4

DRINKING WATER DISTRIBUTION SYSTEMS: BIOFILM MICROBIOLOGY

4.1 INTRODUCTION

Following water treatment by processes mentioned in Chapters 2 and 3, water is distributed through a network of pipes to reach customers. The water distribution system (WDS), an essential component of drinking water treatment, is the "workhorse" that carries drinking water from the plant to the customers. The daily production in the United States is estimated 34 billion gallons of drinking water that flows through 1.8 million miles of distribution pipes. It is estimated that approximately two-third of the pipes are made of plastic, mostly polyvinyl chloride (PVC). Many waterborne outbreaks are attributed to the degradation of water quality in noncovered water reservoirs and in WDSs through cross-connections, main breaks (an estimated 240,000 water distribution main breaks per year in the United States; Lafrance, 2011), contamination during construction or repair, back siphonage (National Research Council, 2006), or negative pressure events (Gullick et al., 2004; NRC, 2006).

Drinking water regulations mostly concerns water quality at the end of the water treatment plant but the water quality can deteriorate during storage and transport through water distribution pipes and in indoor plumbing before reaching the consumer. More recently, US federal regulations addressed water quality in distribution systems through rules covered by the Safe Drinking Water Act (SDWA).

In the long history of water distribution which goes back to 2000 BC, pipes were made of a wide range of materials which include aqueducts, terracotta, wood, copper, lead, bronze, bamboo, and stone. Nowadays, piping materials include cast iron, ductile iron, stainless steel, concrete, asbestos-cement, or PVC, polyethylene, and medium density polyethylene (MDPE) (Bachmann and Edyvean, 2006; Berry et al., 2006; Lafrance, 2011; Walski, 2006; Percival et al., 2000). However, some of

Microbiology of Drinking Water Production and Distribution, First Edition. Gabriel Bitton.

the piping materials lead to corrosion problems (e.g., cast iron and copper pipes) or to leaching of toxic materials (e.g., PVC pipes).

Some major problems concerning WDSs are the following:

- Microbial growth
- Pathogen survival and growth
- Nitrification problems
- Biocorrosion

Deterioration of water distributions systems due to leakage from cracks and joints or back siphonage may lead to drinking water contamination and disease outbreaks. In the United States, approximately 18% of the outbreaks reported for public water systems are due to contamination of the WDS by microbial pathogens and toxic chemicals (Craun, 2001). The contribution of WDSs to acute gastrointestinal illness (AGI) incidence was demonstrated by epidemiological studies which showed that the incidence was higher among consumers who drank from home taps than those who drank treated water bottled at the water treatment plant (Payment et al., 1991a, 1997). This shows that WDS and in-premise plumbing may contribute to disease in the community. The risk of viral AGI from nondisinfected groundwater-based drinking WDSs exceeded the U.S. EPA acceptable risk of 10^{-4} episodes/person-year. An extrapolation of the results to the United States as a whole shows an AGI risk of 470,000 to 1,100,000 episodes/year nationwide (Lambertini et al., 2012; U.S. EPA, 1989).

Drinking water deterioration in WDS has several causes (LeChevallier et al., 2011; Smith, 2002; Sobsey and Olson, 1983):

- Improperly built and operated storage reservoirs which should be covered to prevent airborne contamination and to exclude animals.
- Loss of disinfectant residual.
- Back siphonage and cross-contamination.

Some of the consequences are

- Public health problems due to the excessive growth and colonization of water distribution pipes by bacteria and other organisms, some of which are pathogenic. Microbial growth depends on the availability of nutrients (see Chapter 6)
- Microbial regrowth in storage reservoirs
- Protection of pathogens from disinfectant action
- Taste and odor problems due to the growth of algae, actinomycetes and fungi (see Chapter 5)
- Corrosion problems

Drinking water may contain humic and fulvic acids, as well as easily biodegradable natural organics such as carbohydrates, proteins, and lipids. The presence of dissolved organic compounds in finished drinking water is responsible for several problems,

among which are taste and odors, enhanced chlorine demand, trihalomethane formation, and bacterial colonization of water distribution lines (see Chapter 6 for more details). Water utilities should maintain the distribution system by periodically flushing the lines to remove sediments, excessive bacterial growth, and encrustations due to corrosion.

4.2 BIOFILM DEVELOPMENT IN WDSs

4.2.1 Introduction

Biofilms develop at solid-water interfaces and are widespread in natural environments as well as in engineered systems. They are ubiquitous and are commonly found in trickling filters, rotating biological contactors, activated carbon beds, distribution pipe surfaces, groundwater aquifers, aquatic weeds, tooth surfaces (i.e., dental plaque), dialysis units, dental unit waterlines, and indwelling medical devices (IMDs) such as catheters, pacemakers, orthopedic implants, endotracheal tubes, prosthetic joints, and heart valves (Anwar and Costerton, 1992; Characklis, 1988; Hamilton, 1987; Schachter, 2003; Thomas et al., 2004; Walker and Marsh, 2007; van der Wende and Characklis, 1990). For example, in the United States, the risk of infection from bladder catheters ranges from 10% to 30% while the risk from cardiac pacemakers is 1–5% (Thomas et al., 2004).

Biofilms are relatively thin layers (up to a few hundred microns thick) of attached microorganisms that form microbial aggregates and grow on surfaces. Biofilms formed by prokaryotic microorganisms resemble in some ways tissues formed by eukaryotic cells (Costerton et al., 1995; Wingender and Flemming, 2011). The multicellular behavior is due to the fact that cells within the biofilm interact and coordinate their activities via direct cell-to-cell contact or by releasing signaling molecules (see quorum sensing in Section 4.2.5) (Branda and Kolter, 2004). Observations of living biofilms by scanning confocal laser microscopy have helped in our understanding of biofilm structure. Biofilm microorganisms form microcolonies separated by open water channels (Stoodley et al., 2002; Donlan, 2002). Liquid flowing through these channels allow the diffusion of nutrients, oxygen, and antimicrobial agents into the cells (Donlan, 2002). Some bacteria, such as *Staphylococcus epidermidis*, form honeycomb-like structures (Schaudinn et al., 2007) that provide mechanical stability to the biofilm, help lower the energy costs of individual cells and maximize nutrient absorption. Biofilms also include corrosion by-products, organic detritus, and inorganic particles such as silt and clay minerals. They contain heterogeneous assemblages of microorganisms (Figure 4.1), depending on the chemical composition of the surface, the chemistry of finished water, and oxido-reduction potential in the biofilm. They take days to weeks to develop, depending on nutrient availability and environmental conditions. Biofilm growth proceeds up to a critical thickness (approximately 100–200 μm) when nutrient diffusion across the biofilm becomes limiting. The decreased diffusion of oxygen is conducive to the development of facultative and anaerobic microorganisms in the deeper layers of the biofilm.

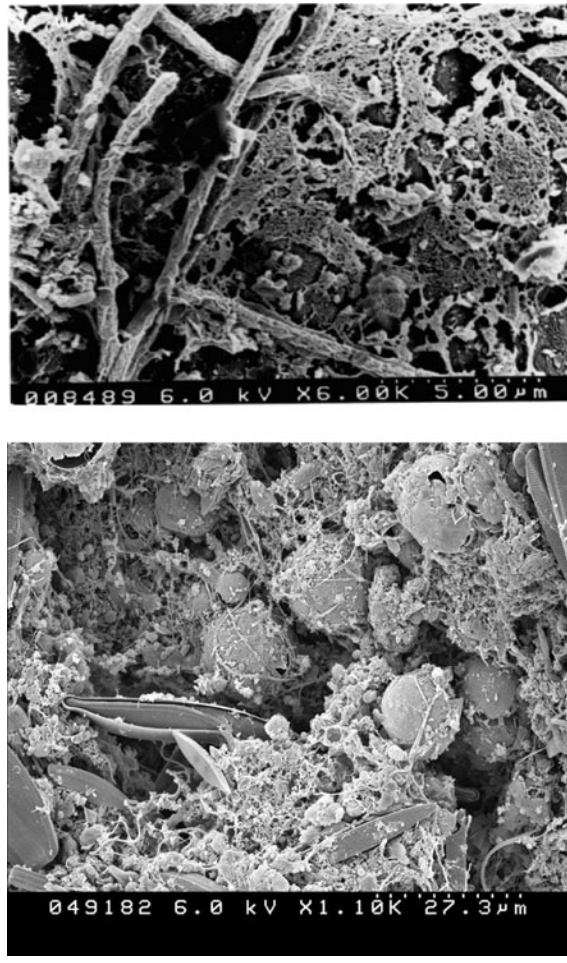

Figure 4.1 SEM pictures of biofilms.

