, which is known as a biostatic effect. Properties an ideal biocide should contain are shown in Table 8.1. There are no biocides that can meet all the requirements, and none of the biocides is suitable for all applications. According to Edwards (1996), the selection of biocides must always be made site specifically. The essential parts of a successful biocide program are proper estimation of reduction in the microbial count and in the number of fungi.

Table 8.1: Properties of an ideal biocide

Performance
Be applicable over a wide range of operating conditions, such as pH and temperature
Not interfere with other paper mill additives
Have a broad spectrum of activity toward microbes
Be efficient and fast-acting
Be environmentally friendly and nontoxic (no organic solvents, heavy metals, dioxins or furans) and also safe for the operator
Be low cost and easy to handle
Be cost competitive
Composition
Free of organic solvents
No smell
Liquid (easy to handle)
Toxicity
High LD50 (i.e., low toxicity with respect to handling)
High LC50 (i.e., low toxicity with respect to effect on aquatic life)
Nonirritant
No influence on biological waste water treatment plants
Easily degradable

Based on Kiuru (2011).

Classification of industrial biocides based on their chemical structures is quite difficult (Allen, 2007). For industrial use, biocides are usually divided into two groups:

Oxidizing biocides

Oxidizing biocides are fast-killing and less costly in comparison to nonoxidizing biocides. The use of oxidizing biocides has continued to increase. An oxidizing biocide attacks microorganisms by oxidizing (an electron transfer reaction) the cell structure, disrupting nutrients from passing across the cell wall.

Nonoxidizing biocides

Nonoxidizing biocides work through various processes. These biocides interfere with reproduction, stop the respiration process, or break the cell wall. They are generally shot-fed to achieve a high enough concentration for a long enough period to kill the bacteria, algae, or fungi. Kill time generally requires several hours up to a day. In some cases, nonoxidizing biocides are found to be more effective and convenient than oxidizing biocides; therefore, both biocides are used together in many conditions such as cooling water systems. Selection of a nonoxidizing biocide depends upon many factors such as water pH, retention time, efficacy against various bacteria, fungus, and algae, biodegradability, toxicity, and compatibility with the other chemistry.

In some case, biocides have been classified on group basis and include a miscellaneous group that do not fit in any major class; sometimes biocides are also classified on the basis of their mode of action. This can be organized based on the target region of the microorganism affected by biocide action.

The types of biocides being used in the papermaking process is heavily regulated (Karsa and David, 2002). They are commonly known as slimicides and are used to reduce the buildup of slime deposits within the water phase of the process. Many changes have taken place over the past 2 decades in the paper industry. Most mills have now changed from acidic sizing processing to alkaline sizing and most of the mills have closed up their processing. In these mills, the water is now recycled within the plant and not discharged. Because of this, microbial contamination has shifted in favor of bacterial strains (because of alkaline conditions) and also increased because of the continuous reintroduction of these species and food sources back into the process. Proper use of biocides and also good plant hygiene procedures are critical for maintaining effective production and low microbiological population to prevent paper failure. The types of biocides commonly used in the papermaking process are shown in Table 8.2. The types of biocides used in the coating and additives for the papermaking process differ from the rest of the pulp and paper industry. These require longer lasting, sometimes up to 1 year's protection in storage tanks, and hence fall into the category of preservatives. The types of preservatives that are commonly encountered in the paper industry are thion, glutaraldehyde, 1,2-benzo-isothiazolin-3-one Bronopol, 1,2-dibromo-2,4-dicyanobutane, and 4,5-chloro-2-methy-4-isothiazolin-3-one.

The mechanisms of action of biocides can be divided into four broad categories: oxidants, electrophiles, lytic, and protonophores (Chapman, 2003). The oxidants—chlorine and peroxides—work directly via radical-mediated reactions to oxidize organic material (Schaechter and Lederberg, 2004; Clapp et al., 1994; Chapman, 2003; Dukan and Touati, 1996). The electrophilic agents include organic biocides such as formaldehyde and isothiazolones and inorganic ions such as silver, copper, and mercury. These biocides react covalently with cellular nucleophiles to inactivate enzymes (Collier et al., 1990; Slawson et al., 1992). They initiate the formation of intracellular free radicals that are responsible for their lethal action (Kimura and Nishioka, 1997; Thurman and Gerba, 1988). Cationic membrane–active biocides such as chlorhexidine and quaternary ammonium compounds and alcohols such as phenoxyethanol destabilize membranes, which result in rapid cell lysis (Broxton et al., 1983; Chawner and Gilbert, 1989; Gilbert et al., 1977). Weak acids, such as sorbic and benzoic acids, interfere with the ability of the cell membrane to maintain a proper pH balance that results in the accumulation of anions and cations inside the cell. Eklund (1985) reports that the inhibition of cell growth by preservatives is the result of different actions: disruption of membrane, inhibition of metabolic reactions, stress on intracellular pH, and the accumulation of toxic anions. Pyrithione is also classified as a weak protonophore (Ermolayeva and Sanders, 1995).

Biocides, both oxidizing and nonoxidizing, when applied properly can be effective in overall biofilm control. The oxidizing microbicides, such as chlorine, bromine, chlorine dioxide, peracetic acid, and ozone, can be extremely effective in destroying both the extracellular polymeric

Table 8.2A: Oxidizing biocides used today in paper industry

Oxidizing biocides
Ammonium bromide
Ammonium sulfate
Chlorine gas
Chlorine dioxide
Hydrogen peroxide
Halogenated alkylhaydantoin
Peracetic acid
Sodium hypochlorite
Sodium bromide

Based on McCoy (1983), Schrijver and Wirth (2007), Paulus (1993), Kitis (2004), Simons and da Silva (2005), Heitz et al. (1996), and Kanto Öqvist (2008).

Table 8.2B: Nonoxidizing biocides used today in paper industry

Nonoxidizing biocides
1,2-Benzisothiazolin-3-on
2-Bromo-2-nitropropane-1,3-diol
5-Chloro-2-methyl-4- isothiazolin-3-on
Diethyldithiocarbamate
N,N-Dimethyl-N,N-didecyl ammonium chloride, benzalkonium chloride
2,2-Dibromo-3- nitrilopropionamide
Glutaraldehyde
Methylene bisthiocyanate
2-Methyl-4-isothiazolin-3-on
Poly(hexamethylene biguanide) hydrochloride
Tetra-(hydroxymethyl)- phosphonium sulfate

Based on McCoy (1983), Schrijver and Wirth (2007), Paulus (1993), Kitis (2004), Simons and da Silva (2005), Heitz et al. (1996), and Kanto Öqvist (2008).

substances (EPS) and the bacterial cells. When using oxidizing microbicides, one must be sure to obtain a sufficient residual long enough to effectively oxidize the biofilm. Unfortunately, there are mills that are overly concerned with the corrosive nature of the oxidizing microbicides and fail to apply the needed residual oxidant required to control biofilm. Low residual oxidant levels are able to significantly reduce the planktonic counts but may not be sufficient to control biofilm. The level of oxidant and duration required is found to vary from system to system. Low-level free residual oxidant will kill planktonic bacteria, yeast, mold, and protozoa. However, medium to high dosages of free residual oxidant are needed to oxidize biofilms. Algal mats particularly require high dosages of free residual oxidant. It is generally more effective to maintain a higher residual for a duration of several hours than it is to continuously maintain a low residual. Continuous low-level feed may not achieve an oxidant concentration sufficient to oxidize the polysaccharide and expose the bacteria to the oxidant. Another misunderstanding is with the use of chlorine at alkaline pH (>8.0). It is often thought that chlorine is ineffective for controlling microorganisms at elevated

pH. This is not completely true. Surely, the hypohalous acid form of chlorine (HOCl) is more effective at killing cells than the hypohalite form (OCl–). However, the hypohalite is actually very effective at oxidizing the extracellular polysaccharide and the proteinaceous attachment structures. Therefore, using chlorine in alkaline cooling waters can still be tremendously effective when applied properly. This is especially true when combining chlorine with bromine or with a compatible nonoxidizing microbicide such as a polyquat. When this is done, one achieves both oxidation of the extracellular material and sufficient kill of the microorganisms. Bromine compounds, such as sodium bromide (NaBr), used to generate free residual hypobromous acid (HOBr) and organobromine biocides, are very effective oxidizing biocides. Bromine is extremely lethal to microbes, and the kinetics of the kill reactions is very quick. Certain nonoxidizing microbicides are also effective in controlling biofilm. Effective control is greatly dependent on the concentration of the product feed, frequency of addition, dosage fed, and resistance of the incumbent population to the product fed. Control cannot generally be achieved by once-a-week additions as is common in "full-service" applications. Typical application for effective control may include a slug addition of product two to five times a week. As with oxidizing microbicides, frequency and dosage will depend on the system conditions. It is generally most effective to alternate nonoxidizing microbicides at every addition to ensure broad spectrum control. Most nonoxidizing microbicides will have little effect in destroying the extracellular polysaccharide found in the biofilm. However, many of these microbicides may be able to penetrate and kill bacteria found within the biofilm, resulting in decreases in the population and weaknesses in the biofilm structure. Thus using the combination of nonoxidizing and oxidizing microbicides is a very effective method of controlling biofilm. When using a nonoxidizing microbicide in combination with an oxidizing agent, there should be a slight to no residual oxidant concentration present in the system at the time of addition. Sufficient time should be given for the nonoxidizing microbicide to work before resuming oxidant feed unless an oxidant compatible microbicide is being used. Using combination biocides has proven very successful in killing unwanted bacterial species. Application of synergistic biocides, in particular, can give improved biocide performance against harmful bacteria.

Biocides show different mechanisms of antimicrobial activity. Paulus (1993) has reported that glutaraldehyde reacts with amino and thiol groups in proteins, causing irreversible cross-links in the cellular constituents. Glutaraldehyde and oxidizing biocides are also effective against bacterial spores (Paulus, 1993). Maillard (2002) has reported that in gram-negative bacteria glutaraldehyde interacts principally with outer components of the cells, particularly lipoproteins. High degree of cross-linking means that the cells are unable to perform their important functions, resulting in a bactericidal effect. Methylene bisthiocyanate (MBT) chelates Fe3+ ions essential for the microbial growth (McCoy, 1983). BCDMH (1-bromo-3-chloro-5,5-dimethylhydantoin) is not found to be biologically active as such, but upon hydrolysis it yields hypobromic and hypochloric acids (Kemira Chemicals Oy, 2003). Isothiazolones (5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in a mixture) and 2,2-dibromo-3-nitrilopropionamide (DBNPA) are electrophilic active compounds. These react with cytoplasmic constituents such as thiol groups of proteins, and inhibit cellular metabolism (Paulus, 1993). Bronopol

(2-bromo-2-nitro-propane-1,3-diol) also contains an active halogen group, but can also release formaldehyde (Paulus, 1993). Dazomet (3,5-dimethyl-1,3,5-2H-tetrahydrothiadiazine-2-tion) is rapidly hydrolyzed in water to methylene isothiocyanate (Kemira Chemicals Oy, 2003), but also releases formaldehyde (Paulus, 1993). Biocides are usually toxic with low biological selectivity. Some biocides such has DBNPA, isothiazolone mixtures, glutaraldehyde, and MBT are also sensitizing (Kemira Chemicals Oy, 2003; Paulus, 1993; Pirttijärvi, 2000). Many of the presently used biocides such as peracetic acid, hydrogen peroxide, BCDMH, DBNPA, glutaraldehyde, or isothiazolone mixtures are reactive molecules that are quickly biodegraded to nontoxic molecules and so are not harmful for the biological wastewater treatment processes. These are also not persistent in the environment (Kemira Chemicals Oy, 2003; Paulus, 1993).

Bleached pulp grades very often involve a combination of treatments with oxidizing biocides, supplemented by toxic organic biocides. It is recommended to treat each of the incoming streams, including the freshwater, filler slurries, chemical additive, and make downstreams. More attention should be given to the starch preparation area because starch is a very good food for the growth of slime. The level of oxidizing agent has to be checked at an adequately low level that there are no problems with the bleaching of dyes or decomposition of starch, etc. A residual of 1 ppm of active oxidizing agent in the paper machine system can be considered a possible starting level. Hydrogen peroxide, when used as a biocide, acts slower but the effect is long-lasting. So, it should be controlled at a higher level of residual activity in the system. The selection of toxic organic biocides can be made based on the temperature of the system and on the relative needs to control bacterial or fungal growth. It is common practice to use the toxic biocide on and off over periods from several minutes to several hours. By this means, a required threshold of activity can be reached and also the cost of the chemicals can be reduced. Such practices should be checked to ensure they do not cause excessive savings in first-pass retention or other problems. Some biocides contain anionic dispersants that interfere with retention. The residual level of oxidizing chemicals is mostly estimated by measuring the redox potential of the furnish. This is done with a platinum(Pt) electrode relative to a standard reference electrode. The effectiveness of a biocide program is best assessed with a combination of measurements which include.

1. Petri-dish cultures of water
2. Tests for the presence of biological deposits as surfaces
3. Slipperiness of wetted surfaces
4. Level of smells within the facility

By the well-organized use of slimicides and preservatives, in many mills working with largely closed water circuits and continuous production of coated papers and boards has been made possible without any problem. There is no single preparation that can solve all the preservation problems occurring in the paper industry as different types of microorganisms have varying degree of resistance to biocides. Biocides should have high activity and cost-effectiveness. The properties demanded of biocides vary according to their specific field of application. Biocides should be used in concentrations that do not upset the papermaking processes, even if they are

added in huge doses. The products should go well together with the many auxiliaries used in papermaking. In the paper used for food packaging application, no substantial amount of biocides should be present in the final product and should possess low ecotoxicity. Filler suspensions and sizes and active ingredients for the antimicrobial finish of paper and board and preservatives for coating mixes, have to meet strict requirements with regard to the absence of odor and color, compatibility, and physiological harmlessness in their use concentrations.

8.3.1 Chlorine

Chlorine is the most widely used disinfectant in public and industrial water supplies, wastewaters, and has many household applications (Kiuru, 2011). Chlorine has been used as a disinfectant since 1846. Despite USEPA regulations to limit chlorine discharge because of toxicity and carcinogen concerns, chlorine continues to be a popular choice of biocide because it is both effective and economical.

Common forms of chlorine compounds are:

- Chlorine gas
- Calcium hypochlorite
- Sodium hypochlorite

Commercial sodium hypochlorite products may contain about 15% free available chlorine. Stability of a sodium hypochlorite solution is affected by concentration, light, pH, and temperature (Casson and Bess, 2003). Factors that negatively affect the strength of sodium hypochlorite solution are:

- High temperature
- Increased hypochlorite concentration
- Storage time

The pH in liquid hypochlorite products vary between 11 and 13. In basic solution, hypochlorite anion decomposes to produce chlorate anion, which is toxic. Transition metals catalyze the decomposition of hypochlorite. Storage conditions and handling of hypochlorite stock are very important for prevention of the losses during the storage period. White (1999) has reported that the most stable hypochlorite solutions are those of low concentrations (10%), with pH of 11, with metal contents of less than 0.5 mg/L, stored in darkness and at cool temperature. Chlorine gas reacts with water to form hypochlorous and hydrochloric acids that lead to reduced pH of the water. The latter determines the biocidal activity. This process takes place according to the following reaction:

$$Cl_2 + H_2O \rightarrow HOCl + HCl$$

Hydrochlorous acid is responsible for the oxidation reactions with the cytoplasm of microorganisms after diffusion through the cell walls. Chlorine that disturbs the production of adenosine triphosphate (ATP), which is an essential compound for the respiration of microorganisms. The bacteria that are present in the water will die as a result of being breathed in,

breathing problems are caused by the activity of the chlorine. The amount of chlorine that needs to be added for the control of bacterial growth is determined by the pH. The higher the pH, the more chlorine is needed to kill the unwanted bacteria in a water system. When the pH values are within a range of 8–9, 0.4 ppm of chlorine must be added. When the pH values are within a range of 9–10, 0.8 ppm of chlorine must be added.

Hypochlorous acid generated by hydrolysis of hypochlorite is the active agent of sodium hypochlorite and has a stronger bactericidal effect than hypochlorite anion. It is uncharged and small and so easily penetrates the bacterial cell membrane. Most of the biocidal activity is provided by hypochlorous acid at pH lower than 7 and higher than pH 8.0, biocidal efficacy is appreciably reduced because of production of hypochlorite anion. Deborde and Gunten (2008) report that the best biocidal activity of hypochlorite is between 6.5 and 7.5. Hypochlorite is less effective than chlorine.

8.3.2 Bromine

Recent environmental restrictions on the usage of chlorine and new alkaline-based chemical treatment programs have increased the application of bromine-based biocides. For effective microbiological control, bromine is always fed with chlorine, either as two separate products or synthesized as a bromo-chloro compound. Hypochlorous acid is required to oxidize the NaBr component and form the biocidal species HOBr. The biocidal efficiency of bromine is similar to that of chlorine (Kiuru, 2011). Bromine is added as a bromide salt and produced by the reaction with chlorine. NaBr must be used together with an activating agent such as chlorine gas, hypochlorite, or ozone because it is not a biocide itself. Elsmore (1995) reports that at pH 8.5, hypobromite has higher biocidal efficiency in comparison to hypochlorite when used at equal concentrations. Several commercially available forms of bromine are:

- Bromine chloride (BrCl)
- Bromine gas
- BCDMH and other brominated hydantoins
- NaBr or isocyanurate/sodium bromide blends
- DBNPA and stabilized bromine products

The most common compounds used in cooling water are BCDMH and mixtures of sodium bromide with sodium hypochlorite or chlorine gas.

Similar to chlorine, bromine hydrolyzes in water and produces HOBr, which has the same oxidizing power as HOCl. HOBr dissociates to form H^+ and OBr^-, but the reaction takes place at a higher pH than chlorine. A bromine solution with a pH of 8.5 will contain close to 60% HOBr, whereas a chlorine solution at the same pH would yield 10% HOCl. In many cases, a smaller dose of bromine will obtain the same microbiological control as using chlorine in a cooling tower system. Another advantage of bromine is that it reacts with

ammonia and other nitrogen compounds to form bromamines that, unlike chloramines, are effective biocides. Bromine is also less corrosive than chlorine to copper alloys. Bromine reacts with iron, manganese, sulfur, and organic matter. Heat and sunlight contribute to bromine demand, but there is less stripping because of lower volatility than chlorine. Its toxicity to aquatic life and possible formation of carcinogens is similar to chlorine and has therefore led to USEPA discharge regulations. Bromine residual can be analyzed using the same N,N′-diethyl-*p*- phenylene-diamine colorimetric method and reagents as chlorine testing as long as other oxidants are absent. Like chlorine, bromine testing should be done at the time of sampling. If a spectrophotometer does not have a bromine program, the result given on the chlorine program can be multiplied by 2.25 to obtain a bromine concentration. It is usually unnecessary to test for free bromine residuals because most combined forms of bromine, such as bromamines, are just as effective as free bromine. If testing for free bromine is required, it should be noted that full color development by the N,N′-diethyl-*p*- phenylene-diamine method will take 2–3 min for stabilized bromine products instead of the directed 30 s.

Hercules Pulp and Paper Division has launched Spectrum® Ammonium Bromide Technology that efficiently controls microorganisms in alkaline systems without the adverse side effects associated with strong oxidizing biocides (Davis and Casni, 2003). This biocide degrades into inert compounds before effluent discharge. It is produced on the site by mixing an ammonium bromide solution with sodium hypochlorite and mill fresh water. Dedicated blending and dosing effluent ensures safe, consistent production of the biocide. Table 8.3 shows the benefits of the new ammonium bromide-based biocide. This biocide is produced onsite using designated dosing equipment. The dosing equipment blends the ammonium bromide solution with sodium

Table 8.3: Benefits of the new ammonium bromide-based biocide

Extremely effective at reducing microbial populations (filamentous bacteria, unicellular bacteria, yeast and mold, and anaerobic bacteria)
Reduced sheet breaks
Reduced sheet defects
Increased time between boilouts
Reduced washups
Exhibits a low oxidizing potential
Reduced corrosion rates
Reduced consumption of costly wet-end additives
Does not damage felts
Reduced halogenated organic compounds
Is not consumed by organics, ammonia, or other compounds that typically act as demand on oxidizers
Prevents oxidizer overfeed, which keeps program costs affordable
Oxidizer residuals remain in system for longer time; improves microbiological population control
Residual is easily measured by total combined chlorine
Simple monitoring can optimize feed rates and prevent excessive program costs
Degrades readily into nontoxic ions
No negative effect on activated sludge plants

Based on Davis and Casni (2003).

Table 8.4: Benefits of ammonium bromide dosing system

Programmable logic controller ensures correct formation of the biocide and monitors for problems
Prevents unnecessary waste of biocide-producing chemicals
System performs automatic shutdown sequence if an interruption of water flow, sodium hypochlorite, or ammonium bromide occurs
Prevents unnecessary waste of biocide-producing chemicals
Final product concentration is 0.25–0.50%
The dilute biocide is not corrosive to skin and does not bleach clothing, which reduces worker exposure concerns
The equipment flushes the feed lines with water after a dosing cycle
No biocide remains in the lines when they are not in use, which reduces worker exposure concerns

Based on Davis and Casni (2003).

hypochlorite and mill freshwater under required reaction conditions to assure 100% conversion of the components. The dosing equipment also strictly controls the reaction to ensure that only the new biocide is produced. The major features and benefits of the dosing system are presented in Table 8.4. Commercial applications have verified the effectiveness of the new biocide at controlling microbial populations. The following case histories highlight some of the key benefits of this technology, which include reduced wet-end deposition, reduced breaks, and reduced papermaking additive usage. A mill producing printing and writing grades from 100% deinked pulp suffered from severe wet-end breaks because of microbiological deposition. To control this deposition, various biocides were dosed to multiple points as follows: sodium hypochlorite in the pulp chest and clear filtrate; HOBr in the short-loop whitewater; several organic biocides in thick-stock feed points; and a biodispersant in the silo. Even with extensive biocide usage, the machine experienced one to two costly breaks per day. An evaluation of the new biocide was recommended to alleviate this problem. Originally, biocide treatments were to be replaced by the new biocide in a stepwise fashion during the first 2 months of the evaluation. Within the first week of startup, the following benefits were observed with the new biocide:

- Total aerobic counts and bioactivity, as measured by ATP, were reduced by more than 99%.
- Alkyl ketene dimer sizing usage was reduced >15%.
- The number of breaks declined from one to two breaks per day to only one break in the entire first week of treatment.

At this point, the mill system was cleaner than any previous experience. These excellent results prompted the mill to replace the entire treatment program with the new biocide after the first week.

A machine making 950 metric tons per day of uncoated printing and writing paper was having problems controlling wet-end deposition. Its original program used an organic biocide to the silo (headbox loop) and broke chest of the machine. The amount of deposition on the machine was measured by an automatic online system based on weight of deposition on a stainless steel coupon. The new biocide replaced the organic biocide program at a similar

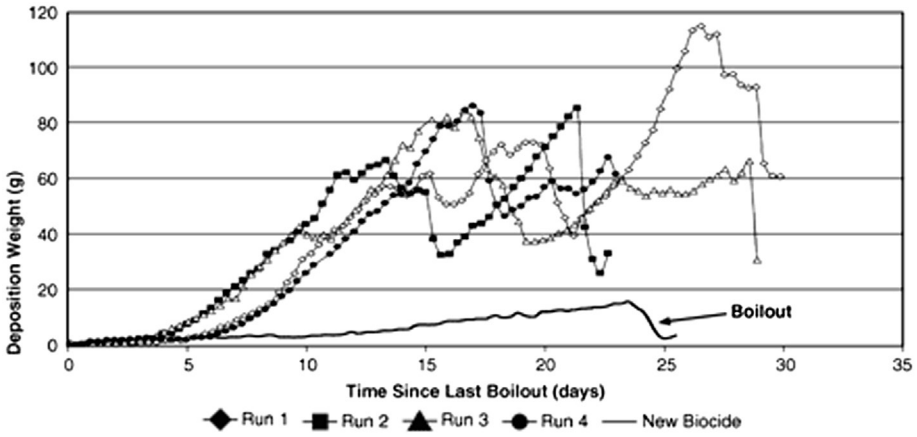

Figure 8.1
Deposition control with new biocide versus previous biocide treatment. *Davis and Casini (2003). Reproduced with permission.*

cost. Shortly after startup of the new program, the total bacterial counts dropped from 10,000,000 to 1000 colony-forming units per milliliter (cfu/mL). Furthermore, the amount of deposition, as measured by the on-line system, was reduced dramatically between boilouts (Figure 8.1). Another mill producing 200 tons per day of alkaline fine printing and writing paper was using a strong oxidizer—HOBr—to control biofilm. The HOBr was produced onsite by blending NaBr with sodium hypochlorite and then fed to the silo. An organic biocide treated the thick stock to control incoming microbiological contamination. The new biocide replaced the HOBr treatment and provided the following benefits:

- On-machine deposition was reduced.
- Sodium hypochlorite usage was reduced from 32.4 to 18.9 lb/h.
- Red and blue dye usage was reduced by 75%.

