Chapter 28

Drinking Water Treatment and Distribution

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Rivers, streams, lakes and underground aquifers are all potential sources of potable water. In the United States, all water obtained from surface sources must be filtered and disinfected to protect against the threat of microbial contaminants. Such treatment of surface waters also improves esthetic values such as taste, color and odors. In addition, groundwater under the direct influence of surface waters such as nearby rivers must be treated as if it were a surface supply. In many cases, however, groundwater needs either no treatment or only disinfection before use as drinking water. This is because soil itself has acted as a filter to remove pathogenic microorganisms, decreasing the chances of contamination of drinking water supplies.

At first, slow sand filtration was the only means employed for purifying public water supplies. Then, when Louis Pasteur and Robert Koch developed the germ theory of disease in the 1870s, things began to change. In 1881, Koch demonstrated in the laboratory that chlorine could kill bacteria. Following an outbreak of typhoid fever in London, continuous chlorination of a public water supply was used for the first time in 1905 (Montgomery, 1985). The regular use of disinfection in the United States began in Chicago in 1908. The application of modern water treatment processes had a major impact on water-transmitted diseases such as typhoid in the United States (Figure 28.1).

28.1 WATER TREATMENT PROCESSES

Modern water treatment processes provide barriers, or lines of defense, between the consumer and waterborne disease. These barriers, when implemented as a succession of treatment processes, are known collectively as a treatment process train (Figure 28.2). The simplest treatment process train, known as chlorination, consists of a single treatment process, disinfection by chlorination (Figure 28.2A). The treatment process train known as filtration entails chlorination followed by filtration through sand or coal, which removes particulate matter from the water and reduces turbidity (Figure 28.2B). At the next level of treatment, in-line filtration, a coagulant is added prior to filtration (Figure 28.2C). Coagulation alters the physical and chemical state of dissolved and suspended solids, and facilitates their removal by filtration. More conservative water treatment plants add a flocculation (stirring) step before filtration, which enhances the agglomeration of particles, and further improves the removal efficiency in a treatment process train called direct filtration (Figure 28.2D). In direct filtration, disinfection is enhanced by adding chlorine (or an alternative disinfectant, such as chlorine dioxide or ozone) at both the beginning and end of the process train. The most common treatment process train for surface water supplies, known as conventional treatment, consists

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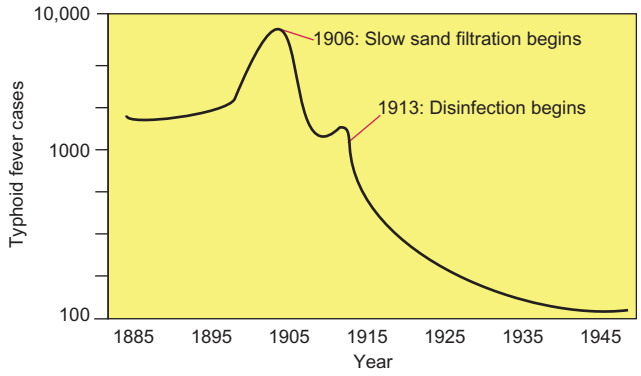

FIGURE 28.1 Impact of water filtration and chlorination on typhoid fever death rate in Albany, New York. From Logsdon and Lippy (1982).

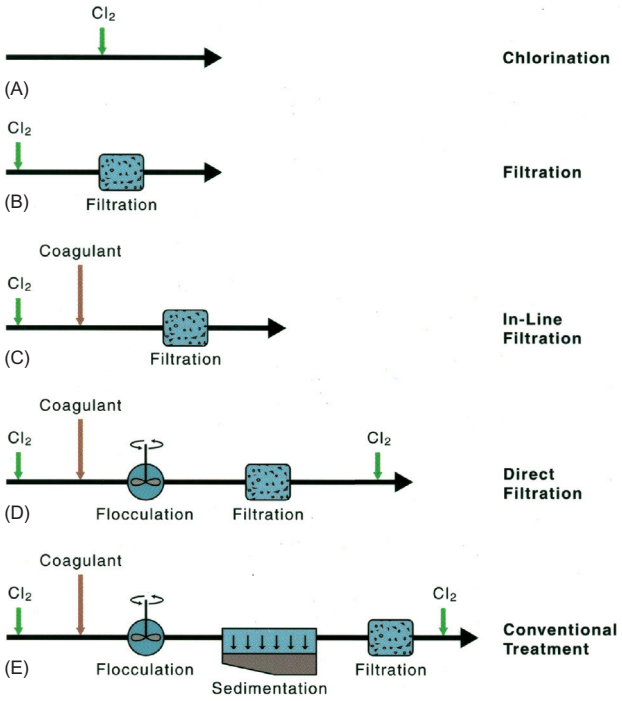

FIGURE 28.2 Typical water treatment process trains.

