DISINFECTION

1. Introduction

Agents that served as disinfectants and antiseptics were known and utilized by ancient peoples. Soldiers disinfected equipment and clothing with fire or boiling water, houses were fumigated with burning sulfur, drinking water was purified by storing it in silver or copper vessels, and food was preserved by drying, salting, acidifying, and treating with spices. These methods worked, but it was not known why they worked.

In the 1500s, Girolamo Fracastoro, a physician in Italy who studied contagion, proposed that infection was caused by the passage from one person to another of minute bodies capable of self-multiplication (1). In the late seventeenth century van Leeuwenhoek, who invented microscopes, was the first to observe bacteria. He reported seeing them become inactive when treated with ordinary vinegar (2). Chlorine and hypochlorite, both first prepared in the 1770s, were tried in France and England in the 1820s for water purification and general sanitation to destroy effluvia believed to cause disease. French doctors reported favorably on the use of hypochlorite in surgery, and Faraday showed that the cowpox virus was inactivated by chlorine. This information, however, was largely ignored or rejected.

In Austria in 1847, Semmelweis demonstrated that washing the hands of doctors with a solution of hypochlorite prevented the spread of childbed fever (3). Louis Pasteur published the results of his experiments proving that bacteria existed in air and were the cause of fermentations in 1861 (4). Joseph Lister, a surgeon in Scotland, met Pasteur and became convinced that bacteria in the air produced infection of wounds. He demonstrated conclusively in 1865 that disinfection with phenol would greatly reduce infection during surgery (5); he later learned that not only the bacteria in the air but those on the hands and clothing were responsible for the infection.

Robert Koch, in Germany in 1881, did scientific laboratory tests on 70 different chemicals, at different concentrations and in different solvents, to assess their ability to kill spores of anthrax bacteria (6). Refinement of the testing methods were made in 1897, 1903, and 1908 (7). They continued to be improved, standardized, and published under the auspices of organizations like the Association of Official Analytical Chemists (AOAC) (now called AOAC International).

In the twentieth century, upon the development of the sulfa drugs and antibiotics, it was expected that microbial diseases could be completely controlled. This did not occur. A survey of hospital infection rates showed that infection rates ran as high as 34% in certain unit areas (8). There are numerous reasons for this: immunity-suppressing agents that make patients much more susceptible to infection are used for some treatments; treatments that control some organisms may allow competing organisms to proliferate; and strains of resistant organisms have developed that elude the treatments meant to control that species. The Environmental Protection Agency (EPA) and the Centers for Disease Control (CDC) reported in the early 1990s that diseases caused by viruses and parasites are on the increase. The cause may be the drinking water supply, because these organisms are not always destroyed by the water-treatment processes (9). Wider, intelligent use of disinfectants and antiseptics can greatly aid in removing microbes to limit the chance of infection. Disinfectants find additional use in preventing spoilage of products such as food, pharmaceuticals, cosmetics, paints, wood, cloth, and even in helping to keep office buildings from becoming uninhabitable (10).

1.1. Definitions. Chemical and physical agents that combat pathogenic and nonpathogenic microorganisms are referred to as disinfectants. Attempts to standardize terminology by health agencies in the United States, such as the CDC, the Food and Drug Administration (FDA), and the EPA, have resulted in the following definitions.

Disinfectant. A disinfectant is a chemical or physical agent that frees from infection and kills bacteria, fungi, viruses, and protozoa, but may not kill or inactivate bacterial spores, and is used only on inanimate objects, not on or in living tissue. A bactericide, fungicide, virucide, etc, is a disinfectant intended to kill the organisms indicated in the term. A germicide claims to kill pathogenic microorganisms, or germs.

Antiseptic. An antiseptic is a chemical substance that prevents or inhibits the action or growth of microorganisms but may not necessarily kill them, and is used topically on living tissue. The distinction between a disinfectant and an antiseptic is that the former is expected to kill all vegetative cells and is used only on inanimate objects, whereas the latter may not kill all cells and is used on the body.

Antiseptics are used in the home for simple cuts and wounds, and in the hospital for treating patient's skin and surgeon's hands prior to operative procedures. Soap, mouthwash, lotions, ointments, nose drops, suppositories, and

vaginal creams that contact the skin and mucous membranes are often treated with germ-killing antiseptics.

2. Disinfection Methods, Means, and Technologies

Disinfection refers to the selective destruction of disease-causing organisms. All the organisms are not destroyed during the process. This differentiates disinfection from sterilization, which is the destruction of all organisms. In the field of wastewater treatment, the three categories of human enteric organisms of the greatest consequence in producing disease are bacteria, viruses, and amoebic cysts. Diseases caused by waterborne bacteria include typhoid, cholera, paratyphoid, and bacillary dysentery; diseases caused by waterborne viruses include poliomyelitis and infectious hepatitis.

2.1. Description of Disinfection Methods and Means. The requirements for an ideal chemical disinfectant are reported in Table 1. As shown, an ideal disinfectant would have to possess a wide range of characteristics. Although such a compound may not exist, the requirements set forth in Table 1 should be considered in evaluating proposed or recommended disinfectants. It is also important that the disinfectant be safe to handle and apply and that its strength or concentration in treated waters be measurable. Disinfection is most commonly accomplished by the use of (*1*) chemical agents, (*2*) physical agents, (*3*) mechanical means, and (*4*) radiation.

Chemical Agents. Chemical agents that have been used as disinfectants include (*1*) chlorine and its compounds, (*2*) bromine, (*3*) iodine, (*4*) ozone, (*5*) phenol and phenolic compounds, (*6*) alcohols, (*7*) heavy metals and related compounds, (*8*) dyes, (*9*) soaps and synthetic detergents, (*10*) quaternary ammonium compounds, (*11*) hydrogen peroxide, (*12*) various alkalis and acids (eg, peracetic acid), and (*13*) antimicrobial nanoemulsions.

Of these, the most common disinfectants are the oxidizing chemicals. Chlorine is the one most universally used. Chlorine dioxide is another bactericide, equal to or greater than chlorine in disinfecting power. Ozone is a highly effective disinfectant, and its use is increasing even though it leaves no residual. Bromine and iodine have also been used for wastewater disinfection. Highly acidic or alkaline water can also be used to destroy pathogenic bacteria because water with pH >11 (basic) or <3 (acidic) is relatively toxic to most bacteria.

Physical Agents. Physical disinfectants that can be used are heat and light. Heating water to the boiling point, eg, will destroy the major disease-producing nonspore-forming bacteria. Heat is commonly used in the beverage and dairy industry, but it is not a feasible means of disinfecting large quantities of wastewater because of the high cost. However, pasteurization of sludge is used extensively in Europe.

Sunlight is also a good disinfectant. In particular, uv radiation can be used. Special lamps that emit uv rays have been used successfully to sterilize small quantities of water. The efficiency of the process depends on the penetration of the rays into water. The contact geometry between the uv-light source and the water is extremely important because suspended matter, dissolved organic molecules, and water itself, as well as the microorganisms, will absorb the radiation.

Table 1. **Comparison of Ideal and Actual Characteristics of Commonly Used Disinfectants**[a]

Characteristic	Properties/response	Chlorine	Sodium hypochlorite	Calcium hypochlorite	Chlorine dioxide	Bromine chloride	Ozone	UV radiation[b]
toxicity to micro organisms	should be highly toxic at high dilutions	high	high	high	high	high	high	high
solubility	must be soluble in water or cell tissue	slight	high	high	high	slight	high	N/A
stability	loss of germicidal action on standing should be low	stable	slightly unstable	relatively stable	unstable, must be generated as used	slightly unstable	unstable, must be generated as used	must be generated as used
nontoxic to higher forms of life	should be toxic to microorganisms and nontoxic to man and other animals	highly toxic to higher life forms	toxic	toxic	toxic	toxic	toxic	toxic
homogeneity	solution must be uniform in composition	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous	N/A
interaction with extraneous material	should not be absorbed by organic material other than bacterial cells	oxidizes organic matter	active oxidizer	active oxidizer	high	oxidizes organic matter	oxidizes organic matter	
toxicity at ambient temperatures	should be effective in ambient temperature range	high	high	high	high	high	high	high
penetration	should have the capacity to penetrate through surfaces	high	high	high	high	high	high	moderate
noncorrosive and nonstaining	should not disfigure metals or stain clothing	highly corrosive	corrosive	corrosive	highly corrosive	corrosive	highly corrosive	N/A
deodorizing ability	should deodorize while disinfecting	high	moderate	moderate	high	moderate	high	
availability	should be available in large quantities and reasonably priced	low cost	moderately low cost	moderately low cost	moderately low cost	moderately low cost	moderately high cost	moderately high cost

[a]See Refs. 11–14.
[b]N/A.

Table 2. **Removal or Destruction of Bacteria by Different Treatment Processes**[a]

Process	Percent removal
coarse screens	0–5
fine screens	10–20
grit chambers	10–25
plain sedimentation	25–75
chemical sedimentation	40–80
trickling filters	90–95
activated sludge	90–98
chlorination of treated wastewater	98–99

[a]See Ref. 14.

It is therefore difficult to use uv radiation in aqueous systems, especially when large amounts of particulate matter are present.

Mechanical Means. Bacteria and other organisms are also removed by mechanical means during wastewater treatment. Typical removal efficiencies for various treatment operations and processes are reported in Table 2. The first four operations listed may be considered to be physical. The removals accomplished are a by-product of the primary function of the process.

Radiation. The major types of radiation are uv, electromagnetic, acoustic, and particle. Gamma rays are emitted from radioisotopes such as Co 60. Because of their penetration power, uv, gamma rays, and high energy electron-beam devices have been used to disinfect (sterilize) water, wastewater or sludge, meat, poultry, fish and other foods, and air borne microorganisms or bioaerosols.

2.2. Mechanisms of Disinfectants. Four mechanisms that have been proposed to explain the action of disinfectants are (*1*) damage to the cell wall, (*2*) alteration of cell permeability, (*3*) alteration of the colloidal nature of the protoplasm, and (*4*) inhibition of enzyme activity (11).

Damage or destruction of the cell wall will result in cell lysis and death. Some agents, such as penicillin, inhibit the synthesis of the bacterial cell wall.

Agents such as phenolic compounds and detergents alter the permeability of the cytoplasmic membrane. These substances destroy the selective permeability of the membrane and allow vital nutrients, such as nitrogen and phosphorus, to escape.

Heat, radiation, and highly acidic or alkaline agents alter the colloidal nature of the protoplasm. Heat will coagulate the cell protein and acids or bases will denature proteins, producing a lethal effect.

Another mode of disinfection is the inhabitation of enzyme activity. Oxidizing agents, such as chlorine, can alter the chemical arrangement of enzymes and deactivate the enzymes.

2.3. Analysis of Factors Influencing the Action of Disinfectants. In applying the disinfection agents or means that have been described, the following factors must be considered: (*1*) contact time, (*2*) concentration and type of chemical agent, (*3*) intensity and nature of physical agent, (*4*) temperature, (*5*) number of organisms, (*6*) types of organisms, and (*7*) nature of suspending liquid (11,12).

3. Disinfection by Chlorination

Disinfection has received increased attention over the past several years from regulatory agencies through the establishment and enforcement of rigid bacteriological effluent standards. In upgrading existing wastewater treatment facilities, the need for improved disinfection as well as the elimination of odor problems are frequently encountered. Adequate and reliable disinfection is essential in ensuring that wastewater treatment plants are both environmentally safe and aesthetically acceptable to the public.

Chlorine is the most widely used disinfectant in water and wastewater treatment. It is used to destroy pathogens, control nuisance microorganisms, and for oxidation. As an oxidant, chlorine is used in iron and manganese removal, for destruction of taste and odor compounds, and in the elimination of nitrogen as ammonia. It is, however, a highly toxic substance and recently concerns have been raised over handling practices and possible residual effects of chlorination. Recent shortages and price escalation of liquid chlorine have also emphasized the need to consider other alternative methods of disinfection (15).

3.1. Background and Properties of Chlorine. Chlorine (Cl_2) is a greenish-yellow colored gas having a specific gravity of 2.48 as compared to air under standard conditions of temperature and pressure. It was discovered in 1774 from the chemical reaction of manganese dioxide (MnO_2) and hydrochloric acid (HCl) by the Swedish chemist, Scheele, who believed it to be compound containing oxygen. In 1810, it was named by Sir Humphrey Davy, who insisted it was an element (from the Greek word chloros, meaning greenish-yellow). In nature, it is found in the combined state only, usually with sodium as common salt (NaCl), carnallite ($KMgCl_3\ 6H_2O$), and sylvite (KCl).

Chlorine is a member of the halogen (salt-forming) group of elements and is derived from chlorides by the action of oxidizing agents and, most frequently, by electrolysis. As a gas, it combines directly with nearly all elements. At 10°C, 1 v of water dissolves ~3.10 v of chlorine; at 30°C, only 1.77 v of Cl_2 are dissolved in 1 v of water.

In addition to being the most widely used disinfectant for water treatment, chlorine is extensively used in a variety of products, including paper products, dyestuffs, textiles, petroleum products, pharmaceuticals, antiseptics, insecticides, foodstuffs, solvents, paints, and other consumer products. Most chlorine produced is used in the manufacture of chlorinated compounds for sanitation, pulp bleaching, disinfectants, and textile processing. It is also used in the manufacture of chlorates, chloroform, and carbon tetrachloride and in the extraction of bromine. Among other past uses, chlorine served as a war gas during World War I.

As a liquid, chlorine is amber colored and is 1.44 times heavier than water. In solid form, it exists as rhombic crystals. Various properties of chlorine are given in Table 3.

Chlorine gas is a highly toxic substance, capable of causing death or permanent injury due to prolonged exposures via inhalation. It is extremely irritating to the mucous membranes of the eyes and the respiratory tract. It will combine with moisture to liberate nascent oxygen to form hydrochloric acid. If both these substances are present in sufficient quantity, they can cause inflammation of

Table 3. **Properties of Chlorine**

symbol (as gas)	Cl Cl_2
atomic no.	17
atomic wt.	35.453
melting point (°C)	− 101
boiling point (°C)	− 34.5
liquid density (0°C and 3.65 atm; g/L)	1.47
vapor pressure (mmHg @ 20°C)	4800
vapor density (@ STP: g/L)	2.49
viscosity (micropoises) at	
$T = 12.7$°C	129.7
= 20°C	132.7
= 50°C	146.9
= 100°C	167.9
= 150°C	187.5
= 200°C	208.5

Table 4. **Acute Toxic Data**[a]

	Conc. (ppm)	Exposure Time(h)
inhalation TC_{LO} (humans)[b]	15	
inhalation LD_{LO} (humans)[c]	430	0.5
inhalation LC_{50} (rats)[d]	293	1

[a]See Ref. 16.
[b]TC_{LO} = lowest published toxic concentration.
[c]LD_{LO} = lowest published lethal dose.
[d]LC_{50} = lethal concentration to 50% of a specified population.

the tissues with which they come in contact. Pulmonary edema may result if lung tissues are attacked.

Chlorine gas has an odor detectable at a concentration as low as 3.55 ppm. Irritation of the throat occurs at 15 ppm. A concentration of 50 ppm is considered dangerous for even short exposures. At or above concentrations of 1000 ppm, exposure may be fatal. Table 4 gives acute toxicity data (16). Chlorine can also cause fires or explosions upon contact with various materials. It emits highly toxic fumes when heated and reacts with water or steam to generate toxic and corrosive hydrogen chloride fumes (17).

4. Fundamentals of Chlorine Chemistry

This section describes the chemistry of chlorine gas molecules and their reactions when dissolved in aqueous solutions. The purpose of this presentation is to show all of the fundamental reactions so that the practical application of chlorine to potable water, industrial process water, and wastewater can be better understood and analyzed.

Our knowledge of the fundamental chemistry of chlorination has been enlarged considerably in the past 50 years, which has contributed to significant advancement in the field. However, the more we learn, the more we realize how fortuitous it is that chlorine, applied in its simplest form (Cl_2), can be a very potent disinfectant. The phenomenon of chemical simplicity must surely be an important contributing factor to its germicidal efficiency. As a disinfectant, it is without equal, despite its shortcomings.

It is well known that the amount and the complexity of pollutants reaching our potable water supplies are increasing at an alarming rate. This has a direct effect on the chemical reactions of chlorine in aqueous solutions. In general, it can be said that the following compounds are of significance in their reactions with chlorine, insofar as water and waste treatment are concerned.

(*1*) Ammonia; (*2*) amino acids; (*3*) proteins; (*4*) total organic carbon (TOC); (*5*) nitrites; (*6*) iron; (*7*) manganese; (*8*) hydrogen sulfide; (*9*) cyanides, and (*10*) organic nitrogen

Before discussing the reactions of these compounds with chlorine, it is desirable to become acquainted with how the chlorine molecule is handled as a disinfectant. Chlorine gas (Cl_2) is dissolved either directly in water by a solution-feed chlorinator to form hypochlorous acid (HOCl), or by a specially controlled process in a solution containing caustic to yield a hypochlorite bleach solution. The exception to these systems occur when a direct gas feed chlorinator is used (14). In these cases, chlorine gas is dispersed directly into the process stream. When the latter solution is used as a disinfectant, it is diluted with water to form hypochlorous acid, as in the first method. The first part of this discussion will concern the fundamental reaction of chlorine and water and the formation of the oxidizing agent HOCl (hypochlorous acid).

4.1. Hydrolysis of Chlorine Gas. When chlorine gas is dissolved in water, it hydrolyzes rapidly according to the following equation:

$$Cl_2 + H_2O \longrightarrow HOCl + H^+ + Cl^- \quad (1)$$

The rapidity of this reaction has been studied by many investigators. Complete hydrolysis occurs in a few tenths of a second at 64°F; and at 32°F, only a few seconds are needed (18). This unusually rapid rate of reaction is best explained if the mechanism is a reaction of the chlorine molecule with the hydroxyl ion rather than with the water molecule. This can be represented as follows:

$$Cl_2 + OH^- \longrightarrow HOCl + Cl \quad (2)$$

The rate constant for this reaction is $\sim 5 \times 10^{14}$, indicating that the reaction occurs at almost every collision of ions (19). This reaction is of great practical importance because it relates to the chemistry of aqueous chlorine solutions discharging from conventional chlorination equipment. The resulting solution in a chlorinator discharge is limited by design to 3500 mg/L. At this concentration, the most highly buffered injector water would result in a pH no >3. At this pH the amount of molecular chlorine in equilibrium with HOCl is substantial.

Table 5. **Percent Molecular Chlorine and Hypochlorous Acid in a Water Solution Buffered from pH 1–6 at 15°C at Atmospheric Pressure**[a]

	Solution Concentration (mg/L)									
	500		1000		1500		2000		3500	
pH	Cl_2	HOCl	Cl_2	HOCl	Cl_2	HOCl	Cl_2	HOCl	Cl_2	HOCl
1	54.30	45.65	64.67	35.25	69.94	29.95	73.29	26.47	78.91	20.89
2	17.66	82.31	27.41	72.52	33.95	65.93	38.78	61.05	49.70	49.97
3	2.48	97.51	4.73	95.25	6.79	93.17	8.68	91.26	13.57	86.28
4	0.26	99.72	0.52	99.46	0.77	99.20	1.02	98.45	1.76	98.19
5	0.03	99.74	0.05	99.71	0.078	99.68	0.10	99.66	0.181	99.58
6	0.00	97.68	0.01	97.67	0.01	97.67	0.01	99.67	0.018	97.66

[a]See Ref. 21.

Concentrations >3500 mg/L cause excessive chlorine gas release at the point of application, which is extremely undesirable. Likewise, if negative pressures exist in the chlorine solution piping, this contributes to the release of molecular chlorine at the point of application. In addition to the degassing effect, the release of gas in the solution piping has been known to adversely affect the hydraulic gradient between the injector and the point of application. Injector systems are usually designed to maintain at least 2 psig at the injector discharge. At this pressure and a temperature of 68°F, the solubility of chlorine in water is only ~7.5 g/L (20).