4.2.2 Processes Involved in Biofilm Development

A number of processes contribute to biofilm development on surfaces exposed to water flow. The processes involved are the following (Bitton and Marshall, 1980; Gomez-Suarez et al., 2002; Olson et al., 1991). The sequence of events is described in Figures 4.2 and 4.3 (Gomez-Suarez et al., 2002; De Kievit, 2009):

4.2.2.1 Surface Conditioning. Surface conditioning is the first step in biofilm formation. Minutes to hours after exposure of a surface to water flow, a surface-conditioning layer, made of ions, proteins, glycoproteins, humic-like substances, and other dissolved or colloidal organic matter, initially adsorb to the surface. This results in a modified surface that is different from the original one (Schneider and Leis,

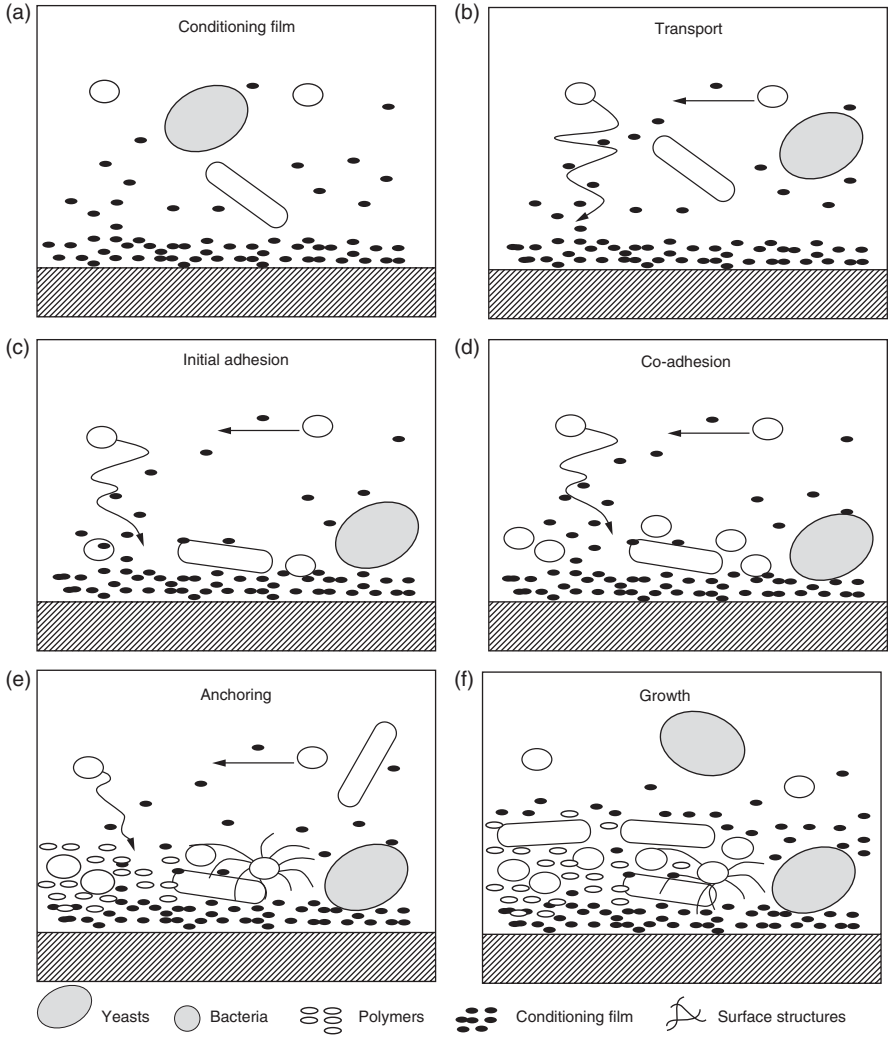

Figure 4.2 Sequence of events in biofilm formation *Source:* Gomez-Suarez et al. (2002). In: *Encyclopedia of Environmental Microbiology*, Gabriel Bitton, editor-in-chief, Wiley-Interscience, N.Y.

2002). The conditioning film is a source of nutrients for bacteria, particularly in oligotrophic environments such as drinking water or groundwater.

4.2.2.2 Transport of Microorganisms to Conditioned Surfaces. Diffusion, Brownian motion, convection, and turbulent eddy transport are involved in turbulent flow regime. Chemotaxis may also enhance the rate of bacterial adsorption to surfaces under more quiescent flow.

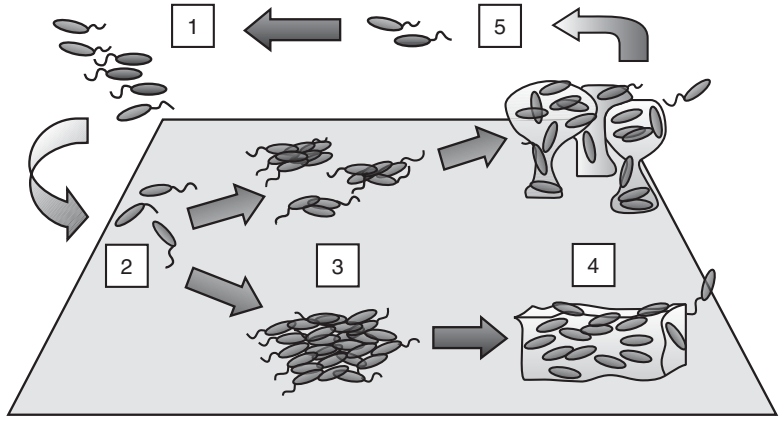

Figure 4.3 *Pseudomonas aeruginosa* biofilm development. Planktonic cells (stage 1) attach onto a solid surface (stage 2) and microcolonies are formed (stage 3). Under conditions that promote bacterial migration (e.g., succinate, glutamate), cells will spread over the substratum, ultimately developing into a flat, uniform mat (stage 4). Under motility-limiting conditions (e.g. glucose), the microcolonies proliferate forming stalk- and mushroom-like structures (stage 4). At various points throughout biofilm maturation, cells can detach and resume the planktonic mode of growth (stage 5). *Source:* De Kievit, T.R. (2009). Environ. Microbiol. 11: 279–288.

4.2.2.3 Adhesion of Microorganisms to Surfaces. According to the thermodynamic theory, adhesion of a microorganism to a surface is favored when the free energy of adhesion is negative ($\Delta G_{adh} < 0$) (Gomez-Suarez et al., 2002). Furthermore, according to the DLVO theory (Derjaguin, Landau, Verwey, and Overbeek theory), adhesion is a balance between Lifshitz-Van der Waals forces and repulsive or attractive electrostatic forces due to electrical surface charges on both microbial and surfaces. Hydrophobic interactions are also involved in microbial adhesion to surfaces.

4.2.2.4 Cell Anchoring to Surfaces. Following adhesion to a given surface, microbial cells are anchored to the surface, using extracellular polymeric substances (EPSs). EPS, made of polysaccharides (e.g., mannans, glucans, uronic acids), proteins, nucleic acids, and lipids help support biofilm structure. EPS display hydrophilic and hydrophobic properties. They help in the adhesion of microorganisms to surfaces and their cohesion within biofilms, and play a structural role in biofilms (Bitton and Marshall, 1980; Characklis and Cooksey, 1983; Costerton and Geesey, 1979; Flemming and Wingender, 2002; 2010; Starkey et al., 2004; Zhu et al., 2010; Xue et al., 2013b). EPS also helps protect microorganisms from protozoan predation, chemical insult, antimicrobial agents, desiccation, and osmotic shock. Moreover, extracellular polysaccharides chelate heavy metals and reduce their toxicity to microorganisms (Bitton and Freihoffer, 1978).

Other attachment organelles include flagella, pili (fimbriae), stalks, or holdfasts (e.g., *Caulobacter*) (Bitton and Marshall, 1980; Olson et al., 1991).

