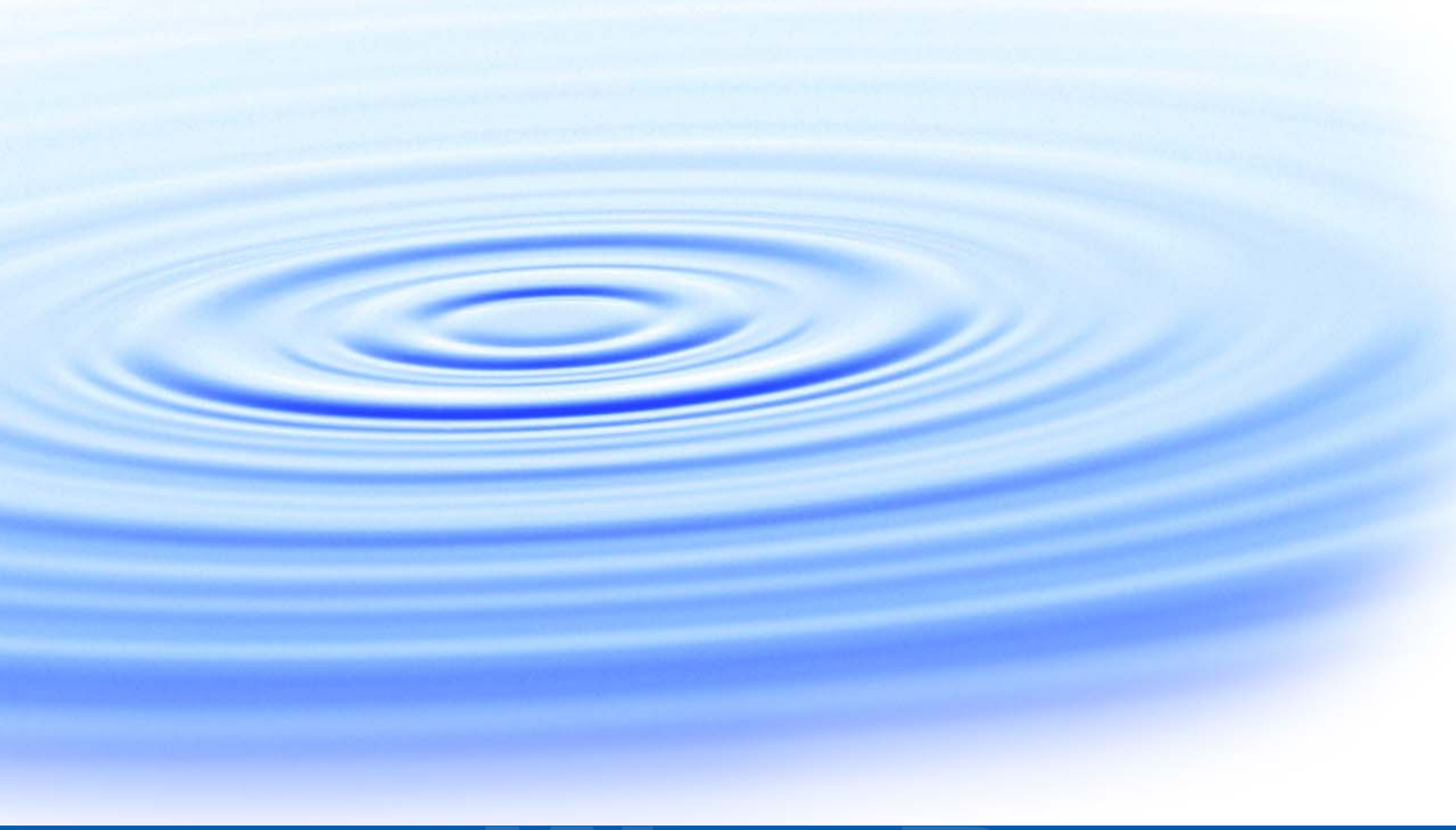
Guidance Document on the Microbiological Quality and Biostability of Reclaimed Water Following Storage and Distribution



WateReuse Foundation

Guidance Document on the Microbiological Quality and Biostability of Reclaimed Water Following Storage and Distribution

About the WateReuse Foundation

The mission of the WateReuse Foundation is to conduct and promote applied research on the reclamation, recycling, reuse, and desalination of water. The Foundation's research advances the science of water reuse and supports communities across the United States and abroad in their efforts to create new sources of high quality water through reclamation, recycling, reuse, and desalination while protecting public health and the environment.

The Foundation sponsors research on all aspects of water reuse, including emerging chemical contaminants, microbiological agents, treatment technologies, salinity management and desalination, public perception and acceptance, economics, and marketing. The Foundation's research informs the public of the safety of reclaimed water and provides water professionals with the tools and knowledge to meet their commitment of increasing reliability and quality.

The Foundation's funding partners include the Bureau of Reclamation, the California State Water Resources Control Board, the Southwest Florida Water Management District, the California Energy Commission, and the California Department of Water Resources. Funding is also provided by the Foundation's Subscribers, water and wastewater agencies, and other interested organizations.

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CONTENTS

TABLES

FIGURES

FOREWORD

The WateReuse Foundation, a nonprofit corporation, sponsors research that advances the science of water reclamation, recycling, reuse, and desalination. The Foundation funds projects that meet the water reuse and desalination research needs of water and wastewater agencies and the public. The goal of the Foundation's research is to ensure that water reuse and desalination projects provide high-quality water, protect public health, and improve the environment.

A Research Plan guides the Foundation's research program. Under the plan, a research agenda of high-priority topics is maintained. The agenda is developed in cooperation with the water reuse and desalination communities including water professionals, academics, and Foundation Subscribers. The Foundation's research focuses on a broad range of water reuse research topics including:

- Definition and addressing of emerging contaminants;
- Public perceptions of the benefits and risks of water reuse;
- Management practices related to indirect potable reuse;
- Groundwater recharge and aquifer storage and recovery;
- Evaluation and methods for managing salinity and desalination; and
- Economics and marketing of water reuse.

The Research Plan outlines the role of the Foundation's Research Advisory Committee (RAC), Project Advisory Committees (PACs), and Foundation staff. The RAC sets priorities, recommends projects for funding, and provides advice and recommendations on the Foundation's research agenda and other related efforts. PACs are convened for each project and provide technical review and oversight. The Foundation's RAC and PACs consist of experts in their fields and provide the Foundation with an independent review, which ensures the credibility of the Foundation's research results. The Foundation's Project Managers facilitate the efforts of the RAC and PACs and provide overall management of projects.

The Foundation's primary funding partners include the Bureau of Reclamation, California State Water Resources Control Board, the Southwest Florida Water Management District, the California Energy Commission, Foundation Subscribers, water and wastewater agencies, and other interested organizations. The Foundation leverages its financial and intellectual capital through these partnerships and funding relationships.

While much attention to reclaimed water has focused on the quality of the water at the treatment plant, that quality can degrade by the time it gets to the point of use. Therefore, a comprehensive understanding of the physical, chemical, and biological factors that affect the microbial quality of reclaimed water within distribution systems is necessary. This report documents changes in water quality in reclaimed distribution systems and provides approaches to minimize deterioration and improve product quality.

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Several utility and wastewater company members across the United States provided us access to their facilities and/or helped us in various ways, including setting up and maintaining the pipe loops and supplying us with operational details of their plants. They include Nelson Alvarez, Charles Barr, J. R. Bauer, Roger Boltrushek, Steve Boynton, Jim Bricker, Michael R. Buchanan, William M. Castle, David W. Choate, Paul D. Cornejo, Ray De Ocampo, Jamie Ginja, Darrell J. Graham, Ronnie H. Graves, Doug Griffith, Michael T. Hamman, Michael Helton, Amy Hertzfield, Robert J. Hitchcock, Ronald Huffman, Jim Huntington, Aaron Kraft, David Kudray, Edward J. Kwityn, Kurt LeGlue, Rich Lutz, Jeff Marlow, Jeff Marshall, William L. McGinty, Greg McGrady, Richard Meloy, Karl Merbach, Wayne M. Nichols, Bobbi Nissen, Roger Parr, Bob Peacock, Thomas Peterson, Efraín Rodríguez, Roger Rowe, Ygnasio Samarripa, Paul Schaffman, Jim Schilling, Don Shields, Kevin Slein, William A. Stewart, John Tekula, Aram Varjabedian, Frank Vogl, Raymond Webb, Karl Weber, Jim Young, and Andre Zinkevich. To all of these individuals, we are truly grateful and acknowledge that our tasks would not have been accomplished without their help. Experimental design and planning were provided in consultation with Jeffrey Rosen of Clancy Environmental Consultants. This work was also funded by the utility subsidiaries of American Water, Voorhees, NJ.

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EXECUTIVE SUMMARY

Freshwater is becoming increasingly scarce as a result of increasing populations, changing precipitation patterns, and/or degradation of existing sources of water, making water reuse a necessity. While much attention to reclaimed water has focused on the quality of the water at the treatment plant, that quality can degrade by the time it gets to the point of use. Therefore, a comprehensive understanding of the physical, chemical, and biological factors that affect the microbial quality of reclaimed water within distribution systems is necessary. This report documents changes in water quality in reclaimed distribution systems and provides approaches to minimize deterioration and improve product quality.

Reclaimed water can be safely used for a variety of purposes: urban (restricted and unrestricted), agricultural (food versus nonfood crops), recreational (restricted versus unrestricted), industrial, environmental, groundwater recharge, and indirect or direct potable reuse. The level of treatment and the monitoring requirements vary by the type of application and by state. Reclaimed water has been used safely for decades. The integrity of the treatment processes is important because sewage and wastewater can contain a variety of pathogenic agents (namely, bacteria, viruses, protozoa, and helminths). In addition, changes in water quality can occur if pathogenic bacteria and indicator organisms subsequently grow in the reclaimed distribution system. Understanding the chemical and physical factors and operational parameters that contribute to bacterial growth in the systems will be central to devising strategies for control.

STUDY APPROACH

To study the chemical, physical, and operational parameters, an intensive yearlong study was conducted to examine the changes in microbial levels in four reclaimed water systems. These systems were located in California (CA), Florida (FL), Massachusetts (MA), and New York (NY) and represented different treatment processes, disinfection practices, storage conditions, and distribution system operations. The treatment technologies ranged from conventional activated sludge with tertiary sand filtration to five-stage Bardenpho with secondary filtration and two variations of membrane bioreactors (MBRs). The processes resulted in variations in organic carbon, nitrate, ammonia, and phosphorus, allowing the evaluation of each of these parameters on microbial growth in distribution systems. Samples were collected from the treated plant effluent, storage reservoir (either an enclosed tank or an open pond), and three points within the distribution system and were examined for a variety of pathogenic and indicator organisms. Samples were collected from each location on four consecutive days during four quarters to evaluate the impact of seasonal factors.

In addition to the full-scale studies, three pipe loop systems were installed at each of the four facilities to examine the effect of various disinfection practices. After a period for biofilm development, one loop was treated with free chlorine, another was treated with a preformed monochloramine residual, and the third loop remained as a control. The loops were operated at various flow rates to simulate detention times and shear stresses in the distribution system.

A new bioluminescence assimilable organic carbon (AOC) test was utilized to study the effect of biodegradable organic matter on bacterial growth in reclaimed water. The

bioluminescence AOC test was faster and easier than the conventional AOC test and permitted insights into changes in the nature of the biodegradable organic carbon (BDOC) as it travelled through the reclaimed systems. A survey of 21 wastewater plants was conducted to examine the impact of various treatment technologies on the levels of BDOC in the treated effluents.

SUMMARY OF RESULTS

The results of the study showed that there were multiple factors that influenced the microbial quality of water in reclaimed distribution systems:

1. In the FL system, different zones of the reclaimed system were operated on alternate days, and the entire system was shut down on Mondays. The occurrence of indicator bacteria, including *Escherichia coli*, and opportunistic pathogens was highest in this system, suggesting that the stagnation of the water and depressurization of the pipeline had a negative impact on microbial quality.

2. The presence of open finished water storage reservoirs also negatively impacted microbial quality in the distribution system. The open reservoirs promoted algal growth, increased BDOC levels, dissipated disinfectant residuals, and contributed to increased bacterial loading of the distribution system, possibly owing to birds roosting on the reservoir.

3. Accumulation of algal cells and particulate material in the distribution system resulted in increases in chlorophyll and turbidity at dead-end locations. The biodegradation of the algal cells could result in releases of AOC, and the sediments could provide habitats for bacterial growth.

4. High levels of biodegradable organic matter had a clear impact on the microbial quality of reclaimed water. BDOC levels averaged between 0.4 and 6.2 mg/L, and average AOC levels ranged between 150 and 1400 μg/L. In general, these levels were about 10 times higher than those found in drinking water systems.

5. High levels of nitrate, phosphate, and ammonia did not affect the occurrence of opportunistic pathogens or indicator bacteria as much as the parameters described above but could be important once levels of biodegradable organic matter were reduced. Detection of sulfide and nitrite levels in some systems suggested that anoxic conditions existed that could result in objectionable odors and discolored water.

6. When a disinfectant residual was maintained in the distribution system, it was effective in controlling microbial occurrence. In the pipe loop studies, free chlorine was more effective than chloramines for heterotrophic plate count (HPC) and *Legionella* control under the conditions studied, but full-scale results could favor chloramines. Additional full-scale studies are needed.

Because of the influence of biodegradable organic matter on reclaimed water, the study paid particular attention to this parameter. The rapid bioluminescence method for AOC was successful in measuring the biostability of reclaimed water and showed that AOC was utilized first. An analysis of 21 wastewater treatment plants showed plant effluent BDOC levels were not dependent upon the treatment technology (Figure Ex-1), suggesting that it may be possible to optimize treatment operations to enhance BDOC removal. The survey showed that 100% of the MBR systems, 58% of the activated sludge, and 25% of the sequencing batch reactor systems had AOC levels lower than the median AOC of 450 µg/L.

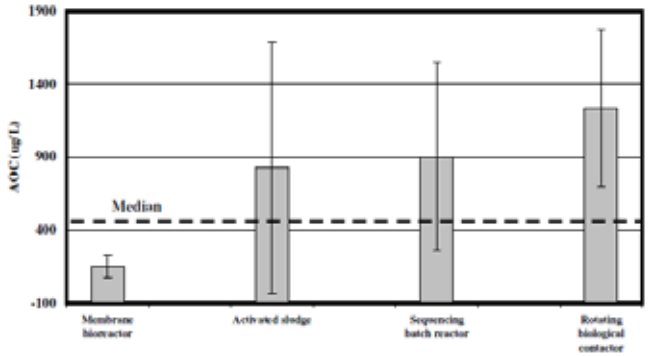


Figure Ex-1. AOC levels in the plant effluent of different wastewater technologies.

Regrowth of microorganisms was especially prevalent in high-AOC systems that lacked a disinfectant residual. High levels of organic carbon, combined with open finished water reservoirs, resulted in rapid depletion of residual disinfectants. However, the pipe loop studies showed that chlorination of reclaimed water typically increased AOC and BDOC levels. It is ironic, therefore, that certain processes to inactivate microbes in plant effluents can also promote bacterial growth in distribution systems. MBR systems with UV disinfection of plant effluent water generally produced the lowest AOC of the 21 wastewater plants surveyed.

The conventional and MBR wastewater treatment systems were generally effective in removing/inactivating microbial pathogens in treated effluents, but regrowth occurred in the distribution systems following a dissipation of the disinfectant residual. However, increased concentrations and frequency of occurrence were observed in reclaimed water systems for nearly all of the microbes monitored (HPC, total coliforms, *E. coli*, *Pseudomonas*, *Aeromonas*, Enterococci, *Legionella*, and *Mycobacterium*). Water temperatures affected the microbiology of reclaimed water, but seasonal changes were apparent in only some systems. *E. coli* O157:H7 was detected only two times in the plant effluent of one conventional system but never showed evidence of regrowth in the distribution system. The absence of common indicator bacteria (total coliform and *E. coli*), however, did not preclude the presence of potentially pathogenic organisms. *Legionella* spp. and *Mycobacterium* spp. were commonly detected in reclaimed water systems (Table Ex-1) and could have public health significance, especially if a disinfectant residual is not maintained.

Table Ex-1. Occurrence of *Mycobacterium* spp. and *Legionella* spp. in reclaimed water[a]

Site	Effluent	Storage	DS1[b]	DS2	DS3
Mycobacterium spp. (CFU/100 mL)					
CA	1 ± 1	5 ± 17	22 ± 15	35 ± 46	30 ± 120
FL	11 ± 20	65 ± 220	55 ± 390	73 ± 600	107 ± 800
MA	170 ± 190	2 ± 1[c]	57 ± 25	320 ± 130	120 ± 80
NY	6 ± 15	50 ± 80	42 ± 110	16 ± 14	31 ± 29
Legionella spp. (10^3 CFU/100 mL)					
CA	<0.3	2.2 ± 4.0	2.3 ± 7.1	0.9 ± 1.5	1.9 ± 3.8
FL	<0.3	3.0 ± 70	2.7 ± 13	3.5 ± 16	8 ± 52
MA	0.4 ± 0.1	<0.3[c]	1.3 ± 2.8	0.7 ± 2.0	0.4 ± 0.7
NY	0.6 ± 2.1	0.7 ± 0.6	0.5 ± 0.6	0.5 ± 0.6	0.5 ± 0.4

[a]Values are geometric means (±SE) based on aggregate densities over the yearlong monitoring.
[b]DS = distribution system.
[c]Disinfection point is at the storage tank for this location.

RECOMMENDATIONS

To minimize deterioration in water quality, seven remedial practices are identified as practical guidance to help administrators of reclaimed water systems manage their distribution systems, notably:

(i) To the extent possible, maintain constant flow in reclaimed distribution systems, avoiding water stagnation and intrusion of untreated groundwater during periods of depressurization.

(ii) Where open storage is practiced, attention should be paid to algal control through reservoir destratification, nutrient (phosphorus and nitrogen) control, chemical treatment, or installation of fine-mesh screens to control entry of the algae and cyanobacteria into the distribution system.

(iii) Avoid accumulation of sediment and debris in reclaimed distribution systems by routinely flushing the network using scouring velocities and practicing unidirectional flushing.

(iv) Evaluate treatment strategies that could improve removal of BDOC, including operation at a longer solid retention time, implementation of biologically activated carbon filtration, application of membrane filtration, or other innovative techniques.

(v) Posttreatment disinfection with UV radiation as this process typically does not lead to an increase in AOC or BDOC. However, the costs associated with UV radiation have to be considered as part of the treatment strategy.

(vi) Because chloramines are more stable and likely to persist longer in reclaimed distribution systems, consider maintaining a monochloramine residual but being careful to minimize any remaining free ammonia that could cause nitrification.

(vii) Consider installing disinfectant booster stations to maintain a residual disinfectant at all points within the distribution system. It would be especially important to disinfect after storage in an open storage reservoirs, since residuals are dissipated in these open ponds.

The project also identified areas for future research:

(i) Examine how to optimize conventional wastewater treatment for improved removal of BDOC and develop design and operational criteria when various treatment processes are used to produce reclaimed water.

(ii) Additional research is suggested to examine the risks from *Legionella* and *Mycobacterium* spp. in reclaimed water. Future studies should evaluate the specific species and serotypes prevalent in reclaimed water. Where possible, virulence determinants and the interaction of these organisms with amoebae, which could increase their public health significance, should be examined.

(iii) The infectivity of *Giardia* cysts, *Cryptosporidium* oocysts, and enteric viruses should be addressed. Ongoing studies are examining the infectivity of cysts and oocysts in reclaimed water, but additional attention should be directed toward risks of enteric viruses.

(iv) This study did not examine the hydraulics of reclaimed distribution systems (it wasn't needed to observe the changes in water quality). However, future studies should examine the impacts of system hydraulics on degradation in water quality.

(v) Because improved operation of reclaimed distribution systems would be the fastest, lowest-cost mechanism to improve water quality, a project to develop best operating practices for reclaimed water distribution system management should be initiated.

CHAPTER 1

INTRODUCTION AND BACKGROUND

Water is the most important natural resource and is quite essential for all life processes. Only a small percentage of the global water supply is considered to be fresh. The WHO estimates that only about one-third of the world's freshwater can serve human needs and that with increasing pollution that amount constantly decreases (WHO, 2001). Freshwater is becoming increasingly scarce in several countries because of increasing populations, changing precipitation patterns, and/or degradation of existing sources of water, making the reuse of water a necessity. Implicit in these observations is the need to reclaim water to alleviate some of the shortages. Thus, reclaiming water for reuse has increasingly become a common practice. Reuse is the deliberate treatment of water for beneficial use, without letting the self-purification process occur naturally through the conventional global hydrologic cycle. The WHO has recently published updates about the use of wastewater, excreta, and grey water for aquaculture and agriculture with the primary aim of maximizing public health protection and the beneficial use of water as a valuable resource (WHO, 2006a and 2006b). Both of these WHO documents emphasize that the choices made in deciding to use reclaimed water for agricultural purposes are not just a simple tradeoff but rather a complex process that defines the risks. They also highlight efforts to design measures to minimize those risks. If properly planned, water reuse projects can have a positive impact on the environment by:

- preventing the pollution of surface water by ensuring effluents with low nutrient content and low levels of microorganisms;
- conserving freshwater, especially in arid and semi-arid areas;
- reducing the need for artificial fertilizers and their associated pollution problems; and
- reducing the high energy demand that is associated with producing those fertilizers.

By definition, reclaimed water refers to effluents that have undergone a combination of physical, chemical, and biological treatments to remove suspended solids, dissolved solids, organic matter nutrients, metals, and pathogens. To date, reclaimed water has rarely been directly used as a source of drinking (namely, potable) water but has increasingly been used for other domestic purposes, such as watering of lawns, laundry, boiler feed in industrial settings, cooling towers, street sweeping, commercial dye houses, and even toilets and urinals. Other uses include irrigation of pasture, arable fields, and golf courses as well as other landscaping water needs, aquaculture, window and vehicle washing, construction (for example, concrete mixing), furnishing of groundwater recharge and supplementation of river flow needs, fire protection, construction material wetting, suppression of dust, and decorative fountains (Narasimhan et al., 2005; Karim and LeChevallier, 2005). Of all these uses, irrigation is the most predominant usage of reclaimed water. In terms of nutrients, reclaimed water is deemed superior to potable water for irrigation purposes.

Florida (FL) prides itself on more than 40 years of using reclaimed water without any documented disease that is associated with the water (FDEP, 2003). A report by Crook (2005) assembled data about the use of reclaimed water at 1600 park, school yard, and playground sites in the United States. The report indicates that reusing water for this purpose under reasonable standards did not present any increase in known health risks to those who frequent those sites from the risks associated with irrigating with potable water. Most of the attention has been focused on the quality of the reclaimed water as it leaves the treatment

facility. However, there is increasing concern about the quality of the water at the point of use. Even in cases where the finished effluent has been certified free of detectable bacteria, some organisms may be detected further along the distribution system. Regrowth has been highlighted as the likeliest source of such organisms in the distribution system. The U.S. Environmental Protection Agency (USEPA) has released several papers that highlight the potential health risks that are associated with distribution system issues, including intrusion, aging infrastructure and the associated corrosion, cross-connection control, decay in water quality over time, repaired water mains, permeation and leaching of materials into the distribution system, and the regrowth/growth of bacteria and biofilms. Thus, treatment plants have to maintain programs that are aimed at controlling bacterial regrowth in distribution systems. However, the factors that control regrowth seem to be numerous and range from the residual disinfectant concentrations, temperature, carbon content (namely, total organic carbon [TOC], dissolved organic carbon [DOC], assimilable organic carbon [AOC], biodegradable organic carbon [BDOC], etc.), operational characteristics (for example, number of storage tanks and pipeline length), turbidity, corrosion levels, dissolved O_2, etc. (LeChevallier et al., 1996). The importance of each of these factors in relation to reclaimed water will be closely examined in this review. This review is part of a larger study aimed at generating a comprehensive understanding of the physical, chemical, and biological factors that affect the microbial quality of reclaimed water within distribution systems. It is the goal of this review to discuss the effects of the quality of source water and how the treatment processes that reclaimed water undergoes affect its biostability. Thus, it provides information about how the quality of reuse water is affected in the distribution system over time.

Within the distribution system, the water can undergo both chemical and biological transformations or encounter changes in the integrity of the distribution system. Thus, the microbiological quality of reclaimed water can change during storage and/or during passage through the distribution system. The parameters that drive the re-emergence and regrowth of pathogens in reclaimed water in storage or distribution systems have not been clearly elucidated. Factors such as temperature, availability of nutrients, concentrations of the residual disinfectant, and the quality of the influent into the distribution system affect biostability and microbial density as well as diversity in distribution systems. These factors are critically examined with recognition of the fact that data on the microbiological status of reclaimed water are generally less extensive than are those on potable water. Thus, where relevant information that is available is based on potable water distribution systems, it will be used to the extent that parallel applicability to reclaimed water allows or is expected or with an effort to highlight its relevance to reclaimed water. A very clear distinction between potable water and reclaimed water is post-treatment quality. The final product of the former after treatment is mandated to meet very stringent quality standards in terms of known pathogens and indicator organisms. In contrast, reclaimed water can, depending on the treatment process, meet more than 95% of the drinking water standards but is allowed to have varied microbiological quality that ultimately depends on its intended use.

CHAPTER 2

PATHOGENS IN WASTEWATER

Human feces may contain microbial constituents as high as 10 to 30% (by weight) and are one of the major sources of infectious agents found in municipal wastewater (Talaro and Talaro, 1999). The four major groups of pathogenic microorganisms found in domestic wastewater are bacteria, viruses, protozoa, and helminths (Bitton, 1994). Many types of bacteria that colonize the human intestinal tract are harmless and are routinely shed in the feces. In addition, pathogenic bacteria, such as *Salmonella*, *Shigella*, *Escherichia coli*, and *Vibrio*, are present in the feces of infected individuals. *E. coli* is a natural inhabitant of the intestinal tract of warm-blooded animals. Its presence in water indicates fecal contamination and signifies the possible presence of enteric pathogens. Finding it in reclaimed water represents the potential risk of gastrointestinal illness associated with the use of such water for operations that are likely to bring humans into close contact with such contaminated water. Other types of bacteria are opportunistic pathogens typically occurring as commensals in healthy individuals but causing diseases in the weak such as young, elderly, and immunocompromised individuals. Such bacteria include *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, and *Legionella*. *P. aeruginosa* is a Gram-negative, aerobic rod belonging to the bacterial family *Pseudomonadaceae.* These bacteria are common inhabitants of soil and water and may cause disease in susceptible humans. *A. hydrophila* is present in all freshwater environments. Some strains are capable of causing illness in fish and amphibians as well as in humans who may acquire infections through open wounds or by ingestion of this organism in food or water. Legionellae are Gram-negative bacteria (≈1 to 3 μm) found in freshwater (Fields et al., 2002) and wastewater (Samadpour, 2003) and cause respiratory diseases in humans when a susceptible host inhales aerosolized water containing the bacteria or aspirates water containing the bacteria (Fields et al., 2002; O'Loughlin et al., 2007). *E. coli* O157:H7 (≈2 μm) is a frank pathogen, which causes abdominal pains, watery diarrhea leading to bloody diarrhea, and low-grade fever. Frank pathogens are those that cause disease in the general population and immunocompromised individuals (Reynolds, 2006). Various strains of *E. coli* O157:H7 can produce Shiga toxin 1 (stx1) and/or Shiga toxin 2 (stx2). Several waterborne outbreaks of *E. coli* O157:H7 have been reported (Hrudey et al., 2003; Swerdlow et al., 1992). A list of pathogens that may potentially be present in untreated wastewater is provided in Table 2.1. Many of the bacterial pathogens are enteric in origin; however, bacterial pathogens that cause nonenteric illness (for example, *Legionella*) have also been detected in wastewaters (Fliermans, 1996).

Viruses that replicate in the intestinal tract of humans are referred to as human enteric viruses. More than 140 different enteric viruses are known to infect man. These viruses are excreted in high numbers, 10^{10} to 10^{12} per g of feces of infected individuals (Flewett, 1983), and are found in large numbers in raw wastewater.

Enteric viruses include enteroviruses, rotaviruses, noroviruses (NVs), hepatitis A virus (HAV), adenoviruses, reoviruses, and others. Enteroviruses are icosahedral viruses approximately 27 to 32 nm in diameter. The genome of these viruses consists of a single strand of RNA. These viruses pose a public health risk because they can be transmitted via the fecal-oral route through contaminated water and because even a single virus particle is capable of initiating an infection in humans. These viruses are capable of causing a wide

range of illnesses, including gastroenteritis, paralysis, aseptic meningitis, herpangia, respiratory illness, fevers, myocarditis, etc.

Table 2.1. Infectious Agents Potentially Present in Untreated (Raw) Municipal Wastewater[a]

Pathogen	Disease
Bacteria	
A. hydrophila	Diarrhea
Campylobacter jejuni	Gastroenteritis, reactive arthritis
E. coli (enteropathogenic)	Gastroenteritis and septicemia
Legionella pneumophila	Legionnaires' disease
Leptospira (spp.)	Leptospirosis
Salmonella typhi	Typhoid fever
Salmonella (2400 serotypes)	Salmonellosis
Shigella (4 spp.)	Shigellosis (dysentery)
Vibrio cholerae	Cholera
Yersinia enterocolitica	Yersiniosis, gastroenteritis, diarrhea, long-term sequelae
Viruses	
Adenovirus (51 types)	Respiratory disease, eye infections, gastroenteritis
Astrovirus (5 types)	Gastroenteritis
Calicivirus (2 types)	Gastroenteritis
Coronavirus	Gastroenteritis
Enteroviruses	Gastroenteritis, heart anomalies, meningitis
HAV	Infectious hepatitis
NV	Diarrhea, vomiting, fever
Parvovirus	Gastroenteritis
Poliovirus	Poliomyelitis
Reovirus	Not clearly established
Rotavirus	Gastroenteritis
Protozoa	
Balantidium coli	Balantisiasis (dysentery)
Cyclospora	Cyclosporidiosis, persistent diarrhea, fever
Cryptosporidium	Cryptosporidiosis, diarrhea
Entamoeba histolytica	Amebiasis
Giardia	Giardiasis
Microsporidia	Diarrhea
Helminths	
Ancylostoma duodenale (hookworm)	Ancylostomiasis
Ascaris lumbricoides (roundworm)	Ascariasis (digestive/nutritional disorders)
Echinococcus granulosis (tapeworm)	Hydatidosis
Enterobius vermicularis (pinworm)	Enterobiasis
Faciola hepatica	Enterobiasis
Necator americanus (roundworm)	Necatoriasis
Schistosoma spp.	Schistosomiasis
Taenia spp.	Taeniasis, cysticercosis
Trichuris trichiura (whipworm)	Trichuriasis

[a]Compiled from Jjemba (2004), Crook (2005), and WHO (2006b).

Noroviruses are the most common cause of nonbacterial gastroenteritis in humans. The genome of NVs possesses a positive-sense, single-stranded RNA of approximately 7.6 to 7.7 kb in length and is composed of three open reading frames (Jiang et al., 1993). The Centers for Disease Control and Prevention determined that NVs account for 93% of reported outbreaks of nonbacterial gastroenteritis in the United States (Fankhauser et al., 2002).

Rotaviruses are the major cause of infantile gastroenteritis throughout the world. Rotavirus is an icosahedral virus about 70 nm in diameter and belongs to the family *Reoviridae*. The genome of the virus consists of 11 segments of double-stranded RNA surrounded by a distinctive double capsid (Midthun and Kapikian, 1996). In the United States, approximately 2.7 million children under 5 years of age contract rotavirus diarrhea annually, leading to 500,000 physician visits and 50,000 hospitalizations (Parashar et al., 1998). In developing countries, it is associated with over 870,000 deaths/year in children under 5 years old (de Zoysa and Feachem, 1985). Recent surveys have shown 3 to 45% of the human population seropositive to rotaviruses in some geographic locations (Teixeira et al., 1998). These viruses are transmitted by the fecal-oral route. Viruses in wastewater vary widely, depending on the detection method used. Sobsey et al. (1995) conservatively estimate an average of 7000 viral particles/L of wastewater. The presence of enteric viruses in reuse water is of particular concern because of their low (<10) infectious dosages (Haas et al., 1999; Murray et al., 2001). Enteric viruses cannot divide and increase in abundance in the absence of their host. In practical terms, this property means that their abundance can only remain stable or even decline in the open environment (including wastewater and reclaimed water) rather than increase, depending on the prevailing conditions.

HAV is an important waterborne virus because of the severity of the disease it may cause in susceptible individuals. The virus is about 27 nm in diameter and contains a single-stranded RNA genome. HAV is the cause of acute infectious hepatitis and has been shown to survive and remain infectious for more than 3 months at both 5 ^{o}C and 25 ^{o}C in water, wastewater, and sediments (Sobsey et al., 1988). HAV is a major cause of acute gastroenteritis, and its symptoms may be the most serious of those caused by the enteric viruses. It has been classified into seven different genotypes, which in humans include genotypes I, II, III, and VII, and in simian genotypes IV, V, and VI (Hussain et al., 2006). HAV infection follows a benign course that is often asymptomatic in children but can develop into acute hepatitis in adults.

Parasites are present in the feces of infected persons. However, they may also be excreted by healthy carriers. Just like viruses, parasites can not multiply in the environment as they require a host to reproduce and are excreted in the feces as environmentally resistant spores, cysts, oocysts, or eggs. G*iardia* and *Cryptosporidium* are protozoan parasites that have emerged as a significant health risk in chlorinated drinking water (USEPA, 2004).

Giardia lamblia is a flagellated protozoan parasite that causes giardiasis (Adam, 2001). The organism causes diarrhea, abdominal pains, nausea, fatigue, and weight loss (Bitton, 1994). *Giardia* is the most commonly isolated intestinal parasite in the world (Gardner and Hill, 2001). *Giardia* exists in two different forms: the environmentally resistant stage cyst and trophozoites. Humans become infected by ingesting the cyst. An infected person may shed up to 1×10^6 to 5×10^6 cysts per g of feces (Jakubowski and Hoff, 1979; Lin, 1985). Once in the environment, the cysts can remain infectious for long periods of time under favorable environmental conditions.

Cryptosporidium parvum is responsible for infections in both humans and animals (Current, 1987; Rose, 1990). The organism causes a profuse and watery diarrhea that is often associated with weight loss and sometimes with nausea, vomiting, and fever (Current, 1987). The duration and the symptoms depend on the immunological status of the host. It can be persistent and potentially fatal in immunocompromised patients. The infective stage of this protozoan parasite is the oocyst, a stage that is very resistant to adverse environmental conditions. Once ingested, the oocyst undergoes excystation and releases infective sporozoites. *Cryptosporidium* has a complex life cycle consisting of both asexual and sexual stages, but it is important that all those stages occur in the host but not in the open environment. Both *Giardia* and *Cryptosporidium* have demonstrated the capability to be transmitted to humans from domestic or wild animals or from other humans by a variety of routes, including water (Current, 1987; Wolfe, 1992).

Numerous outbreaks of giardiasis and cryptosporidiosis have been documented (Moore et al., 1993; Kramer et al., 1996; Herwaldt et al., 1992; MacKenzie et al., 1994). *Giardia* and *Cryptosporidium* are present in high numbers in domestic wastewater and are of particular concern owing to their resistance to disinfectants commonly used in wastewater treatment (Rose et al., 2004; Gennaccaro et al., 2003; Quintero-Betancourt et al., 2003).

Many helminthic parasites occur in wastewater. Examples include the roundworm *Ascaris* as well as other nematodes such as hookworms and pinworms. Depending on the species, the infective stage of helminths could be adult organism, egg, larvae, or ova. The eggs and larvae are resistant to environmental stress and may survive usual wastewater disinfection procedures. Table 2.2 presents the typical concentrations of pathogens found in wastewater; however, the prevalence and concentrations of pathogens in wastewater vary with the health of a community that is served by a wastewater collection system (Rose et al., 2004).

If not properly treated, reclaimed water can thus pose a health risk, especially if it is used for recreational and/or potable purposes. However, very few studies have looked at the proliferation of pathogens in reclaimed water (Rose et al., 1996; Jolis et al., 1999). The most stringent restrictions on reclaimed water use are for unrestricted use on crops that are consumed without processing.

Table 2.2. Concentration of Pathogens Found in Domestic Wastewater[a]

Organism	Concn (CFU/, PFU/, or Cysts/Oocysts per mL)
Fecal coliform	10^4–10^5
Fecal streptococci	10^3–10^4
Enterococci	10^2–10^3
Clostridium perfringens	10^1–10^3
Enteric viruses	10^1–10^2
Giardia cysts	10^{-1}–10^2
Cryptosporidium oocysts	10^{-1}–10^2
Helminth ova	10^{-1}–10^2

[a]Source: Maier et al., 2000.

In most instances, pathogen content restrictions are addressed only in terms of fecal coliform (a popular set of indicators of contamination). Nutrient (namely, ammonia, nitrate, and phosphorus) limits are given only a brief mention, if at all, with no clear acceptable limits highlighted. The only exception to this general observation is FL but only with regard to monitoring *Giardia* and *Cryptosporidium* pathogens in irrigation water.

CHAPTER 3

WASTEWATER TREATMENT PROCESSES

Wastewater treatment systems were first developed in response to the adverse conditions caused by the discharge of raw effluents to water bodies. After municipal wastewater has been collected through a network of mains and pump stations, it flows to a treatment plant, where it is treated to reclaim the water for reuse or release into a receiving body of water. Wastewater treatment is accomplished through a series of physical, biological, and chemical processes, which gradually remove suspended solids, organic compounds, pathogens, and nutrients from the water. Physical components include preliminary treatment and primary treatment to remove organic (and inorganic) solids to protect the treatment plant equipment. Removal of debris at this early stage is effected by screens of prescribed sizes and by settlement. As part of the primary treatment, some solid debris is also allowed to settle in settlement tanks and the removal (by scraping off) of scum and fats that occur as a result of the soaps and oils used in routine personal care. It is estimated that primary treatment removes about 35% of the biochemical oxygen demand (BOD) and 60% of the suspended solids in wastewater (Samadpour, 2003). Thus, contrary to common belief, some level of biological treatment also occurs during this treatment phase, the most notable of which includes the settlement of suspended solids with their associated microbial particles and attached biofilms (Jjemba, 2004). This section briefly describes the most common steps used in wastewater treatment processes. A wastewater treatment system typically includes the components as outlined in the flow diagram underneath (Figure 3.1).

3.1. PRELIMINARY TREATMENT

Preliminary treatment is the process of removing many organic solids from the flow and protecting the works from inorganic solids such as sand and grit. There are commonly two other processes associated with preliminary treatment, storm separation and flow balancing, both of which have an important effect on hydraulic operation of treatment plants.

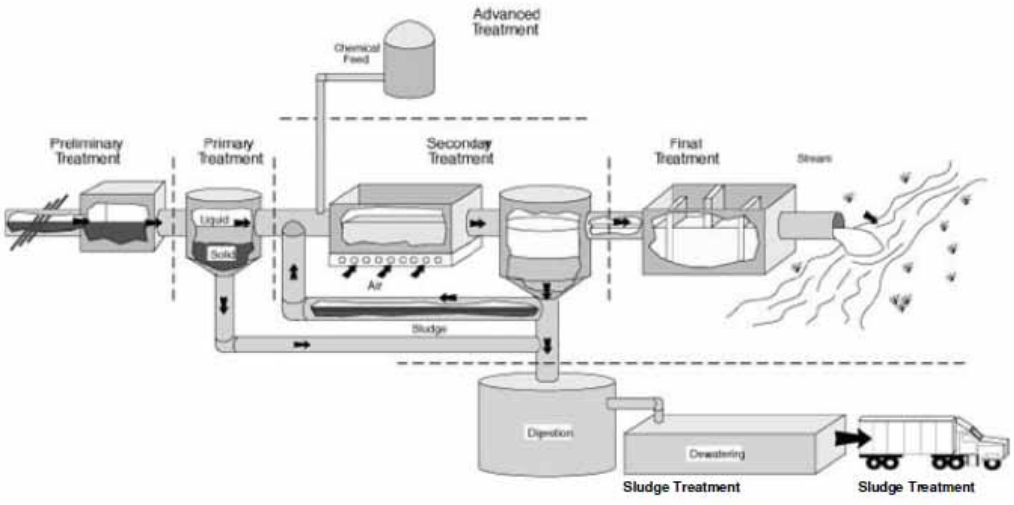


Figure 3.1. Typical components of wastewater treatment system.

Storm water separation is, in some instances, carried out (as opposed to combined sewer systems) after the inlet screening process. This process involves diverting high flows to storm tanks where solid wastes can be settled before being returned either to the works in the event of a short storm or being discharged back to the receiving body of water in the case of a large storm. Flow balancing is adopted where flows to the sites are pumped; hence, the flow arrives at the works in surges. The balancing tank acts to remove the surges and is particularly essential in smaller sewage treatment works.

3.2. SCREENING

The wastewater entering the plant must be screened to remove large objects (roots, rags, glass, rocks, and other large debris) by screening the water through a grate or bar screen. The screens consist of vertical steel bars spaced to catch debris of a certain size. Fine screens remove additional debris from the wastewater stream. Mechanized rakes continuously scrape the screens to remove the debris and deposit the material into hoppers that press the liquid from the material. The screenings are then disposed of as solid waste, their removal greatly reducing the volume of materials to be treated.

3.3. GRIT REMOVAL

Grit consists of sandy materials and other particulates that readily settle from the wastewater. Although some grit may be discharged to the sewer system by users, most grit is washed into the system along with groundwater infiltration. Since grit is inorganic, it cannot be removed in the biological treatment processes. If it is not removed prior to biological treatment, it accumulates in the process units, particularly the sludge digesters, and tends to cause excessive wear on the equipment. The grit is allowed to settle in a grit tank by slowing the velocity of the wastewater flow to approximately 1 ft/s. The inorganic grit settles at this velocity, but the organic material requiring further treatment does not. The grit is removed from the tanks and washed to remove residual organic material. Just as in the screenings, the grit is disposed of as solid waste.

3.4. PRIMARY TREATMENT

The next stage of treatment takes place in the primary sedimentation tanks (Figure 3.1). Primary treatment is the process of settling large particulate material from the flow under gravity, leaving soluble or colloidal material in the wastewater (known as settled sewage). The process is carried out in primary settlement tanks, which can be rectangular or circular. There is normally a deeper section in the tank where the settled particulates are collected and compacted down to form sludge. The tank floors are scraped by using traveling bridge scrapers to move sludge toward the deeper section of the tank. For most wastewater treatment plants, the sludge is automatically pumped away from the primary settlement tanks at regular intervals for further treatment. Primary treatment also involves skimming off surface scum and fats. Scum and fats arise from the soaps used in the bathroom and from the oils and fats that are put down the drain in the kitchen. The primary sedimentation process typically removes around 35% of the BOD and 60% of the suspended solids from the wastewater (Samadpour, 2003).

Figure 3.2. Primary settlement tank.

3.5. SECONDARY TREATMENT

Secondary treatment is the process of removing by biological reaction soluble and colloidal material found in the flow from primary treatment. Most of the biological treatment processes occur during the secondary treatment phase. Most secondary treatment systems are aerobic, although some systems are anaerobic. Some systems alternate between aerobic and anaerobic. The majority of secondary treatment plants are aerobic (anaerobic processes are more commonly used for the treatment of high-strength industrial wastes), although some stages of the more advanced processes can have an anaerobic stage. Percolating filters are primarily a form of packed solid medium such as stones, porous rocks, or plastic material designed to provide for the attachment of microorganisms to support growth as the wastewater flows evenly over the surfaces, supporting the biofilm. Even distribution of the waterway over the percolating filter can be ensured by using a rotating distributor arm to continuously spray the material over the percolating filter. Maturity of the biofilm in such systems is enhanced as more and more microorganisms die off, leaving a percolating surface. The die-off is continuously replaced by new growth. Proper performance of this system requires periodically blowing air through the system as to ensure an adequate supply of oxygen, which in turn supports microbial activity. In essence, the process converts soluble pollutants that do not settle easily into solids that can be separated in settlement tanks. The soluble materials are a substrate for bacteria, becoming converted to biomass. There are two distinct types of secondary treatment, namely:

(i) the attached growth systems, such as percolating filters and rotating biological contactors (RBCs), and

(ii) suspended growth systems (namely, activated sludge).

3.5.1. Attached growth systems

All attached growth systems have a surface where microorganisms can attach and grow in biofilms. For example, in a percolating filter, porous rock is used for a medium. Biofilms will be discussed in detail in Section 6.7.2, but for the present discussion, the traditional and most common attached growth system is the trickling filter (Figure 3.3). This item comprises either a random packed (for example, stones) or structured (made from plastic) medium over which the settled sewage is evenly distributed, typically through use of rotating distributor arms. The air required by the process is induced by natural ventilation into the structure holding the medium. As the biofilm containing the microbial biomass increases, some of the

microorganisms will die, becoming deposited in the flow and leaving the percolating filter. This flow is then passed through a “humus” settlement tank, similar in design to a primary settlement tank, to separate the purified effluent from the dead microorganisms and any inert solids that might have come through the system.

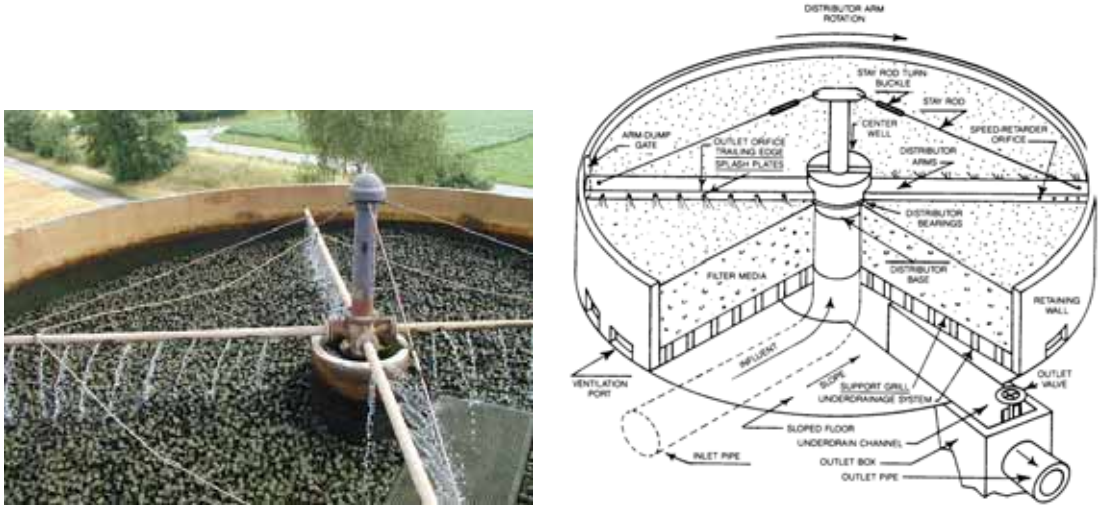


Figure 3.3. A typical trickling filter. The panel on the right is adapted from Natural Primary Drinking Water Regulations (2000).

A typical trickling filter design is shown in Figure 3.3’s right panel. The construction materials should provide a large specific surface area and good permeability to allow good growth of microorganisms within the biological filter. Ideal construction materials include plastic media (namely, bio-towers with the media in various configurations such as vertical flow, cross-flow, etc.), beds of rocks, polyurethane foam, sphagnum, peat moss, gravel, and slag. Some trickling filter systems are actually comprised of a mixture of layers with different combinations of these materials.

Most trickling filters also have a hydraulic or fixed-nozzle system combined with some form of aeration to meet the oxygen demands of aerobes. If well designed and maintained, trickling filters are quite reliable and provide high-quality effluents like those obtained in our survey. Proper operation and maintenance guidelines have been published by the USEPA (2000), and signs of system failure can be suspected if one detects disagreeable odors from the process (because of excessive organic loads that can cause anaerobic zones), ponding on the filter media (owing to excessive biological growth or interference from foreign material, namely, debris on the filter), icing owing to low temperatures, or mechanical strain (namely, slowing, stopping, clogging, etc.) of the distributors as they rotate.