To demonstrate the relationship of the molecular chlorine–hypochlorous acid equilibrium for both buffered and unbuffered water, Tables 5 and 6 have been compiled from a computer printout provided by the Bioengineering Research and Development Lab, U.S. Army, Fort Detrick, Maryland (21). The results are based upon the Cl_2–HOCl equilibrium; the Cl_3^- ion formation from Cl_2 and the chloride ion; a mass balance for all chlorine species; and anion balance on Cl^-. Thus the mole percent for HOCl in the tables is based upon a lengthy and complex cubic equation, which is best described as follows:

$$\text{Percent HOCl} = \frac{100 \times (\text{HOCl})}{[(\text{HOCl}) + (\text{Cl}_2) + (\text{OCl}^-) + (\text{Cl}_3^-)]} \tag{3}$$

Table 6. **Percent Molecular Chlorine and Hypochlorous Acid, and OC1$^-$ Ion in a Water Solution Buffered from pH 6–9 at 20°C**[a]

	Solution Concentration (mg/L)								
	5000			7000			10000		
pH	Cl_2	HOCl	OCl^-	Cl_2	HOCl	OCl^-	Cl_2	HOCl	OCl^-
6.5	0.0063	92.28	7.71	0.0088	92.28	7.71	0.0126	92.28	7.71
7.0	0.0017	79.10	20.89	0.0024	79.10	20.89	0.0034	79.10	20.89
7.5	0.0004	54.84	45.51	0.0005	54.49	49.51	0.0007	54.49	45.51
8.0	0.0001	27.46	72.54	0.0001	27.46	72.54	0.0001	27.46	72.54
8.5	0.0000	10.69	89.31	0.0000	10.69	89.30	0.0000	10.69	89.30
9.0	0.0000	3.65	96.35	0.0000	3.65	96.35	0.0000	3.65	96.35

[a]See Ref. 21.

Table 5 illustrates what happens in the chlorine solution discharge from a chlorinator ranging in feed rates to produce concentrations varying from 500 to 3500 g/L, at 60°F. It also demonstrates the necessity for maintaining a constant high concentration of chlorine (eg, 1500–2000 mg/L at a low pH in the generation of chlorine dioxide). The molecular chlorine present in the solution coming in contact with the sodium chlorite provides the impetus for a fast and complete reaction.

Table 6 demonstrates the stability of a chlorine water solution buffered with either sodium hydroxide or calcium hydroxide. These figures are of interest for on-site manufacture as well as on-site generation of hypochlorite.

In any hypochlorite solution the active ingredient is always hypochlorous acid:

$$\mathrm{NaOCl} + \mathrm{H_2O} \longrightarrow \mathrm{HOCl} + \mathrm{Na^+} + \mathrm{OH^-} \tag{4}$$

$$\mathrm{Ca(OCl)_2} + 2\,\mathrm{H_2O} \longrightarrow 2\,\mathrm{HOCl} + \mathrm{Ca^{2+}} + 2\,(\mathrm{OH^-}) \tag{5}$$

When a chlorine solution, such as the solution discharge of a conventional chlorinator (unbuffered), is subjected to negative pressure conditions, the solubility is reduced, a phenomenon that usually results in the release of molecular chlorine at the point of application, provided the diffuser is in an open body of water such as an open channel.

For example, at atmospheric pressure and 68°F, the maximum solubility of chlorine is ~7395 mg/L. However, if the solution is subjected to a negative head of 9 in. of Hg, the solubility is reduced to ~5560 mg/L (21). Therefore, all systems that are not closed should be designed to avoid negative pressure conditions in the chlorinator solution lines. This prevents off-gassing at the point of application where a diffuser assembly is installed; otherwise, serious corrosion in the surrounding area could occur, as well as offensive chlorine odors would be generated.

4.2. Chemistry of Hypochlorous Acid. *Effect of pH.* The next most important reaction in the chlorination of an aqueous solution is the formation of hypochlorous acid. This specie of chlorine is the most germicidal of all chlorine compounds with the possible exception of chlorine dioxide.

Hypochlorous acid is a "weak" acid, which means that it tends to undergo partial dissociation as follows:

$$\mathrm{HOCl} \longrightarrow \mathrm{H^+} + \mathrm{OCl^-} \tag{6}$$

to produce a hydrogen ion and a hypochlorite ion. In waters of pH between 6.5 and 8.5 the reaction is incomplete, and both species are present to some degree. The extent of this reaction can be calculated from the following equation:

$$K_i = \frac{(\mathrm{H^+})(\mathrm{OCl^-})}{(\mathrm{HOCl})} \tag{7}$$

K_i, the ionization constant, varies in magnitude with temperature. The values of this constant shown in Table 7 have been computed from the acid dissociation

Table 7. **HOCl Ionization Constant**[a]

Temperature (°C)	0	5	10	15	20	25	30
$K_a \times 10^{10.8}$ (mol/L)	1.488	1.753	2.032	2.320	2.621	2.898	3.175

[a]See Ref. 22.

constant, pK_a, based on the formula developed by Morris (22) in 1996 as follows:

$$pK_a = \frac{3000.0}{T} - 10.0686 + 0.0253T \tag{8}$$

where $T = 273 +$ degrees Celcius.

$$\frac{(HOCl)}{(HOCl) + (OCl^-)} = \frac{1}{1 + (OCl^-)/(HOCl)} = \frac{1}{1 + (K_i/[H^+])} \tag{9}$$

Table 8 shows the percent of undissociated HOCl specie for the various temperatures and pH values from 4 to 11.7. The present OCl^- ion is the difference between these numbers and 100 (14).

The percent distribution of the OCl^- ion (hypochlorite ion) and undissociated hypochlorous acid can be calculated for various pH values as follows:

5. Dechlorination with Sulfur Dioxide

The practice of dechlorination has seen dramatic growth in the past decade due to rising concerns over chlorine toxicity and protection of fish and wildlife. Dechlorination removes all or part of the chlorine residual and halogenated organics remaining after chlorination, and reduces or eliminates toxicity harmful to aquatic life in receiving waters.

Sulfur dioxide gas successively removes free chlorine, monochloramine, dichloramine, nitrogen trichloride, and poly-*n*-chlor compounds. When sulfur dioxide is added to wastewater, the following reactions occur:

Reactions with chlorine:

$$SO_2 + H_2O \longrightarrow HSO_3^- + H^+ \tag{10}$$

$$HOCl + HSO_3^- \longrightarrow Cl^- + SO_4^{-2} + 2H^+ \quad \text{(free chlorine)} \tag{11}$$

$$SO_2 + HOCl + H_2O \longrightarrow Cl^- + SO_4^{-2} + 3H^+ \tag{12}$$

Reactions with chloramines:

$$SO_2 + H_2O \longrightarrow HSO_3^- + H^+ \tag{13}$$

$$NH_2Cl + HSO_3^- + H_2O \longrightarrow Cl^- + SO_4^{-2} + NH_4^+ + H^+ \tag{14}$$

$$SO_2 + NH_2Cl + 2H_2O \longrightarrow Cl^- + SO_4^{-2} + NH_4^+ + 2H^+ \quad \text{(combined chlorine)} \tag{15}$$

Table 8. **Mole or Weight Percent Undissociated HOCl Species at Varying Temperature and pH**[a]

	Mole Percent HOCl						
pH	0°C	5°C	10°C	15°C	20°C	25°C	30°C
5.0	99.85	99.82	99.80	99.79	99.74	99.71	99.68
5.5	99.53	99.45	99.36	99.27	99.18	99.09	99.00
6.0	98.53	98.28	98.00	97.73	97.45	97.18	96.92
6.1	98.16	97.84	97.50	97.16	96.82	96.48	96.15
6.2	97.69	97.29	96.88	96.45	96.02	95.60	95.20
6.3	97.11	96.62	96.10	95.57	95.05	94.53	94.04
6.4	96.39	95.78	95.14	94.49	93.84	93.21	92.61
6.5	95.50	94.75	93.96	93.16	92.37	91.60	90.87
6.6	94.40	93.47	92.51	91.54	90.58	89.65	88.78
6.7	93.05	91.92	90.75	89.58	88.43	87.32	86.27
6.8	91.41	90.03	88.63	87.23	85.85	84.54	83.31
6.9	89.42	87.77	86.10	84.43	82.82	81.29	79.86
7.0	87.04	85.08	83.10	81.16	79.29	77.53	75.90
7.1	84.22	81.92	79.63	77.39	75.26	73.27	71.44
7.2	80.91	78.25	75.64	73.11	70.73	68.52	66.52
7.3	77.10	74.08	71.15	68.35	65.75	63.36	61.22
7.4	72.78	69.42	66.20	63.18	60.39	57.87	55.63
7.5	67.99	64.33	60.88	57.68	54.77	52.18	49.90
7.6	62.79	58.89	55.27	51.98	49.03	46.43	44.17
7.7	57.27	53.23	49.54	46.23	43.32	40.77	38.59
7.8	51.57	47.48	43.81	40.58	37.77	35.35	33.30
7.9	45.82	41.79	38.25	35.17	32.53	30.28	28.39
8.0	40.18	36.32	32.98	30.12	27.69	25.65	23.95
8.1	34.79	31.18	28.10	25.50	23.32	21.51	20.01
8.2	29.77	26.46	23.69	21.38	19.46	17.88	16.58
8.3	25.19	22.23	19.78	17.76	16.10	14.74	13.63
8.4	21.10	18.50	16.38	14.64	13.23	12.07	11.14
8.5	17.52	15.28	13.46	11.99	10.80	9.84	9.06
8.6	14.44	12.53	11.00	9.77	8.77	7.97	7.33
8.7	11.82	10.22	8.94	7.92	7.10	6.44	5.91
8.8	9.62	8.29	7.23	6.39	5.72	5.18	4.75
8.9	7.80	6.70	5.83	5.15	4.60	4.16	3.81
9.0	6.29	5.39	4.69	4.13	3.69	3.33	3.05
10.0	0.67	0.57	0.49	0.43	0.38	0.34	0.31
10.5	0.21	0.18	0.15	0.14	0.12	0.11	0.10
11.0	0.07	0.06	0.05	0.04	0.04	0.03	0.03
11.5	0.02	0.02	0.015	0.013	0.012	0.01	0.01
11.7	0.01	0.01	0.01	0.01	0.007	0.007	0.006

[a]See Ref. (15).

For the overall reaction between sulfur dioxide and chlorine (eqs. 10–12), the stoichiometric weight ratio of sulfur dioxide to chlorine is 0.9:1. In practice, it has been found that ~1.0 mg/L of sulfur dioxide will be required for the dechlorination of 1.0 mg/L of chlorine residue (expressed as Cl_2). Because the reactions of sulfur dioxide with chlorine and chloramines are nearly instantaneous, contact time is not usually a factor and contact chambers are not used; however, rapid and positive mixing at the point of application is an absolute requirement.

The ratio of free chlorine to the total combined chlorine residual before dechlorination determines whether the dechlorination process is partial or proceeds to completion. If the ratio is <85%, it can be assumed that significant organic nitrogen is present and that it will interfere with the free residual chlorine process (12).

There is no question that dechlorination removes most of the total residual chlorine from disinfected wastewaters. Consequently, it reduces the toxicity of disinfected wastewater effluent to aquatic wildlife. In most situations, sulfur dioxide dechlorination is a very reliable unit process in wastewater treatment, provided that the precision of the combined chlorine residual monitoring service is adequate. Excess sulfur dioxide dosages should be avoided not only because of the chemical wastage but also because of both biochemical oxygen demand (BOD) and chemical oxygen demand (COD) exerted by the excess sulfur dioxide (23).

6. Chlorine Dioxide

Chlorine dioxide is a highly selective oxidant that is more similar to ozone than it is to chlorine. It is unstable as a compressed gas, and must be generated at the point of use. It cannot be stored in steel containers like chlorine. Historically, chlorine dioxide has been generated on-site as an aqueous ClO_2 solution by reacting to a solution of sodium chlorite with the aqueous solution of a conventional chlorinator injector discharge. New technology, that reacts to chlorine gas with specially processed solid sodium chlorite, is the present (1998) state-of-the-art (CDG Technology, Inc., New York, NY), and has substantially resolved all of the problems historically associated with chlorine dioxide generation, making chlorine dioxide more of a wastewater treatment candidate, especially for tertiary treatment of water intended for reuse. Chlorine dioxide does not combine with the nitrogen as ammonia normally present. Therefore, in a nitrogen-laden wastewater it is reputed to have a disinfection efficiency for both bacterial and viral destruction comparable to that of free chlorine. Experience with chlorine dioxide on wastewaters is limited. It is significantly more expensive than chlorine.

6.1. Disinfection with Chlorine Dioxide. Chlorine dioxide is another bacteriocide, equal to or greater than chlorine in disinfecting power. Chlorine dioxide has proven to be more effective in achieving inactivation of viruses than chlorine. A possible explanation is that, because chlorine dioxide is absorbed by peptone (a protein) and because viruses have a protein coat, adsorption of chlorine dioxide onto this coating could cause inactivation of the virus. In the past, it did not receive much consideration as a wastewater disinfectant due to its high costs.

6.2. Chlorine Dioxide Generation. Chlorine dioxide is an unstable and explosive gas and for this reason it must be generated on site. Generation of chlorine dioxide involves reacting sodium chlorite ($NaClO_2$) with chlorine to produce gaseous chlorine dioxide according to the following reaction:

$$2\,NaClO_2 + Cl_2 \longrightarrow 2\,ClO_2 + 2\,NaCl \qquad (16)$$

Based on equation 16, 1.34 mg sodium chlorite reacts with 0.5 mg chlorine to yield 1.0 mg chlorine dioxide. Because technical grade sodium chlorite is only ~80% pure, ~1.68 mg of the technical grade chlorite would be required to produce 1.0 mg of chlorine dioxide.

6.3. Effectiveness of Chlorine Dioxide. The active disinfecting agent in a chlorine dioxide system is free dissolved chlorine dioxide (ClO_2). The complete chemistry of chlorine dioxide in an aqueous environment is not clearly understood. Chlorine dioxide has an extremely high oxidation potential, which probably accounts for its potent germicidal powers. Because of its extreme high oxidizing potential, possible bacteriocidal mechanisms may include inactivation of critical enzyme systems or disruption of protein synthesis.

6.4. Medical Device Sterilization. Chlorine dioxide gas is effective for the sterilization of packaged medical products (24–26). This use of chlorine dioxide, developed by Rosenblatt and co-workers, was begun as an alternative to gas sterilization processes using ethylene oxide. It was also the application for which the chlorine gas/solid chlorite generation process was originally developed.

6.5. Food Processing. Chlorination of process water in canneries and frozen food packaging became an established practice in the United States about 1946. It was so successful in potato dehydration plants that its use spread rapidly to fruit and other vegetable canning operations.

Originally, the cannery water supply was a once-through system. However, after some 10 years of breakpoint chlorination, canneries attempted to conserve water, primarily to cut down on the cost of treating the wastewater. Thus a recycling system for food processing plants was evolved. It was soon discovered that the nitrogen as ammonia concentration increased significantly in the recycled water, so that it became difficult to control the breakpoint process unless multiple points of application were used. It was found impractical to pursue the free residual process using breakpoint chlorination because of excessive chlorine consumption combined with the generation of intolerable amounts of nitrogen trichloride, which pervaded the working area.

In 1975, the Green Giant Co. decided to try chlorine dioxide (27). Its Blue Earth plant chlorination system was retrofitted to generate chlorine dioxide. The prime objective was to determine the feasibility of water conservation through water recycling while maintaining acceptably low bacteria counts in the complete system. The experiment was a success. The chlorine dioxide treatment of the once-used water was highly effective in the control of bacteria growth and biofouling in pea and corn canneries. The persistent residuals and the lack of reaction with ammonia nitrogen made possible one-point application, rather than the multiple points of application necessary with rechlorination of used water. Chlorine dioxide residuals in the reuse water did not result in the generation of offensive odors in the plant, nor were there any off-flavors produced in the product.

The success at this plant rapidly spread to other food processing plants. Since 1975 a majority of these processing plants have retrofitted their existing chlorination systems or purchased new chlorine dioxide systems. These plants include all types of food processing. The success of chlorine dioxide use in the recycling systems is the result of the chemical characteristics of chlorine dioxide: (*1*) it will not react with nitrogen as ammonia, and (*2*) the residuals persist over a

long period of time, so that a single point of application is sufficient. In most cases the chlorine dioxide dosages vary from 2 to 8 mg/L.

Chlorine dioxide has been proved effective for the disinfection of poultry chiller water, which is known for its high content of organic matter. Recently, the U.S. FDA approved this use (28).

6.6. Summary. *Advantages of Chlorine Dioxide*

- Chlorine dioxide is an effective, fast-acting, broad-spectrum bactericide.
- It is superior as a viricide to chlorine, which makes it a promising candidate for water reuse disinfection.
- It kills chlorine-resistant pathogens—eg, encysted parasites *Giardia* and *Cryptosporidium*.
- It does not react with nitrogen as ammonia or primary amines.
- It does not react with oxidizabale organic material to form trihalomethane (THM).
- It destroys THM precursors and enhances coagulation.
- It is excellent for the destruction of phenols, which cause taste and odor problems in potable water supplies.
- It has a long track record in the removal of iron and manganese. It is superior to chlorine, particularly when the iron and manganese occur in complexed compounds.

Disadvantages of Chlorine Dioxide

- The cost of chlorine dioxide, several times more expensive than chlorine, may make its use prohibitive for certain applications, especially in economically deprived (eg, Third World) regions where even chlorination is not readily affordable.
- Chlorine dioxide cannot be transported as a compressed gas; it must be generated on-site.
- The chlorine dioxide prepared by some processes may contain significant amounts of free chlorine, which could defeat the objective of using ClO_2 to avoid the formation of THMs.

7. Ozone

Ozone is an unstable gas that must be produced at the point of use. It is made commercially by the reaction of an oxygen-containing gas (air or pure oxygen) in an electric discharge. It is a powerful oxidant and has been used since the early 1990s for odor and color removal as well as disinfection of potable-water supplies in Western Europe and Canada. It has been investigated recently for use in a process for polishing tertiary effluents, for both color removal and disinfection. From these investigations it appears that ozone in combination with either chlorine or chlorine dioxide could solve the disinfection problem of both bacterial and viral contamination in tertiary wastewater effluents. This is particularly significant where there is consideration of wastewater reuse.

7.1. Disinfection with Ozone. Ozone was first used to disinfect water supplies in France in the early 1900s. Its use there increased and eventually spread into several Western European countries. Today nearly 1000 ozone disinfection installations exist (primarily in Europe), almost entirely for treating water supplies. A common use for ozone at these installations is to control taste-, odor-, and color-producing agents. Although historically used primarily for the disinfection of water, recent advances in ozone generation and solution technology have made the use of ozone economically more competitive for wastewater disinfection. Ozone can also be used in wastewater treatment for odor control and in advanced wastewater treatment for the removal of soluble refractory organics, in lieu of the carbon-adsorption process. The generation of ozone, the chemistry of ozone, an analysis of the performance of ozone as a disinfectant, and the application of the ozonation process are considered in the following discussion.

7.2. Ozone Generation. Because ozone is chemically unstable, it decomposes to oxygen very rapidly after generation, and thus must be generated on-site. The most efficient method of producing ozone today is by electrical discharge. Ozone is generated either from air or pure oxygen when a high voltage is applied across the gap of narrowly spaced electrodes. The high energy corona created by this arrangement dissociates one oxygen molecule, which re-forms with two other oxygen molecules to create two ozone molecules. The gas stream generated by this process from air will contain $\sim$0.5–3% ozone by weight and from pure oxygen about twice that amount, or 1–6% ozone.

7.3. Ozone Chemistry. Some of the chemical properties displayed by ozone may be described by its decomposition reactions which are thought to proceed as follows:

$$O_3 + H_2O \longrightarrow HO_3^+ + OH^- \quad (17)$$

$$HO_3^+ + OH^- \longrightarrow 2HO_2 \quad (18)$$

$$O_3 + HO_2 \longrightarrow HO + 2O_2 \quad (19)$$

$$HO + HO_2 \longrightarrow H_2O + O_2 \quad (20)$$

The free radicals HO_2 and HO have great oxidizing powers and are probably the active form in the disinfection process. These free radicals also possess the oxidizing power to react with other impurities in aqueous solutions.

7.4. Effectiveness of Ozone. Ozone is an extremely reactive oxidant, and it is generally believed that bacterial kill through ozonation occurs directly because of cell wall disintegration (cell lysis). Ozone is also a very effective virucide and is generally believed to be more effective than chlorine. Ozonation does not produce dissolved solids and is not affected by the ammonium ion or pH influent to the process. For these reasons, ozonation is considered a viable alternative to either chlorination or hypochlorination, especially where dechlorination may be required.