In addition to these benefits, the mill was able to completely shut off the fluorescent dye used at the size press because sheet brightness improved. Because of this success, the new biocide was then applied to an adjacent machine with the following results:

- Total bacterial counts were reduced from 230,000 to 2400 cfu/mL.
- Fluorescent dye usage was reduced at the size press by more than 80%.
- Sodium hypochlorite usage was reduced from 11.6 to 4.0 lb/h.
- Sheet brightness was increased by 2 points.

During this second evaluation, the mill realized that the original HOBr treatment had been reacting with iron in the system, which caused a brown shade in the sheet. When the treatment was switched to the new biocide, the iron was no longer being oxidized, which accounted for the increase in sheet brightness and reduced dye use.

Hootman (2002) has reported a biocide system for use in paper production. This system is effective against slime-forming and sulfate-reducing microorganisms as well as molds and algae. It is also based on the reaction of ammonium bromide and sodium hypochlorite. Unlike conventional biocides, the new system does not require limited-life stocks to be held. Instead, it is dosed into circuits at rates that can be varied for different chemical oxygen demand levels. The mill trials confirm wet-end biocide treatment improves runnability, reduces the incidence of holes and spots in finished paper, and reduces hydrogen sulfide levels. The trials covered closed loop production of unbleached liner from 100% waste paper and also mills producing high-quality bleached graphic papers from chemical pulp. The in situ biocide does not affect adsorbable organic halogen level and there is no accumulation of active biocide or of byproducts.

BromMax is patented single-feed stabilized liquid bromine biocide developed by a US company. It is based on active ingredients of NaBr and sodium hypochlorite and is used to control bacterial, algal, and fungal slime in paper mill process waters. It has the following features and benefits:

- Single-feed, preactivated solution
- Easy to handle and feed
- Enhanced stability over sodium hypochlorite bleach
- Effectiveness of bromine plus stability of nonoxidizing biocides
- Powerful biofilm removal properties
- Compatible with common scale and corrosion inhibitors

8.3.3 Chloramine

The chloramine is a mixture of monochloramine and dichloramine. It is more stable and is a weaker disinfectant than chlorine (Kiuru, 2011). Chloramine is not as reactive as chlorine with organic material in water. Therefore, it produces less disinfection byproducts. Chloramines provide better protection against regrowth of bacteria, which can be important for storage tanks and places with dead ends. But, the slow decay rates may result in higher biocide residues in the final product (Elsmore, 1995; Paulus, 2005).

Chloramines are produced by the reaction between chlorine (Cl_2) and ammonia (NH_3). Chloramines are amines that contain at least one chlorine atom, which is directly bound to nitrogen atoms. Inorganic chloramines are formed when dissolved chlorine and ammonia react. During this reaction, three different inorganic chloramines are produced:

- Monochloramine (NH_2Cl)
- Dichloramine ($NHCl_2$)
- Trichloramine (NCl_3).

Inorganic chloramines, free chlorine, and organic chloramines are chemically related and can change into one another easily. These compounds cannot be found in isolated form. Inorganic

chloramines are not persistent, but these compounds are more persistent than freely available chlorine compounds. Research has shown that depending on the circumstances, the half-lives of inorganic chloramines can vary from 1 min to 23 days.

Chloramines are normally produced by adding ammonia to water containing free chlorine (HOCl or OCl, depending on the pH). The ideal pH value for this reaction is 8.4. This means the water is slightly alkaline. Reaction mechanism is shown as follows:

$$NH_3\ (aq) + HOCI \rightarrow NH_2Cl + H_2O$$

When the reaction takes place, three kinds of inorganic chloramines can be formed. The pH value determines which kind of chloramines is formed. Trichloramines mainly form when the pH value is 3 or below. When the pH value is 7 or above, dichloramine concentrations are highest. The amounts of chlorine and ammonia in the water also influence the origination of chloramines. The chlorine/ammonia rate is ideally 6:1. During chloramine, the rate is usually 3–5:1. When ammonia concentrations are higher, more di- and trichloramines are formed. Organic chloramines can also be formed during these reactions. Organic chloramines cannot be distinguished from other chloramines, using standard chloramine analysis. Table 8.5 shows the properties of various chloramines. Figure 8.2 shows the structures of monochloramine, dichloramine, and trichloramine.

Keegan et al. (2010) did several experiments comparing vapor phase corrosion of different oxidizers using paper machine whitewater and steel plates of different grades. Table 8.6 gives the results from an experiment comparing the vapor phase corrosiveness of water, monochloramine (MCA), and monochloro-5,5-dimethylhydantoin (MCDMH), a partially halogenated hydantoin (Sweeny et al., 2002). Results in Table 8.6 show that MCA was substantially more corrosive in the vapor phase than MCDMH at similar dosage levels on the basis of total active

Table 8.5: Properties of various chloramines

Name	Molecular weight	Preferred pH value	Biocidal effect
Monochloramine NH_2Cl	52	>7	Good
Dichloramine NH_2Cl	85	4–7	Tolerable
Trichloramine NCl_2	119	1–3	Average
Organic chloramines RNHCL	Varies	Unknown	Bad

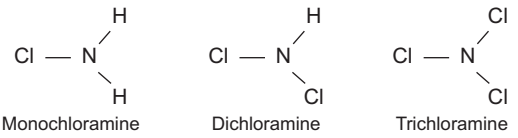

Figure 8.2
Structures of monochloramine, dichloramine, and trichloramine.

Table 8.6: Comparison of vapor phase corrosiveness of monochloramine (MCA) and monochloro-5,5-dimethylhydantoin (MCDMH) on EN10149-2 low carbon steel

Treatment	Dosage as total active chlorine (ppm)	Vapor phase corrosion of the steel coupons after 7 days	
Untreated reference	0	–	4
MCA	5	+++	21
MCDMH	5	–	7
MCA	10	++++	44
MCDMH	10	–	6

Laboratory experiment was performed with authentic paper machine water.
Based on Keegan et al. (2010).

Table 8.7: Halogenated hydantoins

Dichlorodimethylhydantoin	1,3-dichloro-5,5-dimethylhydantoin	$C_5H_6Cl_2N_2O_2$
Bromochlorodimethylhydantoin	1-Bromo-3-chloro-dimethylhydantoin	$C_5H_6BrCl_2N_2O_2$
Dichloroethylmethylhydontoin	1,3-dichloro-5-ethyl-5-methylhydantoin	$C_6H_8Cl_2N_2O_2$
Dibromodimethylhydantoin	1,3-dibromo-5,5-dimethylhydantoin	$C_5H_6Br_2N_2O_2$
Bromochlorodimethylhydantoin	1-bromo-3-chloro-5,5-dimethylhydantoin	$C_5H_6BrClN_2O_2$

chlorine. Tested concentrations of MCDMH did not show any significant difference from the untreated whitewater reference during the experimental period.

Kemira has applied for a patent on the dual use of MCA and MCDMH for corrosion safe microbe control on paper machines. The basis of this technique is to take the advantages of both chemistries along with advanced monitoring technique (PiBa assay) to provide the safest and economical treatment. MCA is added in broke system, save-all, and thick stock streams to lower the general activity of planktonic microbes in the system. MCDMH is added to the short circulation and press section showers. This combination is beneficial for wet-end stability, while at the same time minimizing MCA carryover to the dry section where vapor phase corrosion concerns are the highest. MCDMH is applied to control biofilm formation in a corrosion safe manner.

8.3.4 Halogen-Release Biocides

The halogen-release biocides are organic biocides. In contact with water, these biocides generate hypochlorous and/or HOBr. Hydantoins release chlorine and bromine. A few examples include: 1,3-dibromo-5,5-dimethylhydantoin, BCDMH, and 1,3-dichloro-5,5-dimethylhydantoin. Table 8.7 shows various halogenated hydantoins along with their chemical formula. Figure 8.3 shows the structures of various halogenated hydantoins.

Several hydantoin-based products are available for use in papermaking to control slime development (Syke, 2006). The major advantages of these compounds are their stability and

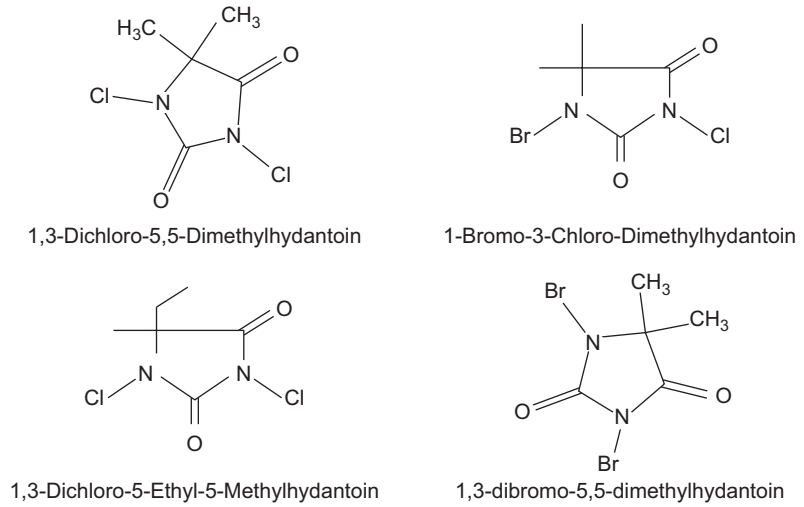

Figure 8.3
Structures of halohydantoins.

being adversely affected by organic matter even though at a lower degree than chlorine (Bloomfield and Miller, 1989). Halogenated hydantoins are used in water cooling tower disinfection, toilet bowl cleanser, and swimming pool and hot tub disinfections.

Interest in using combined halogens has been renewed and papermakers are currently using a number of combined halogen products for the control of microorganisms (Bruce, 2003). Over the past several years, a number of oxidant products have become available in the market (Bruce, 2003; Thomas, 1999). These products consist of halogens, bromine and/or chlorine, combined with an organic or inorganic carrier. One major advantage to combining the halogen is that it can often reduce the negative effect of the oxidant while maintaining its biocidal properties. The objective of combining a halogen with another molecule is to make halogen less aggressive but still biocidal. Bleach or chlorine gas with ammonia was mixed in the 1930s and 1940s to make chlorammonia for microbiological control in papermaking. Even though chloramine treatment was found to be more effective than chlorine, the chemistry was abandoned. The reasons for abandoning chloramine treatment were increased corrosion and increased microbiological activity resulting from the ammonia, which is a good source of nitrogen for bacteria. Later, bleach in combination with sulfamic acid (chlorosulfamate), was recommended as a biocide with low reactivity to process equipment and chemistries but the product was not successful because of the weak biocidal activity of chlorosulfamate. The various trade names often make it confusing to determine the number and nature of the products available.

Papermakers are presently using four types of chemistries:

- Hydantoin
- Sulfamate

- Ammonia/ammonium
- Isocyanurate

Hydantoin group: This group consists of bromine, chlorine, or both attached to a hydantoin molecule. Halohydantoins are not very stable in liquor form, so they are produced and sold as a solid product either in the form of powder, granules, or briquettes. Feeding this product requires the use of a powder feeder or a brominator that consists of a vessel with granules or briquettes. Water is flown through the vessel, which dissolves the product, and is sent to the process. Powder feeders work by making a slurry and delivering that slurry to the process. The product fully dissolves once in the process.

Sulfamate group: This group consists of bromine or chlorine attached to a sulfamate molecule. Halosulfamates can be produced as stable liquid products unlike the halohydantoins. Presently, only bromosulfamate is used for paper mill water treatment; chlorosulfamate is used in some cooling tower applications.

Ammonia/ammonium: This can be produced by mixing chlorine or bleach with ammonia or an ammonium salt. These two products can be premixed before application to the process water or can also be mixed in the process water. These types of haloamines cannot be produced as stable liquid products. A combination of bleach with ammonium bromide has been introduced as a way to produce a new haloamine oxidant.

Isocyanurate group: This consists of chlorine attached to an isocyanurate molecule, but NaBr may also be present, allowing formation of hypobromide. Halocyanurates are produced in solid form like halohydantoins.

Potential benefits of combined halogens are:

- Persistence
- Increased efficacy in high-oxidant demand systems
- Better slime penetration and removal
- Better compatibility with papermaking chemistries and with papermaking equipment

BCDMH is a cost-effective, fast-acting biocide. It is fully compatible with the conditions found in modern papermaking practices. It offers the paper manufacturer a very good solution to microbiological problems by beating the disadvantages of traditional nonoxidizing biocides. This product is found to be three times more effective on filamentous bacteria, which is selectively found in paper machine fresh water and slime deposits. It is a white crystalline compound slightly soluble in water having a melting point of 159–163 °C, 1,3-dichloro-5,5-dimethylhydantoin belongs to the family of imidazolone compounds (Rao et al., 2002). This compound shows low solubility in water, but part per million levels are enough to serve as a good disinfectant and bactericide because it slowly decomposes to produce free chlorine in water (Rao et al., 2002). After this process, the remaining compound (5,5-dimethylhydantoin) can be rapidly decomposed into ammonia and carbon

dioxide by light, oxygen, and microorganisms without leaving any environmentally polluting residues.

A particular combination of halogenated hydantoins has shown better efficacy than other halogen-based slimicides under both acidic and alkaline mill water conditions (Sweeny et al., 2002; Knapick e al., 2003). This combination includes 1-bromo-3-chlor-5,5-dimethyhydantoin, 1,3-dichloro-5,5-dimethylhydantoin, and 1,3-dichloro-5-ethyl-5-methylhydantoin. This combination of hydantoins is referred as BrMEH, for bromine methylethylhydantoin. Knapick et al. (2003) found that BrMEH increases whitewater efficacy in comparison to other oxidizing alternatives. BrMEH is rapidly converted to residual nonhalogenated hydantoin and a halide ion salt. Testing has also indicated that BrMEH hydrolyzes completely in water. Other halohydantoins follow similar pathways, and their degradation products are of a similar low toxicity. BrMEH is easier to handle than liquid oxidative biocides and does not require as much labor. An extensive study of a mid-Atlantic tissue mill resulted in several process recommendations, including an extended BrMEH trial. These trials showed that BrMEH effectively controlled aerobic and sulfate-reducing bacteria and maintained machine cleanliness and product quality. The improved effectiveness of the hydantoins can be explained by the formation of a moderately bound chlorohydantoin species in equilibrium with biocidally active hypochlorite. This equilibrium stabilizes active chlorine in a relatively unreacted combined form, thus releasing biocidally active hypochlorite on demand. So, active halogen lifetime is increased and biocidal efficacy is enhanced. This phenomenon accounts for the superior field results of the product where other inorganic and organic oxidants have not performed well. When the binding affinity of halogen is increased, halogen activity and release is further reduced, but biocide efficacy is also reduced because the release of the active hypochlorite is greatly inhibited. Thus, hydantoins provide an optimum balance of reactivity reduction and hypochlorite release, resulting in a very effective water system biocide for the paper industry. Also, the potential for total organic halogens to find their way into the effluent is reduced because the requirement for applied halogen is reduced. The possibility that the finished paper product will be affected is greatly reduced because less oxidant is used and there is less potential for system metals to corrode. The reduction in corrosion potential decreases any potential effect on the Yankee dryer coating and allows the biocide to be used closer to the wet-end of the machine. The environmental fate of any biocide is a main consideration of any biocide program. A study of papermaking biocides gave halohydantoins a favorable environmental profile. This conclusion was based on the rapid detoxification of active halogen species and the low toxicity of the unhalogenated residuals.

8.3.5 Chlorine Dioxide

Chlorine dioxide is an ideal biocide. It is found to be effective in the control of microbiological growths in paper mills under conditions unfavorable to chlorine. It is particularly effective in systems having a high pH, ammonia-nitrogen contamination, persistent slime problems, or where the microbial contamination is aggravated by contamination with vegetable or mineral

oils, phenols, or other high chlorine-demand producing compounds. Unlike chlorine, chlorine dioxide does not react with organic materials to form trihalomethanes. As a broad-spectrum, oxidizing biocide, chlorine dioxide generated is effective for use in controlling microbiological growth in whitewater paper mill systems. Although chlorine dioxide is nonreactive with ammonia-nitrogen, it may oxidize some sheet additives such as wet strength resins or retention aids. Chlorine dioxide is a strong oxidizing agent and is also widely used as an odor control agent (Baker, 1981; Giatti, 1993; Nelson, 1982; Anonymous, 1990b). It consists of one chlorine atom and two oxygen atoms (Figure 8.4). It is slowly becoming an important tool in disinfection and oxidation in the world today. Physical and chemical properties of chlorine dioxide presented in Tables 8.8 and 8.9 show its amazing capabilities. Chlorine dioxide does not constitute a risk against the environment. The Alliance for Environmental

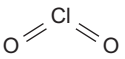

Figure 8.4
Structure of chlorine dioxide.

Table 8.8: Physical properties of chlorine dioxide

Molecular weight of 67.45
Gas at normal temperatures and pressures
Melting point of −59 °C
Boiling point of 11 °C
Yellowish/green and has an odor similar to that of chlorine
Denser than air and is water soluble at standard temperatures and pressures up to 2500 ppm
Explosive in air at concentrations >10%
Prohibited from all form of transport, it is normally generated at the point of application
Decompose in the presence of ultraviolet, high temperatures, and high alkalinity (>pH 12)

Table 8.9: Chemical properties of chlorine dioxide

Chlorine dioxide does not dissociate in water
Chlorine dioxide is an oxidant with a low redox potential
Chlorine dioxide has a few specific chemical reactions
Chlorine dioxide has a very high efficacy against vegetative cells, for example, bacteria, fungi, yeasts, and molds; viruses; algae; and protozoa. It has little to no effect on human, animal and fish cells. It has been shown to have high efficacy against molluscs and acracides with unconfirmed reports suggesting some action against nematodes
The low oxidation potential of chlorine dioxide means that it can penetrate biofilm and indeed chlorine dioxide has been proven as the most effective chemical against biofilm
Chlorine dioxide is a factor lower in dosage for the same efficacy against bacteria and fungi when compared against any other standard disinfectant like chlorine, iodine, bromine, hydrogen peroxide, quaternary ammonium compounds, glutaraldehyde, and phenolic and peroxyacetic acid formulations

Technology has indicated that the "environmental risks of a modern paper mill using chlorine dioxide are insignificant." Alliance for Environmental Technology is a group of 19 North American chemical manufacturers and forest product companies, established to promote proven and practical technologies to raise the environmental awareness.

Chlorine dioxide rapidly kills bacteria, viruses, and *Giardia*, and is also effective against *Cryptosporidium*. Chlorine dioxide also improves taste and odor, destroys sulfides, cyanides, and phenols, controls algae, and neutralizes iron and manganese ions. It is an effective biocide at concentrations as low as 0.1 ppm and over a wide pH range. It is 10 times more soluble in water than chlorine, even in cold water. Unlike iodine, chlorine dioxide has no adverse effects on thyroid function. Chlorine dioxide is widely used by municipal water treatment facilities. Chlorine dioxide is approved and recommended by USEPA as an environmentally friendly drinking water additive to replace chlorine. Chlorine dioxide has been called the "ideal" biocide for a number of reasons:

- It works against a wide variety of bacteria, yeasts, viruses, fungi, protozoa, spores, mold, mildews, and other microbes (Knapp and Bettisti, 2001).
- It exhibits rapid kill of target organisms, often in seconds.
- It is effective at low concentrations and over a wide pH range.
- It biodegrades in the environment.
- Unlike chlorine, it does not generate harmful by-products.

Chlorine dioxide works by penetrating bacteria cell walls and reacting with vital amino acids in the cytoplasm of the cell to kill the organism. The by-product of this reaction is chlorite, which is not known to pose significant environmental or human health risks.

Chlorine dioxide has a lower oxidation potential compared to ozone and chlorine. The optimal pH is between pH 6.0 and pH 10.0 and is generally more effective against microorganisms at pH above 8.0 than chlorine (Knapp and Bettisti, 2001). Chlorine dioxide is converted to chlorite, the predominant end-product (50–70%), and to chlorate and chloride in water (Baribeau et al., 2002). The major advantage of using chlorine dioxide is that it reacts less with ammonia as compared with chlorine. Though chlorine dioxide is the strong oxidizing agent, it is not very effective against established biofilms. For example, Jang et al. (2006) reported that when chlorine dioxide used at a concentration of 25 ppm, it failed to remove a biofilm thicker than 100 μm. Chlorine dioxide is used as a cellulose bleaching agent, for water disinfection at paper manufacturing plants public, and in water treatment facilities. Production of chlorine dioxide onsite can be carried out with Eka chlorine dioxide PurateR technology by Eka Chemicals Systems. Chlorine dioxide is produced from sodium chlorate, hydrogen peroxide, and sulfuric acid in Eka's SVP-PureR generator (Koepenick, 2010). Chlorine dioxide provides broad-spectrum kill of microorganisms. Processing equipment can be kept free of slime buildups by the fast killing rates of chlorine dioxide. Slime control program using chlorine dioxide improves the quality of paper products by reducing defects

such as specks, spots, and holes in the sheet. This reduces sheet breaks and avoids the subsequent production losses. Chlorine dioxide can be used for controlling odors. Chemical spot testing for chlorine dioxide at various points in the system is easily done and can be used to make adjustments in the treatment program to make up for demand changes. This allows slime control by chemical control. Most mills alternately apply several types of biocides to avoid developing resistance by certain troublesome microorganisms to a single product. This possibility is avoided with oxidizing type of chemical. It functions well over a broad pH range. This is important for the mills that operate paper machine at different pH levels, because of the different paper grades produced or because sizing changes have raised the pH in the papermaking processes. The performance of chlorine and some nonoxidizing biocides drop off in alkaline pH environment, but chlorine dioxide does not. The effective dosages of chlorine dioxide are usually low, making chlorine dioxide programs cost competitive with other biocides. Low dosage rates can result in cost reduction for effective slime control and significantly reduce the potential harmful effects to the environment from the mill effluent water. The MD Papéis' Santista mill, located in Cubatão, São Paulo State, Brazil, decided to switch from a monochloramine-based system. The mill produces 60,000 tons per year of printing and writing grades and flexible packaging. Because monochloramine is a persistent chemical and can be harmful to waste treatment, MD Papéis Santista began looking for a way to reduce the toxicity of their effluent. Purate provided the level of treatment efficiency desired without the persistency problems in the effluent, and also helped with paper machine runnability.

Several mills are running trials in the United States and look to be fully commercialized in the near future. A simple conversion to chlorine dioxide, delivered by a compact generator, addresses these issues, and consequently eliminates persistent deposits on forming fabrics, press felts, and equipment. Foul odors, increased calcium levels, and high conductivity can also be drastically reduced. According to Jim Anderson, Purate, Eka Chemicals' "compact chlorine dioxide" is the most cost-effective slime control option for paper machines. Typically, payback is less than 6 months because Eka Chemicals takes responsibility for installing and operating the generator. The initial cost for the customer is limited to polyvinyl chloride piping, electrical and distributed control system connections, including tote bin handling and containment. Figure 8.5 shows the principle of system for Eka Purate application.