4.2.2.5 Cell Growth and Biofilm Accumulation. Biofilms essentially consist of microbial aggregates embedded in EPSs attached to a solid surface (Rittmann, 1995b). It was estimated that biofilms harbor about 95% of the microbial communities in WDSs (Wingender and Flemming, 2004). However, bulk water bacteria may dominate in portions of the distribution system with no chlorine residual (Srinivasan et al., 2008).

4.2.3 Factors Involved in Biofilm Accumulation

The study of single-species biofilms has helped in our understanding of the steps involved in biofilm accumulation which depends on several factors:

4.2.3.1 Concentration of Assimilable Organic Carbon. The presence of even microgram levels of organic matter in distribution lines allows the growth and accumulation of biofilm microorganisms (Block et al., 1993; Ollos et al., 2003; Servais, 1996).

4.2.3.2 Limiting Nutrients. Microbial growth in drinking water can also be highly regulated by the availability of phosphorus, which can act as a limiting nutrient for microbial growth in drinking water (Charnock, and Kjønnø, 2000; Miettinen et al., 1997; Keinänen et al., 2002; Sathasivan and Ohgaki, 1999). For example, in most Finnish drinking waters, microbial growth was well correlated with the concentration of bioavailable phosphorus (Lehtola et al., 2002; Rubulis and Juhna, 2007). The presence of bioavailable phosphorus and iron in water distribution pipes also helps the survival of *Escherichia coli* (Appenzeller et al., 2005; Juhna et al., 2007). Thus, phosphorous removal by water treatment processes may decrease *E. coli* survival in this environment.

4.2.3.3 Trace Elements. Trace elements can stimulate (e.g., Fe, Mn, Zn) or inhibit (e.g., Cu, Ag) bacterial growth in distribution networks.

4.2.3.4 Disinfectant Concentration. Biofilm thickness and activity are inhibited by disinfection with chlorine. For example, the biofilm thickness ($\sim 10^3$ pg ATP/cm^2) in the presence of a chlorine concentration of <0.05 mg/L was two orders of magnitude greater than the thickness ($\sim$10 pg ATP/cm^2) in the presence of a chlorine level of 0.30 mg/L (Hallam et al., 2001). Attached microorganisms are better controlled by monochloramine than by free chlorine (see also Chapter 3). In a bench-scale distribution system, it was found that a free chlorine residual of 0.5 mg/L or chloramine at 2 mg/L reduced biofilm microorganisms by several orders of magnitude (Ollos et al., 2003).

4.2.3.5 Type of Pipe Material. Biofilm thickness also depends on the type of pipe material. Plastic-based materials (polyethylene or PVC) support less attached biomass than iron (e.g., gray iron), cement-based materials (e.g., asbestos-cement, cemented cast iron) (Figure 4.4; Niquette et al., 2000), or stainless steel materials.

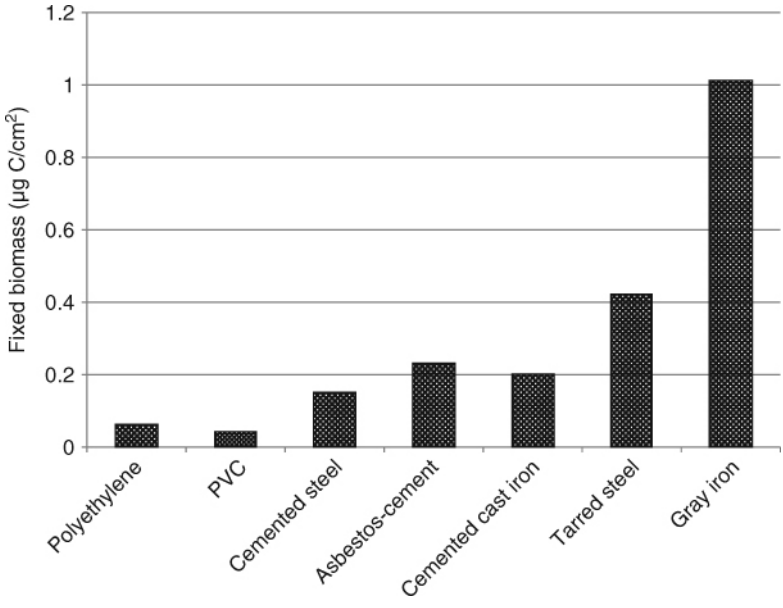

Figure 4.4 Effect of pipe material on biofilm accumulation in a drinking water distribution system. Adapted from Niquette et al. (2000). Water Res. 34: 1952–1956.

Copper pipes showed the lowest biofilm formation potential (Yu et al., 2010). Biofilm formation, as measured by heterotrophic plate counts and ATP content, was higher in galvanized steel pipes than in copper pipes, as the release of Cu from the pipes is toxic to microorganisms (Silhan et al., 2006). Iron corrosion, increased surface roughness, and porosity in iron pipes contribute to increased attached biomass. Iron, as a nutrient, is also a factor in increased biomass in the pipes. Pipe material type can also influence the community composition in the biofilms (Kalmbach et al., 2000). For example, 74% of the bacteria in PVC pipes were *Stenotrophomonas*, whereas iron pipes harbored a more diverse microbial populations which consisted mainly of *Nocardia*, *Acidovorax*, *Xanthobacter*, *Pseudomonas*, and *Stenotrophomonas* (Norton and LeChevallier, 2000). Thus, the use of pipe materials (e.g., stainless steel) that could reduce biofilm development is desirable (Percival et al., 2000).

4.2.3.6 Flow Velocity and Regimen. An increase in flow rate and a change from laminar to turbulent flow can enhance the mass transport of nutrients or biocides into the biofilm, leading to changes in biofilm thickness (see Bachmann and Edyvean (2006) for more details). Sudden changes in water flow leads to detachment of biofilm from the pipe surface with subsequent deterioration of water quality due to increase in suspended bacteria (Choi and Morgenroth, 2003). Hydraulic regime also affects the bacterial community structure in biofilms. A higher diversity was observed at highly varied flow in an experimental WDS (Douterelo et al., 2013).

4.2.3.7 Quorum Sensing. A bacterial communication system, which controls biofilm formation and function (Shrout and Nerenberg, 2012; see more details on this system in Section 4.2.5.

4.2.3.8 Other Factors. A number of other factors (pH, redox potential, TOC, water temperature, hardness) control the growth of microorganisms on pipe surfaces (Olson and Nagy, 1984). Temperature affects directly and indirectly the rate of biofilm formation. A study of biofilm growth in pipes made of different materials showed that biofilm accumulation was higher at 35°C than at 15°C (Silhan et al., 2006). Bacterial growth generally increases when temperature is above 15°C although psychtrophic iron bacteria can grow below this temperature.

4.2.4 Biofilm Ecology

Biofilms allow microorganisms to persist and grow in hostile environments such as WDSs under low nutrient conditions. In this protected environment, EPSs provide a diffusional barrier against disinfectants and other deleterious chemicals. EPS increase the resistance of biofilm and any detached biofilm clusters to chlorine (Xue et al., 2013b). Using model WDSs, it was found that during the initial stages of biofilm formation, the 16S RNA sequences in biofilms are similar to those found in the bulk solution. However, DNA- and RNA-based fingerprints of 20-year-old biofilms show a higher diversity in biofilm communities than in bulk water (Henne et al., 2012). The higher diversity in biofilms than in bulk water was also observed in an experimental WDS. Alphaproteobacteria dominated in the bulk water while beta- and gammaproteobacteria (which include many primary and opportunistic pathogens) were predominant in biofilms (Douterelo et al., 2013). Microcolonies are formed following attachment of single cells to the pipe surface. Species diversity increases thereafter, and biofilms may reach steady state and high ecological diversity, including both heterotrophs and autotrophs, only after 2–3 years (Berry et al., 2006; Martiny et al., 2003). Molecular techniques are now revealing the bacterial diversity in drinking water and biofilms (Kahlisch et al., 2010; Lee et al., 2010; Revetta et al., 2010; Schmeisser et al., 2003). Molecular probing showed the presence of gram-positive bacteria as well as alpha-, beta-, and gamma-proteobacteria. Bacteria generally found in drinking WDSs include *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Alcaligenes*, *Aeromonas*, *Moraxella*, *Enterobacter*, *Citrobacter*, *Sphingomonas*, *Klebsiella*, *Burkholderia*, *Xanthomonas*, *Methylobacterium*, and *Bacillus* (Berry et al., 2006; Block et al., 1997). Examination of biofilms from household water meters showed that the major bacterial genera were *Sphingomonas* (alpha-proteobacteria), *Acidovorax*, *Methylophilus* (beta-proteobacteria), and *Lysobacter* (gamma-proteobacteria). Examination of the Ann Arbor drinking water by 16S rRNA gene sequencing also showed that most of the bacteria were proteobacteria and belonged to *Acidovorax*, *Variovorax*, *Sphingopyxis*, *Ralstonia*, and *Novosphingobium* (Figure 4.5; Lee et al., 2010). Another study in Ohio, using similar techniques, showed the presence of proteobacteria, cyanobacteria, actinobacteria, bacteroidetes, and planctomycetes but 57.6% of the sequences were hard to classify (Revetta et al., 2010).