7.5. Other Benefits of Using Ozone. An additional benefit associated with the use of ozone for disinfection is that the dissolved oxygen concentrations of the effluent will be elevated to near saturation levels as ozone rapidly decomposes after application to oxygen. This may eliminate the need for reaeration of

the effluent to meet required dissolved oxygen water quality standards. Further, because ozone decomposes rapidly, no chemical residual that may require removal, as is the case with chlorine residuals, persists in the treated effluent.

8. Bromine, Bromine Chloride, and Iodine

8.1. Bromine (Br_2). All bromine species used in water and wastewater treatment revert to bromides after being consumed in the oxidation process. This in itself is not an issue. However, when a potable water treatment plant chlorinates water containing bromides, they are oxidized to hypobromous acid and bromamines if any nitrogen as ammonia is present. These compounds react with natural precursors in the water to form yet another series of trihalomethanes, which are considered to be carcinogenic. Therefore, the use of bromine as an alternative or a supplement to chlorination is not considered practical or acceptable from an environmental point of view. The consensus is that there are more than enough bromides occurring naturally in the environment to be dealt with, so that adding more bromides to the waterways only compounds the problem (29).

8.2. Bromine Production. The recovery of bromine from seawater was first achieved on a commercial scale in 1924 by the Ethyl Corporation (30). This process involved treatment of the seawater with chlorine and analine. The first successful bromine plant was put into operation at Kure Beach, North Carolina, 1933, and was capable of extracting 3000 tons of bromine per year. In this plant, the process consisted of adjusting the pH of seawater to 3.5 with sulfuric acid, followed by the application of chlorine. The bromine, liberated by the chlorine, was removed as a dilute bromine gas with a current of air and absorbed in a sodium carbonate solution, from which it was recovered by acidification and stripping with stream. The critical part of this type of bromine extraction is control of the pH at 3.5.

Oxidation of bromide to bromine can be accomplished either chemically or electrochemically. The electrochemical methods are no longer significant for commercial production. Chemical oxidation can be affected by either chlorine compounds or oxygen-containing compounds such as manganese dioxide, bromate, or chlorate.

The extraction of bromine from bromide compounds requires four steps: (*1*) oxidation of bromide to elemental bromine (Br_2); (*2*) separation of the bromine from solution; (*3*) condensation and isolation of the bromine vapor; and (*4*) purification. Current bromine production methods are based on the modified Kubierschky steaming-out process and the H. H. Dow blowing-out process.

Kubierschky Process. In this process, the raw brine is preheated to $\sim 90^{\circ}C$, treated with chlorine in a packed tower, and then placed in a steaming-out tower into which steam and additional chlorine are injected. The outgoing brine is neutralized with caustic and used to preheat the raw brine. From the top of the steaming-out tower, the halogen and steam vapor passes into a condenser and then into a gravity separator. Vent gases from the separator return to the chlorination system, the upper water layer containing Br_2 and Cl_2 is returned to the steaming-out tower, and the lower layer containing crude

bromine passes on to a stripping tower. From the stripping column, bromine is purified in a fractionating column, which produces a 99.8% pure liquid bromine as the final product (29).

The H. H. Dow Process. This process utilizes air instead of steam for the "blowing-out" step in the extraction of bromine. It is a more economical extracting agent than steam, especially when the bromine source is as dilute as in seawater. In the process, the halogens are absorbed from the air in a sodium carbonate solution, or by sulfur dioxide reduction (29);

$$Br_2(Cl_2) + SO_2 + 2H_2O \longrightarrow 2HBr(2HCl) + H_2SO_4 \qquad (21)$$

Bromine can then be separated by chlorinating the mixed acids in the blowing-out tower. The theoretical yield is 2.2 tons of bromine per ton of chlorine (31).

From 1973 to 1975, the estimated total annual bromine production in the United States was ~220,000 tons (32).

In the years following World War I, the demand for bromine was for pharmaceutical bromides, the organic chemical industry, and photography. However, the biggest boon to the bromine industry was the discovery of tetraethyllead as an antiknock ingredient in gasoline to accommodate the powerful high compression automobile engines. But this ingredient posed a serious problem: deposits of lead in the engine. It was found that a mixture of ethylene dibromide and ethylene dichloride added to the tetraethyl lead was an excellent scavenger, which prevented lead deposition in the engine. These lead halides were sufficiently volatile to be expelled in the engine exhausts. It is estimated that ~70% of the 1973–75 production of bromine was used to make ethylene dibromide for gasoline. However, air pollution controls later led to a ban on the use of tetraethyl lead in gasoline, thereby wiping out the major market for bromine production. This might be a boon to the water pollution control industry. This will be discussed later in this chapter. Bromine at a lower price becomes a most interesting disinfectant, particularly for water reuse situations.

8.3. Physical and Chemical Properties. Bromine is a dark brownish red, heavy, mobile liquid. It gives off, even at ordinary temperatures, a heavy, brownish red vapor with a sharp, penetrating, suffocating odor. The vapor is extremely irritating to the mucous membranes of the eyes, nasal passages, and throat, and is extremely corrosive to most metals. Liquid bromine is likewise corrosive and destructive to organic tissues. In contact with the skin, it produces painful burns, which are slow to heal.

Bromine (Br_2; atomic number, 35; molecular weight, 159.83; specific gravity, 3.12) weighs 26.0 lb/gal, and has a boiling point of 58.78°C. Of the metals used to handle bromine, lead is the most versatile (31). Bromine reacts with lead to form a dense superficial coating of lead bromide, which, if not disturbed, prevents further attack. This is similar to the reaction of chlorine and silver. Tantalum is completely resistant to bromine, wet or dry, at temperatures up to 300°F.

Nickel and its alloy, Monel, resist dry bromine and are especially useful as a material for shipping containers. Other nickel alloys, including the hastelloys, are less suitable. Iron, steel, cast iron, stainless steel, and copper are attacked by bromines, either wet or dry. Silver withstands dry bromine.

Bromine handled in lead, nickel, or Monel containers should be dry (<0.003% moisture) and should be protected from ordinary air, from which it can readily absorb enough moisture to make it severely corrosive to these materials.

Bromine is three times as soluble as chlorine (ie, 3.13 g/100 mL water at 30°C), which is an important characteristic when one is considering the physical aspects of applying bromine to a process stream. Dispersion and diffusion is made "easier," and diffuser design to prevent off-gassing is less of a problem than with chlorine.

8.4. Chemistry of Bromine in Water and Wastewater. Bromine is unique in being the only nonmetallic element that is liquid at ordinary temperatures. It reacts with ammonia compounds in solution to form bromamines and displays the breakpoint phenomenon similarly to chlorine.

Bromine in water hydrolyzes:

$$Br_2 + H_2O \longrightarrow HOBr + H^+ + Br^- \tag{22}$$

for which reaction the equilibrium constant is 5.8×10^{-9} mol/L.

Depending on the pH, the proportion of dissociation of hypobromous acid (HOBr) and hypobromite is

$$\frac{[OBr^-][H^+]}{[HOBr]} = K = 2 \times 10^{-9} \tag{23}$$

Like chlorine, bromine reacts with ammonia forming bromamine. Both Galal-Gorchev and Morris (33) and Johnson and Overby (34) have identified and studied the rate reactions of the compounds NH_2Br, $NHBr_2$, and HBr_3 using the ultraviolet absorption spectrophotometry technique. They reported rapid formation of all bromamine species; however, once the bromamines have formed, a series of decomposition reactions take place. The major chemical difference between the bromamine species and the chloramines species is that the formation of the bromamine species is reversible from monobromamine through dibromamine to tribromamine and back again by fast reactions, simply by changing the pH of the solution.

Bromine, like chlorine, displays a breakpoint, and it is the decomposition of the dibromamine that is the basis for this reaction. Tribromamine is the major species of combined residual bromine present beyond the breakpoint. In the pH range of 7–8 it decomposes in accordance with the following equation:

$$2NBr_3 + 3H_2O \longrightarrow N_2 + 3HOBr + 3Br^- + 3H^+ \tag{24}$$

La Pointe and co-workers (35) showed that the breakpoint occurs when the bromine/ammonia nitrogen molar ratio is 1.5. This is precisely the stoichiometric amount of bromine required to oxidize all of the ammonia to nitrogen gas.

For wastewater disinfection, it is of considerable practical significance that the predominant species of bromine compounds is dibromamine over a pH range of 7–8.5. This is so because dibromamine has a germicidal efficiency almost equal to that of free chlorine. Dibromamine is very active and usually displays a rapid

decomposition, reverting to the bromide ion. At this point, the bromine residual is extinguished. This feature of bromine reaction does not always occur in wastewater application, in spite of the presence of excess ammonia N. To date this aberration is thought to be due to the formation of stable organic bromamines resulting from the presence of organic N.

Free bromine residuals (hypobromous acid, HOBr), which would occur in highly nitrified effluents, do not decompose nearly as rapidly as the bromamines. Their persistence increases with decrease in halogen demand of the water being treated.

8.5. Reactions with Chlorine. The reactions with chlorine and the bromide ion are the only ones of particular interest. It is important to realize that free chlorine (HOCl) has the ability to oxidize bromide ions to form hypobromous acid (HOBr) in the pH range 7–9. This phenomenon was the basis of a patent issued to Marks and Strandkov (36). It was put to use in the application of chlorine to recirculated condenser cooling water at gasoline refineries in the 1950s. Bromide salts were added to the cooling water in the atmospheric tower basin. This was followed by intermittent chlorination. The only chemical consumption was that of chlorine. The chlorine added converted the bromides to hypobromous acid. When the bromine residuals disappeared, they reverted to the bromide ion. Thus the intermittent chlorination kept repeating the process to control algae in the towers and tower basins and slime in the condenser tubes.

However, chloramines residuals will not oxidize the bromide ion at pH 4, but free chlorine will, which is the basis of a free chlorine residual analyzer developed by Fischer and Porter in 1968 (37). This analyzer does not suffer from any significant chloramines interference when high concentrations of combined residual are present with free chlorine. A bromide salt is added along with pH 4 buffer to the sample. The free chlorine oxidizes the bromide ion to free bromine, which is measured by the analyzer cell.

Other applications of this phenomenon are described below.

8.6. Use of Bromine in Water Processes. *Potable Water.* The use of free bromine (Br_2) in potable water is probably nonexistent.

The only known use in municipal potable water treatment was at Irvington, California in ~1938. It was discontinued after a reasonable trial period because it did not solve the distribution system problem of water quality degradation. The bromine applied reacted so quickly and completely with the zoological slimes on the walls of pipes that it was impossible to obtain a residual downstream from the point of application. It also imparted a high intensity medicinal taste to the water (30).

As early as 1955, significant efforts were being made to produce solid or dry granular disinfectants using the best attributes of bromine. U.S. Pat. 781,730 was issued to the Diversey Corporation of Chicago, Illinois for the invention of a stable dry product composed of hypochlorite and alkali metal bromide. This product was claimed to have extraordinary disinfectant properties when placed in aqueous solution due to the formation of the hypochlorite–hyprobromite mixture.

In 1967 and 1969 patents were issued to Jack F. Mills and co-workers of the Dow Chemical Co (38,39). The invention described in these patents represents a process for treating water with elemental bromine obtained from the

polybromide form of an anion exchange resin. An effective method for the preparation of this resin is to pass an essentially saturated solution of bromine in aqueous sodium bromide slowly up through a bed of quaternary ammonium anion exchange resin. The resulting polybromide resin in wet form contains ~48% bromine. Development of the polybromide resin system as a practical means for disinfection has been concentrated on units capable of treating small quantities of water—potable water for household use and swimming pools (40). The Everpure Co. of Chicago has developed disposable cartridges containing bromine-impregnated resin to feed predetermined amounts of bromine into water for disinfection (41). The polybromide resin is sealed permanently into the cartridge to prevent its escape into the water system. Polybromide resins with bromine loadings of 25% have a very low acute oral toxicity. Direct contact with undiluted 25% resin is only moderately irritating to the skin but is capable of producing uncomfortable irritation upon direct contact with the eyes.

The disposable cartridge-type brominator has been installed and operated aboard offshore oil well drilling rigs, at some remote land stations, and on ocean-going vessels that use seawater desalination systems as a source for their potable water supply.

Wastewater. The use of bromine in wastewater or water reuse situations is unknown in the United States or Canada as of 1997. The only use that would be acceptable would be in a wastewater discharging into seawater. In the early 1980s, Dow Chemical Co. made a test project of bromine for disinfection of the East Bay Municipal Utility District WWTP effluent that discharges into the San Francisco Bay on the left-hand side of the highway entrance to the San Francisco Bay Bridge en route to San Francisco. The project failed because of the high oxidative power of the bromine. In other words, the bromine demand was "out of site." The required dosages were cost-prohibitive.

However, when a wastewater plant is near an adequate supply of seawater, such as the California coast, if it is convenient, the seawater can be used to operate the chlorinator injectors. The 60–80 mg/L of bromides in the seawater will be converted to bromamines in the chlorine solution. As the oxidative power of bromamines is equivalent to that of free chlorine (HOCl), the total effect by a comparative test at the Santa Cruz, California WWTP was a disinfection efficiency increase of ~15%.

Cooling Water. Around 1983, the electric power industry began an investigation using bromine in condenser cooling water treatment. The objective was to determine whether or not dechlorination could be avoided by using bromine, owing to the expected rapid decay of bromine residuals. The system adopted by some of the steam generating plants amounted to pumping a bromide salt solution into the chlorine solution discharge of existing conventional chlorination equipment. The chlorine oxidizes the bromide ion in the salt solution to free bromine, which goes into solution as hypobromous acid, the active ingredient. Whether or not the resulting bromine residuals will decay fast enough to meet the National Pollutant Discharge Elimination System (NPDES) discharge requirements is unknown at this time.

Swimming Pools. The most widespread use of bromine as a disinfectant began in Illinois during World War II. The Illinois State Department of Health, under the direction of C.W. Klassen, began an investigation of the use of liquid

bromine in the elemental form as a substitute for chlorine when the latter became scarce during the war years. After a trial run in a few swimming pools for a couple of years, it was concluded by bacteriological evidence that it was doing an efficient job of disinfection. Permission to use elemental bromine was granted to a number of additional pools in 1947, and before the end of that year there were about 25 outdoor and 30 indoor pools where bromine was applied instead of the difficult-to-obtain chlorine. However, in order to avoid the hazards of handling liquid bromine, bromine in stick form was introduced about 1958.

9. Bromine Chloride

9.1. Physical and Chemical Properties. Bromine chloride, BrCl, is classified as an interhalogen compound because it is formed from two different halogens. These compounds resemble the halogens themselves in their physical and chemical properties except where differences in electronegativity are noted. Bromine chloride at equilibrium is a fuming dark-red liquid $<5^{\circ}$C. It can be withdrawn as a liquid from storage vessels equipped with dip tubes under its own pressure (30 psig 25°C). Liquid BrCl can be vaporized and metered as a vapor in equipment similar to that used for chlorine.

Bromine chloride is an extremely corrosive compound in the presence of low concentrations of moisture. Although it is less corrosive than bromine (42), great care must be exercised in the selection of materials for metering equipment. Like chlorine and bromine, it may be stored in steel containers—assuming that the BrCl or Br_2 is packaged in an environment when the air is dry (ie, a dew point not higher than -40°F).

When in contact with skin and other tissues, liquid BrCl, like Br_2, causes severe burns. Low concentrations of the vapor are extremely irritating to the eyes and respiratory tract.

Bromine chloride exists in equilibrium with bromine and chlorine in both gas and liquid phases as follows (34):

$$2\,BrCl \longrightarrow Br_2 + Cl_2 \qquad (25)$$

There is little information on the equilibrium in the liquid state. A study by Mills (43) and an earlier investigation by Cole and Elverum (44) indicate less dissociation in the liquid (20%) than in the vapor state. The equilibrium constant for the vapor phase dissociation of BrCl is close to 0.34, which corresponds to a degree of dissociation of 40.3% at 25°C.

The density of BrCl is 2.34 g/cm^3 at 20°C.

The solubility of BrCl in water is 8.5 g/100 cm^3 at 20°C. This is 2.5 times the solubility of bromine and 11 times that of chlorine.

Bromine chloride forms a yellow crystalline hydrate, $BrCl \cdot 7.34\,H_2O$ at 18°C and 1 atm, which compares to the formation of chlorine hydrate ($Cl_2 \cdot 8H_2O$), that forms at 9.6°C. This characteristic is significant because the formation of these hydrates causes operational problems in metering equipment.

9.2. Preparation of Bromine Chloride. Bromine chloride is prepared by adding an equivalent amount of chlorine (as a gas or a liquid) to bromine until the mixture has increased in weight by 44.3%:

$$Br_2 + Cl_2 \longrightarrow 2\,BrCl \tag{26}$$

Bromine chloride may also be prepared by the reaction of bromine in an aqueous hydrochloric acid solution. In the laboratory, it can be prepared by oxidizing a bromide salt in a solution containing hydrochloric acid. This produces the following reaction:

$$KBrO_3 + 2\,KBr + 6\,HCl \longrightarrow 3\,BrCl + 3\,KCl + 3\,H_2O \tag{27}$$

9.3. Chemistry of Bromine Chloride in Water. Bromine chloride vapor appears to hydrolyze exclusively to hypobromous acid and hydrochloric acid:

$$BrCl + H_2O \longrightarrow HOBr + HCl \tag{28}$$

whereas bromine vapor (or liquid) hydrolyzes to hypobromous acid and hydrogen bromide:

$$Br_2 + H_2O \longrightarrow HOBr + HBr \tag{29}$$

The formation of HBr represents a significant loss in the disinfecting potential of the expensive bromine molecules. In the hydrolysis reaction of BrCl, any HBr formed by the dissociation of elemental bromine is presumed to be oxidized quickly to HOBr by the HOCl remaining in solution:

$$HBr + HOCl \longrightarrow HOBr + HCl \tag{30}$$

However, in the case of wastewaters, the nitrogen as ammonia present would immediately convert any HOCl in solution to chloramines in near-neutral pH environments. Chloramines cannot oxidize any HBr formed by hypobromous acid (HOBr) at these pH levels (45). Therefore, the problem of dissociation of bromine chloride is significant in the presence of ammonium ions.

It is of practical significance that the hydrolysis constant for BrCl in water is 2.94×10^{-5} at 0°C, compared with the same constant for chlorine, which is 1.45×10^{-4} at 0°C. It is paradoxical that BrCl is several times more soluble in water than chlorine, yet it hydrolyzes 10 times more slowly. The hydrolysis constant of molecular bromine is 0.7×10^{-9} at 0°C, which is significantly different from that of bromine chloride.

Bromine chloride combines with ammonia in the same manner as molecular bromine to form bromamines. At the usual pH levels encountered in wastewaters (7–8.5), the dominant species will be dibromamine (46). Typical reactions are as follows:

$$NH_3 + HOBr \longrightarrow NH_2Br + H_2O \tag{31}$$

$$NH_2Br + HOBr \longrightarrow NHBr_2 + H_2O \tag{32}$$

$$NHBr_2 + HOBr \longrightarrow NBr_3 + H_2O \tag{33}$$

Bromine chloride is presumed to have a higher speed of reactivity than bromine. In wastewater, the formation of bromamines is probably much faster than the formation of chloramines. Of greater practical significance is the rapid dieaway of the bromamine residuals. The half-life of bromamine residuals in secondary effluents is ~10 min. If organobromamines are formed, the half-life is much longer. In highly nitrified effluents the predominant species would be HOBr. This species of bromine residual persists for a significant length of time in a low-halogen-demand environment.

10. Iodine

10.1. Physical and Chemical Characteristics. Iodine, a nonmetallic element with an atomic weight of 126.92—the heaviest of the halogen group—was discovered by Curtois in 1811. It is the only halogen that is solid at room temperature, and it can change spontaneously into the vapor state without first passing through a liquid phase. It is isolated as a shining blackish-gray crystalline solid of specific gravity 4.93 with a peculiar chlorine-like odor. It melts at 113.6°C to a black mobile liquid and boils at 184°C under atmospheric pressure to produce the characteristic violet-colored vapor (47).