Some attached growth systems are submerged in the effluent. In this case the medium can again be either randomly packed with materials such as sand particulate medium or structured, similar to that found in plastic medium percolating filters. These systems are often referred to as submerged aerated filters or biological aerated filters. The submerged aerated filters are usually of the structured medium type and are followed by a conventional humus settlement tank to effect effluent clarification, while biological aerated filters are random packed medium beds where the bed serves both a treatment and solid separation function. In all these systems, air must be blown into the tanks to provide dissolved oxygen for the bacteria.

An RBC is another example of an attached growth system. It consists of a series of closely spaced, parallel discs mounted on a rotating shaft that is supported just above the surface of the wastewater. The rotating mechanism aerates the wastewater, together with the attached microorganisms (in the form of a biofilm) facilitating the biological treatment process. The rotating packs of discs (known as the media) are rotated at a specific speed (namely, revolutions per minute). The degree of wastewater treatment is related to the extent of medium surface area, rotation speed, hydraulic loading organic loading, and temperature (Al-Ahmady, 2005). Research by Al-Ahmady (2005) shows that rate at which the carbon in the wastewater is removed by the RBC increases with increasing organic loading.

3.5.2. Activated sludge process

The activated sludge process is where microorganisms in suspension break down pollutants such as BOD, ammonia, and phosphorus in the incoming wastewater. The process is carried out in two tanks: an aerated tank, sometimes called “lanes” because of its channel- like geometry (Figure 3.4), and a final settlement tank. Final settlement tanks separate the treated effluent from the existing microorganisms, with the latter settling to form sludge. Some of that sludge is pumped back to the inlet of the activated sludge process. If the microorganism concentration in the aerated tank is too high, then some of the sludge is removed from the system.

Figure 3.4. An activated sludge aeration lane.

The activated sludge system is an ideal example of a suspended growth system as it serves the purpose of decreasing the BOD and reduces inorganic compounds such as ammonia and phosphorus. This process is similarly enhanced by aeration, namely, by actively pumping air through the system. However, removal of ammonia requires either ensuring the presence of anaerobic zones within the tank to enable denitrifiers to use nitrate as an electron donor (instead of oxygen) or, after holding the material for a certain duration, transferring it to another anoxic tank for nitrification to take place. There are many variations of the activated sludge process. The most important distinction is between activated sludge processes that treat nutrients, such as ammonia and phosphorus, and those that treat just carbonaceous material (BOD). Biological nutrient removal is briefly described in the next section.

3.5.3. Biological nutrient removal

An important variant of the activated sludge process is biological nutrient removal. Biological nutrient removal combines the biological removal of BOD, ammonia, and phosphorus in one process. The process is configured as follows:

- Anoxic zone: an unaerated zone where nitrate is removed through denitrification.
- Anaerobic zone: a further unaerated stage where bound cellular phosphate is released into the waste.
- Aerobic zone(s): a large aerated zone where ammonia is oxidized to nitrate and dissolved phosphorus is taken up into the sludge.

The process relies on the fact that more phosphorus is taken up in the aerobic stage than is released in the anaerobic stage. In some cases, part of the effluent from the aerobic stage is recycled to the anoxic stage to ensure complete denitrification of the waste.

Plants that treat ammonia as well as BOD are similar to BOD treatment plants except the waste is retained in the process for a longer duration. Ammonia removal is known as nitrification and involves ammonia converted to nitrate by nitrifying bacteria. In the absence of oxygen, denitrifying bacteria use this nitrate as a substitute for dissolved oxygen for respiration. This process is called denitrification, and the unaerated zone specifically included in the plant is called the "anoxic zone." On a site with no tertiary treatment (see "Tertiary Treatment" underneath), the overflow flow from the final settlement tanks is disinfected and then discharged to the receiving water.

Facultative lagoons (Figure 3.5) are a common form of aquatic treatment-lagoon technology currently in use. The water layer near the surface is aerobic, while the bottom layer, which includes sludge deposits, is anaerobic. The intermediate layer is aerobic near the top and anaerobic near the bottom and constitutes the facultative zone. Aerated lagoons are smaller and deeper than facultative lagoons. These systems evolved from stabilization ponds when aeration devices were added to counteract odors arising from septic conditions. The aeration devices can be mechanical or diffused air systems. The main disadvantage of lagoons is high effluent solid content, which can exceed 100 mg/L. As a means of counteracting this problem, hydrograph-controlled release lagoons are a recent innovation. In this system, wastewater is discharged only during periods when the stream flow is adequate to prevent water quality degradation. When stream conditions prohibit discharge, wastewater is accumulated in a storage lagoon.

The membrane bioreactor (MBR) is an activated sludge process that uses membranes instead of a traditional final settlement tank to separate the sludge from the effluent. Since the pore size of membranes is sufficiently small, the harmful microorganisms are kept within the activated sludge, resulting in a disinfected wastewater prior to discharge. Overall, MBR technology has been shown to substantially remove some nutrients, especially when combined with well-controlled recycling rates and sequencing of aerobic and anaerobic processes (Ahn et al., 2003; Holakoo et al., 2005) or if combined with reverse osmosis (RO) (Comerton et al., 2005). Whereas the biological nitrogen removal requires aerobic-to-anoxic stages, biological removal of phosphorus requires alternating anaerobic-to-aerobic stages (Holakoo et al., 2005). The removal of nitrogen is quite sensitive to the concentration of dissolved oxygen. A dissolved oxygen concentration of 0.5 to 1 mg/L tends to favor nitrification at the expense of ammonification, while below these dissolved oxygen levels, nitrification and ammonification are almost balanced (Holakoo et al., 2005). Whereas nutrient

removal studies in drinking and wastewater processes primarily focus on percent removals with various treatments, the residual concentration of nutrients in the effluent is of equal importance. Thus, Holakoo et al. (2005) removed 36 or 55% total nitrogen from wastewater using MBR technology with a hydraulic retention time of 6 or 4 h, respectively. However, these seemingly high removal rates still left total nitrogen concentrations as high as 19.1 or 17 mg/L, respectively. Even higher removals of phosphorus were attained (namely, >96%; Table 3.1) leaving seemingly low PO_4-P concentrations in the effluent. However, these concentrations are still far above the threshold for preventing bacterial growth. As a basis for comparison, low levels of 0.01 mg of PO_4-P/L and 0.2 mg of NO_3-N/L in drinking water in Raleigh were still nonlimiting to microbial regrowth (Zhang and DiGiano, 2002).

Figure 3.5. Lagoons for wastewater treatment.

Table 3.1. Nutrient Removal from Wastewater with an MBR Operated at Different Hydraulic Retention Times[a]

Nutrient	Retention Time	Nutrient Concn (mg/L)		% Removal
		Influent	Effluent	
Total N	6 h (1–20 days)	29.9	19.1	36.1
Total N	4 h (20–75 days)	30.8	13.8	55.2
PO_4-P	6 h (1–20 days)	5.9	0.1	98.3
Total N	4 h (20–75 days)	6.1	0.2	96.7

[a]Source: Holakoo et al., 2005.

3.6. TERTIARY TREATMENT

The concentration of microorganisms in reclaimed water increases with increasing turbidity, pH, and temperature. Depending on the level of treatment goal required, tertiary (or advanced) treatments are used to improve the physicochemical quality of secondary treatment effluents. Several processes, such as coagulation-flocculation-settling-sand filtration, nitrification and denitrification, carbon adsorption, ion exchange, and electrolysis, can be added to follow secondary treatment in order to obtain high-quality effluents.

The most common tertiary treatment is gravity sand filtration. These filters come in three main types: shallow bed, moving bed, and deep bed. Apart from the obvious differences in the depth of the filter media, the main distinguishing feature is the backwashing method. In

the shallow bed filter, backwashing is achieved by using a hood suspended from a traveling bridge. The filter media are arranged in cells (strips of media separated by using low plastic walls). The hood fits over each cell and final effluent is pumped through the cell being backwashed in the direction opposite to normal operation, with the dirty water being sucked by another pump into the hood, where it is discharged to a launder and returned to the head of the works. Each cell is backwashed in this way once per day. The moving bed type filters use a continuous airlift at the core of the filter to lift sand from the bottom to the top of the filter. Before the sand is returned to the top of the bed, the trapped particles are separated and removed in a waste stream, returning to the head of the works. Finally, deep bed type filters are similar in construction and operation to a rapid gravity filter used in drinking water treatment. Backwashing takes a filter out of service and is achieved by a combination of water and air washing.

3.7. DISINFECTION

After secondary (or, where conducted, tertiary) treatment, the treated water is disinfected in order to significantly reduce the density of microorganisms prior to discharge or reuse. Disinfection is typically achieved through:

(i) chemical treatment,
(ii) UV treatment, or
(iii) filtration (for example, membrane filtration, sand bed filters, etc.).

Disinfection at individual plants can involve all of the above three processes or various combinations of them. For any one treatment plant, the success of disinfection is directly related to the concentration of colloidal and particulate constituents in the wastewater (USEPA, 1998).

3.7.1. Chemical treatment

Disinfection by chemical addition is achieved in much the same way as in drinking water treatment. A strong oxidizing chemical such as chlorine (gas or in liquid form) or ozone (or other disinfectants) is mixed into the main wastewater stream followed by residence in a contacting tank or channel. This process allows time for the chemical(s) to react with the microorganisms and to inactivate pathogenic microbes.

The efficiency of disinfection is influenced by the concentration of the disinfectant, contact time, temperature, and pH. A clear understanding of disinfection kinetics is embedded in the relationship:

$$CT = [\text{concentration of the disinfectant} \times \text{contact time}] \qquad \text{Equation 1}$$

Temperature over the range that is appropriate for reclaimed water affects the rate of disinfection reactions according to the Arrhenius Law, under which the effects of pH largely depend on the disinfectant in solution. Thus, free chlorine increases the disinfection efficiency at lower pH, while chlorine dioxide is more effective at alkaline pH levels (LeChevallier and Au, 2002). Monochloramine is formed instantly in the pH range of 7 to 9 and in chlorine–to–NH_3-N ratios lower than 5:1 at 25 ^{o}C. To a lesser extent, they are also dependent on the temperature and contact time. These outcomes have important implications in reclaimed water since ammonia and TOC levels in such water would produce chloramines and organic chloramines.

Similarly, in chloraminated systems, ammonia is added to the water before, after, or simultaneously with chlorine, forming monochloramine or its derivatives, namely,

$NH_3 + HOCl \rightarrow NH_2Cl$ (monochloramine) $+ H_2O$ Equation 2
$NH_2Cl + HOCl \rightarrow NHCl_2$ (dichloramine) $+ H_2O$ Equation 3
$NHCl_2 + HOCl \rightarrow NCl_3$ (trichloramine) $+ H_2O$ Equation 4

These competing reactions are dependent upon pH and the relative chlorine:nitrogen ratio. Thus, at a pH of 7 to 8 with an equimolar chlorine:N ratio of ≤5:1, monochloramine will predominate. In all these instances the monochloramine formed is considered to be a weak disinfectant that may inactivate coliform bacteria but would not be effective on viruses and protozoa.

Chlorine is very reactive with various bacterial cellular components, upsetting metabolic balance, affecting the synthesis of proteins, and causing genetic defects by modifying pyrimidine and purine bases. However, some bacteria, particularly those that form spores (for example, *Clostridium* spp. and *Bacillus* spp.) and the acid-fast bacteria (for example, *Mycobacterium* spp.) are fairly resistant to disinfection. Similarly, cyst- and oocyst-forming microorganisms are less affected by chlorination. Maintaining a disinfectant residual in the distribution system is intended to impose conditions that are unfavorable to microorganisms. However, disinfectant residual by itself does not guarantee the total elimination of microorganisms in water.

Ozone in aqueous solutions may react with microbes by either direct reaction with molecular ozone or via indirect reaction with the radical species formed when ozone decomposes, although the exact mechanisms by which ozone causes the inactivation of microorganisms are not entirely clear. Ozone is known to attack unsaturated bonds that form aldehydes, ketones, or carbonyl compounds (Langlais et al., 1991). Additionally, ozone can participate in electrophilic reactions, particularly with aromatic compounds, or in nucleophilic reactions with many of the components of the microbial cell. Carbohydrates and fatty acids react only slightly with ozone, but amino acids, proteins, protein functional groups (for example, disulfide bonds), and nucleic acids all react very quickly with ozone (Langlais et al., 1991). It is likely, therefore, that microbes become inactivated through the reaction of ozone with the cytoplasmic membrane (because of the large number of functional proteins), the protein structure of the virus capsid, or destruction of nucleic acids.

Table 3.2. Summary of Ozone Disinfection Results for *Cryptosporidium*[a]

Ozone Concn (mg/L)	Contact Time (min)	CT Product (mg·min/L)	Temp (^{o}C)	% Inactivation
1	5	5	25	90–99
1	10	10	25	>99
0.77	6	4.6	"Room"	>99
0.51	8	4.1	"Room"	>99
0.16–1.3	5–15	7	7	99
0.17–1.9	5–15	3.5	22	99
2.4 (avg.)	2.3	5.5	22–25	99
1.25	15	18.75	10	98.6
1–5	10	10–50	5	18–39
1–5	10	10–50	20	70–>99

[a]Source: LeChevallier and Au (2002).

Ozone is effective for disinfection of *Cryptosporidium* (Table 3.2), *Giardia*, and other indicator organisms except heterotrophic plate count (HPC) bacteria (Wolfe et al., 1989). The inactivation of *G. lamblia* and *Naegleria gruberi* by ozone showed an initial latent phase and had an estimated CT_{99} (a CT for 99% inactivation) of 0.53 and 4.23 mg·min/L, respectively (LeChevallier and Au, 2002). Viruses are generally more resistant to ozone than are vegetative bacteria, although bacteriophage appear to be less resistant to this disinfectant than human viruses do (Langlais et al., 1991). Of the vegetative bacteria, *E. coli* is one of the most sensitive, while Gram-positive cocci (*Staphylococcus* and *Streptococcus*), the Gram-positive bacilli (*Bacillus*), and the mycobacteria are the most resistant (Langlais et al., 1991). *Mycobacterium avium* can be effectively controlled by low doses of ozone (a $CT_{99.9}$ of 0.1 to 0.2 mg·min/L), whereas the organism is highly resistant to free chlorine (a $CT_{99.9}$ of 551 to 1552 mg·min/L for water-grown isolates) (Taylor et al., 2000).

3.7.2. UV irradiation

UV light can be divided into UV-A, UV-B, UV-C, and Vacuum UV categories with wavelengths ranging from about 40 to 400 nm. The UV wavelength effective for inactivating microorganisms resides in the UV-B and UV-C ranges of the spectrum (200 to 310 nm), with maximum effectiveness around 265 nm. Thymine bases on the nucleic acids (DNA and RNA) are particularly reactive to UV light and form dimers (thymine-thymine double bonds) that inhibit transcription and replication of nucleic acids, thus rendering the organism sterile. Thymine dimers can be repaired, a process termed "photoreactivation" in the presence of light or "dark repair" if light is absent (Jagger, 1967). As a result of this repair phenomenon, the strategy in UV disinfection has been to provide a high enough dosage that enough nucleic acid damage occurs to prevent effective repair.

Table 3.3. Typical UV Dosages Required for 4 Log Units' Inactivation of Selected Microorganisms of Importance in Reclaimed Water[a]

Organism	Dose Range (mW-s/cm^2)	Water Source
Bacteria		
Bacillus subtilis spores	31	Lab water
E. coli	20	Lab water
Streptococcus faecalis		Lab water
Salmonella typhi	30	Lab water
Vibrio cholerae	0.65	Lab water
Virus		
MS-2	50	1 groundwater
	64–93	11 groundwater sources
	100	Lab water
Coxsackievirus AZ	30	Lab water
HAV	6–15	3 groundwater sources
	16	Lab water
Poliovirus	23–29	8 groundwater sources
	30	Lab water
Rotavirus—Wa	50	Lab water
Rotavirus SA11	40	Tap water

[a]Adapted from Malley (2002).

Normally the wastewater to be treated is passed through a channel that contains UV lamps. The effectiveness of a UV disinfection system depends on the characteristics of the wastewater, the intensity of UV radiation, the length of time the microorganisms are exposed to the radiation, and the reactor configuration. UV radiation is an effective disinfectant against bacteria and viruses, including coliphage, and the typical effective doses are shown in Table 3.3. UV disinfection is also effective against *Cryptosporidium* oocysts (Bukhari et al., 1999) and *Giardia* cysts (Craik et al., 2000) at doses that are effective against bacteria and viruses.

3.7.3. Disinfecting by filtration

Filtration as part of the treatment and disinfection process has been practiced for many years. It creates a barrier between the microorganisms and the effluent based on size exclusion. Several matrices are used as filtration barriers. They may range from very simple structures such as sand filters to granulated activated carbon (GAC) filters, membrane filters (MF), or ultrafilters (UF). All of these filtration systems rely on simple sieving to remove particles including protozoa, bacteria, viruses, total suspended solids (TSS), and turbidity. Some filtration systems are relatively inexpensive, whereas others such as membrane filtration and ultrafiltration come at a premium but also provide better removal of pathogens and other contaminants. GAC filters are also quite effective. UF can reject the particles to a greater extent than MF can. GAC filters, MF, and UF also have some charge that enables them to exclude more particles than sand bed filters can. The efficiency with which microorganisms are removed by MF and UF can be enhanced even more if they are operated in RO mode, although the high pressure that is required for this mode increases the cost.

3.7.3.1. Simple filtration systems

Constructed wetlands (Figure 3.6), aquacultural operations, and sand filters are generally the simplest and most widely used methods of polishing the treated wastewater effluent from secondary treatment processes. These systems have also been used with more traditional, engineered primary treatment technologies such as Imhoff tanks, septic tanks, and primary clarifiers. Their main advantage is to provide additional treatment beyond secondary treatment where required. In recent years, constructed wetlands have been utilized in two designs: systems using surface water flows and systems using subsurface flows. Both systems utilize the roots of plants to provide substrate for the growth of attached bacteria that utilize the nutrients present in the effluents and for the transfer of oxygen. Bacteria do the bulk of the work in these systems, although there is some nitrogen uptake by the plants. The surface water system most closely approximates a natural wetland. Typically, these systems are long, narrow basins, with depths of fewer than 2 ft, that are planted with aquatic vegetation such as bulrush (*Scirpus* spp.) or cattails (*Typha* spp.). The shallow groundwater systems use a gravel or sand medium, approximately 18 in. deep, which provides a rooting medium for the aquatic plants and through which the wastewater flows.

Two types of sand filters are commonly used: intermittent and recirculating. They differ mainly in the method of application of the wastewater. Intermittent filters are flooded with wastewater and then allowed to drain completely before the next application of wastewater. In contrast, recirculating filters use a pump to recirculate the effluent to the filter in a ratio of 3 to 5 parts filter effluent to 1 part raw wastewater. Both types of filters use a sand layer, 2 to 3 ft thick, underlaid by a collection system of perforated or open joint pipes enclosed within graded gravel. Water is treated biologically by the epiphytic flora associated with the sand and gravel particles, although some physical filtration of suspended solids by the sand grains and some chemical adsorption onto the surface of the sand grains play a role in the treatment process.

Figure 3.6. Constructed wetland.

3.7.3.2. MBR

The MBR process is a modification of the conventional activated sludge process where the clarifier is replaced by a membrane system for the separation between mixed liquor (mixed liquor is a combination of partially treated wastewater and activated sludge) and effluent (Figure 3.7). MBR technology has various advantages that originate from the use of a membrane, including smaller space and reactor requirements, better effluent water quality,

disinfection, increased volumetric loading, and less sludge production (Adham et al., 2001). High biomass concentration can be maintained in the bioreactor, allowing the system to treat high-strength wastewater and be very compact (Nagano et al., 1992; Knoblock et al., 1994). MBRs effectively overcome problems associated with poor settling of sludge in conventional activated sludge processes and permit bioreactor operation with considerably higher mixed liquor solid concentrations. An MBR is typically operated at a mixed liquor suspended solid concentration in the range of 10,000 to 15,000 mg/L, compared to 1000 to 4000 mg/L in a conventional treatment system (Adham and Trussell, 2001). Since MBRs can be operated at an elevated mixed liquor suspended solid concentration, extended solid retention times are easily attainable.

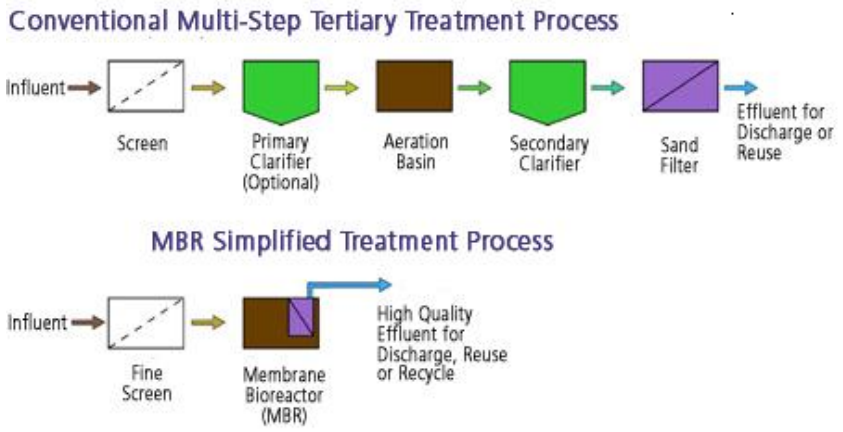


Figure 3.7. Comparison between conventional treatment and MBR processes.

The MBR process can exist in two different configurations, one with low-pressure membrane modules replacing the clarifier downstream of the bioreactor, and the second with the membranes submerged within the bioreactor (Adham et al., 2001). MBRs have been used for treating municipal wastewater, food industry wastewater, industrial wastewater, and landfill leachate and for denitrifying potable water (Delanghe et al., 1994).

Numerous pilot and full-scale studies have demonstrated the ability of MBRs to produce high-quality effluent water with excellent removal of organics and suspended solids (Adham and Trussell, 2001; Chiemchaisri et al., 1993; Cicek et al., 1998; Ueda et al., 1996). Adham and Trussell (2001) evaluated water quality data for two pilot-scale MBRs for 1 year and found that MBRs consistently and reliably produced high-quality water with an average turbidity of 0.1 NTU and a 5-day BOD (BOD_5) below detection level. The MBRs provided an average chemical oxygen demand (COD) and TOC removal of greater than 90 and 80%, respectively. An activated sludge process coupled with a hollow-fiber membrane for solid-liquid separation produced high-quality water with very low TOC and COD levels (TOC < 0.5 NTU and COD = 3 to 5 mg/L) (Chiemchaisri et al., 1993). In a study comparing conventional activated sludge treatment and an MBR process, the MBR system removed more nitrogen and phosphorus than the conventional treatment did (Bodzek et al., 1996).

With increasing interest in the use of reclaimed water for agricultural irrigation and industrial and other nonpotable applications, it is important to assess the effectiveness of the MBR treatment process for control of pathogens. The effectiveness of a bioreactor can be influenced by temperature, type of compounds, contact time, and protocol. Implicit in this observation is the likelihood that MBR performance will slightly vary across seasons,

particularly in regions that have weather extremes making comparison between winter and summer months, for example, absolutely necessary.

Several studies demonstrated the suitability of MBR effluent for direct feed to RO (Adham and Trussell, 2001; Comerton et al., 2005). Comerton et al. (2005) reported production of high-quality reuse water using an MBR-RO system, which provided complete removal (>5.3 logs) of coliphage and total coliforms in the effluent water. MBRs are also considered an effective, nonhazardous alternative to achieve pathogen control in wastewater effluents. Numerous studies have reported microbial reduction by MBRs (Shang et al., 2005; Churchouse and Brindle, 2002; Ueda and Horan, 2000; Chiemchaisri et al., 1993; Cicek et al., 1998; Ueda and Hata, 1999). Churchouse and Brindle (2002) have demonstrated that a full-scale MBR plant produced exceptionally high-quality effluent over years, including 3 to 6 log units' removal of fecal coliform bacteria and 2 to 5 log units' removal of F^+ coliphage. In a study comparing conventional treatment and MBR treatment, the MBR achieved 2 to 6 log units' removal of indigenous bacteriophage and up to 7 log units' removal of fecal coliforms and fecal streptococci, compared to only 2-log removal of the same phage and bacteria by the conventional treatment (Ueda and Horan, 2000). Adham and Trussell (2001) evaluated two pilot-scale MBRs for 1 year and found that the MBR systems were capable of removing greater than 5 log units of total and fecal coliforms and 4 or 5 log units of indigenous coliphage. By contrast, a full-scale tertiary conventional wastewater treatment that treated the same primary effluent removed only 2 log units of total coliforms, 3 log units of fecal coliforms, and 2 or 3 log units of coliphage. Cicek et al. (1998) reported that MBRs effectively removed heterotrophic bacteria and coliphage from wastewater, thereby eliminating the need for effluent disinfection. Most of the MBR membranes have an effective pore size of 0.01 μm to 0.4 μm, and the filtration process of MBRs would physically remove larger microorganisms such as bacteria (2 to 3 μm) and protozoan parasites (4 to 15 μm), but enteric viruses are much smaller (23 to 80 nm) and may pass through the membrane. Thus, the occurrence of viruses in the effluent even in plants where MBR technology is used may not be surprising.

Membrane configuration and pore size vary depending on the manufacturer of the unit. Babcock (2005) compared five different MBRs from five different manufacturers (Enviroquip, Ionics, Zenon, US Filter, and Huber). Each MBR employs somewhat different technologies, including membrane configuration and pore size (Enviroquip: flat panel membranes, 0.4-μm pore size, vertical arrangement in aeration tank, and air scour and relaxation; Ionics: microfiber membranes, 0.4-μm pore size, horizontal arrangement in aeration tank, and air scour and relaxation; Zenon: microfiber membranes, 0.04-μm pore size, vertical arrangement in aeration tank, air scour and relaxation, and backpulsing; US Filter: microfiber membranes, 0.4-μm pore size, vertical arrangement in offline tank, air scour, and backpulsing; and Huber: flat panel membranes, 0.025-mm pore size, vertical arrangement on a rotating shaft in an aeration tank, air scour, and spray wash). All five MBR technologies produced excellent effluent and achieved 6 or 7 log units' removal of fecal coliforms and 5 log units' removal of coliphage. Coliphages are much smaller than the pore size of membranes; however, high removal of coliphage by MBRs occurs owing to physical filtration by the membrane, biomass activity in the aeration tank, and biofiltration by the biofilms that develops on the membrane (Shang et al., 2005). There are differences in permeation cycle times, nitrification/denitrification capabilities, required amount of operator attention, membrane-cleaning frequency, power requirements, and robustness of the systems. It is apparent that many factors other than just water quality are important in the selection of an MBR system.

CHAPTER 4

GUIDELINES AND CATEGORIES OF WATER REUSE

In light of the wide range of pathogens listed previously (Table 2.1), of treatment processes, and of possible uses of reclaimed water, reclaimed water standards vary based on the intended or allowable uses, unlike those of potable water, where a uniform set of parameters automatically deem it unacceptable for drinking. Within the United States, most of the water reuse volumes are in Florida (FL) and California (CA) with Arizona (AZ) and Texas not far behind (Narasimhan et al., 2005). From an international perspective, water reuse is a policy mostly in regions that are chronically deficient in potable water, providing an opportunity to meet some of the water requirements of those respective regions. For example, Israel treats more than 40% of its wastewater to meet the water needs for its agricultural system (Shelef, 2006). Similarly, high proportions of reclaimed water are relied on in other countries such as Saudi Arabia (Al-Aama and Nakhla, 1995). At present, there are no federal regulations for water reuse in the United States, but the USEPA has recently published some guidelines (USEPA, 2004). Some states have regulations, while others have guidelines for water reuse and deal with this issue on a case-by-case basis.

Some states have no rules or guidelines about water reuse. As is expected, the regulations and guidelines greatly vary in those states where they exist. In general, where the intended application of reuse water is likely to be exposed to human activity, the regulations or guidelines are more stringent than where such exposure is minimal or not expected. As a minimum, secondary treatment of the wastewater that is intended for reuse is generally required. The guidelines and restrictions observed by nine states within the United States that lead in water reuse (namely, AZ, CA, Colorado, Florida, Missouri, Nevada, New Mexico, Texas, and Washington [WA]) were discussed by Narasimhan et al. (2005) and are summarized in Appendix I. Also included in that appendix are the general guidelines published by the USEPA (USEPA, 2004) to help guide states, including those that do not have any guidelines of their own. Whether federal or state, the guidelines and regulations have a common theme of minimizing the hazards that may be associated with reclaimed water. Those themes are embedded in the recommended treatment processes, reuse water quality limits, frequency of monitoring, and setback distances. The level of clarity of the regulations and guidelines greatly varies. Some states specify which types of treatments have to be met to suit a particular reclaimed water reuse purpose and also setback distances. Much as these regulations and guidelines are well intentioned, they primarily focus on water reuse parameters at the point of generation without any specific consideration on the status of that water by the time it reaches the point of discharge through the distribution system. In other words, they do not address the potential degradation of the quality of the water in the distribution system. As is noticeable from Appendix I, the reclaimed water is treated to meet certain standards prior to reuse, with secondary treatment being the minimum form of treatment required by all the nine states. In some states (for example, CA), regulators do not permit flushing of reclaimed water distribution systems. However, such a restriction might compromise the maintainance of the sysem.

Reclaimed water treatment processes are, for the most part, similar to those of drinking water. They are mainly aimed at removing organic and inorganic nutrients, reducing turbidity, suspended solids, and pathogens (bacteria, viruses, helminths, and protozoa). Other treatment processes include filtration, disinfection, and advanced oxidation. Mostly targeted by these

processes are turbidity, fecal coliform densities, and BOD. The organic content of the water is not directly addressed by the rules and guidelines. Thus, the high organic matter content and nutrient content in general may lead to regrowth of microorganisms, formation of biofilms, and a general breakdown in the quality of the water. From these guidelines, it is collectively observed that none of them includes any guidance on seemingly important parameters such as AOC, BDOC, and COD.

The use of reclaimed water to a wider extent than is currently the case is possibly limited by the need for dual water distribution lines, with one carrying potable water and another carrying reclaimed water. However, as more and more systems age and undergo replacement, there is increasing interest in setting up dual distribution systems wherever deemed feasible.

Treated wastewater quality greatly varies depending on the treatment method used and the original extent of contamination. This section summarizes the USEPA and WHO guidelines for water reuse.

4.1. CATEGORIES OF REUSE

Current regulations and guidelines in the United States are based on 10 categories (Table 4.1) of reuse. Each reuse category has different regulations that are focused on matching the level of treatment to the intended use, while providing sufficient protection for human health. While a good deal of commonality exists between regulations for each category, details vary from state to state. In addition, not all categories are regulated by each state.

4.2. RECLAIMED WATER QUALITY AND TREATMENT REQUIREMENTS

4.2.1. Urban reuse

Generally, where public access is likely in the reuse application (unrestricted urban reuse), wastewater treatment to a high degree is required prior to application. Where public exposure is not likely (restricted urban reuse), a lower level of treatment is usually accepted. In general, all states that specify a treatment process require a minimum of secondary treatment and disinfection prior to urban reuse. The most common parameters for which water quality limits are imposed are BOD, TSS, and total and fecal coliforms. A limit on turbidity is usually specified to monitor the performance of the treatment facility. Tables 4.2 and 4.3, extracted from the USEPA guidelines, summarize treatment or water quality requirements for seven states that have successful reuse programs and long-term experience.

Currently, no states have set limits on certain pathogenic microorganisms for restricted or unrestricted urban reuse. However, FL requires monitoring of *Giardia* and *Cryptosporidium* for both restricted and unrestricted reuse with a sampling frequency based on treatment plant capacity. For systems with a capacity less than 1 million gal per day (mgd), sampling is required once every 5 years. For systems with a capacity equal to or greater than 1 mgd, sampling is required once every 2 years. For states that do not have specific regulations or guidelines, the USEPA recommends the guidelines outlined in Table 4.4 for urban reuse.

Table 4.1. Categories of Reuse Applications

Reuse Category	Typical Use
Unrestricted urban	Irrigation of areas with unrestricted public access such as parks, playgrounds, school yards, and residences; toilet flushing, air conditioning, fire protection, construction, ornamental fountains, and aesthetic impoundments.
Restricted urban	Irrigation in areas with restricted public access such as golf courses, cemeteries, and highway medians.
Agricultural: food crops	Irrigation of food crops intended for direct human consumption, often further classified as to whether the food crop is to be processed or consumed raw.
Agricultural: nonfood crops	Irrigation not culminating in direct human consumption of product such as irrigation of fodder, pastureland, commercial nurseries, sod farms, etc.
Unrestricted recreational	Impoundment in which no limitations are imposed for body contact recreational activities.
Restricted recreational	Impoundment in which recreational activities are limited to fishing, boating, and other noncontact recreational activities.
Environmental	Creation or enhancement of wetland; augmentation of stream flow.
Industrial	Reclaimed water is used in industrial facilities primarily for cooling system makeup water, boiler-feed water, process water, and general washdown.
Groundwater recharge	Aquifer recharge using infiltration basins, percolation ponds, or injection wells.
Indirect potable	The intentional discharge of highly treated reclaimed water into surface waters or groundwater that is or will be used as a source of potable water.

Table 4.2. Requirements for Unrestricted Urban Reuse[a]

	State						
Variable	**AZ**	**CA**	**FL**	**HI**	**NV**	**TX**	**WA**
Treatment	Secondary treatment, filtration, and disinfection	Oxidized, coagulated, filtered, and disinfected	Secondary, filtration, and high-level disinfection	Oxidized, filtered, and disinfected	Secondary treatment and disinfection	NS	Oxidized, coagulated, filtered, and disinfected
BOD (mg/L)	NS	NS	20 $CBOD_5$	NS	30	5	30
TSS (mg/L)	NS	NS	5	NS	NS	NS	30
Turbidity (NTU)	2 (avg.) 5 (max.)	2 (avg.) 5 (max.)	NS	2 (max.)	NS	3	2 (avg.) 5 (max.)
Coliform	**Fecal**	**Total**	**Fecal**	**Fecal**	**Fecal**	**Fecal**	**Total**
	None detectable	2.2/100 mL (avg.)	75% of samples below detection	2.2/100 mL (avg.)	2.2/100 mL (avg.)	20/100 mL (avg.)	2.2/100 mL (avg.)
	23/100 mL (max.)	23/100 mL (max. in 30 days)	25/100 mL (max.)	23/100 mL (max. in 30 days)	23/100 mL (max.)	75/100 mL (max.)	23/100 mL (max.)

[a]NS, not specified by state regulations; CBOD = carbonaceous BOD. Source: USEPA (2004).

Table 4.3. Requirements for Restricted Urban Reuse[a]

	State						
Variable	**AZ**	**CA**	**FL**	**HI**	**NV**	**TX**	**WA**
Treatment	Secondary treatment and disinfection	Secondary, oxidized, and disinfected	Secondary treatment, filtration, and high-level disinfection	Oxidized and disinfected	Secondary treatment and disinfection	NS	Oxidized and disinfected
BOD_5 (mg/L)	NS	NS	20 $CBOD_5$	NS	30	20	30
TSS (mg/L)	NS	NS	5	NS	NS	NS	30
Turbidity (NTU)	NS	NS	NS	2 (max.)	NS	3	2 (avg.) 5 (max.)
Coliform	**Fecal**	**Total**	**Fecal**	**Fecal**	**Fecal**	**Fecal**	**Total**
	200/100 mL (avg.)	23/100 mL (avg.)	75% of samples below detection	23/100 mL (avg.)	23/100 mL (avg.)	200/100 mL (avg.)	23/100 mL (avg.)
	800/100 mL (max.)	240/100 mL (max. in 30 days)	25/100 mL (max.)	200/100 mL (max.)	240/100 mL (max.)	800/100 mL (max.)	240/100 mL (max.)

[a]NS, not specified by state regulations; CBOD = carbonaceous BOD. Source: USEPA (2004).

Table 4.4. USEPA Suggested Guidelines for Urban Reuse[a]

Suggested Guidelines	Types of Reuse	
	Unrestricted Urban Reuse	**Restricted Urban Use**
Required treatment	• Secondary • Filtration • Disinfection	• Secondary • Disinfection
Reclaimed water quality	• pH = 6–9 • ≤ 10 mg of BOD/L • ≤ 2 NTU • No detectable fecal coliform/100 mL • 1 mg of Cl_2/L residual (minimum)	• pH = 6–9 • ≤ 30 mg of BOD/L • ≤ 30 mg of TSS/L • ≤ 200 fecal coliform/100 mL • 1 mg of Cl_2/L residual (minimum)
Monitoring	• pH - weekly • BOD - weekly • Turbidity- continuous • Coliform - daily • Cl_2 residual - continuous	• pH - weekly • BOD - weekly • TSS - daily • Coliform - daily • Cl_2 residual - continuous
Setback distance	• 50 ft to potable water supply wells	• 300 ft to potable water supply wells • 100 ft to areas accessible to the public

[a]Source: USEPA (2004).

4.2.2. Agricultural reuse and WHO guidelines

Tables 4.5 and 4.6 show the reclaimed water quality and treatment requirements for irrigation of food crops and nonfood crops, respectively, for seven states. Most states require a high-level treatment when reclaimed water is used for edible crops, especially those that are to be consumed raw. Irrigation of nonfood crops requires less stringent treatment and water quality requirements. As is found in other reuse categories, existing regulations on treatment and water quality requirements vary from state to state and depend largely on the type of irrigation employed and the type of food crop being irrigated.

Table 4.5. Reclaimed Water Quality and Treatment Requirements for Agricultural Reuse of Food Crops[a]

	Data per State						
Variable	**AZ**	**CA**	**FL**	**HI**	**NV**	**TX**	**WA**
Treatment	Secondary treatment, filtration, and disinfection	Oxidized, coagulated, filtered, and disinfected	Secondary treatment, filtration, and high-level disinfection	Oxidized, filtered, and disinfected	Secondary treatment and disinfection	NS	Oxidized, coagulated, filtered, and disinfected
BOD_5 (mg/L)	NS	NS	20 mg/L $CBOD_5$	NS	30	5	30
TSS (mg/L)	NS	NS	5 mg/L	NS	NS	NS	30
Turbidity (NTU)	2 (avg.) 5 (max.)	2 (avg.) 5 (max.)	NS	2 (max.)	NS	3	2 (avg.) 5 (max.)
Coliform	**Fecal**	**Total**	**Fecal**	**Fecal**	**Fecal**	**Fecal**	**Total**
	None detectable	2.2/100 mL (avg.)	75% of samples below detection	2.2/100 mL (avg.)	200/100 mL (avg.)	20/100 mL (avg.)	2.2/100 mL (avg.)
	23/100 mL (max.)	23/100 mL (max in 30 days)	25/100 mL (max.)	23/100 mL (max in 30 days)	400/100 mL (max.)	75/100 mL (max.)	23/100 mL (max.)

[a]NS = not specified by state regulations; CBOD = carbonaceous BOD. Source: USEPA (2004).

Table 4.6. Reclaimed Water Quality and Treatment Requirements for Agricultural Reuse of Nonfood Crops[a]

	Data per State						
Variable	**AZ**	**CA**	**FL**	**HI**	**NV**	**TX**	**WA**
Treatment	Secondary treatment and disinfection	Secondary treatment, oxidized, and disinfected	Secondary treatment, basic disinfection	Oxidized, filtered, and disinfected	Secondary treatment and disinfection	NS	Oxidized and disinfected
BOD_5 (mg/L)	NS	NS	20 mg of $CBOD_5$/L	NS	30	5	30
TSS (mg/L)	NS	NS	20 mg/L	NS	NS	NS	30
Turbidity (NTU)	NS	NS	NS	2 (max.)	NS	3	2 (avg.) 5 (max.)
Coliform	**Fecal**	**Total**	**Fecal**	**Fecal**	**Fecal**	**Fecal**	**Total**
	200/100 (avg.)	23/100 mL (avg.)	200/100 mL (avg.)	2.2/100 mL (avg.)	200/100 mL (avg.)	20/100 mL (avg.)	23/100 mL (avg.)
	800/100 mL (max.)	240/100 mL (Max in 30 days)	800/100 mL (max.)	23/100 mL (max.)	400/100 mL (max.)	75/100 mL (max.)	240/100 mL (max.)

[a]NS = not specified by state regulations; CBOD = carbonaceous BOD. Source: USEPA (2004).

Currently, no states have limits on pathogenic organisms for agricultural reuse; however, FL requires monitoring *Giardia* and *Cryptosporidium* for irrigation of food crops with sampling frequency described for restricted and unrestricted urban reuse. For states that do not have specific regulations or guidelines, the USEPA-recommended guidelines are summarized in Tables 4.7 and 4.8.

Table 4.7. USEPA Agricultural Reuse Regulatory Recommendations[a]

	Agricultural Reuse		
Suggested Guidelines	**Food Crops Not Commercially Processed**	**Food Crops Commercially Processed**	**Nonfood Crops**
Required treatment	• Secondary • Filtration • Disinfection	• Secondary • Disinfection	• Secondary • Disinfection
Reclaimed water quality	• pH = 6–9 • ≤ 10 mg of BOD/L • ≤ 2 NTU • No detectable fecal coliform/100 mL • 1 mg of Cl_2/L residual (minimum)	• pH = 6–9 • ≤ 30 mg of BOD/L • ≤ 30 mg of TSS/L • ≤ 200 fecal coliforms/100 mL • 1 mg of Cl_2/L residual (minimum)	• pH = 6–9 • ≤ 30 mg of BOD/L • ≤ 30 mg of TSS/L • ≤ 200 fecal coliforms/100 mL • 1 mg of Cl_2/L residual (minimum)
Monitoring	• pH - weekly • BOD - weekly • Turbidity - continuous • Coliform - daily • Cl_2 residual - continuous	• pH - weekly • BOD - weekly • TSS - daily • Coliform - daily • Cl_2 residual - continuous	• pH - weekly • BOD - weekly • TSS - daily • Coliform - daily • Cl_2 residual - continuous
Setback distance	• 50 ft to potable water supply wells	• 300 ft to potable water supply wells • 100 ft to areas accessible to the public	• 300 ft to potable water supply wells • 100 ft to areas accessible to the public

[a]Source: USEPA (2004).

Table 4.8. Recommended Limits for Constituents in Reclaimed Water for Agricultural Reuse (Food Crops and Nonfood Crops)[a]

	Concn (mg/L) for:	
Constituent(s) or Variable	**Long-Term Use**	**Short-Term Use**
Aluminum	5.0	20
Arsenic	0.10	2.0
Beryllium	0.10	0.5
Boron	0.75	2.0
Cadmium	0.01	0.05
Chromium	0.1	1.0
Cobalt	0.05	5.0
Copper	0.2	5.0
Fluoride	1.0	15.0
Iron	5.0	20.0
Lead	5.0	10.0
Lithium	2.5	2.5
Manganese	0.2	10.0
Molybdenum	0.01	0.05
Nickel	0.2	2.0
Selenium	0.02	0.02
Tin, Tungsten, and Titanium	—	—
Vanadium	0.1	1.0
Zinc	2.0	10.0
Constituents	Recommended limits	
pH	6.0	
TDS	500–2000 mg/L	
Free Chlorine Residual	<1 mg/L	

[a]Source: USEPA (2004).

The WHO recently revised the 1989 guideline for wastewater reuse in agriculture, and a draft version was released in 2005. To better address the appropriate audiences, the WHO decided to present the guidelines for wastewater reuse in three separate volumes: *Guidelines for the Safe Use of Wastewater and Excreta in Aquaculture*, *Guidelines for the Safe Use of Wastewater in Agriculture*, and *Guidelines for the Safe Use of Excreta and Grey Water*. This section briefly summarizes the guidelines developed for the safe use of wastewater in agriculture.

The WHO guidelines are based on tolerable risk and are intended to support the development and implementation of risk management strategies that will facilitate the use of wastewater in different settings while protecting public health. The guidelines are summarized in Table 4.9. The guidelines recommend that treated wastewater should contain:

- ≤1 viable intestinal nematode egg per L (on an arithmetic mean basis) for restricted or unrestricted irrigation; and
- $\leq 10^3$ and $\leq 10^5$ fecal coliform bacteria per 100 mL (on a geometric mean basis) for unrestricted and restricted irrigation, respectively.

Table 4.9. WHO Guideline Values for the Microbiological Qualities of Treated Wastewaters Used for Crop Irrigation[a]

Type of Crop Irrigation	No. of Human Intestinal Nematode Eggs (Arithmetic Mean No. per L)	*E. coli* Count (Geometric Mean No. per 100 mL)
Unrestricted irrigation[b]	≤1	$\leq 10^3$
	Reduced to ≤0.1 (i.e., undetectable) when children under 15 are exposed	Relaxed to $\leq 10^4$ when root crops are not grown
Restricted irrigation	≤1	$\leq 10^5$ (in conjunction with human exposure control techniques)
	Reduced to ≤0.1 (i.e., undetectable) when children under 15 are exposed	Reduced to $\leq 10^4$ when children under 15 are exposed
		Relaxed to $\leq 10^6$ when local agriculture is highly mechanized
Localized	No recommendation	No recommendation

[a]Source: WHO (2005).
[b]Unrestricted irrigation refers to all crops including salad crops and vegetables eaten uncooked; localized irrigation refers to irrigation by drip or trickle irrigation and bubbler irrigation; restricted irrigation refers to irrigation of all crops except salad crops and vegetables eaten uncooked.

Restricted irrigation refers to irrigation of all crops except salad crops and vegetables eaten uncooked, while unrestricted irrigation refers to all crops including salad crops and vegetables eaten uncooked. Effluents complying with both guideline values can be produced by treatment in a well-designed series of waste stabilization ponds. Although fecal coliform levels are much higher than U.S. standards, the recommendations based on risk and measurement of viable nematode eggs point to a trend focusing on risk assessment.

4.2.3. Industrial reuse

Reclaimed water quality and treatment requirements vary based on the final use of the reclaimed water. For example, CA has different requirements for the use of reclaimed water as cooling water, based on whether a mist is created. The guidelines are more stringent where a mist is created than for systems that do not create any mist. Table 4.10 summarizes the regulatory recommendations by USEPA for industrial reuse.

Table 4.10. USEPA Regulatory Recommendations for Industrial Reuse[a]

	Industrial Reuse	
Suggested Guidelines	**Once-Through Cooling**	**Recirculating Cooling Towers**
Required treatment	• Secondary • Disinfection	• Secondary • Disinfection (chemical coagulation and filtration may be needed)
Reclaimed water quality	• pH = 6–9 • ≤30 mg of BOD/L • ≤30 mg of TSS/L • ≤200 fecal coliforms/100 mL • 1 mg of Cl_2/L residual (minimum)	• Variable depends on recirculation ratio • pH = 6–9 • ≤30 mg of BOD/L • ≤30 mg of TSS/L • ≤200 fecal coliforms/100 mL • 1 mg of Cl_2/L residual (minimum)
Monitoring	• pH - weekly • BOD - weekly • TSS - daily • Coliform - daily • Cl_2 residual - continuous	• pH - weekly • BOD - weekly • TSS - daily • Coliform - daily • Cl_2 residual - continuous
Setback distance	• 300 ft to areas accessible to the public	• 300 ft to areas accessible to the public

[a]Source: USEPA (2004).

4.2.4. Groundwater recharge

Groundwater recharge consists of infiltration basins, percolation ponds, or injection wells. Most state regulations allow for the use of relatively low-quality water (namely, secondary treatment with basic disinfection) since these groundwater recharge systems have a proven ability to provide additional treatment. Traditionally, potable water supplies have been protected by requiring a minimum separation between the point of application and any potable supply wells. Hawaii does not specify treatment processes and determines requirement on a case-by case basis, while AZ, Nevada, and Texas do not have groundwater recharge regulations (Table 4.11). CA has recently drafted a regulation to protect public health, while FL has some limited regulations/guidelines. Currently, WA State has the most extensive guidelines for direct groundwater recharge of nonpotable aquifers.

