Chapter 29

Disinfection

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The destruction or prevention of growth of microorganisms is essential for the control of infectious disease transmission and preservation of foodstuffs and biodegradable materials. This is most commonly accomplished by heat, chemicals, filtration or radiation. Heat acts to kill or inactivate by denaturation of essential proteins (enzymes, viral capsids) and nucleic acids. Chemicals may act by many different means to kill organisms or prevent their growth, including destruction of membranes and cell walls, and interference with enzymic action and replication of nucleic acids (Table 29.1). Filtration is a process that acts to remove the organisms physically by size exclusion and does not result in destruction of the organism. Ultraviolet light and gamma radiation act directly on nucleic acids.

Sterilization is a process, physical or chemical, that destroys or eliminates all organisms. A sanitizer is an agent that reduces the number of bacterial contaminants to safe levels as judged by public health requirements. According to the official sanitizer test used in the United States, a sanitizer is a chemical that kills 99.9% of the specific test bacteria within 30 seconds under the conditions of the test (Block, 1991). A disinfectant is a physical or chemical agent that destroys disease-causing or other harmful microorganisms, but does not necessarily kill all microorganisms. Disinfectants are expected to kill more than 99.999% of the test organisms. Disinfectants are usually applied to water and inanimate objects (fomites) to control the spread of pathogenic microorganisms. They can also be used to treat foods and aerosols. A bacteriostat is usually a chemical agent that prevents the growth of bacteria but does not necessarily kill them. For example, silver is often added to activated carbon to prevent the growth of bacteria in home faucet-mounted water treatment devices.

29.1 THERMAL DESTRUCTION

The thermal destruction of microorganisms has been studied in great detail by the food industry because of the importance of this process in killing pathogenic bacteria and preventing foodborne spoilage. The thermal death of microorganisms is generally considered a first order relationship, i.e., linear with time. The time necessary to kill a given number of organisms at a specific temperature is called the thermal death time (TDT). The general procedure for determining TDT by these methods is to place a known number of organisms in a sufficient number of sealed containers to get the desired number of survivors for the test period. At the end of the heating period, the containers are quickly removed and cooled in cold water. Viability of the organism is assessed on standard culture media.

The TDTs of some foodborne and waterborne pathogens are shown in Table 29.2. The D value or decimal

I.L. Pepper, C.P. Gerba, T.J. Gentry: Environmental Microbiology, Third edition. DOI: http://dx.doi.org/10.1016/B978-0-12-394626-3.00029-6

TABLE 29.1 Mechanisms of Inactivation Used by Common Disinfectants

Target	Agent	Effect
Cell wall	Aldehydes Anionic surfactants	Interaction with $-NH_2$ groups Lysis
Cytoplasmic membrane	Quaternary ammonium compounds, biguanides, hexachlorophene	Leakage of low molecular weight material
Nucleic acids	Dyes, alkylating agents, ionizing and ultraviolet radiation	Breakage of bonds, cross-linking, binding of agents to nucleic acids
Enzymes or proteins	Metal ions (Ag, Cu) Alkylating agents Oxidizing agents (chlorine, hydrogen peroxide)	Bind to −SH groups of enzymes Combine with DNA or RNA Damage of bacterial cell membranes; damage of proteins and nucleic acid

From Block (1991).

TABLE 29.2 Thermal Death Times of Water- and Food-borne Pathogenic Organisms

Organism	Temperature (°C)/time (min)	Reference
Campylobacter spp.	75/1	Bandres *et al.*, 1988
Escherichia coli	65/1	Bandres *et al.*, 1988
Legionella	66/0.45[a]	Sanden *et al.*, 1989
Mycobacterium spp.	70/2	Robbecke and Buchholtz, 1992
M. avium	70/2.3[a]	
Salmonella spp.	65/1	Bandres *et al.*, 1988
Shigella spp.	65/1	Bandres *et al.*, 1988
Vibrio cholerae	55/1[a]	Roberts and Gilbert, 1979
Cryptosporidium parvum	72.4/1	Fayer, 1994
Giardia lamblia	50/1[a]	Cerva, 1955
Hepatitis A virus	70/10	Siegl *et al.*, 1984
Rotavirus	50/30	Estes *et al.*, 1979

[a]*In buffered distilled water.*

reduction time is the time required to destroy 90% of the organisms. This value is numerically equal to the number of minutes required for the survivors as a function of time curve to traverse one log (Figure 29.1). This is equal to the reciprocal of the slope of the survivor curve, and is a measure of the death rate of an organism. The temperature at which the D value is determined is given as a subscript. For example, the D value for *Clostridium perfringens* at 250°F is $D_{250} = 0.1$ to 0.2 (Jay, 1996). The z value refers to the degrees Fahrenheit required for the thermal destruction as a function of the temperature curve to traverse one log. This value is equal to the reciprocal of the slope of the TDT curve (Figure 29.2). Whereas D reflects the resistance of an organism to a specific temperature, z provides information on the relative resistance of an organism to different destructive temperatures; it allows the calculation of equivalent thermal processes at different temperatures. If, for example, 3.5 minutes at 140°F is considered to be an adequate process and $z = 8.0$, either 0.35 minutes at 148°F or 35 minutes at 132°F would be considered equivalent processes.

Spore-forming bacteria such as *Bacillus* and *Clostridium* are the most resistant to heat inactivation. Of the nonspore-forming waterborne and foodborne enteric pathogens, enteric viruses are the most heat resistant, followed by the bacteria and protozoa (Table 29.2). Parvoviruses are among the most heat-resistant viruses known (Eterpi *et al.*, 2009). In addition to the type of microorganism, factors that influence TDT in foods include water, fat, salts, sugars, pH and other substances. The heat resistance of microbial cells increases with decreasing humidity or moisture. Dried microbial cells are considerably more heat resistant than moist cells of the same type. Because protein denaturation occurs at a higher rate with heating in water than in air, it is likely that protein denaturation is closely associated with thermal death. The presence of fats and salts increases the heat resistance of some microorganisms. The effect of salt is variable and dependent on the kind of salt, concentration and cation. Cationic salts at molar concentrations greatly increase the thermal resistance of enteric viruses. For this reason, $MgCl_2$ is added to poliovirus vaccine to aid in extending its useful life. The presence of sugars causes an increase in the heat resistance of microorganisms, in part because of decreased water activity. Microorganisms are most

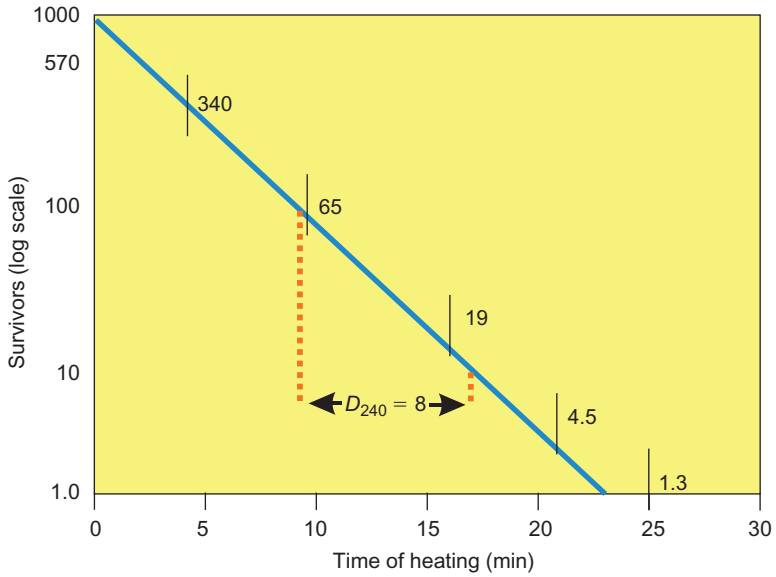

FIGURE 29.1 Thermal inactivation curve for a microorganism. The D value is the time required for inactivation of 90% of the organisms at a given temperature. In this case it required 8 minutes to kill 90% of the organisms at 240°F or $D_{240} = 8$ minutes.

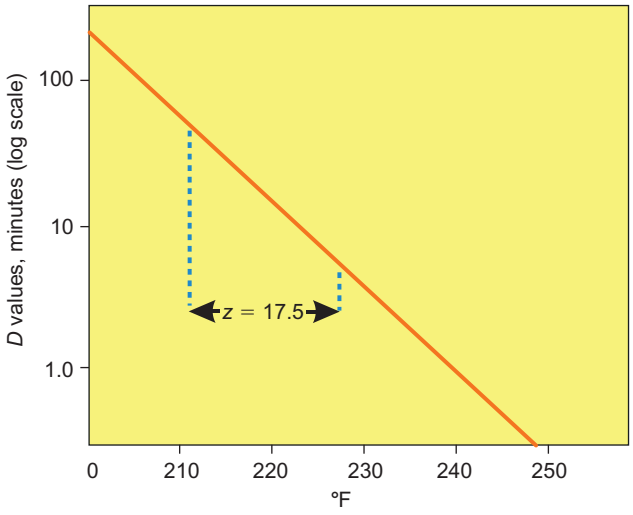

FIGURE 29.2 Thermal death time curve. The z value is equal to the degrees Fahrenheit required for the thermal destruction curve to traverse one log cycle.

resistant to heat at their optimal pH of growth, which is generally about 7.0. As the pH is lowered or raised from the optimal value, there is an increase in heat sensitivity. Thus, acid and alkaline foods require less heat processing than neutral foods. In water, suspended solids or organic matter increase heat resistance (Liew and Gerba, 1980).

29.2 KINETICS OF DISINFECTION

Inactivation of microorganisms is a gradual process that involves a series of physicochemical and biochemical steps. In an effort to predict the outcome of disinfection, various models have been developed on the basis of experimental data. The principal disinfection theory used today is still the Chick–Watson model, which expresses the rate of inactivation of microorganisms by a first-order chemical reaction.

$$N_t/N_0 = e^{-kt} \qquad \text{(Eq. 29.1)}$$

or

$$ln \;\; N_t/N_0 = -kt \qquad \text{(Eq. 29.2)}$$

where:

N_0 = number of microorganisms at time 0
N_t = number of microorganisms at time t
k = decay constant (1/time), and
t = time.