of disinfection, coagulation, flocculation, sedimentation, filtration and disinfection (Figure 28.2E)

Coagulation involves the addition of chemicals to facilitate the removal of dissolved and suspended solids by sedimentation and filtration. The most common primary coagulants are hydrolyzing metal salts, most notably alum [$Al_2(SO_4)_3 \cdot 14H_2O$], ferric sulfate [$Fe_2(SO_4)_3$] and ferric chloride ($FeCl_3$). Additional chemicals that may be added to enhance coagulation are charged organic molecules called polyelectrolytes; these include high-molecular-weight polyacrylamides, dimethyldiallyl-ammonium chloride, polyamines and starch. These chemicals ensure the aggregation of the suspended solids during the next treatment step, flocculation. Sometimes polyelectrolytes (usually polyacrylamides) are added after flocculation and sedimentation as an aid in the filtration step.

Coagulation can also remove dissolved organic and inorganic compounds. Hydrolyzing metal salts added to the water may react with the organic matter to form a precipitate, or they may form aluminum hydroxide or ferric hydroxide floc particles to which the organic molecules adsorb. The organic substances are then removed by sedimentation and filtration, or filtration alone if direct filtration or in-line filtration is used.

Flocculation is a purely physical process in which the treated water is gently stirred to increase interparticle collisions, thus promoting the formation of large particles. After adequate flocculation, most of the aggregates settle out during the 1 to 2 hours of sedimentation. Microorganisms are entrapped or adsorbed to the suspended particles and removed during sedimentation (Figure 28.3).

Sedimentation is another purely physical process, involving the gravitational settling of suspended particles that are denser than water. The resulting effluent is then subjected to rapid filtration to separate out solids that are still suspended in the water. Rapid filters typically consist of 50–75 cm of sand and/or anthracite, having a diameter between 0.5 and 1.0 mm (Figure 28.3). Particles are removed as water is filtered through the medium at rates of 4–24/min/10 dm^2. Filters need to be backwashed on a regular basis to remove the buildup of suspended matter. This backwash water may also contain significant concentrations of pathogens removed by the filtration process. Rapid filtration is commonly used in the United States. Another method, slow sand filtration, is also used. Employed primarily in the United Kingdom and Europe, this method operates at low filtration rates without the use of coagulation. Slow sand filters contain a layer of sand (60–120 cm deep) supported by a gravel layer (30–50 cm deep). The hydraulic loading rate is between 0.04 and 0.4 m/h. The buildup of a biologically active layer, called a *schmutzdecke*, occurs during the operation of a slow sand filter. This eventually leads to head loss across the filter, requiring removing or scraping the top layer of sand. Factors that influence pathogen removal by filtration are shown in Table 28.1.

Taken together, coagulation, flocculation, sedimentation and filtration effectively remove many contaminants as shown in Tables 28.2 and 28.3. Equally important, they reduce turbidity, yielding water of good clarity and hence enhanced disinfection efficiency. If not removed by such methods, particles may harbor microorganisms and make final disinfection more difficult. Filtration is an especially important barrier in the removal of the protozoan parasites *Giardia lamblia* and *Cryptosporidium*. The cysts and oocysts of these organisms are very resistant to inactivation by disinfectants, so disinfection alone cannot be relied on to prevent waterborne illness (see Case Study 22.1). However, because of their smaller size,

FIGURE 28.3 Drinking water treatment plant showing sand filter beds in the foreground and tanks containing alum flocculant in the background. Photo courtesy C.P. Gerba.

TABLE 28.1 Factors Effecting the Removal of Pathogens by Slow Sand Filters

Temperature
Sand grain size
Filter depth
Flow rate
Well-developed biofilm layer

viruses and bacteria can pass through the filtration process. Removal of viruses by filtration and coagulation depends on their attachment to particles (adsorption), which is dependent on the surface charge of the virus. This is related to the isoelectric point (the pH at which the virus has no charge) and is both strain and type dependent (see also Table 19.2). The variations in surface properties have been used to explain why different types of viruses are removed with different efficiencies by coagulation and filtration. Thus, disinfection remains the ultimate barrier to these microorganisms.

Generally, disinfection is accomplished through the addition of an oxidant. Chlorine is by far the most common disinfectant used to treat drinking water, but other oxidants, such as chloramines, chlorine dioxide and ozone, are also used (see Chapter 29). While ultraviolet can be used it does not leave a residual and usually a secondary disinfectant (i.e., chlorine) is added.