Table 4.11. Reclaimed Water Quality and Treatment Requirements for Groundwater Recharge via High-Rate Application System[a]

	Data per State						
Variable	**AZ**	**CA**	**FL**	**HI**	**NV**	**TX**	**WA**
Treatment	NR	Advanced oxidation, but other methods can be approved by the CDPH after addressing public health concerns through public comments and hearings	Secondary treatment and basic disinfection	Case-by-case basis	NR	NR	Oxidized, coagulated, filtered, and disinfected
BOD_5	NR	NS	NS		NR	NR	5 mg/L
TSS	NR	Demonstrated log removal	10 mg/L		NR	NR	5 mg/L
Turbidity	NR	≤2 NTU within a 24-h period	NS		NR	NR	2 NTU (avg.) 5 NTU (max.)
Coliform	NR	Median concn ≤2.2 MPN/100 mL in last 7 days and ≤23 MPN/100 mL in any 30 days	NS		NR	NR	**Total** 2.2/100 mL (avg.) 23/100 mL (max.)
Total nitrogen	NR	<5 in a 24-h composite grab	12 mg/L		NR	NR	NS

[a]NR = not regulated by the state; NS = not specified by the state. Groundwater recharge in CA and in Hawaii is determined on a case-by-case basis. MPN, most probable number. Sources: USEPA (2004), State of California (2000), State of California (2008).

4.2.5. Indirect potable reuse

According to the USEPA guidelines, indirect potable reuse includes the use of reclaimed water to augment surface water sources that are used or will be used for public water supplies or to recharge groundwater used as a source of domestic water supply. Unplanned indirect

potable reuse occurs in many river systems; however, the USEPA guidelines address only the intentional introduction of reclaimed water into the water supply for the purpose of increasing the total volume of water available for potable use. Table 4.12 summarizes treatment and water quality requirements for seven states that are pioneering indirect potable reuse and illustrates the variety of regulatory approaches taken by the states.

Table 4.12. Treatment and Water Quality Requirements for Indirect Potable Reuse[a]

	Data per State						
Variable	**AZ**	**CA[b]**	**FL[c]**	**HI**	**NV**	**TX**	**WA**
Treatment	NR	Case-by-case basis	Advanced treatment, filtration, and high-level disinfection	Case-by-case basis	NR	NR	Oxidized, coagulated, filtered, RO treated, and disinfected
BOD_5	NR		20 mg/L		NR	NR	5 mg/L
TSS	NR		5 mg/L		NR	NR	5 mg/L
Turbidity	NR		NS		NR	NR	0.1 NTU (avg.) 0.5 NTU (max.)
Coliform	NR		**Total** All samples less than detection		NR	NR	**Total** 1/100 mL (avg.) 5/100 mL (max.)
Total nitrogen	NR		10 mg/L		NR	NR	10 mg/L
TOC	NR		3 mg/L (avg.) 5 mg/L (max.)		NR	NR	1 mg/L
Primary and secondary standards	NR		Compliance with most primary and secondary standards		NR	NR	Compliance with most primary and secondary standards

[a]Source: USEPA (2004). NR, not regulated by the state; NS, not specified by state regulations.
[b]Indirect potable reuse in CA and in Hawaii is determined on a case-by-case basis.
[c]FL requirements are for the planned use of reclaimed water to augment surface water sources that will be used as a source of domestic water supply.

Most states specify a minimum time the reclaimed water must reside underground prior to being withdrawn as a source of drinking water. WA State requires that reclaimed water be retained underground for a minimum of 12 months prior to being withdrawn as a drinking water supply. Several states also specify minimum separation distances between a point of recharge and the point of withdrawal as a source of drinking water. FL requires a 500-ft separation distance between the zone of discharge and potable water supply well. See Table 4.13 below.

Table 4.13. USEPA Indirect Potable Reuse Regulatory Recommendations from 2004 Guidelines for Water Reuse[a]

Suggested Guidelines	Groundwater Recharge		Surface Water Augmentation
	Surface Spreading	**Direct Injection**	
Required treatment	• Secondary • Disinfection • Possible filtration or advanced treatment	• Secondary • Filtration • Disinfection • Advanced treatment	• Secondary • Filtration • Disinfection • Advanced treatment
Reclaimed water quality	• Meet drinking water standards after percolation through vadose zone	• Meet drinking water standards • pH = 6.5–8.5 • ≤2 NTU • Total coliform nondetectable/100 mL • 1 mg of Cl_2/L residual (minimum) • ≤3 mg of TOC/L • ≤0.2 mg of TOX/L	• Meet drinking water standards • pH = 6.5–8.5 • ≤2 NTU • Total coliform nondetectable/100 mL • 1 mg of Cl_2/L residual (minimum) • ≤3 mg of TOC/L
Monitoring	• pH - daily • Coliform - daily • Cl_2 residual - continuous • BOD - weekly • Turbidity - continuous • Drinking water standards - quarterly	• pH - daily • Coliform - daily • Cl_2 residual - continuous • Turbidity - continuous • Drinking water standards - quarterly	• pH - daily • Coliform - daily • Cl_2 residual - continuous • Turbidity - continuous • Drinking water standards - quarterly
Setback distance	• 500 ft to extraction wells	• 2000 ft to extraction wells	• Site specific

[a]Source: USEPA (2004).

WA requires the minimum horizontal separation distance between the point of direct recharge and point of withdrawal as a source of drinking water supply to be 2000 ft. FL regulates reclaimed water discharge to surface waters used as potable water sources that are less than 24 h of travel time upstream from the point of withdrawal for potable treatment as indirect potable reuse. Table 4.13 summarizes the recommended guidelines by USEPA for indirect potable reuse.

4.3. RECLAIMED WATER MONITORING REQUIREMENTS

Reclaimed water monitoring requirements vary greatly from state to state and depend on type of reuse. For unrestricted urban reuse, Oregon requires sampling for coliforms daily, while for agricultural reuse of nonfood crops, sampling of total coliforms is required once a week. Oregon also requires hourly monitoring of turbidity when a limit on turbidity is specified. For unrestricted and restricted urban reuse, as well as for agricultural reuse on food crops, FL requires the continuous online monitoring of turbidity and chlorine residual. FL requires that the TSS limit be achieved prior to disinfection and has a minimum schedule for sampling and testing flow, pH, chlorine residual, dissolved oxygen, TSS, carbonaceous BOD (CBOD), nutrients, and fecal coliform based on system capacity. FL also requires an annual analysis of primary and secondary drinking water standards for reclaimed water used in irrigation for facilities greater than 100,000 gpd. Monitoring for *Giardia* and *Cryptosporidium* must also be performed with the frequency dependent on system capacity. Other states determine monitoring requirements on a case-by-case basis depending on the type of reuse.

CHAPTER 5

CATEGORIES OF MICROBES IN RECLAIMED WATER

Evidence of microorganisms can be used as an indicator of the hazards associated with reclaimed water. Besides health concerns, the regrowth of microorganisms in reclaimed water within the storage and distribution systems can clog sprinkler heads and cause aesthetically displeasing color and odors. As summarized in Table 2.1, the microorganisms of concern in reclaimed water belong to four broad categories: namely, bacteria, viruses, protozoa, and helminths. Where storage containers are open and exposed to sunshine, algal and cyanobacterial growth can also be an issue. The occurrence of these categories is discussed in more detail in this section in the context of their effect on the biostability of reclaimed water.

5.1. VIRUSES

Viruses have been detected in wastewater in concentrations as high as 10^3 to 10^4 particles/L (Feachem et al., 1983). They were detectable at concentrations of 0.6 PFU/100 L of postchlorinated reclaimed water and at 0.13 PFU/100 L in a storage tank, compared to the initial 10^3 PFU/100 L at one reclamation facility (Rose et al., 1996). At that plant, virus occurrence was detected in 25% of postchlorinated samples and in 8% of the treated samples in the storage tanks, compared to 100% of the untreated wastewater (Figure 5.1). It is apparent from this figure that viruses occur frequently in wastewater and that they may not be completely removed by routine treatment, including filtration and disinfection. It is also noticeable from this figure that a variety of other microorganisms occur in reclaimed water. They will be discussed individually in the respective sections of this review.

Numerous studies have used viruses as a model organism to determine the fate of microorganisms because viruses are:

(i) quite resistant to disinfection compared to bacteria, and
(ii) quite small, which makes them least affected by filtration.

However, viruses tend to persist in a variety of environments, including inert surfaces such as glass (Mahl and Sadler, 1975), soil (Vaugh et al., 1978), and sometimes reclaimed water (USEPA, 2004). They can be transported through the distribution system by advection, dispersion, attachment, detachment, and inactivation. Just like bacteria, viruses have negatively charged surfaces in most natural environments (Harden and Harris, 1953; Dowd et al., 1998). The negative charge enables them to adsorb onto positively charged surfaces and colloidal material in the distribution system. However, unlike bacteria, viruses do not increase in abundance on their own unless they are in association with their host.

Thus, monitoring viruses in reclaimed water as a sign of quality can be done at a lower frequency than monitoring bacteria requires and from relatively few sampling points, compared to what monitoring bacteria requires. Furthermore, assaying for viruses greatly relies on culture-based techniques either entirely or combined with PCR. It can be a lengthy process that takes about 1 month to ascertain truly positive infectious viruses, a duration that can diminish the usefulness of the results. Furthermore, some viruses of economic importance in water and wastewater (notably rotaviruses, HAV, and NVs, namely, Norwalk and Norwalk-like viruses) are not yet readily culturable on available cell lines. When detected by using PCR amplification of the RNA, their viability or infectiousness still remains

questionable. Still, it can be argued that the detected RNA was from intact viruses, as the RNA from nonintact viruses would be degraded quite rapidly in the reclaimed water and thus not have generated a positive reverse transcriptase-PCR (namely, RT-PCR) signal. This contention underlines the need for studies that examine RT-PCR viral genome signals that correspond to established infectious doses.

Coliphages are viruses that infect *E. coli*. They have many structural similarities (namely, size, morphology, structure, and composition) with enteric viruses. Two types of coliphage are commonly used, namely, male-specific coliphages and somatic coliphages. The former are smaller (24 nm) and infect only male (F-plasmid containing) *E. coli* strains through the sex pili. Somatic coliphages, on the other hand, are 30 nm in diameter and can infect both F^+ and F^- *E. coli*. Coliphages have been widely used as surrogates for enteric viruses, and their assay has a very short turnaround time. Detection of coliphages in the distribution system is indicative of the presence of their host, *E. coli*, and by default, of the presence of fecal contamination. Thus, coliphages have been considered alternative or additional indicators of coliform and other indicator bacteria (Sobsey et al., 1995). They are also considered indicators of enteric viruses because of their physical similarities (in size, structure, morphology, and composition) and because they are present in higher densities than are enteric viruses in wastewater. However, as is shown in Figure 5.1, this assumption may not be entirely correct, as the occurrence of coliphage may, in some instances be more frequent than that of enteric viruses. Those differences may depend on the abundance of natural colloidal materials to which different types of viruses (coliphages versus enteroviruses in this instance) may adhere differently, surviving the imposed treatment regimen. Worthwhile additional information about the presence and abundance of coliphage in reclaimed water can be obtained by sampling more frequently (than one would for enteric viruses) and possibly by using more sampling points.

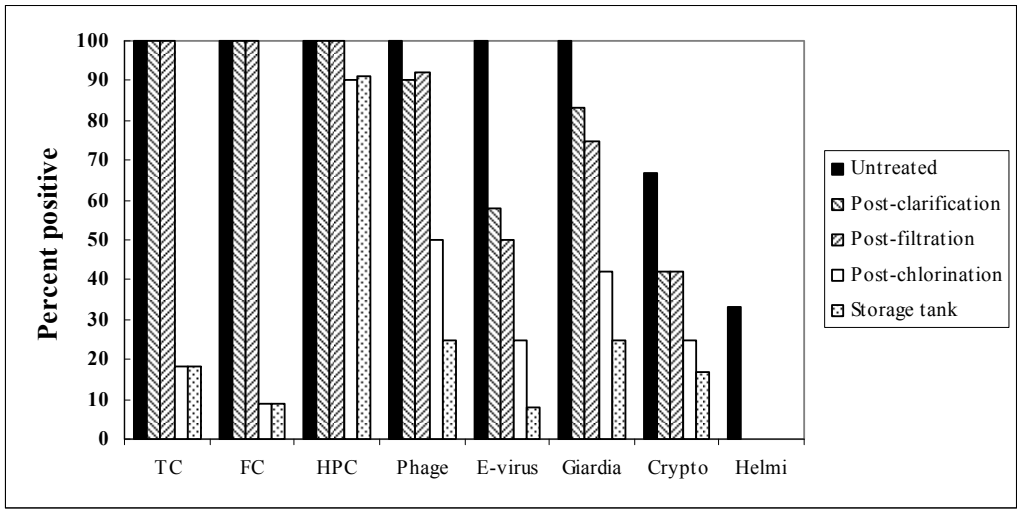


Figure 5.1. Microorganisms detected in wastewater at a single treatment plant in FL through all stages of processing to generate reclaimed water. TC = total coliform, FC = fecal coliforms, Phage = coliphage, E-virus = enterovirus, Crypto = *Cryptosporidium* spp., and Helmi = helminths. The figure is based on data published by Rose et al. (1996).

5.2. PROTOZOA

A clear understanding of the diversity of protozoa requires consideration of their unique traits. Key attributes of some protozoa of economic importance are summarized in Table 5.1. Such attributes range from the types of survival structure that they form, their feeding patterns (parasitism), life cycles, and mobility in the reclaimed water system. Members of interest in reclaimed water among these three phyla are able to survive in nature by forming cysts (for Ciliophora and Sarcomastigophora) or oocysts (for Apicomplexa) once growth conditions become unfavorable (Schuster and Visvesvara, 2004; Hampton et al., 2006). Thus, looking for these survival structures is a signature process for detecting the presence of these organisms in water. Just like viruses, protozoa are generally more resistant to disinfection than are bacteria and can survive longer in the environment than can bacteria. *Giardia* spp. appear to be more prevalent than *Cryptosporidium* spp. in reclaimed water.

Currently, very few states require monitoring the status of protozoa in reclaimed water (see Appendix I). Various reports emphasize the difficulty in inactivating protozoa such as *Giardia* spp. and *Cryptosporidium* spp. by chlorination (Gennaccaro et al., 2003; Quintero-Betancourt et al., 2003). Thus, physical removal through filtration is considered to be more effective than are other treatment methods. The State of Florida mandates testing for protozoa in reclaimed water at a single point postdisinfection, but even then such testing is required only once every 2 years in large treatment facilities and only once every 5 years at the small ones (Gennaccaro et al., 2003). However, recent surveys clearly indicate that both *Giardia* spp. and *C. parvum* oocysts are frequently encountered in reclaimed water even in instances where filtration and disinfection have been conducted (Table 5.2 and Figure 5.1).

Table 5.1. General Classification and Characteristics of Various Protozoa of Public Health Interest in Wastewater[a]

Phylum	Common Name	Distinguishing Characteristics	Representative of Relevance to Reclaimed Water	Remarks
Ciliophora	Ciliates	Have projections called cilia that are similar to flagella in structure but are much shorter. Almost all ciliates have a cytostome through which they feed and possess nuclei of two different sizes, namely, macronucleus and micronucleus. The dual nuclei distinguish this phylum.	*Balantidium coli*	Others include *Paramecium* spp.
Sarcomastigophora	Flagellates (Mastigophora)	Possess flagella that move in a whip-like fashion. Flagella are for movement toward food.	*Giardia* spp.	Others of economic interest but not associated with reclaimed water include *Leishmania* spp., *Trypanasoma* spp., and *Trichomonas vaginalis*.
	Sarcodina (Amoebae)	Have characteristic pseudopodia used for movement.	*Entamoeba histolytica*, *Naegleria* spp.	Not very susceptible to antimicrobial therapy.
Apicomplexa	Sprozoans	Have characteristic special organelles at the tips of their cells that contain enzymes that they use to penetrate their hosts. They have complex life cycles that may involve several hosts to complete the cycle.	*C. parvum*, *Cyclospora cayetanensis*	Other members of economic interest but not directly associated with reclaimed water include *Plasmodium* spp. and *Eimeria* spp.

[a]Source: Modified from Jjemba (2004).

Table 5.2. Occurrence of *C. parvum* Oocysts in Various Phases of Wastewater Treatment[a]

Sample	No. of Samples	% Positive for *C. parvum* Oocysts		Mean Oocysts ± SD (/100 L)	
		Total	Infectious	Total	Infectious
Influent	18	78	33	6910 ± 7731	993 ± 1277
Secondary effluent	18	83	39	112 ± 153	37 ± 28
Postfiltration	17	71	35	37 ± 73	5 ± 5
Final disinfected effluent	15	67	40	28 ± 52	7 ± 9

[a]Gennaccaro et al. (2003).

It is clear from the table above that even the *Cryptosporidium* spp. in the final disinfected effluent can be infectious. Although the mean total number of oocysts in the final disinfected effluents is low, it should be borne in mind that oocyst recoveries in water are rarely above 50% if one uses the common method of concentrating on an Envirochek filter and eluting under the Method 1623 guidelines (USEPA, 1995; Quintero-Betancourt et al., 2003) which was used by Gennaccaro et al. (2003). Recoveries are even much lower with the yarn-wound polypropylene, filter which has been more widely used by various laboratories under the Information Correction Rule survey. Thus, the densities presented in Table 5.3 may be an underestimate of infectious oocysts. It is also worth pointing out that the mean number of oocysts is variable, as is evidenced by the large standard deviations clearly spelling not only the need for more data but also for a clear understanding of the factors in the treatment and distribution systems that enable the oocysts to persist. Quintero-Betancourt et al. (2003) reported a significant correlation (r =0.84; $P < 0.0001$) between the level of indigenous *Cryptosporidium* oocysts and the amount of oxygen required to biochemically oxidize the organic matter present in the water (namely, CBOD).

From a practical perspective, *Giardia* spp. and *Cryptosporidium* spp., just like enteroviruses, do not regrow in the absence of their host. Thus, their status in reclaimed water can be determined by sampling less frequently and at few sampling points. The monitoring of protozoa is problematic because of their random occurrence and poor recovery. Where present, *Giardia* appears to dominate compared to *Cryptosporidium*. However, a recent case study of seven utilities by Narasimhan et al. (2005) also found *Cryptosporidium* spp. more frequently in plant effluents prior to distribution and *Giardia* more frequently within the distribution system. Those authors suggested the need for utilities to monitor these pathogens in reclaimed water. This suggestion has been fully embraced by some states, particularly FL, although the recommended frequency of sampling for these parasites (namely, once every 2 to 5 years depending on the size of the system) is quite low (FDEP, 1999).

5.3. BACTERIA

Typically, the potential presence of pathogens in reuse water is assessed by using indirect measures such as turbidity or suspended solids coupled with regular sampling for indicator organisms, such as coliform bacteria (Rose et al., 2004). However, common indicators such as coliforms and *E. coli* are not considered adequate indicators of viral contamination. Also, there are significant differences among bacteria, viruses, and protozoan parasites in regard to their size, structure, resistance to treatment processes (Figure 5.1), and the ability to regrow in the reuse distribution system.

The detection of pathogenic bacteria, viruses, or parasites requires expensive and time-consuming techniques. Water quality monitoring programs therefore use indicator organisms to identify fecal pollution. The rationale of using an indicator microorganism is that, while it is impractical and currently nearly impossible to test water for all possible pathogens that could be present, an indicator organism that is always found in fecal material could serve as a surrogate for detection of pathogens. Some of the important requirements of indicator organisms are: (i) it should be a member of the intestinal microflora, (ii) it should be present whenever pathogens are present, (iii) it should be present at the same numbers as or at higher numbers than the pathogen, (iv) it should be as resistant as pathogens are to environmental conditions, and (v) it should be detectable by simple, rapid, and inexpensive methods (Grabow, 1996; Britton and Gerba, 1984). Total and fecal coliform bacteria are widely used as microbial indicators for wastewater reuse (USEPA, 2004; WHO, 2005). These organisms have a long history in water quality assessment, mainly because of their association with fecal contamination, and can be identified by relatively simple and rapid detection techniques (Grabow, 1996). The total coliform bacteria include *E. coli*, *Enterobacter*, *Klebsiella*, and *Citrobacter*. Fecal coliforms, part of the total coliform group, are more closely related to fecal pollution and principally include *E. coli* and *Klebsiella pneumoniae*. Other bacterial indicators include total heterotrophs and enterococci. Each of these is discussed underneath in relation to reclaimed water. Other bacterial pathogens of concern are also discussed.

5.3.1. Heterotrophic bacteria

Heterotrophic bacteria are used frequently in the water industry to provide information about the microbiological and aesthetic quality of drinking water and can possibly be adaptable as indicators of the quality of reclaimed water. Heterotrophs have been detected in reclaimed water by various research groups and at a range of facilities (Table 5.3). In instances where sampling was conducted at more than one site in the distribution system, there is some evidence of increased HPC density, showing some regrowth. HPC regrowth was also reported in several utilities studied by Narasimhan et al. (2005).

HPCs are presumed to be a better indicator than counts of coliform bacteria, reflecting the response of naturally occurring organisms in their native state and on disinfection (Lee and Deininger, 2003). Heterotroph is a term that broadly refers to, from a microbiological perspective, any bacteria that obtain energy (and therefore are able to grow) from organic compounds. By that definition, this includes a whole range of bacterial species rather than a homogenous taxonomic group. Their only unifying characteristic is the ability to grow on a specific medium rapidly and under a set of specified environmental conditions. Thus, the quantitative and qualitative composition of bacterial heterotrophs can definitely vary from one distribution system to another and can indeed vary even within different sections of the same distribution system. HPCs can also vary depending on the growth medium (Farnleitner et al., 2004) and incubation temperature (Birks et al., 2005) used for the assay.

Table 5.3. Occurrence of Heterotrophic Bacteria in Reclaimed Water at Various Facilities

Location	Disinfection Practices	Chlorine Residual (mg/L)	HPC in Reclaimed Water (CFU/mL)	Remarks
St. Petersburg, FL	Filtration and chlorination	Not determined	7 to 1.5×10^4	Also detected 1×10^4 to 2×10^4 HPC/mL in the storage tank (Rose et al., 1996)
CA (Site 1)	Chlorination	0.01–1.67	4.8×10^3	Fecal coliform also detected (Ryu et al., 2005)
CA (Site 3)	Chlorination	0.04–5.73	54	No fecal coliform were detected (Ryu et al., 2005)
TX (Site 1)	Chlorination	0.24–0.55	1.5×10^4 to 1.0×10^5	Fecal coliform also detected (Ryu et al., 2005)
TX (Site 2)	Chlorination and UV	1.78–2.84	18 to 1.0×10^3	No fecal coliform were detected (Ryu et al., 2005)
NV	Chlorination and UV	0.3–0.68	1.0×10^3 to 2.4×10^4	No fecal coliform were detected (Ryu et al., 2005)
AZ	Chlorination and UV	0.27–0.82	1.6×10^4 to 2.7×10^4	No fecal coliform were detected (Ryu et al., 2005)
NY	Membrane filtration, ozonation and UV	Not determined	2.5×10^4 (in effluent)	The end of the distribution system had 4.1×10^4 HPC/mL (Karim and LeChevallier, 2005)
MA (A)	Membrane filtration, ozonation and UV	Not determined	4.5×10^3 (in effluent)	The middle and end of the distribution system had 1.3×10^5 and 1.6×10^5 HPC/mL, respectively (Karim and LeChevallier, 2005)
MA (B)	Membrane filtration, UV, and chlorination with intermittent ozonation	Not determined	1.2×10^3 (in effluent)	The middle of the distribution system had 1.2×10^3 HPC/mL (Karim and LeChevallier, 2005)

Despite recent advances in molecular biology, very few laboratories have attempted to understand the diversity of heterotrophic bacteria in water systems under different settings. Farnleitner et al. (2004) recently qualitatively compared the composition of HPCs using 16S-rDNA profiling to study the population dynamics of heterotrophs in drinking water, groundwater, and distribution systems (Figure 5.2).

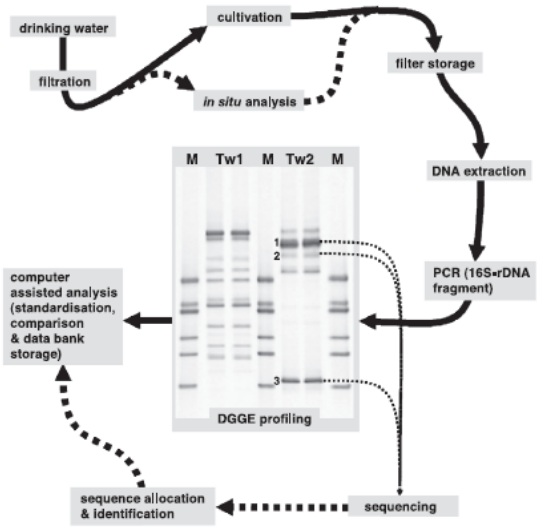


Figure 5.2. Schematic of a 16S-rDNA-based HPC profiling approach (source: Farnleitner et al., 2004, with permission from Elsevier).

A comparison in microbial diversity for two water samples, Tw1 and Tw2, based on denaturing gradient gel electrophoresis is shown in Figure 5.2. Each band in the gel corresponds to a certain operational taxonomic unit (OTU) of the respective HPC community analyzed. Using this approach, Farnleitner et al. (2004) sequenced the most dominant bands and found *Pseudomonas* spp. (band 1), *Aeromonas* spp. (band 2), and *Bacillus* spp. (band 3) as some of the most predominant species that were detected by HPC in one of the types of water they tested. Further work by those authors also showed interesting differences in the type of medium used for determining HPC (namely, 3.9, 3.91, and 8 CFU/mL with ISO, TSA, and R2A, respectively) and a large discrepancy between these HPCs on growth medium versus direct counts of 1.5×10^5 cells/mL in the same water. Those studies also showed a distribution system very dynamically changing with time at different sampling locations (Figure 5.3). The results in Figure 5.3, although of a qualitative, rather than quantitative, nature, clearly show some OTUs emerging or disappearing (both in space and over time), emphasizing the need for temporal sampling to get a better understanding of the microbial dynamics, especially in instances where regrowth is occurring.

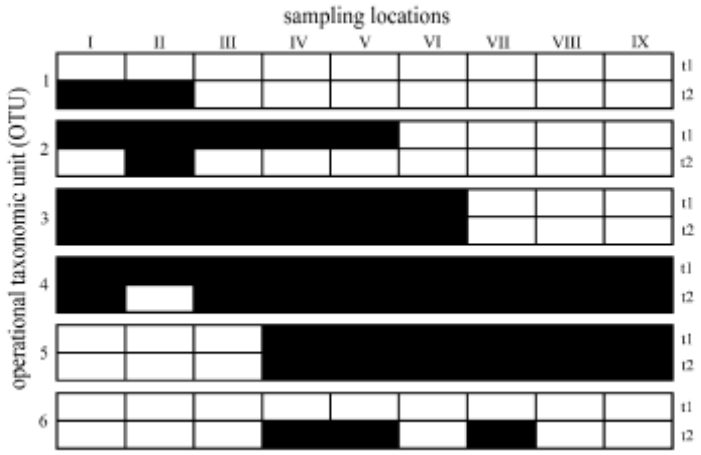


Figure 5.3. Examples of differences in OTUs within different locations (I to IX) of a water distribution system at two different sampling times (t1 and t2). The times t1 and t2 were within two consecutive months (Farnleitner et al., 2004, with permission from Elsevier).

Tokajian and Hashwa (2004) found the majority of heterotrophs recovered on R2A agar from storage tanks to be Gram negative with 85% as α-, β-, or γ-*Proteobacteria*. By comparison, only 60% of the bacteria in the influent belonged to these three subclasses and Gram-positive bacteria constituted only 10% in the storage tanks and 25% in the influent. Overall, α-*Proteobacteria* were most abundant in both the storage tanks (61% abundance) and the influent (32% abundance). Most of them specifically belonged to *Sphingomonas* spp. (*S. rosa*, *S. natatoria*, *S. adhaesiva*, *S. yanoikuyae*, and *Novasphingobium capsulatum*). Most of the γ-*Proteobacteria* subclass members had a high similarity to *Aeromonas* spp. and *Klebsiella oxytoca*, whereas most of the β-*Proteobacteria* subclass members had a high similarity to *Acidovorax* spp. (Tokajian and Hashwa, 2004). The Gram-positive bacteria were mostly *Aeromicrobium*, *Norcadia*, *Arthobacter*, *Micrococcus*, *Staphylococcus*, *Rhodococcus*, *Brevibacillus*, *Mycobacterium*, and *Bacillus* spp. However, there is a lack of clear evidence linking HPC values by themselves to the occurrence of waterborne pathogens and their associated health risks (WHO, 2002). Thus, HPC results give more meaningful interpretation when they are taken in the context of other microbial determinations. Some of those determinations as they relate to potable and reclaimed water are discussed underneath.

5.3.2. Coliforms

Coliform broadly refers to several genera of bacteria that belong to the family Enterobacteriaceae. Coliforms are characterized by their ability to ferment lactose, producing gas and forming acid within 48 h at 35 ^{o}C. Coliforms include members of *E. coli*, *Enterobacter*, *Klebsiella*, and *Citrobacter*. Not all coliforms are of fecal origin, and thus a distinction of fecal coliforms is made by incubating all coliform-positive samples (or colonies) at 44.5 ^{o}C (Eaton et al., 2005). Fecal coliforms specifically include *E. coli* and *Klebsiella*. Coliforms are frequently encountered in reclaimed water. For example, a study by Rose et al. (1996) encountered coliforms in 100% of postfiltered reclaimed water samples at a treatment plant in FL and in 18% of postchlorinated and 18% of storage tank-derived samples (Figure 5.1). A similar trend was observed for coliforms of fecal origin at that time as well. Coliform occurrence in reclaimed water has also been monitored recently by Narasimhan et al. (2005; Figure 5.4) over time. Those temporal data clearly show the erratic occurrence of coliforms in reclaimed water, with the population densities ranging from below detection (as

in most of the months at the site in Figure 5.4A), even though this site had the highest coliform density at some point of all three sites, including a very consistent presence but at low abundance (as shown in Figure 5.4C).

E. coli is usually the most dominant in waters where coliforms are detected and may comprise more than 50% of the coliform population (Tokajian and Hashwa, 2004). Those same authors reported the total and fecal coliforms to be only a small fraction (0.002%) of the HPC in the water storage systems that they studied in Lebanon. However, under low nutrient concentrations, such as those that prevail in drinking water, coliform bacteria may be outcompeted by other heterotrophs (Figure 5.5). By contrast, the nutrient status in reclaimed water is usually much richer than that in potable water and can enable the regrowth and successful competition of coliforms compared to other heterotrophs.

The antagonism between HPC organisms and coliforms has been known for a long time and is believed to be displayed by injury of the coliforms in the presence of other heterotrophs. Coliforms may also not adequately represent the occurrence of pathogens in reclaimed water because they are fairly susceptible to disinfection, compared to some of the pathogens of major concern, including protozoa and viruses (LeChevallier and Au, 2004; Harwood et al., 2005). Thus, alternative surrogates such as enterococcus and coliphage have been proposed.

Despite a widespread reliance on indicator organisms, however, various reports have indicated the discrepancy between indicators and pathogens such as viruses and protozoa in water. Indicator organisms may also fail to represent the extent of regrowth of pathogens in reclaimed water. Furthermore, viruses, helminths, and protozoa can survive various forms of disinfection treatment better than bacteria can and even survive for longer durations in the environment than bacteria can. In practical terms, it is impossible to test water for all possible pathogens. The presence of coliforms in the distribution system reflects either the failures in treatment with the disinfection method used or the regrowth of the bacteria in the distribution system. Failures in disinfection can be associated with biofilm-based microorganisms.

5.3.3. Enterococci

Enterococci are Gram-positive coccus-shaped aerotolerant facultative anaerobes that exist in chains. They are primarily of fecal origin and are characterized by the ability of their colonies to form a greenish or brownish zone (α-hemolysis) on blood agar. In water, they are generally considered fecal contaminants. More than 20 enterococcal species are isolated from human feces, including *E. faecalis* and *E. faecium*. Some enterococci are opportunistic pathogens.

Fecal enterococci in the range of 800 to 1600 CFU/100 mL were detected in untreated grey water samples from a large in-building water recycling facility studied by Birks et al. (2005). Harwood et al. (2005) collected water from several reclaimed water production steps over a 1-year period at different plants in three states, namely, AZ, CA, and FL. The water was collected from the influent, secondary treatment, filtered effluent, and disinfected effluent. Sample collections were conducted every other month for 1 year. Enterococci, coliforms, and coliphage were detected in all of the influent samples. Enterococci were detected in all effluent samples that also had fecal coliforms. Collectively, these results strongly suggest that enterococci can adequately serve as a surrogate for fecal contamination in reclaimed water. However, several reports that have detected enterococci in reclaimed water report qualitative rather than quantitative results (Rose et al., 2001; Harwood et al., 2005; Mazari-Hiriart et al., 2008) or log-removal rates only (Ottoson et al., 2006) for these bacteria.

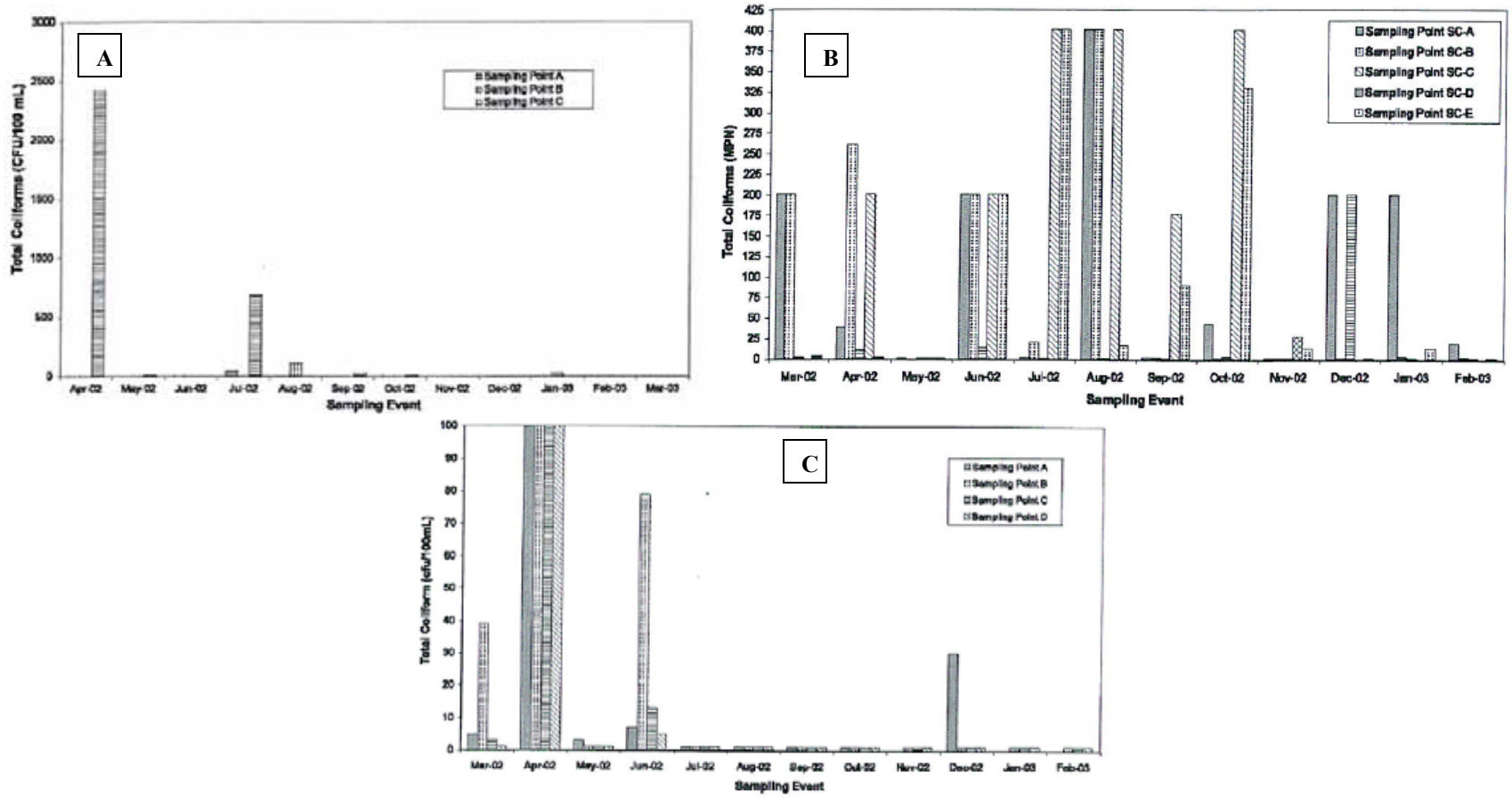


Figure 5.4. Monthly total coliforms in reclaimed water at three different utilities reported by Narasimhan et al. (2005). Sites coded as A, B, and C in this figure were identified as Utilities I, V (LC), and VI by the original authors. Note the difference in the *y* axis scale.

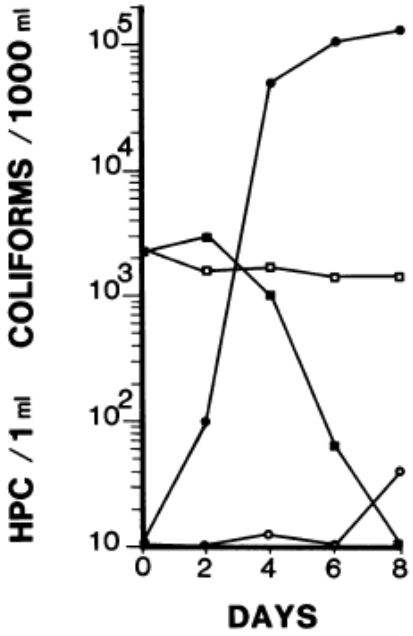


Figure 5.5. Increase in the HPC represented by *Pseudomonas cepacia* (●) with decreasing coliforms represented by *E. coli* (■) in mixtures. *E. coli* in the control treatment (namely, nonmixed) is represented by open square symbols (□), whereas the increase in the background microbial population is represented by open circles (○). (Source: LeChevallier and McFeters, 1985).

5.3.4. *Pseudomonas* spp.

Pseudomonas spp. are some of the most encountered noncoliform bacteria in reclaimed water. As a matter of fact, *Pseudomonas* spp. may be present in potable water that has acceptable levels of coliform, strongly suggesting that these organisms are quite ubiquitous. Table 5.4 shows their presence in all of the locations sampled by Karim and LeChevallier (2005) at three reclaimed water facilities in New York (NY) and Massachusetts (MA). It is also noticeable from those data that they can grow in the distribution system as exemplified by their increasing presence in the distribution system at MA (Site 1). Their frequency of occurrence also increased at the New York site at the end of the distribution system as compared to the total coliform common indicator. Of most concern among the pseudomonads in water are *P. aeruginosa* and *P. paucimobilis* (Rutala and Weber, 1997) although others such as *P. putida* and *P. stutzeri* also occur. Brozel and Cloete (1991) found *P. stutzeri* to be one of the most predominant organisms in cooling tower water samples. Thus, their occurrence in reclaimed water distribution systems on a regular basis is worth studying in order to determine how their abundance is affected by different treatment systems and under different environmental conditions.

Table 5.4. Occurrence of *Pseudomonas* spp., *Aeromonas* spp., and *Legionella* spp. in Reclaimed Water from 3 Sites Sampled Recently

	No. of Positive Samples[a]		
Facility	**Plant Effluent**	**Middle of Distribution System**	**End of Distribution System**
Total Coliform			
NY	3 (5)	NS[b]	1 (4)
MA (Site 1)	3 (5)	5 (5)	5 (5)
MA (Site 2)	2 (5)	1 (5)	NS
Pseudomonas spp.			
NY	4 (5)	NS	3 (4)
MA (Site 1)	1 (5)	2 (5)	3 (5)
MA (Site 2)	2 (5)	1 (5)	NS
Aeromonas spp.			
NY	1 (5)	NS	1 (4)
MA (Site 1)	1 (5)	3 (5)	4 (5)
MA (Site 2)	2 (5)	1 (5)	NS
Legionella spp.			
NY	2 (5)	NS	2 (4)
MA (Site 1)	0 (5)	0 (5)	0 (5)
MA (Site 2)	0 (5)	0 (5)	NS

[a]The numbers in brackets indicate the total number of samples tested from that location. Source: Karim and LeChevallier (2005).
[b]NS = not sampled.

5.3.5. *Legionella* spp.

Legionella spp. are Gram negative, non-spore-forming bacteria that are able to survive for several weeks in water. They are occasionally detected in reclaimed water (Table 5.4). Most species, except *L. oakridgensis*, require iron salts and cysteine for growth. They are isolated on buffered (pH = 6.9) charcoal yeast extract (BCYE) supplemented with cysteine, ferric pyrophosphate, and α-ketoglutarate (Eaton et al., 2005; Lück et al., 2004). To eliminate non-*Legionella* organisms during this selection growth process, the samples are pretreated with acidified potassium chloride (0.2 M KCl/HCl; pH = 2.2). Despite pretreatment, *Legionella* spp. may be outgrown by other bacteria on this selective medium as it grows quite slowly. Its detection is more reliable with PCR or immunofluorescence techniques, although the former is nonquantitative. Both of these alternative detection methods cannot ascertain its viability. Most preferable to confirm the presumptive *Legionella* is the latex agglutination test. The method is more rapid than the direct fluorescence assay, which is time-consuming and quite prone to frequent cross-reactions among various serogroups (Reyrolle et al., 2004).

Just like *Mycobacterium* spp. (see Section 5.3.7), *Legionella* spp. are fairly resistant to disinfection. This attribute is particularly the case with *Legionella* in biofilms, as opposed to those that are planktonic or free-floating (Kim et al., 2002). Part of the difficulty in eliminating *Legionella* spp. with disinfectants is that they can embed into protozoan cells or cysts. Protozoa that have been reported to host *Legionella* spp. include *Acanthamoeba* spp.

(Kilvington and Price, 1990), but a range of other protozoa are also possibly able to harbor *Legionella* spp. Intracellular replication in eukaryotic host cells is probably the major way that *Legionella* spp. multiply in the environment. Under nutrient limitations, *Legionella* spp. can enter a viable but nonculturable state, persisting in biofilms and distribution systems. This survival mechanism has important implications in reclaimed water distribution systems as they are likely to have variable flushes of suitable conditions (for example, temperature, nutrients, etc.) over time.

Each year, between 8000 and 18,000 people are hospitalized with Legionnaires' disease in the United States (CDC, 2005). However, many infections are not diagnosed or reported, so this number may be higher. More illness is usually reported in the summer and early fall, but it can happen any time of year. A recent report by Yoder et al. (2008) shows that, of the 20 waterborne disease outbreaks in the United States during the period 2005 through 2006, half were outbreaks of acute respiratory illness that were attributed to *Legionella*, surpassing the proportion of outbreaks caused by acute gastrointestinal illness. Flannery et al. (2006) showed a 93% reduction in the occurrence of *Legionella* spp. in building plumbing systems in San Francisco after the utility converted from free chlorine to chloramines (Figure 5.6). Amoebae at sampled sites were associated with *Legionella* spp. colonization *only* when chlorine was used for residual disinfection. *Legionella* spp. were cultured from 61 (36%) of 169 samples in which amoebae were present versus 291 (24%) of 1236 samples without amoebae ($p = 0.01$). After conversion to monochloramine, *Legionella* was found in 1 (1%) of 78 samples containing amoebae and 8 (1%) of 866 samples without amoebae ($p = 0.75$). The prevalence of amoebae decreased from 169 (12%) of 1405 samples when chlorine was the residual disinfectant to 78 (8%) of 944 samples collected after conversion to monochloramine ($p = 0.006$). These data demonstrate that occurrence and colonization of amoebae by *Legionella* spp. can be influenced by the type of disinfectant used.

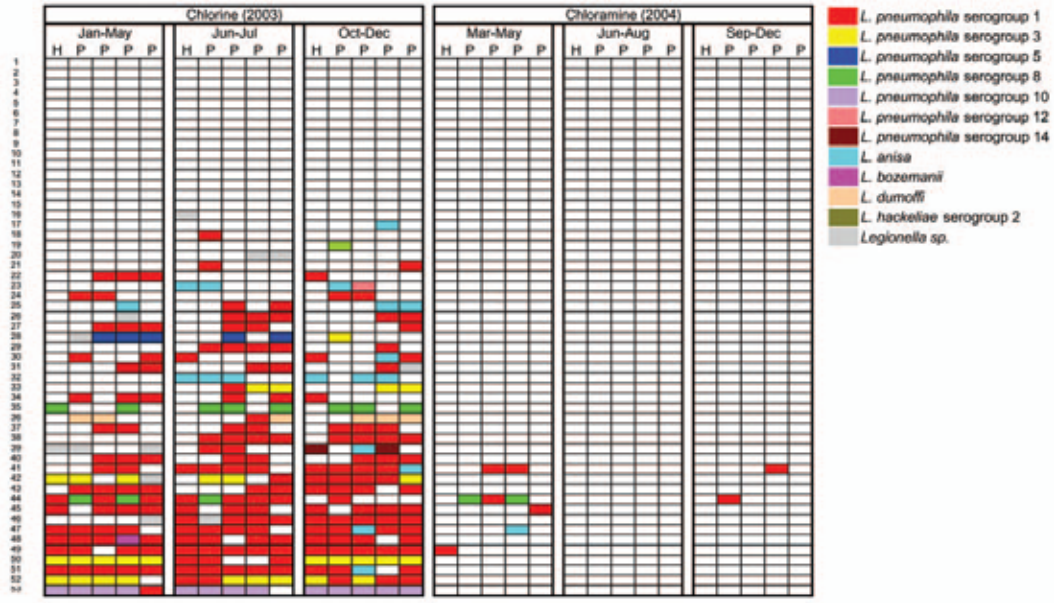


Figure 5.6. Changes in occurrence of *Legionella* in San Francisco buildings after conversion from free chlorine (left) to monochloramine (right). Shaded boxes depict different *Legionella* species (source: Flannery et al., 2006).

5.3.6. *Aeromonas* spp.

Aeromonas spp. are increasingly important indicators of the quality of the water in distribution systems. Their occurrence in reclaimed water is, in some instances, as frequent as that of coliforms (Table 5.4). Ontario (Canada) and The Netherlands have set *Aeromonas* standards at 20 CFU/100 mL for outgoing piped drinking water and 200 CFU/100 mL in distribution systems (Eaton et al., 2005). *Aeromonas* spp. are Gram-negative facultative anaerobes that ferment glucose but not lactose. They are fairly widespread natural inhabitants of the aquatic environment, their abundance being reportedly higher in the warmer months (Eaton et al., 2005). Growth in lactose fermentation tubes without the formation of gas is typically suspected to indicate the presence of aeromonads. They are renowned for colonizing distribution systems (Chauret et al., 2001). They are opportunistic human pathogens, causing red sore disease or hemorrhagic septicemia and water-associated wound infections. Aeromonads are typically isolated from water and have a high ability to regrow in distribution systems (Gavriel et al., 1998; Brandi et al., 1999), even with low concentrations of organic carbon, though regrowth seems to be limited to just a few strains (Kühn et al., 1997). They are also more susceptible to chlorination than is *E. coli* (Sisti et al., 1998; Gavriel et al., 1998).

Aeromonads are assayed by using ampicillin dextrin agar and incubating at 35 ^{o}C (Eaton et al., 2005). Growth on this medium is quite rapid with visible, large, bright-yellow colonies obtainable overnight. The occurrence of false-positive aeromonads in water based on this criterion alone can be quite high, though (see Table 5.5), thus requiring further confirmation of all presumptive *Aeromonas* spp. However, based on the information given by Chauret et al. (2001) about confirmed positives, it is not clear whether what is listed in Table 5.2 as false positives are *Aeromonas* spp. other than *A. hydrophila*, which is the one of main interest in water.

5.3.7. *Mycobacterium* spp.