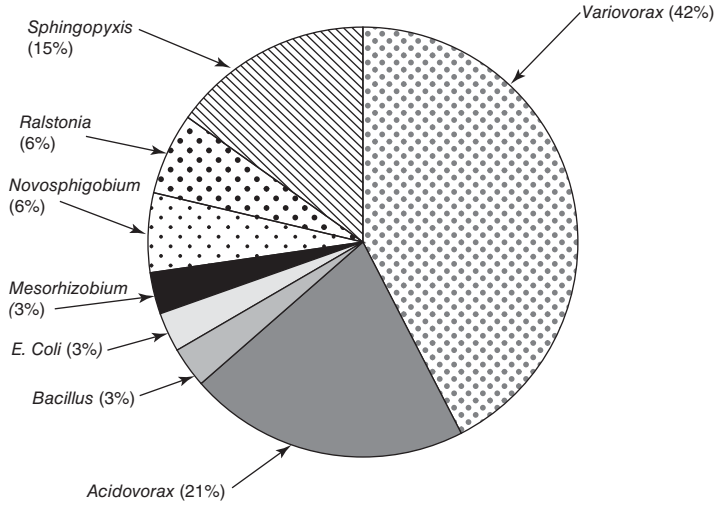

Figure 4.5 Bacterial diversity in drinking water. Pie chart shows the relative diversity of each genus identified by 16S rRNA gene sequencing. *Source:* Lee et al. (2010). Water Res. 44: 5050–5058.

Bacterial community composition changes following chlorine disinfection as alpha-, beta-, and gamma-proteobacteria decreased after chlorination (Poitelon et al., 2010). Community composition is also influenced by the type of disinfectant used (Hong et al., 2010; Poitelon et al., 2010; Williams et al., 2004; Yu et al., 2010). It is known that nutrient-depleted environments, such as marine waters and groundwater, harbor ultramicrocells (UMCs) that pass through 0.2 μm filters (Velimirov, 2001). Such ultramicrocells were detected in chlorinated drinking water and are part of the biofilm community. Analysis of 16S rRNA sequences showed that UMC belong to the Proteobacteria, Firmicules, and Actinobacteria. Some of the UMCs are potential opportunistic pathogens, such as *Stenotrophomonas maltophilia* or *Microbacterium* sp., that cause infections in immunocompromised patients in hospital setting (Silbaq, 2009).

4.2.5 Gene Exchange and Quorum Sensing in Biofilms

The proximity of microbial cells in biofilms offers opportunities for communication and exchange of metabolic products and genetic material (e.g., antibiotic resistance genes) between microorganisms (Cvitkovitch, 2004; Wuertz, 2002; Xi et al., 2009). The use of modern techniques such as confocal laser scanning microscopy and fluorescent-labeled (e.g., tagging with green fluorescent protein) plasmids has facilitated the study of gene transfer in biofilms and in the environment in general. These methods have generally shown that gene transfer rates were much higher than those determined by plating on selective media. In addition to gene transfer by conjugation and transformation, biofilms can also be a suitable environment for gene transfer via

transduction. An example is the transfer of the Shiga toxin (Stx) gene to *E. coli* by an Stx-encoding bacteriophage (Solheim et al., 2013).

Much work is presently being done on the molecular mechanisms of biofilm development. For their survival and interactions in biofilms, bacteria are able to communicate with cells of the same species or with other prokaryotic and eukaryotic species. This cell-to-cell communication mechanism, also called "bacterial twitter," is quorum sensing which requires a threshold cell density for its triggering. It helps microorganisms in biofilm development and function and improves their survival by increasing their access to nutrients and defense against competing microorganisms (Boyle et al., 2013; de Kievit, 2009; Shrout and Nerenberg, 2012; Williams et al., 2007). Several quorum sensing bacteria has been found in water and wastewater treatment systems. These include, among others, *Aeromonas*, *Arcobacter*, *Legionella, Pseudomonas*, *Sphingomonas*, *Vibrio*, and *Nitrobacter* (Shrout and Nerenberg, 2012).

Quorum sensing diffusible signaling molecules (e.g., *N*-acyl homoserine lactone), called autoinducers, play an important role in density-dependent gene expression and biofilm differentiation. Bacteria undergo changes when the signaling molecule has reached a threshold concentration and when the bacterial population has reached a certain density. There are three major classes of signaling molecules: acyl homoserine lactones (AHLs), peptides, and LuxS/autoinducer 2. Antibiotics can also function as signaling molecules at relatively low concentrations (Fajardo and Martínez, 2008). AHL signals are emitted by gram-negative bacteria and their activity was demonstrated in single-species biofilms (e.g., *Pseudomonas aeruginosa*) and in naturally occurring biofilms (McLean et al., 1997). Peptides are the signaling molecules in gram-positive bacteria. Autoinducer-2 (AI-2) is another cell-to-cell signaling molecule that may function in cell communication in some bacteria (Winzer et al., 2002). Some other functions regulated by the AI-2 signal are biofilm formation in *Bacillus cereus*, motility in *Helicobacter pylori*, virulence factor production in *P. aeruginosa*, or susceptibility to antibiotics in *Streptococcus anginosus* (Pereira et al., 2013). In addition to biofilm formation, quorum sensing in both gram-negative and gram-positive bacteria is also known to be involved in the regulation of several activities, such as virulence, motility, EPS production, sporulation, production of secondary metabolites such as antibiotics, conjugation, symbiosis, and interspecies competition and other survival strategies (Miller and Bassler, 2001; Withers et al., 2001).

4.2.6 Biofilm Detachment from Surfaces

Biofilm detachment from surfaces releases microorganisms, including pathogens, which may colonize other surfaces. It affects the occurrence of waterborne diseases resulting from the transport of opportunistic pathogens to the consumer's faucet. Detachment occurs following *erosion* (detachment of single cells), *sloughing* (detachment of large pieces of biofilm, sometimes extending all the way to the substratum), *scouring* following abrasion or scraping and grazing by predators (Percival et al., 2000; Rittmann, 2004; Rittmann and Laspidou, 2002).

Biological processes also involved in biofilm detachment, include, among other factors, a decrease in EPS production, rhamnolipids, anti-biofilm poylysaccharides,

and predation on biofilm microorganisms. Detachment events are genetically regulated (Rendueles et al., 2013; Schooling et al., 2004; Stoodley et al., 2002).

The net accumulation of biofilms on surfaces is described by the following equation (Rittmann and Laspidou, 2002).

$$X_f L_f = \frac{YJ}{b + b_{\text{det}}} \tag{4.1}$$

where;

X_f = biomass density (mg biomass/cm^3)
L_f = biofilm thickness (cm)
Y = true yield of biomass (mg biomass/mg substrate)
J = substrate flux (mg substrate/cm^2-day)
b = specific decay rate of biofilm microorganisms (d^{-1})
b_{det} = specific detachment rate (d^{-1})

Biofilm accumulation increases when the substrate is increased or when detachment is decreased. Under steady state conditions, the average specific growth rate (μ_{ave}) is equal to the specific detachment rate (b_{det}).

Slow growing microorganisms (e.g., methanogens) are protected from detachment because they live in the deeper layers of the biofilm.

There are several factors affecting biofilm detachment (Pedersen, 1990; Rittmann and Laspidou, 2002). Detachment is:

- increased by tangential flow shear stress which is related to the detachment rate (at least for smooth surfaces);
- increased by axial force on the biofilm, due to abrasion and to pressure changes caused by turbulent changes and expressed by the Reynolds number Re;
- decreased by surface roughness;
- affected by physiological parameters such as EPS content (higher detachment at low EPS) or biofilm density (lower detachment for dense biofilms).