10.2. Occurrence and Production. Iodine is always found combined, as in the iodides. It is prepared from kelp and from crude Chile saltpeter. This saltpeter ($NaNO_3$) contains ~0.2% sodium iodate ($NaIO_3$). Historically, the United States has depended on imports for almost all of its iodine needs. Originally from Chile, iodine was produced as a coproduct from natural nitrate production. In recent times, however, Japan has become the dominant factor in iodine world trade. For example, the 1974–1976 United States consumption of iodine was on the order of 6–7 million lb; and during 1975, U.S. imports totaled 5.3 million lb, of which 93% was from Japan and the remainder from Chile (48). Chile's production appears to have stabilized at a maximum of 4.4 million lbs; this is from natural sources.

The small U.S. output in 1975 came entirely from the Dow Chemical Co. in Midland, Michigan, which recovered iodine and several other chemical products from a mixed-salts type of natural well brine. There are some reportedly high-grade brines in northern Oklahoma, which may be exploited by several companies. In the fall of 1975, Houston Chemical Co. broke ground for a 2 million lb/year iodine plant at Woodward, Oklahoma; nevertheless, most of the iodine comes from South America.

10.3. Uses. Iodine and its compounds are used as catalysts in the chemical industry (production of synthetic rubber), and in food products, pharmaceutical preparations, stabilizers (as nylon precursors), antiseptics, medicine (treatment of cretinism and goiter), inks and colorants, and industrial and household disinfectants.

Iodine was first used in medical practice for treatment of goiter by Prout in 1816 (49). The first specific reference to the use of iodine in wounds was in 1839. Iodine was officially recognized by the *Pharmacopoeia* of the United States in 1830 as tincture of iodine. The first official United States tincture was a 5% solution of iodine in diluted alcohol.

10.4. Use in Water Treatment. The first known field use for iodine in water treatment was in World War I by Vergnoux (50), who reported rapid sterilization of water for troops. Investigation on the use of iodine as an alternative method of disinfecting water supplies for United States troops were made in the years following World War I. Hitchens (1922) (51) recommended the use of 5 mL of a 7% tincture of iodine for a Lyster bag (~140 L or 37 gal), and stated that, even with raw Potomac River water, such a treatment rendered the water safe for drinking in 30 min. This was followed by Dunham's recommendation (1930) to increase the dosage to 10 mL of a 7% tincture of iodine per Lyster bag, or 2–3 drops per canteen. Pond and Willard (52) verified in 1937 that 2 drops/L of a 7% tincture of iodine is sufficient to render any water innocuous within 15 min, and that 3 drops/L did not give any better results.

During World War II, a series of studies at Harvard University by Chang and Morris, and Morris and co-workers (53,54) led to the development of globaline tablets for disinfecting small or individual supplies for the U.S. Army. Each tablet contained 20 mg tetraglycine hydroperiodide, of which 40% is I_2 and 20% iodide, combined with 85 mg acid pyrophosphate. One tablet imparts 8 mg I_2 to a liter of water. Chang and Morris (53) list the following advantages of the globaline tablet over the tincture: (*1*) Iodide is present in half the amount of I_2. (*2*) There is a definite dosage. (*3*) Iodine loss is insignificant. (*4*) The presence of an acid salt counteracts high pH. (*5*) The treated water is more palatable (less taste and odor). (*6*) The tablets are convenient for field use. The globaline tablets superseded the Halazone tablets for troop use because one globaline tablet per quarter or liter destroys the cysts of *Entamoeba histolytica* in 10 min, where six Halazone tablets are required to accomplish the same kill (56). Likewise, the human enteric pathogens (bacterial, viral, and protozoan) are satisfactorily destroyed in the same time under ordinary conditions. If the water is very cold, deeply colored, or highly turbid, the dosage should be increased to two tablets per liter and the contact time extended to 20 min (53–55).

Iodination of water supplies has been limited largely to emergency treatment by the military. The use of iodine as a disinfectant for water has been recognized for a long time, but has never generated enough interest to displace the popular use of chlorine. As compared to chlorine, the very high cost of iodine and its possible physiologic effects are the main reasons for its limited use. The military did not give unqualified approval to the use of iodine as a disinfectant for water until a 6-month study was made at a naval installation in the Marshall Islands in 1949–1950. The use of iodine in the drinking water revealed no untoward effects from its ingestion by a large group of service men (56), whose average intake was 12 mg/day for 16 weeks and 19.2 mg/day for 10 weeks.

In 1965, Black and co-workers (57,58) completed a demonstration project, with a grant from the U.S. Public Health Service, to study the effects of prolonged use of iodine at three correctional institutions in Florida. Iodination of these water supplies was carried out over a period of 19 months, serving ~700 persons under carefully planned chemical, medical, and bacteriological controls. Iodine proved to be a satisfactory disinfectant and in doses up to 1.0 ppm did not produce any discernible color, taste, or odor in the water. There was no evidence from the medical investigation that this level of iodine had any adverse effect upon the general health or thyroid function.

11. Peracetic Acid in Wastewater Treatment

Commercially available peracetic acid, also known as ethaneperoxoic acid or peroxyacetic acid, is available in a quaternary equilibrium mixture containing acetic acid, hydrogen peroxide, peracetic acid, and water, as shown in the following equation:

$$CH_3CO_2H + H_2O_2 \longrightarrow CH_3CO_3H + H_2O \quad (34)$$

Where CH_3CO_2H = acetic acid, H_2O_2 = hydrogen peroxide, and CH_3CO_3H = peracetic acid. The biocidal form of the mixture is considered to be undissociated acid, which is predominant at a pH of <4.7. However, hydrogen peroxide also may contribute to disinfection. In surface waters, peracetic acid will be hydrolyzed.

Although peracetic acid is known to be a potent antimicrobial agent, quantitative information on the activity of peracetic acid against typical wastewater microorganisms had been scarce until Solvay (Warrington, Chesire, England) conducted a series of laboratory and field tests to study the effectiveness of Oxymaster (a range of peracetic acid-based disinfectants formulated by the company) in disinfecting wastewaters and effluents (59). The authors concluded that the potential of using peracetic acid for wastewater disinfection application is significant, and cannot be overlooked. Cost-effective, environmentally benign, and easy to use, peracetic acid continues to gain favor abroad and deserves a close look in North America.

12. Antimicrobial Nanoemulsion Technology

The NanoBio antimicrobial nanoemulsions are water/oil emulsions that employ uniformly sized droplets in the 200–400 nm (10^{-9} m) range. These droplets are stabilized by surfactant and are responsible for the cidal activity. In concentrated form, the nanoemulsions appear as a white milky substance with a taste and consistency of cream. They can be formulated in a variety of carriers allowing for gels, creams, liquid products, etc. In most applications, the nanoemulsions become largely water-based, and in some cases such as a beverage preservative comprise 0.01% or less of the resultant mixture. Laboratory results indicated a shelf life of at least 2 years and virtually no toxicity.

The NanoBio nanoemulsions destroy microbes effectively without toxicity or harmful residual effects. The classes of microbes eradicated are [eg, human immunodeficiency virus (HIV), herpes], bacteria (eg, *Escherichia coli, Salmonella*), spores (eg, anthrax), and fungi (eg, *Candida albicans, Byssochlamys fulva*). The nanoemulsions also can be formulated to kill only one or two classes of microbes. Due to a large part to the low toxicity profile, the nanoemulsions are a platform technology for any number of topical, oral, vaginal, cutaneous, preservative, decontamination, veterinary, and agricultual antimicrobial applications.

In case of bioterrorism attacks, the nanoemulsions can potentially destroy pathogens such as anthrax, ebola, and many others. Since it is nontoxic and noncorrosive, it can be used to decontaminate personnel, equipment, terrain,

structure, and water. Recently, the U.S. Army tested the nanoemulsions at Dugway, Utah, against an anthrax surrogate. The emulsion proved to be effective (60,61).

13. Thermal Disinfection

The application of heat is the oldest disinfection technique. Variations in chemical impurities in water do not interfere with the disinfecting efficiency of heat as they do with chlorine and other chemical disinfectants. Capital costs for necessary equipment with automatic controls are low. However, depending on the nature of the application, this can be an energy intensive operation with associated high operating costs. In addition to bacterial pathogens, heat readily destroys a variety of microorganisms that are highly resistant to chemical disinfectants. These organisms include amoebic cysts, worms, and viruses (15).

13.1. Principles of Application. Dry or wet heat can be used to destroy bacteria. These forms of treatment can be used for air and surface disinfection, respectively. Thermally induced deaths of bacteria cells and spores appears to be an exponential function. Chick (62) noted that a monomolecular chemical reaction is needed for the reaction to take on a first-order dependency. Another way of stating this is that the death rate of bacteria subjected to heat is logarithmic; ie, the reduction in the number of viable cells is an exponential function of the exposure time at a constant temperature.

The time needed to destroy an unspecified number of organisms at constant temperature depends on the number of organisms subjected to the heat treatment. Death rate, then, is a function of the population size of cells directly exposed to heat (63).

13.2. Temperature. The effect of temperature on rate of kill can be represented by a form of the van't Hoff–Arrhenius relationship. Increasing the temperature results in a more rapid kill. In terms of the time t required to effect a given percentage kill, the relationship is

$$\ln\frac{t_1}{t_2} = \frac{E(T_2 - T_1)}{RT_1T_2} \tag{35}$$

where

t_1, t_2 = time for given percentage kill at temperatures T_1 and T_2, K, respectively
E = activation energy, J/mol (cal/mol)
R = gas constant, 8.31 J/mol K (1.99 cal/K mol)

Some typical values for the activation energy for various chlorine compounds at different pH values are reported in Table 9 (64).

13.3. The Inate Heat Resistance of the Strain. The more heat resistant a strain, the more likely it is to have a pronounced activation requirement. Spores of *Clostridium thermosaccharolyticum* are a unique example of this having a θ value of 110 at 121°C (65). On this basis, it would require over 11 h to kill

Table 9. **Activation Energies for Aqueous Chlorine and Chloramines at Normal Temperatures**

Compound	pH	E, cal/mol[a]
aqueous chlorine	7.0	8,200
	8.5	6,400
	9.8	12,000
	10.7	15,000
chloramines	7.0	12,000
	8.5	14,000
	9.5	20,000

[a] Cal $\times$ 4.1876 = J.

a population of 1,000,000 spores. This represents 44 times greater resistance over spores ordinarily used as sterility indicators. The survival curve of spores of the thermophile *Bacillus stearothermophilus* is shown in Figure 1. Note that the curve showed both an initial lag and a pronounced hump.

13.4. The Temperature of the Treatment. The lower the treatment temperature, the more pronounced the initial lag is likely to be because of a relatively slow activation rate. Conversely, as treatment temperatures increase, the activation time reduces, the hump becomes larger, and the lag time shorter (eventually approaching extinction because of the inability to observe the effect over very short increments of time). This may partially explain some of the nonlinearity that exists at the initial portion of survival curves.

13.5. The Outgrowth Media. The degree of spontaneous germination and the contribution to germination due to constituents of the growth media

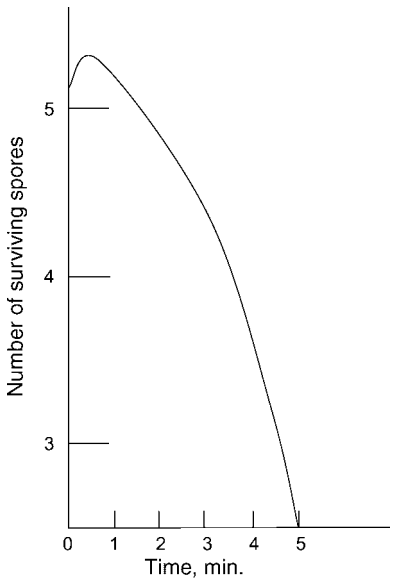


Fig. 1. Typical survival curve for *B. stearothermophilus* spores on filter paper strips exposed to steam >121°C (3).

influences the shape of the initial phase of the survival curve as determined by colony count.

13.6. The Incubation Temperature. The incubation temperature also contributes to the activation of a fraction of the spores in the population. This is not, however, as great a contribution.

13.7. The Past History of the Crop. Drying or wetting, storage temperature, and freeze–thaw cycles all contribute to the shape of the survival curve.

Other factors such as clumping, diffusion, and thermal lags, may also influence the response to heat treatment and the ultimate shape of the survival curves. These factors, however, do not affect the characteristic activation principle which is inherent to the heat-resistant spore.

13.8. Thermal Resistances. Heat resistance varies among microbial species, proteins, and bacterial spores. There are a number of bacteria spore species that are highly resistant to most chemical, physical killing agents, as well as heat. There is a significant difference between the thermal resistances to moist and dry heat. A spore species resistant to one form of heat is not necessarily resistant to the other. A comparison of the spores of thermophile *B. stearothermophilus* with mesophile *B. subtilus* var. *niger* is a good example. These spores are used to monitor sterilization processes for moist and dry heat, respectively. To activate 10^5 spores of *B. stearothermophilus* (in saturated steam at 121°C) requires roughly 12 min of exposure. However, 10^6 spores of *B. subtilus* are inactivated in <1 min under identical conditions.

Amaha and co-workers (66) noted that differences in the degree and rate of thermal destruction could be related to the loss of cations, which enhances the thermoresistance of spores. Also, the rate of loss of viability would be greater in the presence of an increased phosphate concentration or more powerful chelating agents.

Dipicolinic acid (DPA) has been found in all bacterial endospores examined (67). However, it seems to be absent from nonresistant vegetative cells. DPA has been recognized as an agent in establishing thermoresistance (68). Another agent of thermoresistance is calcium. Refractility to heat of the spore and the amount of DPA synthesized have been found to be proportional to the amount of calcium available when calcium is in limiting concentrations.

Zytovicz and Halvorson (69) noted that DPA is a major constituent of bacterial spores. It is believed that its calcium salt is a major contributing factor to heat resistance. Since activation precedes inactivation, the activation process must depend on the dissolution of the Ca^{2+}–DPA complex. Also, the rate of loss of DPA on heating seems to be related to the thermal death of spores, and that heat-resistant strains apparently lose DPA less quickly than heat-sensitive strains. The kinetics of release of the Ca^{2+}–DPA from *B. steroathermophilus* spores is related to time, temperature, and pH.

Bacterial death rates are higher in acid or alkaline media than in neutral suspensions (70). Also, a higher recovery of survivors occurs in the neutral pH zone. Citrate, phthalate, or ammonium buffers reduce thermoresistance of spores compared to those in a phosphate buffer. Spores are more readily inactivated at a low pH, since pH can influence the type of ions that absorb onto the spore surface (this absorption alters the heat stability). Some of the properties of

spores resemble the behavior of ion exchange gels. The spore exchanger resembles a weak cation exchange system. Consequently, the hydrogen ion possessing the greatest exchange potential would displace other cations. This base exchange mechanism allows for the adsorption of calcium in excess of the DPA chelation equivalent as reported by Lechowich (71).

13.9. Spore Biosynthesis of Heat-Resistant Components. Sporulation is a multiphase process. First, the refractile cell is formed; then DPA is synthesized. The final step is the development of the thermoresistant spore.

Warth and co-workers (72) found that the most heat-resistant spores contained the highest amounts of DPA and hexosamine. They concluded that these peptide constituents reside in the cortex and are involved in the basic mechanism of heat stabilization of spores.

Calcium is notably associated with both heat-refractile and relatively water-insoluble compounds. Such an exosporium depleted of calcium and DPA would be expected to act like a porous membrane, which swells under hydrostatic pressure with the absorption of water. Swelling is typical of the germinating spore.

Polypeptides have the capability for extensive intra- and inter-molecular bonding of the hydrophobic type. This plays an important role in the composition of spore coats. Also, increased density is produced by increased binding of heavy atoms, which is presumed to be necessary for sporulation. Tables 10 and 11 list heat resistant characteristics of several bacterial species (73).

13.10. Applications of Heat Treatment. The terms sterilization and disinfection should not be used interchangeably. It is only when heat is used to affect microorganisms that both have the same meaning. Probably the oldest disinfection application is that of boiling water. Most water can be sterilized by simply boiling for 10–20 min. The application of thermal treatment for

Table 10. **Thermal Resistance Characteristics of Bacterial Spore Species**[a,b]

	Moist heat		Dry heat		
Species	D250	z in °F	Heating media	D350[c]	z in °F
B. subtilis			(SHS)	0.57	(42)
B. subtilis			(N+He)	0.17	(31)
B. subtilis			(A,O_2CO_2)	0.13	(31)
B. sterothermophilus	4.0	18	(SHS)	0.14	(26)
B. olymyxa	0.005	14	(SHS)	0.13	(28)
C. botulinum	0.20	15	(A)	0.20	(61)
P. A. 3679	1.50	18	(SHS)	0.13	(60)
P. A. 3679			(He)	0.44	(39)
P. A. 3679			(A)	0.30	(39)

[a]See Ref. 73.

[b](TDT) thermal death time. This is the time in minutes at a particular dry heat temperature to kill all the spores in a given population. z-Value represents the slope of the TDT (log scale) verses temperature curve. D-value is defined as the time in minutes at a particular temperature to reduce the orignal spore by 90%.

[c]Heating media: SHS = superheated steam, N = nitrogen, He = helium, A = air, O_2 = oxygen, CO_2 = carbon dioxide.

Table 11. **Resistance Parameters for Bacteria Spores Exposed to an Ethylene–Dichlorodifluoro–Methane Mixture**[a]

Species	30°C 86°F	40°C 104°F	50°C 122°F	60°C 140°F	70°C 158°F	80°C 176°F	Z
B. subtilis (ATCC 9524)		17.0	7.70	3.55	1.62	0.74	53 °F 29.4 °C
C. Botulinum (Type A-Str.62)		12.2	6.14	3.00	1.48		59 °F 32.8 °C
B. coagulans	10.9	7.00	4.51	2.89			94 °F 53.3 °C

[a]See Ref. 73.

disinfection is not limited to water treatment. There are numerous applications in the canning and food industries as well. There are three specific applications in which thermal treatment is widely used. These are in the processing of dry goods, pasteurization of water, and the thermal conditioning of sludges. Each is discussed below.

13.11. Processing of Dry Goods. The time required to destroy *B. subtilis* var. niger at 121°C is almost 2000 times as long in hot air as it is in steam. Saturated steam at 121°C provides at least seven times more available heat as air. The time needed to kill organisms is not directly proportional to the amount of available heat, but rather depends on different reaction mechanisms which are related to steam versus dry heat.

Steam sterilization is a highly effective method. There are, however, difficulties in its application, viz, moisture penetration, air removal and entrainment, heat and moisture damage, and wetting.

To obtain efficient air removal, it is necessary to use low-temperature steam in conjunction with a high vacuum. A high-vacuum unit will provide a small but steady influx of steam to the sterilization chamber during evacuation. This type of system is prone to air entrainment, which can result from three different causes, viz, a small load in a large volume apparatus, insufficient removal of air by prevacuum, and air leakage into the system.

Air having low translational kinetic energy is driven by relatively high energy steam. If the air is well distributed in a large enough load, no air will be entrained (assuming no air leaks and if the degree of prevacuum is sufficient). The severity of the air entrainment problem depends on the amount of air available relative to the load and its energy state. The greater the energy differential, the worse the air entrainment will be.

The advantages of steam-processing dry goods include a fast cycle time where much larger volumes of material can be processed per day and the fact that damage to materials is minimized because relatively short exposure times are needed.

13.12. Pasteurization of Water and Milk. The success achieved in killing pathogens in milk via pasteurization suggested the use of heat as a means of destroying coliform organisms and eliminating the problems of chemical feeding. Variations in chemical impurities in water do not interfere with the disinfection efficiency of heat as they do with chlorine and many other chemical disinfectants.

In addition to bacterial pathogens, heat readily destroys other organisms, such as cysts, worms, and viruses. These are generally more resistant to chemical disinfectants than vegetative bacteria.

Technology for disinfecting small quantities of water with heat is well established. However, it is difficult, time consuming, and troublesome to disinfect large quantities of water.

Recent efforts have been directed at reducing the required exposure time to achieve sterilization. These efforts have been made in both the heating and cooling phases. The water being treated must be heated to a high temperature very rapidly, followed by a rapid cooling cycle. This, however, requires careful control.