Mycobacterium spp. are acid-fast organisms that are fairly ubiquitous in the environment. *M. avium* is one of the model mycobacterial species that are encountered in water, although other species may be even more prevalent (Le Dantec et al., 2002). It is also fairly prevalent in soil and has been identified as an opportunistic pathogen, affecting immunocompromised individuals (Norton et al., 2004). Available data show that it can occur in drinking water at densities of 1 to 10^3 CFU/100 mL and can grow in water samples to which no additional nutrients have been made available (George et al., 1980). The report by Norton et al. (2004) indicates that it can also grow over a wide range of temperatures (namely, 15 to 45 ^{o}C). *Mycobacterium* spp. are quite resistant to disinfection (Le Dantec et al., 2002), possibly as a result of their peculiar cell wall, which is composed of mycolic acids.

Their lipid cell wall is also believed to enable them to readily colonize surfaces because of enhanced hydrophobicity (Patti and Hook, 1994). Enumeration of *Mycobacterium* spp. on Middlebrook 7H10 agar with aleic/glycerol enrichment (namely, M7H10+OADC) has been successfully used to isolate this organism from water, albeit with a long (21 days at 37 ^{o}C) incubation (Eaton et al., 2005; Norton et al., 2004). Its abundance in those experimental distribution systems largely depended on the type of pipe material used, namely, iron, galvanized metal, copper, or chlorinated polyvinyl chloride (PVC). Although chlorination in this experimental system reduced HPCs, particularly in instances where copper pipes were used, it led to an increased recovery of *M. avium* complex (Figure 5.7).

Table 5.5. Occurrence of *Aeromonas* spp. in Water and the Need to Conduct Confirmation Tests for Presumptive Isolates

Sample	No. of Samples			% Total Samples with Confirmed *A. hydrophila*	% False Out of the Total Presumptive[a]
	Total	Presumptive *Aeromonas* spp.	Confirmed *A. hydrophila*		
Plant intake (raw water)	24	24	18	25	25
GAC-filtered water	22	19	11	50	42
Plant effluent	23	1	0	0	100
Distribution bulk water	60	7	0	0	100
Distribution biofilm	26	11	2	7.7	82

[a]Percent false-positives computed based on data from Chauret et al. (2001).

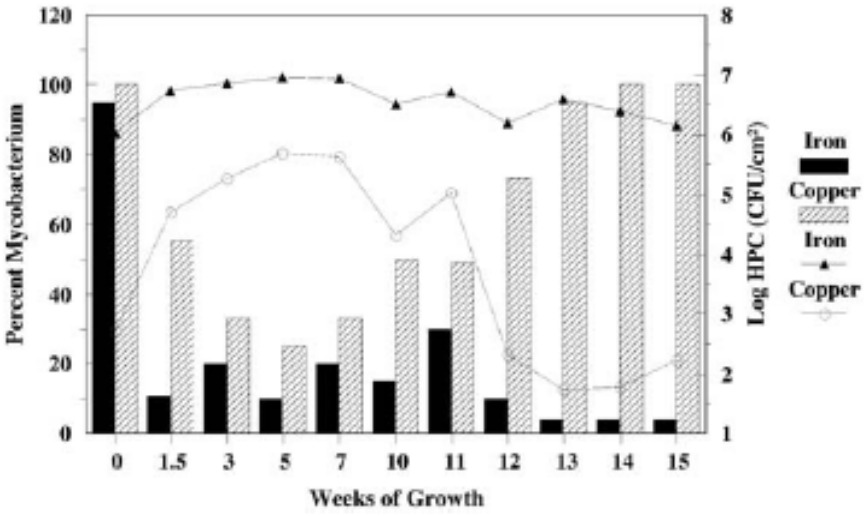


Figure 5.7. HPC (lines) and *M. avium* complex (bars) recovery in copper and iron pipe distribution surface biofilms in an experimental system (source: Norton et al., 2004, with permission from Elsevier).

A recent study by Whittington et al. (2005) showed the continuous presence of *M. avium* subsp. *paratuberculosis* in water troughs kept in a shaded area for 20 weeks. No *M. avium* subsp. *paratuberculosis* was detected in the water after that duration until another 16 weeks. As a plausible explanation of the temporary disappearance and re-emergence of *M. avium* in the water, the authors of that work acknowledge that the water used contained protists that they did not monitor but suspect to have encysted the *M. avium*, later releasing it. This theory is an interesting contention and, together with the known fact of *Legionella* spp. being

protected by protozoa (see next section), justifies looking at other organisms, notably protozoa, besides bacteria in reclaimed water to understand the varied drivers of bacterial regrowth. In terms of clinical impact, it is associated with paratuberculosis or Johne's disease and has also been suspected as the etiological agent for Crohn's disease in humans (Herman-Taylor, 2001; Quirke, 2001).

Other bacteria of interest in reclaimed water include iron bacteria and sulfur bacteria. They will be discussed in the next section as their presence is very much related to corrosion, a physical process that is driven by both chemical and biological events. Cyanobacteria and algal growth increase the AOC and reinfection by vermin and pests, particularly in open storage tanks, and will be discussed later in that context.

5.3. ALGAE AND CYNOBACTERIA

Algae are large, morphologically and physiologically diverse organisms with chlorophyll and the ability to conduct O_2-evolving photosynthesis. Cyanobacteria, also known as blue-green algae or blue-green bacteria, are favored by warm, stable, and nutrient-enriched waters and may constitute an important part of the phytoplankton community in wastewater (Vasconcelos and Pereira, 2001). Algae range from single-celled forms to aggregations of cells or filaments. Many of the unicellular algal forms are motile and can be easily mistaken for protozoa. They are able to grow in areas that are low in carbon, but they have to have light and water. Thus, algae are found throughout the photic (light) zone of bodies of water. Using the energy produced in photophosphorylation, algae convert CO_2 in the atmosphere into carbohydrates and generate O_2 as a by-product.

Most algal growth is not limited by carbon but rather by both nitrogen and phosphorus. Both of these nutrients are typically abundant in reclaimed water. Thus, reducing nutrients does not carry much practical significance as a strategy for controlling algae in reclaimed water. Excessive growth of algae (namely, algal blooms) can cause a condition called eutrophication (Jjemba, 2004). In the long run, the excessive algal bloom is detrimental because when the algae die, the decomposing algal cells not only deplete some of the dissolved oxygen in the water but also increase the TOC, BDOC, and AOC. Degradation of the reclaimed water by algae may increase the need to re-treat the water in order to ensure its desired end use quality, boosting costs. Treatment could be through filtration or by a chemical process. Chitosan has been shown to effectively coagulate some algal species (Chen et al., 1998; Divakaran and Pillai, 2002). Chitosan is a natural coagulant derived from shrimp shells. However, its efficacy largely depends on the water pH, with the most effective removals registered under neutral-to-alkaline-pH conditions. The costlier use of MF membranes has also been shown to effectively remove algae, with average removal of >6 log units (Parker et al., 1999).

CHAPTER 6

PHYSICAL AND CHEMICAL FACTORS THAT AFFECT RECLAIMED WATER QUALITY

The potential presence of pathogens in reuse water is typically assessed indirectly by looking at turbidity, suspended solids, and the density of indicator organisms such as coliform bacteria. Indicators have already been discussed in the previous section, and this first section will focus on the physicochemical parameters. In that regard, Zhang and DiGiano (2002) examined two water distribution systems that supply two neighboring cities, namely, Raleigh (NC) and Durham (NC), from the same water source. They found several similarities in water parameters, namely, HPC (<1 CFU/mL) as well as NO_3-N and NO_2-N levels. Apparent differences in the systems included mostly physical and chemical parameters such as temperature (22 and 17 °C, respectively), pH (8.1 versus 7.1, except if the former used free chlorine as opposed to chloramines), Cl_2 (4 versus 1.9 mg/L), AOC (120 versus 110 µg/L), NH_3-N (0.66 versus 0.01 mg/L), and PO_4-P (0.01 versus 0.02). They used these similarities and differences to decipher the factors that influence bacterial regrowth in the distribution system, clearly demonstrating that physical and chemical factors can be quite crucial in determining the biostability of reclaimed water. Those results are summarized in Table 6.1 underneath with HPC abundance as the basis for determining which parameters were important in the two systems that they investigated. Other parameters of significance in both distribution systems were water residence time and pH. Nutrients in the water were not significant to HPC growth, possibly because they were not limiting.

Table 6.1. Relationship between HPC and Various Water Quality Parameters in Durham and Raleigh Distribution Systems[a]

Value for System Used:					
Durham Distribution System			**Raleigh Distribution System**		
Parameter	***n***	***r***	**Parameter**	***n***	***r***
Chlorine	159	-0.74***	Chloramine	140	-0.63***
Water residence time	159	0.46**	Water residence time	140	0.55***
Temp	150	0.27**	AOC	89	-0.34**
AOC	107	-0.21**	pH	140	-0.29**
pH	140	0.16	Temp	140	0.25*
NO_3-N	159	-0.15	TOC	140	-0.19*
NH_3-N	158	0.04	NO_2-N	139	0.17*
TOC	159	-0.04	NH_3-N	140	-0.13
NO_2-N	147	0.02	NO_3-N	139	0.13
PO_4-P	156	-0.01	PO_4-P	134	-0.1

[a] *n* = number of observations; *r* = Pearson correlation coefficient. Asterisk indicates significance at the 0.0001 (***), 0.001 (**), or <0.05 (*) level (source: Zhang and DiGiano, 2002, with permission from Elsevier).

The results in Table 6.1 clearly show that microbial growth results from a combined influence of several physicochemical parameters that have to be identified and included in models that are designed to predict regrowth. Based on those results, Zhang and DiGiano (2002) used a general linear model to relate the significant parameters as

$$HPC = kC^{a}T^{b}AOC^{c}pH^{d} \qquad \text{Equation 5}$$

where k = model constant; C = concentration of the disinfectant; T = temperature; and a, b, c, and d are the exponents of the independent variables determined by using least-square regression analysis. Further analysis of those data generated a final model shown in Equation 6 for Durham and 7 for Raleigh.

$$HPC\ (CFU\ mL^{-1}) = 5 \times (\text{concentration of disinfectant in mg } L^{-1})^{-1.89} \qquad \text{Equation 6}$$

$$HPC\ (CFU\ mL^{-1}) = 0.00062 \times [\text{disinfectant concn}]^{-1.92} \times [T \text{ in } {}^{o}C]^{-1.92} \qquad \text{Equation 7}$$

Disinfectant concentrations in both equations 6 and 7 are in milligrams per liter, whereas T is temperature in degrees Celsius. The general information about how each of these parameters can influence the quality of reclaimed water on a regular basis is discussed underneath.

6.1. TEMPERATURE

Temperature is perhaps one of the most important factors influencing the regrowth of microorganisms in distribution systems. It either directly or indirectly affects the rate of microbial growth, rate of dissipation of the disinfectants, rate of corrosion, and water velocity through the system as more usage is associated with higher than with lower temperatures. For example, the utilization of reclaimed water is generally much higher during summer than in winter (Rufenacht and Guibentif, 1997). A survey by LeChevallier et al. (1996) of 31 potable water utilities over an 18-month duration showed a significant difference ($p < 0.0001$) between the occurrence of coliform bacteria in water below 15 ^{o}C and their occurrence in water at temperatures higher than 15 ^{o}C. Furthermore, fecal coliform prevalence in the water at those facilities had a predictable trend, with the lowest occurrence being detected in December to April (namely, winter to early spring) and the peak presence in July to October. The findings clearly suggest a season-based temperature effect as the peaks and trough corresponded with the average monthly temperatures. Zhang and DiGiano (2002) also showed an apparent influence of temperature changes across seasons on the occurrence of heterotrophic bacteria in two distribution systems. In that study, HPCs were at least 1 log unit higher in summer and fall than in winter and spring at two North Carolina-based locations. There is no reason to believe that temperature effects in reclaimed water systems would be greatly different from those reported by LeChevallier et al. (1996) and Zhang and DiGiano (2002) for potable water.

This difference in the abundance of microorganisms in the water at different seasonal temperatures can be accounted for, at least in part, by changes in the rate of biochemical reactions (and therefore growth rate). For any type of bacterium, growth increases with temperature until an optimal temperature, above which growth declines or completely stops. Temperatures in reclaimed water within the distribution system are expected to be similar to those that have been encountered in potable water distribution systems. Based on Shelford's law of tolerance, a minimum and maximum temperature set the "tolerance range" for each organism. Unlike eukaryotes, most prokaryotes have a broad temperature tolerance range. Most of the distribution systems, and indeed the global environment, are within the

mesophilic range (namely, 8 to 48 ^{o}C) although because of seasonal extremes some distribution systems can get into the psychrophilic range (namely, -5 to 18 ^{o}C). Optimal growth for psychrophiles occurs around 10 ^{o}C, whereas that of mesophiles occurs around 35 ^{o}C (Madigan et al., 2000). The optimal temperatures for mesophiles may not be frequently reached in distribution systems but are certainly attainable in storage tanks during the hot months of the year. Most studies of waterborne microorganisms are conducted within the mesophilic range of 20 to 37 ^{o}C. Thus, it looks like those study ranges do not address the ecology of psychrophilic organisms in reclaimed water to a reasonable extent.

An activity-versus-temperature relationship (Q_{10}) has been developed based on the fact that enzymatic activity increases with an increase in temperature, within temperature tolerance limits.

$$Q_{10} = \frac{Specific\ activity\ at\ temperature\ (T_o) + 10°C}{Specific\ activity\ at\ temperature\ (T_o)} \qquad \text{Equation 8}$$

It reflects the changes in enzyme activity owing to increases of 10 ^{o}C. Activity in this instance is measured by monitoring respiration rates. Q_{10} values typically range between 1.5 and 3, although higher values have also been reported (Tate, 2000). Q_{10} analyses have been more extensively used in terrestrial systems (for example, Lloyd and Taylor, 1994; Liu et al., 2006) and rarely in marine environments (Bianchi et al., 1997; Rasmussen et al., 2003) but certainly not in potable or reclaimed water distribution and storage systems. To capture the effects of temperature on water biostability, the sampling intervals should preferably be short enough as not to influence the effects in the succeeding sampling cycle. Thus, quarterly sampling events that represent the four seasons, namely, fall, winter, spring, and summer, should be considered ideal to study temperature effects on reclaimed water biostability.

6.2. DISINFECTANT RESIDUALS

The study reported by LeChevallier et al. (1996) showed that the type of disinfection used affected the frequency of occurrence of microbial regrowth in the water and the prevailing water temperature. With chloramines used as the disinfectant, the percentage of coliform-positive samples ranged between 0.15 and 0.69%, whereas the range was 0.12 to 2.1%, with chlorination as the disinfection process (Table 6.2). Furthermore, the density of coliform bacteria was higher in chlorinated than in chloraminated waters (namely, an average of 0.60 CFU/100 mL versus 0.017 CFU/100 mL; $p < 0.0001$). Highest incidences of coliform with chlorination were associated with temperatures above 15 ^{o}C, whereas the positive occurrence of coliform in chloraminated water was less dependent on the temperature. These differences in the abundance and occurrence of coliforms in chlorinated versus in chloraminated waters were, at least in part, attributed to the differences in residual concentrations. Chloramine residual in the effluent averaged 2.5 mg/L, whereas chlorine was at 1.63 mg/L, with the residual possibly affected by temperature. LeChevallier et al. (1993b) showed that monochloramines reduced the density of bacteria in biofilms by 2 log units.

HPC negatively correlated with the disinfectant residual (Zhang and DiGiano, 2002), suggesting that disinfectant residual is an important factor influencing the growth of bacteria in the distribution and storage system. Thus, a decrease in disinfectant residual can result into an increased growth of heterotrophic bacteria. As a matter of fact, disinfectant residual was the most significant parameter in relation to HPC growth in two potable water distribution systems (Table 6.1).

If anything, improper process control and disinfection can enhance microbial growth in the distribution system. For example, microbial growth is enhanced when there is no biologically active filtration step after ozonation (LeChevallier et al., 1996). In such settings the ozone reacts with organic constituents to produce oxidation by-products that have a low molecular weight and are more polar. These by-products also tend to be highly biodegradable, which can lead to biofouling in the distribution system. A net outcome of such an improper series of events is that the ozone can increase AOC levels, which in turn support more microbial growth as it increases the biodegradable material in the ozonated water (Janssens et al., 1984; Price et al., 1993; Escobar and Randall, 2001; Liu et al., 2002; Lee and Deininger, 2003). The increase in biodegradable materials is primarily because of the oxidation of organic constituents like natural organic matter (NOM). Lee and Deininger (2003) reported an increase in the growth of bacteria after ozonation from the bacterial level in nonozonated water.

Similar increases in AOC in drinking water after chlorination have been reported by Polanska et al. (2005) and are attributed to changes in the structure of the organic matter after ozonation. Biofouling may be reduced if ozone-treated water is filtered, through a biologically active medium such as GAC or a slow sand filtration system (LeChevallier et al., 1996). Taking a cue from drinking water systems, AOC is likely to be increased by some disinfectants although not many studies have documented this possibly.

Table 6.2. Relationship between Water Temperature and the Occurrence of Coliforms in 2 Disinfection Systems across Various Sites[a]

	Values for:								
	Free-chlorinated			Chloraminated			Percent coliform positives		
Range (°C)	No. of Samples	No. of Coliform Samples Collected	No. of Coliform-Positive Samples	No. of Samples	No. of Coliform Samples Collected	No. of Coliform-Positive Samples	Total	Chlorinated	Chloraminated
0–5	48	3438	4	30	1416	9	0.268	0.116	0.636
5–10	92	7485	68	55	4561	7	0.623	0.908	0.153
10–15	108	8727	48	58	3776	15	0.504	0.550	0.397
15–20	97	9772	146	62	5458	12	1.037	1.494	0.220
>20	107	7342	154	169	14037	97	1.174	2.098	0.691

[a]Source: LeChevallier et al. (1996).

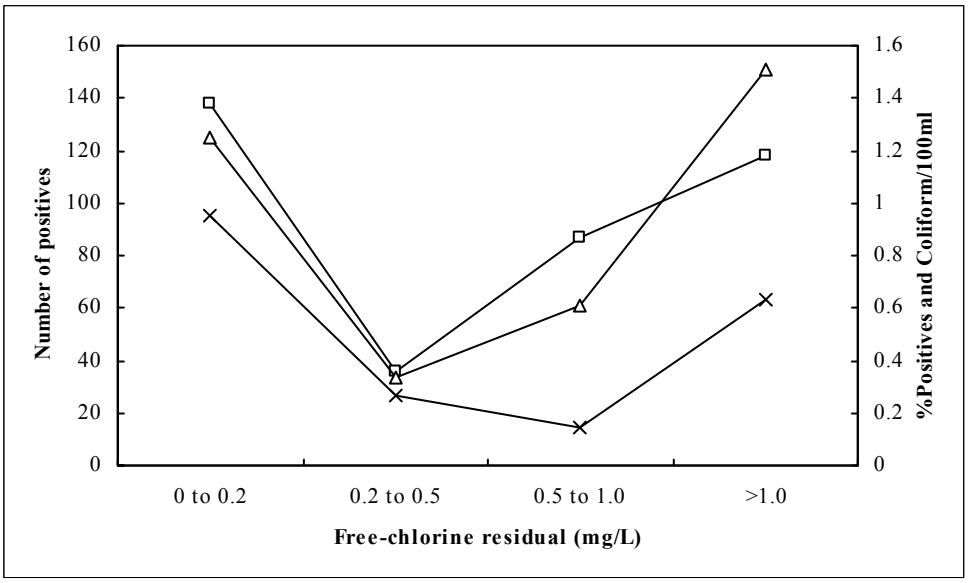
There is a tendency for the disinfectant residual to decrease as the water flows further and further into the distribution system. The decrease is a function of time and also corresponds to the age of the water. Deininger et al. (1992) used fluoride as a tracer to determine the changes in the age of the water in distribution systems. It is noticeable that high levels of disinfectant residual do not necessarily translate into better control of coliform bacteria (Figure 6.1). Thus, there is a U-shaped relationship between disinfection residual and the occurrence of coliform bacteria and also between disinfection residual and the density of coliform bacteria. For the density of coliforms, this relationship was more pronounced with chlorine than with chloramine as a disinfectant. From these results, a minimum residual of 0.5 mg of free chlorine/L or 1 mg of chloramines/L is required for minimal abundance of coliform bacteria in the distribution systems. Chloramines were at an average of 2.5 mg/L, whereas chlorine was at 1.63 mg/L in the effluents. Chloramines are generally believed to provide more stable residual that offers lasting protection than does free chlorine (Zhang and DiGiano, 2002). They also penetrate biofilms more deeply, providing greater inactivation. The U-shaped relationship shown in the figure below strongly suggests that more than one factor, namely, concentration of the disinfectant, is at play in determining the survival and regrowth of bacteria in the distribution system. As a matter of fact, systems with a high concentration of disinfectant also tend to have high levels of AOC, with AOC concentrations of >100 µg/L supporting more coliform-positive samples (LeChevallier et al., 1996). This finding has ramifications, as some plants tend to use excessive amounts of disinfectant with the aim of maintaining a high residual in the distribution system (Narasimhan et al., 2005). Increased distance also lengthens the residence time of the disinfectant residual, owing to an increase in oxidant-demanding reactions providing an opportunity for the existing bacteria to grow. Even though they are more stable in distribution systems than chlorines are, chloramines can also be degraded, and their degradation has both a biological and chemical component. The latter is mainly because of catalysis by NOM (Wilczak et al., 1996; Sathasivan et al., 2005). Because of the importance of nitrification to the degradation of water quality, it is logical to discuss this process in terms of both of its chemical and biological components and how they relate to chloramine residuals, as the two components are not easily separated from each other. Nitrification is a two-step process with ammonia being initially oxidized to nitrite and then ultimately to nitrate (Equations 9 and 10).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \qquad \text{Equation 9}$$

$$NO_2^- + O_2 \rightarrow 2NO_3 \qquad \text{Equation 10}$$

The first step (Equation 9) is facilitated by ammonia-oxidizing bacteria such as *Nitrosospira*, *Nitrosomonas*, *Nitrocystis*, *Nitrosovibrio*, and *Nitrolobus*, whereas the second step is by nitrite-oxidizing bacteria such as *Nitrobacter*, *Nitrospina*, and *Nitrococcus* spp. Thus, ammonia with an oxidation state of -3 is transformed through a +3 intermediate (namely, NO_2^-) to the +5 oxidation state (namely, NO_3^-), an eight-electron difference. Ammonia-oxidizing bacteria and nitrite-oxidizing bacteria can be quantified by using the most-probable-number technique with selective media, but the assay takes 3 to 5 weeks to accomplish (Li et al., 2006). Thus, conventional assaying for nitrifying bacteria is time-consuming and inefficient. The conversion of ammonia to nitrite and nitrate yields energy for the ammonia-oxidizing bacteria and nitrite-oxidizing bacteria and also supports heterotrophic bacteria.

a)



b)

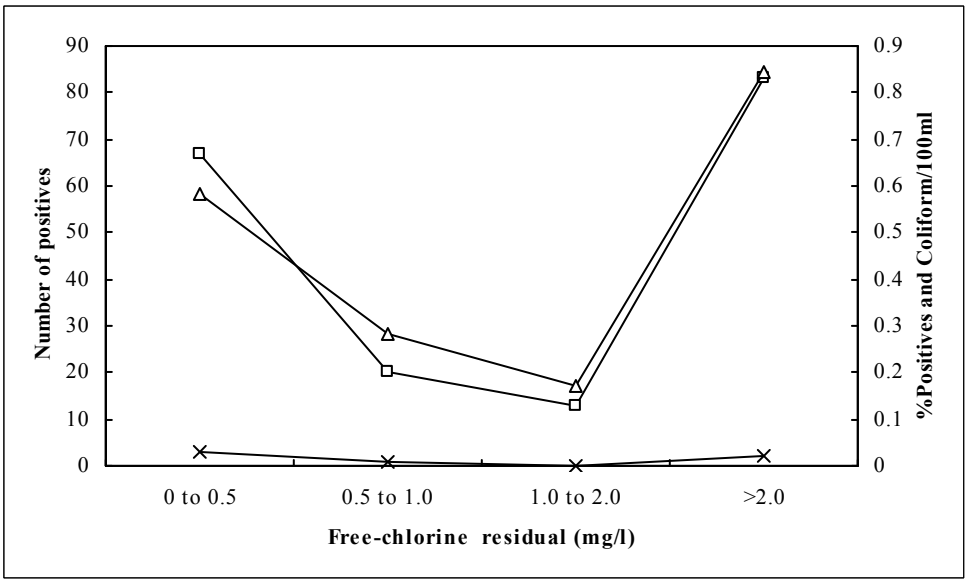


Figure 6.1. Coliform occurrence with (a) chlorination or (b) chloramination after different residual disinfectant concentrations. □ Number of positives, Δ % positive coliform, and × coliforms/100 mL (based on data from LeChevallier et al., 1996).

Once these autotrophs proliferate, they release organic carbon that can serve as a substrate for heterotrophs (Watson et al., 1987). Thus, systems that experience nitrification tend to have elevated HPC and increased concentrations of both nitrite and nitrate. A high concentration of both nutrients tends to make the plants less likely to meet water quality rules and guidelines. Nitrate and nitrite concentrations can affect the regrowth of microorganisms in reclaimed water. Despite this possibility, none of the existing state or federal guidelines/regulations account for the possibility of nitrification in the distribution system.

From the above discussion, therefore, it is apparent that the use of chloramines as disinfectant can be problematic along the way, promoting nitrifying bacteria. Nitrifying bacteria secrete organic compounds, which can in turn stimulate growth. Nitrification can occur under a wide range of pHs (namely, 6.5 to 10) and temperatures that are above 15 ^{o}C. However, nitrification has also been reported to occur at temperatures that are lower than 15 ^{o}C, albeit much more slowly (Wilczak et al., 1996). Sathasivan et al. (2005) recently published a method for separating the two components of the nitrification process by monitoring the concentration of chloramines in parallel water samples that were not filter-sterilized or were sterilized through a 0.2-μm-pore-size filter.

6.3. STORAGE DURATION AND FREQUENCY

Long storage times can allow the existing bacteria to grow and acclimate themselves to the prevailing conditions (for example, pH, temperature, nitrate-N, nitrite-N, etc.). Long storage times also enable sedimentation to build up and ultimately enhance the formation of biofilms. Information about the number of storage tanks versus miles of distribution pipes is useful in predicting the quality of the water. A high incidence of coliform bacteria was associated with a high occurrence of storage tanks in the system (LeChevallier et al., 1996). As water treatment proceeds, the bacteria that survive treatment switch from metabolically active to inactive with such activity resuming only after favorable conditions return. Studies in *E. coli* show the presence of effective mechanisms by which survival during long-term storage occurs (Death and Ferenci, 1994; Notley-McRobb et al., 1997). These same mechanisms may be at play in a number of bacteria other than *E. coli*.

LeChevallier et al. (1992) estimate that a ratio of the number of miles of the distribution pipeline to the number of storage tanks of less than 100 indicates proportionately many storage tanks that can provide an opportunity for the disinfectant residuals to dissipate much faster: a prerequisite for bacterial regrowth to occur. Right after the final disinfection, water is presumed to have an age of zero. However, its age increases as the water is either in storage or in the distribution system. Tracers such as fluorides have been used to estimate the age of the water at various points in respective distribution systems (Deininger et al., 1992; Lee and Deininger, 2003). More recently, Zhang and DiGiano (2002) described, by using both a negative and a positive step, the input of a chemical tracer process for determining the age of the water. In their work, the new water in the system is defined by:

$$\tilde{Y}(t) = 1 - \frac{[T]_t - [T]_{New}}{[T]_{Old} - [T]_{New}} \quad \text{for negative step input, and} \quad \text{Equation 11}$$

$$\tilde{Y}(t) = \frac{[T]_t - [T]_{Old}}{[T]_{New} - [T]_{Old}} \quad \text{for positive step input,} \quad \text{Equation 12}$$

Where $[T]_{Old}$ is the tracer concentration before the step input, $[T]_{New}$ is the tracer concentration after the step input, and $[T]_t$ is the tracer concentration measured at the sampling station. The negative step input in the above equation refers to the duration that it takes for a particular tracer chemical (for example, chlorine, ferric chloride, etc.) concentration to fall to zero at various sampling points that are a known distance away from the point of application after the use/addition of that chemical in the treatment process is discontinued. Similarly, positive step input is the duration it takes for a particular tracer chemical (for example, fluoride, alum, etc.) concentration to be detected at various sampling

points that are a known distance away from the point of application after the use/addition of that chemical in the treatment process is initiated. The distance from the point of treatment (or cessation of treatment) is used in a temporal fashion to determine the duration. Thus, the sampling station in this instance depicts a function of time.

Lee and Deininger (2003) investigated the growth potential of bacteria in water. In that study, water of different ages was sampled from three different locations in the distribution system (Table 6.3) and its HPC was determined. It was then incubated at 20 ^{o}C for 3 days and aliquots taken to determine the HPC again. The 3-day incubation period was adopted, as it had been established in preliminary studies as the duration it takes for bacterial growth in the water to reach the stationary phase. Growth potential was calculated from the relationship:

$$GP = \text{Log [HPC after 3 days/HPC at time zero]} \qquad \text{Equation 13}$$

Those studies showed an increase in turbidity as the water flowed through the distribution system. HPC was also increased in the mid-portion of the distribution system. The growth potential decreased in the samples that were obtained from nearest the finished water but steadily increased as the water moved through the distribution system. The other parameters such as pH, temperature, TOC, and BDOC did not change during this duration. The growth potential was highest in the water that was obtained farthest from the finished water in the distribution system. However, the rate of growth or indeed the density of bacteria in the water obtained from the farthest part of the distribution system after 3 days was not correlated with the initial bacterial populations in the time-zero water. Similarly, the increase could not be explained by the TOC and BDOC concentrations as they barely changed. This finding gives some validity to the argument that regrowth is controlled by a multitude of factors. A most likely factor, which apparently was not considered by Lee and Deininger (2003), is the fraction of organic matter that is assimilable (namely, AOC). Contributions of AOC to microbial growth in water are discussed in Section 6.7.1 ahead.

Table 6.3. Changes in Key Parameters in Drinking Water at Different Sampling Locations in the Distribution System[a]

	Location or Water Type			
Parameter	**Treated Water**	**Close to Treated Water**	**In Mid-Portion of Distribution System**	**Farthest from Treated Water**
Water age (h)	0	20	55–61	>148
Temperature (^{o}C)	13	12.3	12.7	12.8
pH	8.8	8.7	8.7	8.7
Dissolved oxygen (mg/L)	9.3	9.2	9.2	9.2
Conductivity (S/m)	44.3	45.1	44.5	44.6
TOC (mg/L)	3.8	3.7	3.8	3.7
BDOC (mg/L)	1.4	1.3	1.4	1.6
Turbidity (NTU)	0.06	0.18	0.18	0.2
HPC (CFU/mL)	66	10	152	13
Growth potential	2.8	0.6	2.8	3.8

[a]Ozone was used as a disinfectant for the second half of the year in which the study was done.

Variations in water pressure and velocity within the distribution system also lead to the deterioration of water quality as it provides an opportunity for bacterial biofilms to detach, resulting in enhanced regrowth. Tokajian and Hashwa (2004) found that HPCs in the storage tank were more significantly impacted within the 1st day of filling the tank. The HPCs in a cast-iron (log 3.66/mL) storage tank and in one made of polyethylene (log 3.69/mL) did not differ significantly when averaged for the whole duration of the study. However, the HPC was significantly higher in both tanks in summer than in winter, signifying the need to study regrowth trends in distribution and storage systems across seasons. The length of storage of the water in the tank was also quite important in determining the abundance of HPC bacteria in the water. Production by suspended bacteria in a flowing water body is believed to be much lower than that by bacteria that are in biofilms or attached to the surface of the distribution system (for example, pipes) and existing debris. Low production also represents a lower incidence of cell division.

6.4. DISSOLVED OXYGEN

The water within the distribution system contains some dissolved oxygen, the levels following a gradient. Thus, the distribution system extremities that have a low water flow rate also display lower in situ oxygen levels of 7.2 mg of dissolved O_2/L or less (Ridgeway et al., 1981). Dissolved oxygen is an important water quality parameter. Low dissolved oxygen in the water (especially at higher temperatures) is indicative of biological activity in the water (Wilczak et al., 1996). A more informative and easily measurable parameter to reflect oxygen consumption dynamics in reclaimed water is the BOD. BOD represents the oxygen that is required by microorganisms for respiration as they consume the existing substrate. It is simply measured by taking two water samples from the same source, determining the dissolved oxygen in one of the samples and incubating the other sample in the dark for 5 days. The dissolved oxygen in the incubated samples is thereafter determined after 5 days. The difference between the dissolved oxygen at the time of sampling and after the 5-day incubation period is the BOD_5. Obtaining such paired samples along a distribution line and determining the BOD_5 as described above would give a clear quantitative assessment of the biological activity in that water system as the BOD changes with time and distance in the system, with oxygen being used up to oxidize any utilizable substrate that may be present. As the substrate concentration decreases, the oxygen demand also decreases. Clean water should have a BOD of zero. Figure 6.2 shows the apparent increase in the density of coliforms with increasing BOD in the waters of a river in Croatia. Hills et al. (2005) evaluated a single house grey water recycling system in Aylesbury (U.K.). BOD in the water in the five houses evaluated ranged between 22 and 87 mg/L over the 1-year study.

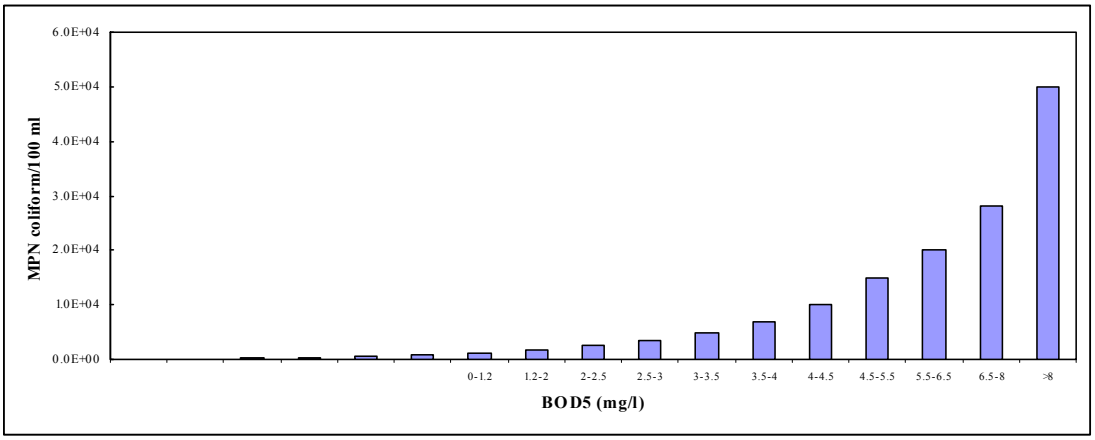


Figure 6.2. The abundance of coliforms in natural water as a function of BOD. The figure is based on data by Štambuk-Giljanović (1999). Notice the similarity of that relationship to that of coliform level with levels of both total N and total P in water, i.e., Figures 6.3 and 6.4.

6.5. pH

Extreme pH values of ≤5 or ≥9 are harmful to most organisms and can hinder microbial growth. The prevailing pH also impacts the quality of reclaimed water as it directly affects the chemical disinfection processes. For example, with chlorination, which has been more widely used as a disinfectant, the chlorine, added as either chlorine gas or hypochlorite salts such as sodium hypochlorite or calcium hypochlorite, exists as hypochlorous acid (pK_a = 7.6 at room temperature). Thus, above a pH of 7.6, it exists as a hypochlorite ion (OCl^-), and below a pH of 7.6, it exists as hypochlorous acid (HOCl). The HOCl is more biocidal than OCl^- (Kim et al., 2002). Thus, determining the pH in the effluent, storage tanks, and the distribution system can enable one to gauge the status of disinfectant residuals and the associated regrowth. Higher pH levels (for example, 8.3) can improve the stability of chloramine residuals, which can in turn suppress nitrifying bacteria, preventing nitrification.

6.6. SEASONALITY

The effects of seasonal variation can be viewed in terms of changes in UV light, which in turn may affect the efficacy of disinfection and/or regrowth in storage reservoirs as well as changes in physicochemical characteristics such as water temperature. If the storage system is an open reservoir, the biostability of the water is also impacted by UV light. Thus, a wide range of organic compounds and some heavy metals have been shown to succumb to phototransformation by UV light in surface water (Lerch et al., 1995; Stangroom et al., 1998; Wurl et al., 2000; Tixier et al., 2002). Photooxidation of the existing compounds can be somewhat enhanced in the presence of humic substances because of the hydroxyl radicals generated by the humates (Tixier et al., 2002). However, photooxidation may also generate some undesirable products in the surface water and even provide ideal conditions for the growth of algae and cyanobacteria. Where algal growth is elevated, the occurrence of algal toxins can be a major issue of concern. Algal toxins are known to adversely affect the liver (hepatotoxins) and nervous system (neurotoxins), causing death in some instances to pets, livestock, and even humans if they come in contact with the alga-contaminated water.

However, it is important that not all algae produce toxins and not all toxin-producing algae always produce toxins. For toxin-producing algae and cyanobacteria, toxin production is influenced by temperature, with optimal production occurring under warm (namely, 20 to 25 ^{o}C) conditions (Gunnarsson and Sanseovic, 2001).

Seasonality can also affect the residence time of the water in the distribution system. The residence time is expected to be shorter during the hot months of the year when water use is highest. This change in turn would affect the extent of regrowth that is likely to occur. Thus, understanding the factors that may influence regrowth should at a minimum include sampling across different seasons to indirectly account for the changes in demand (and supply) of the reclaimed water.

6.7. NUTRIENTS

Unlike in terrestrial systems, the supply of nutrients in aquatic environments is continuously coupled with the ability of microorganisms to turn over the existing nutrients quite rapidly. The high turnover rates are a result of the fact that microorganisms tend to have much shorter generation times than do macroorganisms. Thus, it is imperative to look at nutrient dynamics in distribution systems in a geochemical cyclic fashion, with the cycles being "driven" by microorganisms. Just like terrestrial systems, aquatic distribution systems are also comprised of organic and inorganic material influencing the proliferation of the existing organisms. Thus, nutrients, particularly carbon, nitrogen and phosphorus, play an important role in the regrowth of bacteria in reclaimed water.

Organisms are either oligotrophic or copiotrophic. Oligotrophic environments are defined by low nutrient flux in the range of a fraction of 1 mg of C/L per day (Cavicchioli et al., 2003). Environments such as drinking water are fairly oligotrophic, supposedly with low levels of organic carbon and possibly phosphorus (LeChevallier et al., 1993a; Miettinen et al., 1997; Lehtola et al., 2001). Oligotrophic environments have low nutrient fluxes that are only about 1 to 15 mg of soluble carbon L^{-1} (Poindexter, 1981). Oligotrophs are believed to have an extraordinary ability to scavenge for nutrients efficiently. Thus, despite the low levels of nutrients in such environments, microorganisms on the order of 5×10^4 to 5×10^5 cells mL^{-1} can persist in such oligotrophic waters (Cavicchioli et al., 2003). By comparison, most reclaimed water may be copiotrophic and therefore more suitable to coliforms, as coliform bacteria are copiotrophic, routinely requiring high nutrient concentrations. Copiotrophs are associated with nutrient-rich environments and are generally adapted to using the available resource quite rapidly when such resources are available (Koch, 2001). However, it should be emphasized that copiotrophs can also adapt and survive under poor nutritional environments. Studies with *E. coli* have shown that the synthesis of cyclic AMP reaches a peak prior to the onset of starvation and remains constant for several days during starvation (Death and Ferenci, 1994; Notley-McRobb et al., 1997). It is not clear whether this mechanism is applicable to other coliforms (and to copiotrophs in general), but it would be worth investigating certainly as part of understanding the regrowth of bacteria in water. It is also important that, unlike a chemostat, a reclaimed water distribution system does not remain constant in terms of nutrient status and that, thus, the existing organisms undergo periods of boom and depletion.

It would be expected that all bacterial cells within a particular aquatic environment have similar metabolic activity as they are exposed to similar types and concentrations of nutrients. However, various studies have directly (Lebaron et al., 2001) or indirectly shown this theory not to be the case. Thus, in any environment, there are some cells that are nonculturable as

they are dormant or viable but nonculturable. Although there are apparent discrepancies between the microbial diversity that is displayed by culturing and that which results from molecular techniques, understanding the physiology of regrowth of bacteria in water distribution necessitates isolating the existing organisms. Bruns et al. (2002) showed an increased cultivation efficiency of heterotrophic bacteria in water by providing cyclic AMP and acyl homoserine lactones. However, some oligotrophs are obligates and their cultivation is greatly sensitive to nutrients and thus to culturing on media. The sensitivity, summarized by Cavicchioli et al. (2003), has been attributed to:

(i) intolerance of high nutrient concentrations,
(ii) an absence of specific growth promoters (for example, vitamins),
(iii) inactivation of the cells by neighboring antagonists on plates,
(iv) deleterious effects by lytic phage,
(v) susceptibility to oxidative respiratory bursts, and
(vi) susceptibility to outgrowth in the presence of fresh nutrients.

In conventional cultivation systems, a technique called extinction culture has been used to grow organisms that are already present in an environmental matrix (for example, water) but cannot grow in the presence of other abundant organisms (Button et al., 1993). The exact mechanisms by which this growth after extinction occurs is not yet clear but may have some parallels with the regrowth of bacteria in renewed water.

The presence of microorganisms in reclaimed water can be limited if nutrients are limiting, as is displayed by the abundance of a common indicator organism, coliforms (Figures 6.3 and 6.4). Those two figures together with Figure 6.2 demonstrate an interesting relationship among nitrogen, phosphorus, and BOD with regard to the abundance of microorganisms in water. A typical carbon:nitrogen:phosphorus ratio of 100:10:1 is optimal for microbial activity (Zhang and DiGiano, 2002). Reclaimed water typically contains sufficient levels of phosphorus and nitrogen, and the former can travel substantial distances in the distribution systems at a rate that largely depends on the dissolved organic matter. P limitation in water is very rare, and actually its bioavailabilty in the water can be enhanced by some treatment processes such as ozonation, although the mechanisms by which that increase may occur are not entirely clear (Lehtola et al., 2001).

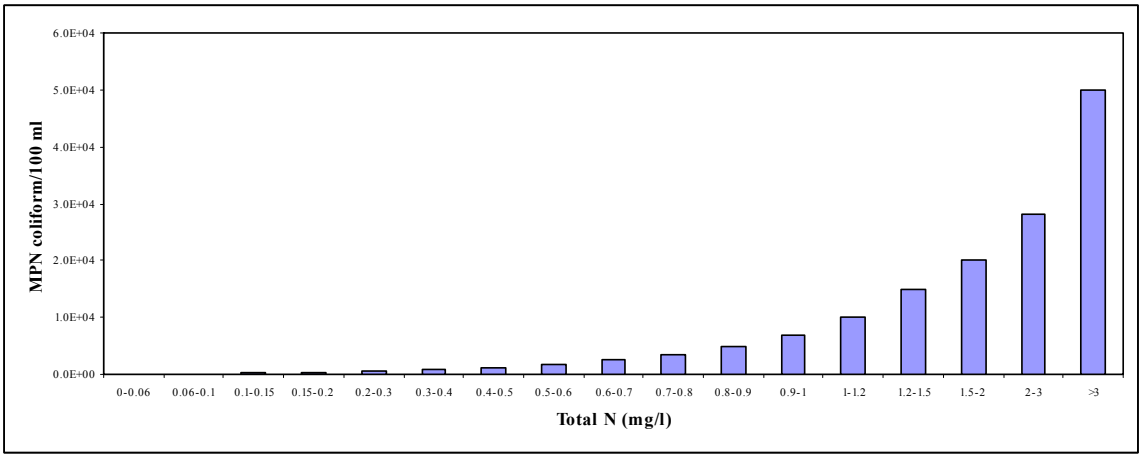


Figure 6.3. Relationship between the abundance of coliforms and total nitrogen in the waters of Dalmatia (Croatia). Figure compiled from data from Štambuk-Giljanović (1999).

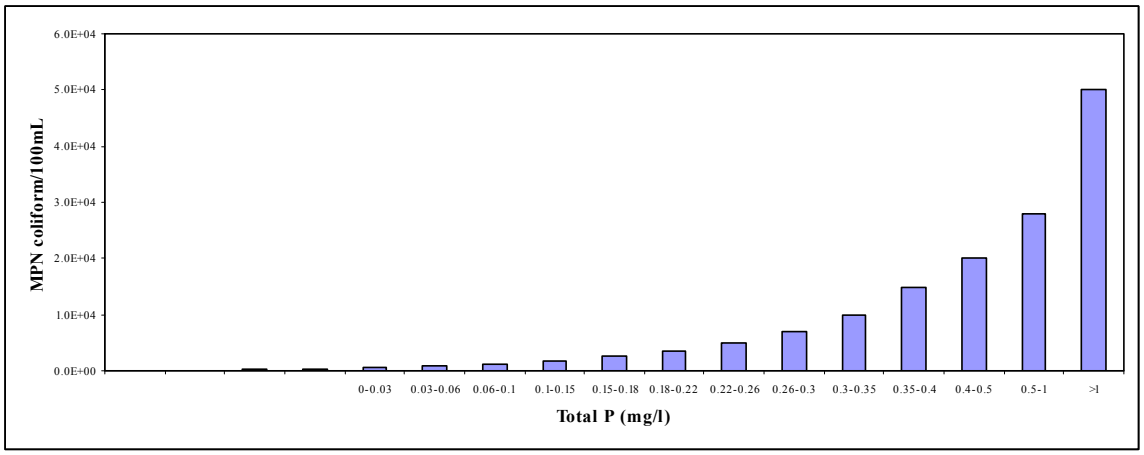


Figure 6.4. Relationship between coliforms and total P in the waters of Dalmatia (Croatia). Figure compiled from data published by Štambuk-Giljanović (1999).

The other key nutrient, namely, carbon, as a factor affecting the quality of reclaimed water is discussed underneath.

6.7.1. Carbon and NOM

Among all the nutrients, carbon is very important because of its relation to energy (and therefore to growth) and its substantial presence in the protoplasm of microbial cells. Thus, its fate in any system also directly affects the fate of the other major nutrients. A key consideration in reclaimed water in relation to regrowth in water would, therefore need a realistic assessment of the existing carbon pools in the water and of how they change. The changes in those carbon pools are actively fully regulated by microorganisms, and high organic carbon levels in distribution systems are associated with bacterial growth. Reclaimed water also contains some detritus and NOM, which is composed of several fractions. As a matter of fact, NOM is found in various concentrations in all natural water sources. It is a complex mixture of compounds that is formed from the breakdown of animal and plant materials, both of which are major components in sewage from which water is reclaimed. NOM spectra show three distinct fractions, namely, (i) carbohydrate, (ii) melanin, and (iii) aromatic rings (Newcombe et al., 1997). Spectral analyses have also demonstated that the nature of NOM in wastewater greatly differs between treatment lots (Hera et al., 2003). Thus, NOM is a complex mixture of dissimilar organic species that are ubiquitous in the environment, including wastewater, reclaimed and potable. However, it can vary greatly from one environment to another. It affects the quality of the water by providing precursor material for disinfection by-products (DBPs) and providing sites for the complexation of heavy metals.

The carbon in the water exists in various forms characterized as TOC, BDOC, and AOC. In water NOM is most commonly represented by TOC, BDOC, and AOC as surrogate measurements, all of which are important substrate indicators for microbial growth in water. Carbon is an essential component of microbial growth and a major ingredient in all metabolic reactions. Other typical surrogates include UV_{254} absorbance and specific UV absorbance (SUVA). SUVA takes into account the concentration of the DOC and is the ratio of UV_{254} to

DOC (in liters/milligram meter). The TOC and related carbon fractions measured in reclaimed water over a 1-year period and how they relate to the microbial densities encountered during that same period are presented underneath. Comparisons to those densities encountered in potable water, which has been more extensively researched, are also made. TOC values for drinking water range between 100 and 25,000 μg/L (Eaton et al., 2005). The upper limit for TOC in reclaimed water has not been exhaustively established but is likely to be even higher than that for drinking water. Previous work by LeChevallier et al. (1991) showed that the occurrence of coliform bacteria in drinking water was increased when TOC was greater than 2.4 mg/L. TOC levels generally tend to be higher in the warmer than in the colder months (Price et al., 1993; Lee and Deininger, 2003).