The logarithm of the survival rate (N_t/N_0) plots as a straight line versus time (Figure 29.3). Unfortunately, laboratory and field data often deviate from first-order kinetics. Shoulder curves may result from clumps of organisms or multiple hits of critical sites before inactivation. Curves of this type are common in disinfection of coliform bacteria by chloramines (Montgomery, 1988). The tailing-off curve, often seen with many disinfectants, may be explained by the survival of a resistant subpopulation as a result of protection by interfering substances (suspended matter in water), clumping or genetically conferred resistance.

In water applications, disinfectant effectiveness can be expressed as $C \cdot t$, where C is the disinfectant concentration and t the time required to inactivate a certain

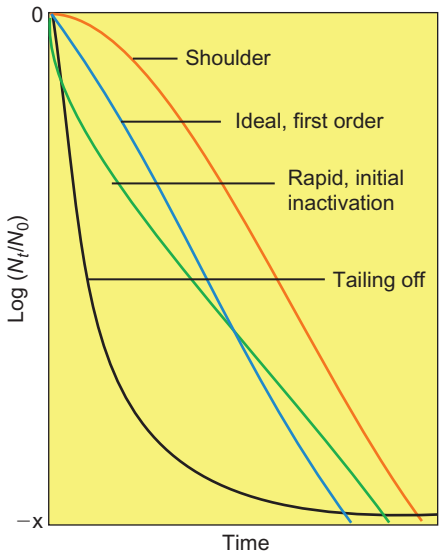

FIGURE 29.3 Types of inactivation curves observed for microorganisms.

percentage of the population under specific conditions (pH and temperature). Typically, a level of 99% inactivation is used when comparing $C \cdot t$ values. The lower the $C \cdot t$ value, the more effective the disinfectant. The $C \cdot t$ method allows a general comparison of the effectiveness of various disinfectants on different microbial agents (Tables 29.3 through 29.6). It is used by the drinking water industry to determine how much disinfectant must be applied during treatment to achieve a given reduction in pathogenic microorganisms. $C \cdot t$ values for chlorine for a variety of pathogenic microorganisms are shown in Table 29.3. The order of resistance to chlorine and most other disinfectants used to treat water is protozoan cysts > viruses > vegetative bacteria.

29.3 FACTORS AFFECTING DISINFECTANTS

Numerous factors determine the effectiveness and/or rate of kill of a given microorganism (Figure 29.4). Temperature has a major effect as it controls the rate of chemical reactions. Thus, as temperature increases, the rate of kill with a chemical disinfectant increases. The pH can affect the ionization of the disinfectant and the viability of the organism. Most waterborne organisms are adversely affected by pH levels below 3 and above 10. In the case of halogens such as chlorine, pH controls the amount of HOCl (hypochlorous acid) and $^{-}$OCl (hypochlorite) in solution (Figure 29.5). HOCl is more effective than $^{-}$OCl in the disinfection of microorganisms. With chlorine, the $C \cdot t$ increases with pH. Attachment of organisms to surfaces or particulate matter in water such as clays and organic detritus aids in the resistance of microorganisms to disinfection. Particulate matter may interfere by either acting chemically to react with the disinfectant, thus neutralizing the action of the disinfectant, or physically shielding the organism from the disinfectant (Stewart and Olson, 1996). The particulate–microbial complex may be thought of as:

- Adsorption of microbes to larger particles
- Adsorption of small particles to the surface of the microbe
- Encasement of the microbe by one or more large particles or many associated small particles

Disinfectant protection is enhanced with decreasing size of the organism and increasing particle availability. Therefore, viruses are afforded greater protection than bacteria. For these reasons, particulate or turbidity removal in drinking water treatment is necessary to ensure the effectiveness of disinfection in the destruction of waterborne pathogens. Dissolved chemical substances that interfere with chemical disinfection include organic compounds, inorganic and organic nitrogenous compounds, iron, manganese and hydrogen sulfide.

Studies have demonstrated that pathogenic and indicator bacteria occurring in the natural environment may be more resistant to disinfectants than laboratory-grown bacteria. This resistance is cell mediated and physiological in nature, requiring that the organism develop adaptive features to survive under adverse environmental conditions (Stewart and Olson, 1996). Cell-mediated mechanisms of resistance to disinfectant agents are poorly understood compared with physicochemical protective effects. Examples of cell-mediated resistance include:

- Polymer or capsule production, which may act to limit diffusion of the disinfectant into the cell
- Cellular aggregation, providing physical protection to internal cells
- Cell wall and/or cell membrane alterations that result in reduced permeability to disinfectants
- Modification of sensitive sites, i.e., enzymes (Stewart and Olson, 1996)
- Efflux pumps to enhance removal of the substance from the bacteria (as in the case of metals and some antibiotics)
- Production of proteins to sequester metal ions

It has been speculated that many of these physiological events are a function of adaptation to low-nutrient conditions in the environment.

Repeated exposure of bacteria and viruses to strong oxidizing agents like chlorine may result in some selection for greater resistance (Bates *et al.*, 1977; Haas and Morrison, 1981). However, the enhanced resistance is not great enough to overcome concentrations of chlorine applied in practice.

TABLE 29.3 $C \cdot t$ Values for Chlorine Inactivation of Microorganisms in Water (99% Inactivation)[a]

Organism	°C	pH	$C \cdot t$
Bacteria			
Escherichia. coli	5	6.0	0.04
E. coli	23	10.0	0.6
Legionella. pneumophila	20	7.7	1.1
Mycobacterium avium (strain A5)	23	7.0	106
Mycobacterium avium (strain 1060)	23	7.0	204
Helicobacter pylori	5	6.0	0.12
Viruses			
Polio 1	5	6.0	1.7
Echo 1	5	6.0	0.24
Echo 1	5	7.8	0.56
Echo 1	5	10.0	47.0
Coxsackie B5	5	7.8	2.16
Coxsackie B5	5	10.0	33.0
Adenovirus 40	5	7.0	0.15
Protozoa			
Giardia lamblia cysts	5	6.0	54–87
Giardia lamblia cysts	5	7.0	83–133
Giardia lamblia cysts	5	8.0	119–192
Naegleria fowleri tropozoites	25	7.5	6
N. fowleri cysts	25	7.5	32.1
Encephalitozoon intestinalis spores	5	7.0	39
Cryptosporidium parvum oocysts	21–24	7.5–7.6	9740–11,300
Toxoplasma gondii	22	7.2	>133,920
Fungi			
Aspergillus fumigatus	25	7.0	946
A. terrus	25	7.0	1404
Penicillium citrirnum	25	7.0	959
Cladosporium tenuissimum	25	7.0	71

From Sobsey (1989); Rose et al. *(1997); Gerba* et al *(2003); Wainwright* et al. *(2007). Shields* et al. *(2008) Sarkar and Gerba (2012); Pereira* et al. *(2013).*

[a]*In buffered distilled water.*

29.4 HALOGENS

29.4.1 Chlorine

Chlorine and its compounds are the most commonly used disinfectants for treating drinking and wastewater. Chlorine is a strong oxidizing agent which, when added as a gas to water, forms a mixture of hypochlorous acid (HOCl) and hydrochloric acids:

$$Cl_2 + H_2O \equiv HOCl + HCl \qquad \text{(Eq. 29.3)}$$

In dilute solutions, little Cl_2 exists in solution. The disinfectant's action is associated with the HOCl formed. Hypochlorous acid dissociates as follows:

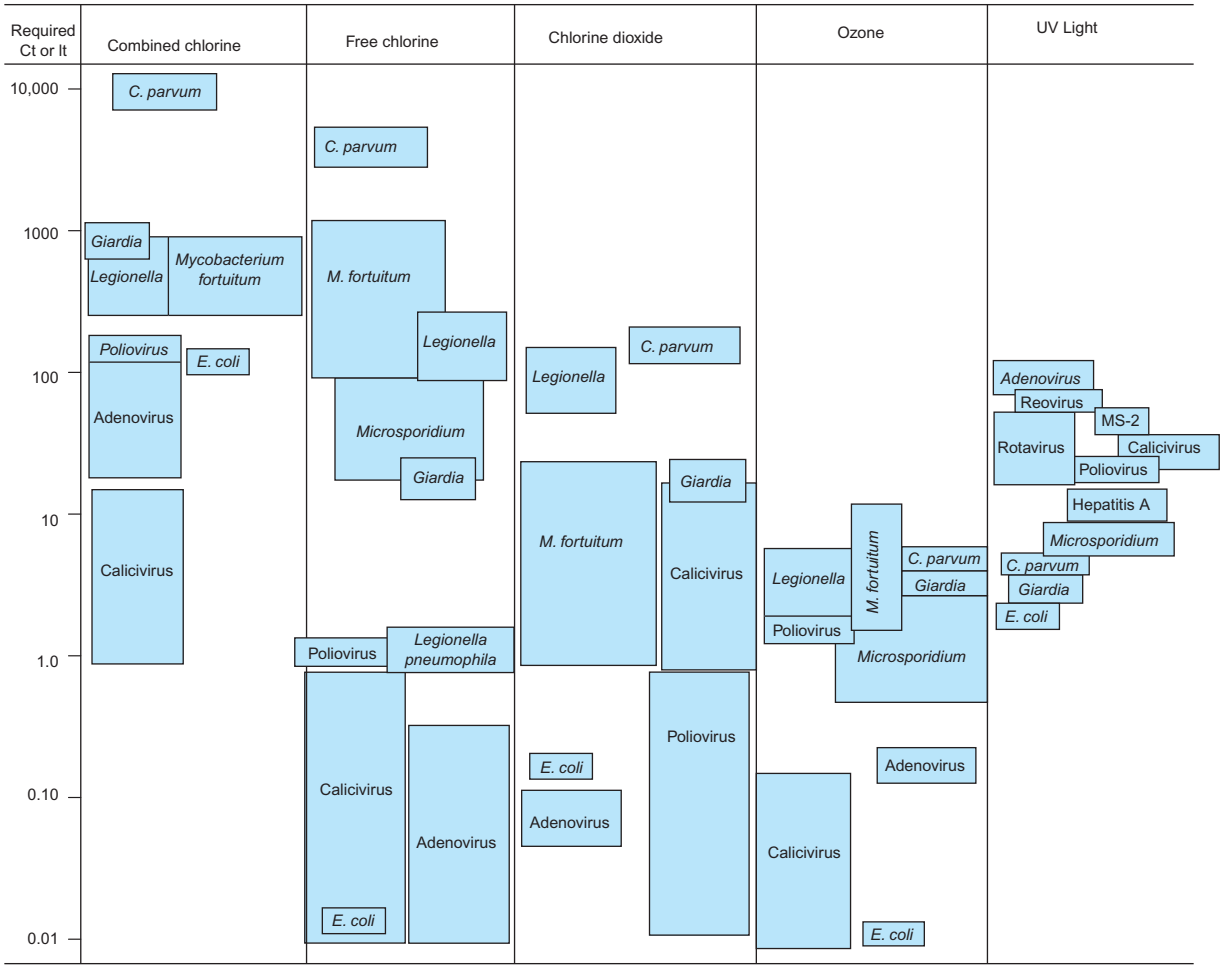

FIGURE 29.4 Overview of disinfection requirements for 99% inactivation of microorganisms. Ct = concentration of disinfectant × time. It = (μW s/cm^2) (time). Adapted from Jacangelo *et al.* (1997).