The small-scale generator enables a chlorine dioxide supply from 0.5 kg/h up to 100 kg/h. This technology is widely employed for many applications throughout Europe and North America, including manganese/iron oxidation and disinfection of drinking water, microorganism control in effluent and cooling water, odor abatement in industrial water, replacement of sodium hypochlorite and chlorine in fresh water, and slime control on paper and board machines. Significant prevention of formation of slime on surfaces in the chlorine dioxide line reduced total aerobic count in the treated clear filtrate. The objective of a full-scale trial was to increase runnability on the paper machine by reducing microbiological activity, thus

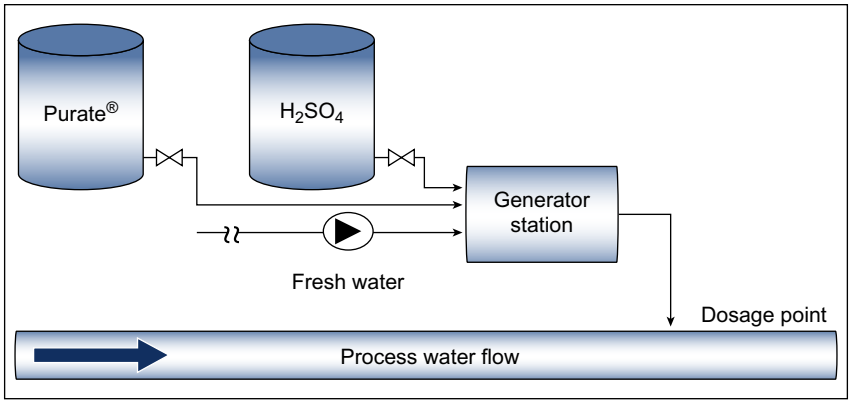

Figure 8.5
Principle of system for Eka Purate application. *Koepenick (2006). Reproduced with permission.*

reducing slime formation. To establish a clear picture of conditions in the water systems of the paper machine, a microbiological survey is always performed before startup.

One important parameter to measure is redox potential (in mV), which can be used as a control signal for chlorine dioxide dosing. Chlorine dioxide is normally added to one or several addition points in the long and short circulation systems (e.g., whitewater, clear filtrate, wire pit).

Early results of the full-scale trial demonstrated:

- Improved runnability
- A reduction in lower quality production
- Significantly reduced microbiological activity
- Significantly reduced formation of pink slime.

The results showed that chlorine dioxide could significantly limit biofilm and slime formation. Figure 8.6 shows results of purate treatment with water of linerboard machine.

Because chlorine dioxide is used for potable water disinfection, it is appropriate to use this versatile disinfectant in food-grade paper applications. Food-grade paper is required to meet higher microbial standards than fine paper. Therefore, the cost of microbiological control is considerably higher than for fine paper. This is because it is difficult to inactivate bacterial spores, particularly the genus *Bacillus*, which survives the extreme temperatures of the dryers in the papermaking process. Chlorine dioxide has been found to be a very effective sporicide in food-grade paper applications (Bendt, 1985; Conkey, 1981) in potable water applications (Ridenour et al., 1949; Sokolova et al., 1969), and in some food processing applications (Foegeding et al., 1986; Ito and Seeger, 1980). Unlike chlorine, chlorine dioxide is relatively nonreactive with most of the organics found in alkaline whitewater. As a result, a large portion of the chlorine dioxide fed will be available for disinfection. Thus the bacterial activity can be

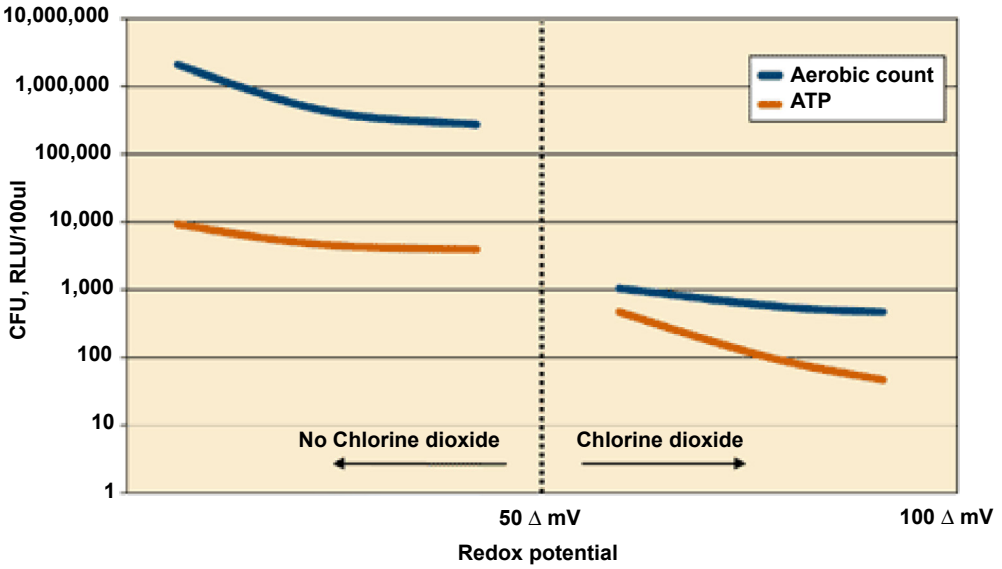

Figure 8.6
Results of purate treatment with water of linerboard machine. Aerobic count and ATP versus redox potential. *Koepenick (2006). Reproduced with permission.*

effectively reduced to almost any desired level by controlling the chlorine dioxide feed rate. A summary of the important benefits of chlorine dioxide is presented in Table 8.10.

The required dosages will vary with water conditions, the severity of contamination, and the degree of control desired. For control of bacterial slime, the required chlorine dioxide residual concentrations range between 0.1 and 5.0 mg/L. Chlorine dioxide may be applied either continuously or intermittently. The typical chlorine dioxide residual concentration range is 0.1–1.0 mg/L for continuous doses, and 0.1–5.0 mg/L for intermittent doses. The minimum acceptable residual concentration of chlorine dioxide is 0.1 mg/L for a minimum 1 min of contact time.

Chlorine dioxide is a gas produced by activating sodium chlorite with an oxidizing agent or an acid source. Sodium chlorite is converted to chlorine dioxide through a chlorine dioxide generator and applied as a dilute solution. Chlorine dioxide solutions should be applied to the processing system at a point, and in a manner, which permits proper mixing and uniform distribution. The feed point should be well below the water level to prevent volatilization of the chlorine dioxide. Coincident feeding of chlorine dioxide with lime or powdered activated carbon should be avoided.

8.3.6 Hydrogen Peroxide

Hydrogen peroxide is much less effective biocide than, for example, hypochlorite. Its chemical formula is H_2O_2 and its structure is shown in Figure 8.7. The bactericidal action of hydrogen peroxide is due to generation of hydroxyl radicals that oxidize thiol groups in proteins (Russell, 1998; Denyer and Stewart, 1998). Hydrogen peroxide produces oxygen from

Table 8.10: Benefits of chlorine dioxide

Chlorine dioxide is a very effective slime control agent.
Chlorine dioxide reacts rapidly and can be applied at a site immediately before the problem area, unlike many conventional antimicrobials, which are generally slow acting.
Chlorine dioxide remains relatively nonreactive with the vast majority of organics, reducing the dose rate necessary to achieve effective control.
Low dose rates result in typically low corrosion rates when compared to other oxidizers. In addition, minimizing or eliminating the slime layer reduces microbiologically influenced corrosion on equipment.
The chlorite ion (chlorine dioxide byproduct) keeps working as both a bacteriostat and slime control agent, even after the chlorine dioxide has reacted.
By effectively controlling slime growth, the frequency of boilouts can be reduced and the potential for unscheduled downtime because of paper breaks can be minimized.
Effectively controlling slime growth minimizes the hole count, maintaining the quality of the finished sheet.
Odors resulting from bacterial fermentation, phenols, sulfides, or mercaptans are virtually eliminated by use of chlorine dioxide.

Based on https://final-test.oxy.com/.../SodiumChlorite/Bacterial%20Slime%20Cont.

Figure 8.7
Structure of hydrogen peroxide.

solution when reacted with organic matter. It reacts with most materials including metals and so it is quickly consumed by organics and nonorganics. Some commercial products have additives that increase bactericidal action of the biocides. At low temperatures or low concentrations, hydrogen peroxide is not a powerful biocide but it exhibits a strong biostatic effect inhibiting growth of many microbiological species (Chiari et al., 1990; Rantakokko et al., 1994; Schirch et al., 1993). The biocidal preparation is found to increase with both temperature and concentration. It has been used to control anaerobic bacteria in the paper industry, as a sterilant for aseptic packaging for milk and also fruit juice containers. Hydrogen peroxide is often used in conjunction with peroxyacetic acid (PAA), existing in an equilibrium mixture designed to specific formulations to achieve the greatest effectiveness for paper industry applications. Hydrogen peroxide generates hydroxyl radicals (HO•), which is highly reactive and responsible for the antimicrobial action. It can attack membrane lipids, DNA, and other cell components. Catalase and peroxidase are enzymes produced in respiring cells to protect the cells from damage by steady-state levels of metabolically generated hydrogen peroxide. Hydrogen peroxide is effective between pH 2 and 10 and active against spores.

8.3.7 Peroxyacetic Acid

PAA is an extremely powerful, fast-acting biocide. PAA is a clear, colorless liquid with no foaming capability. It has a strong pungent acetic acid odor (acetic acid [AA] is the principal

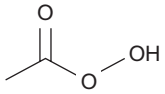

Figure 8.8
Structure of peracetic acid.

component of vinegar) and has an acidic pH of less than 2. It is soluble in water in all proportions and in polar organic solvents. However, it is slightly soluble in aromatic solvents. Peracetic acid or PAA is the peroxide of AA. Figure 8.8 shows the structure of PAA. It has long been used in sewage treatment and in the sugar, dairy, and brewery industries as well as for medical sterilization for renal dialysis machines. PAA is used widely for cold sterilization and disinfection. It is also effective for both drinking and wastewater treatment (Kitis, 2004; Rossi et al., 2007). PAA-based biocides are found to be effective for controlling microbial populations in papermaking process waters (Bjorklund, 2000; Maunuksela, 1995). Because PAA is used rather fast and does not leave any toxic residue, it can be an attractive biocide for mills that produce food-grade paperboard. Peracetic acid is rapidly tidal at low concentrations against a broad spectrum of microorganisms, including gram-positive and gram-negative bacteria, yeasts, molds, and algae under a wide variety of conditions. It is also effective against anaerobic and spore-forming bacteria. Peracetic acid is affective at killing biofilm microorganisms at low concentrations and short contact times. Unlike a number of other biocides, the biocidal activity of peracetic acid is not affected by pH or water hardness and biocidal activity is retained even in the presence of organic matter. For these reasons, peracetic acid is well-suited as a biocide in industrial cooling water and papermaking systems. Peracetic acid is compatible with additives commonly used in these systems. Although peracetic acid is a potent biocide, it is unique in that it does not produce toxic byproducts and its decomposition products, AA, water and oxygen, are innocuous and environmentally acceptable. PAA has a broad spectrum of activity over a wide temperature range. Once reacted, it breaks down to nontoxic end-products—water, oxygen, and AA—which itself breaks down to carbon dioxide and water.

PAA is a strong oxidant and disinfectant. Its oxidation potential is larger than that of chlorine or chlorine dioxide. PAA is commercially available in the form of a quaternary equilibrium mixture containing AA, hydrogen peroxide, PAA, and water as shown by the following equation (Kitis, 2004).

$$CH_3CO_2 + H_2O_2 \rightarrow CH_3CO_3H + H_2O$$

Where

CH_3CO_2H = acetic acid
CH_3CO_3H = peracetic acid
H_2O_2 = hydrogen peroxide

The products of PAA decomposition are AA, hydrogen peroxide, oxygen, and water. There are three reactions in which PAA is consumed in an aqueous solution:

- Spontaneous decomposition
- Hydrolysis
- Transition-metal catalyzed decomposition

In Finland, the PAA mixture Desirox has been successfully used to control microbial growth in waters of the paper mill process (Maunuksela, 1995).

Although it is claimed as an oxidizing biocide, the mode of activity is not merely oxidation, as the molecule penetrates the cell wall to give a greater effect than pure oxidation. There is also no known immunity to PAA, provided sufficiently high active levels are maintained. It is nonfoaming and can reduce chemical and biological oxygen demand in effluent.
The active ingredients can be easily monitored using proprietary electro-optical measuring equipment, giving parts per million concentrations within seconds. The chemistry does have some limitations. It is found to be not much effective on organisms with thicker cell walls such as filamentous bacteria and molds. Certain system chemistries—for instance, high levels of carbonate filler—can mean that higher dosage rates are required to effect control. The control program has been used successfully in UK's Paper New Thames Mill, Kent (Bhattacharjee and Farr, 1977). There are several different commercially available PAA-based biocides. It has rapid bactericidal activity against different vegetative organisms and spores (Baldry, 1983). The bactericidal effectiveness of PAA is affected by temperature and pH (Cords and Dychdala, 1993). The presence of organic compounds adversely affects the biocidal activity of PAA. PAA is effective over a broad pH range (pH 1.0–8.0); however, the optimum antimicrobial activity occurs in acidic environment. The activity of PAA is found to decrease at pH higher than 8 (Cords and Dychdala, 1993; Sanchez-Ruiz et al., 1995). AA present in high amount in PAA-based biocides may have a negative effect on the pH stability of a papermaking process. Another disadvantage associated with PAA disinfection is the increase of organic content and the potential microbial regrowth because of remaining AA (Kitis, 2004).

The sporicidal properties of peracetic acid, hydrogen peroxide, chlorine, and formaldehyde were compared by Alasri et al. (1993) in vitro using a dilution-neutralization micromethod. A combination of peracetic acid and hydrogen peroxide was also tested to assess their interactions. The activities of these agents, which are widely used as disinfectants, were evaluated against *Bacillus* spore isolates found on stored membranes and collection cultures. Peracetic acid and chlorine exhibited an excellent antimicrobial activity, with a destruction of 10^5 spores/mL after 5 min of contact. Generally the effects of the biocides tested were time-dependent. The sporicidal activities of hydrogen peroxide and formaldehyde were the lowest. The combination of peracetic acid and hydrogen peroxide, tested by a checkerboard micromethod, was found to be synergistic. The minimal sporicidal concentration (MSC) was

established in terms of time for each biocide. The lowest MSC values for peracetic acid, hydrogen peroxide, chlorine, and formaldehyde were:

Peracetic acid: 168–336 ppm (1–2 h of contact)
Hydrogen peroxide: 5625–11,250 ppm (5–7 h)
Chlorine: 168–336 ppm (2–3 h)
Formaldehyde: 1875–3750 ppm (5–30 min)

The MSC of a biocide combination of peracetic acid and hydrogen peroxide showed that synergy was maintained with increasing contact time and that the MSC could be reduced by two to eight times when compared with those of the biocides alone. Optimal concentrations and contact times of those chemicals that were promising in vitro were then tested for their ability to disinfect ultrafiltration membranes. The sporicidal activities of peroxide compounds and chlorine were confirmed and the synergism between peracetic acid and hydrogen peroxide was also maintained.

Aquabond Inc. Canada Spotless Sanitize is an effective biocide that uses the strong oxidizing properties of PAA. It is used to prevent biofilm or "slime" formation. In turn, it is a proactive odor eliminator generated by bacteria in paper mills. In addition, the effectiveness of Spotless Sanitize at low temperatures and over a wide pH range makes it an ideal bleaching agent for the pulp and paper industry. The resulting products reach and maintain their brightness goals without yellowing. Spotless Sanitize is an environmentally friendly alternative to aldehydes, bromium, organic sulfur, and quaternary ammonium biocides as well as chlorinated bleaches.

The hydrogen peroxide present reacts with the polysaccharide layer of the biofilm causing it to disrupt, at which point the peracetic acid will destroy the microorganisms present. Solvay's initial trials regarding the application of Proxitane involved the tandem addition of an organic biocide and hydrogen peroxide. The efficiency of that system led to the elimination of all organic biocides replacing them with Proxitane. The other drawback of the single use of most types of organic biocides is the possibility of immune strains of microorganisms developing in whitewater systems that would then require a multibiocide addition. The known wide-spectrum biocidal activity of Proxitane would overcome this. There is no known immunity to peracetic acid. Table 8.11 shows advantages of Proxitane.

8.3.8 Ozone

Ozone (O_3) trioxygen, is a triatomic molecule, consisting of three oxygen atoms (Figure 8.9) It acts as an oxidizer, much like chlorine or bromine, improving system turbidity and removing bioslime (Table 8.12). The biocide action of ozone is a result of its reaction with the double bonds of fatty acids of the bacterial cell wall and membrane. The application of ozone results in a change in bacterial cell permeability and a leakage of cells contents into solution. The action of ozone in water is immediate and after performing its action it reverts back to oxygen. It decomposes in solution producing hydroperoxyl, hydroxyl, and superoxide

Table 8.11: Advantages of proxitane

Effective against a broad spectrum of microorganisms
Operates over a wide temperature range
Removes biofilm
Controls odors
Removes sulfides
Nonfoaming
Reduction of residuals in whitewater system
Safe decomposition products
No disposal problems
Easy to use
Limited investment cost
Cost-effective system
Elimination of the need to clean the machine over a prolonged break

Based on www.solvaychemicals.com.

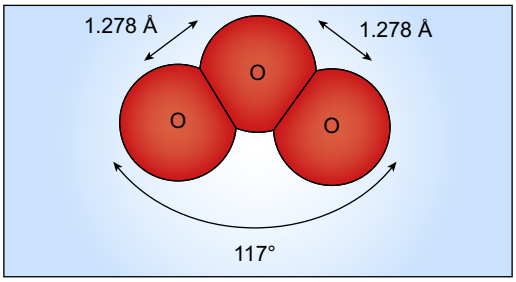

Figure 8.9
Structure of ozone molecule.

Table 8.12: Physical properties of ozone

Ozone is the strongest oxidant; has to be generated onsite because of short half-life
Solubility depends on temperature and ozone concentration in the gas phase
Reacts without residuals resulting in oxygen
Ozone works without formation of undesired byproducts
No formation of trihalomethanes
No formation of adsorbable organic halides

radicals. The reactivity of ozone is due to strong oxidizing power of these free radicals (Kim et al., 1999). The main advantage of ozone use consists of its superiority compared to chlorine for the main reasons that it has been reported to be 1.5 times stronger than chlorine and it is acting 3000 times faster than chlorine without producing harmful decomposition products. In 1995, ozone was declared as generally recognized as safe by the Food and Drug Administration for the treatment of bottled drinking water. Moreover, its generally recognized as safe

status was extended to food processing by experts some years later (Voidarou et al., 2007). Because of its high reactivity, it is required only in low concentrations. Similarly to all oxidizing biocides, ozone has some disadvantages like instability and high corrosion potential. Some factors such as temperature and pH affect solubility, stability, and reactivity of ozone. The pH significantly affects the stability of ozone in aqueous solutions. Stability of ozone in solution is the greatest at pH 5.0 and decreases as pH is increased (Kim et al., 1999). In the paper industry, ozone is mainly used for pulp bleaching, water disinfection, and as a final treatment of effluents. Korhonen and Tuhkanen (2000) found ozone to be very effective for controlling bacteria in recycled whitewater. Ozone was studied as a biocide to control microbial growth in a printing paper machine whitewater system from Stora Enso newsprint Varkaus Mills in Finland. Two samples (a cloudy discharge from a disc-filter save-all and a clear filtrate from the same save-all) were treated by ozone dose; about 80% of the aerobic heterotrophic bacteria in the disc save-all cloudy discharge and 90% removal of the aerobic heterotrophic bacteria in the clear filtrate were destroyed (Voidarou et al., 2007). The use of ultrasound with ozone is found to be of great interest because the use of ultrasound in conjunction with biocides offers a greener alternative. Ultrasound is presently employed in several industries such as surface cleaning, medical scanning ultrasonic therapy, food and beverage technology, materials science nanosynthesis (nanotechnology), mineral processing, industrial welding, nondestructive testing, and environmental. Though the energies required for ultrasonic disinfection alone are high there is now commercial equipment available using lower powers that is often combined with ozone (Eadaoin and Timothy, 2008). The ozonation costs are US$ 1–2/kg O_3 produced, depending on if the ozone equipment already exists. Then, a 99% destruction of the aerobic heterotrophic bacteria will cost from US$ 0.04–0.15 per m^3 whitewater treated. Ozone also functions as a micro flocculating agent to "polish" the water and improve clarity.

Ozone-treated water will maintain better heat transfer efficiencies through reduced biological fouling and increased water clarity. Ozone cannot be stored or transported like other industrial gases because it rapidly decays into diatomic oxygen and should be produced onsite. Because ozone is a short-lived gas molecule that is formed when oxygen reacts with other oxygen molecules to form three parts oxygen (O_3). This reaction requires energy. Ozone generators form ozone by passing dry, clean air through a high-voltage electric discharge (i.e., corona discharge), creating ozone at a concentration of approximately 1%. The corona discharge method is the most common type of ozone generation; these units usually work by means of a corona discharge tube. They are typically cost-effective and do not require an oxygen source other than the ambient air to produce ozone. Temperature and humidity plays a large role in how much ozone is being produced. The important parameter affecting ozone generation efficiency is the gas temperature, which is controlled by cooling water temperature and/or gas velocity. The ozone synthesis is better when the water is cooler. The lower the gas velocity, the higher the concentration, but the lower the net ozone produced. In typical industrial conditions, almost 90% of the effective power is released as heat and needs to be removed by a sufficient cooling water flow.

Because ozone is generated at and injected directly into the water stream, there are no containers of hazardous biocide that can leak, spill, or otherwise cause danger to employee safety. The oxidation power of ozone is actually greater than chlorine (bleach). Although ozone does not remove all minerals or particles, it is extremely effective at containing and eliminating costly microbiological growth, killing bacteria on contact 3100 times faster than chlorine. Ozone's short reaction time also makes it environmentally friendly. Very little residual is maintained within the system because the short-lived reaction takes place immediately after injection into the water stream. This allows for a cleaner and more environmentally friendly discharge to the environment and also an easier path to compliance with discharge permitting.

8.3.9 Glutaraldehyde (1,5-Pentanedial)

Glutaraldehyde is a broad-spectrum biocide and is found to be effective against bacteria, fungi, yeasts, molds, algae, and protozoa. It is extensively used as an antimicrobial agent in a variety of applications such as in cooling water systems, paper-pulp industry, oil field operation, leather tanning industry, poultry industry, cosmetic field, microbiological field, food industry, and the medical area. The extensive use of this biocide is due to being noncorrosive to stainless steel, soft metals, rubber, and glass (Banner, 1995; Herbert, 1995; Lutey, 1995; Walsh et al., 1999). Glutaraldehyde is useful in preventing the formation of slime in Lutey, 1980 all the areas of the papermaking process (Purvis and Tomlin, 1991). Glutaraldehyde is an amber-colored liquid usually supplied in solutions of acidic pH. As with other aldehydes, the two aldehyde groups react readily under suitable conditions, particularly with proteins. It is miscible with water and having melting and boiling points −14 °C and 187 °C, respectively. It is found to be readily biodegradable and is effective against the aerobic and anaerobic microorganisms including sulfate-reducing bacteria. It is found to be fully compatible with commonly used wet-end additives and significantly reduces the level of microorganisms in both acidic and alkaline systems. It shows more than 90% reduction at 25 ppm and essentially complete kill at 50 ppm in ASTM (American Society for Microbiology) paper slimicide test. It considerably reduces the amount of sulfate-reducing bacteria present in the solutions at any time point Figure 8.10 shows structure of glutaraldehyde. It achieves its biocidal activity by cross-linking the outer proteinaceous layers of the cell in such a way that cellular permeability is changed. The bacterial cell is unable to undertake most, if not all, of its essential functions. The ability of the outer covering of the cell to transport nutrients to the cell and to

Figure 8.10
Structure of 1,5-pentanedial (glutaraldehyde).

remove waste products from the cell is hampered and cell death results (Russell and Chopra, 1996; Simons et al., 2000). The cell walls of all living organisms contain free amine groups (lysine and arginine) that serve as the reactive site for glutaraldehyde attack. Complex cross-links are formed on the cell surface, and as essential cellular functions are disrupted, the cell dies. With increasing pH, more reactive sites for glutaraldehyde attack are formed, and the cidal reaction accelerates. Although glutaraldehyde kills most quickly at alkaline pH levels, it is still effective under acidic conditions. It is also effective against anaerobic bacteria, notably sulfate-reducing bacteria, because it is not inactivated by sulfide.