The fraction of NOM that is biodegradable and can be used by microorganisms for growth is commonly referred to as BDOC (Volk and Chauret, 2002). However, it should be noted that both NOM and BDOC are defined by measurement methodologies rather than by some "inherent chemical quality." Thus, measurement of the BDOC fraction is dependent on the method comprised of different microbial flora. The existing microorganisms are able to use the existing DOC differently. Therefore, BDOC tests measure the fraction of organic carbon in the water that is biodegradable by the existing indigenous microorganisms. In essence therefore, it is the difference in DOC before and after a specific duration. The procedure is performed in several different ways by different laboratories. Servais et al. (1987) measured the change in DOC after a 30-day incubation with an indigenous bacterial suspension. Joret (1988) shortened the duration of the assay by incubating the water sample with 100 g of precolonized sand. The authors reported that the sand method produced levels of BDOC equal to or higher than those yielded by the suspension method. Frias et al. (1992) developed a rapid method for determination of BDOC utilizing a flowthrough column. A water sample is circulated through a glass column containing sintered glass beads on which biofilm bacteria have been permitted to develop. As the water flows through the column, the biofilm bacteria consume the biodegradable organic material. The difference in the inlet and outlet DOC levels is the BDOC. Kaplan and Newbold (1995) further refined the glass column technique by standardizing the flow rate, column size, and contact time. They report that the repeatability of the column bioreactor was greater than 93%. Because the biofilm organisms must take time to adapt to changes in nutrient composition, the researchers found that the columns were sensitive to changes in source waters. Therefore, the method works best if one uses the same source water all the time, a situation that is well adapted for potable water utility application rather than for reclaimed water.

In order to standardize utilizable C-related measurements of growth potential in water, the AOC assay has been used as an alternative. AOC collectively reflects the fraction of labile DOC that is most readily used by bacteria for growth. Unlike BDOC, the AOC test determines how much microbial biomass can be generated by known microorganisms (namely, *P. fluorescens* P17 and *Spirillum* strain NOX) utilizing the DOC. It is composed of low-molecular-weight compounds such as sugars, peptides, fatty acids, and amino acids (Haddix et al., 2004; Hammes et al., 2005) and acts as a surrogate, in addition, for other low-molecular-weight organics that may not be detectable or quantifiable with current analytical techniques. AOC reveals the growth potential of the cells in terms of carbon equivalents. The two organisms used in determining AOC were selected because of the differences in their nutritional capabilities. To determine AOC, the water sample is dechlorinated with sodium thiosulfate, initially pasteurized at 70 ^{o}C, and then spiked with *P. fluorescens* P17 and *Spirillum* strain NOX. Growth of each of the two organisms at room temperature (20 to 23 ^{o}C) is then quantified over a predetermined interval (for example, 3, 4, and 5 days). The maximum cell yield of P17 and NOX is converted to acetate-C equivalents (Eaton et al.,

2005) through the use of a carbon-limited standard curve. However, it is noteworthy that the conditions under which the AOC is determined (for example, growth medium, temperature, etc.) influence the AOC values obtained. For example, the values would be different if R2A rather than TSA was the medium. Similarly, the growth rates of P17 and NOX differed after incubating at 28 ^{o}C from those after incubating at 21 ^{o}C (Hammes et al., 2005) and at 25 ^{o}C versus 15 ^{o}C (LeChevallier et al., 1993a).

Van der Kooij et al. (1982) estimated that AOC levels have to be less than 10 µg/L to limit bacterial growth in water. The BDOC that is quantified by using the AOC method is associated with NOM of low molecular weight (namely, less than 1000 Da) that corresponds to about 16 to 38% of the TOC (Hem and Efraimsen, 2001) and represents only about 0.1 to 9% of the total DOC (Van der Kooij and Hijnen, 1985; cited by Narasimhan et al., 2005). The use of single and pure bacterial strains, namely, *P. fluorescens* P17 and *Spirillum* strain NOX in determining AOC, has some limitations that have been highlighted by several research groups (for example, Hammes and Egli, 2005). Most notable of these is that it is labor-intensive, tedious, and reliant on pure bacterial strains that have been conditioned to grow on a single compound rather than on the complex growth milieu that microorganisms encounter in the natural environment. To address some of these criticisms, some modifications such as monitoring growth by flow cytometry (Hammes and Egli, 2005) or using ATP determinations as an indicator of metabolic activity (LeChevallier et al., 1993a) have been suggested. Another significant modification is the use of genetically engineered, luminescent derivatives of the standard P17 and NOX (Haddix et al., 2004). This assay is unique because the AOC quality of the water is determined through measurements of growth rate data as opposed to those of growth rate potential. Bacterial luminescence growth rates are monitored by using instrument-based luminometry. This method is more cost-effective and less labor-intensive than previous modifications. Data are converted to AOC using acetate-C and peak bioluminescence conversion relationships.

AOC can be useful in predicting the growth of coliforms and heterotrophic bacteria in water, with HPC growth being limited at AOC levels that are less than 20 µg/L (LeChevallier et al., 1993a). AOC concentrations of <50 µg/L are deemed biologically acceptable in water (LeChevallier et al., 1993a), but the actual AOC value is more informative if it is confirmed that C, and not any other nutrient such as N or P, is the most limiting to microbial growth in the system. LeChevallier et al. (1996) found the normal AOC in drinking water to average 86 µg/L (range, 18 to 189 µg/L; n = 31). More recently, Karim and LeChevallier (2005) reported AOC concentrations of 918, 615, and 505 µg/L at a NY- and two MA-based reclaimed water facilities, respectively. Those AOC concentrations in the reclaimed water are five to nine times higher than what is typically encountered in drinking water and suggest a high propensity for bacterial regrowth in reclaimed waters. Data based on quarterly sampling of reclaimed water are presented in Table 6.4 and confirm the high concentrations of AOC in such waters. From that study, it is also clear that such high AOC concentrations are associated with an abundance of heterotrophic bacteria. In most instances at all the five utilities sampled, the density of heterotrophic bacteria increased in the distribution system compared to what was detected in the treatment plant effluents. Total coliforms also increased in some instances but not to the same extent as heterotrophs, possibly because of competition for the AOC.

Table 6.4. Coliform and HPC at a Range of AOC Concentrations in the Treatment Plant and Reclaimed Water Distribution System[a]

	Values for:					
	Treatment Plant			Distribution System		
Location and Sampling Date	**Coliforms (CFU/100 mL)**	**HPC (CFU/mL)**	**AOC (µg of C/L)**	**Coliforms (CFU/100 mL)**	**HPC (CFU/mL)**	**AOC (µg of C/L)**
Utility I						
08/22/2002	1	80	774	<1	60	716
11/07/2002	2	37,000	1705	10	1302	535
01/16/2003	<1	40,750	536	1	40,750	387
03/07/2003	<1	24,750	301	<1	75	394
Utility II and IIA						
08/22/2002	NA	10,000	726	128	67,000	NA
10/16/2002	NA	38,000	611	150	67,200	319
01/16/2003	10,400	7975	567	44	193,750	278
02/20/2003	6	3475	472	24	71,750	439
Utility IV						
08/22/2002	<1	200	365	<1	1500	464
11/07/2002	7	131,000	1842	7	34,000	451
01/16/2003	<1	<5	NA	<1	1283	150
03/07/2003	<1	<5	611	<1	>5,000	173
Utility V						
08/22/2002	NA	10,000	726	128	67,000	NA
10/16/2002	NA	38,000	611	150	67,200	319
01/16/2003	10,400	7975	567	44	193,750	278
02/20/2003	6	3475	472	24	71,750	439
Utility VI						
08/22/2002	<1	2	1718	<1	40	220
11/07/2002	NA	NA	NA	NA	NA	NA
01/16/2003	<1	35	492	<1	10	779
03/07/2003	<1	3000	433	<1	<5	916
Geometric mean	**34**	**3500**	**650**	**31**	**6,100**	**380**

[a]NA = not analyzed. Table based on data from Narasimhan et al. (2005).

The information about the sources of AOC and how to control it is still limited. AOC has also been controversial in some studies. For example, Zhang and DiGiano (2002) reported an unexpected relationship between AOC and HPC in the sense that water with lower AOC concentrations in two distribution systems displayed high HPCs and vice versa (Figure 6.5). However, on closer scrutiny, this unexpected relationship is explained by the fact that with a constant supply of AOC into the distribution system over time, the areas with high HPC (namely, high growth) are expected to consume more substrates, which in turn locally reduce the AOC. Similarly, areas with low microbial growth would end up with a higher accumulation of AOC, leading to a negative correlation between these two parameters. This explanation will not hold true in instances where the supply of AOC in the distribution system is not constant, though, and underscores the need for one to develop a comprehensive understanding of the system one is working with (for example, through several sampling events and with a wide range of parameters) rather than relying on a snapshot assessment.

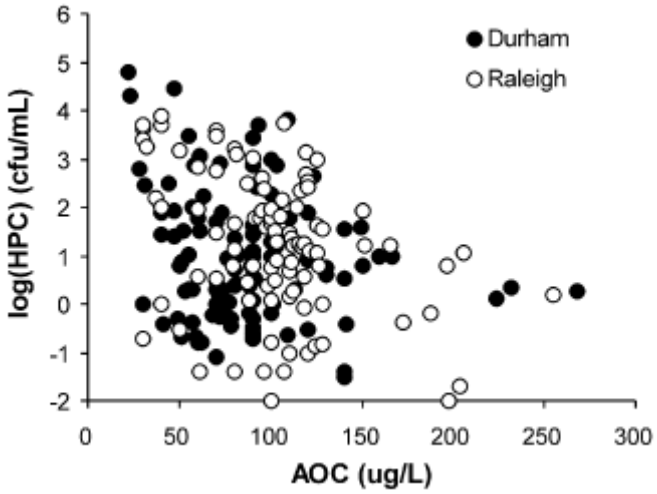


Figure 6.5. Relationship between HPC and AOC in the Durham and Raleigh distribution systems (source: Zhang and DiGiano, 2002, with permission from Elsevier).

6.7.2. Biofilms and nutrient dynamics in reclaimed water

It is widely known that most bacteria in water systems are in biofilms attached to surfaces and piping material (MacDonald and Brözel, 2000; Lazarova and Manem, 1995). A biofilm is a consortium of microorganisms that are attached onto a surface. The attached organisms produce extracellular polysaccharides that enable them to attach to each other and to the surface creating a rich milieu that over time becomes self-sustaining as some cells that die off generate debris and nutrients that support more growth. Such aggregation of the cells increases the resistance to disinfection with chlorine or chloramines severalfold (LeChevallier and Au, 2002). Some of the cells slough off the biofilm and shed into the aquatic system. As a matter of fact a study by van der Wende et al. (1989) showed that most suspended bacteria in drinking water may originate from biofilms. Such sloughing off can result from changes in flow rates, pH, nutrient status, disinfectant concentration, or disinfectant type.

Biofilm formation is governed by at least four main factors, notably:

(i) the deposition and adsorption of both living and dead microorganisms from the aqueous to the solid phase,

(ii) the continued erosion of the biomass by the flowing water,

(iii) growth of the attached microbes at the expense of the available DOC, and

(iv) death of the attached microorganisms.

If the integrity of the biofilm is not disrupted by, for example, sanitization or flushing, the biofilm bacteria are likely to remain in place, but detachment and attachment of microorganisms periodically occur in the distribution system (Figure 6.6).

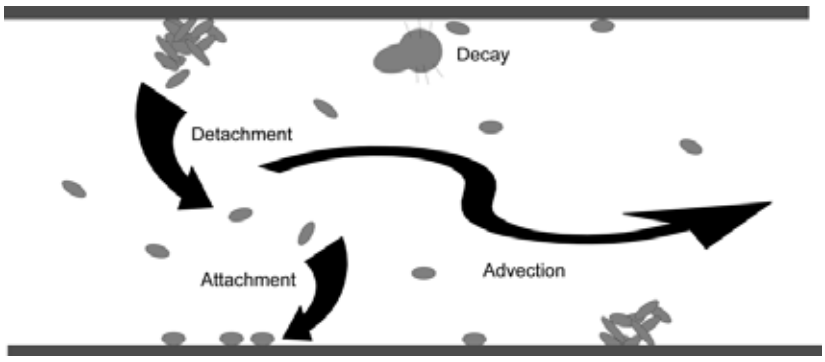


Figure 6.6. Dynamics of microbial attachment and detachment in a typical pipe flow system (adapted from Boe-Hansen, 2002).

Research shows that biofilms are not uniform structures but rather non-uniform, with voids and channels through which nutrients are transported. Sloughing off is effected by increases in shear stress (Choi and Morgenroth, 2003), which in turn influence the morphology of the biofilm. Pang and Liu (2006) noted the presence of different morphotypes of microcolonies within the larger biofilm aggregates, indicating the recruitment of secondary microorganisms in the biofilm development process.

Biofilms are presumed to represent the extent of microbial growth in the water distribution system. Direct microscopy has shown that the architecture of biofilms is very adaptive to the changes in carbon concentrations (Stoodley et al., 1998; Hall-Stoodley et al., 2004) and the nature of available carbon (Pang and Liu, 2006). In the study by the latter group, which is directly relevant to reuse water, biofilm formation in a flow channel fed by secondary effluents (without any additional treatment) was compared with biofilm formations in a flow channel that had been pumped through a biofilter. The biovolume and thickness of the biofilm were lower in the biofilter-treated secondary effluent. Since the effluents were initially identical (namely, initially drawn from the same secondary effluent), the only difference between them being imposed by filtration versus nonfiltration, the differences in biofilm formations were possibly attributable to the biofiltered effluents being less nutritious than the secondary effluent counterpart that had not been filtered. Furthermore, the biofilms formed by the organisms in the nutrient-poorer biofiltered effluents were more open in

structure, maximizing the influx of nutrients into the biofilm. The microbial composition of the biofilms in both treatments, as determined by using florescent in situ hybridization, also changed over time with the initially dominant β-proteobacteria being gradually replaced by the α-proteobacteria. The percentage of *Actinobacteria* also decreased as α-proteobacteria increased (Pang and Liu, 2006).

Pang and Liu (2006) also used T-RFLP to show the selective proliferation of *Aquabacterium* phylotypes and *Legionella* spp. in the biofilms that developed in the distribution system with biofiltered secondary effluents, compared to results for the system that was receiving nonfiltered secondary effluents (Figure 6.7). Those results demonstrated the ability of these phylotypes that are quite physiologically well adapted to poor nutrition to proliferate. Microorganisms that do well under such nutrient-limited conditions also tend to be more metabolically versatile. For example, organisms that can fix their own nitrogen from the atmosphere (for example, *Azospira*, *Azoarcus*, etc.) can have a better edge over nonfixers (Pang and Liu, 2006). Such organisms will still have the ability to grow even in environments where readily utilizable nitrogen such as ammonium and nitrates is limited.

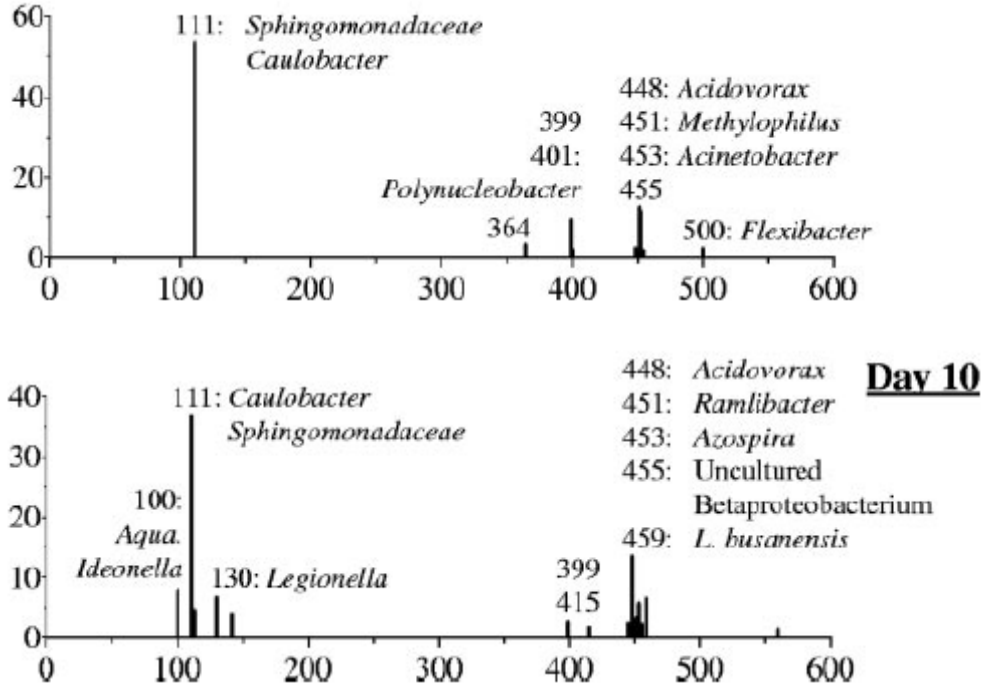


Figure 6.7. T-RFLP profiles from 10-day-old biofilm samples digested with a restriction enzyme (MspI). The relative abundance of each fragment was computed by expressing the associated peak area as a percentage of the total peak area of all fragments. Aqua = *Aquabacterium* (source: Pang and Liu, 2006).

6.7.3. Iron bacteria in bacterial regrowth and pipe corrosion

All types of water have some electrolytic component that gives them some degree of corrosiveness, the extent of the resultant corrosion depending on the physicochemical characteristics of the water and the nature of the corroding material (namely, piping). The rate of corrosion is quantified by using the Larson index, which is primarily the ratio of chloride and sulfates to bicarbonates. Increases in sulfate and chloride ions result in enhanced rates of corrosion and increased levels of soluble iron. Thus, Larson ratios of <1 are recommended as to control corrosion and, in turn, the regrowth of bacteria. Chlorine as part of the disinfectant is a strong oxidant that can increase the corrosion rate of steel. Similarly, monochloramines can increase the deterioration of rubber products. Corrosion products are also suspected to react and inactivate the disinfectant (LeChevallier et al., 1993b). Thus, corrosion of pipes also contributes to the problem of regrowth in distribution systems. The pitting and crevices on corroding surfaces also provide physical protection to the attached organisms against disinfection. Corrosion also reduces the efficacy of disinfection and can interfere with the detection of some pathogens such as *Mycobacterium* spp. (Norton et al., 2004).

Traditionally, corrosion has been depicted as an aerobic process, but it is important that it can occur under anaerobic and anaerobic conditions. On attaching to the distribution system to form biofilms, the bacteria can produce differential aeration cells through which oxygen is depleted in an uneven fashion on the metal surface. As growth continues, the bacterial microcolonies become anodic relative to the portions of the metal that are still exposed to oxygen, generating an electrochemical gradient and thus a current that facilitates further corrosion (Jjemba, 2004). As a matter of fact, the spots where corrosion is occurring can have anaerobes predominate. Under aerobic conditions, oxygen serves as the electron acceptor, forming oxides and hydroxides. Under anoxic conditions, on the other hand, protons become the electron acceptors, yielding H_2 and other reduced products (Equation 14).

$$Fe^0 + 2H^+ \rightarrow Fe^{2+} + H_2 \quad G^0 \approx 0 \qquad \text{Equation 14}$$

The tubercles that are created during corrosion increase the surface area of the pipe, increase hydraulic mixing, precipitate organic compounds, and also offer some physical protection to some of the existing microorganisms in the system against the disinfectant. Corrosion also releases nutrients from the pipe material that support microbial growth (Morton et al., 2005). Electron microscopy shows that the distribution pipe is a complex ecosystem with a variety of environments and a whole range of organisms (LeChevallier et al., 1993b). Micrographs from that study show areas within a pipe that had numerous nodular projections with an array of chemical peaks (for example, Fe, Cu, Si, S, K, and C) adjacent to another smooth area that was rich in iron oxide. Iron-oxidizing bacteria were detected in densities of 2×10^3 to 9×10^3 cells L^{-1} in Neva River water (St. Petersburg, Russia) and were estimated to contribute to about 30% of the corrosion rate in that city's distribution system (Borschevskii et al., 1994).

Gallionella spp. (popularly referred to in the water industry as iron bacteria) have been found in a variety of environments, including soil and aquatic systems. They are characterized by appendages or bacterial stalks, making their detection under the microscope in samples derived from corroded environments easy. However, recent reports indicate that other bacteria that are rod-shaped, other than the conventional appendaged bacteria (namely, bacteria with stalks), can also oxidize iron (Emerson and Moyer, 1997; Hauck et al., 2001). Furthermore, iron-oxidizing bacteria are fairly widespread in the environment, putting in question the common misconception that they are restricted to freshwater and low-temperature environments. However, their proliferation tends to be limited to

physicochemical conditions that have a low redox potential (E_h range, +200 to +320 mV) and a neutral pH (namely, pH = 6 to 7.6). Iron can also be oxidized anaerobically by phototrophic (Heising and Schink, 1998) or chemotrophic (Benz et al., 1998) denitrifying bacteria. *Gallionella* spp. are always associated with iron and are morphologically kidney-shaped mycoplasmoidal cells that are deficient in peptidoglycans. The deficiency in peptidoglycans in their cell wall deprives them of a typical rigid structure (Ridgway et al., 1981). *Gallionella* spp. can proliferate in the distribution system under favorable conditions, discoloring the water owing to the accumulated insoluble iron salts. Such conditions include, among others, the presence of at least 0.2 mg Fe L^{-1}. This accumulation can in turn alter the physicochemical properties of the surface of the pipe and serve as nutritional substrates for various chemoorganotrophic microorganisms. Of course the nature and extent of corrosion will depend on the distribution pipe material. Materials that have been commonly used for pipes include steel, concrete, galvanized iron, and asbestos-concrete (LeChevallier et al., 1993b; Chauret et al., 2001). According to McNeill and Edwards (2001) citing a 1996 AWWA report, the majority of the distribution pipes in the United States are composed of cast iron (38%), ductile iron (22%), and steel (5%).

Many other types of bacteria (for example, *Leptothrix*, *Sphaerotillis*, *Pseudomonas*, *Mycobacterium*, and *Enterobacter* spp.) can utilize ferric ions as electron acceptors under anaerobic conditions, producing soluble ferrous ions. As a matter of fact, a study by LeChevallier et al. (1993b) showed the predominance of *Pseudomonas* spp. in the pipe biofilm, although their abundance was reduced to only 18 to 33% of the population when monochloramine was the disinfectant. The pseudomonads were mostly replaced by *Hydrogenophaga* spp. Besides iron bacteria, other anaerobes such as sulfur-reducing bacteria can also enhance corrosion through the utilization of H_2. Thus, sulfur-reducing bacteria have been detected in various drinking water distribution systems.

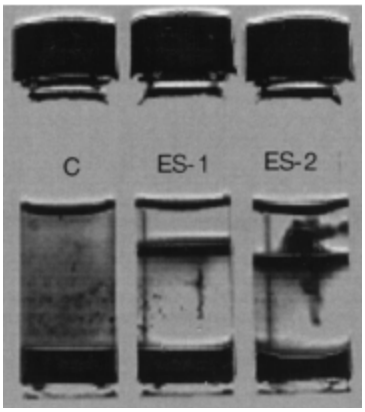


Figure 6.8. Noticeable distinct black horizontal band of iron oxides (from associated Fe-oxidizing bacterial growth) in the semisolid overlay medium in ES-1 and ES-2. The tube on the left is the control (C), which was not inoculated with any bacteria. The black layer at the bottom in all of the tubes is the FeS-agarose plug (source: Emerson and Moyer, 1997).

The well-studied iron-oxidizing bacteria such as *Gallionella* spp. show gradient growth that prospers neither under strongly reducing conditions nor in a highly oxidizing environment but rather between those two extremes. These conditions are quite prevalent in reclaimed water distribution systems, which are also characterized by low dissolved oxygen levels. Iron-oxidizing bacteria can be easily enriched and enumerated by the most-probable-number technique using an anoxic bicarbonate-buffered mineral medium with 1 mM sulfate as the sulfur source (Hauck et al., 2001) using a gradient tube method described by Emerson and Moyer (1997). This approach was initially developed for enriching iron-oxidizing bacteria from sediments but can be modified for our purpose to quantify these organisms in reclaimed water. Under this setup, the slush medium at the top allows a diffusion gradient to form, providing a transition between oxygenated and anoxic environments during the descent from the air medium interface downward into the tube (Emerson and Moyer, 1997; Edwards et al., 2003). Essentially, the bottom of the gradient tube has a synthetic plug which serves as a source of reduced iron (Fe^{2+}) and a reductant to the overlying slush. Briefly, an FeS precipitate prepared as described by Hanert (1992) can be mixed in a 1:1 ratio with modified Wolfe's mineral medium (MWMM) (Hanert, 1992) in 1% (w/v) agarose, forming an iron plug. The iron plug is then covered with an overlay medium containing a 0.15% (w/v) agarose combined with MWMM and vitamins. The tube contents can then be inoculated with a known volume of water (after concentrating by centrifuging for example) to detect and enumerate the iron-oxidizers. When this method is used in sediment studies, typical results obtained are shown in Figure 6.8 above.

With all of the information gaps reviewed in Chapter 2 to this point, a study was conducted to identify the key chemical and physical water quality parameters that influence changes in microbial populations in reclaimed water distribution systems. A secondary objective was to evaluate a novel AOC assay in reclaimed water. The comprehensive results from this study were used to develop guidance for system operators to better understand and control microbiological growth in reclaimed water systems.

CHAPTER 7

MATERIALS AND METHODS

7.1. DESCRIPTION OF THE PLANT SURVEY

A set of four reclaimed water plants were selected for a 1-year intensive sampling campaign. The respective locations are shown in Figure 7.1, and their related characteristics are summarized in Table 7.1. The process flow diagrams for all four locations are presented in Appendix II.

Figure 7.1. Locations in the continental United States where intensive annual sampling took place.

Table 7.1. Characteristics of the 4 Reclaimed Water Sites Studied

	Data for State:			
Variable	**CA**	**FL**	**MA**	**NY**
Treatment Process	Trickling filters with tertiary sand filtration	Activated sludge with Bardenpho and secondary filtration	Single anoxic; MBR (Zenon)	Anoxic and aerobic; MBR (Zenon)
Disinfection	Chlorine	Chlorine	Chlorine	UV/ozone
Storage	Open pond	Open pond	1×10^6-gal tank	20,000-gal tank
NH_3-N[a]	High	Intermediate	Intermediate	Low
NO_3-N[a]	Intermediate	Low	Low	High
P[a]	Intermediate	Low	High	Low
Reuse Type	Irrigation (landscaping)	Irrigation (residential, golf, schools, etc.)	Toilet flushing	Irrigation, toilet flushing, cooling tower

[a]Nutrient classification for the four plants was based on some preliminary survey information from previous studies.

7.1.1. CA

The wastewater treatment plant in CA performs or contains screening, flow equalization, trickling filters, primary and secondary clarification, flocculation, denitrification, filtration, a disinfection system, and two reclamation storage reservoirs. The treatment system has a combined capacity of approximately 235,000 gpd. Treated wastewater is discharged to reclamation storage reservoirs (Figure 7.2) and is used for irrigation by spraying throughout the surrounding residential development.

Figure 7.2. The CA plant site. The panel on the left shows the sampling tube submerged in the storage reservoir (pool), the center panel shows the chlorine contact basin, and the panel on the right shows the pumping station for moving water up to the storage reservoir and out to the distribution system.

7.1.2. FL

The plant has an activated sludge process with 9 mgd at full capacity. It is operated in five-stage Bardenpho advanced wastewater treatment mode with an average flow of 7.7 mgd and runs 24 h a day throughout the year. It has approximately 150 collection system pump stations, and all of the wastewater is primarily processed for reuse for irrigation purposes (Figure 7.3). The process generates about 1650 dry tons of biosolids per annum (>400 semitrailers) that is dried and used as a soil conditioner (namely, fertilizer). Its average operating cost is approximately $1500 per 1 million gal. A schematic of its process flow is presented in Appendix II. The reclaimed water is stored in a 22.45-ft-deep pond that can accommodate approximately 55.85 million gal of usable storage volume.

Figure 7.3. The FL plant. The panel on the left shows the plant from the outer perimeter of the grounds, the center panel shows the clarifier, and the panel on the right shows finished and postchlorinated water. The plant has a total of six clarifiers.

7.1.3. MA

This single anoxic MBR (Zenon) with a nitrification and denitrification system is a 250,000-gpd wastewater treatment plant that was initially designed to expand to 1.1 mgd, a capacity that is attainable during high-flow seasons (namely, full-scale activity and use of the sports stadium that it serves [Figure 7.4]). However, plant management switched the membranes from Zenon to Torray flat plate membranes after our summer sampling. Thus, the fall sample was impacted by that change. It has a 680,000-gal equalization tank with a 3500-gal-per-min submersible lift station. It provides on-site wastewater discharge and recharge to the local aquifer, and the reclaimed water is exclusively used for flushing toilets in the stadium.

Figure 7.4. The reclaimed water facility in MA (center) serves a football stadium (left). The excess reclaimed water is periodically discharged in a vault to contribute to groundwater (right). The holding tank during peak seasons is shown in the center panel, right next to the treatment plant.

7.1.4. NY

The anoxic and aerobic MBR (Xenon membrane; Figure 7.5) facility is located on Manhattan Island (New York City) within the basement of a 293-unit high-rise building. It generates 25,000 gpd of reclaimed water, of which 9000 gpd are for toilet flushing and 11,500 gpd are for the cooling tower. The rest of the water is used for landscape irrigation. Overall, the building uses 50% less potable water than do other high-rise buildings of the same size. Its MBR system uses 35% less energy overall and 65% less energy at peak demand.

Figure 7.5. A high-rise apartment in NY that meets all its toilet and landscaping needs with reclaimed water. The treatment plant is located in the basement (center), and the finished water is disinfected with UV/ozone treatment.

7.2. SAMPLING PLAN AND ANALYTES

At each location, the reclaimed water was sampled from the plant effluent, storage tank (or pond), and three points in the distribution system on four consecutive days in winter (December 2006 to March 2007), spring (March to May 2007), summer (June to August 2007), and fall (September to October 2007). The water temperature and conductivity were

instantly determined on site by using a portable probe (SympHony SP80PC). The water pH was determined by using a portable Hach meter (HQ40d). Both probes were operated as described by their manufacturers. Disinfectant residuals were measured as free and total chlorine according to Standard Method 4500 - Cl G. AOC, BDOC, and TOC samples were collected in sterile, carbon-free, graduated, borosilicate KIMAX® bottles with black polypropylene caps welded to a PTFE/silicone liner (Kimble/Kontes, Vineland, NJ). All other samples for bacteria and algal analysis were collected in sterile 1-L polypropylene, wide-mouth Nalgene® bottles (Nalge Nunc Corp., Rochester, NY). Prior to shipping to the field, 2% sodium thiosulfate was added to all bottles designated for bacterial analyses and AOC and BDOC determinations to quench residual chlorine in accordance with Standard Method 9060A (Eaton et al., 2005).

7.2.1. Nutrients

The following nutrients were measured in the field by using a portable spectrophotometer (model DR 2400; Hach Co., Loveland, CO) immediately following sample collection. The preloaded methods contain factory-generated standard curves used to determine the sample concentrations. Wavelength calibration was completed upon startup of the instrument. Inherent to the following methods are directions for evaluating method performance using standard solutions of the analyte being tested.

7.2.1.1. Determination of NO_3-N

The NO_3-N concentration of the water sample was measured by the cadmium reduction method (Hach method 8039). Ten milliliters of sample was poured into the 25-mL Hach sample cell; then one NitraVer 5 Nitrate Reagent Powder Pillow was added and thoroughly mixed. Cadmium metal reduced nitrates in the sample to nitrite. The nitrite ion reacted in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt coupled with gentisic acid to form an amber solution. After a 5-min reaction period, the absorption at the wavelength of 500 nm was selected to determine the NO_3-N concentration.

7.2.1.2. Determination of NO_2-N

The NO_2^- concentration of the water sample was measured by the ferrous sulfate method (Hach method 8153). Ten milliliters of sample was poured into the 25-mL Hach sample cell; then one NitriVer 2 Nitrite Reagent Powder Pillow was added and thoroughly mixed. The method used ferrous sulfate in an acidic medium to reduce nitrite to nitrous oxide. Ferrous ions combined with the nitrous oxide to form a greenish-brown complex in direct proportion to the nitrite present. After a 10-min reaction period, the absorption at the wavelength of 585 nm was selected to determine the NO_2^- concentration.

7.2.1.3. Determination of NH_3-N

The NH_3-N concentration of the water sample was measured by the salicylate method (Hach method 8155). A 0.1-mL sample was transferred to an AmVer Diluent Reagent High Range vial. Then one Ammonia Salicylate Reagent Powder Pillow and one Ammonia Cyanurate Reagent Powder Pillow were added and thoroughly dissolved. Ammonia compounds combined with chlorine to form monochloramine. Monochloramine reacted with salicylate to form 5-aminosalicylate. The 5-aminosalicylate was oxidized in the presence of a sodium nitroprusside catalyst to form a blue compound. The blueness was masked by the yellowness

from the excess reagent present to give a green solution. After a 20-min reaction period, the absorption at the wavelength of 655 nm was used to determine the NH_3-N concentration.

7.2.1.4. Determination of PO_4^{3-}

The PO_4^{3-} concentration of the water sample was measured by the molybdovanadate method (Hach method 8114). Twenty-five milliliters of sample was poured into the 25-mL Hach sample cell; then 1.0 mL of molybdovanadate reagent was added and thoroughly mixed. Orthophosphate reacted with molybdate in an acid medium to produce a phosphomolybdate complex. In the presence of vanadium, yellow vanadomolybdophosphoric acid was formed. The intensity of the yellow was proportional to the phosphate concentration. After a 5-min reaction period, the absorption at the wavelength of 430 nm was used to determine the PO_4^{3-} concentration.

7.2.1.5. Determination of S^{2-} (liquid)

The sulfide concentration of the water sample was measured by the methylene blue method (Hach method 8131). Twenty-five milliliters of sample was poured into the 25-mL Hach sample cell; then 1.0 mL of sulfide 1 reagent and 1.0 ml of sulfide 2 reagent were added and thoroughly mixed. Hydrogen sulfide and acid-soluble metal sulfides reacted with *N*,*N*-dimethyl-*p*-phenylenediamine sulfate to form methylene blue. The intensity of the blue was proportional to the sulfide concentration. After a 5-min reaction period, the absorption at the wavelength of 665 nm was used to determine the sulfide concentration.

7.2.1.6 Determination of SO_4^{2-}

The SO_4^{2-} concentration of the water sample was measured by the SulfaVer 4 method (Hach method 8051). Ten milliliters of sample was poured into the 25-mL Hach sample cell; then one SulfaVer 4 Sulfate Reagent Powder Pillow was added and swirled into the mix. Sulfate ions in the sample reacted with barium in the SulfaVer 4 and formed a precipitate of barium sulfate. The amount of turbidity formed was proportional to the sulfate concentration. After a 5-min reaction period, the absorption at the wavelength of 450 nm was used to determine the sulfate concentration

7.2.2. Dissolved oxygen and turbidity

The dissolved oxygen was measured with a Hach HQ40d Dual-Input Multi-parameter Meter. Turbidity was measured with a nephelometer (Hach 2100N Turbidimeter) as specified by the manufacturer against a set of standards.

7.2.3. Color of reclaimed water

Both true color and apparent color were measured by Hach method 8025 (platinum and cobalt color units) with a spectrophotometer (model DR 4000U, Hach Co.) after the samples warmed to room temperature. Apparent color was measured on nonfiltered samples, whereas true color was measured on a sample that passed through a 0.45-µm-pore-size membrane filter.

7.2.4. Reclaimed water alkalinity

Alkalinity was determined by using the titration method (Method 2320-B; Eaton et al., 2005) on 100-mL aliquots of reclaimed water. Titration to pH = 4.5 using 0.02N Sulfuric Acid (J.T. Baker, Phillipsburg, NJ) was conducted with a Brinkman digital Buret II 05R3988 (Brinkman Instruments, Inc., Westbury, NY). The volume of titrant was used to compute the total alkalinity from the formula:

Total alkalinity (mg of $CaCO_3$/L) = (A × B × 50,000)/mL of sample

where A = amount of titrant used and B = normality of titrant.

7.2.5. UV-absorbing organic constituents

UV absorbance at 254 nm (UV_{254} in centimeters^{-1}) was measured in duplicate by Hach method 10054 with a spectrophotometer (model DR 4000U, Hach Co.). Specific UV absorbance (SUVA in number of liters/[milligram meter]) was calculated as the ratio of UV_{254} and DOC concentration (SUVA = UV Abs [cm^{-1}] × 100/DOC [mg/L]).

7.2.6. Biochemical analyses

7.2.6.1. Glassware preparation

Glassware was prepared for organic carbon analysis. Borosilicate collection bottles were washed with detergent (neodisher ® Laboclean F; Miele, Princeton, NJ) in Mielabor G 7783 (Miele) and then baked in a muffle oven for 6 h at 550 °C. Screw caps were also detergent washed, rinsed with MilliQ water (Milli-Q Academic; Millipore Corp., Billerica, MA) and soaked for 1 h in a bath containing 10% nitric acid (ACS Grade; EMD, Gibbstown, NJ). TOC vials were cleaned in the same manner following the detergent wash with an additional acid wash step. After the acid bath, caps and TOC vials were rinsed five times with MilliQ water and air dried. TOC vials were wrapped in aluminum foil and muffled for 6 h.

7.2.6.2. TOC determination

TOC was measured as nonpurgeable organic carbon according to Standard Method 5310B (high temperature platinum-catalyst) by using a Shimadzu TOC-5000 (Columbia, MD) with ASI-5000A autosampler. Samples were collected in glassware described above and were transferred upon receipt into 10 mL of TOC vials in duplicate and acidified (pH ≤ 2). Samples prepared in this manner could be stored at 4 °C up to 28 days, though they were generally analyzed immediately. Laboratory-fortified blanks were analyzed once per analytical run as verification standards. Acceptance criteria were ± 25% of the true value. Sample analysis was performed in triplicate and reported as milligrams per liter.

7.2.6.3. BDOC determinations

BDOC was measured in reclaimed water according to the BDOC-sand method (Joret and Levi, 1986; Volk et al., 1994). The samples were collected in acid-washed glass bottles, shipped overnight to the laboratory, and analyzed upon receipt. Biological sand was prepared from 3 kg of bagged sand in 10 L of settled water from the Delaware River Regional Water Treatment Plant (Delran, NJ) for at least 2 weeks under gentle aeration. Once colonized, sand was stored in dechlorinated tap water and washed until the DOC released from the washings was < 0.1 mg/L prior to use. In a 500-mL flask, 300 mL of the sample was inoculated with 100 g of the biologically active sand and incubated (aeration at 20 ± 2 °C under 4 L h^{-1}). DOC values in the water were analyzed on a daily basis until a minimum DOC value was reached as described for TOC analysis. The difference between the starting DOC level and the lowest

DOC level observed during incubation was the BDOC level for the sample, expressed in micrograms or milligrams per liter.

A positive control that consisted of 2000 µg of sodium acetate carbon per liter of mineral salt solution (per liter of Milli-Q water, 17.1 mg of K_2HPO_4, 76.4 mg of NH_4Cl, and 144 mg of KNO_3) was performed with a 92% recovery rate to verify the procedure and bacterial colonization of the sand.

7.2.6.4. AOC

The conventional bioassay (Van der Kooij, 1990) for determining AOC using an inoculum of *P. fluorescens* P17 and *Spirillum* strain NOX was conducted in initial studies. Dechlorinated samples were pasteurized at 70 °C for 30 min and then cooled. P17 and NOX inocula were added to the samples, and bacterial growth was monitored by spread plating. Through the use of this approach, the maximum growth (N_{max}) observed was converted into micrograms-per-liter acetate carbon equivalents.

In corresponding studies, *P. fluorescens* P17 and *Spirillum* strain NOX mutagenized with *luxCDABE* operon fusion and inducible transposons to produce bioluminescent strains (Haddix et al., 2004) that were used to determine AOC. The luminescence was determined at specific intervals, and the maximum growth and growth rate of these bioluminescent strains were also monitored over time using a sensitive, photon-counting luminometer (Figure 7.6) with a programmable 96-well microtiter plate format. In preliminary studies, AOC results for the luminescent mutagens compared well to those for the parent strains (p = 0.97 and 0.99 for P17 and NOX, respectively). Standard curves resulted in an r^2 between luminescence units and acetate carbon of 0.95 for P17 and 0.89 for NOX as seen in Figure 7.7.

Figure 7.6. LMax II luminometer (Molecular Devices, Sunnyvale, CA).

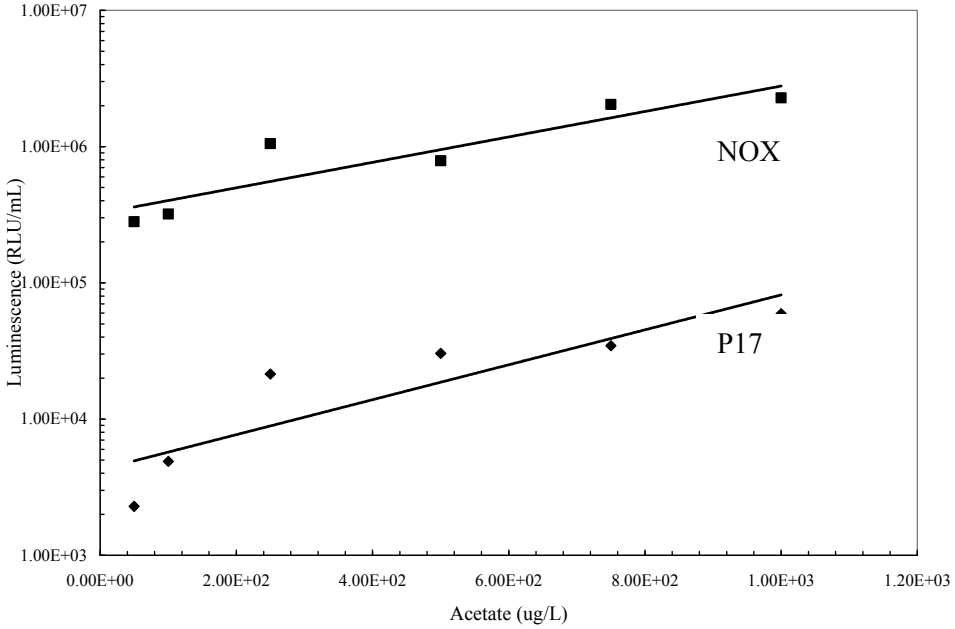


Figure 7.7. Standard curve results of AOC luminescence.

Samples were inoculated with luminescent strains of the AOC organisms and monitored postinoculation on day 0 and then again on days 3 through 5 in order to compare luminescence results to colony counts. To validate the bioluminescence test, parallel analysis of reuse samples was conducted by using both the bioluminescence and conventional plate count assays. Luminescence was converted to acetate carbon equivalents using the Monod model (from standard curve) and maximum growth yield (CFU) values were evaluated and compared.

7.2.7. Bacteria

7.2.7.1. HPC bacteria

Heterotrophic bacteria is a broadly defined term referring to any bacteria that obtain energy (and therefore are able to grow) from organic compounds (see Section 5.3.1). They were enumerated by the spread-plate method (Standard Method 9215C [Eaton et al., 2005]) using R2A medium (pH = 7.2). Dilution of the water samples was with phosphate buffer solution prepared by dissolving 34.0 g of potassium dihydrogen phosphate (KH_2PO_4), in 500 mL of reagent-grade water, adjusting to a pH of 7.2 ± 0.5 with 1 N sodium hydroxide (NaOH), and diluting to 1 L with reagent-grade water (solution A). We added 1.25 mL of stock phosphate buffer solution and 5.0 mL of magnesium chloride solution (81.1 g of $MgCl_2 \cdot 6H_2O$/L of reagent-grade water) to 1 L of reagent-grade water. The samples were processed within 24 h by inoculating 0.1 mL per plate (or dilutions thereof), thus ensuring that the inoculum was completely absorbed before incubating. The plate contents were incubated at 28 °C for 7 days, and humidity within the incubator was maintained. All bacterial colonies were counted, and the counts were used to compute the density of bacteria per milliliter based on the following equation:

$$\text{CFU/mL} = \frac{(Colonies\ \ counted)}{(mL\ volume\ of\ \ sample\ \ plated)}$$

If plates from all dilutions of any sample have no colonies, report the count as <1 divided by the corresponding largest sample used.

7.2.7.2. Total coliform

Coliform belong to the family Enterobacteriaceae. They are characterized by their ability to ferment lactose, producing gas and forming acid within 48 h at 35 °C (see Section 5.3.2). They were determined from aliquots of 1, 10, and 100 mL of the water. The respective aliqouts were filtered (white gridded nitrate cellulose, 0.45-µm pore size) and a vacuum applied. The filter was then mounted onto m-Endo agar LES, and its contents were incubated for 24 h at 35 °C. The shiny/metallic colonies were presumptive coliforms. From these, a maximum of five colonies per plate were randomly selected for confirmation in fermentation tubes by a search for ability to ferment lactose (brilliant green lactose bile broth) at 35 °C within 24 to 48 h (Standard Method 9222B [Eaton et al., 2005]).

7.2.7.3. Fecal coliforms

Fecal coliforms were determined by the membrane filtration method (Standard Method 9222D) on m-FC medium containing 10 mL of 1% rosolic acid per L of medium. Aliquots of 100 mL of reclaimed water were filtered through a 0.45-µm-pore-size gridded membrane and mounted onto m-FC plates. *E. coli* was used as a positive control. The plate contents were incubated at 44.5 ± 0.2 °C for 24 h. Colonies of various shades of blue were counted as presumptive fecal coliforms. Verification and confirmation were done by inoculating (maximum of five colonies per plate) into brilliant green lactose bile broth fermentation tubes and incubating at 44.5± 0.2 °C for 24 ± 2 h. Tubes that produced gas (as a sign of lactose fermentation) were confirmed as positive for fecal coliforms. Failure to produce gas (with little or no growth) indicated negative for fecal coliforms. The results from a maximum of five colonies were extrapolated to represent the total number of colonies originally identified as presumptive.

7.2.7.4. Aeromonas *spp.*

Aeromonas spp. are Gram-negative facultative anaerobes that ferment glucose but not lactose (see Section 5.3.6). They were detected following Standard Method 9260L using m-*Aeromonas* selective agar base prepared from 5 g of tryptose, 11.4 g of dextrin, 2 g of yeast extract, 3 g of NaCl, 2 g of KCl, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.06 g of $FeCl_3 \cdot 6H_2O$, and 0.08 g of bromethyl blue. Adjustment to pH = 8 as solution A followed. Solution B was prepared by dissolving 100 mg of sodium deoxycholate to 10 mL of water. Solutions A and B were added and thereafter 13 g of agar added. The medium was autoclaved for 15 min and cooled to 50 °C. Before dispensation, 10 mg of ampicillin sodium salt and 2 mg of vancomycin hydrochloride (each antibiotic initially dissolved in 10 mL of water and filter-sterilized [0.2-µm pore size]) were added. Aliquots of 0.1, 1, 10, and 100 mL of the reclaimed water sample were filtered (0.4-µm pore size) and the filters mounted on the ampicillin dextrin agar plate. *A. hydrophila* ATCC 7966 and sterile water were used as the positive and negative controls, respectively. The plate contents were incubated aerobically overnight at 35 °C, and the distinct bright yellow colonies of 1- to 1.5-mm diameter were scored as *Aeromonas* spp.