28.2 WATER TREATMENT REQUIREMENTS

Production of safe drinking water requires a holistic approach that considers the source of water, treatment processes and the distribution system. A multiple-barrier approach is used to ensure that if one barrier fails, the remaining barriers minimize pathogen presence in the water delivered to the consumer's tap. The essential barriers are:

- Source water protection
- Water plant processes
- Disinfection
- Distribution system residual disinfection
- Security

Source water protection means ensuring the highest water quality source possible before treatment by controlling use of the watershed, including minimizing sewage and domestic animal (e.g., cattle) contamination (Fox *et al.*, 2006). The treatment processes and disinfection must be adequate to ensure that the concentrations of pathogens are reduced to levels that minimize risk (see Chapter 26). This is dependent upon the concentrations expected in the water source. Thus, water sources which have significant sewage discharges or runoff from farm

TABLE 28.2 Coagulation, Sedimentation, Filtration: Typical Removal Efficiencies and Effluent Quality

Organisms	Coagulation and Sedimentation (% Removal)	Rapid Filtration (% Removal)	Slow Sand Filtration (% Removal)
Total coliforms	74–97	50–98	>99.999
Fecal coliforms	76–83	50–98	>99.999
Enteric viruses	88–95	10–99	>99.999
Giardia	58–99	97–99.9	>99
Cryptosporidium	90	99–99.9	99

From U.S. EPA (1988).

TABLE 28.3 Removal of Virus by Coagulation–Settling–Sand Filtration

Virus	Viral Assays, PFU (% Removal)		
	Input	Settled Water	Filtered Water
Poliovirus	5.2×10^7	1.0×10^6 (98)	8.7×10^4 (99.84)
Rotavirus	9.3×10^7	4.6×10^6 (95)	1.3×10^4 (99.987)
Hepatitis A virus	4.9×10^{10}	1.6×10^9 (97)	7.0×10^8 (98.6)

Adapted from Rao et al. (1988).

areas with large numbers of cattle may need to include processes that can remove greater numbers of pathogens. In developing the Surface Treatment Rule, the United States Environmental Protection Agency recognized this issue, and now requires utilities to assess the concentration of pathogens in water sources (U.S. EPA, 2003). Minimum requirements for water treatment are based upon concentrations of pathogens in the raw water (Table 28.4). Under these rules, a system must remove at least two logs (99%) of *Cryptosporidium* oocyts. However, because of concerns that some water treatment plants may draw water from poor quality sources with elevated levels of *Cryptosporidium*, additional treatment is required if monitoring shows oocyst concentrations of 0.075/liter or greater. The actual treatment required depends upon how many oocysts are detected in the raw untreated water.

TABLE 28.4 Disinfection and Process Credits (Log Removal[a]) under the U.S. Environmental Protection Agency Surface Water Treatment Rule (U.S. EPA, 1991, 2003)

Process Credits	Viruses	*Giardia*	*Cryptosporidium*
Total log removal/ inactivation required	4.0	3.0	2.0 to 5.5[b]
Conventional treatment; sedimentation and filtration credit only	2.0	2.5	3.0
Disinfection required	2.0	0.5	0 to 2.5
Direct filtration credit	1.0	2.0	2.5
Disinfection required	3.0	1.0	0 to 3.5
No filtration	0	0	0
Disinfection required	4.0	3.0	2.0 to 5.5

[a]*log 10 removal: each log is a 90% removal of the original concentration in the source water.*
[b]*Requirement depends on concentration of Cryptosporidium oocysts in source water.*

As the treated drinking water travels though the distribution pipe system to the consumer, the microbial quality slowly degrades. The degradation is caused by several factors including the loss of disinfectant residual, biofilm sloughing, stirred-up pipe sediments caused by rapid changes in flow, pipe breaks, intrusions of contaminants into the pipe network from pressure drops and cross connections. Regrowth of bacteria that survived the treatment processes, and growth of bacteria in biofilms on pipe walls and surfaces in storage tanks and reservoirs, can also occur (Fox *et al.*, 2006). Heterotrophic bacterial growth or regrowth usually occurs when the free chlorine residual drops below 0.2 mg/L, the water temperature exceeds 10°C, and assimibile organic carbon (AOC) is greater than 50 μg/L. Because of this, chloramines are sometimes added to water to provide a residual disinfectant for water within the distribution system. In addition, new real-time monitoring systems for both biological and chemical contaminants are currently being developed not only to ensure water quality, but also to protect against water intrusion via terrorist activities.