Glutaraldehyde was first synthesized by Harries and Tank in 1908 (Gorman and Scott, 1980). As with other aldehydes, the two aldehyde groups react readily under suitable conditions, particularly with proteins (Richards and Knowles, 1968). The ratio of monomer to polymer and type of polymer present has been the subjects of numerous publications. The dialdehyde existed as a monomer (25%) in equilibrium with the cyclic hemiacetal. It has been reported that the presence of free aldehyde groups is essential for biocidal activity. From an H-NMR study on commercial (aqueous) acid glutaraldehyde, it has been suggested that the protein cross-linking reactions are possible because of α, β-unsaturated aldehydes (Richards and Knowles, 1968). The pure acid glutaraldehyde underwent very rapid hydration on dissolution in water to give three hydrates in equilibrium. An acetal-like polymer similar to that suggested by Aso and Aito was also shown to exist in acid solution (Aso and Aito, 1962). A scheme depicting glutaraldehyde polymerization in acid and alkaline solutions has been suggested by Gorman and Scott (1980). An increase in temperature produces more free aldehyde in acid solution, whereas in alkaline solution loss of reactive aldehyde groups is possible. Progression to the higher polymeric form could occur with increased time and pH because it has been shown that there is an extensive loss of aldehyde groups from polymerization in alkaline solution (Bowes and Cater, 1966). Therefore loss of reactive aldehyde groups could be responsible for the rapid loss of biocidal activity of alkaline solutions in storage. Increased biocidal activity in heated acid solutions can also be explained by displacement of equilibrium toward the monomer. Glutaraldehyde is an agent that acts as a protein cross-linker and is used as a biocide. It is able to bridge amino acids or H- bonds, thereby modifying the folding of the proteins and stopping its activity (Gorman and Scott, 1980). It is thus likely to react and be consumed by wet-end additives that carry an amine function (Wolf and Sterner, 1972). The dialdehyde reacted with 30–50% of the E-NH_2 groups in the isolated peptidoglycan and it was proposed that two tripeptide side chains could be joined when free and amino groups are available (Hughes and Thurman, 1970). Cell wall peptidoglycan (murein, glycopeptide, mucopeptide) contains many chemical groupings capable of reaction with glutaraldehyde. The effect of lysozyme on the isolated wall peptidoglycan of *Bacillus subtilis*, was examined and it was found that although splitting of the lysozyme-sensitive bond occurred, glutaraldehyde-treated peptidoglycan was less sensitive than the untreated polymer to lysis by lysozyme (Hughes and Thurman, 1970). The effect of glutaraldehyde on different

microorganisms is presented in Table 8.13. The stability of glutaraldehyde is affected basically by pH and temperature as shown:

- Glutaraldehyde can be used very effectively up to pH 10. At very high pH (>10.5) glutaraldehyde is still effective, but a shortened half-life may necessitate more frequent dosing.
- Glutaraldehyde is efficacious through a broad temperature range; at higher temperatures glutaraldehyde works faster, although its half-life can be shortened.
- The optimum pH for glutaraldehyde, in terms of rate of efficacy and half-life, is in the range of 7–9, which encompasses most use conditions.
- Adverse storage conditions may impact product quality in a nonhazardous way.

Glutaraldehyde is supplied as follows:

- As an aqueous solution in concentrations ranging from 14% to 50% actives.
- As a blend with quaternary amines combining two unique biocides with two different modes of action to deliver synergy and even greater efficacy.
- As freeze-protected blends that offer high performance in extreme conditions.

Aqueous solutions of glutaraldehyde do not contain or require stabilizers, salts, or heavy metals, which may concentrate in closed systems. Table 8.14 shows the physical properties of 50% aqueous glutaraldehyde.

Table 8.13: Effect of glutaraldehyde on different microorganisms

Microorganism	Time for complete kill (hours)	ppm
Escherichia coli	1.0	50
Enterobacter aerogenes	1.5	45
Pseudomonas aeruginosa	1.0	25
Klebsiella pneumoniae	1.0	50
Staphylococcus aureus	1.0	50
Candida albicans	7.0	100

Control cfu for all organisms 10^5; pH = 7.0.
Based on www.prirodni-akvarium.cz/clanky/Glutaraldehyde.pdf.

Table 8.14: Physical properties of 50% aqueous glutaraldehyde

Specific gravity	1.129 ($H_2O = 1$)
Boiling point	100.5 °C
Freezing point	−21 °C
Vapor pressure at 20 °C	0.20 mm Hg (active ingredient)
Solubility in water	100%
Flash point	None
Exposure limits	0.1 ppmv ceiling (Union Carbide) 0.05 ppmv ceiling (ACGIH)

ACGIH, American Conference of Governmental Industrial Hygienists; ppmv, parts per million volume.
Based on www.prirodni-akvarium.cz/clanky/Glutaraldehyde.pdf.

Glutaraldehyde-based formulations are extremely effective in controlling the growth of unwanted organisms. However, the minimal impact of glutaraldehyde on the natural environment is just as important as its biocidal efficacy. Several studies have been conducted to determine the acute toxicity of glutaraldehyde to aquatic organisms, both fresh water and marine/estuarine. In acute toxicity studies of glutaraldehyde in three aquatic species, the no observable effect concentrations ranged from 2.5 to 0.029 mg/L (algae) to 9–24 mg/L (Daphnia magna) in freshwater and marine studies, respectively. In chronic studies, the no observable effect concentrations ranged from 0.31 mg/L (algae) to 4.25 mg/L (Daphnia magna). This suggests that the environmental toxicity of glutaraldehyde does not increase significantly with repeated exposure. Glutaraldehyde belongs to the aldehydes chemical class whose properties clearly differ. Unlike formaldehyde, all available long-term animal data clearly reveal that glutaraldehyde is not carcinogenic. Several regulatory and advisory agencies have set occupational exposure limits for glutaraldehyde. Users should ensure that any exposure does not exceed the limit applicable. However, a limit as such does not prevent the use of glutaraldehyde-based products in any application. Use as a high-level disinfectant on medical devices has led to cases of eye, nasal, respiratory, and skin irritation and dermal sensitization basically because of poor control of exposure following spills. In some cases, occupational asthma has been reported, although the available data do not suggest that exposure up to the limit value induces such effects. Products based on glutaraldehyde are effective against gram-positive and gram-negative bacteria, fungi, and a variety of viruses (including infectious bursal disease, porcine reproductive and respiratory syndrome virus, hog cholera virus, human corona virus, Newcastle disease virus, avian reovirus, avian rotavirus, and strains of avian influenza virus). This wide spectrum of biocidal activity supports the many diverse applications. Tables 8.15 and 8.16 show features and limitation of glutaraldehyde, respectively.

Protectol GA 50 biocide marketed by BSF is a 50% solution of glutaraldehyde. It is very effective against a broad spectrum of bacteria and fungi common to the papermaking industry and is useful in the prevention of slime buildup in all areas of the manufacturing process. Protectol GA 50 has a rapid speed of kill, is cost-effective, and easy to use. In paper processing, the principal benefit of Protectol GA 50 is that it can be used as a stand-alone product.

Table 8.15: Features of glutaraldehyde

Broad-spectrum efficacy
Quick kill under alkaline conditions
Highly effective against biofilm, sulfate-reducing bacteria and *Legionella*
Compatible with dispersants, surfactants and most WT chemicals, including CMIT/MIT
Compatible with halogens and other WT additives
Readily biodegradable at concentrations <5 ppm
Does not contain or release formaldehyde
Kills via cross-linking proteins in cell wall

Based on Mirrico seminar, Kazan, September 2011.

Table 8.16: Limitations of glutaraldehyde

Polymerizes under alkaline and high temperature conditions
Shows weak efficacy versus fungi and algae
Stability with ammonia and alkaline pH
Deactivated by bisulfites
Evaporation potential increases with temperature and/or aeration

Based on Mirrico seminar, Kazan, September 2011.

Table 8.17: Minimum inhibitory concentrations (MIC) of Protectol GA 50 biocide

Test organism	MIC (ppm)
Staphylococcus spp.	50
Bacillus spp.	1250
Desulfovibrio spp.	60
Pseudomonas spp.	150–250
Candida spp.	1250
Aspergillus spp.	475

*www2.basf.us/**biocides**/pdfs/PIB_Brochure.pdf.*

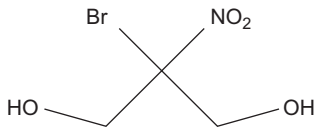

Figure 8.11
Structure of 2-bromo-2-nitropropane-1,3-diol (Bronopol).

It is extremely effective against slime-forming bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Micrococcus* spp. found in fouled papermaking systems. The ability of Protectol GA 50 to rapidly reduce the level of microorganisms present in a typical paper making process is excellent. The minimum inhibitory concentrations (MIC) of Protectol GA 50 biocide are presented in Table 8.17.

8.3.10 Bronopol (2-Bromo-2-nitropropane-1,3-diol)

Bronopol (2-bromo-2-nitropropane-1,3-diol) is a white crystalline odorless substance melting at about 130 °C. It is soluble in water, lower alcohols, AA, diethyl ether, and ethyl acetate, but weakly soluble in chloroform and acetone, and practically insoluble in hydrocarbon solvents (Legin, 1996). Figure 8.11 shows the structure of 2-bromo-2-nitropropane-1,3-diol (Bronopol). Its solubility values in some of the solvents are as follows (wt./vol.% at 22–25 °C):

- Water, 28
- Methyl alcohol, 89
- Ethyl alcohol, 56

- Isopropyl alcohol, 41
- Ethylene glycol, 61
- Methyl carbitol, 54
- 1,2-propylene glycol, 52
- Dipropylene glycol, 48
- Polyethylene glycol, 300
- Diethyl sebacinate, 10
- Isopropyl myristate, mineral oil, and vegetable oils, less than 0.5

Aqueous solutions of the pure compound have a pH of 5.0–5.5. This is explained by the mobility of hydroxyl hydrogen atoms. The compound in the solid state can be stored for 3 years and longer, and it is not affected by factors such as daylight, air humidity (up to relative humidity of 90%), and temperature (up to 45 °C) (Legin, 1996; Bryce et al., 1978). However, aqueous solutions of Bronopol are stable only in the cold, provided that the acidity is sufficiently high. An increase in the pH and temperature leads to decomposition of the compound. This is due to the splitting of formaldehyde. The initial process in the decomposition of Bronopol appears to be a retro-aldol reaction with the liberation of formaldehyde and the formation of bromo nitroethanol (Bryce et al., 1978). Bromonitro ethanol itself is significantly less stable than Bronopol and in the range of conditions investigated its maximal concentration did not exceed 0.5% of the initial Bronopol concentrations. Simultaneously a second-order reaction involving Bronopol and formaldehyde occurs to give 2-hydroxymethyl-2-nitro-1,3-propanediol. The antimicrobial activity of Bronopol is mostly because of the presence of electron deficient bromine atoms in the molecules, which shows oxidation properties rather than the ability to liberate formaldehyde (Legin, 1996). The mechanism of the antimicrobial effect of Bronopol consists of the cross-linking of sulfohydride groups of dehydrogenase enzymes occurring on the surface of microbial cells. The disulfide bridges block microbial metabolism.

BSF has launched Myacide AS biocide. It is the industrial grade of 2-bromo-2-nitropropane-1,3-diol or Bronopol. It provides highly effective antimicrobial activity for use in diverse and demanding industrial biocide applications, combining well-proven efficacy with important environmental safety characteristics. The main benefits of Myacide AS in the paper industry are its established performance as a slimicide active ingredient in mill process water and as an effective preservative for mill additives. The ideal physical and microbiological properties of Bronopol place it in a new generation of products, which can be seen replacing older chemistries. The product contains a minimum of 98% 2-bromo-2-nitropropane-1,3-diol. Bronopol is highly effective, particularly against aerobic slime-forming bacteria such as *Pseudomonas* spp., *Klebsiella* spp., *Bacillus* spp., and the *Enterobacter* spp. Table 8.18 compares the MIC of Bronopol against six key microorganisms. Figure 8.12 shows the efficacy of Bronopol in preserving a typical paper mill additive based on calcium carbonate. The procedure used was ASTM E 723-91 at pH 7.5 with a mixed inoculum of *P. aeruginosa*, a second (1,2-benzisothiazolin-3-one–resistant) *Pseudomonas* species, *Enterobacter cloacae*, and *Klebsiella* aerogenes. Myacide AS at a level of >20 ppm was able to control this mixed inoculum for 6 weeks following a single initial challenge

Table 8.18: Minimum inhibitory concentrations (MIC) of Bronopol

Test organism MIC (ppm)	MIC (ppm)
Staphylococcus spp.	12.5–50
Bacillus spp.	12.5–50
Desulfovibrio spp.	12.5–50
Pseudomonas spp.	12.5–50
Candida spp.	400
Aspergillus spp.	3200

*www2.basf.us/**biocides**/pdfs/PIB_Brochure.pdf.*

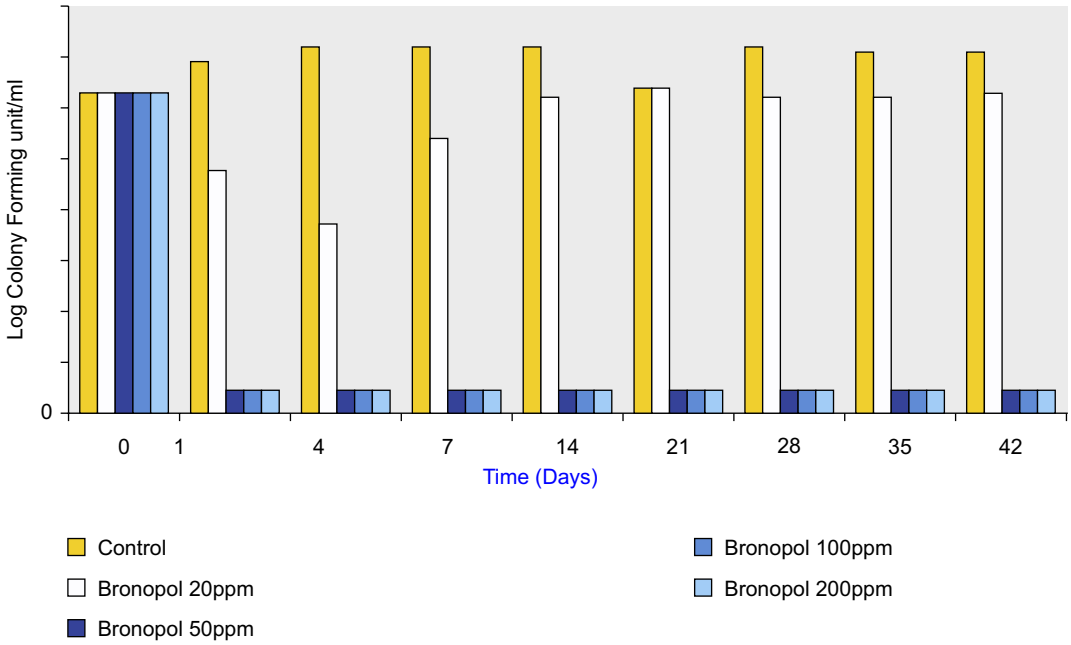

Figure 8.12
Efficacy of Bronopol in preserving a typical paper mill additive based on calcium carbonate. *Based on Specialty Chemicals by BASF, Paper industry biocides* www2.basf.us/biocides/pdfs/PIB_Brochure.pdf.

of 106 cfu/mL and a reinoculation at 21 days. 1,2-benzisothiazolin-3-one at levels of up to 200 ppm active was unable to control the *Pseudomonas* growth.

8.3.11 5-Chloro-2-Methyl-4-Isothiazolin-3-One/2-Methyl-4-Isothiazolin-3-One

5-chloro-2-methyl-4-isothiazolin-3-one (CMIT) and 2-methyl-4-isothiazolin-3-one (MIT) are active ingredients of Kathon WT biocides. These biocides are marketed by Dow Chemical Company. Figures 8.13 and 8.14 show the structures of CMIT and MIT. It is a high-performance paper mill slimicide with a broad spectrum of activity that can cope with the rapid changes in microbial flora that occur in different papermaking systems. It penetrates and kills

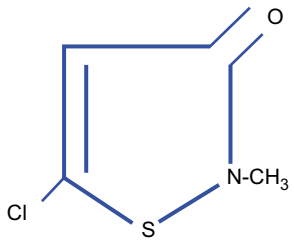

Figure 8.13
Structure of 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT).

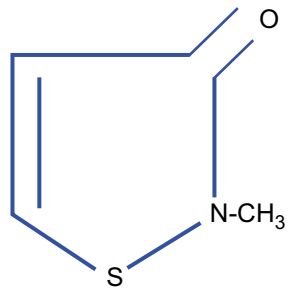

Figure 8.14
Structure of 2-methyl-4-isothiazolin-3-one (MIT).

microorganisms in the biofilm and is not inactivated by the high level of suspended organic solids found in paper mill water. It also provides cost-effective microbial control. Kathon WT has amber-gold color, is completely soluble in water, and has a mild odor and specific gravity of 1.32 at 20 °C. Kathon is stable over a wide range of conditions found in cooling water and paper mill applications. It is an extremely effective, broad-spectrum microbiocide that causes an immediate inhibition of growth on coming in contact with a microorganism. It is effective over a wide pH range and is therefore ideal for use in the alkaline conditions that exist in multicycle cooling towers and modern papermaking (Divkovic et al., 2005). It is found to be compatible with chlorine, corrosion, and scale inhibitors and most anionic, cationic, and nonionic formulations at normal-use levels. It causes immediate inhibition of growth on coming in contact with a microorganism. The growth inhibition rapidly becomes irreversible and results in cell death. Even before death occurs, the Kathon-treated organism is unable to synthesize degradative enzymes or the exopolymers that facilitate adhesion and biofilm formation. Growth inhibition rapidly becomes irreversible and results in cell death when essential proteins are progressively oxidized. It controls the wide variety of algae, bacteria, and fungi found in industrial water systems. Such a broad-spectrum product reduces inventory and handling costs, lowers operator training expenses, and reduces the risk of dosing error. Effective control of such a wide variety of microorganisms at levels as low as 1 ppm active ingredient provides an unrivalled and cost-effective treatment. It readily penetrates the surface of adhering biofilm to give effective control of sessile microorganisms. When diluted below use concentrations, it is readily

Table 8.19: Features and benefits of CMIT/MIT

Fast-acting
Provides immediate control
Broad-spectrum activity
Effective against bacteria, algae, and fungi. Effective versus *Legionella*, biofilm, sulfate-reducing bacteria
Stable over a wide range of pH and temperature
Effective under conditions typically encountered in most processes
Clear, water soluble liquid
Fully water soluble at use levels and easy to dose
Broad chemical compatibility
Compatible with most cooling water and paper mill additives and biocides
Low use rates
Cost-effective
Biodegradable and does not produce adsorbable organic halides or formaldehyde
Environmentally friendly

Based on Mirrico seminar, Kazan, September 2011.

Table 8.20: Limitation of CMIT/MIT

Poor stability above pH 9 and >40 °C
Poor stability with nucleophiles and reducing agents
Poor stability above pH 9 and at temperature higher than 40 °C
Perceived weakness versus sulfate-reducing bacteria
Slow killing
Safe handling concerns/sensitization/burns
New solid version will address safety issues

Based on Mirrico seminar, Kazan, September 2011.

biodegradable. Their decomposition does not lead to the presence of chlorinated organics in the environment. Tables 8.19 and 8.20 show features and benefits of CMIT/MIT.

Figure 8.15 presents a case history of biocide treatment in a newsprint mill where biocide addition was at the broke towers. Using carbamate, bacterial counts in the broke pulp were unacceptably high. After changing to a cost equivalent level of Kathon WT, bacterial counts in the broke were significantly reduced and downtime from contamination was minimized.

8.3.12 2,2-Dibromo-3-Nitrilopropionamide

DBNPA is a powerful biocide with two exceptional properties: it kills microorganisms immediately upon addition and it degrades rapidly (Exner et al., 1973). It is a white crystalline powder having melting point of 124.5 °C, water solubility 15,000 mg/L at 20 °C, and vapor pressure 9.00 E^{-4} mm Hg at 20 °C (Norstrom et al., 2009). Figure 8.16 shows the structure of DBNPA. It is intended for commercial use in pulp, paper, and paperboard mills; industrial cooling water systems; industrial air-washer systems; enhanced oil and gas

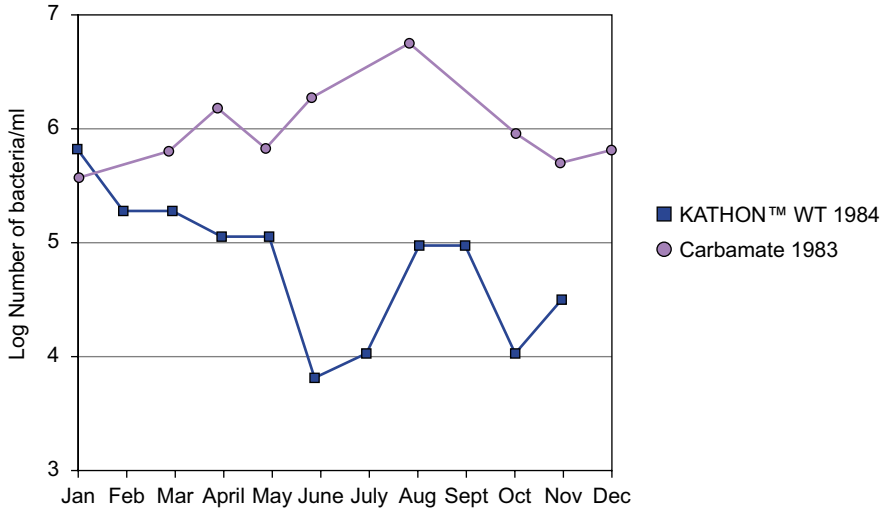

Figure 8.15
Comparative efficacy of KATHON WT and carbamate in a paper mill producing newsprint. *From KATHON™ WT water treatment microbicide. Reproduced with permission from the Dow Chemical Company.*

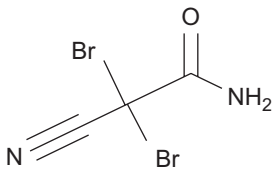

Figure 8.16
Structure of 2,2-dibromo-3-nitrilopropionamide (DBNPA).

recovery systems; metal-working fluid systems; and the paint and coatings industries. It can also be added to finished products as a preservative enhancer. It controls algae, bacteria, and fungal growth. DBNPA has low vapor pressure and high water solubility that makes the compound be retained mainly in the water phase. But, DBNPA has a short half-life and is rapidly degraded in water by hydrolysis. DBNPA is not an oxidizing biocide and is bromine releasing. DBNPA does act similarly to the typical halogen biocides. The liquid formulated DBNPA is an oxidizer, and the solid formulated DBNPA as a tablet is not an oxidizer. The liquid formulation is oxidizing because of the HOBr in the formulation. DBNPA readily degrades under alkaline conditions. It is sensitive to ultraviolet light and nucleophilic substances. It is uncharged and non–surface-active and it seems unlikely to interact with wet-end additives (Huber et al., 2010). DBNPA dissolves in water, forming a relatively stable solution in an acid pH range. Its unusual solubility and stability in polyethylene glycol (average molecular weight, 200 Da) make this glycol a preferred solvent. Aqueous solutions hydrolyze under alkaline conditions with the rate of decomposition increasing with the alkalinity.

However, the rate of hydrolysis is not fast enough to interfere with the antimicrobial activity of fresh, alkaline (pH 7–9.5) solutions (Wolf and Sterner, 1972).

Although DBNPA is compatible with many chemical classes, including oxidizing agents, it will react readily with nucleophilic agents and sulfur-containing reducing agents. The facile reaction of DBNPA with sulfur-containing nucleophiles common to microorganisms, such as glutathione or cysteine, is the basis of its mode of antimicrobial action. DBNPA is therefore not a typical oxidizing or halogen-releasing biocide. Unlike other thiol-reactive biocides, its action is such that thiol-based amino acids, like cysteine, are oxidized beyond the formation of disulfide species. This reaction irreversibly disrupts the function of cell-surface components, interrupting transport across cell membranes and inhibiting key biological functions. DBNPA degrades rapidly by both nucleophilic and hydrolytic pathways to relatively nontoxic products. The rate of hydrolysis of DBNPA is strongly pH-dependent: at pH 6.0 and 25 °C, the DBNPA molecule has a half-life of 155 h (about 6.5 days), but at pH 8.0 and 25 °C, its half-life is about 2 h. The ultimate degradation products of DBNPA are ammonia, carbon dioxide, and bromide ion. The mechanism for the environmental degradation of a DBNPA has been described by Exner et al. (1973). There are two competing pathways:

1. DBNPA ~ DBAM (dibromoacetamide)
2. DBNPA ~ CAM (cyanoacetamide).

The second pathway was defined as occurring in the presence of nucleophiles or sunlight. Table 8.21 and 8.22 show features and limitations of DBNPA. It can be considered rapidly degradable, would be removed by wastewater treatment facilities, and would not persist in the environment. It does not accumulate in the food chain. However, it is highly toxic (US classification)/very toxic (EU classification) to aquatic organisms on an acute basis.