4.2.7 Some Methods Used in Biofilm Study

Several physical, chemical, biochemical, molecular, and microbiological methods are available for biofilms study. Some of these methods, including molecular techniques, are summarized in Table 4.1 (Broschat et al., 2005; Characklis et al., 1982; Lazarova and Manem, 1995; Neu and Lawrence, 2002; Percival et al., 2000; Schaule et al., 2000; Schmidt et al., 2004).

In the cultivation-based method, a low nutrient medium, designated as R2A agar, is recommended to determine the heterotrophic plate count (HPC) in environmental samples such as drinking water or groundwater. However, this approach detects only from 0.1% to 10% of the total bacterial counts in most aquatic environments (Pickup, 1991). Microbial biomass in biofilms can be measured via determination of specific

TABLE 4.1 Some methods used for biofilm study

Type	Analytical Method
Microscopy	Use of light microcopy, fluorescence microscopy, scanning confocal laser microscopy, scanning electron microscopy, environmental scanning electron microscopy, transmission electron microscopy, atomic force microscopy.
Image analysis	Image structure analyzer, time lapse imaging
Direct measurement of biofilm quantity	Biofilm thickness (using optical methods, image analysis, thermal resistance, quartz crystal microbalance)
	Biofilm mass (total cell count via staining with Acridine Orange or DAPI, crystal violet assay)
Indirect measurement of biofilm quantity	Extracellular polymeric substances (EPS)
	Specific biofilm constituents (polysaccharides, proteins, nucleic acids)
	Total organic carbon (TOC)
	Total proteins
	Peptidoglycans
	Lipid biomarkers
Microbial activity within biofilms	Viable cell count (plate counts, preferably in low nutrient medium such as R2A agar, Live/Dead BacLight^{TM})
	Active bacteria: direct viable count (DVC)
	DVC-FISH
	ATP
	Lipopolysaccharides
	Substrate removal rate
	Dehydrogenase activity (e.g., TTC, INT, or CTC dyes) or esterases such as carboxyfluorescein diacetate (CFDA).
	Oxygen uptake rate (OUR)
	DNA
	rRNA
	mRNA
Microbial observation and identification	Immunofluorescence (monoclonal or polyclonal antibodies)
	FISH (fluorescent *in situ* hybridization)
	mRNA amplified by polymerase chain reaction
	Green fluorescent protein (GFP)
	Use of commercial fluorochromes such as DAPI, acridine orange, SYTOX Green, PicoGreen and propidium iodide, or FUN-1 (for fungi)
Microenvironment	Fluor conjugates for determining diffusion and permeability in biofilms
	Use of microelectrodes for determining pH, temperature, O_2, NH_4, NO_3, NO_2, H_2S, and CH_4 within the biofilm
Other methods	Mass spectroscopy
	Infrared spectroscopy
	X-ray spectroscopy

Source: Adapted from Broschat et al. (2005); Characklis et al. (1982); Lazarova and Manem (1995); McLean et al. (2004); Mezule et al. (2013); Neu and Lawrence (2002); Percival et al. (2000); Schaule et al. (2000); Schmidt et al. (2004).

cell biochemical constituents such as ATP, DNA, RNA, proteins, lipid biomarkers, bacterial cell wall components, or photosynthetic pigments (Sutton, 2002).

Total cell counts in biofilms are difficult due to the presence of aggregated cells. This task is made easier by using modern techniques such as scanning confocal microscopy (SCLM) which gives information about the biofilm structure. The non-destructive SCLM in conjunction with 16S rRNA-targeted oligonucleotide probes help to show the heterogeneous structure of biofilms and their complex biodiversity by giving information on the distribution and activity of specific groups of microorganisms in biofilms (Costerton et al., 1995; Schramm et al., 1996). rRNA-targeted, fluorescently labeled oligonucleotide probes are useful in identifying biofilm microorganisms and community composition (Kalmbach et al., 2000). Other nondestructive microscopic techniques include epifluorescence microscopy using fluorogenic dyes (e.g., acridine orange or 6-diamidino-2-phenylindol known as DAPI), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and environmental SEM (ESEM) which allows the observation of the specimen in its own hydrated state.

Microelectrode probes give information on the physicochemical properties (pH, temperature, O_2, NH_4, NO_3, NO_2, H_2S, CH_4) of the biofilm microenvironment (Burlage, 1997). These microelectrodes have helped shed light on the distribution of nitrifiers and sulfate reducers along the depth of biofilms. Microelectrodes for biofilm study will be replaced in the future with fiber-optic microsensors (Beyenal et al., 2000).

Microbial activity within biofilms can also be assessed by measuring enzyme activity (e.g., esterase or dehydrogenase activity), reduction of tetrazolium salts such as cyanoditolyl tetrazolium chloride (CTC), 2-*p*-(iodophenyl)-3-(*p*-nitrophenyl)-5-tetrazolium chloride (INT), triphenyl tetrazolium chloride (TTC), or resorufin by biofilm microorganisms. Fluorescein diacetate (FDA) or carboxyfluorescein diacetate (CFDA) are used to measure esterase activity and the active fluorescent cells are counted under a fluorescent microscope. Cell viability can also be determined by the LIVE/DEAD BacLight kit, or special dyes which measure membrane integrity (e.g., SYBR Green propidium iodide). The fluorescence intensity can be determined via flow cytometry (Berney et al., 2007, 2008; Cerca et al., 2011).

Several techniques have been proposed for gaining information about biofilm formation and thickness. Some of the methods are (Broschat et al., 2005; Milferstedt et al., 2006; Nivens et al., 1995; Reipa et al., 2006):

- *Quartz crystal microbalance* (QCM). This nondestructive technique measures biomass changes on a quartz crystal. Both the quartz oscillator frequency (f) and resistance (R) are monitored during biofilm formation and the data are expressed as $\Delta R/\Delta f$ which reflects changes in the viscoelastic properties of the biofilms. QCM was used in the long-term monitoring of biofilm formation by *Pseudomonas cepacia* and *P. aeruginosa*.
- *Optical methods*: Early on, biofilm thickness was estimated by focusing a light microscope on the top and bottom layers of a biofilm. A more recent approach

consists of exposing the biofilm to a desktop scanner and then following with image analysis. The gray level of the images is well correlated with the biofilm biomass. A reflectance assay was considered to determine biofilm formation of 14 *Enterococcus* isolates on opaque and nonopaque surfaces

- *Crystal violet assay:* This method consists of staining dry biofilms with 0.1% crystal violet, washing with buffer to remove excess dye, releasing the dye from the biofilm with 95% ethanol and measuring the absorbance at 595 nm.

4.3 GROWH OF PATHOGENS AND OTHER MICROORGANISMS IN WDSs

Pathogen accumulation in biofilms of WDSs is an important public health issue as it may contribute to the spread of waterborne diseases. Pathogens enter the WDS through insufficient water treatment or through leaks. They grow in WDSs and colonize pipe inner walls, connections, tubercles, and dead ends. However, biofilm growth over surfaces is not continuous as noncolonized spots are observed. Bacterial accumulation preferably occurs in the colonized area and increases with biofilm age (Paris et al., 2009).

4.3.1 Earlier Studies on the Microbiology of Distribution Systems

The presence of a wide range of microorganisms (eubacteria, filamentous bacteria, actinomycetes, diatoms) was demonstrated in distribution pipes by SEM. Some of the bacteria were seen attached to the surfaces by means of extracellular fribrillar materials (Ridgway and Olson, 1981; Ridgway et al., 1981). Turbercles provide a high surface area for microbial growth and protect microorganisms from the lethal action of disinfectants. Turberculated cast iron pipe sections from the WDS in Columbus, Ohio, metropolitan area harbored high numbers of aerobic and anaerobic bacteria (e.g., sulfate reducing bacteria). Some samples contained up to 3.1×10^7 bacteria per gram of tubercle material (Tuovinen and Hsu, 1982). Several investigators have also reported the proliferation of iron and manganese bacteria that grow attached to pipe surfaces and, subsequently, cause a deterioration of water quality (e.g., pipe clogging and color problems). Attached iron bacteria such as *Gallionella* have *stalks* that are partially covered with iron hydroxide. This was confirmed by X-ray energy-dispersive microanalysis (Ridgway et al., 1981).