$$HOCl \equiv +H^+OCl^- \qquad \text{(Eq. 29.4)}$$

The ratio of hypochlorous acid and $^-$OCl (hypochorite ion) depends on the pH of the water (Figure 29.5). The amount of HOCl is greater at neutral and lower pH levels, resulting in greater disinfection ability of chlorine at these pH levels. Chlorine as HOCl or $^-$OCl is defined as free available chlorine. HOCl combines with ammonia and organic compounds to form what is referred to as combined chlorine. The reactions of chlorine with ammonia and nitrogen-containing organic substances are of great importance in water disinfection. These reactions result in the formation of monochloramine, dichloramine, trichloramine, etc.

$$NH_3 + HOCl \rightarrow \underset{\text{monochloramine}}{NH_2Cl} + H_2O \qquad \text{(Eq. 29.5)}$$

$$NH_2Cl + HOCl \rightarrow \underset{\text{dichloramine}}{NHCl_2} + H_2O \qquad \text{(Eq. 29.6)}$$

$$NHCl_2 + HOCl \rightarrow \underset{\text{trichloramine}}{NCl_3} + H_2O \qquad \text{(Eq. 29.7)}$$

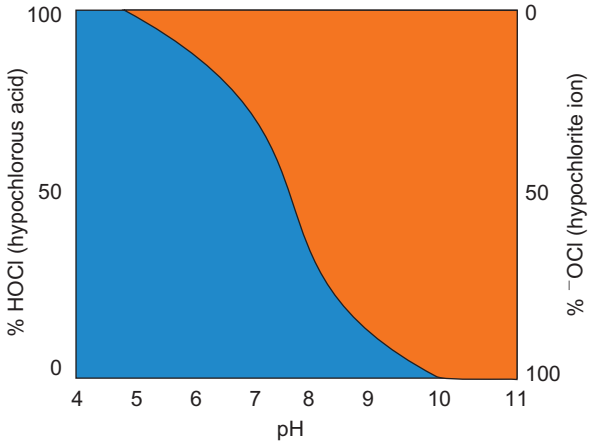

FIGURE 29.5 Distribution of HOCl and $^-$OCl in water as a function of pH. From Bitton (2011).

Such products retain some disinfecting power of hypochlorous acid, but are much less effective at a given concentration than chlorine.

Free chlorine is quite efficient in inactivating pathogenic microorganisms. In drinking water treatment, 1 mg/L or less for about 30 minutes is generally sufficient to reduce significantly bacterial numbers. The presence of interfering substances in wastewater reduces the disinfection efficacy of chlorine, and relatively high concentrations of chlorine (20−40 mg/L) are required (Bitton, 2011). Enteric viruses and protozoan parasites are more resistant to chlorine than bacteria (Table 29.3) and can be found in secondary wastewater effluents after normal disinfection practices. *Cryptosporidium* and *Toxoplasma* oocysts are extremely resistant to chlorine. A chlorine concentration of 100 mg/L is necessary to cause 99% inactivation of *Cryptosporidium* following a 100-minute contact time (Table 29.3). Chloramines are much less efficient than free chlorine (about 50 times less efficient) in inactivation of viruses.

Being a strong oxidizing agent, chlorine will react with any organic molecule including proteins, lipids, carbohydrates and nucleic acids to disrupt their structure. Bacterial inactivation by chlorine may result from (Stewart and Olson, 1996):

- Altered permeability of the outer cellular membrane, resulting in leakage of critical cell components
- Interference with cell-associated membrane functions (e.g., phosphorylation of high-energy compounds)
- Impairment of enzyme and protein function as a result of irreversible binding of the sulfyhydryl groups
- Nucleic acid denaturation

The actual mechanism of chlorine inactivation may involve a combination of these actions, or merely the effect of chlorine on a few critical sites. It appears that bacterial inactivation by chlorine is primarily caused by impairment of physiological functions associated with the bacterial cell membrane.

Chlorine may inactivate viruses by interaction with the viral capsid proteins or/and the nucleic acid (Figure 29.6). The site of action may also depend on the concentration of chlorine and the type of virus. It has been found that at free chlorine concentrations of less than 0.8 mg/L, inactivation of poliovirus RNA occurs without major structural changes, whereas chlorine concentrations in excess of 0.8 mg/L result in damage to the viral RNA and protein capsid (Alvarez and O'Brien, 1982) (Figure 29.11). The protein coat appears to be the target for the double-stranded RNA rotaviruses (Vaughn and Novotny, 1991). The protein involved in the binding to the host bacterium was found be involved with the loss of infectivity in MS 2 phage (Wigginton *et al*., 2012) (Figure 29.7).

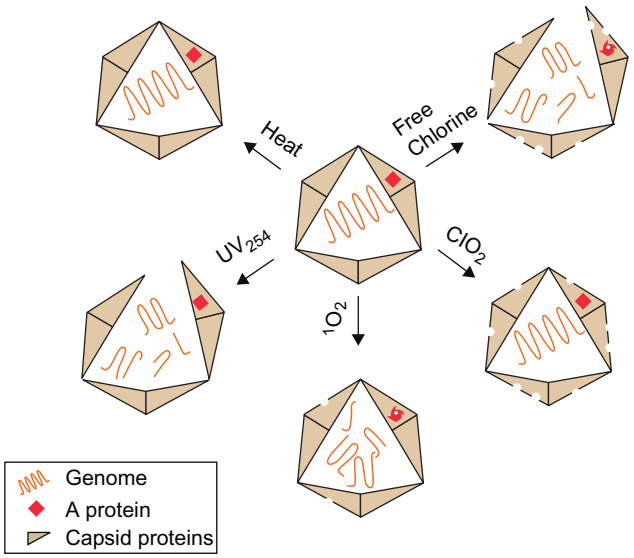

FIGURE 29.6 Mechanisms of MS 2 virus inactivation by disinfectants. Wigginton *et al*. (2012).

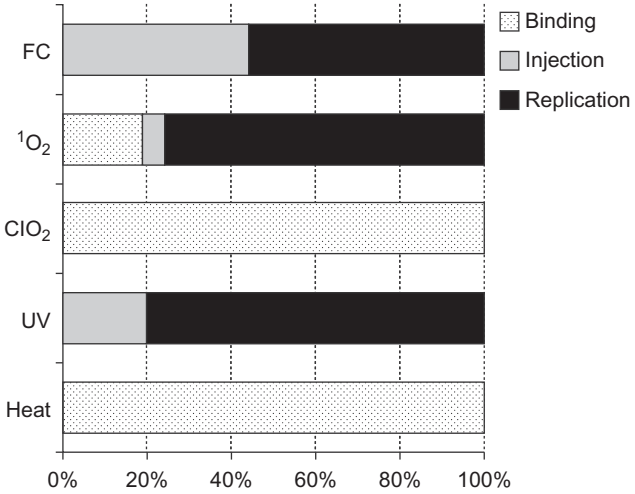

FIGURE 29.7 Relative contributions of MS 2 host binding protein, nucleic acid injection and replication to overall inactivation by different disinfectants. Wigginton *et al*. (2012).

29.4.2 Chloramines

Inorganic chloramines are produced by combining chlorine and ammonia (NH_4) for drinking water disinfection. The species of chloramines formed (see Eqs. 29.5 through 29.7) depends on a number of factors, including the ratio of chlorine to ammonia-nitrogen, chlorine dose, temperature and pH. Up to a chlorine-to-ammonia mass ratio of 5, the predominant product formed is monochloramine, which demonstrates greater disinfection capability than other forms such as dichloramine and trichloramine. Chloramines are used to disinfect drinking water by some utilities in the United States, but because they are slow acting, they have mainly been used as secondary disinfectants when a residual in the distribution system is desired. For example, when ozone is used to treat drinking water,

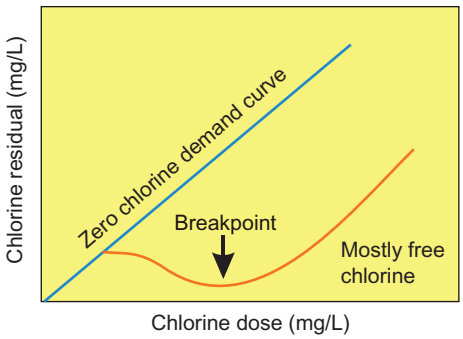

FIGURE 29.8 Dose–demand curve for chlorine.

no residual disinfectant remains. Because bacterial growth may occur after ozonation of tap water, chloramines are added to prevent regrowth in the distribution system. In addition, chloramines have been found to be more effective in controlling biofilm microorganisms on the surfaces of pipes in drinking water distribution systems because they interact poorly with capsular polysaccharides (LeChevallier *et al.*, 1990).

Because of the occurrence of ammonia in sewage effluents, most of the chlorine added is converted to chloramines. This demand on the chlorine must be met before free chorine is available for disinfection. As chlorine is added, the residual reaches a peak (formation of mostly monochloramine), and then decreases to a minimum called the breakpoint (Figure 29.8). At the breakpoint, the chloramine is oxidized to nitrogen gas in a complex series of reactions summarized in Eq. 29.8:

$$2NH_3 + 3HOCl \rightarrow N_2 + 3H_2O + 3HCl \qquad \text{(Eq. 29.8)}$$

Addition of chlorine beyond the breakpoint ensures the existence of free available chlorine residual.