8.3.13 2-n-Octyl-4-Isothiazolin-3-One or Kathon 893

OIT (2-n-octyl-4-isothiazolin-3-one) is marketed by Dow Chemical Company. It is a yellow liquid miscible in water and oil and stable in light and pH 9.5. OIT exhibits excellent fungistatic and fungicidal activity against fungi, including yeasts, mold, and gram-positive bacteria, and limited activity against gram-negative bacteria. OIT also belongs to the isothiazolinone group which in general all are electrophilic molecules containing an activated N-S bond that enables them to react with nucleophilic cell entities thus exerting an antimicrobial action (Alaxender, 2002). Figure 8.17 shows the structure of OIT. This biocide makes use of a two-step mechanism that involves rapid growth inhibition leading to a loss of cell viability. Growth inhibition is the result of rapid disruption of the central metabolic pathways of the cell by inhibition of several specific enzymes, including dehydrogenases. The essential enzymes that are affected are associated with the nutrient metabolism, Krebs cycle, and energy generation. The main physiological activities that are rapidly inhibited in microbial cells are respiration (oxygen consumption), energy generation (ATP synthesis), and growth (assimilation). Most of these major

Table 8.21: Features of DBNPA

Shows broad-spectrum efficacy
Extremely fast-acting
Effective at lower dose levels
Highly effective against biofilm and *Legionella*
Easy to dose liquid
Noncorrosive at in-use concentration
Low environmental impact
Short half-life at highly alkaline pH
Kills via disruption of respiration and metabolism; reactions with sulfydryls

Based on Mirrico seminar, Kazan, September 2011.

Table 8.22: Limitation of DBNPA

Limited shelf life (6 months)
Weak versus algae and fungi
Not compatible with strong nucleophiles and reducing agents
Low solubility in water
Not ultraviolet stable
Occasionally referred to as an oxidizer

Based on Mirrico seminar, Kazan, September 2011.

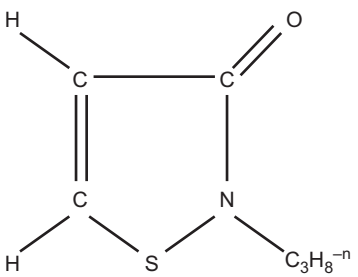

Figure 8.17
Structure of 2-n-octyl-4-Isothiazolin-3-one (OIT).

enzymes are present in both aerobic and anaerobic microorganisms, which shows the broad spectrum nature of this biocide. Inhibition of cellular activity and growth is rapid within minutes. However, cell death (cidal activity) is observed after several hours of contact. Generally, the higher the concentration of biocide, the shorter the contact time needed for more complete kill. Cell death results from the progressive loss of protein thiols in the cell from one of the multiple pathways. As cell metabolism is disturbed, free radicals are produced, which also results in cell death. This exceptional mechanism results in its broad spectrum activity.

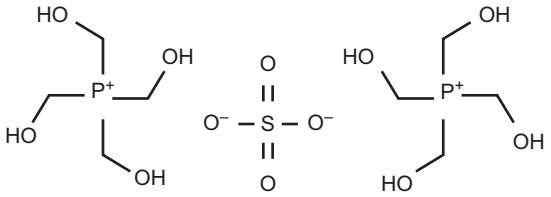

Figure 8.18
Structure of tetrakis (hydroxymethyl) phosphonium sulfate (THPS).

8.3.14 Tetrakis (Hydroxymethyl) Phosphonium Sulfate

The USEPA registered a new class of antimicrobial chemistry for use in papermaking. This is based on the biocidal molecule tetrakis hydroxymethyl phosphonium sulfate (THPS) (Hack et al., 1997). THPS biocides are classified by Department of Transportation as nonhazardous because they have very low acute toxicity in the environment. THPS is fully water soluble and a clear colorless liquid. It is found to be highly effective against sulfate-reducing bacteria having an odor that resembles aldehyde and is stable for 14 days at temperatures 21–54 °C (WHO, 2002). THPS is a quaternary phosphonium salt having the structure shown in Figure 8.18 (Haack and Downward, 1997). Aqueous solutions of THPS are acidic (pH 3.2) because of the small dissociation of THPS to tris(hydroxymethyl)phosphine, $P(CH_2OH)_3$, formaldehyde, and sulfuric acid (Thomas et al., 2007). THPS is also readily biodegradable and has no potential to bioaccumulate. Another environmental benefit is that THPS is rapidly oxidized in the environment to trishydroxymethylphosphine oxide (THPO), which has a very low aquatic toxicity and is not considered to present an environmental hazard (Haack and Downward, 1997). The following data show the low toxicity of THPO.

- Rainbow trout, 96 h LC50: >5000 mg/L
- Daphnia magna, 48 h EC50: >1000 mg/L
- Skeletonema costatum EC50: 2090 mg/L

Both THPS and THPO will also photodegrade in the environment. Based on these and other data, compared with conventional biocides, THPS offers genuine environmental benefits. Table 8.23 shows effect of THPS against *Enterobacter aerogenes* and sulfate-reducing bacteria.

THPS degrades rapidly on discharge to a molecule that is virtually nontoxic, thus reducing the risk of pollution and/or harm to biological effluent treatment plants. These biocides are fast-acting and are effective against sulfate-reducing bacteria and biofilms. The most exceptional property of THPS is its ability to combine broad spectrum antimicrobial effectiveness with a relatively benign human and environmental toxicity profile. The nonfoaming THPS molecule can be monitored on-site with a simple and rapid titration procedure to ensure proper dosing levels. Laboratory tests showed that up to 200 ppm of THPS formation could safely be dosed to the paper mill without adversely affecting the effluent treatment plant

Table 8.23: Effect of THPS against *Enterobacter aerogenes* and sulfate-reducing bacteria

Enterobacter aerogenes			
THPS concentration (ppm a.i.)	Surviving bacteria per milliliter after stated exposure time[a]		
	2 h	6 h	24 h
0 (control)	2.3×10^5	1.3×10^5	1.9×10^6
15	4.0×10^4	1.0×10^0	0
37.5	1.0×10^0	1.0×10^0	0
75	0	0	0
150	0	0	0

Sulfate reducing bacteria		
	Surviving sulfate reducing bacteria per milliliter after stated exposure time[b]	
THPS concentration (ppm a.i.)	6 h	24 h
0 (control)	8.8×10^6	1.4×10^7
10	1.1×10^5	1.4×10^7
25	2.0×10^0	0
50	0	0
100	0	0

[a]The initial bacterial level was 1.8×10^6.
[b]The initial SRB level was 2.0×10^6.
Based on Haack et al. (1997).

Table 8.24: Effect of THPS on activated sludge in the biological effluent treatment plant

THPS dose (ppm)	Oxygen demand (%O_2/min)
0 (control)	40
100	44
200	39
300	20

Based on Haack et al. (1997).

(Haack et al., 1997) (Table 8.24). This indication was confirmed during the plant trial, which demonstrated that microbial control could be achieved in the process at a dose rate of only 9.6 ppm THPS. Hydrogen sulfide levels were controlled at acceptable levels. There were no detrimental effects in the effluent treatment plant. Table 8.25 shows features and benefits of THPS and Table 8.26 shows limitation of THPS.

8.3.15 3.8 Dazomet (3,5-Dimethyltetrahydro-1,3,5-Thiadiazine-2-Thione)

Dazomet, a heterocyclic compound, is used as a slimicide in paper mills; a material preservative treatment for coatings, adhesives, epoxy flooring compounds, slurries, and high viscous suspensions; a biocide treatment used during petroleum operations; a biocide treatment to

Table 8.25: Features and benefits of THPS

Active against sulfate-reducing bacteria, algae, and *Legionella* Useful for a wide range of industrial applications
Broad-spectrum and fast-acting biocide Control of wide range of microorganisms
Dissolves iron sulfide Reduces iron sulfide related problems, fouling of equipment
Low dosages Cost-effective
Favorable aquatic toxicity Very low impact on ecology and minimal effect on environment
No organic solvents Safety in use; completely water miscible
Nonfoaming Easy to use in high-flow system

Based on Mirrico seminar, Kazan, September 2011.

Table 8.26: Limitation of THPS

Not compatible with oxidizing biocides
Cationic properties react with anionic inhibitors
Releases formaldehyde rapidly
Unstable at high pH
Issues with use of THPS in high calcium waters

Based on Mirrico seminar, Kazan, September 2011.

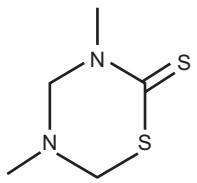

Figure 8.19
Structure of Dazomet.

recirculating cooling water systems; and a remedial wood treatment to utility poles. Dazomet is considered moderately toxic on an acute oral basis to both birds (lethal dose 50% [LD50] = 424 mg/kg) and mammals (LD50 = 415 mg/kg). It has a melting point of 104–105 °C, flash point 156 °C, water solubility <0.1 g/100 mL at 18 °C, and storage temperature 0–6 °C. Its structure is shown in Figure 8.19.

Dazomet is degraded by hydrolysis in an aqueous solution within a period of days (Chervenak et al., 2005). Dazomet, a biocide with good activity in principle, suffers in particular in products adjusted to an alkaline pH (e.g., talcum, calcium carbonate slurries) rapid

Table 8.27: Minimum inhibitory concentrations (MIC) of Dazomet biocide

Test organisms	MIC (ppm)	
	20 °C	40 °C
Staphylococcus aureus	500	3.9
Escherichia coli	500	62.5
Proteus mirabilis	250	15.6
Pseudomonas aeruginosa	250	31.3
Candida albicans	500	7.8

*www2.basf.us/**biocides**/pdfs/PIB_Brochure.pdf.*

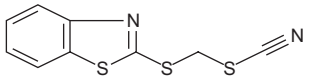

Figure 8.20
Structure of TCMTB (2-(thiocyanomethylthio)benzothiazole).

degradation with release of toxic and highly odorous gases (Sismanoglu et al., 2004). Dazomet is rapidly degraded in aqueous media to carbon disulfide, formaldehyde, and methylamine, with half-lives in the order of 3–20 days. However, in moist soil it degrades mainly to methyl isothiocyanate. It is unstable and decomposes to methylamine in water, probably via thiocarbamic acid. Multiple products are observed including methyl isocyanide, sulfur dioxide, hydrogen sulfide, N-methyl formamide methylamine, and carbonyl sulfide. Methyl isocyanide in turn degrades to methyl isocyanate (Ruzo, 1982).

Protectol DZ biocide marketed by BSF is the trade name for Dazomet. Within the pulp and paper industry, it is used both as a preservative for mill additives and as an active slimicide to control growth in the process water. It boasts excellent antimicrobial activity. Protectol DZ is particularly active against slime-forming organisms and sulfate-reducing bacteria, which can be responsible for corrosion. The most important benefits of Dazomet in the pulp and paper industry are its rapid speed of kill and broad-spectrum activity. Dazomet is extremely effective against slime-forming and spoilage organisms such as *Pseudomonas* spp., *Klebsiella* spp., *S. aureus*, *Aspergillus niger*, and *Candida albicans*. The MIC of Dazomet is displayed in Table 8.27.

8.3.16 2-(Thiocyanomethylthio)Benzothiazole

TCMTB (2-(thiocyanomethylthio)benzothiazole) is used as a slimicide and paper coating preservative for controlling bacteria, fungi, and yeasts that cause deterioration of paper and paperboard products and used to preserve paper-adhesive formulations. In its pure form, TCMTB is a white crystalline, whereas technical grade TCMTB is a black viscous liquid. Figure 8.20 shows the structure of TCMTB. It forms the active ingredient of the commercial fungicide Woodstate 30WBR. It has low aqueous solubility having boiling point: >120 °C,

melting point: <−10 °C, relative density (water = 1): 1.4, solubility in water, 100 g/mL: 0.0033 (Cserjesi and Johnson, 1982). The substance decomposes on heating in the presence of sulfide or when exposed to sunlight producing toxic fumes including hydrogen cyanide, nitrogen oxides, and sulfur oxides (Meneses et al., 2005). Hydrolysis and/or photolysis of TCMTB results in 2-MBT, which can photolyze to benzothiazole and 2-hydroxybenzothiazole or undergo biomethylation to 2-(methylthio)benzothiazole. TCMTB has the following properties.

- Stable at pH 5.0
- Slowly hydrolyzed at pH 7.0
- Rapidly hydrolyzed at pH 9.0

The major breakdown pathway of TCMTB results from photolysis (Nawrocki et al., 2005). TCMTB products are used in commercial/institutional premises and residential and public access areas. As an antimicrobial pesticide, TCMTB is used largely as a materials preservative (e.g., leather products and hides, pulp/paper products, latex, wallpaper, paints, carpets). TCMTB is a slimicide regulated by the Food and Drug Administration (21 CFR 176.300) for controlling bacteria, fungi, and yeasts, which cause deterioration of paper and paperboard products. TCMTB is also used as a fungicide for commercial and on-farm seed treatment. It is used as pulp/paper slimicide, paper-adhesive preservative, and in paper coating. It belongs to chemical family thiazole and its common name is TCMTB or TCMB; its empirical formula is C9H6N2S3; and another of its names is 2-(benzothiazolythio)methyl thiocyanate.

8.3.17 2-Bromo-4-Hydroxyacetophenone

BHAP (2-bromo-4-hydroxyacetophenone) is an organobromine compound and is a good biocide for bacterial slimes. Its common name is 2-bromo-4′-hydroxyacetophenone and its chemical name is ethanone 2-bromo-1-(4-hydroxyphenyl). There are three products—Busan 90, 93, and 1130—containing the active ingredient bromohydroxyacetophenone. Buckman Laboratories, Inc., manufactures these three products. They are used in the control of slime-forming bacteria, deterioration/spoilage bacteria, fungi, and slime-forming fungi in paper mills and water systems. They have also been used to inhibit the growth of bacteria and fungi that cause the microbiological degradation of papermaking chemicals. It is a reddish brown, viscous, odorless liquid, having a boiling point of 139.1 ± 0.7 °C at 737.9 mm Hg and solubility 0.248 ± 0.07 g/100 mL water. It is found to be stable under all storage conditions. At a pH 5.0, 7.0, and 9.0, BHAP has a hydrolytic half-life of 272, 250, and 173 h, respectively. Figure 8.21 shows the structure of BHAP. It is useful for situations requiring continuous or semicontinuous dosing at low levels, such as once-through cooling systems, where the dose rate is only 1–3 mg/L. It is supplied as a 30% active biocide. The dose rate for recirculating cooling systems is typically 10–20 mg/L, but could possibly reach up to 80 mg/L in fouled systems. Considerably higher dose rates are required for algal and fungal slimes. Because BHAP is not pH-dependent, it is effective at high pH levels. However, BHAP has a relatively long half-life, typically 175–250 h, which may affect its potential for cooling system bleed discharge. Other organobromine group products with similar biocidal mechanisms include:

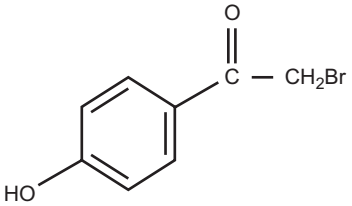

Figure 8.21
Structure of 2-bromo-4-hydroxyacetophenone (BHAP).

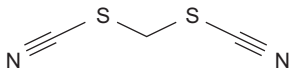

Figure 8.22
Structure of methylene bis(thiocyanate).

bisbromo acetyl butene and β-bromo-β-nitrostyrene. An example of BHAP is BRM10 from Buckman Laboratories, Inc. (Frayne, 2001). BHAP photodegrades in water with a half-life of less than 2 days. Also, BHAP's half-life in aerobic aquatic environments is 2.5 days.

8.3.18 Methylene Bis(Thiocyanate)

MBT is used as a biocide in a number of applications. Its major use is in water-cooling systems and paper mills as an inhibitor of algae, fungi, and bacteria. It is also used for preservation of paints, adhesives, synthetic polymer lattices, and latex emulsions; antimicrobial treatment of water-thinned products, thickeners, and slurries; preservation of wood and wood products; and preservation of leather and leather products and oil well brines and drilling mud. MBT is a yellow to light orange solid that melts at 105–107 °C. It has limited solubility in water (<1 mg/mL) but is soluble in organic solvents and therefore it is usually formulated with dispersants or supplied as a water slurry. Its molecular formula is $CH_2(SCN)_2$ and molecular weight is 130. Figure 8.22 shows the structure of MBT. It maintains long-term effects and is applicable to broad pH value and temperature ranges. MBT is pH-sensitive and rapidly hydrolyzes in the pH range above 8.0. For this reason, it is not recommended for use in systems where the recirculating water pH generally exceeds 8.0 (Mass-Diepeveen and Van Leeuwen, 1988). Not much is known about the mechanism of MBT responsible for biocidal activity. It has been suggested that it inhibits cell respiration. Because it is a competitive microbiocide, it inactivates the electron transfer cytochromes of the microorganisms. The thiocyanate fragment of the methylene ester of the thiocyanic acid reacts to blocking the transfer of electrons in the microorganism, which results in cell death.

8.3.19 Other Biocides

Patent WO 1999,043209 A1 by Buckman Laboratories (King et al., 1999) describes potentiation of biocide activity using a diethanolamide. In the method, at least one biocide and at least one

diethanolamide are applied to a substrate or aqueous system subject to the growth of microorganisms. The diethanolamide is applied in an amount effective to increase the biocidal activity of the biocide. Biocidal compositions are described where the biocide and the diethanolamide are present in a combined amount effective to control the growth of at least one microorganism. The combination of the biocide and the diethanolamide is particularly useful as a biocide in the leather industry, the lumber industry, the papermaking industry, the textile industry, the agricultural industry, and the coating industry as well as in industrial process waters.

Pereira et al. (2001) studied reduction of biofouling in paper production processes by using a carbamate-based biocide as a retention agent. This biocide was used to modify the surface properties of the microbial cells to promote their attachment to the cellulose fibers and to prevent the bacteria from developing biofilms on the equipment. About 75% of the cells were retained by the fibers. The effect of glutaraldehyde, another traditional biocide in pulp mills, was also tested and it was concluded that this biocide did not modify the surface charge of the bacteria. Contrary to the glutaraldehyde, the carbamate-based biocide modifies the surface electrical charge of the microbial cells, making them more positive. Therefore, this biocide may promote cell aggregation when the electrostatic repulsion becomes low or nonexistent (i.e., when the zeta potential is close to zero). This happens for carbamate concentrations of 100 mg/L and 200 mg/L, when the pH is 5.9, and for concentrations of 200 mg/L, when the pH is 6.7.

FennoClean performic acid (PFA) is a halogen-free biocide program from Kemira. It is highly effective against primary-biofilm forming bacteria. PFA is a reaction product of formic acid and hydrogen peroxide, and its biocidal activity is based on active oxygen. PFA is thus halogen-free, corrosion safe, and also fully biodegradable. This highly efficient biocide improves paper machine cleanliness while also not generating any persistent adsorbable organic halide compounds in wastewater that could be harmful for the environment. PFA also leaves no biocidal residuals in the paper product, making it safe for end-users and the environment. Kemira PFA technology is currently available in Europe only. It is supplied with Kemira's proprietary monitoring methods to enable accurate dosing based on true needs (e.g., PiBa Assay). Table 8.28 shows features and benefits of FennoClean PFA.

Table 8.28: Features and benefits of FennoClean performic acid (PFA)

Kills microorganisms within a very short contact time
Fully biodegrading to water and carbon dioxide
Effective microbe control, provides good runnability
Cleaner felts and extended felt lifetime, improved operations and profitability
Leaves no biocide residuals in the finished product, safe for end-users
No environmental concerns
Corrosion safety, reduces machine maintenance costs
Improved environmental footprint

Based on http://www.kemira.com/en/industries-applications/Pages/fennoclean-pfa.aspx.

Kurita Water Industries Japan has developed a biocide sold by the name Fuzzicide. It is produced by the reaction of two chemicals—an inorganic compound with no inherent antimicrobial activity and sodium hypochlorite. Among organic slime control agents commonly used in paper mills, current methods require raising dosing concentrations to enhance antimicrobial activity, bringing to the fore the issue of proportionate increases in environmental loads. Comparatively, newly released Fuzzicide is an innovative slime control agent with high resistance to bacteria, fungi, and yeast, assuring eco-friendliness and safety. This agent has been verified to have no effect on papermaking chemicals such as dyes. Kurita Water Industries has taken the initiative in incorporating Fuzzicide into approximately 40 machines centering on fine paper for 5 years since receiving its dealership in Japan from AY Labs, an Israeli venture firm. The benefits of Fuzzicide have since been substantiated. The company further aims to expand the use of Fuzzicide in neutral-pH paper machines—such as paperboard and newsprint machines that require slime control because of changes in paper types—in addition to fine paper machines. Fuzzicide features include availability in intended water systems with high organic matter content, low metal corrosivity, and no generation of toxic waste such as trihalomethane. Fuzzicide is not supplied in containers because of easy degradation and a lack of long-term stability. It requires the installation of dedicated dosing equipment near the intended system, and the agent can be added while being constantly generated in the equipment. The dosing equipment is outfitted with a computer to control the proportions and reactions of the above two chemicals when forming Fuzzicide and to add it in appropriate quantities. Sodium hypochlorite is commonly used for sterilizing tap water. However, it tends to assign priority to dissolved organic matter rather than reacting to bacteria. Thus it is seldom used in papermaking, a process with significant dissolved organic matter that decreases antimicrobial activity. Table 8.29 shows key features of Fuzzicide.

Mills producing food packaging board use a microbial deposit control program. A multi-ply Fourdrinier board machine running at 420 m/min was using a nonoxidizing microbial control program (Simons et al., 2003). A program was proposed combining a nonhalogenated oxidant with a nonoxidizing biocide. After optimization, the mill reported: spore counts in the finished paper reduced to low levels vital in food-packaging grades (average of more than 90% reduction); total aerobic microbial counts reduced to levels below mill goals (Figure 8.23); no taints, taste, or residual biocide in the final paper; improved production by 393 tons/month a cleaner machine; and improved production and provided a high-quality product. The mill also enjoyed an 854% return on investment. Another mill running under alkaline conditions at 1200 m/min producing 1440 tons/day of coated wood-free paper was experiencing runnability problems

Table 8.29: Key features of Fuzzicide

Superior bactericidal effect in a short time and in small concentrations
Effective against bacteria and a wide range of microorganisms such as fungi and yeast
Little consumption by dissolved organic matter, which furthers osmosis into slime and inhibits its growth
No rise in corrosion of metals such

Based on https://www.kurita.co.jp/english/aboutus/.

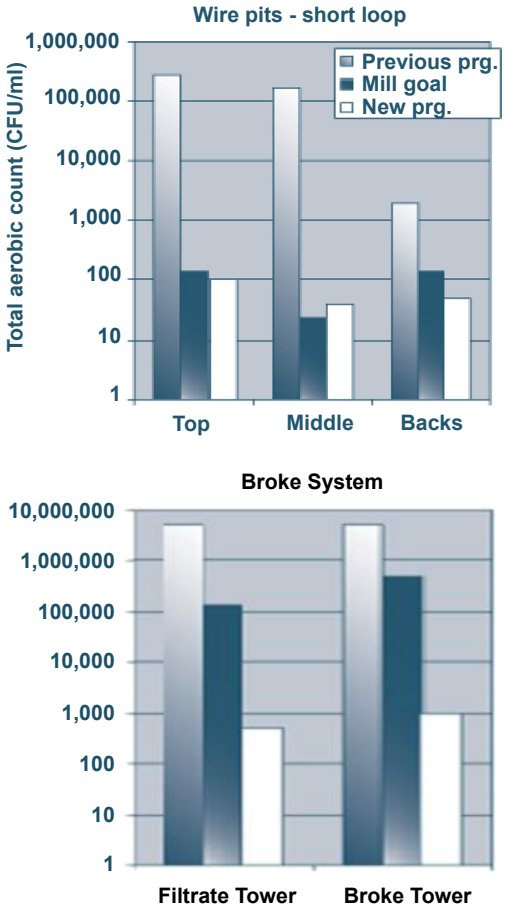

Figure 8.23
Food packaging board machine average total aerobic counts. *Simons et al. (2003). Reproduced with permission.*

because of slime buildup at the wet-end. The paper machine was using a nonoxidizing deposit control program based on MBT, DBNPA, and glutaraldehyde. The mill's fresh water was not microbiologically treated. A paper machine system survey (BioAudit) was conducted, including: understanding of system flows and volumes; system pH, temperature and oxidation-reduction potential measurements; plating studies to understand the system's microbial population; TRA-CIDE toxicity and bioactivity studies to find the best deposit control program. A program was proposed to the mill combining a nonhalogenated oxidant with a nonoxidizing biocide (Simons et al., 2003). The fresh water was brominated with Ondeo Nalco's ACTI-BROM to improve the control of filamentous bacteria and other microorganisms that mainly enter through this route. After startup and optimization on-site by performing TRA-CIDE cycle studies to achieve the correct feeding strategy and concentration, the mill reported the following benefits:

reel breaks reduced by 14%, from 0.53 to 0.46 breaks per day; paper holes reduced by 19%, from 2.36 to 1.89 holes per day; chemical oxygen demand(COD) decreased by 37%, from 628 to 396 mg O_2; and production increased by 3.6%, from 1152 to 1194 tons/month (Figure 8.24).