4.3.2 Opportunistic Bacterial Pathogens

As mentioned in Chapter 1, this group includes genera such as *Pseudomonas*, *Aeromonas*, *Klebsiella*, *Flavobacterium*, *Enterobacter*, *Citrobacter*, *Serratia*, *Acinetobacter, Proteus, Providencia, L. pneumophila, S. maltophilia*, and nontubercular mycobacteria (NTM) (Sobsey and Olson, 1983; van der Wielen and van der Kooij,

2013). They are of health concern to the young, the elderly, and immunocompromised consumers. The routine occurrence of these opportunistic pathogens in distribution systems have been documented in the literature (Keevil, 2002). For example, most *Aeromonas* isolates from 13 Swedish drinking WDSs produced virulence factors such as cytotoxins, suggesting potential public health problems (Kuhn et al., 1997). *Sphingomonas* spp. can also be found in water distribution biofilms. They are often overlooked due to their slower growth than other members of the HPC (Koskinen et al., 2000). They have been isolated from activated carbon and sand filters, and are potential reservoirs of antibiotic-resistant bacteria in drinking water (Vaz-Moreira et al., 2011). Biofilms can also be a potential reservoir for *H. pylori* which can survive in the viable but nonculturable (VBNC) state (Linke et al., 2010; Percival and Thomas, 2009). The VBNC state is a bacterial response to stress (e.g., nutritional stress, exposure to disinfectants and other toxicants such as heavy metals). A wide range of bacteria are now known to enter in the VBNC state when exposed to environmental stress (Oliver, 2005). Biofilms may also harbor antibiotic-resistant bacteria (Schwartz et al., 2003; see also Chapter 1).

4.3.3 Nontubercular Mycobacteria (NTM)

The genus *Mycobacterium* includes pathogenic species such as *M. tuberculosum* and *M. leprae*, and NTM which are found in soil and aquatic environments and are considered as opportunistic pathogens (Falkinham, 2002; Falkinham et al., 2001). NTM were detected around the world in 21% to more than 80% of samples from WDSs (see review by Vaerewijck et al., 2005). A more recent survey in two chloraminated WDSs in Florida and Virginia showed that the percent occurrence of *Mycobacterium* spp. was 93.7% and 94.4%, respectively. The percent occurrence of *M. avium* was 10% and 8.9%, respectively (Wang et al., 2012b). In Germany and France, mycobacterial species were found in 90% of biofilm samples taken from water treatment plants (Schulze-Robbecke et al., 1992). A survey of seven water treatment plants in the Netherlands showed that NTM were detected in all distributed drinking water samples and their levels were much higher in distributed water than in treated water (van der Wielen and van der Kooij, 2013). Approximately one-third of the 100 known mycobacterial species have been detected in WDSs. In the Netherlands, several mycobacterial species (*M. peregrinum, M. salmoniphilum, M. llatzerense, and M. septicum*) were identified in distributed water and in shower water (van Ingen et al., 2010). The primary *Mycobacterium* species detected in biofilms of WDSs in China (Guangzhou and Beijing) were *M. arupense* and *M. gordonae* (Liu et al., 2012a). *Mycobacterium avium* subsp. *paratuberculosis* (MAP), a possible causative agent of Crohn's autoimmune disease in humans, was detected, via PCR, in finished drinking water (Aboagye and Rowe, 2011; Beumer et al., 2010). This opportunistic pathogen persists for several weeks in biofilms and its survival is much higher than that of *E. coli* (Lehtola et al., 2007; Torvinen et al., 2007). The growth of *M. avium* in WDSs depends on the level of available organic carbon (Falkinham et al., 2001; LeChevallier, 2004).

4.3.4 *Legionella* in Hot-Water Tanks and Distribution Systems

Due to airborne transmission through showering, much work has been carried out on the survival and growth of Legionellae in potable WDSs and plumbing in hospitals and homes (Colbourne et al., 1988; Felfoldi et al., 2010; Muraca et al., 1988). A recent survey showed that the percent occurrence of *Legionella* spp. was 67.5% in a Florida WDS and 30% in a Virginia system (Wang et al., 2012b). Furthermore, legionellae appear to be more resistant to chlorine than *E. coli* (States et al., 1989), and small numbers may survive in distribution systems that have been judged to be microbiologically safe. Under high shear turbulent-flow conditions, survival of *L. pneumophila* and *Mycobacterium avium* is much higher than that of *E. coli* which is traditionally used as an indicator (Lehtola et al., 2007). Legionellae may also grow to detectable levels inside hot-water tanks in hospitals and homes and thus pause a health threat (Stout et al., 1985; Witherell et al., 1988). The *Legionella* isolates responsible for nosocomial Legionnaires' disease corresponded to isolates from potable water (Garcia-Nunez et al., 2008). *Legionella* survives well at 50°C and may even grow and multiply in tap water at 32–42°C (Dennis et al., 1984; Yee and Wadowsky, 1982). The enhanced survival and growth in these systems have also been linked to stagnation (Ciesielski et al., 1984), stimulation by rubber fittings in the plumbing system (Colbourne et al., 1988), and trace concentrations of metals such as Fe, Zn, and K (States et al., 1985, 1989). It was found that sediments found in WDSs and tanks (i.e., scale and organic particulates) and the natural microflora significantly improved the survival of *Legionella pneumophila*. Sediments indirectly stimulate *Legionella* growth by promoting the growth of commensalistic microorganisms (Stout et al., 1985; Wadowsky and Yee, 1985). For example, *Mycobacterium chelonae*, an opportunistic bacterial pathogen, can have a positive effect on the cultivability of *L. pneumophila* and *H. pylori* in biofilms (Gião et al., 2011).

Protozoa, mostly amoebas and ciliates, play a role in the survival of *Legionella* in water distribution pipes. Biofilms and the presence of protozoa (e.g., *Acanthamoeba* spp.) play an essential role in the survival, proliferation, and pathogenicity of *Legionella* in drinking water (Corsaro et al., 2010; Lau and Ashbolt, 2009). Biofilms growing in in-premise plumbing harbor *Legionella* cells as well as protozoan hosts which help in the propagation of this pathogen. The biofilm-associated *Legionella* is detached and aerosolized during showering and can be subsequently inhaled.

Several methods have been considered for controlling *Legionella* in water: superchlorination (chlorine concentration of 2–6 mg/L), chloramination, maintaining the hot-water tanks at temperatures above shock treatment at 70°C for 30 minutes, ultraviolet irradiation (Antopol and Ellner, 1979; Knudson, 1985; Kool et al., 2000; Muraca et al., 1987; Stout et al., 1986), biocides (Fliermans and Harvey, 1984; Grace et al., 1981; Soracco and Pope, 1983), and alkaline treatment (States et al., 1987). There are less *Legionella* nosocomial infection outbreaks in hospitals that use monochloramine than those using free chlorine (Flannery et al., 2006; Heffelfinger et al., 2003; Kool et al., 1999). *Legionella* can, however, become heat-resistant after repeated heat treatments (Allegra et al., 2011). The control of legionellae in potable water systems

may also be achieved via the control of protozoa which may harbor these pathogens (States et al., 1990).

4.3.5 Antibiotic-Resistant Bacteria and Antibiotic Resistance Genes

Antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARG) to several antibiotics (amoxilline, chloramphenicol, ciprofloxacin, gentamicin, rifampin, sulfisoxasole, tetracycline) were found in several finished and tap water samples. The ARB levels were often higher in tap water than in finished water, indicating that WDSs can serve as a reservoir for ARB and ARG (Xi et al., 2009). Antibiotic resistance genes have been detected in biofilms from drinking WDSs. Enterobacterial *amp*C β-lactam-resistance and enterococcal *van*A vancomycin-resistance genes were detected in drinking water biofilms although no enterobacteriaceae or enterococci were detected in those samples. However, the Staphylococcal *mec*A methicillin-resistance gene was not found in drinking water biofilms (Schwartz et al., 2003).

Several studies have shown that chlorination of water and wastewater induces selection for ARB (Murray et al., 1984; Shrivastava et al., 2004). The mechanism of this selection is not well known.

However, at the present time, we do not know if increased resistance to antibiotic present a significant risk to consumers, especially the young, the elderly, and immunocompromised people.

Table 4.2 lists some human pathogens and opportunistic pathogens detected in biofilms of domestic plumbing systems (Eboigbodin et al., 2008).