Although numerous studies have been conducted to determine the mode of microbial inactivation by free chlorine, there have been fewer studies concerning chloramine inactivation mechanisms. It should be noted, however, that because of the poorly controlled experimental conditions employed by early investigators, many of the postulated chlorine inactivation mechanisms may have involved the action of chloramines rather than free chlorine. Research to date indicates that chloramines primarily inactivate microorganisms by irreversible denaturation of proteins (Stewart and Olson, 1996). Chloramine inactivation of bacteria is caused primarily by the oxidation of sulfyhydryl-containing enzymes, and to a lesser extent, a reaction with nucleic acid. In contrast to chlorine, there are no existing data to suggest that chloramines can modify the permeability state of the cell. Viral inactivation by chloramines is similar to the mechanism of inactivation by chlorine, in which primary targets consist of both capsid proteins and nucleic acid.

29.4.3 Chlorine Dioxide

Chlorine dioxide is an oxidizing agent that is extremely soluble in water (five times more than chlorine) and, unlike chlorine, does not react with ammonia or organic compounds to form trihalomethane, which is potentially carcinogenic. Therefore, it has received attention for use as a drinking water disinfectant. Chlorine dioxide must be generated on site because it cannot be stored. It is generated from the reaction of chlorine gas with sodium chlorite:

$$2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2NaCl \qquad \text{(Eq. 29.9)}$$

Chlorine dioxide does not hydrolyze in water, but exists as a dissolved gas.

Studies have demonstrated that chlorine dioxide is as effective as, or more effective in inactivating bacteria and viruses in water than, chlorine (Table 29.4). As is the case with chlorine, chlorine dioxide inactivates microorganisms by: denaturation of the sulfyhydryl groups contained in proteins (Stewart and Olson, 1996); inhibition of protein synthesis (Bernarde *et al.*, 1967); denaturation of nucleic acid; and impairment of permeability control.

Studies with bacteriophage have suggested that the protein in the capsids is irreversibly damaged by chlorine dioxide (Figure 29.7). However, studies with poliovirus have suggested that the viral RNA is separated from the capsid during treatment (Vaughn and Novotny, 1991). The viricidal efficiency of chlorine dioxide increases as the pH is increased from 4.5 to 9.0 (Chen and Vaughn, 1990).

29.4.4 Bromine and Iodine

Bromine undergoes reactions in water similar to those of chlorine. However, its disinfecting capacity and mode of action differ from those of chlorine. The primary use of bromine is limited to hot tubs or spas and certain industrial applications (cooling towers). It is not as fast acting as chlorine, but is effective against bacteria (*Legionella*), viruses and protozoan parasites (*Entamoeba histolytica*). Bromine appears primarily to attach to the protein of viruses without causing structural damage (Keswick *et al.*, 1981). It does not appear to be able to penetrate the protein coat to inactivate the viral RNA.

Iodine has been used as a disinfectant primarily for small-scale water treatment needs such as those of campers, the space shuttle and small water treatment systems. On a comparative mg/L basis, more iodine than chlorine is required for a comparative bacterial kill. Iodine reacts in water as follows:

$$I + H_2O \approx \underset{\text{(iodine hydrolysis)}}{HOI} + H^+ + I^- \qquad \text{(Eq. 29.10)}$$

$$3I_2 + 3H_2O \approx \underset{\text{(iodate formation)}}{IO_3^-} + 5I^- + 6H^+ \quad \text{(Eq. 29.11)}$$

Iodide and iodate are primarily formed above pH 8.0, and have no viricidal action and little action against bacteria. Thus, at pH 9.0 HOI is the dominant form, whereas at pH 5.0, I_2 is dominant. At low pH iodine is more effective against some protozoan cysts because they are more sensitive to I_2 than to HOCl (Gottardi, 1991). This behavior is explained by the higher diffusibility of molecular iodine through the cell walls of cysts.

Iodine is not effective against all protozoa. For example, while *Giardia* cysts can be inactivated by iodine, *Cryptosporidium* oocysts are very resistant (Gerba *et al.*, 1997). In contrast to protozoa, viruses are more readily inactivated at pH levels above 7.0 because of the stronger oxidizing power of HOI. Iodine displays first-order inactivation kinetics, indicating single-site inactivation. Iodine oxidizes sulfhydryl groups and tryptophan and, perhaps more importantly, substitutes tyrosyl on histidyl moieties at neutral pH and room temperature. Structural changes in viral integrity have been noted by electron microscopy after treatment with iodine, and thus infectious RNA could be released into the environment.

29.5 OZONE

Ozone (O_3), a powerful oxidizing agent, can be produced by passing an electric discharge through a stream of air or oxygen. Ozone is more expensive than chlorination to apply to drinking water, but it has increased in popularity as a disinfectant because it does not produce trihalomethanes or other chlorinated byproducts, which are suspected carcinogens. However, aldehydes and bromates may be produced by ozonation, and may have adverse health effects. Because ozone does not leave any residual in water, ozone treatment is usually followed by chlorination or addition of chloramines. This is necessary to prevent regrowth of bacteria because ozone breaks down complex organic compounds present in water, into simpler ones that serve as substrates for growth in the water distribution system. The effectiveness of ozone as a disinfectant is not influenced by pH and ammonia.

Ozone is a much more powerful oxidant than chlorine (Tables 29.3 and 29.6). The $C \cdot t$ values for 99% inactivation are only 0.0011–0.2 for enteric bacteria and 0.04–0.42 for enteric viruses (Bitton, 2011). Ozone appears to inactivate bacteria by the same mechanisms as chlorine-based disinfection: by disruption of membrane

TABLE 29.4 $C \cdot t$ **Values for Chlorine Dioxide Inactivation of Microorganisms in Water (99% Inactivation)**

Microbe	ClO_2 Residual (mg/L)	Temperature (°C)	pH	% Reduction	$C \cdot t$
Bacteria					
Escherichia coli	0.3–0.8	5	7.0	99	0.48
B. subitilis spores		21	8.0	99	25
Viruses					
Polio 1	0.4–14.3	5	7.0	99	0.2–6.7
Rotavirus SA11					
Dispersed	0.5–1.0	5	6.0	99	0.2–0.3
Cell-associated	0.45–1.0	5	6.0	99	1.7
Hepatitis A virus	0.14–0.23	5	6.0	99	1.7
Adenovirus 40	0.1	5	7.0	99	0.28
Coliphage MS2	0.15	5	6.0	99	5.1
Protozoa					
Giardia muris	0.1–5.55	5	7.0	99	10.7
Giardia muris	0.26–1.2	25	5.0	99	5.8
Giardia muris	0.21–1.12	25	7.0	99	5.1
Giardia muris	0.15–0.81	25	9.0	99	2.7
Cryptosporidium parvum		21	8.0	99	1000

Adapted from Sobsey (1989); Rose et al. *(1997); Charuet et al. (2001); Gerba* et al. *(2003).*

TABLE 29.5 $^{\circ}C \cdot t$ values for chloramine inactivation of microorganisms in water (99% inactivation)[a]

Microbe	°C	pH	$C \cdot t$
Bacteria			
Escherichia coli	5	9.0	113
Mycobacterium fortuitum	20	7.0	2667
Viruses			
Polio 1	5	9.0	1420
Echo 11	5	8.0	880
Hepatitis A	5	8.0	592
Adeno 2	5	8.0	990
Adeno 40	5	8.0	360
Coliphage MS2	5	8.0	2100
Rotavirus SA11			
Dispersed	5	8.0	4034
Cell-associated	5	8.0	6124
Protozoa			
Gardia muris	3	6.5–7.5	430–580
Gardia muris	5	7.0	1400
Cryptosporidium parvum	1	8.0	64,600
C. parvum	20	8.0	11,400

Adapted from Sobsey (1989); Rose et al. *(1997); Driedger* et al. *(2001); Cromeans* et al. *(2010).*
[a]*In buffered distilled water.*

TABLE 29.6 $C \cdot t$ values for Ozone Inactivation of Microorganisms in Water (99% Inactivation)

Organism	°C	pH	$C \cdot t$
Bacteria			
Escherichia coli	1	7.2	0.006–0.02
Viruses			
Polio 1	5	7.2	0.2
Polio 2	25	7.2	0.72
Rota SA11	4	6.0–8.0	0.019–0.064
Coxsackie B5	20	7.2	0.64–2.6
Adeno 40	5-7	7.0	0.02
Protozoa			
Giardia muris	5	7.0	1.94
Giardia lamblia	5	7.0	0.53
Encephalitozoon intestinalis	5	7.0	0.30.–0.04
Cryptosporidum parvum	1	—	40.0
C. parvum	7	—	7.0
C. parvum	22	—	3.5
Toxoplasma gondii	20	7.7–7.8	>69

From Sobsey (1989); Rose et al. *(1997) ; Gerba* et al. *(2003).*

permeability (Stewart and Olson, 1996) (Figure 29.6); impairment of enzyme function and/or protein integrity by oxidation of sulfyhydryl groups; and nucleic acid denaturation. The effect of ozone on destruction of the cell wall of bacteria and protozoan oocysts is dramatic (Figures 29.9 and 29.10). *Cryptosporidium* oocysts can be inactivated by ozone, but a $C \cdot t$ of 1–3 is required. Viral inactivation may occur by breakup of the capsid proteins into subunits, resulting in release of the RNA, which may then be damaged (Figure 29.11).

29.6 METAL IONS

Heavy metals such as copper, silver, zinc, lead, cadmium, nickel and cobalt all exhibit antimicrobial activity; however, because of toxicity to animals, only copper and silver have seen widespread application. Copper and silver have seen use as swimming pool and hot tub disinfectants. Copper has been used to control the growth of *Legionella* in hospital distribution systems. Surfaces containing 65% or more copper have been approved as self-sanitizing surfaces (U.S. EPA, 2012). Silver has been used as a bacteriostat added to the activated carbon used in faucet-mounted water treatment devices for home use. Concentrations of copper used in water disinfection range from 200 to 400 μg/L. Silver exhibits greater antimicrobial action and concentrations of 40–90 μg/L give the same effectiveness. The effectiveness of metal ions is influenced by pH, presence of anions and soluble organic matter. Unlike halogens and other oxidizing disinfectants, metals remain active for long periods of time in water. The rate of inactivation is slow compared with oxidizing agents (Figure 29.12); however, their action is enhanced in the presence of low concentrations of oxidizing agents such as chloramines (Straub *et al.*, 1995). The enhanced rate of inactivation is due to a synergistic interaction of both disinfectants.