7.2.7.5. Enterococci

Enterococci are Gram-positive coccus-shaped aerotolerant facultative anaerobes. At a microscopic level, they exist in chains (see Section 5.3.3). Enterococci were enumerated by using the membrane filtration method (Standard Method 9230C) with mE agar composed of 10 g of peptone, 15 g of NaCl, 30 g of yeast extract, 1 g of esculin, 0.15 g of sodium azide, and 15 g of agar per L. The medium was amended with 0.05 g of cycloheximide (Actidione)/L and 0.25 g of nalidixic acid/L as well as 0.15 g of 2,3,5-triphenyl tetrazolium chloride/L after autoclaving. Aliquots of 100 mL were filtered (0.45-µm pore size), and the membrane was aseptically mounted onto the mE agar. The plate contents were incubated at 41 ± 0.5 ^{o}C for 48 h. Membranes with presumptive enterococci were thereafter mounted onto EIA substrate plates composed of 1 g of esculin, 0.5 g of ferric citrate, and 15 g of agar per L (pH = 7.1 ± 0.2) and incubated at 41 ± 0.5 ^{o}C for 20 min. The pink/red colonies that turned to black or reddish-brown precipitate on underside of the filter were scored as enterococci.

7.2.7.6. E. coli

E. coli is one of the best-known (and most-studied) coliforms. It colonizes the gut, and therefore, its presence is associated with fecal contamination. *E. coli* bacteria were enumerated on m-TEC agar (Standard Method 9213D [Eaton et al., 2005]) by filtering 100-mL reclaimed water aliquots (0.45-µm-pore-size nitrate cellulose). The plate contents were incubated briefly (namely, 2 h) at 35 ± 0.5 ^{o}C to rejuvenate injured bacteria and then transferred to 44.5 ± 0.2 ^{o}C for 22 h. The membrane was then treated with a urea substrate (namely, 2 g of urea with 10 mg of phenol red/100 mL of water) and incubated for 15 min. The resulting yellow/yellowish brown colonies under UV radiation were counted as *E. coli*.

7.2.7.7. E. coli *O157 enrichment and detection*

E. coli O157 is one of the *E. coli* strains that possess virulence factors, causing distinct syndromes of diarrhea. It was detected by using the IMS-Reveal method (Bukhari et al., 2007). Specifically, 100 mL of reclaimed water was filtered (0.45-µm pore size membrane). The captured cells were eluted in 10 mL of 1× trypticase soy broth in Leighton tubes and incubated at 42 ^{o}C for 5 h. A positive control using *E. coli* O157:H7 ATCC 35150 and a negative control using *E. coli* ATCC 13706 were also tested. An aliquot of 100 µl of Dynabeads anti-*E. coli* O157 was added, and the culture was incubated for one more hour. The bead-bacterial complex was separated by using a magnet and then resuspended in 1 mL of phosphate-buffered solution with further incubation in a water bath at 60 ^{o}C for 10 min. The samples were cooled on ice, and then 100 µl was inoculated into the Reveal testing device (Neogen Corp., Lansing, MI). The reaction on the device was read after 10 to 15 min.

7.2.7.8. P. aeruginosa

Pseudomonas spp. are some of the most common noncoliform bacteria. Of most concern to public health in this genus is *P. aeruginosa*. *P. aeruginosa* bacteria were enumerated following Standard Method 9213E (Eaton et al., 2005) on modified m-PA agar. A known aliquot of the water was filtered through a sterile 0.45-µm-pore-size membrane (Whatman), and the membrane was mounted onto a modified m-PA agar plate. The plate contents were incubated at 41.5 ± 0.5 ^{o}C for 72 h (namely, 3 days) after which all colonies of approximately 0.8- to 2.2-mm diameter that have a flat appearance with light outer rims and brownish to greenish-black centers were counted.

7.2.7.9. Legionella *spp.*

Legionella spp. are Gram-negative non-spore-forming bacteria that are occasionally detected in water (see Section 5.3.5). *Legionella* spp. were determined by filtering 100 mL of the water (white gridded nitrate cellulose 0.45-µm pore size), and the filter was aseptically submerged in 10 mL of phosphate-buffered solution. The solution was vortexed for 30 s, and an aliquot of 0.1 mL was mixed with an equal amount of acid (namely, HCl-KCl, pH = 2.2; Standard Method 9260). The mixture was incubated at room temperature for 15 min, and then 0.1 mL of a KOH-KCl base was added to neutralize the acid. An aliquot of 0.1 mL (and its dilutions) was then introduced onto BCYE plates supplemented with *Legionella* agar enrichment (BD Difco, Sparks, MD) which primarily contains cysteine, an essential amino acid for *Legionella* spp. A PAV supplement (Remel, Lenexa, KS) that contains polymyxin B, anisomycin, and vancomycin was also added. The plate contents were incubated at 35 ^{o}C, and growth was monitored for up to 1 week. Randomly selected presumptive *Legionella* spp. (a maximum of five colonies from each plate) were streaked on BCYE without any cysteine (NHS, 2007). Failure to grow in the absence of cysteine was regarded as confirmatory for *Legionella* spp.

7.2.7.10. Mycobacterium *spp.*

Mycobacterium spp. are acid-fast organisms that are fairly ubiquitous (see Section 5.3.7). They were enumerated by initially decontaminating a known aliquot of the sample with cetylpyridinium chloride (CPC) to a final concentration of 0.005% to avoid overgrowth of nontarget organisms. While most Gram-negative bacteria are susceptible to CPC, *Mycobacterium* is relatively resistant. The CPC-treated sample was then filtered (0.45-µm pore size), and the filter was mounted onto Middlebrook 7H10 agar plates and its contents incubated up to 21 days at 37 °C. Representative colonies that had a variety of appearances such as smooth opaque, smooth transparent, and tan and irregularly shaped were subjected to acid-fast staining as described by Seeley et al. (1991). The cells from colony smears that retained a characteristic redness under the microscopy were scored as *Mycobacterium* spp.

7.2.7.11. Sulfur bacteria

Sulfur bacteria oxidize or reduce organic sulfur compounds, the latter being favored under anaerobic settings, reducing sulfate to hydrogen sulfide. They can contribute to corrosion and odor problems in reclaimed water. Sulfate-reducing bacteria were enumerated following Standard Method 9240D (Eaton et al., 2005) on a medium composed of 5 g of agar, 4 g of 60% sodium lactate, 2 g of magnesium sulfate, 2 g of ferrous ammonium sulfate, and 40 g of trypticase soy agar per L. The medium was adjusted to pH = 7.2 to 7.4 prior to autoclaving. All plates were used within 4 h after preparation to minimize saturation with oxygen. Aliquots of 100 mL of reclaimed water were filtered through a gridded membrane (0.45-µm pore size) and were carefully mounted onto the plate. The plate contents were then incubated (in an inverted position) in an anaerobic jar (PML Microbiologicals, Portland, OR) with a palladium catalyst (BBL GasPak^{TM} Plus) at room temperature (21 to 24 ^{o}C) for up to 21 days. Colonies that were blackened around the edges were enumerated as sulfate-reducing bacteria.

7.2.7.12. Iron bacteria

Iron is a critical substance for life, playing an important role in energy metabolism. Iron bacteria function under different redox conditions and utilize a variety of substrates for growth. They were enumerated by using the Biological Activity Reaction Test (BART®)

biodetection system, a patented bacterial testing system that detects biological activity by examining reaction patterns and time lags (Cullimore, 1999). The organisms are identified as iron-related bacteria based on a reaction pattern signature in which selective nutrients comprised of ferric ammonium citrate in a crystallized deposit and diffuse upward, encouraging iron-oxidizing bacteria. The growth at the bottom of the tube is attributed to iron-reducing bacteria. The ferric ammonium citrate provides carbon (as citrate), nitrogen (as ammonium), and iron. These specific zones within the nutrient column are provided by the presence of a floating intercedent device, which restricts the entry of oxygen into the sample below, creating the anaerobic zone. In this fashion the system provides a range of environments from aerobic to anaerobic, with a transitional redox zone in the middle. The assay was conducted by aseptically adding 15 mL of reclaimed water to the IRB-BART detector vial (Figure 7.8) purchased from the LaMotte Co. (Chestertown, MD). The vials were sealed and incubated at 22 to 24 ^{o}C for 15 days without disturbance. A vial that received sterile water was included as a quality control measure. The vials were examined daily, and the iron bacterium populations were estimated based on the kit manufacturer's guidelines (Table 7.2).

Table 7.2. Interpretation of Days of Delay to 1st Reaction and Possible Population Density[a]

Days of Delay	Aggressivity	Possible Population (log CFU/mL)
1	Very high	6.2 ± 1.4
2	High	5.4 ± 0.9
3	High	4.5 ± 1.2
4	Moderate	4.1 ± 1.2
5	Moderate	3.8 ± 1.4
6	Moderate	3.3 ± 1.4
7	Background	3.1 ± 1.5
10	Background	2.5 ± 1.2
15	Very low	<2.0

[a]Source: LaMotte Co.

Figure 7.8. IRB-BART® system for iron-related bacteria.

7.2.7.13. Bacteriophage

The male-specific (F^+) and somatic coliphage were assayed by the single agar layer procedure (EPA Method 1602) using aliquots of 100 mL of reclaimed water (USEPA, 2001). *E. coli* F_{amp} and *E. coli* CN-13 were used as the male-specific and somatic hosts, respectively. The PFU were counted after incubation of the plate contents overnight at 35 ^oC.

7.2.8. Protozoan parasites

Giardia spp. and *Cryptosporidium* spp. in the reclaimed water were assayed once from the effluent each location per season. Collection was conducted by running a known volume of reclaimed water (ranging between 38 and 49 L) through a sterile Envirochek HV sampling capsule (Pall Life Sciences). The filter was shipped to the American Water commercial laboratory (Belleville, IL) overnight on ice and eluted within 24 h using Laureth 12 following the guidelines specified under EPA Method 1623 (USEPA, 2005). Briefly, the filter was eluted by using three 5-min washes on a laboratory shaker at 900 rpm. The elution buffer contains Laureth 12, EDTA, Tris, and antifoam A. The eluted sample was concentrated at 2000 × *g* for 15 min. The volume of the concentrated pellet was measured and 0.5 mL of pellet resuspended per subsample. The sample/subsample was purified by using immunomagnetic separation with Dynal Dynabeads GC Combo. The purified sample was disassociated by using 0.1 N HCl and placed on Dynal Spot-On slides. The slides were stained by using Waterborne Aqua-Glo and 4',6'-diamidino-2-phenylindole (DAPI). The slides were read on an Olympus BX50 (Olympus America Inc., Lake Success, NY), with any potential detection characterized by fluorescein isothiocyanate (FITC) staining, DAPI staining, and differential interference contrast microscopy. FITC is a reagent that is commonly used to derivatize proteins with a fluorescein group (often an antibody,

immunoglobulins, lectins and other proteins, peptides, nucleic acids, nucleotides, and oligo- and polysaccharides). It is commonly used in the hybridization staining of sections, for living cells, and as stains. It fluoresces apple green when excited with near-UV light. DAPI is another stain that is extensively used in fluorescence microscopy. It forms fluorescent complexes with natural double-stranded DNA.

7.2.9. Algae and cyanobacteria

To determine the concentration of these photosynthetic pigments, aliquots of 500 mL of reclaimed water were filtered (0.45-µm pore size) (Whatman). In some instances, more than one filter was necessary for these large volumes. The filter(s) for each aliquot was inserted into a glass tissue grinder (Kontes, Vineland, NJ) and then dissolved in a mixture of acetone with $MgCO_3$ (Eaton et al., 2005). The $MgCO_3$ mixture was made by initially adding 1 g of $MgCO_3$ to 100 mL of distilled water and then combining 90 parts of acetone with 10 parts of saturated $MgCO_3$ solution. The dissolved mixture was stored (4 ^oC) in the dark for at least 4 h and thereafter centrifuged (500 × *g* for 20 min) to remove the debris, and thereafter the supernatant was used to determine the absorbance at 664 nm. Because pheophorbide *a* and pheophytin *a*, two common chlorophyll *a* degradation products, can interfere with the determination of chlorophyll *a* as they absorb light and fluorescence in the same region as chlorophyll *a*, determinations can be optimized by acidification. Such acidification leads to loss of the magnesium atom in chlorophyll *a*, generating pheophytin *a*. The OD_{664} of the acidified mixture was then determined by taking 3 mL of the mixture, determining the OD_{664}, and then adding 0.1 mL of 0.1 N HCl and finally reading the OD_{664} within 90 s. The volume assayed and the length of time that elapsed after acidification before the reading was taken are highly critical in this process for accurate and consistent results. To standardize the OD_{664} readings, chlorophyll stocks of 0, 0.0185, 0.034, 0.05, 0.1, and 0.32 mg/mL were made and their OD_{664} determined. That determination generated a correlation coefficient (R^2) of 0.9947 (Figure 7.9).

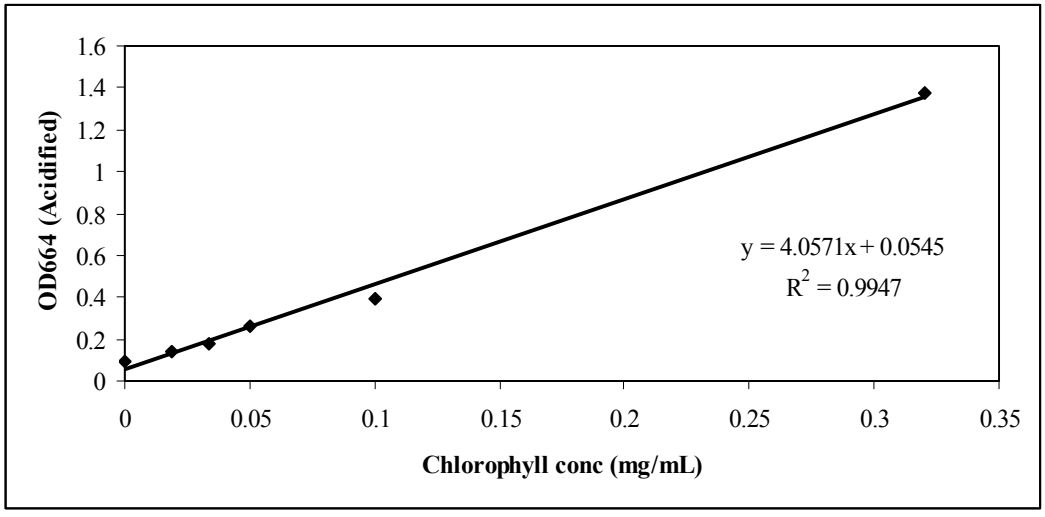


Figure 7.9. Standard curve for chlorophyll concentration versus OD_{664}.

7.2.10. Viruses

Viral pathogens in the reclaimed water were assayed once from each location per season. Collection was conducted by running a known volume of reclaimed water (ranging between 151.4 and 231 L) through a sterile Virosorb® 1MDS Cartridge (CUNO Filtration, Carlstadt, NJ). The filter was shipped to the laboratory overnight on ice and eluted within 72 h by using 1 L of a 1.5% beef extract (BBL Microbiology Systems; pH = 9.5). The eluted viruses were concentrated as described under Information Correction Rule guidelines (USEPA, 1995) by specifically adjusting the pH to 3.5 ± 0.1 and stirring for 30 min. The mixture was then centrifuged (3100 rpm) for 15 min, and the supernatant was discarded. The pellet was disrupted with a 30-mL sterile solution of $Na_2HPO_4 \cdot 7H_2O$ (0.15 M; pH 9.5) and centrifuged at 4500 rpm (15 min). The supernatant was adjusted to pH = 7 to 7.5, its final volume determined, and it was then filtered (0.2-µm pore size) and aliquoted into two equal fractions. Each of the fractions was stored at -80 °C until needed for RNA extraction.

7.2.10.1. RNA extraction

RNA was isolated from the sample using the SV Total RNA Isolation System (Promega catalog no. Z3100) with some modifications. Specifically, an aliquot of 125 µl of the sample was mixed with 175 µl of SV RNA lysis buffer mixed with β-mercaptoethanol. This initial mixture has been shown to be effective in isolating HIV RNA in plasma using the Promega SV Total RNA Isolation system (Eva Belloso, personal communication). The mixture was vortexed for 5 s, and then 350 µl of the SV RNA dilution buffer was added. The mixture was then added to 300 µl of isopropanol in an SV spin column and incubated at room temperature for 5 min. It was thereafter centrifuged at 12,000 × *g* for 1 min. The liquid in the collection tube was discarded, and 600 µl of the RNA Wash (RWA) solution (with ethanol) was added to the spin column. The column was centrifuged again at 12,000 × *g* for 2 min, and the eluate was discarded. To eliminate DNA, DNase (50 µl) mix was added to the membrane of the column and incubated at room temperature for 15 min. This reaction was stopped by adding 200 µl of DNase Stop Solution (DSA) to each column and centrifuging at 12,000 × *g* for 1 min after which 600 µl of RWA solution was added. The addition was followed by centrifuging (12,000 × *g* for 1 min). This process was repeated with 250 µl of RWA solution and centrifuging (12,000 × *g*) for 2 min. RNA was finally eluated with 100 µl of nuclease-free water into a collection tube and stored at -20 °C until needed for PCR. Parallel reclaimed water samples were spiked with poliovirus and processed like the nonspiked ones. As an additional control, an aliquot of 125 µl of a 20-PFU stock of poliovirus was also handled as the samples and processed by using the SV total RNA extraction kit.

7.2.10.2. RNA primers and probes

Based on the literature, the primers shown in Table 7.3, which are specific for enteroviruses, HAV, rotavirus, and NV, were adapted for this study. For detection of the amplification signal, the probe was labeled with the fluorescent dyes 5-carboxyfluoroscein (FAM) on the 5' end and *N*,*N*,*N'*,*N'*-tetramethyl-6-carboxyrhodamine (TAMRA) on the 3' end. When the two dyes are near each other, as is the case with an intact oligonucleotide probe, TAMRA acts as a quencher for FAM by absorbing at the FAM emission spectra (DesJardin et al., 1998). As the PCR progresses, the 5' exonuclease activity of Taq polymerase degrades the probe, enabling the fluorescence signal to be detected. The primers were synthesized by Operon Biotechnologies (Huntsville, AL), and the characteristics provided by the manufacturer are summarized in Table 7.3. An aliquot of TE buffer (Promega; catalog no. V6231) reaching the

volume specified in the 100 µM column in Table 7.3 was added to the respective primer to attain a 100 µM primer concentration.

7.2.10.3. RT reaction

Figure 7.10. Roche LightCycler 480 System II RT-PCR device.

The reagents for RT-PCR were purchased from Promega (Madison, WI), and the Access RT-PCR system (catalog no. A1250) was used as stipulated by the manufacturer's protocol with the employment of 25-µl reaction volumes. To synthesize the first strand cDNA, the mixture was incubated at 45 ^{o}C for 40 min (or as indicated in Table 7.4). The template was then denatured at 94 ^{o}C for 2 min and thereafter subjected to various temperature cycles as summarized in Table 7.4 by using the Roche LightCycler 480 system II RT-PCR device (Roche Diagnostics, Indianapolis, IN; Figure 7.10). A schematic of the PCR process with a labeled probe is shown in Figure 7.11. RT-PCR has repeatedly been demonstrated to be effective in detecting viruses in environmental and clinical samples (Oberste et al., 2006). The RT-PCR product curves were examined, and their threshold cycle (namely, the number of cycles at which the fluorescence generated within a reaction crosses the threshold, referred to as the crossing point [Cp] value) was evaluated. A 3-µl aliquot of each product was added to a PCR Super Mix^{TM} (Promega; catalog no. M7502) in 25-µl reaction volumes together with the FAM-TAMRA probe upstream, and the downstream primer was reamplified (40 cycles) under conditions that were similar to those described above and the curves as well as Cp values determined. The PCR products were stored at 4 ^{o}C until analysis by agarose gel electrophoresis. The gel was composed of 1.6% agarose containing 1.5 µg of ethidium bromide/mL and was run for 1 h at 100 V. The gel was then observed under a UV transilluminator (UVP, Upland, CA) for the presence of a characteristic band.

Table 7.3. Characteristics of the Oligonucleotide Primers and Probes Used[a]

Target	Primer or Probe	Sequence (5'-3')[b]	Location	Reference	Product Characteristics						
					OD	No. of pmol	MW	No. of µg	E260	Tm	No. of µl for 100 µM
Enteroviruses	EV-F	CCCCTGAATGCGGCTAATC		Wang et al., 2002	3.5	20090.86	5748.78	115.5	174208.6	62.32	200.91
	EV-R	GATTGTCACCATAAGCAGC			3.55	18813.15	5796.84	109.06	188697.8	58	188.13
	EV-probe	FAM-CGGAACCGACTACTTTGGGTGTCCGT-TAMRA			5.16	17990.36	9434.65	169.73	286820.3	69.32	179.9
HAV	HAV1	TTTCCGGAGCCCCTCTTG		Costa-Mattioli et al., 2002	4.36	29334.59	5417.57	158.92	148630	62.18	293.35
	HAV2	AAAGGGAAATTTAGCCTATAGCC			4.24	17532.27	7080.71	124.14	241839.7	59.2	175.32
	HAV3	AAAGGGAAAATTTAGCCTATAGCC			3.91	15296.33	7393.92	113.1	255616.9	59.44	152.96
	HAV probe	FAM-ACTTGATACCTCACCGCCGTTTGCCT-TAMRA			12.84	47131.55	9289.56	437.83	272429	67.75	471.32
Rotavirus	Rota NVP3-F	ACCATCTACACATGACCCTC	963–982	Pang et al., 2004	1.88	10249.2	5965.93	61.15	183429	60.4	102.49
	Rota NVP3-R	GGTCACATAACGCCCC	1034–1049		1.93	13041.49	4811.15	62.74	147989.2	59.28	130.41
	TagMen probe	ATGAGCACAATAGTTAAAAGCTAACACTGTCAA	984–1016		4.24	10588.41	11603.15	122.86	400437.7	63.42	105.88
NV	Cog 2F (GII)	CARGARBCNATG TTYAGRTGGATGAG	5003	Trujillo et al., 2006	10.69	39654.52	8097.94	321.12	369578.33	64.86	396.55
	Cog 2R (GII)	TCG ACG CCA TCT TCA TTC ACA	5100		11.62	61371.53	6301.17	386.71	189338.6	60.61	613.72
	Probe Ring 2 (GII)	FAM-TGGGAGGGCGATCGCAATCT-TAMRA	5048		2.39	9811.45	7654.48	75.1	243593	64.5	98.11

[a]All primers and probes were synthesized and purified by using high-performance liquid chromatography by Operon Biotechnologies and had a scale of 50 nmol.
[b]R = A or G, Y = C or T, N = any.

Because the band of the positive control was very faint, it was deemed necessary to take a 10-µl aliquot of the RT-PCR products and combine it with a PCR Super Mix™ as well as 4 µl of the upstream and downstream primer. This mixture was then subjected to another 40 cycles of PCR under conditions that were similar to those described above. The RT-PCR conditions for each type of virus are summarized in Table 7.4.

The reamplified products were also analyzed on 1.6% agarose containing 1.5 µg of ethidium bromide/mL run for 1 h at 100 V. The gel was then observed under a UV transilluminator (UVP) for the presence of a characteristic band. The presence of the expected band in the reamplified products served as the ultimate criterion for scoring the sample as positive for enteroviruses.

Table 7.4. RT-PCR Conditions for Virus Assays

Target Virus	Primer Quantity	RT-PCR Conditions	Reference
Enterovirus	300 nM	Incubate at 65 °C (2 min); 48 °C (40 min); 95 °C (10 min); [60 cycles of denaturation at 94 °C (15 s) and amplification at 58–61 °C (1 min)]	Wang et al., 2002
HAV	300 nM	Incubate at 65 °C (2 min); 45 °C (40 min); 95 °C (5 min); [60 cycles of denaturation at 94 °C (15 s) and amplification at 60–62 °C (1 min)]	Modified from Costa-Mattioli et al., 2002
Rotavirus	200 nM	Incubate at 65 °C (2 min); 45 °C (40 min); 95 °C (10 min); [40 cycles of denaturation at 94 °C (15 s) and amplification at 57–61 °C (1 min)]	Pang et al., 2004
NV	400 nM	Incubate at 65 °C (2 min); 55 °C (30 min); 95 °C (10 min); [45 cycles of denaturation at 94 °C (30 s) and amplification at 57–61 °C (1 min)]	Modified from Trujillo et al., 2006

7.2.10.4. Viral RT-PCR QA/QC

RT-PCRs for an HAV stock and its dilutions were done after extracting the viral RNA using the SV Total RNA extraction kit. Amplification was positive for the 10-, 100-, and 1000-fold dilutions (Figure 7.12). In that assay, an aliquot of the reclaimed water samples from each of the four locations collected per season was spiked with 20 µl of an HAV stock containing $3.1 \times 10^4 \pm 0.4 \times 10^4$ copies/µL. RNA of each of the spiked samples was extracted as described above for nonspiked samples using the Promega SV Total RNA Isolation system. The RNA was eluted from the column by using 100 µL of RNase-free water. Aliquots of 1, 2, 4, and 5 µL of RNA were amplified in a final volume of 25 µL prepared in a single tube containing 12.5 µL of AccessQuick™ RT-PCR (Promega; catalog no. A1702) combined with 0.5 µL of AMV RT (Promega) and 300 nM HAV1, HAV2, and HAV3 primers. To enable detect of the

reaction, the mixture also contained 0.01 µM HAV-probe. To optimize the reaction, the respective RNA aliquots of 1, 2, 4, and 5 µL were amplified by initially heating at 65 ^{o}C for 2 min, reverse transcribing the RNA into cDNA (40 min at 45 ^{o}C), and terminating the RT reaction by heating to 95 ^{o}C for 5 min. Denaturation and amplification of the cDNA were then conducted at 94 ^{o}C for 15 s and 1 min at 60 to 62 ^{o}C (namely, touchdown PCR) for 60 cycles. Both RT-PCR and subsequent amplification were conducted by using a LightCycler 480 System II RT-PCR device. Results for this optimization analysis are displayed in Figure 7.12, and the detected concentrations are summarized in Table 7.5. From those data, it is apparent that RNA aliquots of 1 µL or less were insufficient for obtaining successful amplification, even in the HAV stock solution. On the other hand, aliquots of 4 µL of RNA led to inconsistent results and to underestimation of the RNA copies. Aliquots of 5 µL were even less reliable (Table 7.5), and those with 10 µL (data not presented) completely failed to detect any HAV copies in the reaction. Those data also show that the quantitative RT-PCR assay is quite sensitive and able to detect a single RNA copy number (Table 7.5; Figure 7.12). This preliminary analysis served as the basis for adapting a 2- to 3-µL aliquot used for quantitative RT-PCR for all of the other viruses tested.

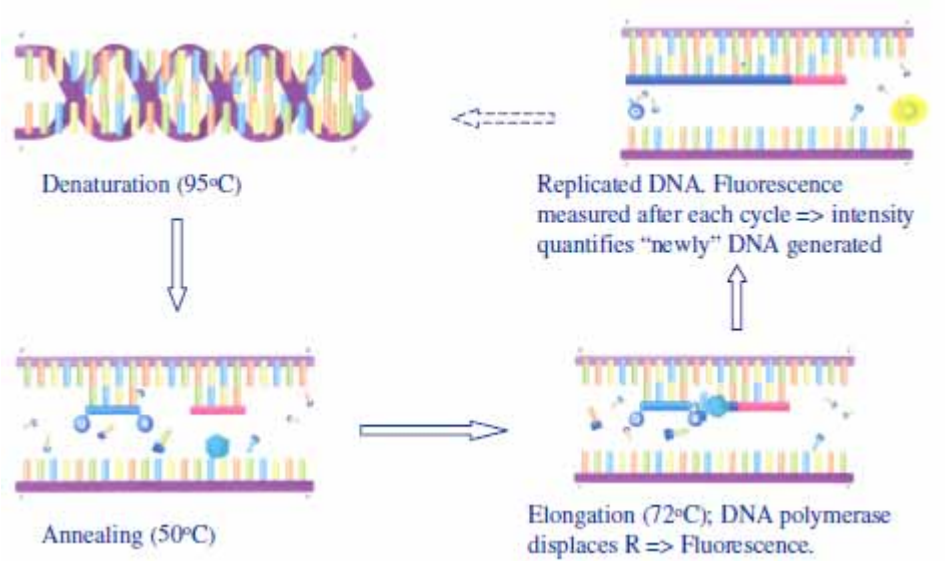
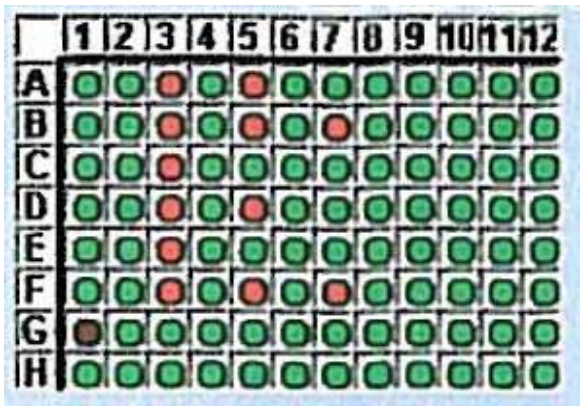


Figure 7.11. Schematic of the quantitative PCR process used in the present analysis. Not shown in the above schematic: the initial RT phase in which the single-stranded (viral) RNA is used to generate double-stranded DNA (namely, cDNA) that is used in the denaturation and process. Q = Quencher (namely, TAMRA), and R = Reporter (namely, FAM).



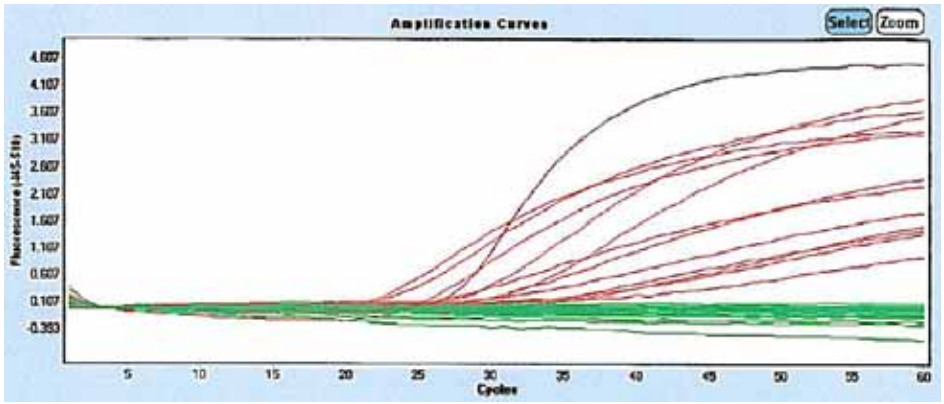


Figure 7.12. The microplate output for a QA/QC analysis for samples spiked with HAV at different concentrations. Different aliquots of RNA from HAV were introduced in columns B1-E1, B3-E3, B5-E5, B7-E7, and B9-E9. Cells G1 and G2 are the Roche positive and negative controls. Wells A1, A3, A5, A7, and A9 contained a winter FL sample spiked with HAV, whereas F1, F3, F5, F7, and F9 contained a winter NY sample spiked with HAV. Aliquots of 1, 2, 4, 5, and 10 µL were used as template in columns 1, 3, 5, 7, and 9 of the microplate. Cell identifiers and related copy numbers are summarized in Table 7.5.

Table 7.5. Results of the Quantitative RT-PCR QA/QC Analysis[a]

Position	Sample ID	Vol of Template (1 µL)	Cp[b]	Copies/µL	Position	Sample ID	Vol of Template (1 µL)	Cp	Copies/µL
A1	FL spike (winter sample)	1	-	N/D[c]	A5	FL spike (winter sample)	4	30.62	40
B1	HAV stock	1	-	N/D	B5	HAV stock	4	28.58	1.4×10^2
C1	HAV stock (10^{-1} dilution)	1	-	N/D	C5	HAV stock (10^{-1} dilution)	4	-	N/D
D1	HAV stock (10^{-2} dilution)	1	-	N/D	D5	HAV stock (10^{-2} dilution)	4	37.61	1
E1	HAV stock (10^{-3} dilution)	1	-	N/D	E5	HAV stock (10^{-3} dilution)	4	-	N/D
F1	NY spike (winter sample)	1	-	N/D	F5	NY spike (winter sample)	4	29.18	1×10^2
G1	Standard	N/A	27.61	1×10^3					
G2	Negative control		N/A	N/D					
A3	FL spike (winter sample)	2	23.78	4.3×10^3	A7	FL spike (winter sample)	5	-	N/D
B3	HAV stock	2	23.56	4.9×10^3	B7	HAV stock	5	36.57	1
C3	HAV stock (10^{-1} dilution)	2	28.65	2.76×10^2	C7	HAV stock (10^{-1} dilution)	5	-	N/D
D3	HAV stock (10^{-2} dilution)	2	32.14	2.85×10	D7	HAV stock (10^{-2} dilution)	5	-	N/D
E3	HAV stock (10^{-3} dilution)	2	35.01	3	E	HAV stock (10^{-3} dilution)	5	-	N/D
F3	NY spike (winter sample)	2	21.54	1.52×10^4	F7	NY spike (winter sample)	5	24.46	1.2×10^3

[a]The positions identified correspond to those displayed in Figure 7.12 above.
[b]Cp = Crossing point.
[c]N/D = None detected.

7.2.11. Pipe loop assembly and sampling

7.2.11.1. Assembly and biofilm formation

The purpose of the pipe loops is to: (i) provide a test system for examining different disinfection alternatives under controlled conditions with different initial qualities of the reuse water and (ii) provide data that can be used to model the regrowth of HPC, total coliform, and *Legionella* bacteria in reclaimed water. The pipe loops (Choose-A-Color PVC tubing, 1/8-in. inner diameter, 1/4-in. outer diameter, 1/16-in. wall thickness; catalog no. 9446K251; McMaster-Carr, Robbinsville, NJ) were designed to examine three different disinfection schemes using three parallel 150-ft branches (LeChevallier et al., 1993b) (Figure 7.13). The loops were installed for durations specified in Table 7.6. The flow rate in the loops was selected to provide a shear velocity similar to that for distribution systems.

Table 7.6. Acclimation of Biofilm Development at Each Location

Location	Duration of Loop Study
CA	41 days
FL	27 days
MA	73 days
NY	75 days

Figure 7.13. The three coiled branches of the pipe loop installed at the NY location. Each branch is 150 ft long and has a flow rate of 1.54 mL/s. The loop branches are color-coded in increments of 5 ft to permit easy identification of the sampling points.

7.2.11.2. Disinfection and sampling

After the acclimation period (for formation of the biofilm) the three branches of the pipe loop were separated so that each was fed by its own reservoir (Figure 7.14) filled with plant effluent water. The water in one reservoir was disinfected with free chlorine added as sodium

hypochlorite (namely, Clorox®), whereas another reservoir was treated to form monochloramine. The monochloramine stock was prepared by reacting free chlorine with ammonia in a carbonate buffer (to maintain pH) at a chlorine-to-ammonia ratio of 3:1. Water in each reservoir was replenished with new disinfected water (free and total chlorine) after 24 h. The third pipe loop received unamended reuse water and served as a control to represent the normal distribution system.

The pipe loops were treated with disinfected water (either free or monochloramine) or unamended effluent for 48 h prior to examination. The loops were sampled at four locations (1, 50, 100, and 150 ft) by collecting the bulk water at these lengths. To minimize disturbance of the biofilm and water flow during the sampling process, sample collection started from the end of the loop (namely, 150 ft) and moved toward the inlet (at the beginning). To obtain baseline water quality information, a sample of the bulk water in each of the three reservoirs was also obtained. To collect the biofilm sample, a 1.5-ft section of the tubing was cut at each location and was clamped at each end to maintain the water in the tube and to prevent dehydration of the biofilms. Each clamped piece was inserted into a Ziploc® bag, and both the bulk water and biofilm loop piece were shipped to the lab on ice for further analysis. The physicochemical analyses (namely, pH, free chlorine, total chlorine, PO_4^-, NO_3-N, NO_2-N, and NH_4-N) for the bulk water were conducted on site as described in previous reports, whereas all biological analyses were conducted within 24 h after sampling. The biofilm samples were processed within 48 h. In the laboratory, the bulk water was analyzed for total coliform, HPC, and *Legionella* spp. as described previously.

Figure 7.14. Sampling from the three pipe loop treatments. The reservoirs allowed free chlorinated or chloraminated water to be continuously pumped (using a peristaltic pump) through different sections of the pipe loop.

7.2.11.3. Biofilm extraction and analysis

Biofilms were extracted by using a 1/8-in. diameter brush, and the material was rinsed into a sterile plastic centrifuge tube (Corning, NY). The final volume was adjusted to 25 mL, and a 10-mL aliquot was used for total solids as well as carbon content determinations. Total solids were measured according to Standard Method 2540C. The rest of the material was adjusted back to 25 mL with Zwittergent 3-12 solution (Camper et al., 1985). The final solution contained Zwittergent 3-12 (10^{-6} M), ethylene glycol-*bis*(β-aminoethyl ether)-*n,n,n',n'*-tetraacetic acid (EGTA; Sigma-Aldrich, St. Louis, MO) (10^{-3} M), Tris (0.01 M), and peptone (0.1%). The mixture

was homogenized at 13,000 rpm (Polytron PT1200; Kinematica, Littau-Lucerne, Switzerland) for 30 s, and the HPC, total coliform, and *Legionella* spp. were determined. These determinations were made as described for bulk water samples with the exception of *Legionella* spp. from the biofilm extract without filtration. However, the mixture was acidified with HCl-KCl for 15 min and then neutralized with 0.1 mL of KOH-KCl. By use of the inner diameter of the pipe loop (1/8 in.) and the 1-ft length of the pipe loop piece from which the biofilm material was extracted, the volume of the tubing was determined to be 2.45 cm^3. The internal surface area of the 1-ft segment was similarly established to be 30.6 cm^2. The density of bacteria per square centimeter was calculated and then used to determine the equivalent density of bacteria per milliliter of biofilm based on the assumption that since 1 cm^3 can accommodate 1 mL, the volume of 2.45 cm^3 of tubing contained 2.45 mL of water. Thus, the density of bacteria in the biofilm material obtained from 1 ft of volume represented the equivalent density of the biofilm population in 2.45 mL. This conversion enabled a direct comparison of the density of microorganisms in the bulk water and the density in the biofilm for each disinfectant.

CHAPTER 8

RESULT-BASED GUIDANCE DEVELOPMENT

Because of the large number of data collected in this study, it was easiest to organize the results in a series of conclusion statements. This approach can be used to effectively convey information to a broad cross-section of people in the reuse industry to communicate complex sets of data. Our data set enabled us to come up with 15 different conclusions, which are outlined underneath. Each is supported by the relevant results from the study.

8.1 THE RAPID BIOLUMINESCENCE METHOD FOR AOC WAS SUCCESSFUL IN MEASURING THE BIOSTABILITY OF RECLAIMED WATER

8.1.1 Comparison between conventional plate count and rapid bioluminescence AOC methods indicated that the latter was suitable for application to reclaimed waters

Traditionally, the AOC method has been applied to monitor optimization and treatment of organic carbon removal to reduce the potential for bacterial regrowth. For example, predicting coliform occurrence was related to a combination of factors including disinfectant residual level, temperature, and AOC (LeChevallier et al., 1996). Other factors such as filtration, disinfectant type, water chemistry, and system maintenance are also important considerations.

Reclaimed wastewater can have high levels of organic carbon and relatively high levels of BDOC and AOC. In this study, biodegradable organic matter levels in reclaimed water averaged four to five times higher than levels typically seen in drinking water supplies (Geldreich and LeChevallier, 1999). Therefore, control of biodegradable organic matter in reclaimed water is important for limiting bacterial regrowth and risks from opportunistic pathogens. In this study, AOC proved to be a useful tool for predicting regrowth in reclaimed water systems. Previous work using the conventional AOC method revealed that AOC levels in MBR wastewater effluents ranged between 500 and 900 μg/L (Karim and LeChevallier, 2005). The conventional AOC method would have been impossible to use in the current study because of the complexity and time-consuming nature of the standard test. The extent of AOC analysis would not have been feasible if it were not for the availability of the bioluminescence AOC test.

This study showed that reclaimed water matrices varied considerably in organic carbon levels. To verify that the luminescence method was suitable for reuse waters, parallel analysis was performed to compare the conventional plate count method and the rapid AOC luminescence method (Figure 8.1). The correlation coefficient (R^2) between the two techniques was 0.92 (P17 + NOX), and for the individual organisms the R^2 values were 0.83 for P17 (not shown) and 0.96 for NOX (Figure 8.2).

Figure 8.1 shows a strong relationship between the plate count and luminescence methods. This comparison indicates that 92% of the data show a strong linear relationship. Moreover, the slope of the data is 0.98, indicating that AOC values determined by either method share nearly a 1:1 relationship. When one is comparing two analytical methods, often a plot of the difference between the methods against their mean can also be informative (Bland and Altman, 1986; Figure 8.3). Assuming that the differences are normally distributed, 95% will lie between the mean

difference ± 1.96 times the standard deviation. This analysis enables a clearer view of the two methods' measurement errors. Based on the data presented in Figures 8.1 and 8.2, the conventional method underestimated AOC (Figure 8.3). The only outlier occurred near the upper range as a result of a lower AOC concentration determined by the conventional method. One issue that is not encountered in the bioluminescence test but continues to be a major drawback of the conventional method was inaccurate capture of the maximum growth yield during incubation and delay in results due to the comparatively long culture durations required.

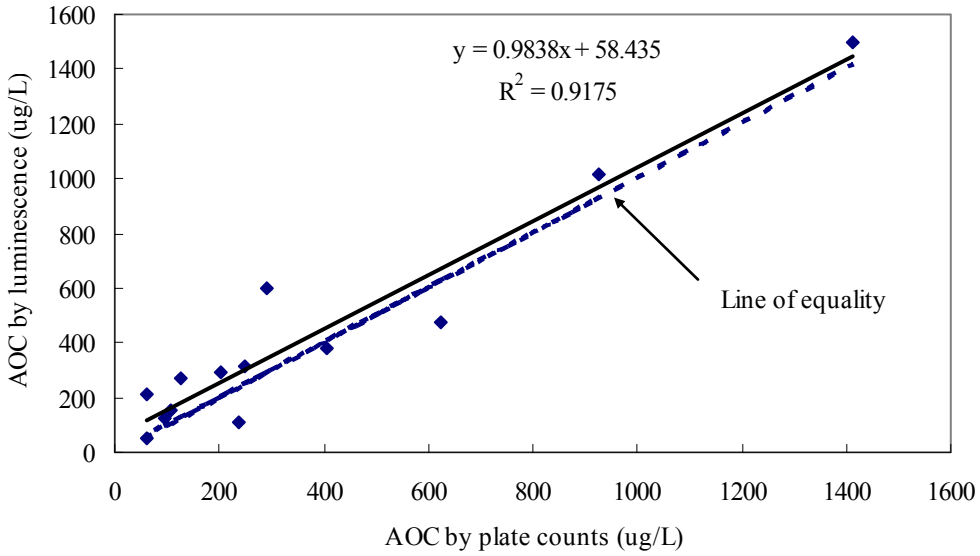


Figure 8.1. AOC method comparison. The line of equality represents equivalent results for both methods.

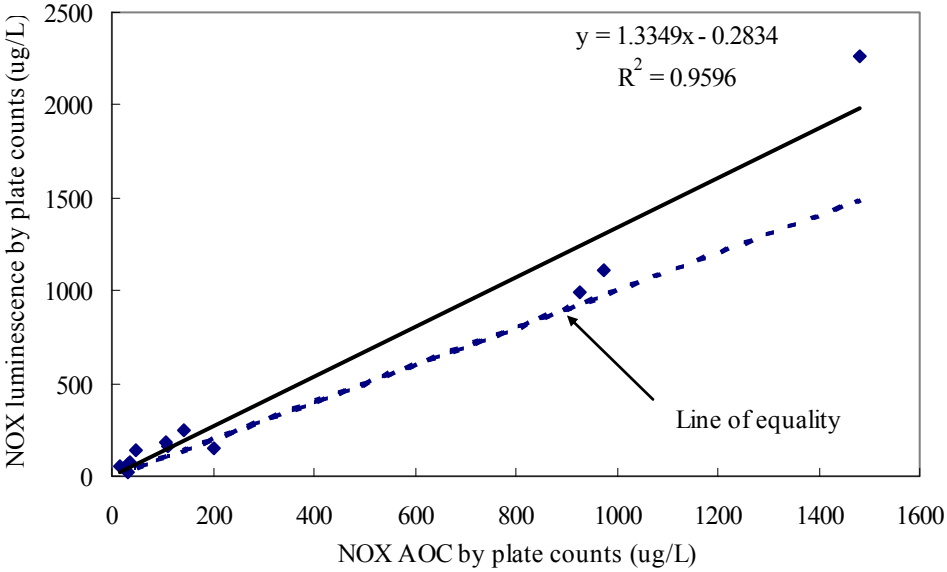


Figure 8.2. NOX AOC method comparison. The line of equality represents equivalent results for both methods.

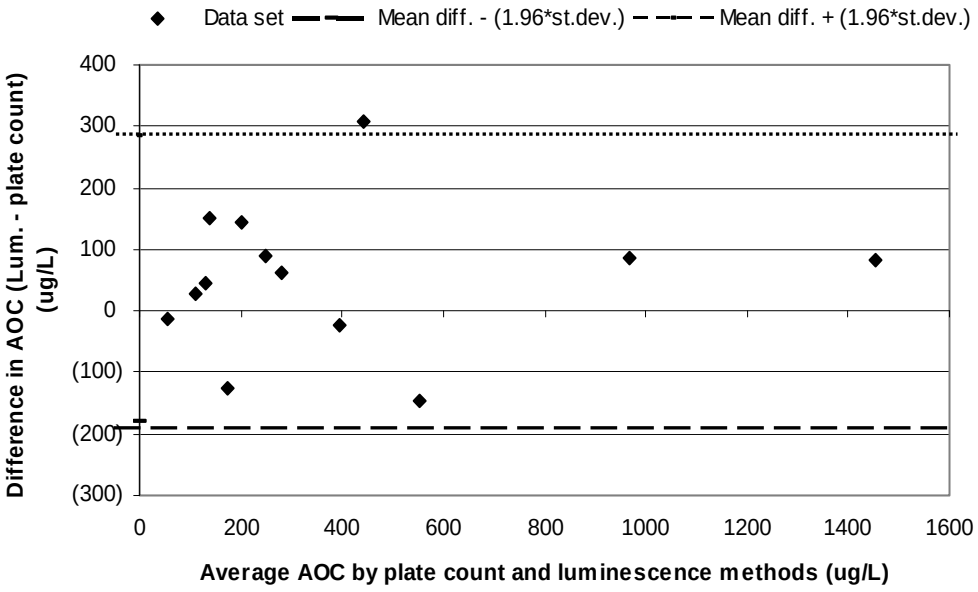


Figure 8.3. Analysis of AOC methods' mean difference.

8.1.2 Modeling AOC was possible through the application of the Monod growth curve

The acetate standard curve results were fitted to the classic Monod growth curve as described in Materials and Methods. Modeling acetate in this way permits the estimation of both the maximum growth (N_{max}) and growth rate (μ_{max}). Calculation of the growth parameters μ_{max} and substrate saturation constant (K_s) provides additional information on the characteristics of the biodegradable carbon. When the model was applied to reclaimed waters, maximum luminescence generally occurred within 3 days—faster than the acetate standard curves (a two-carbon-source compound). Reclaimed wastewater is likely comprised of a mixture of energy-intensive carbohydrates and amino acids that results in rapid bacterial growth. While the Monod curve is an accepted way of modeling the substrate, we found that the actual water samples do not follow the same pattern that acetate, a single substrate, does. For example, data for plant effluent (Figure 8.4) and storage pond (Figure 8.5) showed that the measured luminescence (—■—) peaked sooner (around 2 days) and faster than the model prediction (—+—).

If one assumes that reclaimed water contains a variety of substrates available as AOC, applying a multiple-substrate model provided a better fit for the observed data in actual reclaimed samples. A single-substrate model accurately measured the peak yield of AOC on acetate carbon during method development; however, growth on AOC present in reclaimed water occurred more rapidly and more precisely matched the output of the multiple-substrate model. Both models have similar maximum growth kinetics and similar AOC yield (Figure 8.6). The multiple-substrate model provides information on the substrate utilization: for example, how easily biodegradable (or persistent) the AOC substrates were in the sample.