28.3 WATER DISTRIBUTION SYSTEMS

28.3.1 Microbial Growth

Once drinking water is treated, it must often travel through many miles of pipe or be held in storage

TABLE 28.5 Problems Caused by Biofilms in Distribution Systems

Frictional resistance of fluids
Photoreduction of H_2S because of anaerobic conditions
Taste and odor problems
Colored water (red, black) from activity of iron- and manganese-oxidizing bacteria
Resistance to disinfection
Regrowth of coliform bacteria
Growth of pathogenic bacteria (i.e., *Legionella*)

reservoirs before it reaches the consumer. The presence of dissolved organic compounds in this water can cause problems, such as taste and odors, enhanced chlorine demand, and bacterial colonization of water distribution systems (Bitton, 2011). Bacterial concentrations in distribution system water vary from < 1 colony forming units (CFU)/ml to as high as 10^5 to 10^6 CFU/ml in water from slow flow or stagnant areas in the distribution system. The density of bacteria in pipe biofilms, however, may be several orders of magnitude higher, 10^5 to 10^7 per sq cm (Berger *et al*., 2012). Biofilms of microorganisms in the distribution are of concern because of the potential for protection of pathogens from the action of residual disinfectant in the water, and the regrowth of indicator bacteria such as coliforms (Table 28.5).

Biofilms may appear as a patchy mass in some pipe sections or as uniform layers (see Section 6.2.4). They may consist of a monolayer of cells in a microcolony or can be as thick as 10 to 40 mm, as in algal mats at the bottom of a reservoir (Geldreich, 1996). These biofilms often provide a variety of microenvironments for growth that include aerobic and anaerobic zones because of oxygen limitations within the biofilm. Growth of biofilms proceeds up to a critical thickness, at which nutrient diffusion across the biofilm becomes limiting. Biofilm microorganisms are held together by an extracellular polymeric matrix called a glycocalyx. The glycocalyx is composed of exopolysaccharides (EPS) including glucans, uronic acids, glycoproteins and mannans. The glycocalyx helps protect microorganisms from predation and adverse conditions (e.g., disinfectants) (see Section 2.2.4).

The occurrence of even low levels of organic matter in the distribution system allows the growth of biofilm microorganisms. Factors controlling the growth of these organisms are temperature, water hardness, pH, redox potential, dissolved carbon and residual disinfectant.

It has been demonstrated that biofilms can coexist with chlorine residuals in distribution systems (Geldreich, 1996). *Escherichia coli* is 2400 times more resistant to chlorine when attached to surfaces than as free cells in the water, leading to high survival rates within the distribution system (LeChevallier *et al*., 1988a). The health significance of coliform growth in distribution systems is an important consideration for water utilities, because the presence of these bacteria may mask the presence of indicator bacteria in water supplies resulting from a breakdown in treatment barriers. Total numbers of heterotrophic bacteria growing in the water or biofilm may interfere with the detection of coliform bacteria. High heterotrophic plate counts (HPC) are also indicative of a deterioration of water quality in the distribution system. It has been recommended that HPC numbers not exceed 500 organisms per milliliter.

Biofilms in distribution systems are difficult to inactivate. Chlorine levels commonly used in water treatment are inadequate to control biofilms. Free chlorine levels as high as 4.3 mg/L have proved inefficient in eliminating coliform occurrence. It has been suggested that monochloramine may be more effective in controlling biofilms because of its ability to penetrate the biofilm (LeChevallier *et al*., 1988b).

Water-based pathogens such as *Legionella* may also have the ability to grow in biofilms and the distribution system. *Legionella* is known to occur in distribution systems and colonize the plumbing, faucet fixtures and taps in homes (Colbourne *et al*., 1988). *Legionella* are more resistant to chlorine than *E. coli*, and small numbers may survive in the distribution system. Hot-water tanks in homes and hospitals favor their growth. *Legionella* survives well at 50°C, and it is capable of growth at 42°C (Yee and Wadowsky, 1982). It has been found that sediments in water distribution systems and the natural microflora favor their survival. *Legionella* associated with hot-water systems in hospitals have caused numerous outbreaks of disease in immunocompromised patients. Sporadic cases in communities show a strong association with the colonization of household taps. Forty percent of sporadic cases of this illness may be related to household taps or showers.

28.3.2 Organic Carbon and Microbial Growth

Bacterial growth in distribution systems is influenced by the concentration of biodegradable organic matter, water temperatures, nature of the pipes, disinfectant residual concentration and detention time within the distribution system (Bitton, 2011). Bacteria such as *Pseudomonas aeruginosa* and *P. fluorescens* are able to grow in tap water at relatively low concentrations (μg/L) of low-molecular-weight organic

substrates such as acetate, lactate, succinate and amino acids. The amount of biodegradable organic matter available to microorganisms is difficult to determine from data from dissolved organic carbon (DOC) or total organic carbon (TOC) measurements. These measurements capture only the bulk water portion of organic matter, of which only some is biodegradable (Figure 28.4).