The comparisons of the performance of various biocides are presented in Table 8.30.

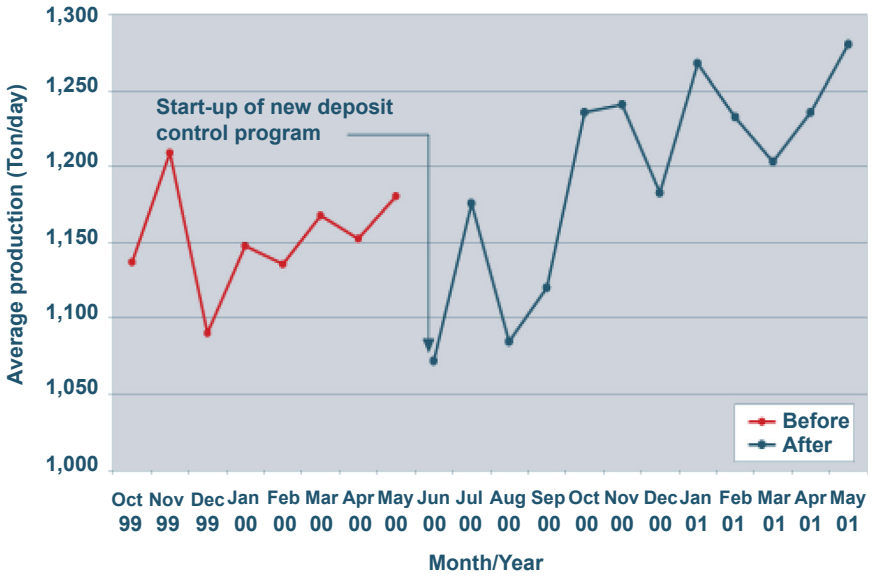

Figure 8.24
Coated wood-free paper machine monthly production counts. *Simons et al. (2003). Reproduced with permission.*

Table 8.30: Comparison of the performance of various biocides

	Oxidizing biocides (Cl_2/ HOCl BCDMH ClO_2)	THPS	Glutaraldehyde	DBNPA	CMIT/MIT
Persistence	No	Yes	Yes	No	Yes
Thermal resistance	Excellent	Good	Excellent	Medium	Medium
Rate of kill	Very, very fast	Fast	Fast	Very fast	Slow
pH	4–7.5	Up to 9.5	Up to 9.5	Up to 8.5	2 to 9
Microorganisms	A, B, F	A, B	A, B, (F) biofilm +++	A, B, F biofilm ++	B, F, Y, M
Biodegradability	NA	Inherently biodegradable	Very fast	Fast	Inherently biodegradable
FA release	No	Yes	No	No	No

Based on Mirrico seminar, Kazan, September 2011.

8.4 Enzyme Use

Increasingly restrictive environmental regulations make it necessary to take the environmental issues into account in the selection of a suitable treatment. Most of the biocides conventionally employed are hazardous substances. The use of these substances is regulated by legal requirements (N.N. Directive 98/8/EC, 1998; REACH, 2006), being a potential source of pollution problems in effluents and in the environment (Blanco et al., 1996; Blanco, 2003; Johnsrud, 1997, 2000; Schenker, 1996; Schenker and Gould, 1996; Schenker et al., 1998; Van Haute, 1999; Bott, 1998). The use of substances that reduce reliance on antimicrobial agents, such as enzymes also known as "green chemicals," would be an attractive strategy (Bajpai, 2012; Simoes et al., 2010). Enzymes have been evaluated by several researchers in both the laboratory and in paper process streams for control of microbiological slime deposits (Anonymous, 1984, 1990a; Anstey et al., 1998a,b; Fischer and Baurich, 1999; Freis, 1984; Galon, 1997; Gould, 1998; Hagelsieb et al., 1996; Hagelsieb et al., 1999; Hart, 2001; Jaquess, 1994; Kanto and Brutar, 1996; Kupfer and Baurich, 1999; Lindvall, 1998b; Siika-aho et al., 2000; Schuetz and Wollenweber, 1999; Van Haute, 1997b; Benard, 2010; Rivera and Jara, 2007; Loosvelt and Datweiler, 2007; Xu, 2005; Paice and Zhang, 2005; Buchert et al., 2004; Bajpai, 2012; Bajpai and Bajpai, 2001; Grant, 1998; Johnsrud, 1997; Torres et al., 2011, 2012; Schenker et al., 1997; Moor and Hatch, 1984; Patterson, 1986; Colasurdo and Wilton, 1988; Hatch and Moore, 1984; Augustin et al., 2004). Table 8.31 shows the characteristics and advantages of enzymatic "green" biocide.

Enzymes have been used for several years in many industries, but have had limited use and acceptance until recently in the pulp and paper industry. Some of the more common industrial enzymes are alpha amylases, cellulases, lipases, proteases, and xylanases. Enzymes contain polypeptide chains consisting of amino acids. The structure and function of enzymes are determined by the sequence of amino acids in the polypeptide chains. The molecular weights of enzymes range from around 10,000 to a million or more. The most

Table 8.31: Advantages of enzymatic biocide

Biodegradable product that does not pose any risk to mill workers or to the environment
Nonvolatile, nonreactive, and is stable during transportation
Totally replaces standard "chemical" biocides at paper mills
Shows bacteriostatic and bactericidal properties
Active against gram-positive and gram-negative bacteria
Has residual effect; it can be applied by shock loads
Does not allow bacterial strains to create microbial resistance
Eliminates biological slime in piping and equipment doing a permanent boilout
Reduces paper breaks at the PM and increases stability of the PM
Allows closure of mill water circuits without increasing water corrosivity
Reduces bad odors in the water circuits and final products

Based on Cotrino and Ordonez (2011).

important property of enzymes is their specificity. Enzymes can catalyze one particular reaction on a particular substrate without affecting other elements. The enzyme specificity can be pictured like a lock and key where polypeptide chains fold and arrange in such a way as to form a unique binding active site for the substrate. The chemical reaction occurs at this active site.

Enzymes may affect colonization and adhesion of microorganisms in following four different ways (Longhi et al., 2008; Oulahal et al., 2007; Kristensen et al., 2008):

- Firstly, they may attack the adhesiveness of settling organisms, thus preventing the settlement event
- Second, enzymes may degrade the polymers in the biofilm matrix formed by proliferating settled organisms
- Third, enzymes may catalyze the release of antifouling compounds from the surface. These compounds may be nontoxic or toxic, but they can be much less stable than conventional biocides, what should prevent the problem of bioaccumulation of harmful chemicals
- Last, the intercellular communication during colonization of a surface may be hindered by specific enzymes

The accepted theory behind enzymatic slime control is that the enzymes degrade the extracellular polysaccharides by cleaving a specific bond in the EPS and dissolve the slime. The cells are directly exposed to the biocides once the slime gets dissolved. Biocides are now effective at lower concentration. The slime is a fructan polymer and is broken down to monomers by carbohydrase type of enzymes. Enzymes involved in the degradations of fructans are hydrolases and transferases:

> Hydrolases: These are hydrolytic enzymes. According to their mode of action, they are either endo- or exo-enzymes. These enzymes produce oligofructans or only fructose.
> Transferases: These enzymes split off fructose dimers and give rise to difructose anhydride by simultaneous transfructosylation.

Levan is produced by different types of bacteria, most of which are attached to surfaces. Levanase enzymes from *Streptomyces*, *Bacillus*, and *Rhodotorula* have been purified. Their molecular weights are found to be 54, 135, and 39 KDC, respectively. Levanase from *Bacillus* sp. produces levan heptose as the predominant product. It begins forming when colonizing bacteria cement to a surface and creates a biofilm by covering themselves with layers of levan polysaccharides. This creates a protective niche that completely covers the cells. It eventually grows into the large, complex slime deposits by trapping fines, fibers, mat-forming organisms, and general debris that create productivity problems for paper mills. The enzyme of *Streptomyces* sp. No. 7-3 and *Streptomyces* exfoliatus F3-2 have been found to hydrolyze levan to produce levanbiose.

The enzymatic antifouling mechanisms include the following (Torres et al., 2012; Oulahal et al., 2007; Van Houdt and Michielis, 2005; Kristensen et al., 2008; Cordeiro and Werner, 2011):

- Cell lysis by degradation of components of the cell membrane
- Degradation of compounds anchoring cells to the surface of adhesives produced during settlement and anchorage and of the extracellular matrix secreted by proliferating adhered organisms
- Disruption of intercellular communication
- Quorum sensing (i.e., bacterial cell–cell communication)
- Degradation of environmental substances that are fundamental for the survival of the fouling organisms or generating antifouling compounds

Verhoef et al. (2005) report two major approaches to develop effective enzyme products for application in the paper industry. The first approach involves directly identifying the polysaccharides present in the slime and looking for specific enzymes able to degrade them. An example of this case is levan, which is produced by several species of *Bacillus* and *Pseudomonas* bacteria that can grow in paper machines, especially those manufacturing fine paper, where the level of inhibiting compounds is low. The enzyme levan hydrolase hydrolyzes levan to low-molecular-weight polymers that are water-soluble and therefore clean the slime out of the system. Another case where it was applied is a family of products called Darazyme, developed by Grace Dearborn's group; this work was based on the preliminary identification of slime components and the subsequent application of specific enzymes or combination of enzymes depending on the polysaccharides found (Bajpai, 1999). The other approach is indirectly testing to identify enzymes that show activity on the biofilm by studying their effects on slime development. The specificity in the way enzymes interact with the biofilm makes this a complex technique, being somewhat difficult to identify enzymes that are effective against different types of slimes. According to Simoes et al. (2010), formulations containing several types of enzymes seem to be fundamental when it comes to applying this slime control approach successfully.

The use of enzymes in the control of microbiological slime deposits has proved useful under modern mill conditions (Hatcher, 1983; Grussenmeyer and Wollenweber, 1992, 1993). EDC-I (enzymatic deposit control) is an enzyme patented by Economics Laboratory (Hatcher, 1973) that hydrolyzes and depolymerizes the fructose polysaccharide levan, which has been identified in paper mill slime (Colasurdo and Wilton, 1988). The product is environmentally safe and has no effect on the operation of activated sludge effluent plants. EDC-I has been successfully applied in systems with wide range of temperature and pH. It is used by paper mills in the United Kingdom, Scandinavia, Japan, and the United States. Applications have been on machines producing writing and printing, fine paper grades, and paperboards. This enzyme is produced during an aerobic fermentation of a common nonpathogenic, non–spore-forming soil bacterium. Personal safety is greatly enhanced through the use of an enzyme product

rather than conventional toxic biocides. The enzyme is nontoxic, does not release dangerous fumes and is not corrosive and also does not require any special handling. Spills can be flushed away with water. The exact period for which the enzyme is stable depends on a number of factors peculiar to any paper machine system, including the temperature and pH profiles as well as any other additive.

Leroy et al. (2008) and Molobela et al. (2010) have reported that proteases, and particularly serine proteases, are most efficient in removing *Pseudoalteromonas* and *Pseudomonas fluorescens* biofilms. Lequette et al. (2010) showed that serine proteases were efficient in removing *Bacillus cereus* biofilms, whereas glycosidases were efficient on *P. fluorescens* biofilms when used as a mix of α- and β-polysaccharidases. Marcato-Romain et al. (2012) characterized biofilms from the paper industry and evaluated the effectiveness of enzymatic treatments in reducing them. The EPS extracted from six industrial biofilms were studied. EPS were mainly proteins, the protein to polysaccharide ratio ranged from 1.3 to 8.6 depending on where the sampling point was situated in the papermaking process. Eight hydrolytic enzymes were screened on a 24-h multispecies biofilm. The enzymes were tested at various concentrations and contact durations. Glycosidases and lipases were inefficient or only slightly efficient for biofilm reduction, whereas proteases were more efficient: after treatment for 24 h with pepsin, Alcalase1, or Savinase1, the removal exceeded 80%. Savinase1 was tested on an industrial biofilm sample. This enzyme led to a significant release of proteins from the EPS matrix, indicating its potential efficiency on an industrial scale.

Torres et al. (2011) studied the effectiveness of 17 commercial enzymatic products on biofilm formed by the flora present in the process water obtained in the sheet forming zone of the wire section from a 100% recycling paper mill using mixed recovered paper as raw material and producing paper for board. The results showed that Pectinex Smash and its fraction Novoshape were the best formulations in the prevention of biofilm formation (Blanco et al., 2011). Pectinex Smash® has a mixture of pectinolitic activities and Novoshape is an enzymatic solution of a microbial pectin methylesterase. The gene encoding of the esterase enzyme is derived from fungus *Abdopus aculeatus* and is transferred into a strain of the food grade organism *Aspergillus oryzae* for commercial production. The Novoshape preparation has a declared activity of 10 PEU/mL and an optimal temperature of 50 °C. This enzyme belongs to the carbohydrate esterase family, catalyzes the hydrolysis of methyl ester groups, and has high specificity for pectin substrates, a property widely used in food industry and in plant science.

The combination treatment of enzyme and selected biocides has a substantial effect on cell survival. Ferris et al. (1989) reported that in all cases, the combination treatment resulted in significantly greater population reduction compared with the use of biocide alone at the same concentration. The enzyme alone showed no effects. Further, it was shown that on

application of enzyme (0.10 kg/metric ton) on machines producing printing grade paper, the biocide concentration could be reduced from 0.15 to 0.02 kg/metric ton (Ferris et al., 1989). The amount of biocide was reduced to ~15% of what had been used before using the enzyme. Hatcher (1983) also achieved a similar level of reduction in biocide concentration in the whitewater system of a paper mill when an enzyme preparation was used. Patterson (1986) has reported that with the use of enzyme, the concentration of biocide may be reduced by 50% and slime breaks are reduced from three per day to three per month. Colasurdo and Wilton (1988) reported that with the use of an enzyme preparation at Sonoco Products Co., Hartsville, Georgia, the slime breaks were almost eliminated which resulted in increased productivity. The Levanase enzyme produced by *Rhodotorula* sp. has been found to reduce the required biocide concentration by 25% without negatively affecting the paper properties (Chaudhary, 1992; Chaudhary et al., 1998). In spite of the strong indications that exopolysaccharides are involved in adhesion, enzyme treatment has shown that proteases are more effective than carbohydrate-degrading enzymes in removing bacteria from surfaces.

Microorganisms have cell walls, which give rigidity. If the cell wall is removed in some manner, the cell usually dies because of lysis, which results from osmotic balance. In microorganisms, the cell walls contain substances like cellulose, chitin, mucopeptide, and β-1,3-glucan. In addition to these, many microorganisms have capsules, slime layers, or other surface components, which are polysaccharides or proteins attached to the outside of the rigid layer conferring additional rigidity and/or protection. There are enzymes which will attack all of these polymers. The use of lytic enzymes with gluconase and protease activity in paper machine process water to kill microorganisms causing precipitates and/or forming slime and/or adversely affecting product quality, has been promoted. Enzymatic dispersion of biological slimes has been studied with pentosanase-hexosanase enzyme, Rhodozyme HP-150. This enzyme is especially effective in treating industrial waters used in the operation of cooling towers to disperse slimes and slime-forming masses within such waters to prevent the deposit of such slimes on the heat exchange surfaces of cooling towers and surface associated with such units. In his patent, Orndorff (1983) has described a method of killing and inhibiting the growth of microorganisms in industrial process streams using peroxidase- or lacasse-catalyzed oxidation of various phenolic compounds to generate microbial oxidation products (e.g., poisonous quinines).

Enzyme-based antislime—Betzdearborn's Spectrum—has been developed in response to such pressures of the European biocide directive ecolabeling, ISO 1400, and mill water system closure. This is available in the market and among its tool kit of products is a gluconase enzyme, which catalyzes the attack of glucose, which represents a significant proportion of the polysaccharides in the EPS on paper machines. In addition, formation of this troublesome polysaccharide coating is inhibited by new and nonenzymatic materials in Spectrum, which interfere with slime formation mechanism (Bajpai, 2012).

The use of a mixture of enzymes has been found to be more effective in treating microbially produced extracellular polysaccharides in cooling water and in papermaking broke water than the use of a single enzyme. The composite enzyme system tested consisted of cellulase, alpha amylase, and protease. According to some manufacturers and service companies, the enzymes available are too species-specific and can be used only on a smaller scale or as a supplement to conventional biocidal methods.

Weyerhaeuser Inc., Dryden, Ontario, a fine paper mill in Canada, put an increased emphasis on paper machine shutdown cleaning and deposit control. This helped the mill to substantially reduce breaks and increase efficiency (Daignault and Jones, 2003). A coordinated chemical deposit-control program was supplemented by cleaning programs focused on difficult to reach areas that were suspected as possible contributors to breaks. The big breakthrough in starch system cleaning has been the introduction of enzyme-cleaning products. The enzyme used is amylase, an enzyme that breaks down the starch deposits and thus allowing them to be easily washed away. The enzyme is so powerful and persistent that it is necessary to deactivate the enzyme with hypochlorite or peroxide after the cleaning. The Dryden mill has used the starch enzymatic cleaner for the past few years with very good success. In the early enzymatic starch cleanings, the system was found to have leaks after the enzyme boilout. Starch deposits that had plugged the leaks were completely cleaned away. In addition to the enzymatic cleaning, regular caustic cleanings of the system were done. These were found to be effective but do not match the enzyme cleaning. The size press area itself is cleaned with hot water.

The lytic activity of biological catalyst enzymes reportedly prevents biofilm formation. Steve patented a method that implemented plant dehydrogenase enzymes such as peroxidase and laccase to kill or inhibit the growth of microorganisms in industrial processes (Steve, 1983). Similarly, studies were proposed to use microbial or plant enzymes in the presence of oxidants to degrade the bacterially produced polysaccharides in biofilms (Ratto et al., 2001; Jedrzejas, 2000). A new group of biocides based on the cell wall lytic enzymes obtained from microorganisms, such as β-1,3-glucanases, chitinases, and proteases, attack bacterial, fungal, and yeast cell walls (Stepnaya et al., 2008; Shastry and Prasad, 2005; Bar-Shimon et al., 2004; Nagaraj Kumar et al., 2004; Brito et al., 1989). Tables 8.32–8.34 show the results of applying the new enzymatic biocide at pulp and paper mills (Cotrino and Ordonez, 2011).

Klahre et al. (1998), however, report poor performance of enzymes alone in antifouling efforts in paper mills, particularly in long-term applications. The enzymes themselves are rapidly degraded by extracellular proteases. Brisou (1995) already showed that there were a vast variety of target structures that enzymes had to interact with, indicating that there is no single enzyme or enzyme mixture to effectively remove biofilms.

The specificity in the enzymes mode of action makes it a complex technique, increasing the difficulty of identifying enzymes that are effective against all the different types of biofilms.

Table 8.32: Bacterial control at a tissue paper mill starting the use of the enzymatic biocide

Day	Bacterial count at wire pit (cfu/g)
0	60 million
5	15 million
10	4–5 million

Based on Cotrino and Ordonez (2011).

Table 8.33: Downtime reduction because of removal of dirt and detachment of slime at paper machine using enzymatic biocide at an OCC mill

1. *Downtime reduction because of elimination of dirt detachment*
   a. Reduction from 5.6 h/day to 0.4 h/day on PM 1 (reduction 93%)
   b. Reduction from 2.7 h/day to 0.2 h/day on PM 2 (reduction 92%)
2. *Downtime reduction because of elimination of slime detachment*
   a. Reduction from 0.5 h/day to 0.1 h/day on PM 1 (reduction 74%)
   b. Reduction from 0.8 h/day to 0.3 h/day on PM 2 (reduction 70%)

Based on Cotrino and Ordonez (2011).

Table 8.34: Bacterial count at the machine chest of an OCC recycling mill using the enzymatic biocide. Monthly average values of total bacterial count at the machine chest in an OCC recycling mill

Month	Bacterial count (cfu/mL × 10^6)
Initial	21
April	9
May	7
June	7
July	6.8
August	5.69

Based on Cotrino and Ordonez (2011).

Formulations containing several different enzymes seem to be fundamental for a successful biofilm control strategy. Basically, proteases, and polysaccharide hydrolyzing enzymes may be useful (Meyer, 2003). Moreover, the use of enzymes in biofilm control is still limited because of the low prices of the chemicals used today compared with the costs of the enzymes. In fact, the technology and production of these enzymes and the enzyme-based detergents are mostly patent-protected. Moreover, the low commercial accessibility of different enzyme activities limits their current usage (Johansen et al., 1997).

Orgaz et al. (2006) in their study on biofilm removal, concluded that on biofilm removal of *P. fluorescens*, the enzyme pectin esterase produced by *Trichoderma viride* (which belongs to the same family as pectinase methylesterase), could possibly deacetylate a polysaccharide in the biofilm matrix, making it softer and possibly more porous (Orgaz et al., 2006, 2007). Many microbial EPS have different substituent groups as ketal-linked pyruvate or ester-linked

acetyl groups. The removal of acyl groups, especially the acetate, can significantly affect the physical properties of polysaccharides. The removal of one of the substituents such as the acetyl group, in particular the acetate, influences the physical properties of EPS (Sutherland, 1999). Several fungi can degrade complex plant cell-wall material, by secreting a large variety of enzymes. This versatility makes commercial polysaccharide-degrading enzyme mixtures have a widespread use in multiple fields, such as fruit processing (McKay, 1993) or wastewater treatment (Wesenberg et al., 2003). They could also be used to degrade bacterial biofilm matrices or prevent and control the formation of biofilm in the piping system of the paper mill wastewater. Because of the EPS heterogeneity, a mixture of enzymes might be necessary for efficient biofilm degradation.

8.5 Biological Equilibrium

Biological equilibrium allows biocide-free operation and is applicable to all paper grades. This process is based on the principle of natural biological equilibrium. In this process, nontoxic, modified lignosulfonates are used with specific components that are chelating agent different from those generally used in papermaking. The lignosulfonate is water soluble. With controlled bacterial activity, efficient system cleaning and the reduction of chemical deposits is obtained. This process maintains the organisms and the suspended matter in the whitewater in a colloidal state and prevents the bacteria from forming deposits and agglomerating. The bacteria adheres to the modified lignosulfonate. It carries a net negative charge. The material becomes more flocculent as the bacteria digest the organic substances in the colloids. The lignosulfonate is drained off on the wire press and the bacteria enter the finished paper. Counterindications are open water systems, use of recycled fibers, unbleached kraft pulp, and an increase in the organic matter (Oberkofler, 1987, 1989, 1992; Oberkofler and Braunsperger, 1994; Braunsperger et al., 1996).