The same time–temperature relationships hold for water and water solutions as for dry goods in steam. However, the time necessary to attain a specified temperature must be added to the process. The dairy and canning industries have used automated processes integrating for time–temperature requirements over the heating and cooling phase of the treatment.

For water pasteurization to be economical, it is necessary to use the heat of the treated water to warm the water to be treated. Also, such a system must be operated at a nearly steady flow rate to reduce heat losses. This requires proper surge tanks and reservoirs from which treated water can be recycled for treatment of incoming streams.

In a typical treatment system, untreated water would enter a heat exchanger. An electric heater would raise the water temperature to the pasteurization temperature. A 15-s retention time at the pasteurization temperature is typically needed. This would be done in a retention tube. An air relief valve would permit escape of gases liberated at the higher temperature. One of the drawbacks with such a system is that water temperatures can fall $<161^{\circ}$F. To prevent this, a solenoid valve and a thermostat switch must be properly arranged. When the water is hotter than 161°F, the water flows through the heat exchanger, where it supplies its heat to the incoming water and is then cooled. The heat exchanger is generally the most expensive component in this system.

For proper evaluation of such a system, the following areas should be evaluated.

- The effectiveness of the unit in killing bacteria.
- The power consumption required for the unit.
- Maintenance requirements and reliability of the equipment.
- The significance of scale or corrosion in reducing the operating efficiency or life of the equipment.

The removal and destruction of coliform bacteria for an operation similar to the one described is given in Table 12. At no time during normal operation were coliform organisms isolated from the finished water. On one occasion, the unit was challenged by adding coliform organisms to the clear well to produce a concentration of 950,000/100 mL. One coliform organism was isolated from the 50-mL portion, for an average count of 0.75/100 mL. Thus, even with a very high concentration in the unpasteurized water, the final product was satisfactory.

Table 12. **Data on Coliform Destruction by Pasteurization**[a]

Date of samples		Max. treated water temperature (°F)	Retention time at max. temperature (s)	Number density in raw water (MPN/100 mL)	Number density in clear well (MPN/100 mL)
Jan.	22	166	2	1,600	540
	27	164	2	110,000	1400
	29	166	2	14,000	2300
Feb.	3	165	2	7,900	110
	19	164–174	2	4,900	130
	24	164–176	2	1,700	33
	26	164–176	2	7,900	490
Mar.	10	166–176	2	4,900	3300
	12	164–176	2	79,000	3300
	17	164–176	2	460,000	700
	19	164–178	2	23,000	330
	24	162–170	2	1,400	130
Apr.	7	164–179	15	13,000	79
	9	168–178	15	1,700	13
	14	161–170	15	11,000	13
	16	165–175	15	2,200	220
	21	161–170	15	490	6.8
	23	161–170	15	33,000	79
May	5	163–170	15	460	49
	12	164–177	15	22,000	100
	21	164–171	15	11,000	4.5
	23	168–180	15	1,100	33

[a]See Ref. 16.

14. Disinfection with Sound

Sound waves are an alteration in pressure, stress, particle displacement, particle velocity, or a combination of the above that is propagated in an elastic medium. Sound waves thus require a medium for transmission (ie, they cannot be transmitted in a vacuum). The sound spectrum covers all possible frequencies. The human ear responds to frequencies in the range of 16 Hz to 16 kHz. Frequencies >20 kHz are ultrasonic. Sound waves in the 50–200-kHz range are employed for cleaning and degreasing.

In water purification, ultrasonic waves have been used to effect disintegration by cavitation and mixing of organic materials. The waves themselves have no germicidal effect. However, when used with other treatment methods they can provide the necessary mixing and agitation for purification (15).

14.1. Ultrasonic Generators and Bubble Dynamics. Among the possible applications that can be derived from ultrasonics are

- Faster rate of coagulation of suspended particles in water and sewage.
- Sterilization of water and sewage effluents.
- Lower treatment costs for sewage and industrial wastes.

To assess these benefits, it is necessary to examine the usual methods of sound generation and the manner in which they are applied to the liquid media.

Sound wavelengths, like wavelengths of light, may be arranged in a linearly structured spectrum. The sound spectrum may be divided into three portions, only one of which is audible to the human ear. Anything <20,000 cycles per second (cps) is considered to be subsonic. Above 20 cps is ultrasonic. To have a bactericidal effect, it is necessary to produce high intensity sound above the subsonic frequencies. Because the ear does not respond to these frequencies, it is possible to work without discomfort in the presence of sound intensities billions of times as great as those in the audible range.

The effects of ultrasonic vibrations are not generally attributed to the high acoustic frequency, but rather to the high sound intensity. Frequencies >xs18,000 cps are used to increase the acoustic intensity far beyond that which is tolerable at audible levels without endangering the human hearing mechanisms.

There are various types of generators used to produce high generators sound waves. One type utilizes the magnetostrictive properties of nickel and cobalt. These metals are placed in an alternating magnetic field that causes the metal to expand or contract in a direction parallel to the magnetic field. The basic mechanism of this generator consists of a rod that oscillates to generate sonic energy. The generated energy is produced by a resonant magnetic field that in turn is created by the coordination of an electric circuit, density, modulus of elasticity, and mass of the metal.

Acoustic generators are used primarily to produce cavitation in liquids. There are only a few cases in which the observed effects of ultrasonic vibrations have not been attributed to cavitation. Normally, a high intensity sound is passed through water causing the liquid to experience alternating tension and compression. When the acoustic intensity is sufficiently high, the tensile stress placed on the water becomes sufficiently large to rupture the liquid's molecular structure. This will occur at a weak point in the liquid, such as a gas bubble or a dust particle site. The acoustic pressure variations do not necessarily have to place the liquid under tension. This is only true when the instantaneous pressure is decreased low enough that the vapor pressure of the dissolved gases and of the liquid itself is reached. Here, the liquid is disrupted by the formation of gas bubbles. In general, there are low and high pressure zones created by sound waves. The bubble formation may be visualized as a localized boiling phenomenon.

During cavitation, it is possible to see that the liquid is subjected to successive low and high pressure zones making bubbles form in the low pressure phase to collapse very quickly when followed by a high pressure region. On a continuous basis, the formation and collapse of bubbles occurs very rapidly. Thus, individual bubbles are relatively small when they collapse. Under this circumstance, the implosion may be related to a water hammer phenomenon, with localized liquid pressures reaching the order of hundreds of atmospheres. High intensity shock waves formed by the collapse of the cavitation bubbles are responsible for destructive effects of ultrasonic vibrations. Figure 2 illustrates the bubble dynamics involved in cavitation (15).

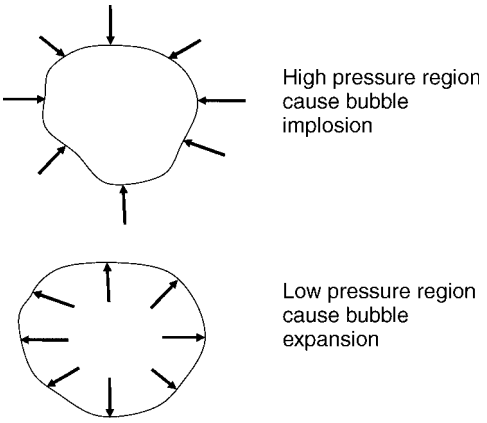


Fig. 2. Bubble dynamics.

It is common practice to describe cavitation by a dimensionless number known as the cavitation number.

$$\sigma_c = \frac{P - P_v}{\rho V^2 / 2g_c} \tag{36}$$

where

P = static pressure (absolute in undisturbed flow) (lb-force/ft^2)
P_v = liquid vapor pressure (absolute) (lb-force/ft^2)
ρ = liquid density (lb/ft^3)
V = free-stream velocity of liquid (ft/s)
g_c = dimensionless constant = 32.17 (lb) (ft)/(lb-force) (s^2)

The cavitation number is the ratio of the net static pressure available to collapse the bubble to the dynamic pressure available to initiate the formation of the bubble. The value of σ_c for incipient cavitation for a specific boundary condition is a characteristic of the system geometry.

14.2. Application to Microorganism Destruction. Induced cavitation through ultrasonics can be used to destroy bacteria, provided the cavitation implosion occurs in the immediate vicinity of the bacteria cell. The effective range of a shock wave generated by the implosion is $\sim$1–2 μ.

To assure an adequate bacteria kill rate, a cavitation nucleus must exist in the molecular structure of the water surrounding the bacterial cell. The weaker this nucleus becomes, the greater the probability that cavitation will occur; thus, the greater the probability that a bacteria cell will be destroyed.

The sonoration causing cavitation enhances inactivation by reducing the high surface tension caused by organic material (74). Although the sonication process reduces the time needed for a complete inactivation, by itself it would

not have the desired effect. It is desirable to combine sonication and ozonation. This combination results in a synergistic effect that inactivates microorganisms.

14.3. Factors Contribution to Bactericidal Destruction. Horton and co-workers (75) reported the effects of ultrasound on various bacteria. Studies on various aged cultures (1-, 3-, 7-, and 8-day-old culture samples) showed that the older the sample, the more susceptible microorganisms were to ultrasonic vibrations. For the oldest sample, sterility was achieved after only 25 min of sonoration. The standard 1-day culture sample (youngest culture) was sonorated for 60 min under comparable conditions and found to contain 0.05% of the original number of organisms. Although this appears to be a small concentration, it represents ~400 organisms/mL due to the high initial concentration of microorganisms used in the tests. Horton's study showed culture age to play an important role in the lethal effects of ultrasonic vibrations on bacteria.

Another important and obvious parameter is temperature. The temperature of the microorganisms' environment directly impacts on the death rate. Figure 3 shows the effect of temperature of the fraction of organisms (*E. coli*) surviving after exposure to ultrasonic waves. The death rate of *E. coli* increases with increasing temperature. Figure 3 shows that as temperature increases, a shorter exposure time is needed to achieve the same degree of microorganism destruction.

Other major parameters significantly affecting the efficiency of ultrasonic destruction are surface tension and pH. It is worthwhile to examine some of the experimental studies of these parameters in detail.

Surface Tension. The surface tension of an aqueous solution is a good indication of the intermolecular strength of weakness of the system. For weakly structured systems, cavitation plays the dominant role in microorganisms

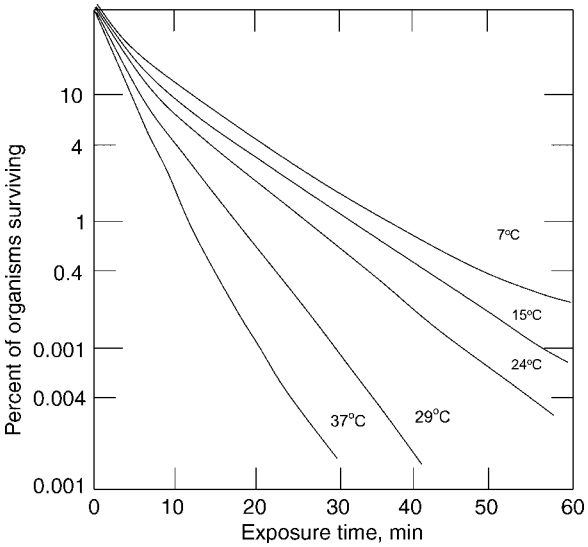


Fig. 3. Effects of temperature on the ultrasonic death rate of *E. coli*.

Table 13. **Data of Horton and Co-Workers Showing Killing Rate of *E. coli* for Different Surface Active Agents**[a,b]

		Exposure time (min)					
Compound	Conc (ppm)	6	15	25	40	Surface tension (dyn/cm)	Killing rate (min^{-1})
distilled H_2O		110.0	58.3	35.8	0.132	72.8	0.047
NaCl	8.500	80.9	44.4	10.5	0.154	76.6	0.057
$NaNO_3$	200	67.0	28.5	3.72	0.91	73.1	0.063
	600	67.0	32.9	5.01	0.119	71.4	0.092
sodium benzoate	400	93.8	43.6	8.58	0.026	69.9	0.041
	1.200	75.7	35.5	11.7	0.103	72.8	0.088
leucine							
	600	107.1	61.3	36.1	12.3	63.7	0.027
	1.000	128.2	81.0	52.6	25.8	66.4	0.020
glycine	400	95.4	43.9	17.8	2.33	71.0	0.045
	1,200	98.5	47.3	16.3	2.94	70.4	0.044
	2.000	96.8	47.6	16.1	4.32	69.1	0.042
peptone (bacto)	25	71.8	30.2	6.2	1.18	71.8	0.056
	75	73.0	29.2	14.1	3.60	70.1	0.037
	125	68.2	31.2	14.8	4.23	68.7	0.035
gelatin	200	132.6	93.8	61.5	14.9	66.6	0.031
	600	85.0	65.0	40.8	22.0	61.6	0.018

[a]Ref. 76.
[b]All data at 20°C.

destruction. For this reason, studies have been directed at examining the effects of surface active agents on ultrasonic destruction efficiency.

Horton and co-workers (75) used sodium chloride, sodium nitrate, sodium henzoate, leucine, glycine, and gelatin as surface active agents in their study. These materials were added in various concentrations to bacterial suspensions. Surface tension of resultant systems were measured with a du Nouy platinum ring torsion meter. Control suspensions (those that were not sonorated) were observed in all cases. Whenever a significant change in bacterial numbers occurred because of the presence of the surface tension alterant, that suspension was eliminated from the experiment. Test bacterial suspensions were sonorated for a specific time and samples were withdrawn at appropriate intervals for analysis.

Horton and co-workers developed a correlation between the surface tension of the bacterial suspension and the rate of bacterial destruction. Table 13 presents the results of some of their data for *E. coli*. The study showed that surface tension plays a significant role in the ultrasonic killing rate. As the surface tension increases, the killing rate increases exponentially.

A statistical analysis of their data using linear regression gave the following general correlation between killing rate and surface tension.

$$\eta = -4.6 + 0.05\sigma \tag{37}$$

where

η = the logarithm to the base 10 of the ultrasonic killing rate in units of reciprocal minutes (min^{-1}).

σ = the surface tension of the suspension (dyn/cm).

The coefficient of determination for their data was 0.82.

14.4. Destruction of *Endamoeba Histolytica*. Cysts of *Endamoeba histolytica* (*E. histolytica*) are highly resistant to disinfection by chlorine. Studies of *E. histolytica* are quite different from those of *E. coli* because of the nature of these organisms. The culture is normally carried on a Boeck-Drbohlav egg medium, on which the organisms exist as active trophozoites.

For testing purposes, cysts can be grown in culture tubes. When an adequate culture has been obtained, samples are centrifuged and the sediment placed in sterile saline. This can then be aseptically placed in the test chamber.

Cyst enumeration is accomplished by removing a sample of the suspension with a wide-mouthed pipet and transferring a quantity to a microscope slide. Cysts are then stained with iodine and placed under a cover slip. The sample can be studied under a microscope and the population size counted. Generally, there is no difficulty in distinguishing between viable and destroyed cysts. The latter are no longer discernable under the microscope.

Ultrasonics prove to be a better disinfectant than chlorination for *E. histolytica*. Furthermore, *E. histolytica* is much more easily destroyed by ultrasonics than other microorganisms. Figure 4 compares the ultrasonic death rates of *E. histolyca* to *E. coli* and yeast. Cysts can be destroyed in a much shorter time than *E. coli*. The time required for sterilization is slightly >30 s. Also, the active form of the protozoan (trophozoite) can be destroyed even more rapidly than the resistant encysted form.

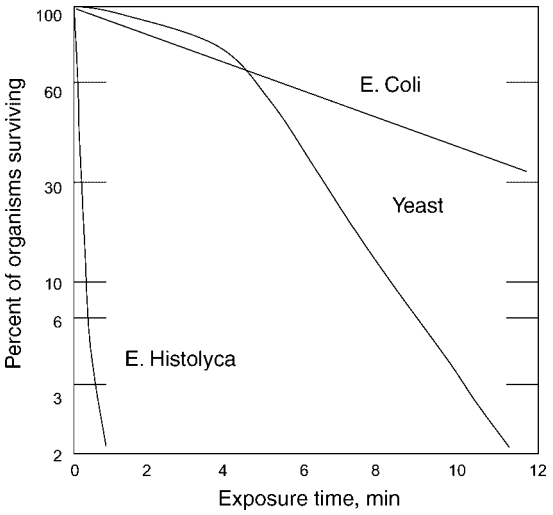


Fig. 4. Compares the ultrasonic death rates of *E. histolytica*, *E. coli* and yeast.

14.5. Miscellaneous Wastewater Applications. Since ultrasonics can be used to facilitate coagulation, it makes a perfect method for hardness removal. Ultrasound can be used to precipitate calcium oxide (CaO) and magnesium oxide (MgO), which are major constituents contributing to hard water.

Ultrasound can be used in conjunction with ozonation. Ultrasound will break particle aggregates into discrete particles. This results in a much greater surface area exposed for ozone to oxidize contaminants. In terms of design requirements for ozonation vessels, smaller volume requirements are needed to achieve the same degree of germicidal destruction than a system without ultrasonics. Land requirements for a plant of this type can require as little as one-fifth of that for a conventional treatment plant.

Ultrasound can be effectively used in the bacteriological and viricidal fields. It can also reduce turbidity if used with a $CaSO_4$ solution. Ultrasound is an effective method for wastewater disinfection. It provides residual disinfection much as chlorine does. That is, even 72 h after sonification, microorganism regrowth is not observed. However, ultrasound does have disadvantages. Production of bubbles or cavities is needed for this technique to be a powerful disinfection. A relatively high cost is associated with this technique.

15. Ultraviolet Disinfection of Wastewater

15.1. Introduction. In rural areas of India, Bangladesh, Brazil, Peru, China, and other underdeveloped and developing nations, waterborne diseases such as typhoid, cholera, hepatitis, and gastroenteritis infect and kill many infants and children each day, according to the WHO (76). Wastewaters or water can contain an incredibly large variety of microorganisms, although some are harmless, many however, are disease causing. These microorganisms must be destroyed before the water is safe to discharge into a receiving body of water or to reclaim and reuse it. With increasing emphasis on promoting a sustainable ecological future and concern over introducing a toxic chemical in the water, the design of the disinfection process is increasingly leaning toward technologies that destroy the pathogens while balancing the effects of this disinfected wastewater on the population of aquatic biota or a drinking water supply.

Due to current fire codes and public health concern, some municipalities are limited to a certain amounts of chlorine they can store at any given time on the plant which makes it difficult to manage chlorination process and meet the NPDES permit limits for a major treatment plant in the United States. Furthermore, the U.S. regulatory concern over toxic chemicals in water, limiting on the amount of chlorine discharging into the receiving water or establishment of total residual chlorine (TRC) limits on the wastewater effluent. These limits could be, and usually were met by designing an additional processing step, called dechlorination, into the disinfection process. However, the dechlorination process uses yet another chemical, sulfur dioxide, to remove chlorine from the effluent before it discharged to receiving water. However, the chlorination and dechlorination of wastewater were not environmentally acceptable because they produce possible carcinogens and destroy the aquatic biota in receiving waters. Across the United

States the EPA started to look for an alternative wastewater disinfection system. Various governments, municipalities (77–80) and corporations sponsored research (14,81–84) to show that the uv disinfection of wastewater was effective and economical. Another most important development was the parallel flow open channel modular uv system. This new design of the uv system for wastewater in the early 1980s opened up uv disinfection for both the retrofit market and new wastewater treatment plants. To promote a friendlier discharge to the marine environment, designers have begun to prefer alternative disinfection technologies, which emphasize sustainable and clean ecological disinfectants—such a clean technology is uv disinfection. Since uv irradiation is not a chemical additive, it does not leave or produce any toxic compounds in the wastewater. Therefore, the use of uv does not affect a drinking water supply or the aquatic biota in receiving waters. In most areas of the United States and Canada, the use of uv light irradiation for the disinfection of wastewater has become the accepted alternative to chlorination or chlorination/dechlorination.

15.2. What Is UV Light and the Mechanism of Germicidal Action? The power of sunlight to destroy microbial life has long been known and appreciated. Effective disinfection in air, on surfaces and in water has been accomplished by exposure to the direct rays of the sun. Sunlight is an important factor in the self-purification of water in streams and in impounding reservoirs. The effect of sunlight at destroying bacteria, particularly intestinal bacteria has been reported many times. The ordinary rays of sunlight play little part in this bactericidal action. The results are caused by uv rays. Sources of high intensity ultraviolet light have been developed that can be used to disinfect water, wastewater, air, etc.