Several bioassay tests have been proposed using either pure cultures of selected bacteria or mixed flora from the source water for assessment of the assimilable organic carbon (AOC) in water (Geldreich, 1996). Measurements of bacterial action in the test sample over time are determined by plate counts, direct cell count, ATP, turbidity, etc. (see Example Calculation 28.1). It is estimated that the assimilable organic carbon in tap water is between 0.1 and 9% of the total organic carbon (van der Kooij *et al.*, 2003), although this fraction may be higher if the treatment train involves ozonation, which breaks down complex organic molecules and makes them more available to microorganisms (Table 28.6 and Figure 28.5). In fact, rapid regrowth of heterotrophic plate counts usually occurs after ozonation of tapwater. Addition of a secondary disinfectant is required to control this regrowth.

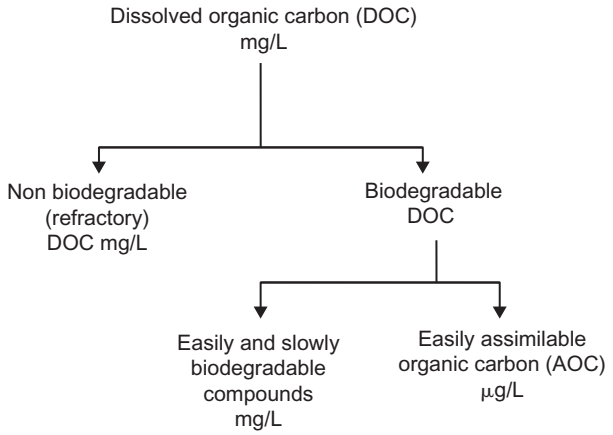

FIGURE 28.4 Fraction of organic matter in drinking water distribution systems. Based on Volk and LeChevallier (2000).

Because determination of AOC levels based on *P. fluorescens* does not always appear to be a good indicator of the growth potential for coliforms, a coliform growth response (CGR) test has been developed (Geldreich, 1996) (Figure 28.5). This procedure uses *Enterobacter cloacae* as the bioassay organism. Changes in viable densities of this organism in the test over a 5-day period at 20°C are used to develop an index of nutrients available to support coliform biofilm growth. The CGR result is calculated by log transformation of the ratio of the colony density achieved at the end of the incubation period, to the initial cell concentration:

Thus:

$$\text{CGR} = \log (N_5/N_0) \qquad \text{(Eq. 28.1)}$$

Example Calculation 28.1 Calculation of Assimilable Organic Carbon

Determination of assimilable organic carbon (AOC) involves a single bacterial species, usually either *Spirillum* NOX or *Pseudomonas fluorescens* P-17. The organism is inoculated into a sample of water which has been pasteurized by heat to kill the indigenous microflora and then the test bacterium in stationary phase is added. Growth is monitored (usually 7 to 9 days) until stationary phase is reached. The number of organisms at the stationary phase is assumed to be the maximum number of organisms that can be supported by the nutrients in the sample, and the yield (numbers of the test bacterium) on acetate carbon is assumed to equal the yield on naturally occurring AOC. When acetate is used as the carbon source in determination of yield, AOC concentrations may be reported as acetate-carbon equivalents. Reporting AOC as micrograms carbon per liter assumes that the yield (total number of bacteria after incubation for 7–9 days) on acetate is equal to the yield on naturally occurring AOC. In theory, the concentration of less than 1 µg carbon per liter can be detected. In practice, organic carbon contamination during glassware preparation and sample handling imposes a limit of detection of approximately 1–10 µg AOC/liter.

The AOC can be calculated as follows:

$$\text{AOC}\ (\mu\text{g carbon/liter}) = (N_{max} \times 1000)/Y$$

where

N_{max} = maximum colony counts (CFU/ml) and
Y = yield coefficient(CFU/mg carbon).

The AOC concentration is expressed as micrograms acetate-carbon equivalents per liter.

When using *P. fluorescens* strain P-17, $Y = 4.1 \times 10^6$ CFU/µg carbon. Thus, if the final yield of the test organism is 5×10^6/ml after 9 days of incubation,

$$\text{AOC of sample} = 1.22\ \mu\text{g acetate-carbon equivalents/liter}$$

where N_5 = number of CFU per milliliter at day 5 and N_0 = number of CFU per milliliter at day 0. Any sample that demonstrates a 1-log or greater increase is interpreted as supporting coliform growth. Calculated values between 0.51 and 0.99 are considered to be moderately growth supportive, and those less than 0.5 are regarded as not supportive of coliform growth.