BIM Kemi AB has marketed biocide-free slime control since 1981, and its own product, Bimogard, since 1991 (Anonymous, 1986; Bjorklund, 1999, 2000, 2001a,b; Bjorklund, 2002a,b; Gavelin, 1996). Bimogard is a multifunctional, multicomponent product consisting of chemically modified lignosulfonates and is fortified with various surfactants. It is not toxic to microorganisms at concentrations used in mills. It is the first completely biocide-free slime control system in Europe. This process has been successfully used in low as well as neutral to high pH conditions, and at small, old, as well as large, modern, fast-moving paper machines. It is also used as intermittent board machines in pulp mills. The Bimogard system appears similar to the Biochem Process (Morros, 1995). This product is based on modified lignins and surfactants. It functions by:

- Cleaning the surfaces and thus reducing the adhesion of bacteria
- Reducing microorganism activity by inhibiting the production of extracellular polysaccharides
- Reducing bacterial growth and spore formation

Several Bimogard systems are in operation on paper machines for lightweight coated fine paper, newsprint, tissue, greaseproof paper, and paperboard. Results show that the system has brought about considerable improvements in the efficiency:

- Hole frequency declines
- Time between washups increases
- Cleaning becomes easier
- Lesser breaks in the wet-end; lesser production of broke
- Cost for using Bimogard for slime control is the same or most often less than using biocides
- Bacterial count is usually 10^5–10^7/mL in mills using Bimogard. Most of these bacteria do not cause any harm and die in the drying section without causing slime problems
- Does not trigger bacterial defense mechanisms unlike biocides

Bjorklund (2001b) studied the effect of Bimogard on the amount of EPS after introduction to a mill before using biocides. The amount of EPS decreased after introduction of biocide-free slime control in paper mill using 100% deinked pulp (DIP).

Bimogard has many other advantages (Table 8.35). Table 8.36 shows the effect of Bimogard on the amount of EPS after introduction to a mill previously using biocides. It can be clearly seen that the amount of EPS decreases after introduction of biocide-free slime control in paper mill using 100% DIP. The bacteria count is usually 10^5–10^7/mL in mills using Bimogard. Most of these bacteria are harmless and die in the drying section without causing slime problems.

Bimogard is currently running on several machines in Scandinavia using a variety of different pulps for production of tissue, newsprint, and carton as seen in Table 8.37. In Sweden, this has decreased the use of toxic slimicides with 20–25%. Also, this slime control has been successfully used in low as well as neutral to high pH conditions, and at small, old as well as large, modern, fast-moving paper machines. Bimogard is also used at intermittent board machines in pulp mills.

Table 8.35: Advantages of Bimogard

Keeps surfaces clean and smooth thereby preventing biofouling
Decreases the production of EPS (“slime”), thereby preventing growth of biofilm
Delays the growth of bacteria and decreases the formation of spores, thereby preventing bacteria in ready-made paper or carton
pH-stable
Temperature-stable
Environmentally friendly
Does not endanger the health of the staff
Is proven to be a satisfactory or even better alternative to biocides
Is cost-effective

Based on Bjorklund M (2001b).

Table 8.36: Effect of Bimogard on EPS after introduction to a mill previously using biocides

	EPS (mg/g)			
Sample	Before introduction of Bimogard	2 weeks after introduction of Bimogard	4 weeks after introduction of Bimogard	16 weeks after introduction of Bimogard
Deposit from section under forming roll	13.0	6.1	5.5	0.0
Back water	6.3	3.1	1.2	0.0
Process water from wire pit	3.8	1.3	0.0	0.0
Fresh water	0.0	0.0	0.0	0.0

Based on Bjorklund M (2001b).

Table 8.37: Mills using Bimogard

Paper grade	Type of pulp
Tissue	Sulfate
Tissue	DIP
Tissue	DIP
Tissue	DIP
Tissue	Sulfate
Tissue	Sulfite, sulfate
Printing and writing paper	Sulfite, sulfate
Printing and writing paper	Sulfite, sulfate
Greaseproof	Sulfate
Newsprint	DIP, TMP
White-lined chipboard	Waste paper
Tissue	Sulfate
Newsprint	DIP, TMP
Newsprint	DIP, TMP
Newsprint	DIP, TMP
Newsprint	DIP, TMP
Wood-containing printing paper	Sulfite, SGW-pulp
Folding box board	SGW-pulp, sulfate
Printing and writing paper	Sulfite
Printing and writing paper	Sulfite

Based on Bjorklund M (2001b).

A method for closed water systems from Petromontan uses a multifractionated and modified lignosulfate called Petrodis (Anonymous, 1986). This reduces the requirement of biocides when added to the system and also results in better functioning of the paper machine. This is attributed to:

- More efficient biological control
- Reduced chemical scaling

- Reduction of toxic substances in the system and in the effluent
- Safer for workers
- Reduces costs for the control of slime and scale

8.6 Biodispersants

Biofilm control programs can be made more effective through the use of biopenetrant/dispersant products. The use of biodispersants offers an ecologically attractive method of controlling slime in the pulp and paper industry. They eliminate or reduce the needs for biocides (Anstey et al., 1998a; Blankenburg and Schulte, 1997, 1999; Gould, 1998, 2001; Pauly, 2001; Robertson and Taylor, 1994; Saner, 1998; Stenqvist, 1992; Van Haute, 1997a,b; Van Haute, 1999; Weisshuhn et al., 2000; Wright, 1997; Schenker, 1996). These are nonbiocidal surface-active agents that penetrate and loosen the biopolymer matrix. This not only aids in sloughing the biofilm, but will also expose the microorganisms to the microbicides. They can be continuously applied or periodically slug-dosed. Because these products effectively mobilize solids, they can cause clogging in downstream and down-gradient locations, and this potential must be taken into account when designing proper application. Biodispersants are particularly effective when dealing with systems that have a high total organic carbon loading and a tendency to foul. These products are typically fed in slug additions before microbicide feed. Low-level continuous feed may also be effective. However, in some cases, this may not be as effective because it may not reach a certain threshold amount required to produce the desired effect. Developments in biodispersant technology are making this approach more effective and popular than ever before. This technology is based on nonionic polymers, which are nontoxic, nonfoaming, colorless, and free of organic solvents. Because of their nonionic character, they will not increase the system anionicity and also not interfere with other papermaking chemicals. Table 8.38 shows biodispersants used in the paper industry.

Biodispersants dissolve and disperse deposits, preventing the biofilm from reestablishing itself. These dispersants act as biopenetrants opening the biofilms and allowing biocides to penetrate the exopolysaccharides. It has been also reported that dispersants facilitate

Table 8.38: Biodispersants used in the paper industry

Synonym	Chemical basis
Amides	Alkylamides
Cationic, hybrid ionic	Fatty acids
Glycols	Alcohols
Lignosulfonates	Salts of lignosulfonic acid
Non ionic, anionic	Alcohol
Terpenes	Terpenoids

Based on Schrijver and Wirth (2007).

penetration of biocides into the cell or that they trigger sloughing of biofilms. Biodispersants have no pH limitations and are suitable for use in both acidic and alkaline papermaking.

Surface-active agents for slime control may be prepared by chemical synthesis, obtained as a byproduct of industrial processes (e.g., lignosulfonates), manufactured from natural raw materials (e.g., paraffin wax, terpenes from oranges, produced by cultivated microorganisms (Johnsrud, 1997). The industrial dispersants can be divided in three categories:

- Anionic dispersants: alkyl and aryl sulfonates are the most widely used dispersants of this group. They consist of benzene sulfonic acids with alkyl chains of 10–15 carbon atoms in the para position (Atwood and Florence, 1983). These surfactants can be used as the sodium salt or in conjunction with other surfactants (Blanco, 2003).
- Cationic dispersants: some of the chemicals, used commonly as cationic surfactants can also have biocidal properties (Blanco, 2003). An example of this combined action is that of quaternary ammonium compounds.
- Nonionic dispersants: examples of this group of surfactants are n-octyl glucoside and polyethylene oxide (10) cetyl ether (Blanco, 2003).

The nonionic products, which have seen large-scale commercial use, are of the four types—alcohol ethoxylates, alkylphenol ethoxylates, polyoxyethylene esters, and polyoxyethylene-polyoxypropylene derivatives. The latter are mixed polymers with hydrophobic groups derived from propylene oxide, further reacted with ethylene oxide until the desired properties are achieved. Generally, they are of rather high molecular mass, often with much more than the usual eight to 15 molecules of ethylene oxide characteristics of the other nonionics. Dispersants do not kill or do not always even inhibit the growth of microorganisms. Therefore, their dosage or evaluation of their effect cannot be based on cell counts in circulation waters and new efficient methods to evaluate their efficiency in mill conditions are required (Blanco, 2003).

The chemistry of dispersing agents can be designed to give various dispersing/solubilizing and emulsifying properties to the product, which may disturb the biofilm, stabilize the emulsion with cells and biofilm components, and form a protective layer on the hydrophobic surfaces that delays the attachment of biofilm (Blankenburg and Schulte, 1996). Different dispersants chemistries can be designed according to the propose use (Kanto et al., 2001).

The use of organic natural products has reported a few problems such as the high concentrations required and the microorganisms may use the natural products as a nutrient source (Blanco, 2003). For example, lignosulfonates can increase the growth of microorganisms under certain conditions because of the presence of sugars that can be used as nutrients by the microorganisms (Cardoso, 1992; Robertson and Taylor, 1994).

The dosage and design of the treatment system depends on the problem to be solved. Recommended addition points are the short circuit before the headbox or to clarified water (Johnsrud, 1997). The dispersants treatments may be applied either to obtain biocide-free microbial control programs or in combination with biocide in order to reduce the dosage needed (Schenker, 1996).

The mechanism by which conventional dispersant additives function in papermaking systems is by contributing a high negative ionic charge to the surfaces upon which they get adsorbed (Ashraf et al., 2014). The increased negative charge of the particle surfaces increases the electrostatic potential energy barrier and reduces contact with the surfaces because the bacteria are negatively charged. To be effective, the amount of dispersant must be sufficient to overpower any coagulants that may be present (e.g., aluminum sulfate, high-density cationic polymers). There are some problems with the use of dispersants. These products effectively mobilize solids but they can cause clogging in downstream and down-gradient locations; therefore, this potential must be considered when designing an appropriate application that will not lead to an uncontrolled accumulation of dispersed materials in a paper production system. Particularly cationic retention aids tend to be deactivated by dispersants. However, in some cases, dispersants have helped papermakers overcome specific deposit problems (Hubbe et al., 2006). Under some circumstances, slime deposits may be responsible for up to 70% of all breakages, blockages, and pump failures in pulp and papers mills (Safade, 1988).

A deposit control program was proposed to a paper mill producing uncoated wood-free and specialty papers with the goal of reducing the use of biocides while maintaining or improving the overall performance at similar costs (Simons et al., 2003). The recommended program involved the continuous application of a biodispersant while reducing feeding frequency of the same nonoxidizing biocides used previously by the mill. After optimization, the mill reported reduced nonoxidizing biocide use by 60%, fewer deposits on the paper machine, sheet breaks reduced from an average of 5.8–2.6 breaks per day, and deposit control program costs reduced by 20%. This demonstrates that selected dispersants can significantly reduce the amount of nonoxidizing biocides needed to obtain good slime control in a cost-effective manner. Poor microbial deposit control is the main cause of poor paper machine runnability and loss of efficiency. The use of new monitoring tools allows mills to optimize slime control programs to achieve the best performance in a cost-effective way. The use of traditional toxic nonoxidizing biocides can be significantly reduced by the use of new nonhalogenated oxidants in combination with new nontoxic deposit control agents. An optimized microbial deposit control program can give papermakers a high return on investment, because program costs can be offset by improved runnability, decreased rejects, and reduced biological oxygen demand (BOD) in the final effluent.

A new biofilm cleaner has been developed that penetrates and destroys biofilms (http://www.industrialchemtex.com/docarchive/pub/TT-32%20Biofilms.pdf). This technology destroys the biopolymer matrix, but it also attacks the attachment structures and completely removes the biomass from the metal surface. The penetrating action of the cleaner exposes the microorganisms growing there to the effects of the microbicide being used. This will reduce the amount of biocide required to achieve a complete kill of algae and bacteria in the system. By leaving a bare metal surface, it also allows the corrosion inhibitor to reestablish protection. The biofilm cleaner should be slug-fed to a point in the system where good mixing is

obtained. Addition should be made 30 min to 1 h before biocide addition. The level of cleaner required will be dependent on the amount of biofilm present. As with the biocides themselves, frequency of addition depends on the rate of reinfection, the level of nutrients available, and the ambient conditions.

Buckman Laboratories has developed new technologies to control biological deposits for more than 50 years. Buckman researchers, microbiologists, and application engineers have developed the Neoteric Product Line for deposit control. Neoteric biodispersants are new, specially designed Buckman products for use in preventing and slowing biofilm development in paper machine systems without sacrificing papermaking operations. These products can penetrate existing biofilms, loosening and dispensing them, which allows biocides to penetrate and reach the organisms, and were developed with safety and the environment in mind (Van Haute, 1999; Hart, 2001; Koopmans and de Vreese, 2002). The programs have been successfully used in acidic, neutral, and alkaline conditions and in the manufacture of all types of paper, paperboard, tissue, and toweling (Van Haute, 1999, 2000). Buckman reports that several paper machines in Europe use Neoterics in their short circulation loop, with Busperse and Buzyme. Neoteric programs are designed to increase the effectiveness of traditional biocides, reducing and in some cases eliminating the use of biocides in wet-end. Neoteric programs consist of dispersants, enzymes, and potentiators added to the wet-end of the paper machine:

- Neoteric dispersants are a select group of chemicals that prevent agglomeration and deposition on machine surfaces of common deposit causing components in the paper machine furnish.
- Neoteric enzymes are biodegradable and nonbiocidal, have relatively low level of toxicity to test animals, and are Food and Drug Administration–allowed. These enzymes include amylase enzymes and protease enzymes in a formulation that attacks starches and proteins.
- Potentiators are nonbiocidal products that increase the performance of biocide. These products allow the use of less organic biocide while still achieving the same effect as the original dosage of biocide. The potentiators simply lower the effective concentration of biocide required for a given application. These products, when used alone, do not reduce or slow microbial growth or metabolism.

Neoteric deposit control helps to improve sheet quality, reducing holes, breaks, and off-specification production. It has no negative impact on sizing, color, retention, drainage, or other machine operations when used as directed. Neoteric deposit control can help reduce lost production by keeping machines running longer between washups and can provide better utilization of resources such as energy, water, and manpower. Jacquelin et al. (1994) also reported the synergistic action of enzymes in combination with surfactants and phenolic antimicrobials. The combination of proteolytic enzymes with surfactants was found to enhance the efficiency of cleaning on *Bacillus* spp. biofilms (Parkar et al., 2004).

Nalco recommends lignosulfonate-based biodispersants plus thorough machinery and system cleansing; elimination of static areas and rough surfaces; fresh water and additive contamination controls; and good retention and prevention of anaerobic bacteria in storage tanks through oxygenation for closed paper machine systems. The name biodispersant suggests the activity on a biological entity, but laboratory study has shown that many commercial biodispersants, in the absence of a microbicide, have little or no ability to remove an existing biofilm or prevent their formation. In fact, most of the commercial biodispersant applications include the use of a microbicide. Biodispersant technology can increase the overall efficiency of a given microbicide program.

Buckman Laboratories have developed an enzyme-based product line of biodispersants for boilouts and other applications (Wolfanger, 2001). This patented enzyme stabilization process promotes greater shelf life and reduced temperature instability concerns. It also reduces significant safety and health hazards in paper machine operations that are present during caustic boilout. It is also possible to release nontoxic cleaning solutions without any treatment before discharge. These products were found to be successful in mill operation. A fine, coated paper mill wanted to enhance safety by eliminating the use of caustic during traditional paper machine boilouts. Buckman began an enzyme-based Neoteric boilout program that matched the effectiveness of the traditional boilout and in some areas—especially the headbox—exceeded it. The mill now completes boilouts in less time and has a cleaner system, with fewer related startup deposits than experienced with caustic boilouts. Another fine paper mill using softwood and hardwood kraft furnish had a problem with starch deposits in its size press system. Buckman replaced their caustic boilout with its Neoteric program that quickly and completely removed the starch deposits. Another mill producing 100% recycled paperboard was concerned about caustic safety issues and had a problem with paper machine system deposits. Buckman's Neoteric enzymatic boilout program eliminated deposits in tanks and lines and reduced safety and health concerns.

Enzyme-based boilouts remove the compounds such as starch, slime, pitch, adhesives, latex, and other synthetic binders that hold the deposits together. The type of enzyme used and the dispersant depends on the type and amount of deposit present in the system. Starch slurry contains deposits that are microbiological and/or starch protein based. Boilouts using a product that contains a stabilized protease enzyme are found to be effective in these systems. For the cooked starch system, an alpha amylase product is used to remove deposits comprised mainly of cooked starch. In both cases, a preboilout system flush is essential. This removes the cooked starch and allows the enzyme to work particularly on deposits. An ideal starch system boilout uses the following:

- 0.2–0.5% of the boilout product
- Temperature of 120–150 °F
- Recirculation time of 1–2 h

Enzymatic boilouts are pH neutral and can be dumped directly to the sewer without neutralizing the solution and without any upset at the waste treatment facility. Also, it removes the safety concerns associated with working with and around the caustic solution. It eliminates the requirement for excessive rinsing to purge caustic from the system. Each of these factors contributes to a reduction in the downtime necessary for a boilout and maintenance outage, which shows cost savings.

Starch-based coating systems can be successfully cleaned via a boilout using the alpha amylase enzyme. The parameters for this boilout are similar with the enzyme-based boilout product as mentioned previously. It is required to use a neutral dispersant/penetrant capable of removing latex and other binders that may be present in the coating formulation in addition to the enzyme-based product. Also a higher temperature range (130–150 °F) and a longer recirculation time (2 h) will be beneficial for a starch-based coating system boilout. Enzyme boilouts are not recommended for the coating systems that do not contain starch. Enzyme boilouts have been successfully applied in systems that use both starch-based and non–starch-based coatings. Enzyme-based chemistries are found to be successful for different types of paper mills ranging from acid to alkaline papermaking, recycled to virgin, board to fine paper to tissue, including all variety of additives linked with these various furnishes. The question arises about how this is possible given the specific mode of action of enzymes. This appears logical, if the majority of the machine system deposits are microbiological slime. A protease breaks down these deposits. But in a system that has deposits containing various fiber, fillers, and hydrolyzed additives, how does the protease enzyme work? Actually, most deposits in the paper machine system have microbiological growth in, on, and around the deposit. When the enzyme breaks down this matrix, the deposit sloughs off and is carried away in the boilout solution. This is based on the ability of bioengineered enzymatics to attack specific deposits by eating the organisms that compose them, making them easy to rinse away. There are certain pH-neutral additives that can be added to help in penetrating, breaking down, and removing deposits that are nonmicrobiological in nature. These additives in fact help the protease-based enzyme product enter into the deposit where microbiological growth is helping to anchor and hold a deposit together. This is a cleaning effect and is found to be better than caustic boilouts.

Paper companies are currently striving toward environmental stewardship, worker health, and safety and producing a product of the highest quality. Neutral boilouts are a major step forward in application technology to help the papermaker in meeting these difficult objectives. The future is sure to bring new and varied used of enzyme technology to further benefit the paper industry through the further development of stabilized enzyme products.

8.7 Use of Competing Microorganisms

Some nonconventional approaches have been used in solving slime problems. The literature shows the existence of multiple interspecies interactions or the simple production of a

metabolite can interfere with biofilm formation and development (Carpentier and Chassing, 2004; Kives et al., 2005; Røssland et al., 2005; Tait and Sutherland, 1998; Valle et al., 2006). Competition for substrates is one of the major evolutionary driving forces in the bacterial world, and several experimental data obtained in the laboratory, under controlled conditions, show how different microorganisms may effectively outcompete others because of their better utilization of a given energy source (Christensen et al., 2002; Simoes et al., 2007; Lindvall, 1998a).

The inoculation of non–slime-forming organisms to outcompete the slime formers has been proposed. A slime control process has been patented by Oberkofler (1993). This is based on the introduction of a consortium of bacteria commercially available in freeze-dried or liquid form. The bacteria are pregrown before inoculation of the circuit water, and the amount added is calculated on the basis of total organic carbon present. The additives may be introduced along with the bacteria, thus favoring their growth. These additives include tensides to avoid the adhesion of bacteria, lignosulfonate to increase the nutrients, and enzymes to catalyze the breakdown of organic substances. It has been also suggested aeration of the circulating water with oxygen or air or the addition of oxygen releasing compounds such as hydrogen peroxide. The invention is not limited to bacteria, but the fungi alone or the mixture of fungi and bacteria can also be used. The patent describes an indiscriminate use of bacteria and fungi, of which the mixed culture of freeze-dried bacteria used contains genera associated with slime formation in pulp and paper mills but also contains such genera undesirable for paper and board intended to come into contact with food stuffs. Plant-scale trials showed that the addition of the selected microbes to the circulating water reduced the buildup of slime on solid surfaces and in the liquid phase.

Many bacteria synthesize and excrete biosurfactants with antiadhesive properties (Desai and Banat, 1997; Nitschke and Costa, 2007; Rodrigues et al., 2004; van Hamme et al., 2006). Rodrigues et al. (2004) reported that biosurfactants produced by *Lactococcus lactis* 53 impaired biofilm formation on silicone rubber Surfactin from *B. subtilis* breaks biofilms without affecting cell growth and prevents biofilm formation by microbes such as *Salmonella enterica*, *E. coli*, and *Proteus mirabilis* (Mireles et al., 2001). Other biosurfactants showed biofilm control potential (Davey et al., 2003; Walencka et al., 2008; Rivardo et al., 2009). Microbial molecules, used as biopreservatives, such as nisin, lauricidin, reuterin, and pediocin, have been reported for their biofilm control potential against microbes generally found in dairy processing facilities, including *Listeria monocytogenes* (Dufour et al., 2004; Garcia-Almendarez et al., 2008; Mahdavi et al., 2007; Zhao et al., 2004). Valle et al. (2006) showed that *E. coli* expressing group II capsules release a soluble polysaccharide into their environment that induces physicochemical surface alterations, which prevent formation of biofilm by gram-positive and gram-negative bacteria. Davies and Marques (2009) observed that *P. aeruginosa* produces *cis*-2- decenoic acid, which induces the dispersion of established biofilms and of inhibiting biofilm development. This molecule was tested, when applied exogenously, against *B. subtilis*, *E. coli*, *S. aureus*, *Klebsiella pneumoniae*, *P. aeruginosa*,

P. mirabilis, *Streptococcus pyogenes*, and the yeast *C. albicans*. It has been suggested that this molecule is functionally and structurally related to the class of short-chain fatty acid signalling molecules.

8.8 Biofilm Inhibitors

Biofilm inhibitors prevent biofilm formation at an earlier stage than biodispersants and enzymes and are an effective environment friendly alternative treatment for short loops. Sulfosuccinates, a family of molecules, are very effective in inhibiting deposit formation (Davis et al., 1999; Scharpf, 1998; Schenker et al., 1998). An exclusively designed sulfosuccinate molecule is found to act at an earlier stage than biodispersants and enzymes in the inhibition of biofilm deposit formation. The mechanism of biofilm inhibitors differs from that of biodispersants and enzymes in that bacterial attachment and biofilm formation is prevented by hindering the formation of a concentrated extracellular EPS layer around a bacterial cell. Bacteria lacking EPS have less capability to permanently attach to surfaces, cannot easily protect themselves from the harmful effects of microbicides, and do not provide "glue" to hold wet-end deposits together. Thus, in the idealized model of biofilm formation, sulfosuccinate works in stage 2, actually preventing the bacteria from forming an intact EPS matrix. The cells are then only loosely associated with the surface. Because of the absence of the concentrated EPS layer around the bacterial cell, sulfosuccinates are described as biofilm inhibitors to distinguish them from microbicides, biodispersants, and enzyme.

The first generation of biofilm inhibitors were studied in a European alkaline fine paper mill, a Scandinavian board mill, a European tissue mill, a North American tissue mill, and two North American alkaline fine paper mills (Bunnage et al., 2000). Before running any of the field trials, lithium tracer studies were conducted from which the system cycle-up was determined and the product application rates were accordingly determined. The control data from a European trial location reflect deposition on a machine with a conventional proprietary microbicide (dodecylguanidine hydrochloride and methylene bis (thiocyanate) and biodispersant in the short loop whitewater and a conventional proprietary microbicide (Bronopol and quaternary ammonium chloride) in the save-all. The control consisted of the paper machine running with a commercial biocide program. The treated system received a program consisting of the biofilm inhibitor. This conventional program was found to be effective at keeping the machine clean and providing the mill with a desired 4-week interval between boilouts. The microbicide and biodispersant feeding the short loop were completely replaced with a biofilm inhibitor program on an equal cost per ton basis. A side-stream monitoring device was used to measure visual appearance of the biofilm and the growth of certain biofilm constituents over a 2-week period. Both measurements showed a significant decrease in biofilm growth with the biofilm inhibitor program. Over an 11-month period, testing on this fine paper machine trial indicated that the biofilm inhibitor program reduced the number of breaks by 40%.