The term "ultraviolet light" or simply "ultraviolet (uv)" is applied to electromagnetic radiation emitted from the region of the spectrum lying beyond the visible light and before X-rays. The upper wavelength limit is 400 nm ($1\ \text{nm} = 10^{-9}\ \text{m}$) and the lower wavelength limit is 100 nm, below which radiation ionizes virtually all molecules. The narrow band of uv light lying between the wavelengths of 200 and 300 nm has often been called the germicidal region because uv light in this region is lethal to microorganisms including: bacteria, protozoa, viruses, molds, yeasts, fungi, nematode eggs and algae. Figure 5 shows that the most destructive wavelength is 260 nm, which is very close to the wavelength of 254 nm produced by germicidal low-pressure uv lamps. Figure 5 also shows the similarity between uv light's ability to kill the fecal coliform, *E. coli* and the ability of its genetic material (ie, nucleic acid) to absorb uv light. The uv light causes molecular rearrangements in the genetic material of microorganisms and this prevents them from reproducing. If a microorganism cannot reproduce then it is considered to be dead.

15.3. How Does UV Light Work? Ultraviolet light disrupts the dividing of the DNA (genetic material, chromosomes) and the production of enzymes by the following mechanism (Fig. 6).

The components within the DNA that absorb the uv light are the nucleotide bases: adenine, guanine, thymine, and cytosine. Although proteins fulfill many vital functions in cells, their uv absorption compared with that of DNA is of minor consequence. Although the nucleotide bases in DNA are strong uv absorbers their contributions to biological effects are grossly different. This is

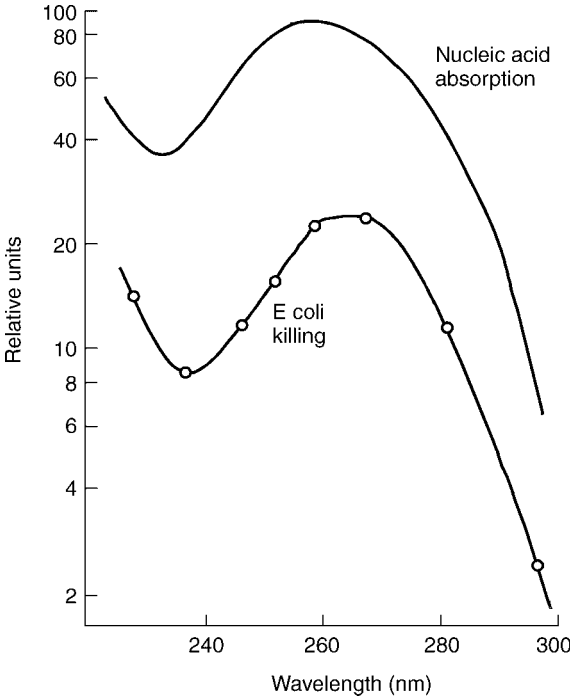


Fig. 5. Comparison of the action spectrum for inactivation of *E. coli* to the absorption spectrum of nucleic acids (85).

because the following must occur to have a photochemical reaction and the nucleotides vary with respect to these qualifications:

1. The radiation must be absorbed by the molecule.
2. The molecule must possess a chemical bond which is of importance to the function of the organism.
3. A sufficient amount of the excitation energy of the absorbed uv photon must reach this vulnerable bond to alter it.
4. After the chemical change the new configuration must endure.

The nucleotides differ in their ability to absorb uv light and undergo a permanent chemical change. The pyrimidines (thymine and cytosine) are ten times more sensitive to uv light than the purines (adenine and guanine). Of the pyrimidines, thymine undergoes change the most readily and the chemical changes are very stable.

Ultraviolet light reacts with two adjacent thymine molecules to produce a thymine dimer (Fig. 6). Microorganisms are inactivated by photochemical uv damage to cellular ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). This absorption of uv energy forms new bonds between adjacent nucleotides, creating double molecules, or dimers. Dimerization of adjacent pyrimidine molecules, particularly thymine, is the most common photochemical damage.

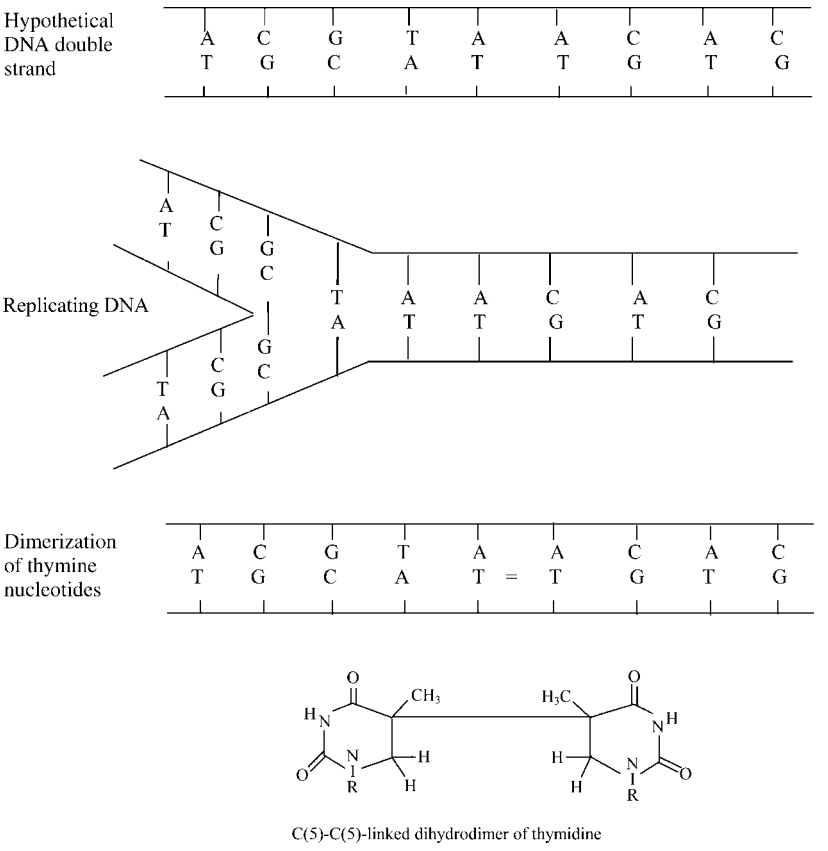


Fig. 6. The disruption of the DNA molecule by uv light that produces thymine dimmers (80).

Formation of numerous thymine dimers in the DNA of bacteria and viruses prevents replication and results in cell death.

If these thymine dimers are produced in vital areas of the organism's DNA, it cannot produce important enzymes or it cannot produce a functional copy of its DNA when it wants to reproduce.

15.4. How Is UV Damaged DNA Repaired? Photochemical damage caused by uv may be partially repaired by some organisms. Studies show that the amount of cell damage and subsequent repair is directly related to the uv dose. The amount of repair will also depend on the dose (intensity) of photoreactivating light. For low uv dose, the resulting minimal damage can be more readily repaired than for high doses where the number of damaged sites is greater. There is an enzymatic photoreactivation process in which the photochemical damage can occur in presence of a specific enzyme. This process is brought about by illumination of the cell with visible blue light. Such photoreactivation requires a specific enzyme that binds to the defective site on the DNA. Illumination results in the absorption of light energy by the enzyme. The absorbed energy in some manner promotes cleavage of chemical bonds at the defect in a single

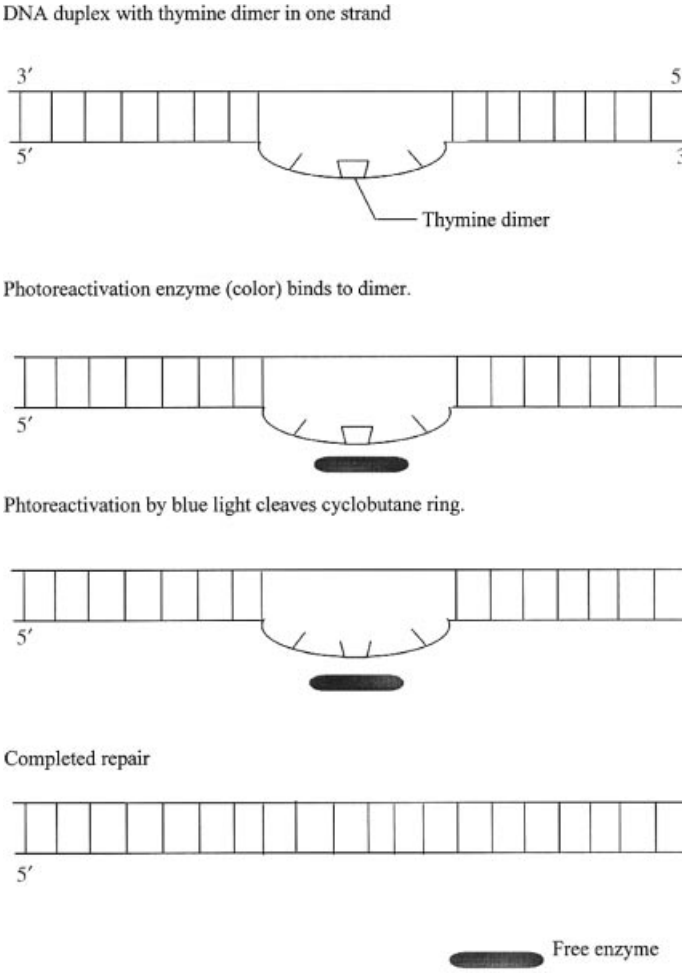


Fig. 7. Photo repair of uv damaged DNA (80).

DNA strand (Fig. 7). The most common photoreactivation process is the cleavage of the thymine dimer to yield two free thymine residues. In this way, visible light can be used to repair DNA damaged by uv light.

Sunlight exposed organisms have a special need for this genetic repair system in which one region of the sun's spectrum is used to repair damage induced by another region without direct involvement of the cell's metabolic processes.

Photoreactivation has been observed in bacteria and their phages (viruses), as well as in species of blue-green algae, fungi, higher plants, protozoa, chinoderms, arthropods and in all major groups of vertebrates and mammals.

The selections of uv lamps, ballast/power supplies to uv lamps, power distribution and control, the design of an open channel modular uv system, uv lamp rack, uv bank, and wastewater level control over the uv lamps all have effects on germicidal efficiency. The effects of all these parameters are described by Das (86).

Table 14. **Major Parameters Affecting the UV Disinfection of Wastewater**

Parameters	Acceptable values
percent transmittance (T) or absorbance	35–65
total suspended solids (TSS), mg/L	5–10
particle size, μm	10–40
flow rate/condition or hydraulics	ideal plug flow
iron, mg/L	<0.3
hardness, mg/L	<300

15.5. Effects of Wastewater Quality Parameters on UV Efficiency. The efficiency of a uv disinfection system strongly depends on effluent quality that acts to decrease the uv intensity in wastewater. Table 14 shows the major parameters that must be taken into consideration when a uv disinfection system is being designed for wastewater. The customer or the consultant must provide this information to the uv manufacturer because each uv system is designed on an individual basis.

15.6. UV Transmission or Absorbance. Ultraviolet lights' ability to penetrate wastewater is measured in a spectrophotometer at the same wavelength (254 nm) that is produced by germicidal lamps. This measurement is called the *Percent Transmission* or *Absorbance* and it is a function of all the factors that absorb or reflect uv light. As the percent transmission gets lower (higher absorbance) the ability of the uv light to penetrate the wastewater and reach the target organisms decreases.

It is imperative that the uv transmission of wastewater is measured because it is impossible to estimate the uv transmission by simply looking at a sample of wastewater with the naked eye. The range of effective transmittances (T) will vary depending on the secondary treatment systems. In general, suspended growth-treatment processes produce effluent with T varying from 60–65%. Fixed-film processes range from 50–55% T and lagoons 35–40% T. Industries that influence uv transmittance include textile, printing, pulp and paper, food processing, meat and poultry processing, photo developing, and chemical manufacturing.

The system designer must obtain samples of wastewater during the worst conditions or carefully attempt to calculate the expected uv transmission by testing wastewaters from plants that have a similar influent and treatment process. The designer must also strictly define the disinfection limits because this determines the magnitude of the uv dose.

15.7. Suspended Solids. Suspended solids in biologically treated effluents are typically composed of bacteria-laden particles of varying number and size. Some of the suspended solids in wastewater will absorb or reflect the uv light before it can penetrate the solids to kill any occluded microorganisms. The uv light can penetrate into suspended solids with longer contact times and higher intensities but there is still a limit to killings of pathogens. If wastewaters were devoid of suspended solids uv disinfection could be used almost universally.

Obtaining the proper information about the level of suspended solids is very important for the sizing of the uv system. If a wastewater treatment

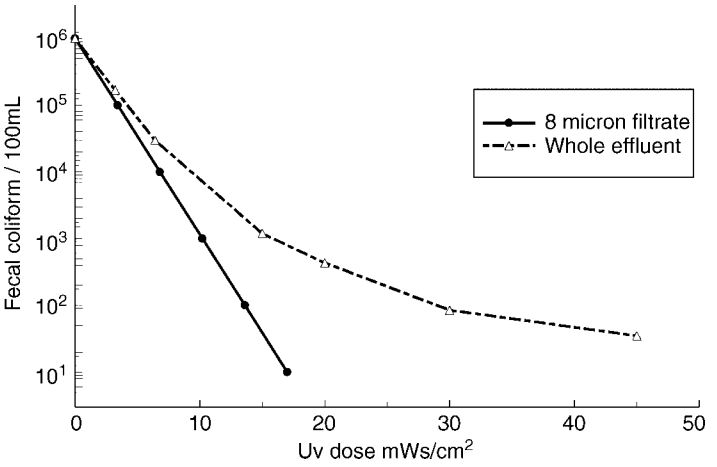


Fig. 8. Typical uv dose-response curve for filtered and unfiltered wastewater.

plant producing high levels of suspended solids is already in operation, a pilot study will show the frequency of cleaning of the quartz sleeves as a result of the fouling of the quartz sleeves by the suspended solids. Pilot testing will also determine whether the fecal coliform limit can be attained.

Figure 8 illustrates that the protection provided by TSS (particulates) results in a high uv dose demand for the whole effluent. Filtration resulted in reducing TSS levels, decreased in particle sizes and numbers, and as a result, lowered in the uv demand to achieve a given disinfection target (87).

15.8. Particle Size Distribution. Particle size distribution measurements of wastewater effluent are used as an indicator of filter and clarifier performance. Typically, particle sizes are related to the type of wastewater process and level of treatment, which in turn results in a decrease in both the number and mean size of particles. Table 15 illustrates the effect of large particle size on uv demand (87).

15.9. Flow Rate/Condition or Hydraulics. The US EPA provides an in-depth analysis of the effect of hydraulics on the uv disinfection of wastewater (77).

The number of microorganisms that are inactivated within a uv reactor is a function of the multiplication of the average intensity and residence time.

That is, the uv dose (D) is equal to the intensity (I) times the exposure time (t)

$$D = It$$

Table 15. **An Increase in Particle Size Directly Affects the UV Demand**

Particle size (μ)	UV demand
<10	easily penetrated, low uv demand
10–40	can be penetrated, uv demand increased
>40	will not be completely penetrated, high uv demand

As the flow rate increases the number or size of the uv lamps must be proportionately increased to maintain the same disinfection requirements. Therefore the uv system must be designed for the maximum flow rate at the end of lamp life.

The uv unit must be designed so that it provides as much sideways motion as possible with very little forward mixing. This makes sure that every microorganism is exposed to the average dose of uv light, which is especially important when the water has a low uv transmission or high suspended solids. The open channel uv system where the wastewater flows parallel to the submerged lamps has a very good hydraulic profile (78).

The height of the wastewater above the top row of uv lamps must be rigidly controlled by a flap gate or weir at all of the flow rates. Therefore the uv system must be designed for the maximum flow rate. This is especially important if the wastewater treatment plant receives storm water runoff.

The uv system must also be designed for the minimum flow rate. Many smaller wastewater treatment plants approach zero flow at night. During this period of time the wastewater has a greater chance to warm up around the quartz sleeves and produce deposits on the sleeves. There is also the possibility of exposing the quartz sleeves to the air. Because the lamps are warm any compounds left on the sleeves will bake onto them. Water splashing onto these exposed sleeves will also result in uv absorbing deposits. When the flow returns to normal a layer of water will now be passing through the uv unit without being properly disinfected. The designer must be very careful with the selection of the flow control device for the above situation. A flap gate has a normal flow range of 1:5 and they all leak at low flow rates. It is possible to reach 1:10 ratio but it is better to use two or more channels. A weir that keeps the lamps fully submerged at zero flow may be a much better solution (78).

15.10. Iron. Iron affects uv disinfection by absorbing uv light. It does this in three ways. If the concentration of dissolved iron is high enough in the wastewater, the uv light will be absorbed before it can kill any microorganisms. Iron will precipitate out on the quartz sleeves and absorb the uv light before it enters the wastewater. The third mechanism that is just being investigated is the adsorption of iron onto suspended solids, clumps of bacteria, and other organic compounds. This adsorbed iron will prevent uv light from piercing the suspended solids, etc, and killing the entrapped microbes. The maximum solubility of iron (Fe^{2+}) in carbon-bearing waters occurs within the common pH range of 6–9 as reported by Fair and co-workers (88).

The uv industry has adopted a level of 0.3 ppm as the maximum allowable level of iron, but there is no data to substantiate this limit. The level of iron should be measured in the wastewater, and if it approaches 0.3 ppm, a pilot study should be instituted to determine whether the desired disinfection level can be attained and what the cleaning frequency should be. An in-place cleaning system can be incorporated in the uv design. If possible a wastewater treatment plant should be designed with a non-iron method of precipitating phosphate.

Examples of non-iron methods for removing phosphates are biological phosphorus removal and alum. Studies showed that dissolved aluminum salts have no effect on uv transmittance, and flocculated solids containing aluminum do not show an increased resistance to uv disinfection (87).

Table 16. **Classification of Water Hardness**[a]

Hardness range (mg/L as $CaCO_3$)	Hardness description
0–75	soft
75–150	moderately hard
150–300	hard
>300	very hard

[a]See Ref. 89.

15.11. Hardness. Calcium and magnesium salts, which are generally present in water as bicarbonates or sulfates, cause water hardness.

A problem with hard water is the formation of mineral deposits. For example, when water containing calcium and bicarbonate ions is heated, insoluble calcium carbonate is formed.

$$Ca^{2+} + 2\,HCO_3^- \longrightarrow CaCO_3(\text{precipitate}) + CO_2 + H_2O$$

This product precipitates and coats on any warm or cold surfaces. The optimum temperature of the low pressure mercury lamp is 40°C or 104°F. At the surface of the protective quartz sleeve there will be a molecular layer of warm water where calcium and magnesium salts will be precipitated. These precipitates will prevent the uv light from entering the wastewater.

With the electronic ballast, the electrodes of the lamp do not produce the large quantities of heat that were experienced with the core/coil ballast. Field data will determine whether hardness will be less of a problem with the electronic ballast.

Unfortunately, no rule exists for determining when hardness will become a problem. Table 16 shows the classification of water hardness reported by Sawyer and MaCarty (89). Waters that contain hardness ~300 mg/L may require pilot testing of a uv system. This testing is especially important if very low flow or no flow situations are experienced because the water will warm-up around the quartz sleeves and produce excessive coating.

Experience has shown that it is very difficult to get operators to clean the quartz sleeves at a frequency greater than every 2–3 weeks (78).

15.12. Wastewater Source. It should be determined whether the wastewater treatment plant receives periodic influxes of industrial wastewater that may contain uv absorbing organic compounds, iron or hardness that may affect uv performance. These industries may be required to pretreat their wastewater.

For example, a textile mill may be periodically discharging low concentrations of dye into the municipal wastewater system. By the time this dye reaches the treatment plant it may be too diluted to detect without using a spectrophotometer. Dye can readily absorb uv light thereby preventing uv disinfection.

15.13. Degree of Inactivation. The degree of inactivation by uv radiation is directly related to the uv dose applied to the water or wastewater. Dose is

described as the product of the rate at which the energy is emitted (intensity) and the time the organism is exposed to the energy.

$$D = It \tag{38}$$

where; D = dose, μW s/cm^2; I = irradiation/intensity, μW/cm^2; and t = time, s.