It is important to note that the CGR test responds only to the concentrations of assimilable organic materials that support growth of coliforms characteristic of regrowth in biofilms. In fact, parallel assays comparing *E. coli* response with that of *Enterobacter cloacae* indicate a significant difference in growth response between these two coliforms. *Ent. cloacae* growth can occur in nutrient concentrations far below those required by *E. coli*.

TABLE 28.6 Concentrations of Assimilable Organic Carbon (AOC) in Various Water Samples

Source of Water	DOC (mg C/L)[a]	AOC (mg C/L)[a]
River Lek	6.8	0.062–0.085
River Meuse	4.7	0.118–0.128
Brabantse Diesbosch	4.0	0.08–0.103
Lake Yssel, after open storage	5.6	0.48–0.53
River Lek, after bank filtration	1.6	0.7–1.2
Aerobic groundwater	0.3	<0.15

Adapted from van der Kooij (2003).
[a]*DOC, dissolved organic carbon; AOC, assimilable organic carbon; mg C/L, milligrams carbon per liter.*

Detecting any changes in the dissolved organic concentrations is another approach to obtaining a measure of assimilable organic carbon. Rather than using pure cultures, these procedures utilize the indigenous microflora of the raw surface-water source of the biomass washed from sand used in the sand filter during drinking water treatment (van der Kooij, 2003).

The biodegradable dissolved organic carbon (BDOC) is given by the following formula:

$$\text{BDOC (mg/L)} = \text{initial DOC} - \text{final DOC} \quad \text{(Eq. 28.2)}$$

The general approach is as follows. A water sample is sterilized by filtration through a 0.2-μm pore size filter, inoculated with indigenous microorganisms, and incubated in the dark at 20°C for 10–30 days until DOC reaches a constant level. BDOC is the difference between the initial and final DOC values (Servais *et al.*, 1987). The advantage of using the consortium of heterotrophic microorganisms that occurs in these aquatic habitats is in their acquired proficiency to degrade a diverse spectrum of dissolved organics that may be in test samples. Whether the inoculum is from the raw source water or the sand filter biofilm has not been found to be critical for optimal test performance.

28.3.3 Microbial Community Structure

Genomic-based molecular methods have greatly increased our ability to understand microbial community dynamics in drinking water distribution systems. Recent studies indicate that these communities are complex and influenced by the source water (ground vs. surface), chemical properties of the water, treatment and type of disinfectant residual. Studies have shown Alphaproteobacteria, Betaproteobacteria or Gammaproteobacteria are in predominance (Hwang *et al.*, 2012). The abundance of different groups of bacteria has

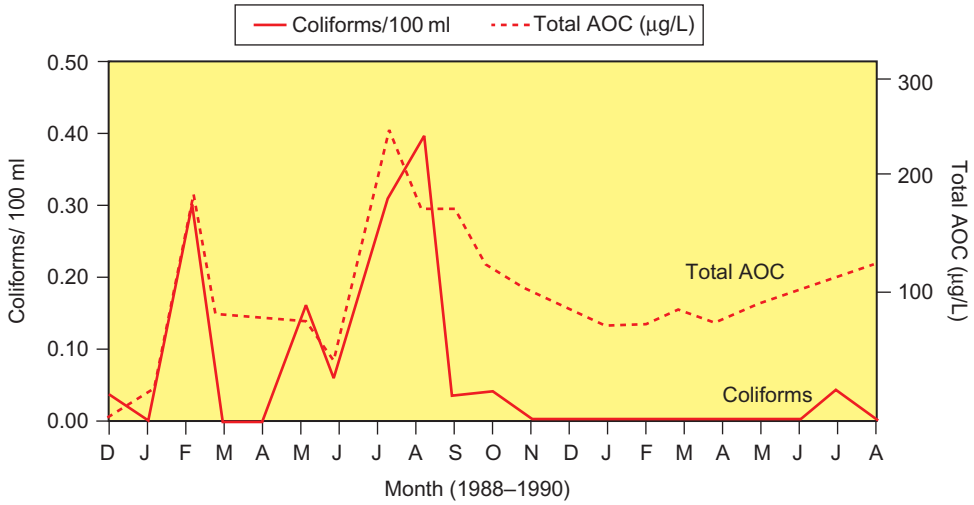

FIGURE 28.5 Relationship between mean coliform densities and total assimilable organic carbon (AOC) levels. From LeChevallier *et al.* (1992).