Metal ions may inactivate bacteria or viruses by reacting outside or inside the cell or virus either directly or indirectly. It has been suggested that the inactivating capacity of heavy-metal ions is due to their oxidation

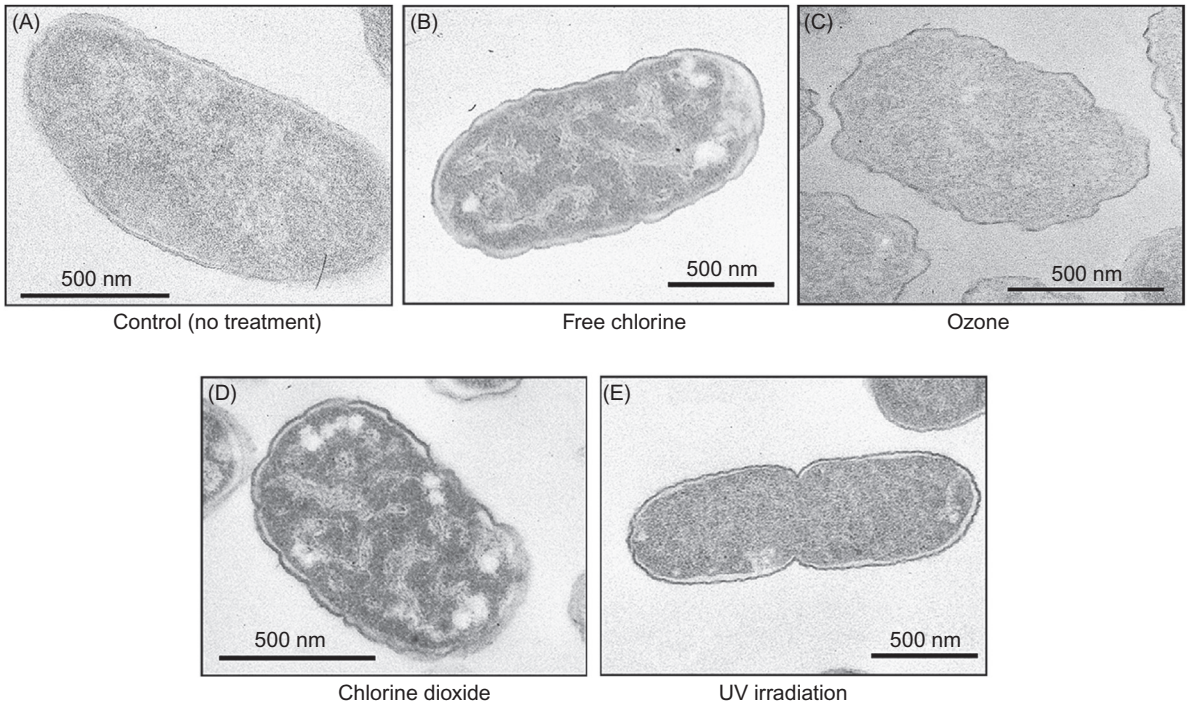

FIGURE 29.9 Transmission electron micrographs of *E. coli* before and after 90% inactivation by various disinfectants. Choi *et al.* (2010).

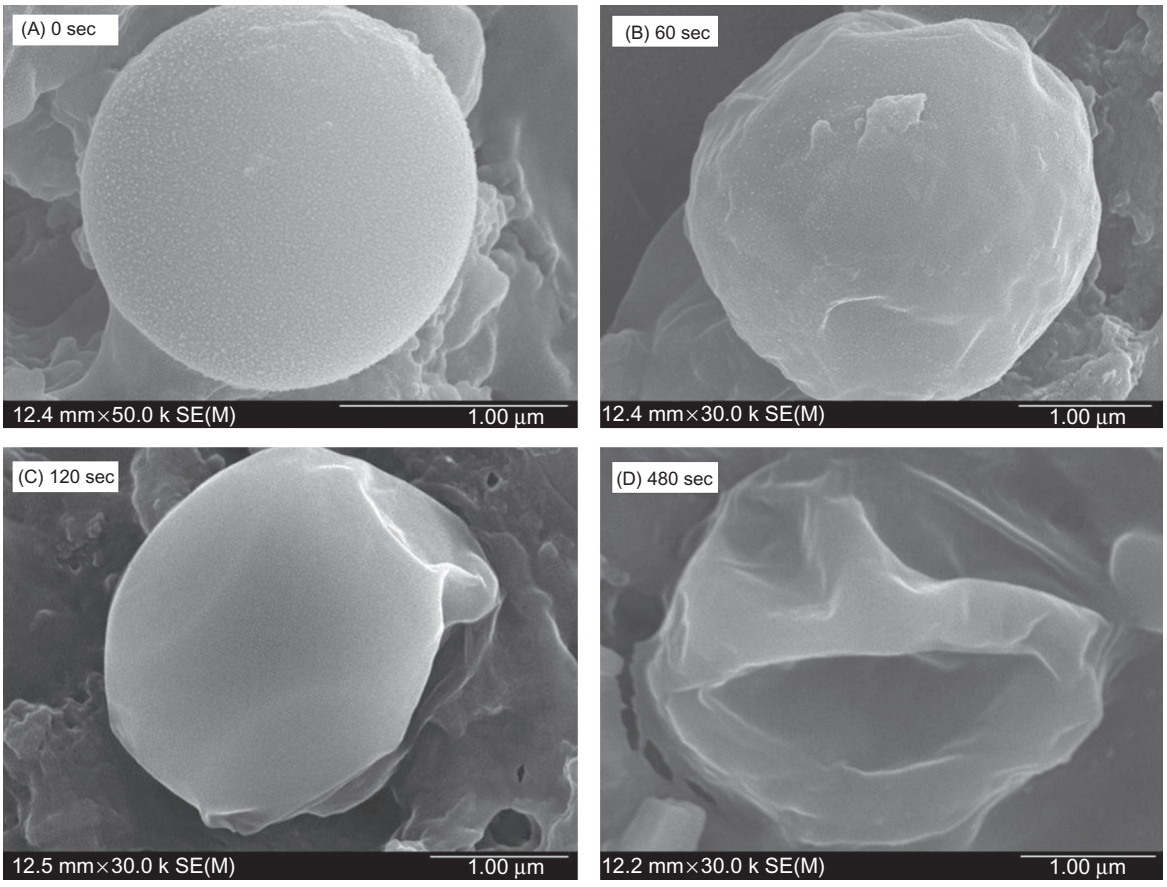

FIGURE 29.10 Scanning electron micrographs of *Cryptosporidium* oocysts after various time exposures to ozone. Ran *et al.* (2010).

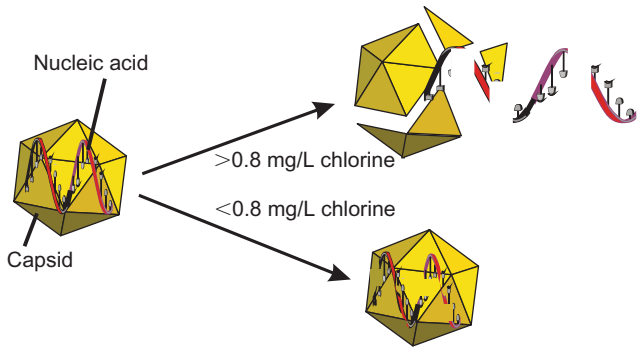

FIGURE 29.11 Virus inactivation by chlorine.

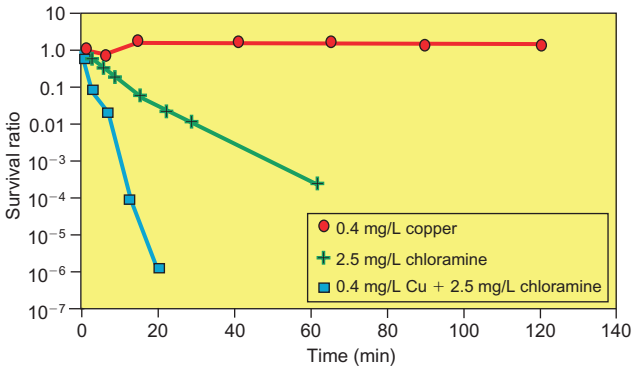

FIGURE 29.12 Synergistic inactivation of *Escherichia coli* by chloramines and copper. From Straub *et al.* (1995).

power, and that a functional relationship exists between the inactivation rate and the oxidation potential of the ion (Thurman and Gerba, 1989). Inactivation of the macromolecules (proteins or nucleic acids) is thought to involve site-specific Fenton mechanisms. It is assumed that the metal ion binds to a biological target and is reduced by superoxide radicals or other reductants and subsequently reoxidized by H_2O_2, generating hydroxide radicals (Figure 29.13). Repeated cyclic redox reactions may result in multi-hit damage as radical formation occurs near the target site. Copper and silver may bind to proteins, interfering with the normal function of enzymes, resulting in cell death. Silver readily reacts with sulfyhydryl groups in proteins. Metals may also bind to the nucleic acids, forming complexes that interfere with replication.

The action of metals is slow and may be reversed by addition of chelating agents. For example, assay of samples containing silver for bacteria will give lower counts if the silver is not first neutralized by addition of sodium thiosulfate–sodium thioglycolate to inhibit the bacteriostatic effect of silver (Chambers *et al.*, 1962).

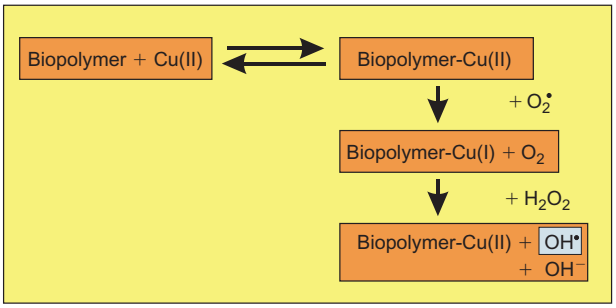

FIGURE 29.13 Modified site-specific Fenton mechanism. From Thurman and Gerba (1989).

29.7 ULTRAVIOLET DISINFECTION

The use of ultraviolet disinfection of water and wastewater has seen increased popularity because it is not known to produce carcinogenic or toxic byproducts or taste and odor problems, and there is no need to handle or store toxic chemicals. Unfortunately, it has several disadvantages including higher costs than halogens, no disinfectant residual, difficulty in determining the UV dose, maintenance and cleaning of UV lamps, and potential photoreactivation of some enteric bacteria (Bitton, 2011). However, advances in UV technology are providing lower cost, more efficient lamps and more reliable equipment. These advances have aided in the commercial application of UV for water treatment in the pharmaceutical, cosmetic, beverage and electronics industries in addition to municipal water and wastewater application.