Therefore the principal design factors for any uv system for disinfection are the intensity of radiant energy that is able to reach the organism and the time of exposure.

15.14. Germicidal Efficiency. Various investigations have shown a wide range of sensitivity of different microorganisms to uv energy. Kawabata and Harada (91) reported the following contact times required to achieve a 99.9% kill (3-log reduction) at a fixed uv intensity for the following organisms:

Organisms	Time (s)
E. coli	60
Shigella	47
S. typhosa	49
Streptococcus faecalis	165
B. subtilis	240
B. subtilis spores	369

Ultraviolet radiation has also been shown to be effective in the inactivation of viruses. Huff and co-workers (91) reported satisfactory results that included studies of several strains of polio virus, Echo 7, Coxsackie 9 viruses. The intensities varied from 7000 to 11,000 μW s/cm^2.

Hill and co-workers (92) reported that the infectivity loss of Polio 1 virus exceeded 3 logs after 15.7 s of uv exposure in continuous flowing seawater. Based upon these findings the use of uv should be highly effective in the disinfection of flowing seawater for use in artificial shellfish purification systems.

Current designs are for high intensity and lower exposure times—6-10 s. There is no doubt that the germicidal efficiency of uv is predictable for a given species of organism on the basis of the uv intensity-exposure time product. In practice, it will be necessary to prove and evaluate a given installation to confirm the design parameters (14).

15.15. UV Dose versus Bacterial Kill. This relationship is characterized by a mathematical model that assumes second-order kinetics when the coliform concentrations are in the range where disinfection usually takes place, as follows:

$$\frac{dN}{dt} = -kN^2I \tag{39}$$

Integrated, this becomes

$$\frac{1}{N} - \frac{1}{N_0} = kIt \tag{40}$$

where

N = coliform counts, MPN/100 mL, at time t
N_0 = influent coliform concentration, MPN/100 mL
k = rate constant, counts/s
I = the average uv intensity in the exposure chamber
t = exposure time, s

The influent coliform concentration is usually so much greater than the final concentration that the term $1/N_0$ becomes negligible and equation 40 can be simplified to

$$\frac{1}{N} = kIt \quad \text{or} \quad N = (\text{k}D)^{-1} \tag{41}$$

Equation 41 can be used to calculate coliform count at the end of uv exposure at a varying uv intensity and time of exposure for a known rate constant.

On the basis of 350 samplings conducted throughout a 1-year pilot program Scheible and Bassell (93) have been able to show quite favorable correlation between uv dose and coliform kill by the following empirical equation:

$$\text{Effluent fecal coliform} = (1.26 \times 10^{13})(\text{uv dose})^{-2.27} \tag{42}$$

Loge and co-workers (84,85) used a probabilistic design approach in their pilot studies and developed an empirical formula that can be used to calculate the fecal coliform density after expose to uv light, and the correlation is given as follows:

$$N = A(\text{SS})^a(N_0)^b(\text{UFT})^c(I)^n(t)^n \tag{43}$$

Where N = coliform density after exposure to uv light, MPN/100 mL; SS = suspended solids, mg/L; UFT = unfiltered % transmittance at 253.7 nm; N_0 = influent coliform density, MPN/100 mL; I = average intensity of uv light, nW/cm^2; t = exposure time, s (assuming approximate plug flow conditions); and A, a, b, c, n = empirical coefficients (102.919, 1.947, 0.3233, 0, −2.484, respectively). This correlation can be used to predict reasonably well the number of lamps necessary to meet the NPDES permit requirements for wastewater treatment plants.

15.16. Summary. The germicidal efficiency of an uv disinfection system strongly depends on effluent quality—high TSS concentration in effluent will reduce the uv transmittance and as a result higher coliform count. If dissolved iron concentration in effluent is >0.3 ppm, iron will absorb uv light, as well as will precipitate out on the sleeve and absorb uv light before it transmit to the wastewater that would limit the germicidal efficiency. However, studies reported that dissolved aluminum salts have no effect on uv transmittance and germicidal efficiency. Waters that contain hardness >300 mg/L will have an adverse effect on the overall uv germicidal efficiency. Higher uv dose (product of light intensity and exposure time) will increase higher germicidal efficiency (86).

16. Ultraviolet Germicidal Irradiation in Inactivating Airborne Microorganisms

The recent resurgence and current epidemics of tuberculosis (TB) in many developed countries have focused attention on transmission in high-risk settings (94–97). The prevalence of HIV infection, the high case fatality rate of multidrug-resistant TB especially among AIDS patients, and transmission from unsuspected TB patients support the importance of environmental control measures in high risk settings (98–100). Ultraviolet germicidal irradiation (UVGI) that occurs in the upper portion of air in a room has been considered an environmental control measure that could economically reduce exposure to *Mycobacterium tuberculosis* (MTB) droplet nuclei (101,102). For this approach, air above people's heads (usually >2.1 m) is subject to 254-nm germicidal ultraviolet C (UVC), whereas lower room air, where people actually stay and breathe, is not irradiated. A large volume of air can be disinfected without overexposing people to UVC. Currently, upper room UVGI is recommended by the Centers for Disease Control and Prevention as a supplemental approach for preventing transmission of TB (103).

Three factors are important in the efficacy of upper room UVGI: the upper room disinfection rate, air volume ratio for the irradiated upper room and the nonirradiated lower room, and the air mixing rate between the upper and lower room (104). The disinfection rate in the upper room depends on uv dose and uv susceptibility of the microorganism. The uv dose is the product of uv irradiance and exposure time (85). Susceptibility of microorganisms to uv depends on the complexity of the microorganism's structure, its repairability, and its general sensitivity (105). As the uv dose becomes higher and microbial susceptibility to UVGI increases, the efficacy of upper room UVGI increases. The desirable scenario is to maintain the maximum amount of uv irradiance in the upper part of the room while minimizing people's exposure to UVC in the lower part of the room. The American Conference of Governmental Industrial Hygienists currently recommends that measured uv irradiance in the lower room should be $\leq$6 mJ/cm^2 (0.2 μW/cm^2) for 8-h exposure (106). The current criterion for installing upper room UVGI is one 30-W (input) lamp or two 15-W lamps for each 200 s ft^2 (19 m^2) of floor area (107–109). A 30-W lamp for every seven occupants has been recommended for crowded conditions.

The mixing rate between air in the upper and the lower part of the room is also important in the efficacy of upper room UVGI (110). This air mixing occurs mainly by convection caused by a vertical temperature gradient in nonmechanically ventilated rooms. When a room has a mechanical ventilation system, ventilation type and locations of supply and exhaust also play important roles in vertical air mixing. The presence of a mixing fan (or ceiling fan) also affects vertical air mixing. The volume of upper room irradiated air and lower room nonirradiated air depends on the room dimensions (especially ceiling height), the type and number of uv fixtures, and reflection characteristics of room surfaces (111,112).

The investigators have found that upper room UVGI can significantly reduce the concentration of airborne microorganisms (*S. marcescens* and BCG)

aerosolized in a saliva stimulant in a typical mechanically ventilated hospital isolation room. Because upper room UVGI varied significantly with environmental factors such as temperature, air mixing, and air exchange rate, it is important to optimize environmental conditions to produce the best and most persistent effect in reducing the risk of TB in high risk settings (94,113).

17. Electromagnetic Radiation Techniques

Electromagnetic radiation is the propagation of energy through space by means of electric and magnetic fields that vary in time. Electromagnetic radiation can be specified by frequency, vacuum wavelength, or photon energy. Goldblith's (114) early studies on the potential uses of electromagnetic radiations for the destruction of microorganisms was initiated almost immediately after Roentgen's discovery of radioactivity in 1896.

Early studies on the application of uv light for microorganism destruction was conducted by Gates (115) during the 1930s. The maximum bactericidal effect in the uv region of the spectrum was determined to be between 2600 and 2700 Å.

After World War II, large amounts of ionizing energy became available. Considerable work has been done to attempt to employ the bactericidal effects of ionizing energy for the preservation of food and, to a lesser extent, the preservation of pharmaceuticals (15).

17.1. Effects of Ionizing Radiation on Microorganisms

- Radappertization—the commercial sterilization of materials.
- Radurization—the reduction of organisms, mainly vegetative cells, to a very low level and subsequent storage of the foodstuff at temperatures above freezing to ensure longer shelf life. Normally, a dose of $<10^6$ rad is used. An equivalent term is radiopasteurization.
- Radicidation—the removal of pathogens or organisms significant in public health (eg, Salmonella, from a food or foodstuff) by substerilizing doses of radiation.

Ionizing energy affects microorganisms both directly and indirectly. Theory describing direct affects is referred to as the target theory and was developed largely by Lea (116). Here, the organism is visualized as a target that is hit directly by an ionizing particle or ray. The mathematical analyses developed by Lea shows that, in general, when targets (in this case microorganisms) are destroyed by direct hits, this involves a probability concentration depending solely on the number of particles or rays (ie, the dose) and the number of targets (ie, number of microorganisms). This result is described by the following relationship:

$$N/N_0 = e^{-D/D_0} \tag{44}$$

where

N_0 = the initial number of microorganisms.
N = the number of microorganisms surviving a dose D.

D_0 = the slope of the inactivation curve, also known as the 37% dose or that dose needed for 63% kill/37% survival.

That target theory assumes that the rate of destruction of the microorganisms cannot be influenced by the concentration of organisms, medium temperature, or dose rate. Equation 44 states that the rate of destruction is only influenced by the number of ionizing particles (ie, the dose).

The destroying action of ionizing irradiation on a molecule is caused by energy released within the molecule itself. In contrast, indirect action results in cell destruction because of diffusion of radicals produced in the adjacent liquid. The diffusion distance is ~40 Å. Indirect effects, in a sense, still take place within an organism, but inactivate the organism by diffusion to and reacting with a sensitive site. This should not be confused with a solute effect in which radicals and other radiation produced compounds are formed extracellularly in the menstruum. Certain effects of an indirect nature occur in the menstruum and are still able to be lethal to a cell. The production of compounds such as hydrogen peroxide, organic peroxides, and radicals will affect the structure and contents of the microbial cell. Thus, ionizing energy can also exert its effects on microorganisms through the reaction products of the radiolyses of water (indirect action) diffusing into the cell and causing its demise.

Sine water is the major constituent of a cell, its radiolyses is also important. The radiolyses of water results in hydroxyl, hydrogen, and hydroperoxyl radicals as well as some hydrogen peroxide if oxygen is present. The result in indirect action is that of medium, temperature, and concentration of solute having a profound effect.

The overall reactions involved in the mechanism of the radiolyses process are outlined below:

$$H_2O^+ \longrightarrow OH^- + H^+ \quad (45)$$

$$e^- + H_2O \longrightarrow OH^- + H^+ \quad (46)$$

$$H_2O \longrightarrow H^+ + OH^- \quad (47)$$

The free radicals produced in the more densely ionized portion of the track (photons of energy ionizing the material and also producing free radicals, excited atoms, etc) can recombine as follows:

$$2\,H^+ \longrightarrow H_2 \quad (48)$$

$$2\,OH^- \longrightarrow H_2O_2 \quad (49)$$

$$H^+ + OH^- \longrightarrow H_2O \quad (50)$$

In other portions of the track where diffusion occurs, these radicals may react with the solute. Therefore, the extent of the interactions between the solute and those produced by radiolyses are dependent upon solute concentration.

In the presence of oxygen, significant quantities of the hydroperoxyl radical HO_2 are formed.

$$H^+ + O_2 \longrightarrow HO_2^+ \quad (51)$$

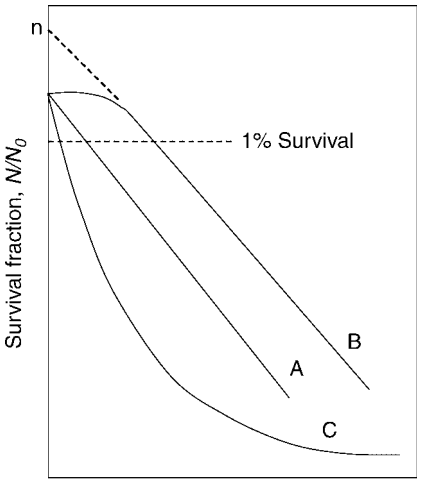
$$2\,HO_2^+ \longrightarrow H_2O_2 + O_2 \quad (52)$$

The longevity of the H_2O_2 molecule can be appreciable. Oxygen can also be formed by other reactions involving the OH radical and H_2O_2. Hydrogen peroxide and the hydroperoxyl radical may act as oxidizing or reducing agents. In acid media, and HO_2 radical is undissociated and is a strong oxidant and a weak reducing agent, whereas the O_2 ion obtained from the dissociation of the HO_2 radical can act as a reducing agent.

The indirect effects of radiations on microorganisms may be minimized by freezing a suspension to minimize migration of free radicals, by using dried preparations to restrict moisture content and thus prevent formation of the radicals, or by the addition of other solutes to compete for the free radicals.

Ionizing radiation is capable of causing a wide variety of physical and biochemical effects in microorganisms. Most investigations indicate that the primary cellular target is the DNA molecule of the cell. The radiosensitivity of several organisms ranging from viruses to higher plants and animals are correlated with the chromosome volume. The larger the volume, the more sensitive the biological unit is to ionizing radiation. It also appears that extreme difference in radiosensitivity with many microorganisms is the result of a given organism's ability to repair DNA damage rather than an inherent radiation resistance of the DNA.

Survival curves characterize the destruction of organisms and the suspended material in which they are irradiated. Survival curves are prepared by a plot of the logarithm of the survival fraction versus radiation dose. Figure 9 illustrates a typical family of survival curves. The three curves shown in the figure each impart different information. Curve A is an exponential relationship. This type of curve suggests that some site within the cell is the sensitive volume or target which when inactivated by radiation, results in the inability of the cell



N = Survivors of irradiation dose.
No = Original number of microorganisms
n = Extrapolated value of survival fraction

Fig. 9. Typical survival curves.

to divide and to form a visible colony when placed on a growth medium. The exponential curve lends itself to an analysis of inactivation by the target theory. The primary target appears to be the DNA. This has proved extremely useful in determining the shape and size of defined targets such as enzymes and viruses. Curve A is best expressed by equation 44.

A unit of value for expressing survival is the D_{10} value (D value), which is the number of rads required to reduce the population by one log cycle. In curve A, the D_{10} value can be obtained from any portion of the straight line, but in other types of curves its interpretation is more difficult.

Curve B is referred to as a multitarget curve. At low doses, the slope of the curve is flat. The intercept is normally obtained by extrapolating over the linear portion of the curve, which gives a relationship that is exponential at higher doses. With some organisms such as *Micrococcus radiodurans* (a radiation-resistant coccusm) and radiation-resistant mutants of *Salmonella*, the magnitude of the flat portion of the curve is very significant in comparison to the exponential portion. This distortion of the exponential curve is likely due to repairing mechanisms (117). In examining Figure 9, note that for each of the three curves, a different dosage is required to inactivate 99% of the cells. Also, curves B and C will not be characterized by any one survival fraction.

One equation that has been proposed to account for the flat portion in curve B is the following (117):

$$\text{Fraction surviving} = 1 - (1 - e^{-x})^n \qquad X = KD \tag{53}$$

where K is the slope of the linear portion of the survival curve expressed as reciprocal of kiloroentgens (kr^{-1}), and D is the dose. The parameter K should be independent of n (the extrapolated intercept value). The value of n should be greater than or equal to unity.

Curve C is believed to arise from microbial populations having nonhomogenity with respect to resistivity. A higher portion of the less resistant cells are first inactivated, leaving the more resistant cells to "tail out."

17.2. Characterization of Dose Requirements. The dosage of ionizing radiation required for a specific effect depends on a number of parameters. These parameters are the species of microorganism, population size, medium properties, temperature, the gas phase, water activity, and the presence of sensitizing compounds. Among these, microorganism species plays the dominant role.

Species. The relative radiation resistance among species is classified by D_0 (ie, dosage for 37% survival) and the D_{10} dose (90% inactivation of a population). Spores of bacteria are typically the most resistant organisms to radiation. Gram-negative rods are the most sensitive. Yeast and fungi are intermediate. An exception is *M. radiodurans*, which is a highly radiation resistant coccus that is heat sensitive. Hugo (119) reports radiation sensitivities of a number of organisms.

Population Size. The initial level of microorganisms does not play a dominant role in effecting radiation dose requirements. The death rate of microorganisms resulting from exposure to ionizing energy appears to be the first order. Hence, any logarithmic increase in the initial concentration of organisms will only result in a linear increase of the dose.

Medium. Since part of the effect of ionizing radiations on an organism is due to indirect action mediate through free radicals and activated molecules, the nature of the medium or menstruum in which the organisms are suspended obviously plays an important role in the dose requirements for a given microbicial effect.

Generally, the more complex the medium, the greater the competition of the components of the medium for the free radicals and activated molecules produced by the radiation, thus sparing the organisms or protecting them. Conversely, the greatest sensitivity usually occurs when the organisms are suspended in buffer or physiological saline. With natural foodstuffs, it is difficult to predict in which foodstuff the contaminant will be more radiosensitive or radioresistant. In attempting to determine the dosage required for the sterilization of a given food material, it is essential that the food be inoculated with a sufficient and realistic number of the most radioresistant organism likely to be found in the particular foodstuff.

Gas Phase. The role of the gas phase has long been recognized as a modifying factor in the radiation sensitivity of bacteria. Figure 10 illustrates this effect with *S. faecalis*. The exact mechanism of the oxygen effect is not known, although several explanations by Hollander (119) and Powers (120) have been presented. The atmosphere during irradiation can, with most organisms, play an important role with respect to their relative radiosensitivity.

Temperature. The medium temperature can affect dose requirements significantly. Organisms are more sensitive in liquid solution than when suspended in the frozen state. This is due to the interaction with free radicals in liquid solution and the immobilization of the free radicals and prevention of their diffusion when the medium is frozen.

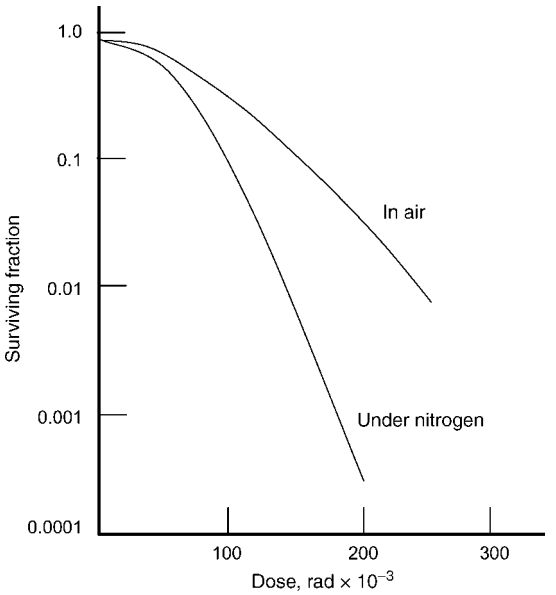


Fig. 10. Shows the effect of irradiation with high voltage electrons on *S. faecalis* in a phosphate buffer, under air and nitrogen.

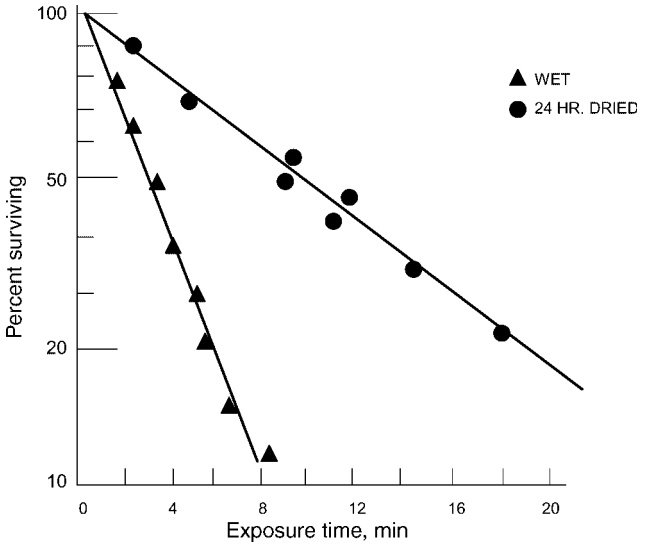


Fig. 11. Compares the ultrasonic death rates of *E. histolytica*, *E. coli* and yeast.