TABLE 28.7 Distribution of Opportunistic Pathogens in Distributions with Different Disinfection Residuals

Organism	Percent of Total in	
	Free Chlorine	Chloramine
Mycobacterium	1.29	19.65
Legionella	0.31	0.09
Amoeba	0.03	>0.0001

TABLE 28.8 Distribution of Members of Bacteria Domain Determined via Taxonomic Identifications of Annotated Proteins at the Class Level

Domain	Free Chlorine %[a]	Chloramine %
Actinobacteria	6.2	27.8
Cytophaga	0	2.3
Flavobacteria	0	2.3
Sphingobacteria	0	2.0
Chlamydiae	0.4	0.1
Chlorobia	1.4	0
Chloroflexi	1.3	0
Gloeobacteria	1.3	0
Cyanobacteria	9.0	0
Bacilli	4.1	0
Clostridia	5.6	0
Planctomycetacia	1.3	0
Alphaproteobacteria	35.1	22.5
Betaproteobacteria	6.2	24.1
Deltaproteobacteria	11.9	10.5
Gammaproteobacteria	0.5	0.1
Other classes representing <1%	15.4	8.4
Total	**100**	**100**

Source: Gomez-Alvarez et al. (2012).
[a]*Each number in brackets = % total sequences in each group.*

been found to vary between distribution systems that have a free chlorine residual and those that use chloramines (Gomez-Alvarez *et al*., 2012). Such changes in community structure can be significant to protection of community health as it was found that disinfection type can cause changes in abundance of opportunistic pathogens (Tables 28.7 and 28.8).

28.3.4 Intrusion Events

Intrusion of water into drinking water distribution systems can result in exposure to consumers after the water has been treated. Intrusion can occur from cross connection with water containing wastes, changes in water pressure or intentional addition from terrorist-motivated events. Transient negative pressure events (which create suction into the pipes) can occur in pipelines, and if leaks are present, provide a potential portal for water into the distribution systems. Karim *et al*. (2003) examined 66 soil and water samples immediately adjacent to drinking water pipes from eight utilities in six states of the United States. About 56% of the samples were found positive for human enteric viruses. In addition, total fecal coliform levels in some soil samples were greater than 1.6×10^4 CFU/100 g of soil, suggesting that the sampling locations were potentially under the influence of leaking sewage pipes. Several epidemiological studies have found that the greater the distance a person lives from the treatment plant, the greater the incidence of gastroenteritis. The use of point-of-use treatment devices at consumers' taps has also found a decrease in illness for gastroenteritis for the young and elderly. To better understand contamination in distribution systems, real-time monitoring systems are receiving increased attention.

28.4 REAL-TIME MONITORING OF MICROBIAL CONTAMINANTS IN WATER DISTRIBUTION SYSTEMS

Even in developed countries, microbial contamination of drinking water is a major issue, and over the past decade, the number of waterborne outbreaks associated with water distribution systems has increased. Contaminated drinking water in the distribution system can result from inadequate treatment, or from leaks or breaks in the distribution pipes, which result in accidental intrusion events that allow microbial or chemical contamiants to enter the potable water. In addition, EPA is concerned about deliberate intrusion events that could occur through bioterrorist activities. Traditionally, utilities have utilized indicator tests for fecal pollution to monitor for the potential presence of pathogens. However, such cultural assays can take up to 48 hours to complete, during which time contaminated water could be delivered to consumers.

Recently, utilities have been evaluating new online monitoring systems to augment traditional monitoring. Specifically, the goal has been to integrate software for

Case Study 28.1 Real-time Detection of *E. coli* and *Bacillus* Spores using Multi-angle Light Scattering

Multi-angle light scattering (MALS) is a technology that utilizes laser light scattering to detect intrusion of *E. coli* cells within a water distribution system (Miles *et al.*, 2011). Real-time detection was successful in tap water over a concentration range of 10^3–10^6 cells per ml. Cell numbers as determined by MALS were similar to those obtained by conventional assays such as dilution and plating (see Chapter 10), and acridine orange direct counts (AODC) (see Chapter 9).

MALS was also utilized to detect *Bacillus thuringiensis* spores (a surrogate for *Bacillus anthracis* spores) introduced into a distribution system over a concentration range of 10^2–10^5 spores per ml (Sherchan, 2013) (Figure 28.6).