Microbial inactivation is proportional to the UV dose, which is expressed in microwatt-seconds per square centimeter (μW-s/cm^2) or:

$$\text{UV dose} = I \cdot t \qquad \text{(Eq. 29.12)}$$

where $I = \mu\text{W/cm}^2$ and t = exposure time.

In most disinfection studies, it has been observed that the logarithm of the surviving fraction of organisms is nearly linear when it is plotted against the dose, where dose is the product of concentration and time ($C \cdot t$) for chemical disinfectants or intensity and time ($I \cdot t$) for UV. A further observation is that constant dose yields constant inactivation. This is expressed mathematically as:

$$\log \frac{N_s}{N_i} = \text{function}(I_i t) \qquad \text{(Eq. 29.13)}$$

where N_s is the density of surviving organisms (number/cm^3) and N_i is the initial density of organisms before exposure (number/cm^3). Because of the logarithmic relationship of microbial inactivation versus UV dose, it is common to describe inactivation in terms of log survival, as expressed in Eq. 29.14. For example, if one organism in 1000 survived exposure to UV, the result would be a -3 log survival, or a 3 log reduction:

$$\log \text{survival} = \log \frac{N_s}{N_i} \qquad \text{(Eq. 29.14)}$$

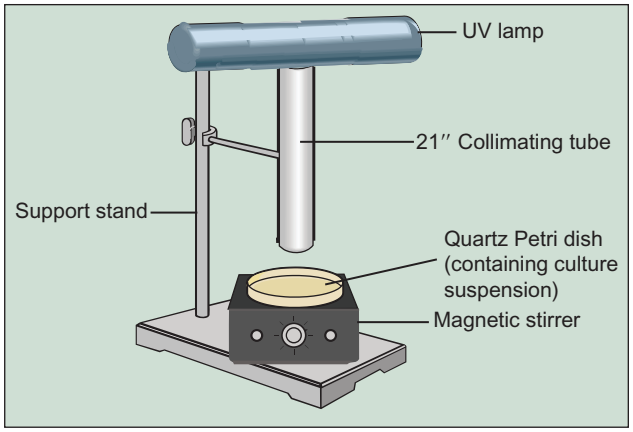

FIGURE 29.14 Collimating tube apparatus for UV dose application.

Determining the UV susceptibility of various indicator and pathogenic waterborne microorganisms is fundamental in quantifying the UV dose required for adequate water disinfection. Factors that may affect UV dose include cell clumping and shadowing, suspended solids, turbidity and UV absorption. UV susceptibility experiments described in the literature are often based on the exposure of microorganisms under conditions optimized for UV disinfection. Such conditions include filtration of the microorganisms to yield monodispersed, uniform cell suspensions, and the use of buffered water with low turbidity and high transmission at 254 nm. Thus, in reality, higher doses are required to achieve the same amount of microbial inactivation in full-scale flow through operating systems.

The effectiveness of UV light is decreased in wastewater effluents by substances that affect UV transmission in water. These include humic substances, phenolic compounds, lignin sulfonates and ferric iron. Suspended matter may protect microorganisms from the action of UV light; thus filtration of wastewater is usually necessary for effective UV light disinfection.

UV inactivation data are usually collected by placing a suspension of organisms in a stirred, flat, thin-layer dish in water with low UV light absorbance. In UV batch reactors, there are uniform UV intensities and contact time can be controlled. To deliver UV to these reactors, a collimating beam apparatus should be used (Figure 29.14). The light emitted at the end of the collimating beam is perpendicular to the batch reactor surface, thus creating a uniform, constant irradiation field that can be accurately quantified by means of a radiometer and photodetector calibrated for detecting 254-nm light. In general, the resistance of microorganisms to UV light follows the same pattern as the resistance to chemical disinfectants, i.e., double-stranded DNA viruses > MS 2 coliphage > bacterial spores > double-stranded RNA enteric viruses > single-stranded RNA enteric viruses > vegetative bacteria (Table 29.5).

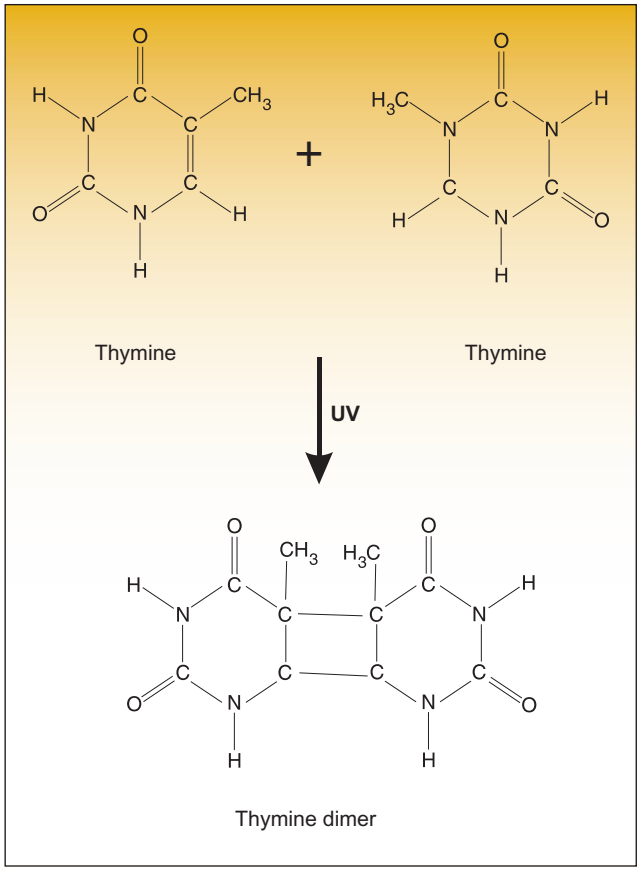

FIGURE 29.15 Formation of thymine dimers in the DNA of irradiated nonsporulating bacteria.

Ultraviolet radiation damages microbial DNA or RNA at a wavelength of approximately 260 nm. It causes thymine dimerization (Figure 29.15), which blocks nucleic acid replication and effectively inactivates microorganisms. The initial site of UV damage in viruses is the genome, followed by structural damage to the virus protein coat. Viruses with high molecular weight, double-stranded DNA or RNA are easier to inactivate than those with low-molecular-weight, double-stranded genomes. Likewise, viruses with single-stranded nucleic acids of high molecular weight are easier to inactivate than those with single-stranded nucleic acids of low molecular weight. This is presumably because the target density is higher in larger genomes. However, viruses with double-stranded genomes are less susceptible than those with single-stranded genomes because of the ability of the naturally occurring enzymes within the host cell to repair damaged sections of the double-stranded genome, using the nondamaged strand as a template (Roessler and Severin, 1996) (Figure 29.16).

A phenomenon known as photoreactivation occurs in some UV light-damaged bacteria when exposed to visible wavelengths between 300 and 500 nm. The UV light

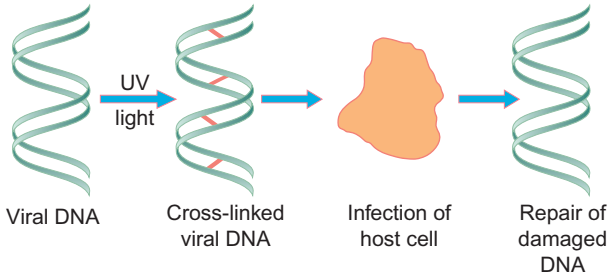

FIGURE 29.16 Viral repair in double-stranded DNA viruses using host cell repair enzymes. From Pepper *et al.* (2006).

damage is repaired by activation of a photoreactivating enzyme, which binds and then splits the thymine dimers. DNA damage can also be repaired in the dark by a mechanism that excises dimerized pyrimidine base pairs, and allows the reinsertion of undimerized bases by other enzymes. The regenerative capacity of any organism is dependent on the type of organism. Total and fecal coliforms are capable of photoreactivation, but fecal streptococci are not. To prevent photoreactivation, sufficient doses must be applied or exposure to direct sunlight prevented.

A minimum dose of 16,000 μW s/cm^2 has been recommended for treating drinking water, as this results in a 99.9% reduction in coliforms. However, this level is not enough to inactivate enteric viruses and some protozoan cysts (Table 29.7) (Abbaszadegan *et al.*, 1997). *Cryptosporidium* oocysts and *Giardia* cysts are both very sensitive to UV light irradiation.

There are three types of UV light sources in use today. These include low pressure lamps, medium pressure lamps and pulsed UV light. Differences in the source lamp characteristics of these three types of UV result in different spectral outputs of UV light and photo densities that vary in their action on microorganisms. Low pressure UV lamps are the ones used most commonly for disinfection, and produce essentially monochromatic UV light at a wavelength of 253.7 nm. Medium pressure UV lamps emit polychromatic UV light ranging from 200 to 1400 nm with several peaks at 185 and 300 nm. Pulsed UV emits intense pulses of light in high photon densities, rather that the continuous, lower wavelength of low and medium pressure lamps. Since low pressure UV emits very near the 260 nm absorbance maximum for DNA, it inactivates microorganism largely by damaging their DNA/RNA. Medium and pulsed UV emit wavelengths which can damage other cellular components such as proteins, amino acids, lipids and small molecules such as carboxylic and ketone compounds (Eischeid *et al.*, 2011). An advantage to medium and pulsed UV is that it prevents photoreactivation of bacteria and adenoviruses, allowing the use of lower doses.