Water Activity. Bacteria are generally more radiation resistant when dry than in the presence of water. As in the case of temperature, this is due to the absence of indirect action when the solvent medium is removed and thus only the direct effect persists. Powers (120) showed that with spores, water can be a powerful protective and sensitizing agent simultaneously (note that one may be a physical phenomenon and the other a chemical one). With both foods and medical supplies, bacteria are more resistant in the dry state than when hydrated in suspension, which is due to minimization of indirect action in the dry state. An example of the difference of radiosensitivity in a wet versus a dry state is given in Figure 11.

Sensitizing Compounds. Considerable work has been done in the field of radiation biology on using chemical compounds to sensitize or protect microorganisms from radiation. The following compounds have been found to be helpful in modifying the viability of bacteria subjected to ionizing energy: iodoacetic acid, phenylmercuric acetate, and vitamin K_5, its derivatives and analogues.

It was also demonstrated by Chandler (121), working with eggs, that food materials reduce the radiosensitizing efficiency of these compounds, presumably by competing for the free radicals by the radiation in the solute.

In addition to the above parameters, other factors' effect on ionization doses include culture age, preirradiation growth medium, and post irradiation storage. These generally play secondary roles.

17.3. Summary. Although the main effect of ionizing energy on microorganisms is direct, much of the lethal effect is also due to indirect action of the radiation mediated through free radicals and activated molecule formation. As a result, the nature of the medium or menstruum in which the organisms are suspended, gaseous environment, water activity, temperature of the substrate,

etc—in fact all factors that influence indirect effects—are important in determining the degree of effect on the microorganism and, thus, on the sterilizing doses of radiation needed.

In studies directed at determining required sterilizing doses for a given process, materials should be inoculated with a large enough number of the most radioresistant species one would expect to encounter. Also, the material should be packed under conditions that are commercially encountered.

18. Electron Beam Technologies

18.1. "Ancient" History. The origins of electron beam processing can be traced back to 1895, when a paper published by Wilhelm Conrad Roentgen described the production of X-rays. Within 1 year, an article appeared that reported on the ability of X-rays to kill bacteria. In 1948, results of experiments on 22 species of bacteria with electrons and X-rays prompted interest from medical products manufacturers. This led to the development of the first commercial irradiation sterilizer, a small Van de Graaff accelerator. The first commercial irradiation of medical devices took place in Scotland in 1955 at the Ethicon division of the Johnson & Johnson facility, where catgut sutures were sterilized. Ethicon determined that their sutures were breaking during surgical procedures because steam and/or heat sterilization methods caused them to become brittle. The use of irradiation sterilization eliminated the breakage, and the company parlayed the technological breakthrough into a mass conversion of market share. Ethicon's suture market share grew from 5% in 1955 to >90% by the early 1970s.

18.2. Early Designs. Early accelerator systems provided poor penetration due to the low energies that the equipment produced. Those accelerators produced only a 2-MeV beam that could barely penetrate the suture material packages irradiated at Ethicon. These "ancestral" machines were also very difficult to control and were unreliable because no specific industry base was established for their use—the same machines used for medical products were also used in research laboratories. Thus, original hopes that electron beam technology could be commercialized were short lived. The irradiation market was taken over by Co-60 (gamma) equipment that did not share in the early technological shortcomings of electron beam.

18.3. E-Beam Equipment Today. During the 1970s, several companies, including Varian Associates, Phillips, and Siemens, took a new look at the application of X-ray technology for radiographic and oncology therapy equipment. Their involvement in the improvement of durability and reliability of accelerated electron technology raised performance parameters to a new level. Figure 12 shows a schematic diagram of a high energy electron beam device for sterilized wastewater, sludges, meat, poultry, and so on (122).

Today, healthcare manufacturers can benefit from such improvements as

- Higher energy (10 MeV) and thus better penetration.
- High duty cycles (7000–8000 h/year).

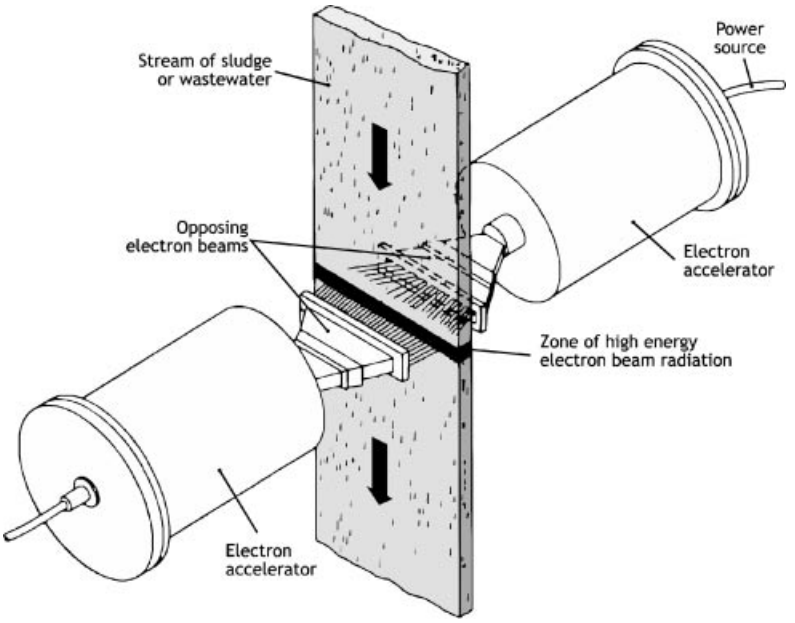


Fig. 12. Schematic diagram of high energy electron-beam device (122).

- Fully automated electronic control systems featuring programmable logic controllers (PLCs).
- Fully integrated turnkey designs of tightly controlled processing and documentation parameters.
- Full facility and process validation.

19. Other Sterilization Methods

19.1. Ethylene Oxide. Ethylene oxide (EtO) is, for many products, an effective sterilization agent. It is especially useful for custom procedure kits containing unit dose drugs in hermetically sealed packages and for products that discolor, distort, or otherwise degrade when processed with radiation. The EtO sterilization process normally requires a product conditioning step in which product is placed in a highly humidified area for a specified period of time. This process humidifies the product, allowing the sterilizing agent, ethylene oxide gas, to penetrate more effectively. After prehumidification, products are exposed to the EtO by being placed in a chamber for several hours. After exposure, products must be "aerated" by sitting in another chamber in which residual gasses which may have clung to the product are allowed to disperse. This step may take up to several days, depending on the product and the amount of gas absorbed. After EtO processing, products may not be released for use until a laboratory test on biological indicators has been performed. This test may delay release of product by an additional 3–7 days.

19.2. Ethylene Oxide—Processing Considerations. Each load of product that is sterilized with EtO has a biological indicator (BI) or "spore" strip included in the product. Upon removal from the EtO chamber, the product cannot be released until a sterility test is performed upon the BI strip to verify destruction of the indicator organism.

Products sterilized with ethylene oxide must be packaged in breathable packaging to allow gasses to escape after EtO exposure. While this type of packaging is readily available, it is quite costly. The process of EtO sterilization requires careful control of several parameters for every lot processed. These parameters include time of exposure, humidity, temperature, pressure, EtO gas concentration, and vacuum. Should an abnormal reading in any of these parameters occur, effectiveness of the process may be questioned. Probably the greatest concern for EtO users and providers is evidence of potential health hazards of EtO to plant employees, the environment, and patients. It is suspected that EtO presents a hazard due to its potentially carcinogenic properties. Furthermore, out-gassing of EtO from processing facilities has come under close governmental scrutiny in recent years. The resulting regulations have increased costs for EtO processing providers, and those costs have been necessarily passed along to EtO customers.

19.3. Gamma Rays (Co-60). Gamma irradiation involves exposure of products in their final shipping containers to a radioactive isotope known as Co-60. Cobalt-60 is processed almost exclusively worldwide by the Canadian government and provided to gamma irradiation plants by Nordion International, who processes and packages the isotope in cobalt "pencils." The pencils are shipped, under extremely tight control and under "hazardous materials" shipping regulations, to gamma plants as needed.

The cobalt pencils are housed in "source racks" within the gamma facility. The racks are placed in the gamma cell, and products in their final shipping configurations travel through the cell on a conveyor system. This process takes several hours to complete. Cobalt-60 decays with time, and appropriate cycle timer setting adjustments must be made at the gamma plant to account for the current cobalt inventory quantity. When the cobalt source is sufficiently decayed, the pencils must be replaced. This "resourcing" procedure takes several days to complete, during which time the affected gamma cell is rendered inoperable. Upon completion of resourcing, dose mapping must be performed on all products which will be irradiated in the cell.

The effect of gamma irradiation to the product microbial population (bioburden) is the same as the effect of electron beam processing. The fundamental difference between gamma and e-beam sterilization is the manner in which the radiation energy is delivered to the material being irradiated.

19.4. Cobalt-60—Processing Considerations. The irradiation dose provided by a gamma plant is a function of exposure time of the product to the irradiation source (Co-60), and is thus controlled by a timer setting. Products that can be scheduled to run within the same timer setting are most convenient for a gamma plant to process. The dose range which is most often set in gamma plants is $\sim$25–35 kilogray (kGy). Products that require lower or higher doses than this range frequently must wait until the timer setting is adjusted to accommodate atypical doses. Gamma facilities cannot easily adjust up or down to

produce specific dose ranges, and frequently, products that do not necessarily require a 25 kGy minimum to destroy their inherent bioburden are processed at the higher rate anyway. Because products are exposed to the gamma source for a time period of 4–8 h, the possibility of product degradation in the form of discoloration and/or embrittlement, is increased over other forms of radiation sterilization such as electron beam.

19.5. Other Sterilization Methods. Several other sterilization methods, such as gas plasma, steam, and others are available and/or under development. At this writing, however, these methods do not accommodate large volumes of product packaged in final shipping configurations which are destined for finished goods inventory at the completion of the sterilization process.

19.6. Advantages and Benefits of Electron Beam. *Electron Beam Compared with Ethylene Oxide.*

- **Significantly faster turnaround time**. Electron beam requires no prehumidification, no poststerilization aeration, and no sterility testing after the product is processed.
- **Reduced inventory carrying costs**. Faster turn-around time requires less product to be held in inventory.
- **Product consistency**. Electron beam processing variables consists of only "dose delivered;" other processing variables inherent to EtO, (eg, gas concentration, vacuum, pressure, etc) are not variables in electron beam processing.
- **Lower cost of sterilization**. Electron beam processing is extremely cost competitive; furthermore, biological indicator testing is not necessary in electron beam processing, thus eliminating an additional testing charge.
- **Safety**. Electron beam processing does not utilize potentially dangerous chemicals or gases, nor is there the potential that chemicals or gases might remain on or in the product.
- **Cost savings in product packaging**. Electron beam does not require the special "breathable" packaging necessary for EtO to allow for noxious gas aeration; nonpermeable plastic packaging is less expensive and easily penetrated with electron beam.

19.7. Irradiation Processing of Food. Foodborne illness continues to be a significant health issue in the United States today. The CDC estimate that 9000 Americans die each year from diseases caused by pathogenic bacteria such as *Camphylobacter, E. coli* O157:H7, *Listeria monocytogenes, Salmonella, and S. aureus*. Additionally, the Council for Agricultural Science and Technology (CAST) and the CDC estimate that 33–81 million Americans suffer from foodborne diarrheal diseases each year. The United Nations Food and Agriculture Organization estimates that up to 25% of the world's food supply is lost every year to pests and bacteria.

Irradiation processing (sometimes referred to as cold pasteurization) when used in concert with proper food handling and processing techniques can greatly reduce the probability that foodborne pathogens associated with poultry, meat, produce, and other types of food will reach consumers, without compromising the nutritional quality of such foods.

Serious investigations regarding utilization of ionizing radiation for cold pasteurization were initiated in the early 1950s. However, in the 1958 Food Additive Amendment to the Federal Food, Drug, and Cosmetic Act irradiation sources were explicitly defined as food additives, rather than processes. This effectively delayed commercialization of food irradiation for several decades, since authorizing regulation prescribing safe use conditions and premarket review, as well as acceptance by the U.S. FDA were required for each specific food use. Consequently, it was not until the mid-1980s that approvals for the first applications of irradiation for microbial control were granted (herbs, spices, seasonings, dehydrated enzymes, etc) (123).

In December of 1997, the FDA amended its food additive regulations to provide for the safe use of ionizing radiation for the treatment of fresh or frozen uncooked meats to control foodborne pathogens and extend product shelf life. The three approved sources of radiation were (*1*) gamma rays (typically produced by radioisotopes of cobalt and cesium), (*2*) X-rays [with the maximum energy of 5 million electron volts (MeV)], and (*3*) beams of accelerated electrons (e-beams), with the maximum energy of 10 MeV. Previously, in 1990, the FDA made a similar amendment to allow for irradiation of fresh or frozen poultry. The Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) is in the process of amending its meat and poultry inspection regulations so as to take into account the above mentioned FDA actions.

19.8. Irradiation Processing. For >60 years, the physical and chemical changes induced by absorption of radiation sufficiently high in energy to produce ionization have been the subject of both university and industrial research. Early work dealing with chemical effects of ionizing radiation utilized the natural radioisotopes of radium and radon as radiation sources. At this time, the most common commercial sources of ionizing radiation are ^{60}Co and ^{137}Cs for gamma irradiation, and electron accelerators for e-beam irradiation. When the electron beam generated by an accelerator is directed at a target consisting of a high atomic number metal, such as tungsten or gold, X-rays with a broad spectrum of energies are produced. The amount of energy absorbed, also known as the dose, is measured in units of kilograys (kGy), where 1 kGy is equal to 1000 J/kg, or megarads (MR or Mrad), where 1 MR is equal to 1,000,000 ergs/g. With respect to food processing, irradiation applications can be categorized by dose level effects as follows: (*1*) low dose (up to 1 kGy): sprout inhibition of tubers, ripening delay of fruits, insect deinfestation; (*2*) medium dose (~1–10 kGy): reduction of pathogenic and spoilage bacteria and parasites; and (*3*) high dose (>10 kGy): complete sterility. Maximum doses approved for poultry and meat cold pasteurization are 3 and 7 kGy, respectively. Foods currently irradiated to high doses (eg, 44 kGy min) include those for use by astronauts during space flight, and for consumption by hospital patients with severely compromised immune systems.

While the ionizing radiation provided by e-beams is in the form of electrons, in the case of X-rays and gamma rays, it is provided by photons. The latter have no mass and are thus able to penetrate deeper into materials. Electrons, on the other hand, have a small mass, and are characterized by more limited penetration. Conversely, throughput efficiencies of gamma and X-rays are significantly lower than those of e-beams. For example, a typical 10 MeV, 50 kW e-beam

accelerator can cold pasteurize 31,500 kg of food per hour at a dose of 2 kGy. Even a low power (1-kW) 10-MeV accelerator will have a dose rate in excess of 450 kGy/h. The low dose rate characteristic of the natural radioisotope decay means that in the case of gamma irradiation dose rates on the order of 5–10 kGy/h are typical. Similarly, the throughput efficiency for X-rays is limited by the fact that in addition to generating photons, heavy metal targets generate considerable heat. In fact, while X-ray target conversion efficiencies vary with the atomic number of the metal used, they are typically no higher than 5–8%. In practice this means that in order for an X-ray to process products with the same speed as a 10 MeV, 50 kW e-beam, it will need to have 625 kW of power.

All forms of ionizing radiation interact with matter by transferring energy to the electrons present in the nuclei of target materials. These electrons may then be either released from the atoms, yielding positively charged ions and free electrons, or moved to a higher energy atomic orbital, yielding and excited atom or molecule. These ions, electrons, and excited species are the precursors of any chemical changes observed in irradiated material (124–126).

19.9. Effect of Ionizing Radiation on Foods. The effect of ionizing radiation on living matter is characterized by cellular destruction stemming from the disruption of the genetic material. That is, the radiation-induced cleavage of chemical bonds in the cell's DNA results in the inability of the cell to reproduce. On the organism level, the cellular inability to reproduce results in death of the organism. The breaking of chemical bonds described above involves the formation of stable radiolytic products from the reactive ions or free radicals that are formed when molecules absorb ionizing radiation. These radiolytic species, including glucose, formic acid, and carbon dioxide, are generally the same as those that are formed when food is treated by heat (ie, cooked). In fact, in >30 years of intensive investigation, no radiolytic products specifically unique to irradiated foods have been identified. The FDA estimates the maximum theoretical level of such products at a dose of 1 kGy to <3 mg/kg of food (3 ppm).

The overall retained nutritional quality of irradiated food depends on a number of factors, including irradiation dose, temperature, food composition, and the presence or absence of oxygen (vacuum vs atmospheric irradiation). However, scientists believe that irradiation produces no greater nutritional loss than what occurs in other food processing methods, such as cooking or canning. Additionally, nutrient losses can be reduced by irradiating foods in an oxygen-free atmosphere, or while frozen (127–129).

19.10. Treatment of Medical and Infectious Waste by e-Beam. Approximately 800 million lb of infectious and hazardous biomedical waste are generated by the 7000 hospitals and medical facilities in the United States each year. Medical/infectious waste comes from many sources. The major generators of medical waste are hospitals, medical laboratories, research laboratories, commercial diagnostic laboratories, animal experimentation units, and industrial laboratories. Other sources of medical waste include outpatient medical clinics, dental clinics, nursing homes, and veterinary hospitals and schools. This waste must be properly treated and sterilized to prevent accidental infection of the health professionals who handle it as well as the general public through inadvertent exposure.

Environmentalists have long been concerned about the important environmental problems created by the most common form of disposal of medical waste, incineration. When burned, hospital waste and medical/infectious waste emit various air pollutants, including hydrochloric acid, dioxin/furan, and toxic metals (lead, cadmium, and mercury). These toxins find their way to people through meat and dairy products after their release from incinerators. Insistent pressure and lobbying from concerned citizens lead to the Clean Air Act of 1997. As a result of this act, EPAs air emission standards and guidelines will reduce air emissions from medical waste incinerators (MWIs) by 75–98% from current levels. The regulations will substantially reduce emissions in highly populated urban areas, as well as in more rural areas. As a result of many facilities switching to other methods of waste disposal, EPA expects the standards to apply to between 10 and 70 new MWIs by the year 2002. EPA expects the guidelines to result in the discontinued use of as many as 50–80% of the almost 2400 existing MWIs.

Various attempts to develop or adapt other treatment and remediation technologies have also shown very limited promise and little success until Bio Sterile Technology's BIOSIRIS electron beam medical waste sterilization system became available (http://www.biosterile.com).

Electron beam technology has been used for decades in industrial applications, however, it has previously been too costly and complex, relative to incineration, for use at hospital sites for medical waste sterilization. After 5 years of research and engineering, the BioSterile Technology team has eliminated both of these barriers with the introduction of BIOSIRIS—the first compact, self-contained electron beam waste treatment system.

19.11. Anthrax Sterilization. Anthrax is an acute infectious disease caused by the spore-forming bacterium *Bacillus anthracis*. Anthrax most commonly occurs in hoofed mammals and can infect humans. Symptoms of disease vary depending on how the disease was contracted, but usually occur within 7 days after exposure. The serious forms of anthrax are inhaled anthrax, cutaneous anthrax, and intestinal anthrax. Initial symptoms of inhalation anthrax infection may resemble a common cold. After several days, the symptoms may progress to severe breathing problems and shock. Inhalation anthrax is often fatal.

Direct person-to-person spread of anthrax is extremely unlikely, if it occurs at all. In persons exposed to anthrax, infection can be prevented with antibiotic treatment.

19.12. New Methods and Technology. Anthrax decontamination is a rapid evolving field, with new methods and technologies continually being developed and tested. Several different antimicrobial pesticides and devices are being currently used by qualified experts under carefully controlled conditions in anthrax clean up being done across the country. Currently, research efforts are under way to effectively sanitized anthrax by using chemical and radiation technologies, including chlorine dioxide, ethylene dioxide, decontamination foam, e-beam, etc. The use of these chemical and irradiation treatment for anthrax can be obtained from the U.S. Army Medical Research Institute of Infectious Diseases in Fort Detrick in Maryland, and the U.S. EPA websites (USAMRID, http://www.usamriid.army.mil/; http://www.epa.gov/epahome/hi-anthrax. htm).

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