4.3.6 Protozoan Parasites

Biofilms can also serve as reservoirs for protozoan parasites cysts and oocysts. The trapping and concentration of *C. parvum* oocysts and their survival in biofilms was demonstrated under laboratory conditions (Rodgers and Keevil, 1995). Free-living

TABLE 4.2 Some human pathogens detected in biofilms from domestic plumbing systems

Pathogen	Comments
Legionella pneumophila	Causes Legionnaires' disease
Legionella bozemanii	Causes human pneumonia
Helicobacter pylori	Causes gastric diseases including cancer
Pseudomonas sp.	Some species (e.g., *P. aeruginosa, P. maltophilia*) are human pathogens
Sphingomonas sp.	Some species cause nosocomial infections in humans
Micrococcus kristinae	Found in catheter-associated bacteremia
Corynebacterium sp.	Part of human skin flora. Some species are pathogenic
Acanthamoeba keratitis	Can cause infection in the cornea

Source: Adapted from Eboigbodin et al. (2008). Amer. Water Works Assoc. J. 100 (10): 131–137.

pathogenic protozoa, such as *Naegleria fowleri*, may also find refuge in biofilms, particularly in stagnant areas of the distribution network. There is experimental evidence that *N. fowleri* can colonize and persist for months in pipe biofilms, although none was detected in two full-scale distribution networks (Biyela et al., 2012).

The cysts and oocysts can subsequently be resuspended in WDSs by biofilm sloughing following an increase in water flow (Helmi et al., 2008; Puzon et al., 2009; Searcy et al., 2006).

4.3.7 Enteric Viruses

Enteric viruses are occasionally detected in treated drinking water (Bitton et al., 1986). Laboratory studies have shown that, following their entry into the WDS, viruses may adsorb to biofilms where they may survive for relatively long periods. A similar phenomenon was observed in wastewater systems where viruses (noroviruses, enteroviruses, FRNA phages) were found to attach and persist in biofilms for longer periods than in wastewater (Skraber et al., 2009). However, only one of three field studies of virus monitoring in biofilms showed the presence of infectious enteroviruses (Vanden Bossche, 1994). Similarly to other pathogens, pathogenic viruses may contaminate drinking water through sloughing of biofilm pieces.

4.4 SOME ADVANTAGES AND DISADVANTAGES OF BIOFILMS IN DRINKING WATER TREATMENT AND DISTRIBUTION

The development of biofilms on surfaces can be beneficial or detrimental to processes in water and wastewater treatment plants. Trickling filters and rotating biological contactors are examples of processes that rely on microbial activity in biofilms to treat wastewater.

4.4.1 Advantages

Some advantages offered by biofilms are (Rittmann, 1995b; Shrout and Nerenberg, 2012)

- The fact that anytime a solid surface is exposed to water is followed by biofilm formation demonstrates well the benefits of biofilms to microorganisms.
- Biofilms act as biocatalysts in natural systems for the self-purification of surface waters and groundwater, and in engineered fixed-film processes (e.g., trickling filters, rotating biological contactors, biofilters in water purification, riverbank filtration, membrane-based biofilm reactors) for the treatment of water and wastewater.
- They are desirable whenever microorganisms must be retained (i.e., need for a high mean cell residence time) in a system with a short hydraulic retention time,

as is the case for biological treatment of drinking water which involves the processing of large volumes of water with very low concentrations of biodegradable compounds.

- Many of the processes used in drinking water treatment allow biofilm formation and include, among others, granular activated carbon, slow and rapid sand filters, and biological water treatment.

4.4.2 Disadvantages

However, biofilms can cause problems in water treatment and distribution systems (Rittmann, 1995b, 2004; Smith, 2002; van der Wende and Characklis, 1990):

- Because of mass-transport resistance, bacteria are exposed to lower concentrations of substrates than in the bulk liquid.
- Biofilm accumulation increases fluid frictional resistance in distribution pipelines (Figure 4.6; Bryers and Characklis, 1981), leading to an increase in pressure drop and to reduced water flow if the pressure drop is held constant. The microbial community structure of the biofilm also affects frictional resistance. A predominantly filamentous biofilm appears to increase frictional resistance (Trulear and Characklis, 1982).
- Biofouling decreases flow rates in membrane-based water treatment processes. The biofouling is due to the overproduction of EPSs mainly composed of polysaccharides.

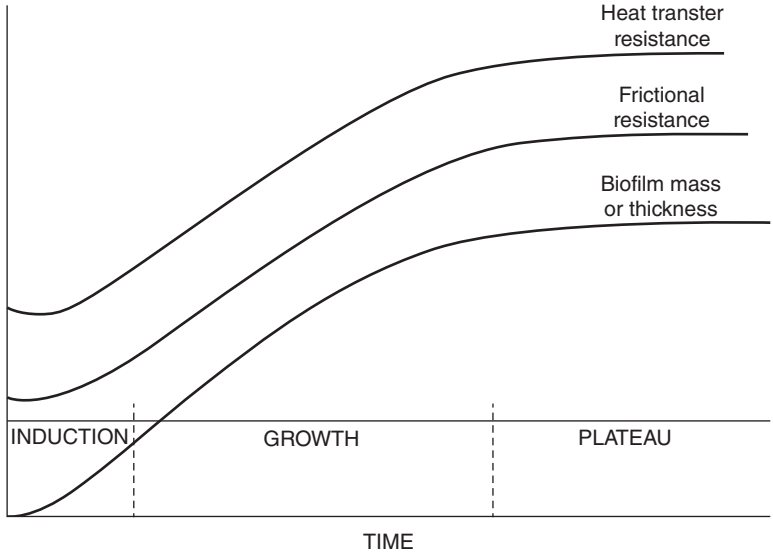

Figure 4.6 Increase in heat transfer resistance and frictional resistance resulting from biofilm growth. *Source:* Bryers and Characklis (1981). Water Res. 15: 483–491.

- Anaerobic conditions lead to the production of H_2S, a toxic gas characterized by a rotten-egg odor.
- Biofilms have an impact on public health and are the cause of persistent infections (Costerton, 1999). They are conducive to the accumulation of pathogens and parasites in distribution pipes biofilms which are habitats for opportunistic pathogens such as *Legionella* and *Mycobacterium* and lead to infections related to implants and dental plaques. Biofilms are involved in 80% of chronic inflammatory and infectious diseases due to pathogenic bacteria (Sauer et al., 2007). Biofilms developing on implanted medical devices harbors microbial pathogens such as *Candida albicans*, *Staphylococcus aureus*, *P. aeruginosa*, *Kelbsiella pneumoniae*, and *Enterococcus* spp., and *E. coli*. Most of the circulatory and urinary tract infections are due to biofouled implanted devices. These infections increase patient morbidity and mortality and the cost of medical care. Biofilms associated with medical implants are often resistant to antibacterial and antifungal drugs (Anwar and Costerton, 1992; Donlan, 2002; Thomas et al., 2004).
- Corrosion problems: Biocorrosion of pipes is associated with biofilm growth. Biofilm accumulation is associated with corrosion of iron pipes where Fe^{2*} serves as an electron donor. Complaints about red and black waters, due to the activity of iron- and manganese-oxidizing bacteria (e.g., *Gallionella*, *Hyphomicrobium*) (Rittmann, 2004).
- Resistance of biofilm microorganisms to biocides: Biofilm microorganisms can be up to 1500 times more resistant to biocides and antibiotics than freely suspended bacteria (Sauer et al., 2007). Increase in resistance to chlorine disinfection contributes to the regrowth of indicator and pathogenic bacteria in distribution systems. It was suggested that this increased resistance may be due to diffusional resistance of the biofilm matrix as measured with a chlorine microelectrode (de Beer et al., 1994), the protective effect by extracellular polymeric materials, the presence of efflux pumps that export biocides, antibiotics, and other toxic substances to the bacterial surrounding medium (Marquez, 2005), selection of biofilm microorganisms with enhanced resistance to the disinfectant, attachment of bacteria to biological (e.g., macroinvertebrates and algal surfaces) and nonbiological surfaces (particles, activated carbon) or cell surface hydrophobicity (Steed and Falkinham, 2006). Furthermore, multispecies biofilms appear to be more resistant to disinfection than single-species ones (Simoes et al., 2010). As shown for activated carbon, biofilm bacteria appear to be more resistant to residual chlorine and monochloramine than suspended bacteria (Berry et al., 2009; LeChevallier et al., 1988). The protective effect of biofilms toward pathogens exposed to chlorine was also demonstrated for *Mycobacterium avium* and *Mycobacterium intracellulare* (Steed and Falkinham, 2006). Particulates, including particulate organic matter, protect pathogenic microorganisms from disinfectant action (Hoff, 1978). It was found that *Enterobacter cloacae*, in the presence of particulates from WDSs, is protected from chlorine action as a result of its attachment to the particles (Herson et al., 1987). Chloramines appear to