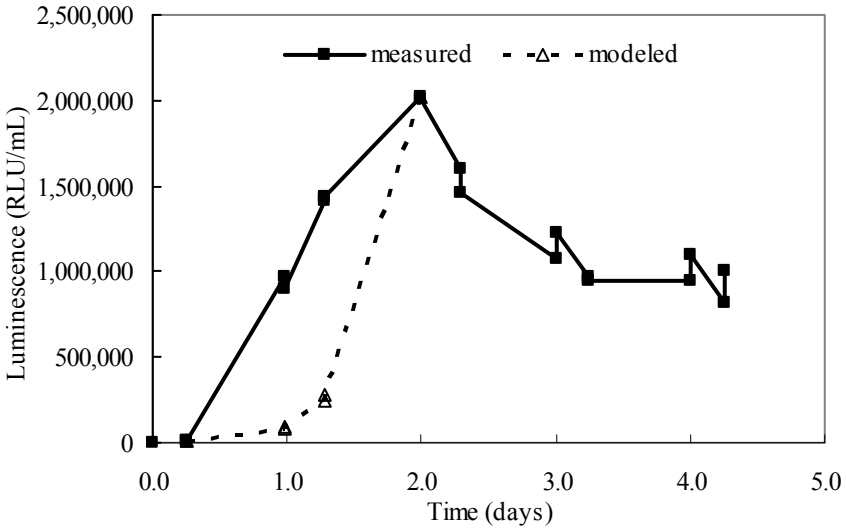


Figure 8.4. FL effluent sample—AOC luminescence data.

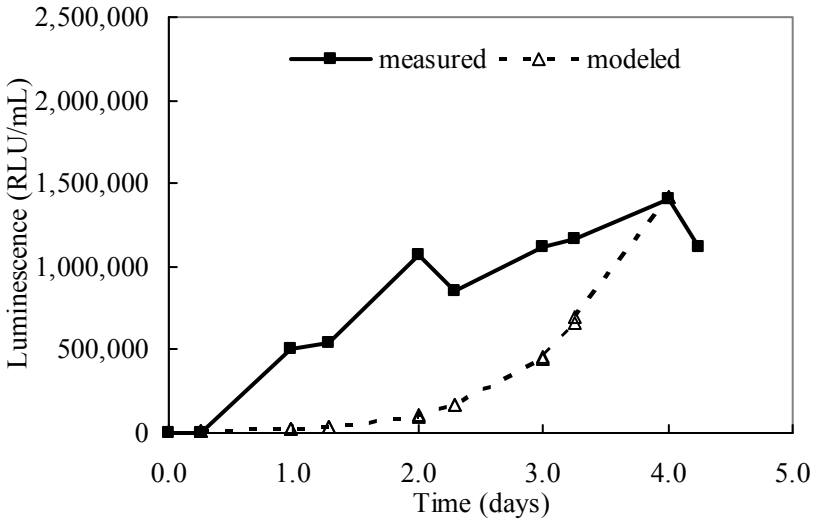


Figure 8.5. FL storage sample—AOC luminescence data.

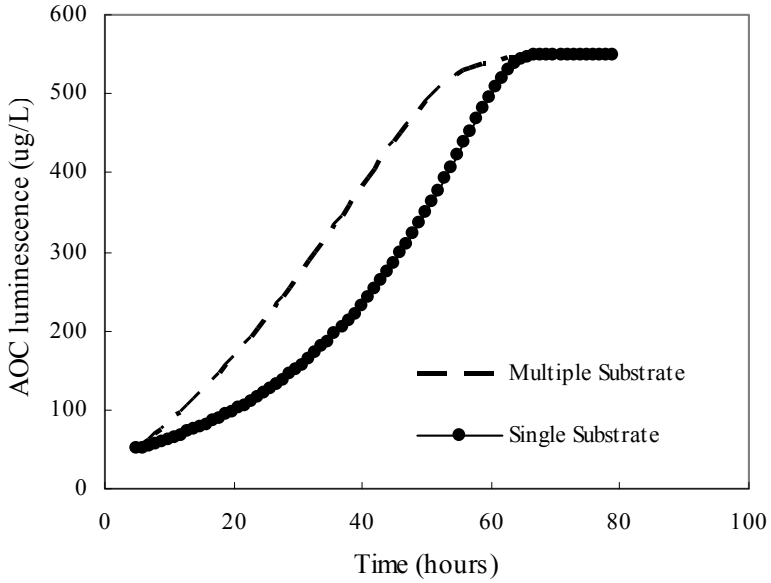


Figure 8.6. Substrate model comparison.

8.2. DISINFECTANT RESIDUALS WERE RAPIDLY DEPLETED IN THE DISTRIBUTION SYSTEM

Three of the four utilities examined used chlorine as a disinfectant. However, after application the disinfectant residuals were rapidly depleted, and very little disinfectant persisted within the distribution system. The point of chlorine application was at the effluent in CA and FL and at the storage tank in MA. CA did not practice breakpoint chlorination, and therefore the elevated levels of ammonia nitrogen in the effluent (Table 8.1) reacted with chlorine to form high total chlorine residuals (Table 8.2). FL practiced breakpoint chlorination, thereby chemically oxidizing ammonia nitrogen and maintaining free chlorine residual in the effluent. Only a fraction of the disinfectant residual data from CA, MA, and FL had values above the detection limit of 0.02 mg/L (Table 8.2), and in the cases where residuals were measurable, only about 25% of the free chlorine measurements were greater than 0.2 mg/L—a limit typically accepted to provide adequate protection against bacterial growth. In CA and FL, 28% and 26% of the free chlorine residuals were less than the detection limit, respectively, and 70% of the chlorine residual data were less than the detection limit in MA.

Table 8.1. Survey Data: Mean Ammonia (mg/L) Nitrogen Concentrations and Quartile Analysis from Survey of 4 Utilities[a]

Site	N	N>D	Mean	Stdev	Min	25%ile	50%ile	75%ile	90%ile	95%ile	Max	DL
CA	94	94	7.80	10	0.40	1.45	4.85	10.00	16.70	23.35	54.00	0.01
FL	91	67	0.10	0.2	0.00	0.01	0.02	0.04	0.33	0.55	0.93	0.01
MA	100	45	0.04	0.2	0.00	0.00	0.01	0.04	0.07	0.09	2.00	0.01
NY	100	21	0.01	0.0	0.00	0.00	0.01	0.01	0.02	0.03	0.07	0.01

[a]N = number of observations; DL = detection limit; Stdev = standard deviation.

Measureable disinfectant levels typically occurred at the point of application, but the residual was completely diminished in the distribution system (Figure 8.7). This depletion occurred in the open storage reservoirs in CA and FL but also in the closed distribution system in MA. None of the systems examined was able to maintain a low but stable disinfectant residual at the ends of the reclaimed distribution networks. The phenomenon of loss of disinfectant has been documented in potable water systems (see discussion in Section 6.2), but its importance in reclaimed water distribution systems seems to be even more paramount.

Table 8.2. Survey Data—Mean Chlorine Residual Concentrations and Quartile Analysis from Survey of 3 Utilities[a,b]

Site	N	N>DL	Mean	Stdev	Min	25%ile	50%ile	75%ile	90%ile	95%ile	Max	DL
Total chlorine (mg/L as Cl_2)												
CA	95	92	8.0	19.3	0.0	0.3	0.3	5.85	24	55.6	127	0.02
FL	91	81	1.4	2.1	0.0	0.4	0.4	1.7	5.1	6.75	9.6	0.02
MA	100	28	0.2	0.4	0.0	0.0	0.0	0.1	0.91	1.1	1.2	0.02
Free chlorine (mg/L as Cl_2)												
CA	100	72	0.4	0.8	0.0	0.0	0.1	0.15	1.842	1.988	4.1	0.02
FL	91	67	0.8	1.7	0.0	0.0	0.1	0.4	3.4	5.2	8	0.02
MA	100	30	0.1	0.1	0.0	0.0	0.0	0.1	0.3	0.3	0.4	0.02

[a]N = number of observations; DL = detection limit; Stdev = standard deviation.
[b]NY did not use chlorine for disinfection.

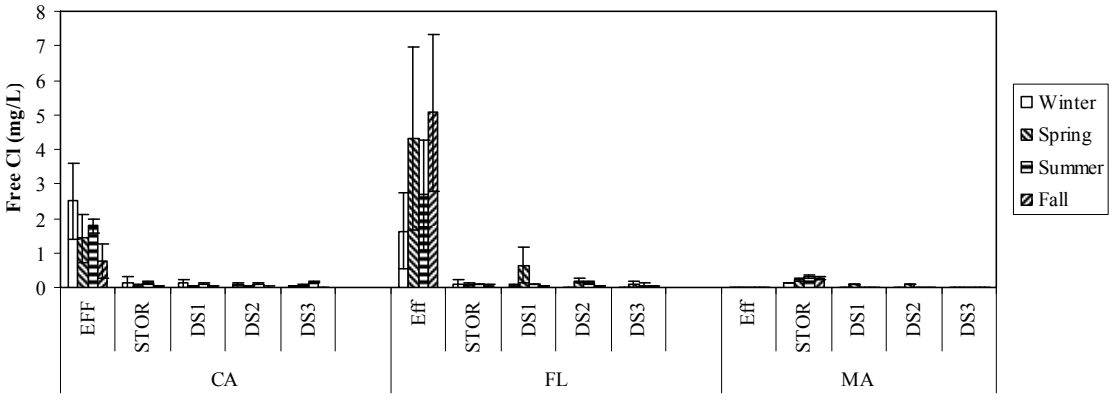


Figure 8.7. Residuals measured in the distribution systems where chlorine was used as a disinfectant. Bars show mean ± SD of the 4-day sampling period per season.

Free chlorine is often used as an inexpensive disinfectant in a wide range of applications to minimize bacterial growth. So we could further investigate the phenomenon of chlorine disinfectant loss in the systems surveyed, a pipe loop study was set up at each location as to specifically examine the effectiveness and stability of free chlorine and chloramine disinfectant residuals under controlled conditions. Generally, residuals were present at a concentration of 0.02

to 0.1 mg/L in the three plants that chlorinate but were absent in the NY system. The NY system does not chlorinate. After imposition of the pipe loop treatments, the residuals in the inlet were all > 0.2 mg/L. However, disinfectant residual loss occurred throughout the length of most pipe loop systems, although the extent of loss greatly differed (Table 8.3). The most significant loss of free chlorine occurred in the NY loop, from 3.6 mg/L to 0.04 mg/L throughout the length of the pipe loop system (Figure 8.8). A modest loss of chlorine was also detected in the CA pipe loop system. This trend was similar to that seen in the survey results, in which plant effluent residuals from 2 to 6 mg/L as Cl_2 were often completely diminished in the distribution system.

Table 8.3. Chlorine Residual Measurements at Inlet and throughout 1- to 150-ft Sections[a]

		Chlorine Residual (mg/L as Cl_2)				
Treatment to Loop	**Site**	**Inlet**	**1 ft**	**50 ft**	**100 ft**	**150 ft**
Control	CA	0.1	0.1	ND[b]	ND	ND
	FL	0.02	0.03	0.03	ND	ND
	MA	0.03	0.05	0.04	0.02	0.03
	NY	ND	ND	ND	ND	ND
Free chlorine	CA	0.8	0.8	0.6	0.2	0.1
	FL	13.5	13.4	18.0	12.9	13.1
	MA	1.30	1.13	1.03	1.02	1.14
	NY	3.60	3.90	1.76	0.43	0.04
Chloramine	CA	6.5	6.6	6.0	5.5	5.7
	FL	1.70	1.78	1.69	1.62	1.61
	MA	1.87	1.82	1.80	1.78	1.83
	NY	1.88	1.80	1.64	1.41	0.65

[a]Free chlorine residuals reported for control and free chlorine loops; total chlorine residuals reported for the chloramine-treated loop.

[b]ND = not detected.

Because of their highly reactive nature, free chlorine residuals are often not stable and will rapidly react with organic matter to form chlorinated DBPs such as trihalomethanes (Rook, 1974). DBPs have long been a major public health concern in the drinking water industry and have recently been measured in reclaimed effluents (Matamoros et al., 2007). DBPs in reclaimed effluents are likely to continue undergoing investigation as reclaimed waters are used for an increasing number of applications and as risks associated with exposure routes are addressed. Combined chlorine residuals, specifically monochloramine, offer more stable disinfectant protection in distributed waters because of a lesser tendency to react with organic carbon. Evidence from Funamizu et al. (2004) indicated that combined chlorine was more stable than free chlorine in reclaimed waters with various levels of organic carbon. However, issues surrounding disinfection are not nearly this straightforward, and various factors influence disinfection success and residual stability.

Through the process of breakpoint chlorination, enough chlorine was added to loop inlet water to react with ammonia nitrogen past the point at which the oxidative destruction of combined residual chlorine occurs (Haas, 1999). Owing to water quality fluctuations in FL, the breakpoint was exceeded, resulting in particularly high residual levels in the free chlorine loop (Table 8.3).

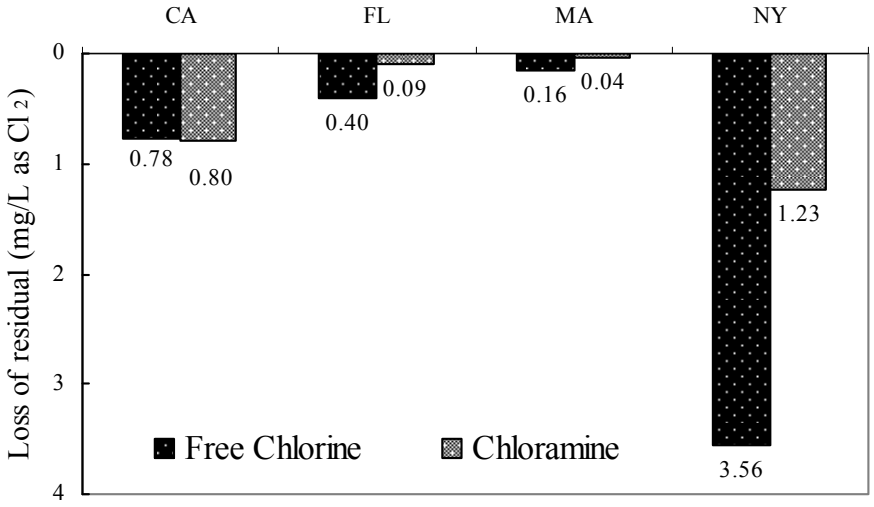


Figure 8.8. Residual loss in pipe loops as indicated by the difference between the starting concentration at the inlet and the final concentration at the end of the loop.

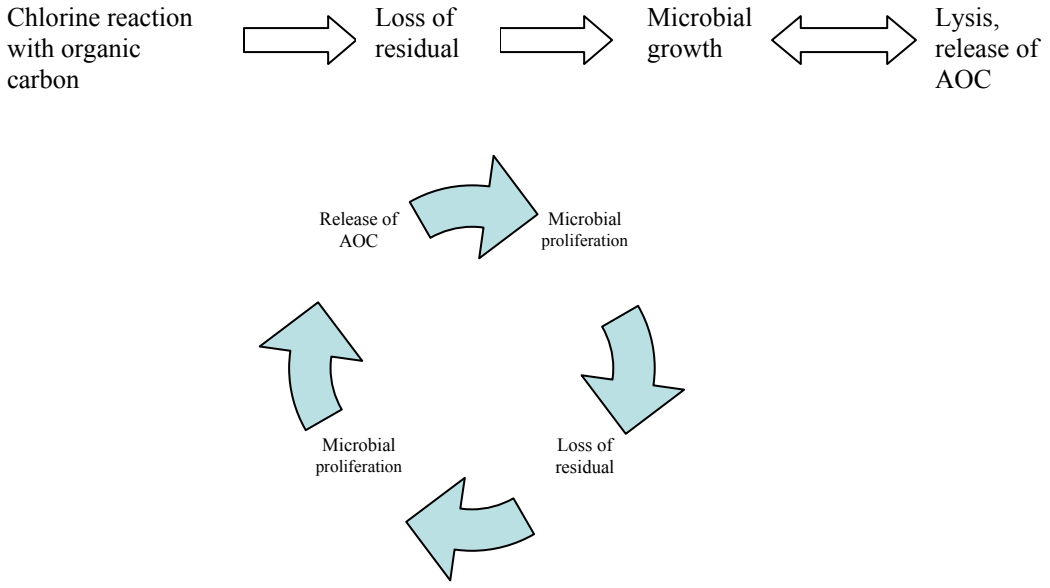


Figure 8.9. Simple diagram indicating circular contribution of AOC causing increased microbial proliferation.

Loss of a disinfectant residual may result from various factors, such as disinfectant demand from organic matter, ammonia, corrosion products, temperature, pH, etc. It is unlikely that the chlorine residual in the pipe loop systems was depleted through a typical reaction pathway with ammonia, because breakpoint chlorination was performed to remove ammonia from the bulk water being fed into the loops and because NY had no measurable levels of ammonia in the inlet water (data not shown). The rapid loss of chlorine residuals in the NY system (Figure 8.8) may have been the result of reactions between chlorine and organic carbon where the disinfectant can oxidize the carbon into smaller, more assimilable components that can be used for bacterial growth. In addition, natural microbial cell lysis can release organic matter into the water, stimulating both the chlorine demand and bacterial regrowth. This bacterial growth in turn further depletes disinfectant, and once the disinfectant is depleted, there is no hindrance to further microbial proliferation (Figure 8.9).

Owing to the extended incubation time, biofilm development was greatest in CA and NY, and the elevated amount of biofilm material (measured as total solids; Figure 8.10) present on the loop walls may have resulted in demand reaction(s) between chlorine and either attached bacteria (biofilm surface/layers attached to the loop walls) and/or suspended bacteria in the water column (sloughing off from the walls). Suspended bacteria in the water phase were shown to increase 10-fold in the absence of chlorine (Codony et al., 2005).

Reactions between chlorine and organic matter specifically in the reclaimed wastewater matrix have been modeled by Funamizu et al. (2004). The models showed that low-molecular-weight organic matter (namely, <3000 Da) was most reactive to chlorine as compared to medium- and high-molecular-weight fractions (3000 to 10,000 Da). AOC has been associated with molecular weights in the 300- to 1000-Da range. It is possible that reactions between the disinfectant and organic carbon occurred. BDOC and AOC concentrations provided a pool of reactive carbon (generally, BDOC is associated with chlorine demand) and carbon available for consumption (AOC is readily used by microbes).

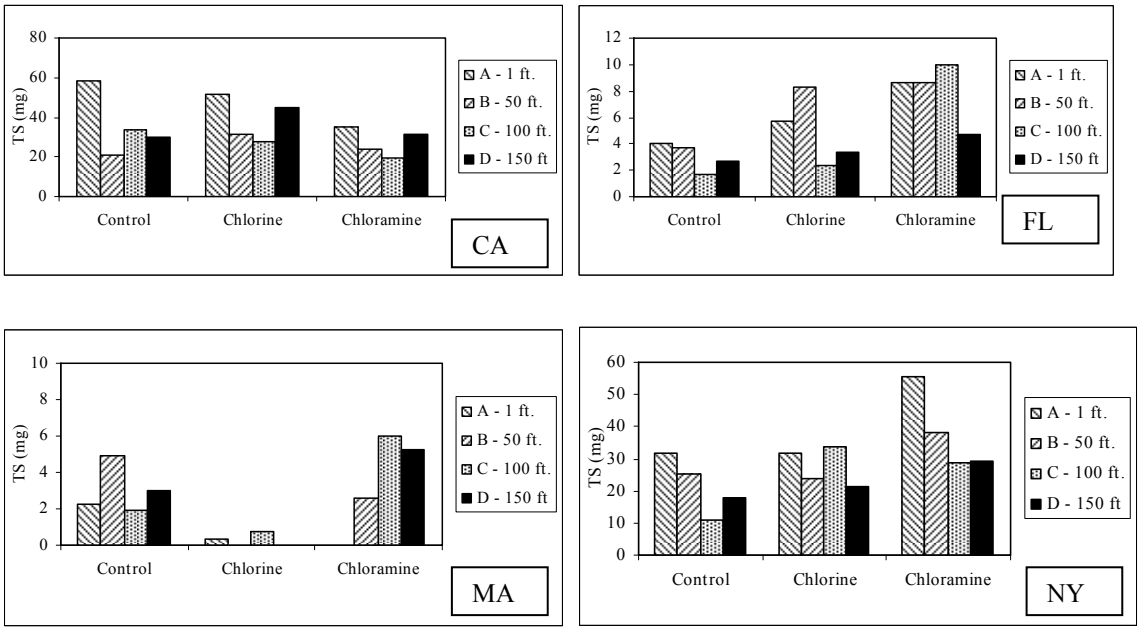


Figure 8.10. Biofilm total solids (TS) in a 1-ft section of a 1/8-in.-diameter pipe loop. The biofilm accumulated over a 1- to 2-month period and thereafter disinfected with chlorine or chloramine. Note the difference in the *y* axis scale.

The results presented in this section confirmed that disinfectant residuals did not persist in reclaimed water distribution systems under the current operations. The loop tests corroborated the findings and suggested that reactions with biofilm material will result in further loss of residual. The following sections will investigate the impact of disinfectant residuals on microbial growth as well as the impact of BDOC and loss of disinfectant residuals on the growth of microorganisms in the systems.

8.3. MAINTENANCE OF A DISINFECTANT RESIDUAL WAS EFFECTIVE IN CONTROLLING THE INOCULUM FROM BIOFILMS, WITH FREE CHLORINE MORE EFFECTIVE THAN CHLORAMINATION FOR HPC AND *LEGIONELLA* CONTROL UNDER THE CONDITIONS STUDIED

Disinfectant residual levels have an important role in controlling the occurrence and distribution of microbes in the systems, and subsequent data will clearly indicate the need for a sufficient and stable disinfectant residual. As already documented in Section 8.2, a significant loss of chlorine residual in the full-scale systems and controlled pipe loop studies occurred. Additional analysis of the pipe loop data shows that the decrease in disinfectant typically corresponded with an increase in heterotrophic bacteria in the bulk water (Figure 8.11a and b). The decrease in disinfectant was more rapid with chlorine than with chloramine. Figure 8.11 represents data from only two locations, but the trend was similar at all four sites. Although the HPC bacteria were still present in the biofilm on disinfection (Figure 8.12a and b), their prevalence in the bulk water remained under control. As indicated in Section 6.7.2, most bacteria in water systems are attached to surfaces and piping material. However, some of the biofilm-based organisms can be introduced into the bulk water if the integrity of the biofilm is disrupted, and this introduction can create a constant source of inoculum for water that has been disinfected.

The loss of disinfectant was associated with an increase and re-emergence of microbes. Disinfection of the pipe loops (Table 8.3) with free chlorine consistently reduced the HPCs in the bulk water and kept them low throughout the length of the CA and MA loops. However, in the other two loops, HPC bacterial levels increased at locations farther away from the intake. With chloramination, HPC bacterial densities in the bulk water also decreased significantly (by >1 log unit) (Table 8.4). However, these decreases were not always consistent and may have been a result of the relatively short duration of exposure (3 days) or sloughing of the biofilm during sample collection. Given the short duration of the experiment and the short contact time, the superior performance of free chlorine is not unexpected (LeChevallier et al., 1988). HPC bacterial levels in the inlet of the chloramine loops were generally higher than in free chlorinated systems, and the microbes grew to levels generally 10 times higher than in the free chlorinated systems (Table 8.4). Chloramine residuals were generally < 2 mg/L in the pipe loops (Table 8.3), except in the CA system, where the chloramine residual averaged about 6.1 mg/L. At this level chloramines effectively prevented the regrowth of HPC bacteria. Thus, a free chlorine residual more efficiently controlled the occurrence of suspended HPC bacteria in the pipe loop systems than did a chloramine residual.

Legionella spp. were also detected in the loop systems, albeit at lower levels and less frequently then the HPC bacteria were (Table 8.5). Chlorine significantly reduced *Legionella* occurrence, which was detected in only two samples from the NY loop. *Legionella* was detected frequently in the chloraminated loops in FL, MA, and NY; however, there was a significant amount of variability. *Legionella* was not detected in the chloraminated CA loop, possibly because of the higher chloramine residual.

In the pipe loop disinfection studies, the loops at all four locations were negative for coliform bacteria in both bulk water and the biofilm samples (data not shown), but there was an abundance of HPC bacteria in all locations (Table 8.4), with the highest bacterial densities occurring in the inlet of the undisinfected loops. Because biofilms were allowed to develop prior to disinfection for various lengths of time (41, 27, 73, and 75 days in CA, FL, MA, and NY, respectively), comparison of bacterial levels between different loop systems may not be appropriate. In addition, the disinfectant residuals maintained in each system varied (Table 8.3), further complicating analysis.

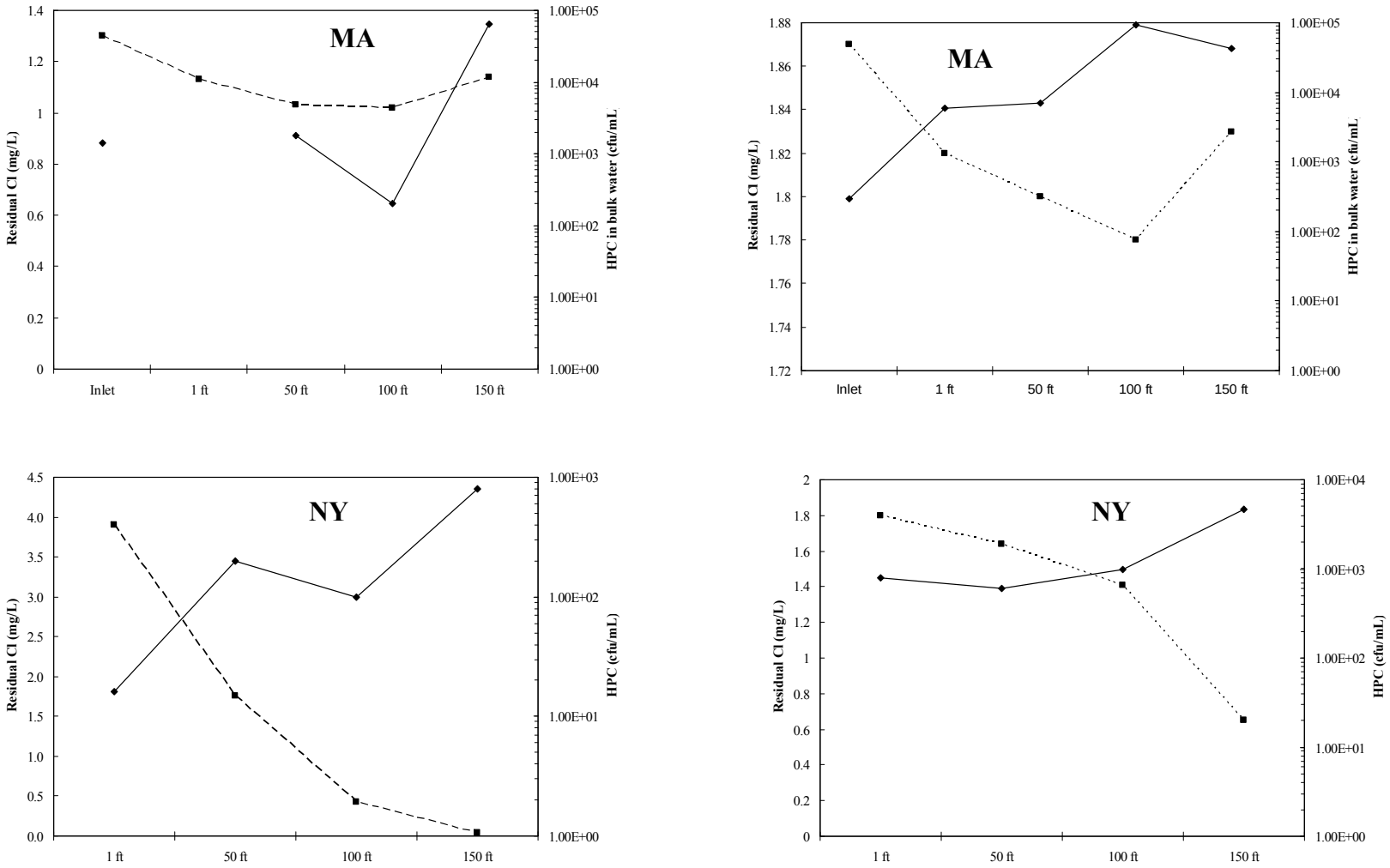


Figure 8.11a. Consistent increase in HPCs (solid lines) in the bulk water of two MBR systems as the residual disinfectant (broken lines) in the pipe loop diminished. Both panels on the left depict disinfection with chlorine, whereas those on the right were with chloramine as the disinfectant.

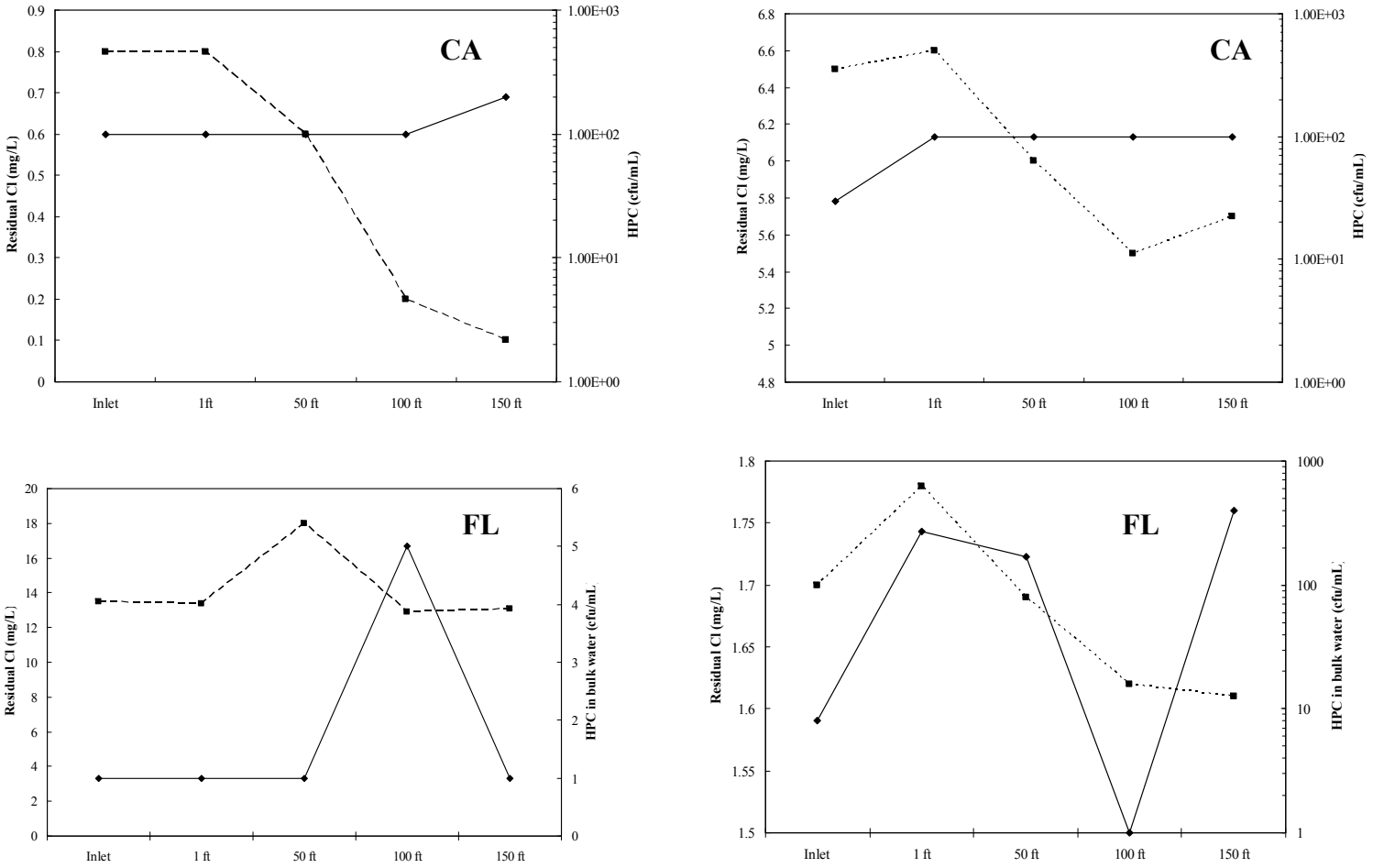


Figure 8.11b. Consistent increase in HPCs (solid lines) in the bulk water of two conventional treatment systems as the residual disinfectant (broken lines) in the pipe loop diminished. Both panels on the left depict disinfection with chlorine, whereas those on the right were with chloramine as the disinfectant.

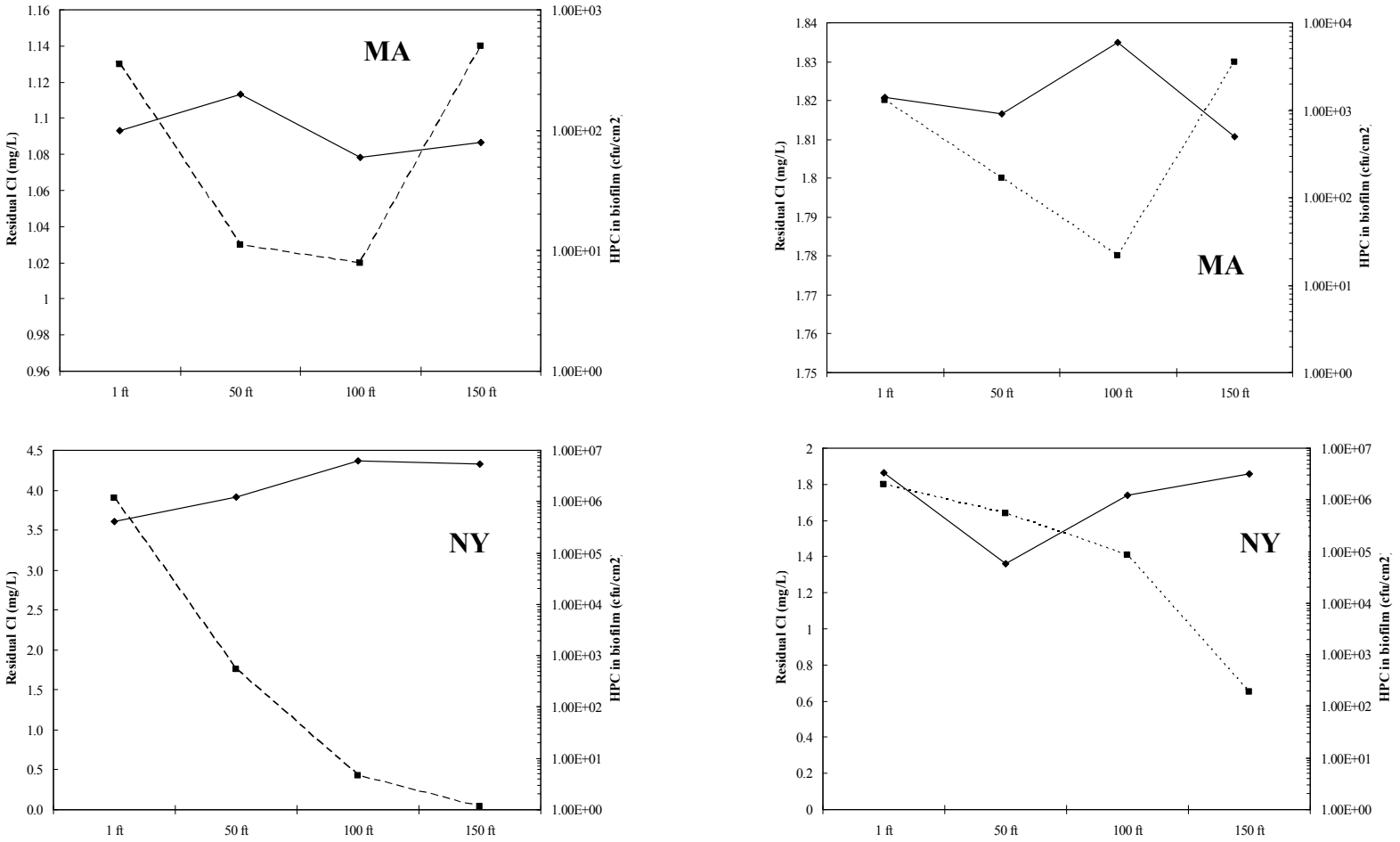


Figure 8.12a. Effect of chlorine (the two panels on the left) and chloramine (the two panels on the right) on HPCs in the biofilm at different residual disinfectant levels in MBR-derived pipe loops. HPCs are represented with solid lines, whereas the disinfectant is represented with broken lines.

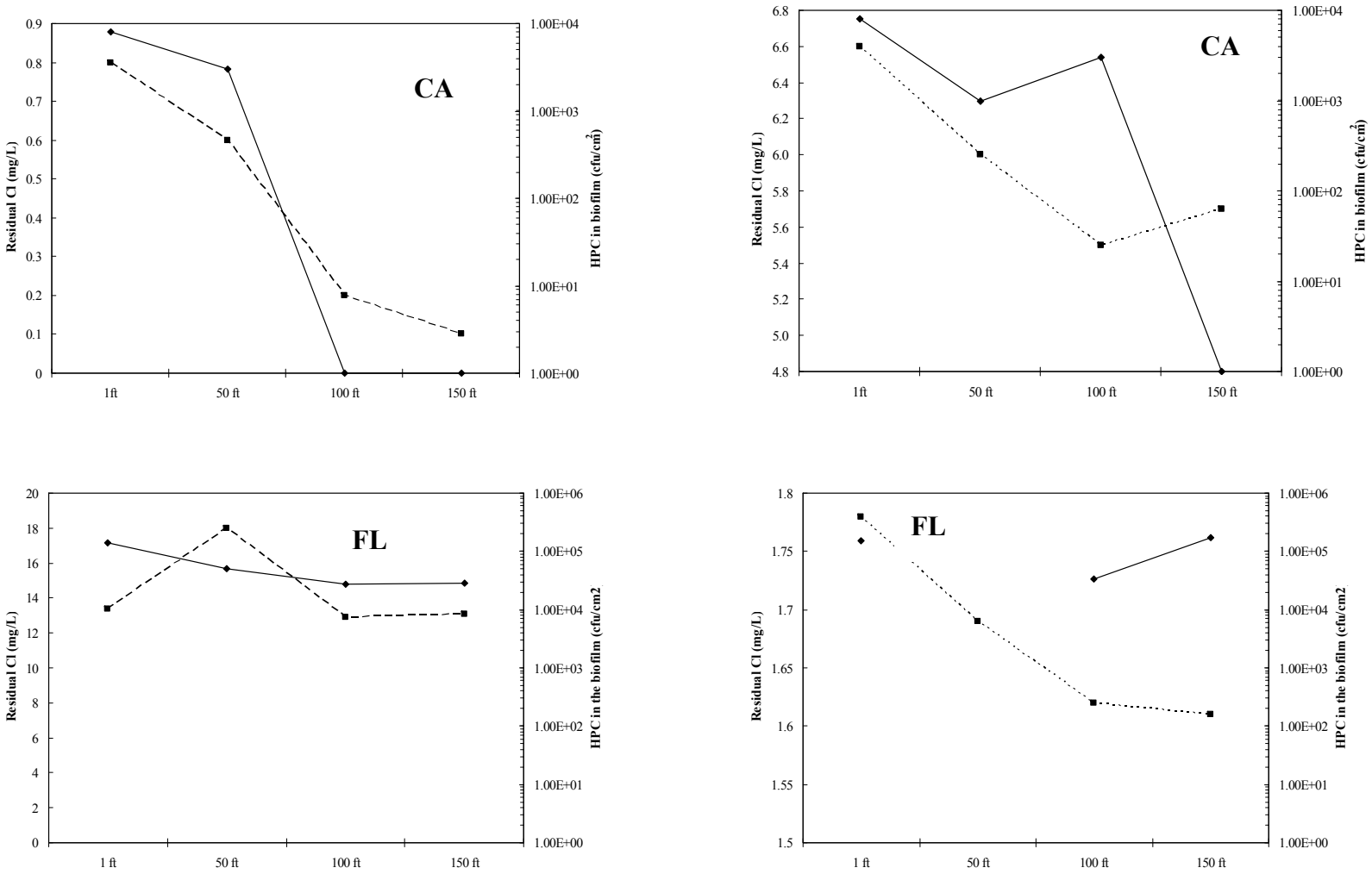


Figure 8.12b. Effect of chlorine (the two panels on the left) and chloramine (the two panels on the right) on HPCs in the biofilm at different residual disinfectant levels in conventional treatment-derived pipe loops. HPCs are represented with solid lines, whereas the disinfectant is represented with broken lines.

Table 8.4. HPCs in Bulk Water and Biofilm in the Pipe Loops[a,b]

Site	Inlet			At 1-ft length			At 50-ft length			At 100-ft length			At 150-ft length		
	Density of HPC bacteria in bulk water (10^3 CFU/100 mL)														
	Control	**Chlorine**	**Chloramine**	**Control**	**Chlorine**	**Chloramine**	**Control**	**Chlorine**	**Chloramine**	**Control**	**Chlorine**	**Chloramine**	**Control**	**Chlorine**	**Chloramine**
CA	3800 ± 600	0.1	0.03	2900 ± 71	ND[c]	0.1 ± 0.07	4200 ± 900	0.1	0.1 ± 0.07	3600 ± 350	0.1 ± 0.1	0.1 ± 0.07	3000 ± 700	0.2 ± 0.4	0.1 ± 0.07
FL	0.26 ± 0.1	ND	0.008 ± 0.002	0.14 ± 0.01	0.001 ± 0.005	0.27 ± 0.060	0.14 ± 0.03	ND	0.17 ± 0.01	0.008 ± 0.005	0.005 ± 0.005	ND	0.2 ± 0.1	ND	0.4 ± 0.02
MA	1000 ± 7	1.4 ± 0.3	0.3 ± 0.07	1200 ± 50	ND	6.0 ± 1.1	2500 ± 80	1.8 ± 0.3	6.9 ± 1.1	5200 ± 3700	0.2 ± 0.05	95 ± 8	3500 ± 1000	64 ± 10	42 ± 20
NY	810 ± 120	ND	ND	75 ± 11	0.016 ± 0.009	0.8 ± 0.1	33 ± 5	0.2 ± 0.002	0.6 ± 0.2	72 ± 0.9	0.1 ± 0.02	1.0 ± 0.2	22 ± 8.5	0.8 ± 0.02	4.6 ± 0
	Density of HPC bacteria in biofilm ($\times 10^4$ CFU/cm^2)[b]														
CA	NA[d]	NA	NA	38 ± 13000	0.8 ± 0	0.8 ± 0	100000 ± 16000	0.3 ± 0.1	0.1 ± 0.1	7200 ± 500	ND	0.3 ± 0.2	3800 ± 6	ND	ND
FL	NA	NA	NA	4.7 ± 0.5	14 ± 2.5	15 ± 56	6.0 ± 1.1	5.1 ± 0.3	TNTC[e]	10 ± 3	2.7 ± 2.4	3.4 ± 4.8	50 ± 13	2.9 ± 0.9	17 ± 55
MA	NA	NA	NA	43 ± 11	0.01 ± 0.003	0.14 ± 0.04	53 ± 7	0.02 ± 0.005	0.09 ± 0.003	96 ± 13	0.006 ± 0.001	0.6 ± 0.03	110 ± 58	0.008 ± 0.002	0.05 ± 0.005
NY	NA	NA	NA	6500 ± 120	410 ± 60	3400 ± 520	4700 ± 0	1200 ± 3	58 ± 30	820 ± 120	6200 ± 1600	1200 ± 60	1100 ± 8	5400 ± 500	3200 ± 640

[a]The respective treatments had reclaimed water that was disinfected with chlorine (HOCl) or chloramine (NH_2Cl) or was not treated at all (namely, control).
[b]Values are geometric means ± SD based on at least two replicates.
[c]ND = not detected (namely, <3 CFU/100 mL for bulk water and <1 CFU/cm^2 for biofilms).
[d]NA = not applicable.
[e]TNTC = too numerous to count.

The occurrence and survival of *Legionella* spp. in the loops are summarized in Table 8.5. Just like the HPC bacteria, *Legionella* spp. were largely eliminated from the bulk water by free chlorine and remained absent throughout the length of the loops. Chloramination was also quite effective against *Legionella* spp. in the CA and FL loops. By contrast, the *Legionella* data are less consistent for the MA and NY loops, but these loops were allowed to develop for the longest time (73 and 75 days) and the short period of chloramination (3 days) probably was insufficient for complete control. In the control loops, the biofilm closest to the inlet generally had the highest *Legionella* density, with levels declining downstream of the inlet. Overall, disinfection with free chlorine in the present study reduced the *Legionella* spp. to below detection in the biofilms, particularly in the CA and MA loops. Chloramination was also effective for *Legionella* control, but low levels were still detected in biofilm samples. It is possible that longer exposures are needed to yield more consistent results in chloraminated systems.

It is also important that the pipe material for the loops was PVC and that the effectiveness of free chlorine and chloramine disinfection is dependent upon the nature of the pipe materials. Therefore, the performance of disinfection in full-scale systems could be different from performance in the pipe loop studies. Accumulation of corrosion products on metallic pipes can greatly affect the efficacy of disinfection.

To facilitate comparison between the density of bacterial cells in the bulk water and the density in the biofilm, the cells in the biofilm recovered per unit volume of loop tubing were, based on the volume of water in the 1-ft length of the loop, converted to a per-volume basis. The results from that computation are presented in Figures 8.13 and 8.14. Because the loops were installed and run for different durations, the data cannot be directly compared. However, HPCs in the bulk water in the control treatment were generally lower than those in the biofilm. This difference was especially more prominent in the FL, CA, and NY loop systems, with log differences of 4 to 5 log units, whereas those in the MA system were only 1 log unit lower than those in the biofilm (Figure 8.13a and b). Disinfection with chlorine or chloramine did not significantly reduce the biofilm-based HPCs in the FL and NY loops but reduced them by more than 3 log units in the CA and MA loops, with chlorine providing a slightly better HPC removal from the biofilm than chloramine did. In contrast, Clark and Sivaganesan (1999), using epifluorescence (namely, direct counting as opposed to growth on conventional media), did not find any significant difference between disinfection of microorganisms with chlorine and disinfection with chloramine in the biofilm and bulk water from three different pipe systems (namely, PVC, cement, and polyethylene).