TABLE 29.7 UV Dose to Kill Microorganisms

Organism	Ultraviolet Dose (μW-s/cm^2) Required for 90% Reduction
*Bacillus subtilis**	56,000
*Clostridium perfringens**	45,000
Campylobacter jejuni	1100
Escherichia coli	1300–3000
Klebsiella terrigena	3900
Legionella pneumophila	920–2500
Salmonella typhi	2100–2500
Shigella dysenteriae	890–2200
Vibrio cholerae	650–3400
Yersinia enterocolitica	1100
Adenovirus	23,600–56,000
Coxsackievirus	11,900–15,600
Echovirus	10,800–12,100
Poliovirus	5000–12,000
Hepatitis A	3700–7300
Rotavirus SA11	8000–9900
Coliphage MS-2	18,600
Cryptosporidium parvum	3000
Toxoplasma gondii	7000
Giardia	2000
Acanthamoeba	40,000
Naegleria fowleri (trohpozite)	6500
Naegleria fowleri (cyst)	31,500
Encephalitozoon intestinalis	2800

From Roessler and Severin (1996); John et al. *2003; Hijnen* et al. *(2006); Gerba* et al. *(2003); Sarkar and Gerba (2012).*
**Environmental strains (spores)*

29.8 PHOTODYNAMIC INACTIVATION AND PHOTOCATAYLYSTS

The usefulness of photoreactive dyes for inactivating microorganisms and oxidizing toxic compounds and organic matter in wastewater has been demonstrated. Photodynamic action may be defined as the sensitization of microorganisms to inactivation by visible light through the action of certain dyes (e.g., methylene blue). The dye combines with the nucleic acid or another critical site, and the complex absorbs light energy and attains an

excited energy state. The excited complex then combines with oxygen as the energy is released in a reaction that results in disruption of chemical bonds and loss of infectivity of the organism. Titanium dioxide is a photocatalyst that has a similar effect in the presence of UV light, causing strong oxidizing reactions at the surface of the metal oxide (Watts *et al.*, 1995). Different materials can be added to titanium dioxide to use visible light energy in the generation of free radicals.

29.9 OTHER CHEMICAL DISINFECTANTS

There are a few other chemical disinfectants that have seen widespread use primarily in consumer, institutional and industrial products. These include spray and wipe disinfectants available to the consumer and are widely used in the food industry. Quaternary ammonium compounds (Quats) are surfactants having both hydrophobic (water-repelling) and hydrophilic (water-attracting) properties. The basic structure of a quat is shown in Figure 29.17. The cation (positively charged) portion is a central nitrogen with four attached groups, which can contain a variety of structures, and is the functional part of the molecule. The anion (negatively charged) portion (X^-) is usually chlorine (Cl^-), and is linked to the nitrogen to form a quat salt. Benzalkonium chloride and cetylpyridinium chloride (Figure 29.18) are two of the most common basic quats structures in use. Benzalkonium chloride includes an aromatic ring, two methyl groups and a long chain ethyl ($CH_2{}^-CH_3$)/methyl chain, which can vary in length from C_{12} to C_{16}. Quats vary in their antimicrobial activity depending on the type and their formulations. They are effective against most common bacteria, but they are not sporicidal, although they may inhibit sporulation. They are effective against enveloped viruses (influenza), and specific formulations are effective against nonenveloped viruses (norovirus). Quats appear to act by adsorbing to and disrupting structure and function, eventually leading to leakage of cytoplasmic material (McDonnell, 2007). Direct interaction with viral and spore surface proteins may also cause prevention of growth, loss of function and disintegration. The presence of low-level residues after continued application may allow the selective development of bacterial strains with greater tolerance to quats over time (e.g., *Pseudomonas*). Tolerance is defined as the need for greater concentrations of an antimicrobial to kill the target organism, whereas resistance is defined as inability of the antimicrobial to kill the target organism. Thus, development of increased tolerance does not limit the practical application of a disinfectant, but may require increased concentrations to kill a target organism. It is difficult for microorganisms to develop resistance to disinfectants because they act nonspecifically on organic molecules, unlike

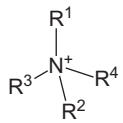

FIGURE 29.17 The basic structure of a quaternary ammonium compound.

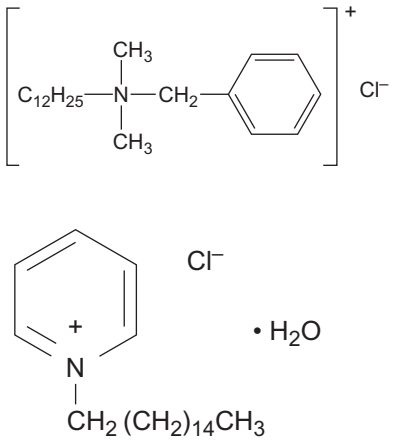

FIGURE 29.18 Structure of benzalkonium chloride (upper) and cetylpyridinium chloride (lower).

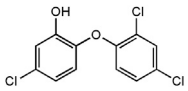

FIGURE 29.19 Triclosan.

antibiotics which act on specific sites in microorganisms. Thus, chlorine has been used for more than 100 years without microorganisms evolving a resistance to it (Rusin and Gerba, 2001).

Triclosan is also an antibacterial and fungal agent used in a wide variety of consumer products including hand soaps, mouth washes, shampoos and toothpastes, and via incorporation into materials (e.g., cutting boards). Triclosan is a bisphenol compound (Figure 29.19) and is known for its mildness to the skin. Mechanisms of triclosan action have received a great deal of study. Its action is much more specific than the other antimicrobials discussed in the chapter. Triclosan at concentrations used in products acts on multiple cytoplasmic and membrane targets (Russell, 2004). However, at lower concentrations, triclosan appears bacteriostatic, and is seen to target bacteria mainly by inhibiting fatty acid synthesis. Triclosan binds to bacterial enoyl–acyl carrier protein reductase enzyme (ENR). This binding increases the enzyme's affinity for nicotinamide adenine dinucleotide (NAD^+) resulting in the formation of a stable complex of ENR-NAD^+-triclosan,

which is unable to participate in fatty acid synthesis. Fatty acids are necessary for reproducing and building cell membranes. Some bacterial species can develop low-level resistance to triclosan at its lower bacteriostatic concentrations, which results in a decrease of triclosan's effect on ENR-NAD$^+$ binding (Health *et al.*, 1999). Some bacteria have innate resistance to triclosan at low, bacteriostatic levels, such as *Pseudomonas aeruginosa*, which possesses multi-drug efflux pumps that "pump" triclosan out of the cell (Chuanchuen *et al.*, 2003). Other bacteria, such as some of the *Bacillus* genus, have alternative *FabI* genes (*FabK*) to which triclosan does not bind, and hence are less susceptible. Although increased tolerance to low levels of triclosan has been reported in numerous laboratory studies, this has not been enough to limit its use in practical applications.

29.10 GAMMA AND HIGH-ENERGY IRRADIATION

Ionizing radiation generated by radioactive materials such as cesium 127 or cobalt 60 and high-energy electron beams can inactivate microorganisms either directly or indirectly by production of free radicals. Nucleic acids are the main targets of ionizing radiation. Ionizing radiation has been studied in great detail for preservation of foods and for wastewater and sewage sludge treatment. Factors that influence the effectiveness of ionizing radiation include the type of organism (generally, the smaller the organism the more resistant); composition of the suspending medium (organic material offers protection); presence of oxygen (greater resistance in the absence of oxygen); and moisture (greater resistance of dried cells and radiolysis of water). The unit of dose is the rad, which is equivalent to the absorption of 100 ergs per gram of matter. A kilorad (krad) is equal to 1000 rads. Typical doses to produce a *D* value of 90% inactivation are shown in Table 29.8. Viruses are the most resistant to ionizing irradiation in water and sludge.

Sludge irradiators have been built in Europe and experimental electron beam irradiators in the United States. The electron beams are generated by a 750-kV electron accelerator. The unit treats a thin layer ($\approx$ 2 mm) of liquid sludge spread on a rotating drum. Such systems are costly for waste treatment and require thick concrete shielding.

TABLE 29.8 Sludge Irradiation: *D* Values for Selected Pathogens and Parasites

Organism	*D* value (k rad)
Bacteria	
Escherichia coli	$<$22–36
Klebsiella spp.	36–92
Enterobacter spp.	34–62
Salmonella typhimurium	$<$50–140
Streptococcus faecalis	110–250
Viruses	
Poliovirus	350
Coxsackievirus	200
Echovirus	170
Reovirus	165
Adenovirus	150
Parasites	
Ascaris spp.	$<$66

Modified from Ahlstrom and Lessel (1986).

QUESTIONS AND PROBLEMS

1. Of the non-spore-forming bacteria, which microbial group is the most resistant to thermal inactivation in water?
2. What is thermal death time? *D* value?
3. Why are all microorganisms not inactivated according to first-order kinetics?
4. How long would you have to maintain a residual of 1.0 mg/L of free chlorine to obtain a $C \cdot t$ of 15? A $C \cdot t$ of 0.1?
5. Why is chlorine more effective against microorganisms at pH 5.0 than at pH 9.0?
6. Which chlorine compound is most effective against biofilms? Why?
7. What factors interfere with chlorine disinfection? Ultraviolet disinfection?
8. What is the main site of UV light inactivation in microorganisms? What group of microorganisms is the most resistant to UV light? Why?
9. At what pH is iodine most effective against protozoan parasites? Why?
10. What is photoreactivation? Are all microorganisms capable of photoreaction? If not, why?
11. What are two sources of ionizing radiation? How does ionizing radiation kill microorganisms?
12. Why does suspended matter interfere with the disinfection of microorganisms?
13. Chlorine has been in use for the disinfection of drinking and waste water for more than 100 years, yet no water- or foodborne bacteria or virus has developed resistance to chlorine. Why?

14. What dose of UV light would you need to kill 99.9% of the poliovirus in water?
15. What is a photocatalyst? How does it work?

REFERENCES AND RECOMMENDED READING

Abbaszadegan, M., Hasan, M. N., Gerba, C. P., Roessler, P. F., Wilson, B. R., Kuennen, R., *et al*. (1997) The disinfection efficacy of a point-of-use water treatment system against bacterial, viral and protozoan waterborne pathogens. *Water Res.* **31**, 574–582.

Ahlstrom, S. B., and Lessel, T. (1986) Irradiation of municipal shudge for pathogen control. In "Control of Sludge Pathogens" (C. A. Sorber, ed.), Water Pollution Contol Federation, Washington, DC.

Alvarez, M. E., and O'Brien, R. T. (1982) Effects of chlorine concentration on the structure of poliovirus. *Appl. Environ. Microbiol.* **43**, 237–239.

Bandres, J. C., Mawhewson, J. J., and Du Pont, H. L. (1988) Heat susceptibility of bacterial enteropathogens—implications for the prevention of travelers diarrhea. *Arch. Intern. Med.* **148**, 2261–2263.