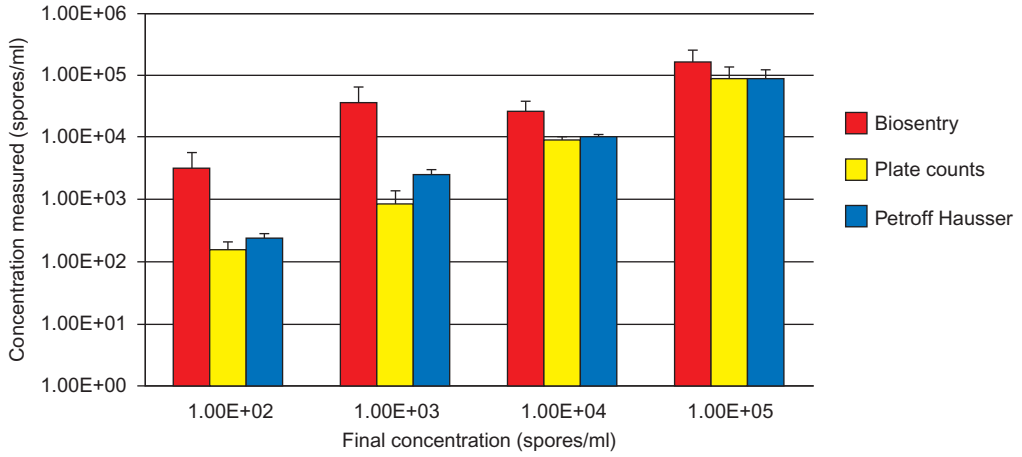

FIGURE 28.6 MALS response to *B. thuringiensis* spores in unfiltered tap water.

data management with new real-time sensor technologies to provide an early warning monitoring program via a supervisory control and data acquisition system (SCADA), installed at critical points within the distribution system. Use of such real-time technologies allows for a rapid response to contamination that safeguards the public from consuming contaminated water. Although utilities are concerned with both chemical and microbial contaminants, here we focus on real-time detection of microbial contaminants.

28.4.1 Real and Near Real-Time Technologies Form Monitoring Microbial Contaminants

Physical microbial characteristics can be used to detect microbial concentrations via several methodologies including vibrational spectroscopy and multi-angle light scattering technologies. Vibrational spectroscopy utilizes spectra that are emitted following excitation of molecules by laser light. These technologies include Raman spectroscopy and Fourier transformed infrared spectroscopy (Driskell *et al.*, 2005; Rule and Vikesland, 2009).

Multi-angle light scattering (MALS), which results from laser light that strikes particulates, can also be used to detect microorganisms. The specific light scattering that results allows for the differentiation of bacterial cells and spores in real time. Such technologies can also be used to detect increases in microbial cells in water, in essence functioning as a cell counter. To date, viruses cannot be detected in real time. In addition, the sensitivity of detection still needs to be improved to enable the technology to be used in water.

Near real-time assays can be defined as those assays that require approximately 2 hours to complete. These include quantitative PCR; ATP measurements via bioluminescence assays; antibody assays; flow cytometry; and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Many of these assays are still in their infancy, and many still have issues related to sensitivity and specificity (Sherchan, 2013). Biosensors are also being developed that rely on recognition of specific biological targets including proteins or nucleic acid. Immunosensors rely on the interaction between antigens on target cells and antibodies (Shirale *et al.*, 2010). Viruses can be detected in near real time through the use of qPCR with a detection time of approximately 2 hours.

However, a major limitation of qPCR detection is that it does not discriminate between infectious and noninfectious virus. Very recently, aptamers have been utilized to detect microbes (Liu *et al*., 2012). Aptamers are single-stranded nucleic acids (RNA or DNA) with defined tertiary structures for selective binding to target molecules. Aptamers can be readily produced by chemical synthesis and modified to result in aptamers that can bind to a broad range of biological targets with high affinity and specificity comparable to those of antibodies (Li *et al*., 2012).

Overall, the field of real and near real-time detection of microbes is advancing rapidly, but many challenges remain, including the need for a real-time assay for viruses.

QUESTIONS AND PROBLEMS

1. Why is it important to reduce the amount of biodegradable organic matter and nutrients during water treatment?
2. Describe the major steps in the conventional treatment of drinking water.
3. What group of waterborne pathogens is most effectively removed by filtration? Why?
4. What methods can be used to assess the growth of bacteria in water?
5. Which pathogenic microorganisms are the most difficult to remove by conventional water treatment and why?
6. Why is coliform regrowth in distribution systems a problem?
7. Why does the HPC increase after ozonation of drinking water?
8. A water treatment plant is required to remove 3 $\log_{10}$ of *Cryptosporidium* oocysts from their water source. What treatment processes do you recommend? What if they have to remove 5 $\log_{10}$? See Chapter 25 for *Cryptosporidium* resistance to disinfectants.
9. To determine the AOC of drinking water, two 40 ml flasks are inoculated with 500 colony forming units (CFU) of *Pseudomonas fluorscens* strain P-17. After 9 days the concentration in one flask has reached 2.4×10^5 CFU/ml and 1.2×10^5 CFU/ml. Calculate the average AOC in this drinking water sample.
10. The type of residual disinfectant used in distribution systems affects the microbial community living in those systems. Why?

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