This provided the mill with a 103% return on investment. Also, the mill totally removed microbicides from the short loop of the machine, thus providing a much safer program. Results showed that, for the specific paper machine, continuous feed is preferential over semicontinuous. The trial consisted of various production runs with boilouts in between runs.

Olofsson et al. (2003) reported that *N*-acetyl-L-cysteine (NAC) may be an interesting candidate for use as an agent to reduce and prevent biofilm formation on stainless steel surfaces in environments typical of paper mill plants. Using 10 different bacterial strains isolated from a paper mill, the researchers found that the mode of action of NAC is chemical as well as biological in the case of bacterial adhesion to stainless steel surfaces. The initial adhesion of bacteria is dependent on the wettability of the substratum. NAC was shown to bind to stainless steel, increasing the wettability of the surface. Moreover, NAC decreased bacterial adhesion and even detached bacteria that were adhering to stainless steel surfaces. Growth of various bacteria, as monocultures or in a multispecies community, was inhibited at different concentrations of NAC. No detectable degradation of extracellular polymeric substances (EPS) by NAC was found, showing that NAC reduced the production of EPS, in most bacteria tested, even at concentrations at which growth was not affected. Altogether, the presence of NAC changes the texture of the biofilm formed and makes NAC an interesting candidate for use as a general inhibitor of formation of bacterial biofilms on stainless steel surfaces.

http://www.ncbi.nlm.nih.gov/pubmed?term=Olofsson%20AC%5BAuthor%5D&cauthor=true&cauthor_uid=12902275 US Patent 20,060,018,945 A1 (Britigan and Singh, 2006) provides a gallium-containing composition for coating/impregnating a device or device surface to prevent biofilm growth formation. The present invention also provides a method of preventing or inhibiting biofilm growth formation and for killing established biofilms.

Biosurfactants produced from the microbes were also found to impair biofilm-forming abilities. Biosurfactants produced by *Lactococcus lactis*–impaired biofilm formation on silicone rubber (Rodrigues et al., 2004). The ability of biosurfactant obtained from the probiotic bacterium *L. lactis* 53 to inhibit adhesion of four bacterial and two yeast strains isolated from explanted voice prostheses to silicone rubber with and without an adsorbed biosurfactant layer was investigated in a parallel-plate flow chamber. The microbial cell surfaces and the silicone rubber with and without an adsorbed biosurfactant layer were characterized using contact-angle measurements. Water contact angles indicated that the silicone-rubber surface with adsorbed biosurfactant was more hydrophilic (48°) than bare silicone rubber (109°). The results showed that the biosurfactant was effective in decreasing the initial deposition rates of *Staphylococcus epidermidis* GB 9/6 from 2100 to 220 microorganisms cm(−2) s(−1), *Streptococcus salivarius* GB 24/9 from 1560 to 137 microorganisms cm(−2) s(−1), and *S. aureus* GB 2/1 from 1255 to 135 microorganisms cm(−2) s(−1), allowing for a 90% reduction of the deposition rates. The deposition rates of *Rothia dentocariosa* GBJ 52/2B, *C. albicans* GBJ 13/4A, and *Candida tropicalis* GB 9/9 were far less reduced in the presence of the biosurfactant compared with the other strains.

Surfactin from *B. subtilis* was found to disrupt biofilm without affecting cell growth and prevent biofilm formation of *Salmonella* enteric and *E. coli* (Mireles et al., 2001). Microbial molecules such as nisin, reuterin, and pediocin have been reported on their abilities to control biofilm formation by *L. monocytogenes* (Dufour et al., 2004). However, the use of biological control is not a cost-effective method in comparison to the chemical used. Because chemical disinfectants have been widely used to eliminate biofilms, the properties of the chemical have been concerned based on effectiveness, safety, easy applicability, easy rinsing off from the surfaces, no toxic residues are left that can affect the health properties and sensory values of the final products. In the past, efficiencies of biological and chemical disinfectants were previously tested on planktonic (free cell) rather than biofilm mode of growth. Biofilms have been reported to be 100–1000 times resistant to disinfectants (Gilbert et al., 2002). Thus, to identify the efficiency of disinfectant in the elimination of biofilm must be evaluated in the biofilm mode of growth.

A conventional microbicide strategy is still recommended for incoming furnish to maintain a consistent microbiological population level and as treatment for mill fresh water.

8.9 Bacteriophage Use

Phages are ubiquitous in nature. Bacteriophages are viruses that infect bacteria and may provide a natural, highly specific, nontoxic, feasible approach for controlling several microorganisms involved in biofilm formation (Kudva et al., 1999; Duckworth, 1976). Bacterial viruses or bacteriophages (phages) are hypothesized to be the predominant lifeform in the biosphere, clearly outnumbering their host bacterium. Bacterial viruses were discovered about 100 years ago (d'Hérelle, 1922; Hauduroy, 1925) and their discovery is attributed to Félix d'Hérelle, a French-Canadian microbiologist and Frederick Twort, an English bacteriologist (Twort, 1915; d'Hérelle, 1917). d'Hérelle named these viruses as "bacteriophages" from the words "bacteria" and "phagein," which in Greek means to eat or devour. d'Hérelle performed extensive trials in humans and animals (Barrow and Soothill, 1997) and tried to shed a light on their nature and ability to function as therapeutic agents (d'Hérelle, 1922, 1917). He isolated bacteriophages for a number of bacterial hosts causing diseases. The phage products were produced in large scale at d'Hérelle's laboratory in Paris (today known as L'Oréal), and by several pharmaceutical companies (Eli Lilly & Co., Parke-Davis, Squibb & Sons, and Swan-Myers division of Abbott Laboratories, etc.) (Straub and Applebaum, 1993; Sulakvelidze et al., 2001; Summers, 1999). Twenty years after the official finding of phages, the first antibiotic, penicillin, was discovered. This fact, allied with some early clinical failures (Eaton and Bayne-Jones, 1934) and theoretical concerns did not continue with the phage therapy in the United States and most of the Western Europe. However, research and therapeutic use of phages continued in the Eastern European countries and the former Soviet Union (Alisky et al., 1998; Chanishvili et al., 2002; Slopek et al., 1987; Weber-Dabrowska

et al., 2000). In these countries, phages continued to be regarded as a good treatment method against a wide range of bacterial infectious diseases (Sulakvelidze et al., 2001; Ho, 2001).

Phages are currently suggested as possible alternatives to antibiotics for the treatment of bacterial diseases in humans and animals and widely explored to minimize the pathogen loads in food products of animal and plant origin. These phages do have a variety of advantages over chemical agents:

- Isolation is fast and simple
- Production is inexpensive
- Specific against a host or host range and thus do not affect the normal microflora of the environment where they can be applied
- Environmentally friendly
- Self-replicate at the infection site as long as the host bacterium is present
- No serious side effects reported

Not much information is available on the action of bacteriophages on biofilms (Hughes et al., 1998a,b; Sillankorva et al., 2004; Sutherland et al., 2004). The infection of biofilm cells by phages is extremely conditioned by their chemical composition and the environmental factors, such as temperature, growth stage, media, and phage concentration (Chaignon et al., 2007; Sillankorva et al., 2004). The major advantage of using phages in combating bacteria is their bactericidal (killing) nature, selectivity, and nontoxicity to man and the environment. The main drawback is that the phages have to be isolated for each harmful bacterium, the type of which may vary between paper mills. When phages make contact with biofilms, further interactions take place, depending on the susceptibility of the biofilm cells to the phage and to the accessibility of the receptor sites. The integrity of the biofilm may be quickly destroyed if the phage also contains polysaccharide-degrading enzymes or if significant cell lysis is affected by the phage. These microbes are noted for their high activity in the presence of host cells and selective lytic action. But, the industrial application of this technique has been slow. Viruses lack an independent metabolism and multiply only inside the living cells, using the metabolic machinery of the host cells. A bacteriophage can propagate only by coming into contact with its specific host bacterium. When this happens, the phage lyses the bacterium completely. Some of the bacteriophages that attack *E. coli* (ψ, λ, and T-4 phage) are very well known for their extensive contribution to development in molecular biology. The bacteriophage contains an icosahedral head of 50–100 nm in diameter, a long tail of about 100 nm in length with or without a sheath around it and six tail fibers at the end of its tail. The bacteriophage contains only protein with nucleic acid as gene. The bacteriophage selectively attacks its particular host bacterium. The gene of the bacteriophage is placed into the host bacterium, where it multiplies. The host cells also begin to produce the specific protein which constitutes the bacteriophage. A large number of bacteriophages are formed in the host cell, when the phages are released from the host, the cycle begins once again. The bacteriophage formed in

the host cell has the same properties and morphology as the original. The reproduced phages thus can constantly lyse the same host bacteria. The number of bacteriophages formed after one lytic cycle is called "burst size" and the time required for the cycle is called latent period. These values are reported for evaluating the activity of bacteriophage.

Bacteriophages are found to induce a wide range of polysaccharide-degrading enzymes in their hosts. Dispersion by induction of a prophage, followed by cell death and subsequent cell cluster disaggregation has been observed by Webb et al. (2003). However, phage enzymes are very specific and rarely act on more than a few closely related polysaccharide structures. Phages and bacteria can coexist symbiotically within biofilms, suggesting that they would make poor tools for the control of biofilm formation. Combinations of phage enzymes and disinfectants have been recommended as possible control strategies under certain conditions (Tait et al., 2002) with the phage added before addition of disinfectant being more effective than either of these alone. Mixtures of enzymes are commonly used, composed on arbitrary base.

Japanese researchers conducted investigations using *Pseudomonas* sp. (S−1) and its corresponding bacteriophage (pS−1) (Araki and Hosomi, 1990). The growth of slime-forming bacteria was inhibited in the presence of its corresponding bacteriophage. Results with bacteriophage and conventional biocides showed that after addition of 10 ppm of MBT to the test solution, the colony count of the slime-forming bacteria increased from 87×10^5 cfu/mL to 110×10^5 cfu/mL after 24 h at 28 °C showing that MBT was effective in keeping the activity of the slime-forming bacteria to a low level. However, the simultaneous addition of MBT and bacteriophage PS-1 reduced the colony count of the slime-forming bacteria from 100×10^5 cfu/mL to 2.4×10^5 cfu/mL. Results showed that addition of bacteriophage to mill whitewater was an effective technique for slime control and addition of bacteriophage with conventional biocides was also effective. Practical application of this technique on a commercial scale awaits completion of fundamental studies in several key areas. Bacteriophages will not impair the activity of the sludge used in waste treatment systems unlike conventional biocides.

Vaatanen and Harjy-Jeanty in 1986 reported the use of bacteriophages in paper mill whitewater systems. Their concept was based on the idea of isolating harmful bacterial streams of process waters and thereafter searching for virulent, lytic bacteriophage for these bacteria. They studied bacteriophages lytic for the bacteria *Enterobacter* and *Klebsiella* in model systems and found that phage activity against *Enterobacter agglomerans* stopped its growth for more than 19 h. When *K. pneumoniae* was dosed with phage at 3-h intervals, bacterial growth was no more restricted than when the culture was injected only at the beginning of the test. It was concluded that further studies to determine the efficacy of bacterial control in process waters should take into consideration the expected development of bacterial resistance to phage attack and the variation in bacterial strains among paper mills.

Sharma et al. (2005) observed the synergistic effect of an alkaline cleaner and a bacteriophage in the inactivation of *E. coli* O157:H7 biofilms formed on stainless steel. Lu and Collins (2007) engineered a bacteriophage to express a biofilm degrading enzyme. This enzymatic phage was found to have the ability to attack the bacterial cells in the biofilm and the biofilm matrix, considerably reducing the biofilm cell counts; more than 99.9% of removal was achieved. Hughes et al. (1998a) working on the control of *E. agglomerans* biofilms by the use of phages observed that the cells were killed and the biofilms were degraded by the bacteriophage. The phage then lysed the biofilm cells and the polysaccharide polymerase enzyme degraded the EPS and caused biofilm to slough off. Sillankorva et al. (2004) used bacteriophages to remove *P. fluorescens* cells, showing that phages were effective in the removal of biofilms in the early stage of development and 5-day-old biofilms under optimal conditions; up to 80% biofilm removal was obtained. Hanlon et al. (2001) have reported that in *P. aeruginosa* biofilms the bacteriophage migration through the biofilms is facilitated by the reduction in alginate viscosity. This phenomenon is related to the exopolysaccharide degradation by enzymes produced by the bacterial host. Hibma et al. (1997) reported that a bacteriophage (*L. monocytogenes* phage ATCC 23074-B1) was used successfully in *L. monocytogenes* biofilm inactivation. *E. coli* biofilms have been shown to be susceptible to bacteriophage T4 (Doolittle et al., 1995).

8.10 Electrochemically Activated Biocides

Electrochemically produced biocides can be a green alternative for organic biocides in use today (Ullah, 2011; Ashraf et al., 2014). These biocides are produced by electrolysis of diluted salt solutions in an electrolysis cell (Kiuru et al., 2010). The oxidation-reduction potential was found to be an important factor that defines the biocidal activity of electrolyzed anode water. These electrochemically generated biocides are environmentally friendly, nontoxic, hypoallergenic, chemical residue-free, fast-acting powerful biocide agents that do not require special handling and can be safely disposed of in municipal sewage systems (Robinson et al., 2010). Inactivation of microbes by electrochemical oxidation may happen in following two different ways:

- Direct anodic oxidation: Microorganisms are destroyed at the electrodes surface by hydroxyl radicals produced from water by electrolysis. Hydroxyl radical is the most powerful oxidant and it can cause oxidative stress against cell of bacteria.
- Indirect electrochemical oxidation: Microorganisms are destroyed by electrochemically produced ozone or hydrogen peroxide.

In paper and board machines, broke towers are usually the places where anaerobic conditions develop. Anaerobic bacteria are more sensitive to oxidizing compounds than aerobic bacteria and therefore electrochemically produced biocides are found to be powerful against anaerobic

bacteria. Bacterial spores are highly resistant to many conventional organic biocides. Oxidizing biocides such as hypochlorite are found to be more effective against spores. Electrochemically produced hypochlorite had a bleaching effect and that was evident as an ISO brightness increase of approximately 1 unit.

Electrochemical oxidation has been applied successfully to degrade different organic pollutants, such as phenols or industrial wastewaters, textile wastewaters, and olive mill wastewaters (Särkkä et al., 2008). Several terms are being used to represent solutions that are produced by the electrolysis of salts from passing an electric current (Ashraf et al., 2014). They are called

- Electrochemically activated solutions (ECAS)
- Electrolyzed oxidizing water
- Mixed oxidants (mioxs)
- Electrochemically activated water

The main molecule produced by electrolysis of sodium chloride solution of a given strength with biocidal potential is HOCl. It is an unstable hydroxy radical. The redox potential of these solution is between +800 and +1200 mV; pH ranges from 2 to 5 (Cloete et al., 2009; Thorn et al., 2012). Electrochemically produced biocides can be obtained using other salts, such as potassium chloride and magnesium chloride. These solutions have been extensively used in many industries. The primary oxidant is chlorine, produced from electrolysis of the salts in the aqueous media (Thorn et al., 2012; Buck et al., 2002). The next candidate used to make ECAS in the halogen series is bromine. It is an effective biological control agent. It rapidly degrades to harmless bromide. Therefore, it is not a persistent pollutant. Bromine is produced by electrolysis of a nonhazardous aqueous solution of NaBr and chloride. The electrolytic bromine can completely replace hazardous/toxic chemical biocides for the control of microorganisms in cooling towers (Timothy, 2007). Anaerobic bacteria are more sensitive to oxidizing compounds in comparison to aerobic bacteria, and therefore electrochemically generated biocides are powerful antianaerobes. Bacterial spores are highly resistant to many traditional organic biocides, whereas these ECAS have been effective against a number of pathogens, including *Bacillus atrophaeus*, *B.* cereus, *Bacillus anthracis*, human norovirus, hepatitis B virus, HIV, *Aspergillus flavus*, *C. albicans*, *S. aureus*, *P. aeruginosa*, *Enterococcus faecalis*, *Clostridium difficile, Cryptosporidium parvum* oocysts, and staphylococcal enterotoxin (Venczel et al., 1997; Shetty et al., 1999; Kim et al., 2000; Rogers et al., 2006; Park et al., 2007; Tagawa et al., 2000; Xiong et al., 2010; Morita et al., 2000; Zeng et al., 2011; Buck et al., 2002; Holcomb et al., 2005; Robinson et al., 2010; Raad and Sherertz, 2001).

In a study by Gareth et al. (2013), acidic ECAS were stored at temperatures of 4 and 20 °C for 398 days to examine the changes in the free chloride content, pH,

oxidation-reduction potential, and antimicrobial activity. The results showed a faster degradation at the higher temperature than at the lower temperature, with a concomitant decline in bactericidal capacity, whereas the pH did not change. The use of high concentrations of biocides can be avoided by adding greener biocide enhancers, such as ethylenediamine disuccinate (EDDS); its usage in the laboratory allowed the application of less glutaraldehyde to the test biofilms formed by sulfate-reducing bacteria. EDDS, which is a biodegradable chelator, increases the effectiveness of glutaraldehyde in its treatment of sulfate-reducing bacteria biofilms. This concept was initially used by Raad and Sherertz (2001) to treat biofilms on catheters (Raad et al., 2003, 2007) but with the new concept of green materials, ethylenediaminetetraacetic acid (EDTA) was a noncompatible molecule because of its slow biodegradability, and it was replaced with environmentally compatible chelating agents. The activity of biocides such as THPS can be increased by the use of EDDS.

Eversdijk et al. (2012) designed a biocide controlled-release system that consisted of modified nanoclay particles incorporated with different model biocides, and studied their release and antifungal activities under different environmental conditions. This composite strategy allowed the production of a tunable system to control the release rate and extend its effectiveness to 45 days during an artificially generated rain test. This approach appears promising for the prolonged protection of different construction materials and waterborne paints. Routine coatings last for an average of half a year or a maximum of 2 years, whereas the desired service life in buildings is at least 10 years. The effect of a controlled-release system on biocide concentrations over time was studied. Increasing the dose of a biocide was found to result in a minor prolongation of protection, but increased in-house environmental pollution. To meet the challenges to material performance and environmental safety, such durable composite materials are a beam of hope for safer green applications (BPD, Directive 98/8/EC, 2012).

Eguıa and Trueba (2007) applied marine biotechnology to produce environmentally benign water-based coatings using lower-toxicity elements and bacteria isolated from surfaces immersed in marine water. These promising coatings were able to reduce the problem of biofouling while having no adverse effects on the surrounding environment.

Electrochemically generated biocides showed an effective way to control microbial problems at a paper mill (Ullah, 2011). They can be added to water or pulp and they have hardly any negative effect on the process or end-product. For paper makers, this study is of great interest because onsite-generated biocides are low cost solutions based on actual biocide need. Onsite-generated biocides also eliminate the storage and transportation of biocides and provide a basis for building a new control program. These electrochemically generated biocides are fast-acting, powerful biocide agents, used during all stages of disinfection and

cleaning, applied in liquid, aerosol or frozen forms, chemical residue-free, generated onsite or in concentrated amounts for imminent use, eliminating handling and storage issues, and produced from municipal tap water and salt.

Flores et al. (2013) studied the ability of citrate-capped silver nanoparticles (AgNPs) to stop the formation of bacterial biofilms by *S. aureus* and *P. aeruginosa*. These are clinically important gram-positive and gram-negative bacteria. They used two different approaches. The first approach involved the dispersal of AgNPs to study the bactericidal effect on planktonic bacteria, whereas the second approach was to adsorb AgNPs on a titanium substrate to test their bactericidal effect on sessile bacteria. Planktonic *P. aeruginosa* was found to be more susceptible to nanoparticles than was *S. aureus*, which can be attributed to their different cell wall structures. Similar results were obtained for the sessile species. The results also revealed that performance was improved by using titanium-based implants, even at very low concentrations.

Hussein et al. (2013) produced chitosan (a biopolymer), by extracting the exoskeletons of crustaceans found in seafood waste. It was 85.2% deacetylated and had an average molecular weight of 109 kDa. Chitosan was derivatized, forming 2-N,N-diethylbenzene ammonium chloride N-oxoethyl chitosan, called compound I, and 12-ammonium chloride N-oxododecan chitosan, ecofriendly inhibitors of carbon steel corrosion in low-pH media. The results demonstrated that compound I was the more efficient corrosion inhibitor. Comparing the antibacterial activity of chitosan and its derivatives indicated that chitosan (compound II) was more effective than its derivatives against several bacteria: *C.* albicans, *E. coli*, *E. faecalis*, and *S. aureus*.

Yuan et al. (2012) attempted to reduce biocorrosion by sulfate-reducing bacteria. This approach involved a combination of surface-initiated atom transfer radical polymerization and in situ chemical oxidative graft polymerization techniques. Stainless steel was tethered with a poly(4-vinylaniline)–polyaniline (PVAn-PANI) bilayer layer coating. This method involved three steps: in the first step, a trichlorosilane coupling agent was immobilized on the stainless steel surface, providing sulfonyl halide groups for the ATRP of PVAn; in the second step, PANI was grafted onto the PVAn in situ; in the third step, the PVAn-PANI bilayer reduced bacterial adhesion and biofilm formation on the stainless steel surface, as showed by electrochemical test results, confirming that their anticorrosion and antibacterial properties were suitable for severe environments.

Hakkinen et al. (2004) has reported electrochemical microbial antifouling technology for the prevention of biofilm and inorganic deposits on stainless steel surfaces. This is based on polarization of the steel surface. A preventive current is passed from an electrode to the (metal) surface to be protected through an electrolyte, leading to oxidation and reduction reactions. These reactions temporarily change the pH of the metal surface. This creates

conditions that prevent microbial attachment. Several UPM mills are running full-scale microbial antifouling technology systems successfully.

8.11 Other Techniques in Biofilm Treatment

Research work for exploring possibilities of photocatalytic TiO_2 coating for reducing biofilms on nonliving surfaces has been investigated by Raulio et al. (2006). The model organism, *Deinococcus geothermalis*, known to initiate growth of durable, colored biofilms on machine surfaces in the paper industry, was allowed to form biofilms on stainless steel, glass and TiO_2 film-coated glass or titanium. When biofilms on photocatalytic TiO_2 surfaces, submerged in water, were exposed to 20 W h m^{-2} of 360 nm light, both kinds of adhesion threads were completely destroyed and the *D. geothermalis* cells were extensively removed (from >107 down to below 106 cells cm^{-2}). TiO_2 films prepared by the sol–gel technique were slightly more effective than those prepared by the ALD technique. Doping of the TiO_2 with sulfur did not enhance its biofilm-destroying capacity. The results show that photocatalytic TiO_2 surfaces have potential as a self-cleaning technology for warm water using industries.

Also antifouling potential of electric polarization combined and not combined with biocides was studied in nonsaline warm water with high organic content by Peltola et al. (2011). When *D. geothermalis* biofilms grown for 24 h in simulated paper machine water were exposed to cathodic or cathodically weighted pulsed polarization at least 60% ($P<0.05$) of the biofilms were removed from stainless steel (AISI 316L). Biofilm removal by 25 ppm (effective substances 5–25 ppm) of oxidizing biocides (bromochloro-5,5-dimethylhydantoin, 2,2-dibromo-2-cyanoacetamide, peracetic acid) increased to 70% when combined with cathodically weighted pulsed polarization. Using a novel instrument that allows real-time detection of reactive oxygen species, these researchers showed that the polarization program is effective in antifouling generated reactive oxygen species in a pulsed manner on the steel surface. They suggested that the observed added value of oxidative biocides combined with polarization depended on reactive oxygen species. This suggestion was supported by the finding that a reductive biocide, methylene bisthiocyanate, counteracted the antifouling effect of polarization (Table 8.39).

Table 8.39: Comparison of different methods used for biofilm prevention at paper mills

Methods	Effectiveness	Ease of use at mill	Cost/ton of paper
Physical cleaning	High	Challenging and slow	1–2 euros
Biocide treatment	High	Easy and fast	1–4 euros
Biodispersants	Low	Easy and slow	1–4 euros
Electrochemical treatment	High	Challenging and slow	5–10 euros

Based on https://noppa.lut.fi/noppa/opintojakso/.../biocides_case_study.pdf.

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