Bates, R. C., Shaffer, P. T. B., and Sutherland, S. M. (1977) Development of poliovirus having increased resistance to chlorine inactivation. *Appl. Environ. Microbiol.* **33**, 849–853.

Bernarde, M. A., Snow, N. B., Olivieri, V. P., and Davidson, B. (1967) Kinetics and mechanism of bacterial disinfection by chloride dioxide. *Appl. Microbiol.* **15**, 257–265.

Bitton, G. (2011) "Wastewater Microbiology," 4th ed. Wiley-Liss, New York.

Block, S. S. (1991) "Disinfection, Sterilization, and Preservation," 4th ed. Lea & Febiger, Philadelphia, PA.

Cerva, L. (1955) The effect of disinfectants on the cysts of *Giardia intestinalis*. *Cesk. Parasitol.* **2**, 17–21.

Chambers, C. W., Procter, C. M., and Kabler, P. W. (1962) Bactericidal effect of low concentrations of silver. *J. Am. Water Works Assoc.* **54**, 208–216.

Chauret, C. P., Radziminski, C. S., Lepu, M., Creason, R., and Andrew, R. C. (2001) Chlorine dioxide inactivation of *Cryptosporidium parvum* oocysts and bacterial spore indicators. *Appl. Environ. Microbiol.* **67**, 2993–3001.

Chen, Y.-S., and Vaughn, J. (1990) Inactivation of human and simian rotaviruses by chlorine dioxide. *Appl. Environ. Microbiol.* **56**, 1363–1366.

Choi, M., Kim, J., Kim, J. Y., Yoon, J., and Kim, J. H. (2010) Mechanisms of *Escherichia coli* inactivation by several disinfectants. *Water Res.* **44**, 3410–3418.

Chuanchuen, R., Karkhoff-Schweizer, R. R., and Schweizer, H. P. (2003) High-level triclosan resistance in *Pseudomonas aerugginosa* is solely a result of efflux. *Am. J. Infect. Control.* **31**, 124–127.

Cromeans, T. L., Kahler, A. M., and Hill, V. R. (2010) Inactivation of adenoviruses, enteroviruses, and murine norovirus in water by free chlorine and monochloramine. *Appl. Environ. Microbiol.* **76**, 1028–1033.

Driedger, A. M., Rennecker, J. L., and Marinas, B. J. (2001) Inactivation of *Cryptosporidium* oocysts with ozone and monochloramine at low temperature. *Water Res.* **35**, 41–48.

Eischeid, A. C., Thurston, J. A., and Linden, K. A. (2011) UV disinfection of adenovirus: present state of research and future directions. *Crit. Rev. Environ. Sci. Technol.* **41**, 1375–1396.

Estes, M. K., Graham, D. Y., Smith, E. R., and Gerba, C. P. (1979) Rotavirus stability and inactivation. *J. Gen. Virol.* **43**, 403–409.

Eterpi, M., McDonnell, G., and Thomas, V. (2009) Disinfection against parvoviruses. *J. Infect. Hosp. Infect.* **73**, 64–70.

Fayer, R. (1994) Effect of high temperature on infectivity of *Cryptosporidium parvum* oocysts in water. *Appl. Environ. Microbiol.* **60**, 2732–2735.

Gerba, C. P., Johnson, D. C., and Hasan, M. N. (1997) Efficacy of iodine water purification tablets against *Cryptosporidium* oocysts and *Giardia* cysts. *Wilder. Environ. Med.* **8**, 96–100.

Gerba, C. P., Nwachuku, N., and Riley, K. R. (2003) Disinfection resistance of waterborne pathogens on the United States Environmental Protection Agency's Contaminant Candidate List (CCL). *J. Wat. Supply Res. Technol.* **52**, 81–94.

Gottardi, W. (1991) Iodine and iodine compounds. In "Disinfection, Sterilization, and Preservation" (S. S. Block, ed.), 4th ed., Lea and Febiger, Philadelphia, pp. 152–166.

Haas, C. N., and Morrison, E. C. (1981) Repeated exposure of *Escherichia coli* to free chlorine: production of strains possessing altered sensitivity. *Water Air Soil Pollut.* **16**, 233–242.

Health, R. J., Rubin, J. R., Holland, D. R., Zhang, E., Snow, M. E., and Rock, C. O. (1999) Mechanism of triclosan inhibition of bacterial fatty synthesis. *J. Biol. Chem.* **274**, 11110–11114.

Hijnen, W. A. M., Beerendonk, E. F., and Medada, G. J. (2006) Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Res.* **40**, 3–22.

Jacangelo, J., Patania, N., Haas, C., Gerba, C., and Trussel, R. (1997) Inactivation of waterborne emerging pathogens by selected disinfectants. Report No. 442, American Water Works Research Foundation, Denver, Colorado.

Jay, J. M. (1996) "Modern Food Microbiology," 5th ed. Chapman & Hall, New York.

John, D. E., Nwachuku, N., Pepper, I. L., and Gerba, C. P. (2003) Development and optimization of a quantitative cell culture infectivity assay for *the microsporidium Encephalitozoon intestinalis* and application to ultraviolet light inactivation. *J. Microbiol. Methods* **52**, 183–196.

Keswick, B. H., Fujioka, R. S., and Loy, P. C. (1981) Mechanism of poliovirus inactivation by bromine chloride. *Appl. Environ. Microbiol.* **42**, 824–829.

LeChevallier, M. W., Lowry, C. H., and Lee, R. G. (1990) Disinfecting biofilm in a model distribution system. *J. Am. Water Works Assoc.* **82**, 85–99.

Liew, P., and Gerba, C. P. (1980) Thermo stabilization of enteroviruses on estuarine sediment. *Appl. Environ. Microbiol.* **40**, 305–308.

McDonnell, G. E. (2007) "Antisepsis, Disinfection, and Sterilization," ASM Press, Washington, DC.

Montgomery, J. M. (1988) "Water Treatment and Design," John Wiley & Sons, New York.

Pepper, I. L., Gerba, C. P., and Brusseau, M. L. (eds.) (2006) 2nd ed., Elsevier Science/Academic Press, San Diego, CA.

Pereira, V. J., Marques, R., Marques, M., Bernoliel, M. J., and Crespo, M. T. B. (2013) Free chlorine inactivation of fungi in drinking water sources. *Water Res.* **47**, 517–523.

Ran, Z., Li, S., Huang, J., *et al*. (2010) Inactivation of cryptosporidium by ozone and cell ultrastructures. *J. Environ. Sci. (China)* **22**, 1954–1959.

Robbecke, R. S., and Buchholtz, K. (1992) Heat susceptibility of aquatic Mycobacteria. *Appl. Environ. Microbiol.* **58**, 1869–1873.

Roberts, D., and Gilbert, R. J. (1979) Survival and growth of noncholera vibrios in various foods. *J. Hyg. Cambridge* **82**, 123–131.

Roessler, P. F., and Severin, B. F. (1996) Ultraviolet light disinfection of water and wastewater. In "Modeling Disease Transmission and Its Prevention by Disinfection" (C. J. Hurst, ed.), Cambridge University Press, Cambridge, UK, pp. 313–368.

Rose, J. B., Lisle, J. T., and LeChevallier, M. (1997) Waterborne Cryptosporidiosis: incidence, outbreaks, and treatment strategies. In "Cryptosporidium and Cryptosporidiosis" (R. Fayer, ed.), CRC Press, Boca Raton, FL, pp. 93–109.

Rusin, P., and Gerba, C. P. (2001) Association of chlorination and UV irradiation to increasing antibiotic resistance in bacteria. *Rev. Environ. Contamin. Toxicol.* **171**, 1–52.

Russell, A. D. (2004) Whither triclosan? *J. Antimicr. Chemother.* **53**, 693–695.

Sanden, G. N., Fields, B. S., Barbaree, J. M., and Feeley, J. C. (1989) Viability of *Legionella pneumophila* in chlorine-free water at elevated temperatures. *Curr. Microbiol.* **18**, 61–65.

Sarkar, P. S., and Gerba, C. P. (2012) Inactivation of *Naegleria fowleri* by chlorine and ultraviolet light. *J. Am. Water Works Assoc.* **104**, 95–96.

Shields, J. M., Hill, V. R., Arrowood, M. J., and Beach, M. J. (2008) Inactivation of *Cryptosporidium parvum* under chlorinated recreational water conditions. *J. Water Health* **6**, 513–520.

Siegl, G., Weitz, M., and Kronauer, G. (1984) Stability of hepatitis A virus. *Intervirology* **22**, 218–226.

Sobsey, M. D. (1989) Inactivation of health-related microorganisms in water by disinfection processes. *Water Sci. Technol.* **21**, 179–195.

Stewart, M. H., and Olson, B. H. (1996) Bacterial resistance to potable water disinfectants. In "Modeling Disease Transmission and Its Prevention by Disinfection," Cambridge University Press, Cambridge, UK, pp. 140–192.

Straub, T. M., Gerba, C. P., Zhou, X., Price, R., and Yahya, M. T. (1995) Synergistic inactivation of *Escherichia coli* and MS-2 coliphage by chloramine and cupric chloride. *Water Res.* **24**, 811–818.

Thurman, R. B., and Gerba, C. P. (1989) The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. *CRC Crit. Rev. Environ. Control* **18**, 295–315.

USEPA (2012) United States Environmental Protection Agency. Test Method for Efficacy of Copper Alloy Surfaces as a Sanitizer.

Vaughn, J. M., and Novotny, J. F. (1991) Virus inactivation by disinfectants. In "Modeling the Environmental Fate of Microorganisms" (C. J. Hurst, ed.), American Society for Microbiology, Washington, DC, pp. 217–241.

Wainwright, K. E., Miller, M. A., Barr, B. C., Gerdner, I. A., Meli, A. C., Essert, T., *et al*. (2007) Chemical inactivation of *Toxoplasma gondii* oocysts in water. *J. Parasitol.* **9**, 925–931.

Watts, R. J., Kong, S., Orr, M. P., Miller, G. C., and Henry, B. E. (1995) Photcatalytic inactivation of coliform bacteria and viruses in secondary wastewater effluent. *Water Res.* **29**, 95–100.

Wigginton, K. R., Pecson, B. M., Sigstam, T., Bosshard, F., and Kohn, T. (2012) Virus inactivation mechanisms: impact of disinfectants on virus function and structural integrity. *Environ. Sci. Technol.* **46**, 12069–12078.