be more efficient in biofilm control than free chlorine (HOCl or OCl^-) (see Chapter 3). This may be due to the lower affinity of chloramines for bacterial polysaccharides (LeChevallier et al., 1990; van der Wende and Characklis, 1990). It was proposed that, for biofilm control, free chlorine should be used as a primary disinfectant but the residual should be converted to chloramine (LeChevallier et al., 1990). The increased resistance of biofilm microorganisms to chlorine applies to other antibacterial agents such as antibiotics. The free chlorine residual decreases as the water flows through the distribution system. In a laboratory study, silver (100 μg/L) used as a biocide did not have any significant effect on preventing biofilm formation on PVC and stainless steel surfaces (Sylvestry-Rodriguez et al., 2008).

4.5 BIOFILM CONTROL AND PREVENTION

Biofilms can be controlled or removed by mechanical, chemical, or enzymatic means (Brisou, 1995). The drinking water industry has a few options for controlling biofilm growth in WDSs (Camper et al., 2003; Rittmann, 2004):

- Mechanical cleaning: It is combined with the use of biocides when the biofouling is severe.
- Maintain a proper level (1 mg/L as Cl_2) of chlorine residual throughout the WDS to reduce biofilm accumulation. The chlorine level cannot exceed 4 mg/L as Cl_2. Maintaining a chlorine residual is, however, difficult to achieve in large systems and cannot prevent biofilm growth in the presence of high levels of electron donors.
- Obtain a biostable water by removing or drastically reducing the level of organic and inorganic electron donors in treated water. This can be accomplished by using membrane filtration, enhanced coagulation, or biological filtration (see Chapter 6).
- Consider the effect of the pipe material: Replace deteriorating iron pipes with other materials (e.g., PVC or CPVC pipes). However, one should be concerned about the leaching and accumulation of the monomer, vinyl chloride, a proven human carcinogen (Walter et al., 2011).
- Potential "jamming" of quorum sensing in biofilms (Barraud et al., 2009; Hentzer et al., 2004; Shrout and Nerenberg, 2012): Attempts to interrupt quorum sensing in biofilms are potentially a useful strategy in the control of infectious diseases. As a result of the emergence of antibiotic resistance in bacteria, there is presently a quest for quorum sensing inhibitors to attenuate and control bacterial infections (Hentzer et al., 2003). Laboratory studies have shown that several chemicals can control biofilms by interrupting quorum sensing. Some examples are 2,4-dinitrophenol, a furanone produced by red algae, nitric oxide to disperse single- or multi-species biofilms, or protoanemonin, a natural compound which inhibits quorum sensing in *P. aeruginosa*. The application of this research

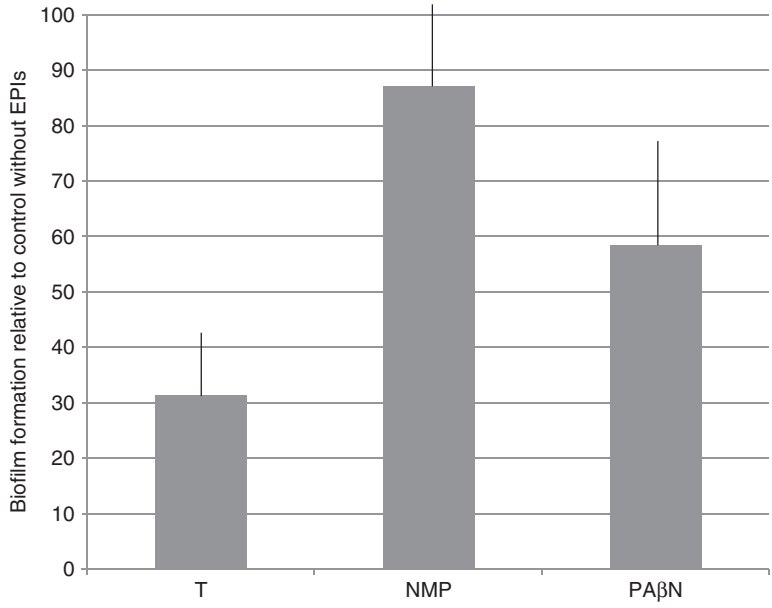

Figure 4.7 Effect of efflux pump inhibitors (T, NMP and PAβN) on biofilm formation in *Staphylococcus aureus*. T = Thioridazine; NMP = 1-naphthylmethyl piperazine; PaβN = Phe-Arg-β Naphthylamide. Adapted from Kvist et al. (2008). Appl. Environ. Microbiol. 74: 7376–7382.

to WDSs under field conditions awaits further investigation. Quorum sensing interruption by chemicals could be used in combination with other control measures to improve biofilm control. Some microorganisms make endogenous nitric oxide (NO) which is generated by NO-synthase under the control of the *nos* gene. The presence of NO helped decrease the ability of *P. putida* to form biofilms (Bobadilla Fazzini et al., 2013; Liu et al., 2012b; Njoroge and Sperandio, 2009). There are also quorum enzymes or bacteria that release enzymes that inhibit signaling in biofilms, leading to control of biofouling in membrane bioreactors (Jiang et al., 2013; Kim et al., 2013b). As an example, a recombinant *E. coli* that produces *N*-acyl homoserine lactonase, inhibited biofouling in a membrane bioreactor (Oh et al., 2012; Yeon et al., 2009a, 2009b). The feasibility of this approach for controlling biofilms is yet to be demonstrated.

- As regard the formation of biofilms on medical devices, great efforts are being made to prevent their formation and to develop drugs to treat existing biofilms. One original control method is to block quorum-sensing pathways in order to prevent the formation of biofilms on medical devices. Bacteria also have several chromosome-encoded efflux pumps that help export antibiotics, antiseptics, heavy metals, solvents, detergents, and other biocides to the surrounding medium (Martinez et al., 2009). These efflux pumps would lead to increased resistance of biofilms to toxic chemicals, including disinfectants. The

addition of efflux pumps inhibitors (e.g., thioridazine, phe-arg β-naphthylamide, 1-naphthylmethyl piperazine) helped reduce biofilm formation by *Staphylococcus aureus* (Figure 4.7; Kvist et al., 2008). A wide range of chemicals are being screened by biotechnological companies to find a way to control biofilms. Some of these chemicals are the halogenated furanones produced by marine algae (Schachter, 2003).

- Antibiofilm polysaccharides: Some bacteria are able to produce antibiofilm polysaccharides which can inhibit biofilm formation or destabilize preformed biofilms. They potentially have useful applications in industrial and medical fields and can help reduce the incidence of infections caused by biofilms on medical devices (Rendueles et al., 2013).
- Other biofilm control approaches are surface modification or application of an electric current (Chiang et al., 2009; Wellman et al., 1996).

WEB RESOURCES

http://www.personal.psu.edu/faculty/j/e/jel5/biofilms/
(biofilm online manual, Amer. Soc. Microbiology)

http://www.personal.psu.edu/faculty/j/e/jel5/biofilms/primer.html
(biofilm primer)

http://www.erc.montana.edu/
(Center for Biofilm Engineering, Montana State University, Bozeman)

http://www.nesc.wvu.edu/ndwc/articles/OT/SP01/History_Distribution.html
(history of drinking water distribution)

http://www.nottingham.ac.uk/quorum/
(general information about quorum sensing)

http://www.mrc-lmb.cam.ac.uk/genomes/awuster/wecb/
(gives many references on quorum sensing)

http://www.epa.gov/safewater/dwh/index.html
(Drinking water: EPA consumer information)

http://www.epa.gov/safewater/sdwa/index.html
(SDWA: Safe Drinking Water Act)

http://www.epa.gov/safewater/standards.html
(General information on drinking water)

http://www.cdc.gov/safewater/
(Safe Water System, Center for Disease Control and Prevention)

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