Table 8.5. *Legionella* spp. in Bulk Water and Biofilm in the Pipe Loops[a]

	Density of *Legionella* spp. in bulk water (CFU/100 mL)														
	Inlet			At 1-ft length			At 50-ft length			At 100-ft length			At 150-ft length		
Site	**Control**	**Chlorine**	**Chloramine**	**Control**	**Chlorine**	**Chloramine**	**Control**	**Chlorine**	**Chloramine**	**Control**	**Chlorine**	**Chloramine**	**Control**	**Chlorine**	**Chloramine**
CA	300	ND[c]	ND	ND	ND	ND	$3 \times 10^4 \pm 1.7 \times 10^4$	ND	ND	ND	ND	ND	ND	ND	ND
FL	300	ND	300	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MA	ND	ND	$1.0 \times 10^3 \pm 1.9 \times 10^3$	ND	ND	$2.6 \times 10^3 \pm 16 \times 10^3$	$2.6 \times 10^3 \pm 16 \times 10^3$	ND	ND	$1.3 \times 10^3 \pm 3.6 \times 10^3$	ND	$5.2 \times 10^3 \pm 64 \times 10^3$	$11.4 \times 10^3 \pm 9.5 \times 10^3$	ND	ND
NY	$20 \pm 8.1 \times 10^4$	ND	ND	$1.8 \times 10^4 \pm 8.0 \times 10^5$	ND	$1.8 \times 10^3 \pm 1.4 \times 10^3$	$3.8 \times 10^5 \pm 4.4 \times 10^5$	300	300	$3.3 \times 10^5 \pm 3.9 \times 10^5$	ND	$9.3 \times 10^3 \pm 3 \times 10^3$	$3.0 \times 10^5 \pm 4.5 \times 10^5$	540 ± 470	$1.5 \times 10^5 \pm 4 \times 10^3$
	Density of *Legionella* spp. in biofilm (10^3 CFU/cm^2)[b]														
CA	NA[d]	NA	NA	55 ± 1600	ND	ND	640 ± 20	ND	0.04 ± 0.007	450 ± 320	ND	0.9 ± 0.3	300 ± 100	ND	ND
FL	NA	NA	NA	0.05	0.43 ± 0.35	0.15	0.12 ± 0.1	ND	0.14 ± 0.08	0.08 ± 0.07	ND	0.09 ± 0.5	0.11 ± 0.04	ND	ND
MA	NA	NA	NA	0.13 ± 0.002	ND	0.1 ± 0.04	0.25 ± 0.03	ND	0.07 ± 0.07	0.37 ± 0.10	ND	0.32 ± 0.43	0.13 ± 0.01	ND	0.18 ± 0.07
NY	NA	NA	NA	240 ± 150	1.7 ± 0.5	150 ± 110	23 ± 12	51 ± 54	1.4 ± 7	0.8 ± 1	ND	ND	120 ± 190	ND	ND

[a]The respective treatments had reclaimed water that was disinfected with chlorine (HOCl) or chloramine (NH_2Cl) or not treated at all (namely, control).
[b]Values are geometric means ± SD based on at least two replicates.
[c]ND = Not detected (namely, <300 CFU/100 mL for bulk water and <1 CFU/cm^2 for biofilms).
[d]NA = Not applicable.

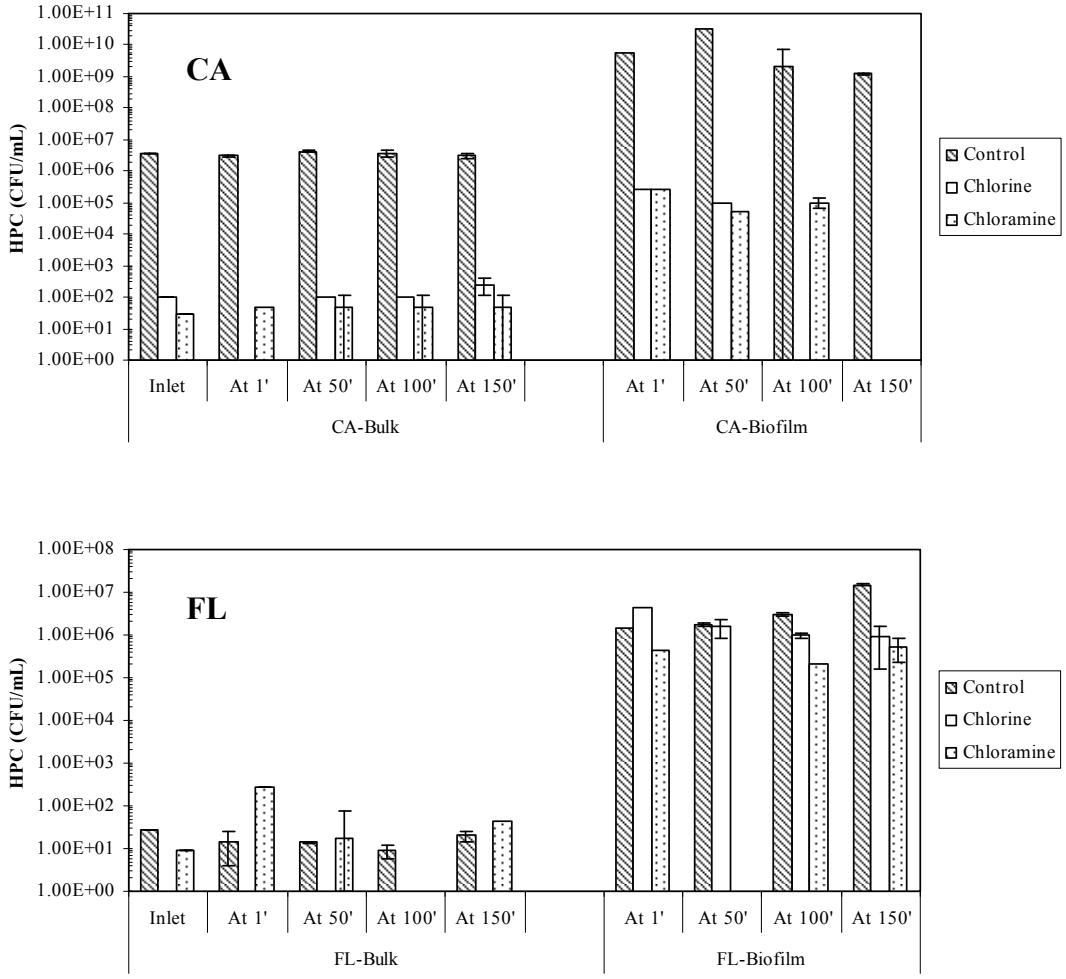


Figure 8.13a. HPC in the bulk water and biofilm with different disinfectants. Note that the HPC bacteria in chloraminated biofilm for FL at 50 ft were present but too numerous to count at the maximum dilution plate. HPCs in the FL bulk water were comparatively lower because the loop intake was very close to the plant's point of chlorine contact. The density of bacteria in the biofilm was converted to numbers of CFU per milliliter by considering the density per unit area (cm^2) in relation to the total volume of water in the 1-ft section of the pipe loop sampled (see Materials and Methods for details).

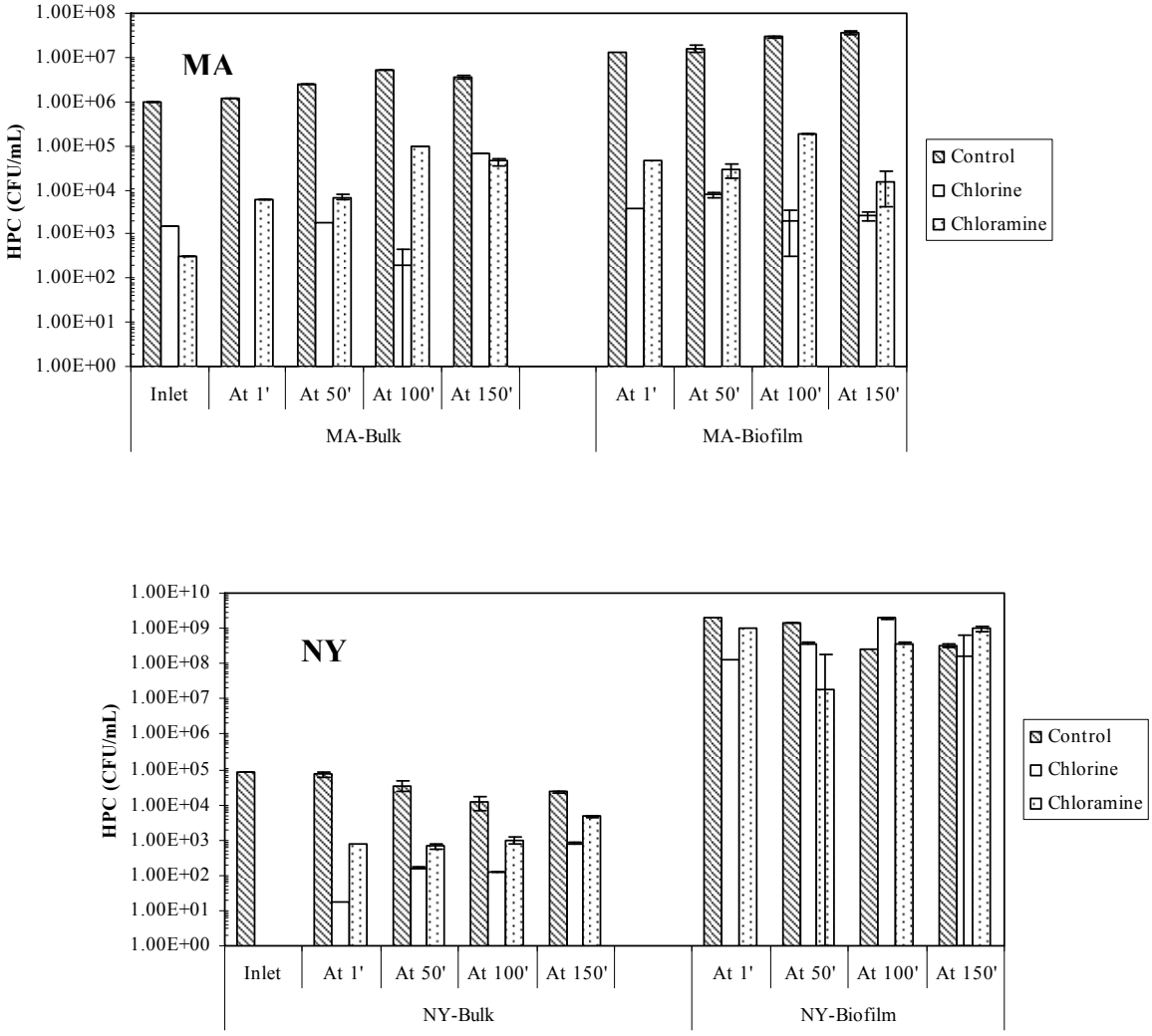


Figure 8.13b. HPC in the MA and NY bulk water and biofilm with different disinfectants. The density of bacteria in the biofilm was converted to numbers of CFU per milliliter by considering the density per unit area (cm^2) in relation to the total volume of water in the 1-ft section of the pipe loop sampled (see Materials and Methods for details).

A similar comparison of the density of *Legionella* in the bulk water and the density in the biofilm also showed that *Legionella* spp. in the bulk water intake were 2 to 5 log units lower than that in the control treatment biofilm (Figure 8.13a and b). However, in the case of CA, the headwaters did not contain detectable levels of *Legionella*, although these organisms ultimately built up in the control biofilm. Chlorine was effective in preventing *Legionella* in the bulk water and biofilm in the FL and MA loops. *Legionella* disinfection in the biofilm was successful in the NY loop but only at the extreme end of the loop. The chlorine-treated loop prevented *Legionella* from establishing in the CA biofilm, but chloramination was

inconsistent in preventing these organisms. These findings were contrary to those of Flannery et al. (2006), who reported a reduced frequency of occurrence of *Legionella* spp. in municipal hot-water systems upon treating the water with chloramine (see Figure 5.6; Section 5.3.5). The reasons for the difference between our findings and those of Flannery et al. (2006) are not entirely clear, but disinfectants are known to be less effective against biofilm bacteria, with higher disinfectant concentrations being necessary to affect biofilm-based organisms (de Beer et al., 1994).

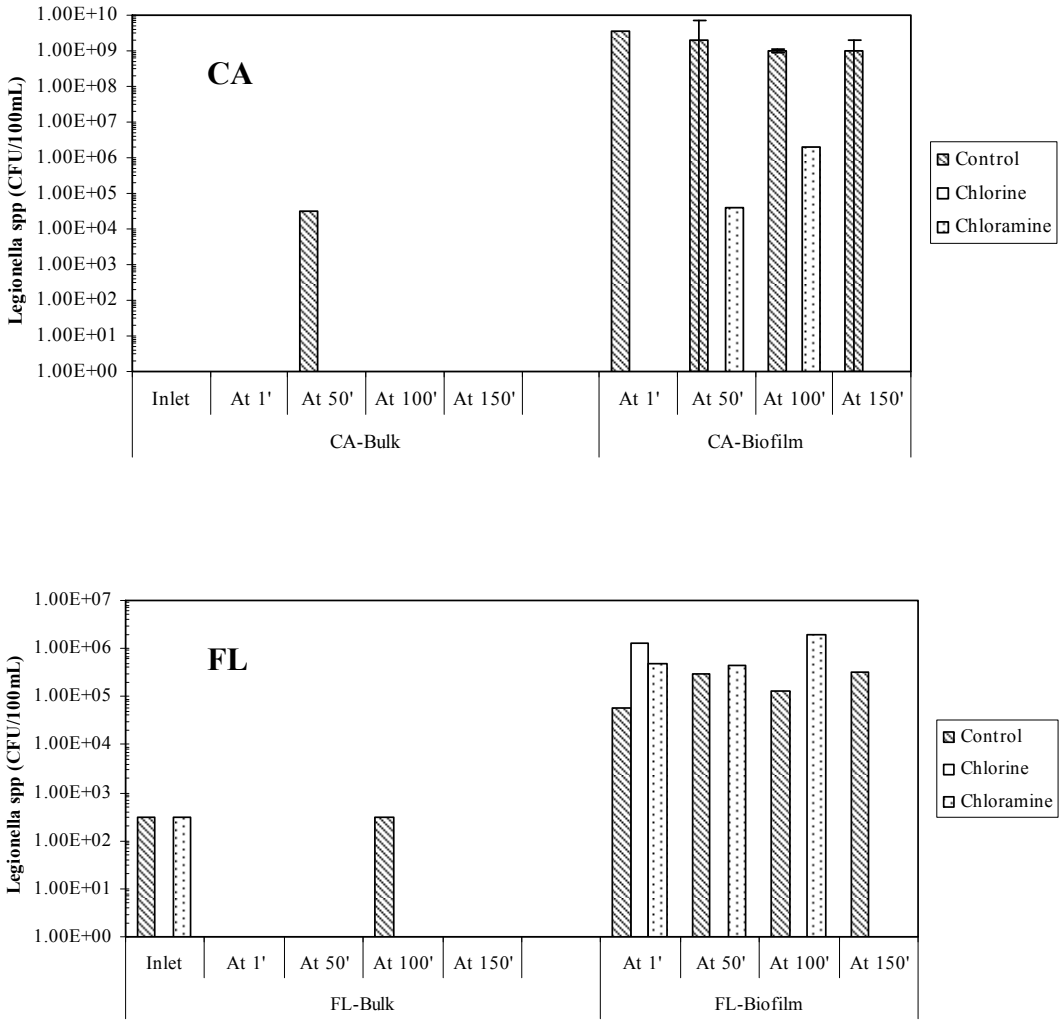


Figure 8.14a. *Legionella* in the bulk water and biofilm in the conventional CA and FL loops with different disinfectants. The density of bacteria in the biofilm was converted to numbers of CFU per milliliter by considering the density per unit area (cm^2) in relation to the total volume of water in the 1-ft section of the pipe loop sampled (see Materials and Methods for details).

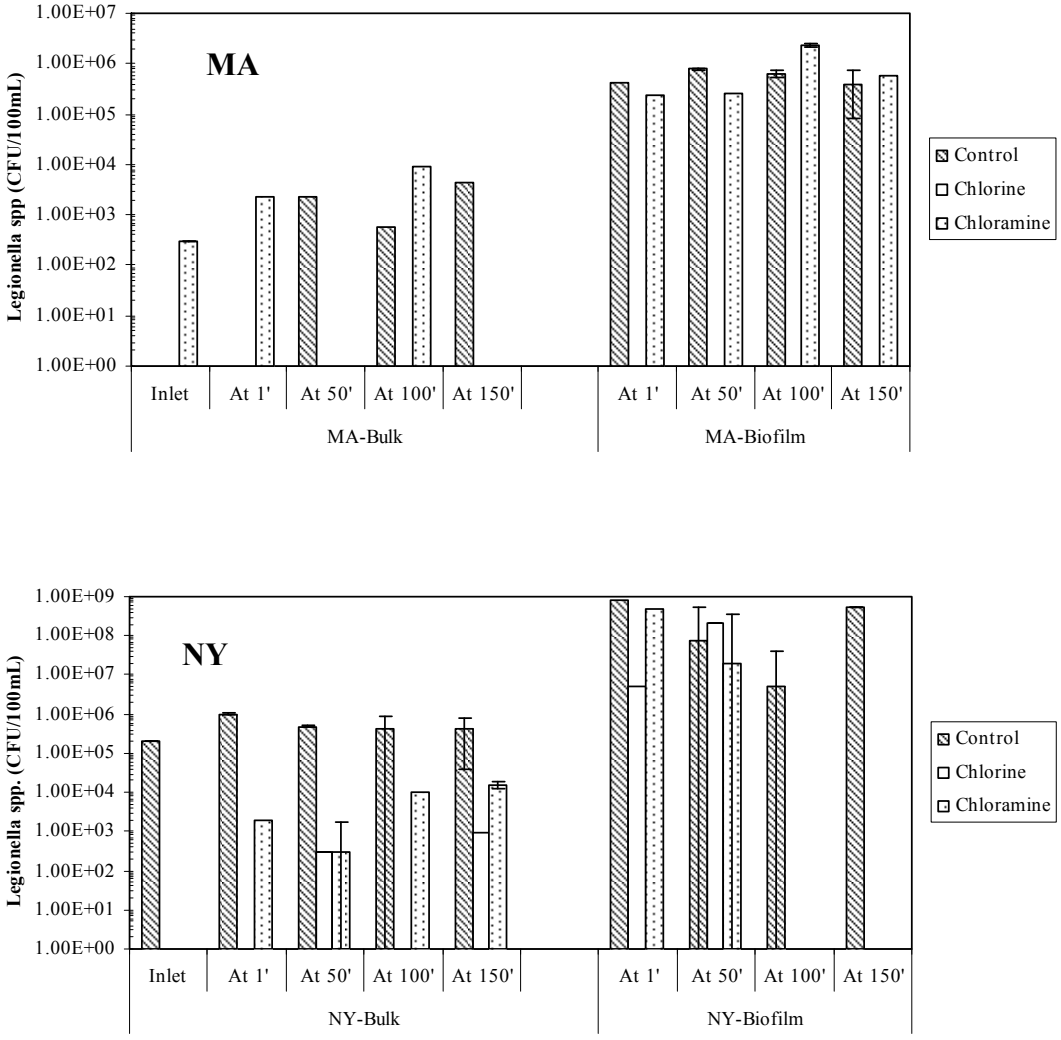


Figure 8.14b. *Legionella* in the bulk water and biofilm in the MBR MA and NY system loops with different disinfectants. The density of bacteria in the biofilm was converted to numbers of CFU per milliliter by considering the density per unit area (cm^2) in relation to the total volume of water in the 1-ft section of the pipe loop sampled (see Materials and Methods for details).

8.4. THE CONVENTIONAL AND MBR WASTEWATER TREATMENT SYSTEMS WERE EFFECTIVE IN REMOVING MICROBES IN THE EFFLUENT, BUT REGROWTH OCCURRED IN THE DISTRIBUTION SYSTEMS

From a microbial perspective, the primary purpose of wastewater treatment systems is to remove and/or inactivate pathogenic microbes. The systems studied were effective in this regard, but potentially pathogenic bacteria regrew in the distribution system (Figure 8.15 and

Table 8.6). Changes in HPC bacterial levels and *Mycobacterium* spp. from plant effluent through the distribution system are summarized in Figures 8.15a and 8.15b below. The trickling filter and chlorination system in CA reduced HPCs in the effluent during spring and summer, when HPC densities in the effluent were $\leq 10^3$ CFU/mL. These organisms increased at least 10-fold in the distribution system. Similar or even larger increases in HPCs were observed in the other three systems irrespective of whether conventional treatment or MBR practices were used.

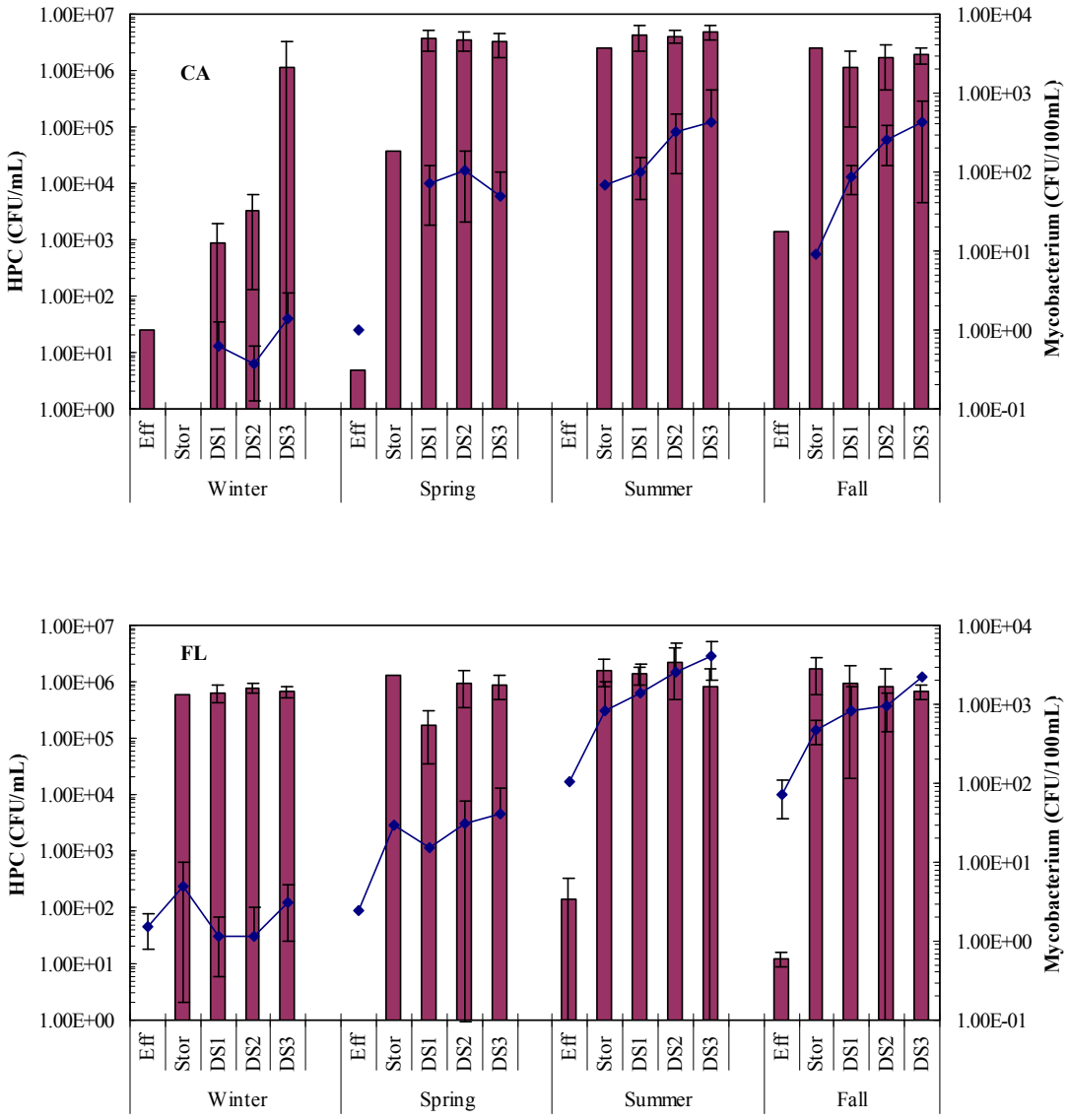


Figure 8.15a. Regrowth of HPC bacteria (columns) and *Mycobacterium* spp. (lines) in two reclaimed water distribution systems with conventional treatment. Eff = effluent and Stor = storage pond, whereas DS1, DS2, and DS3 = distribution system points 1, 2, and 3.

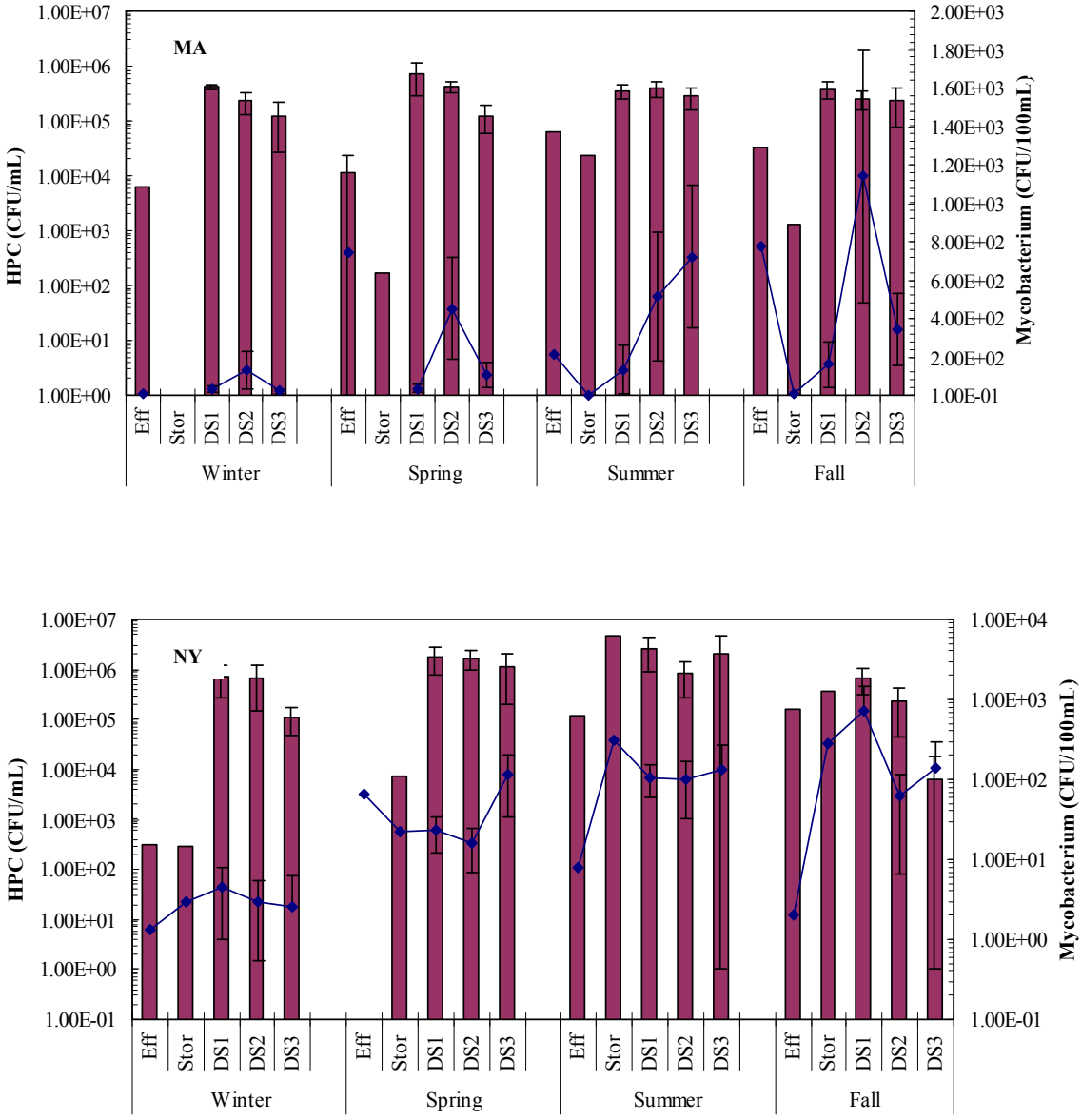


Figure 8.15b. Regrowth of HPC bacteria (columns) and *Mycobacterium* spp. (lines) in two reclaimed water distribution systems with MBR treatment technology. Eff = effluent and Stor = storage tank, whereas DS1, DS2, and DS3 = distribution system points 1, 2, and 3.

Wastewater treatment also reduced the abundance of *Mycobacterium* spp. in the disinfected effluent. It is worth noting that for the system in MA, the point of disinfection was the storage tank and that reduction of all of the microorganisms assayed in this system occurred at this point of disinfection. However, in all of the four systems, these organisms re-emerged in the reclaimed water as it flowed through the system. In general, the regrowth of *Mycobacterium* spp. were least prominent in winter and spring. Most of the other organisms assayed were also effectively removed by the treatment system (conventional or MBR) but effectively regrew in reclaimed water.

Table 8.6. Abundance of Various Microorganisms in Treated Effluents and Their Regrowth in the Distribution System[a]

Organism	Site	Effluent	Storage	DS1[b]	DS2	DS3
Total coliform (CFU/100 mL)	CA	<1	1 ± 1	1 ± 1	1 ± 1	1 ± 6
	FL	1 ± 1	11 ± 7	3 ± 6	9 ± 46	7 ± 17
	MA	2 ± 1	<1[c]	1 ± 1	1 ± 1	1 ± 1
	NY	3 ± 23	<1	<1	1 ± 1	<1
Fecal coliform (CFU/100 mL)	CA	1 ± 1	<1	<1	<1	<1
	FL	1 ± 1	12 ± 9	1 ± 3	4 ± 4	4 ± 5
	MA	<1	<1[c]	<1	<1	<1
	NY	2 ± 6	<1	<1	1 ± 1	<1
E. coli (CFU/100 mL)	CA	1 ± 1	1 ± 1	1 ± 1	<1	1 ± 1
	FL	1 ± 1	7 ± 8	2 ± 2	2 ± 3	4 ± 2
	MA	<1	<1[c]	<1	1 ± 1	1 ± 1
	NY	3 ± 10	1 ± 1	<1	1 ± 1	1 ± 1
Enterococci (CFU/100 mL)	CA	<1	<1	<1	1 ± 1	<1
	FL	1 ± 0	9 ± 26	3 ± 9	27 ± 36	10 ± 18
	MA	<1	<1[c]	<1	<1	<1
	NY	3 ± 34	<1	<1	<1	<1
Pseudomonas spp. (CFU/100 mL)	CA	<1	2 ± 2	2 ± 5	3 ± 4	6 ± 12
	FL	<1	8 ± 4	2 ± 2	9 ± 10	4 ± 2
	MA	1 ± 1	<1[c]	2 ± 5	2 ± 3	2 ± 5
	NY	1 ± 1	2 ± 1	1 ± 1	1 ± 3	6 ± 130
Aeromonas spp. (CFU/mL)	CA	1 ± 1	2 ± 7	6 ± 3	20 ± 200	66 ± 900
	FL	1 ± 1	210 ± 480	91 ± 170	120 ± 700	300 ± 410
	MA	1 ± 1	<1[c]	6 ± 46	5 ± 57	1 ± 33
	NY	1 ± 2	1 ± 1	10 ± 18	32 ± 42	1 ± 1
Legionella spp. (10^3 CFU/100 mL)	CA	<0.3	2.2 ± 4.1	2.3 ± 2.0	0.9 ± 1.5	1.9 ± 1.6
	FL	<0.3	3.0 ± 70	2.7 ± 13	3.5 ± 16	8 ± 52
	MA	0.4 ± 0.2	<0.3[c]	1.3 ± 2.8	0.7 ± 2.0	0.4 ± 0.7
	NY	0.6 ± 2.1	0.7 ± 0.6	0.5 ± 0.6	0.5 ± 0.6	0.5 ± 0.4

[a]Geometric mean (SE) aggregate of four seasons.
[b]DS = distribution system.
[c]Disinfection location.

In the present study, no or very low densities of coliforms, *E. coli*, enterococci, *Pseudomonas*, *Aeromonas*, or *Legionella* spp. were detected in the effluent from the conventional plants (namely, CA and FL) and from the MBR in MA. Some densities of coliforms, *E. coli*, and enterococci were detected in the NY MBR system, but this detection was very rare (namely, once in summer 2007) possibly owing to damage in the membrane system. Generally, our results were similar to those of de Koning and van Nieuwenhuijzen (1999), who did not detect any coliforms in the membrane filtration systems tested in The Netherlands. Membranes are increasingly being used in the reclaimed water industry to remove nutrients and microbial contaminants. Jolis et al. (1996) documented the removal of more than 4 log units of coliforms and a 1.9-log removal of MS2 phage by membrane filtration.

Satisfactory removal of microorganisms by trickling filters, as is practiced at the CA plant, has also been documented, especially if the filters are well constructed and operated (Natural Primary Drinking Water Regulations, 2000; Filipkowska and Krzemieniewski, 1998). Proper construction and operation of these systems were discussed earlier (see Section 3.5.1). Successful activated sludge treatment as was practiced at the FL plant depends on the concept of bubbling air (or pure O_2) through the wastewater to promote bacterial activity to metabolize organic carbon. The activated sludge system in FL also had a component for removing phosphorus (namely, five-stage Bardenpho) from the sludge. A schematic of this process is shown in Figure 8.16. Such enhanced biological phosphorus removal systems are designed to couple a front-end anaerobic zone with a subsequent aerobic zone that selectively enriches for microbes that are capable of biologically removing phosphate from the wastewater. The system promotes the synthesis and consumption of intracellular polymers of phosphate (as polyphosphate) and carbon (as polyhydroxylkanoates or cellular carbohydrates). The microorganisms responsible for removing phosphate utilize energy derived from polyphosphate hydrolysis for carbon uptake and storage during the anaerobic stage. In the aerobic stage, they use the previously stored carbon for growth and polyphosphate formation (Liu et al., 2000; Cloete and Oosthuizen, 2001).

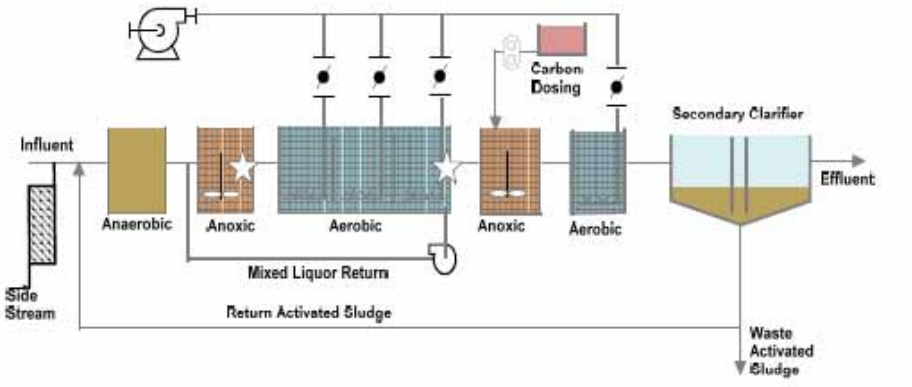


Figure 8.16. Schematic showing the principle behind phosphorus removal in enhanced biological phosphorus removal systems.

Despite the superb ability of the conventional and MBR systems at removing microbes in the effluent, regrowth occurred, as evidenced by the abundance of microorganisms in the distribution system. It is worth noting, however, that the common indicators, notably coliforms, *E. coli*, and enterococci, generally remained rare in the system compared to *Aeromonas* and *Legionella* spp. As will be discussed later (see 8.14), other factors could also contribute to the microbial occurrence. For example, the FL system had an intermittent supply of water, and the practice of shifting the supply from one zone of the system to the other could have contributed to the intrusion of contaminants when the system was depressurized.

8.5. THE ABSENCE OF COMMON INDICATOR BACTERIA (COLIFORMS AND *E. COLI*) DID NOT PRECLUDE THE PRESENCE OF POTENTIALLY PATHOGENIC ORGANISMS

Because there are so many types of microorganism in water that can cause disease, it is impossible to test for the presence of each of them individually. Public health, environmental microbiology, and environmental regulators utilize the concept of indicator organisms that can be used to signal the potential for fecal contaminants in water. In the present study, the abundance of these indicators, together with some potentially pathogenic species, was monitored to determine their changes in reclaimed water systems. The most commonly used indicator organisms include total and fecal coliforms and *E. coli* (Eaton et al., 2005; Harwood et al., 2005; USEPA, 2004), and these usually nonpathogenic organisms are abundant in untreated sewage. Testing for these indicator bacteria is widespread because the tests are easy and inexpensive (Eaton et al., 2005).

The frequency at which the opportunistic pathogens occurred in reclaimed water in the absence of key common indicator organisms was quantified and is summarized in Table 8.7. To make these trends more understandable, the data are presented by season, location, and sampling site (plant effluent, after storage, and three points in the distribution system—all of which were tested on four consecutive days). For example, in the FL system coliform bacteria were detected in only 10% of the winter samples where *Mycobacterium* spp. were found. Likewise in the NY system, coliform bacteria were absent in 71.4% of the winter samples where *Mycobacterium* was detected. Similarly, *E. coli* was not detected in any of the instances when *Aeromonas* spp. were detected in the CA system in the summer (Table 8.7). The Indicator Index is the number of times (out of 16 episodes) in which the indicator completely missed signaling the presence of the potential pathogen. The higher the index value (up to a maximum of 16), the less reliable the indicator was for predicting the presence of the respective pathogen.

Based on the Indicator Index results, it is apparent that enterococci were the least useful indicator of reclaimed water quality with regard to predicting the occurrence of potential pathogens such as *Mycobacterium*, *Legionella*, *Aeromonas*, and *Pseudomonas* spp. Because the presence of the opportunistic pathogens was a result of regrowth and not necessarily owing to "recontamination" of the reclaimed water, it is probably not a surprise that total coliform bacteria had the best overall Indicator Index score; although they still failed to predict pathogen occurrence 12 to 44% of the time. Various studies have reported a lack of relationship between *Aeromonas* incidence and that of coliforms, *E. coli*, or HPCs (Landre et al., 1998). Total coliform bacteria are more environmentally robust and would be expected to grow under conditions that would be limiting to *E. coli*, for example. It is also interesting that

the reliability of the Indicator Index did not differ with respect to whether the water was derived from MBR or conventional treatment systems.

Many states regulate reclaimed water quality based on the absence of total or fecal coliforms in treated effluents, and these organisms are typically susceptible to chemical disinfection (Miescier and Cabelli, 1982; Payment, 1999). This study showed that the systems were typically capable of meeting these standards. However, the presence of potentially opportunistic pathogens in reclaimed water systems in the absence of indicator organisms means that these indicator organisms should not be relied upon to make public health assessments. The majority of environmental mycobacteria are nonpathogenic. However, some of their members, particularly the *M. avium* complex, which comprises *M. avium* and *M. intracellulare*, are considered opportunistic pathogens (Peterson et al., 1989; Chege et al., 2008). However, the present study did not determine the virulence of the *Aeromonas*, *Pseudomonas*, *Legionella*, or *Mycobacterium* spp. detected in reclaimed waters. Many of these species are of low or no virulence.

This study also examined several frank pathogens, including enteropathogenic *E. coli* O157:H7, which is associated with severe diarrhea infections (Powell et al., 2000). This strain causes hemolytic-uremic syndrome, a rare kidney disorder whose symptoms include bloody diarrhea, followed by renal failure. The organism is particularly dangerous to children whose immune system is not fully developed, as well as for elderly and immunocompromised individuals. From an ecological perspective, the organism is able to proliferate in the rumen and is prevalent in about 5% of dairy cows in temperate regions (Pell, 1997). It can survive adverse environmental conditions such as low temperatures (< 8 ^{o}C), pHs lower than 4, and high levels of salt (Clavero and Beuchat, 1996).

This study used an enrichment procedure, immunomagnetic capture, and antigen detection to screen for *E. coli* O157 occurrence in treated effluents (Figure 8.17). In previous studies, this approach was found to detect *E. coli* O157 at concentrations of <1/100 mL with recovery rates of 71 to 111% (Bukhari et al., 2007). In this study, *E. coli* O157 was encountered only twice in the reclaimed water, both times in the same system, namely, FL in spring and fall (Table 8.8). Its presence was detected only in the effluent, and the organism was never detected in the storage pond or the distribution system, indicating that *E. coli* O157 was not able to grow in the reclaimed water distribution system.

Table 8.7. Percentage of Instances When Opportunistic Pathogens Were Present in Reclaimed Water in the Absence of a Common Indicator Organism[a]

Season and Location	*Mycobacterium* spp. in Absence of:			*Legionella* spp. in Absence of:			*Aeromonas* spp. in Absence of:			*Pseudomonas* spp. in Absence of:		
	Coliform	***E. coli***	**Enterococci**	**Coliform**	***E. coli***	**Enterococci**	**Coliform**	***E. coli***	**Enterococci**	**Coliform**	***E. coli***	**Enterococci**
Winter												
FL	10 (*n* = 10)	40 (*n* = 10)	0 (*n* = 4)	10 (*n* = 10)	30 (*n* = 10)	0 (*n* = 4)	0 (*n* = 4)	50 (*n* = 4)	0 (*n* = 4)	0 (*n* = 5)	60 (*n* = 5)	0 (*n* = 4)
CA	40 (*n* = 5)	80 (*n* = 10)	100 (3)	50 (*n* = 4)	66.7 (*n* = 7)	100 (*n* = 1)	50 (*n* = 4)	75 (*n* = 4)	100 (*n* = 4)	40 (*n* = 5)	80 (*n* = 5)	100 (*n* = 3)
MA	71.4 (*n* = 7)	92.3 (*n* = 13)	100 (*n* = 4)	60 (*n* = 5)	90 (*n* = 10)	100 (*n* = 2)	66.7 (*n* = 3)	100 (*n* = 3)	100 (*n* = 3)	0 (*n* = 1)	100 (*n* = 1)	100 (*n* = 1)
NY	71.4 (*n* = 7)	92.3 (*n* = 13)	100 (*n* = 5)	66.7 (*n* = 3)	83.3 (*n* = 6)	100 (*n* = 2)	75 (*n* = 4)	75 (*n* = 4)	100 (*n* = 4)	50 (*n* = 2)	66.7 (*n* = 3)	100 (*n* = 2)
Spring												
FL	50 (*n* = 2)	28.6 (*n* = 7)	50 (*n* = 2)	28.6 (*n* = 7)	30.8 (*n* = 13)	50 (*n* = 4)	40 (*n* = 5)	40 (*n* = 5)	60 (*n* = 5)	40 (*n* = 5)	40 (*n* = 5)	33 (*n* = 3)
CA	85.7 (*n* = 7)	100 (*n* = 11)	100 (*n* = 4)	75 (*n* = 4)	100 (*n* = 7)	100 (*n* = 3)	100 (*n* = 5)	100 (*n* = 5)	100 (*n* = 5)	100 (*n* = 1)	100 (*n* = 1)	100 (*n* = 1)
MA	85.7 (*n* = 7)	100 (*n* = 13)	100 (*n* = 4)	100 (*n* = 2)	100 (*n* = 4)	0 (*n* = 0)	100 (*n* = 4)	100 (*n* = 4)	100 (*n* = 4)	100 (*n* = 2)	100 (*n* = 2)	100 (*n* = 2)
NY	100 (*n* = 8)	100 (*n* = 14)	100 (*n* = 5)	100 (*n* = 7)	100 (*n* = 11)	100 (*n* = 4)	100 (*n* = 5)	100 (*n* = 5)	100 (*n* = 5)	100 (*n* = 2)	100 (*n* = 2)	100 (*n* = 1)
Summer												
FL	11 (*n* = 9)	16.7 (*n* = 12)	40 (*n* = 5)	0 (*n* = 6)	0 (*n* = 8)	0 (*n* = 2)	0 (*n* = 4)	25 (*n* = 4)	25 (*n* = 4)	0 (*n* = 6)	16.7 (*n* = 6)	25 (*n* = 4)
CA	85.7 (*n* = 7)	92.3 (*n* = 13)	75 (*n* = 4)	100 (*n* = 6)	90 (*n* = 10)	75 (*n* = 4)	100 (*n* = 4)	100 (*n* = 4)	75 (*n* = 4)	85.7 (*n* = 7)	85.7 (*n* = 7)	75 (*n* = 4)
MA	87.5 (*n* = 8)	85.7 (*n* = 14)	100 (*n* = 5)	100 (*n* = 8)	81.8 (*n* = 11)	100 (*n* = 5)	100 (*n* = 3)	100 (*n* = 3)	100 (*n* = 3)	100 (*n* = 5)	100 (*n* = 5)	100 (*n* = 2)
NY	87.5 (*n* = 8)	71.4 (*n* = 14)	80 (*n* = 5)	100 (*n* = 6)	77.8 (*n* = 9)	100 (*n* = 4)	100 (*n* = 4)	60 (*n* = 5)	80 (*n* = 4)	0 (*n* = 1)	0 (*n* = 1)	0 (*n* = 1)
Fall												
FL	66.7 (*n* = 6)	75 (*n* = 12)	50 (*n* = 4)	60 (*n* = 5)	100 (*n* = 7)	25 (*n* = 4)	75 (*n* = 4)	100 (*n* = 4)	25 (*n* = 4)	66.7 (*n* = 3)	80 (*n* = 5)	33 (*n* = 3)
CA	57 (*n* = 7)	70 (*n* = 10)	100 (*n* = 4)	60 (*n* = 5)	60 (*n* = 10)	100 (*n* = 4)	50 (*n* = 2)	50 (*n* = 2)	100 (*n* = 2)	0 (*n* = 3)	66.7 (*n* = 3)	100 (*n* = 1)
MA	87.5 (*n* = 8)	100 (*n* = 14)	100 (*n* = 5)	100 (*n* = 5)	100 (*n* = 9)	100 (*n* = 3)	100 (*n* = 2)	100 (*n* = 2)	100 (*n* = 2)	80 (*n* = 5)	80 (*n* = 5)	100 (*n* = 3)
NY	100 (*n* = 8)	100 (*n* = 14)	100 (*n* = 5)	100 (*n* = 8)	100 (*n* = 13)	100 (*n* = 5)	0	0	0	100 (*n* = 2)	100 (*n* = 2)	100 (*n* = 1)
Indicator index[b]	**2**	**5**	**10**	**7**	**6**	**10**	**7**	**8**	**9**	**5**	**6**	**10**

[a]The numbers in brackets (*n*) represent the total number of times that the respective analyte was detected at that location in the season.
[b]Indicator index reflects the number of instances (out of 16 episodes) in which the indicator completely missed pathogens. The higher the index (up to a maximum of 16), the less reliable the indicator is in predicting the presence of the respective pathogen.

Table 8.8. Occurrence of *E. coli* O157 in Distribution Systems over a 1-Year Duration

		Sampling Location within System				
Site	**Season**	**Effluent**	**Storage**	**DS1**	**DS2**	**DS3**
CA	Winter	-	-	-	-	-
	Spring	-	-	-	-	-
	Summer	-	-	-	-	-
	Fall	-	-	-	-	-
FL	Winter	-	-	-	-	-
	Spring	+	-	-	-	-
	Summer	-	-	-	-	-
	Fall	+	-	-	-	-
MA	Winter	-	-	-	-	-
	Spring	-	-	-	-	-
	Summer	-	-	-	-	-
	Fall	-	-	-	-	-
NY	Winter	-	-	-	-	-
	Spring	-	-	-	-	-
	Summer	-	-	-	-	-
	Fall	-	-	-	-	-

Figure 8.17. Samples coded PC1 (from effluent), PC2 (from the storage pond), PC3A, PC3B, and PC3C (from three points in the distribution system) during testing for the presence/absence of *E. coli* O157:H7 using an immunoassay kit (Reveal®, Lansing, MI). Respective samples were determined to be positive for *E. coli* O157:H7 if both the test and control bands were visible, as is the case in PC1 (topmost panel) and positive control (bottom panel). All of the other samples are negative, but the test strip is viable as signified by the presence of a single band.

The Reveal® testing system for detecting *E. coli* O157:H7 is certified by the Association of Analytical Communities and operates by wicking the sample through a reagent zone that contains antibodies that are specific to *E. coli* O157:H7 conjugated to colloidal particles. If O157:H7 antigens are present, they bind to the gold-conjugated antibodies. The antigen-antibody complex travels along the wick through a nitrocellulose membrane that contains a zone of anti-*E. coli* O157:H7 antibody where it is captured, in the presence of an appropriately colored indicator, forming some visible aggregation. As part of quality control, all viable testing panels form at least one line (in the control area) to ensure that the test is working properly (Figure 8.19). The biochemical testing methods that are routinely used for detecting *E. coli* are not effective in detecting *E. coli* O157:H7 and other serotypes that are enteropathogenic (Bukhari et al., 2007). This fact was confirmed in the present study, in which, for both instances where *E. coli* O157:H7 was detected, the samples were negative for conventional *E. coli* indicators (Table 8.8).

Table 8.9. Occurrence of Protozoa and Virus Pathogens in Reclaimed Water in Relation to Common Bacterial Indicators

Site and Sampling Location	Season	Common Bacterial Indicators				Protozoa		Bacteriophage		Enteric Viruses						
		Coliform								Enterovirus		HAV			NV	
		Total	Fecal	*E. coli*	Ent[a]	Crypto[a]	*Giardia*	MS[a]	Somatic	Presence	Copies/L	Presence	Spike	Rotavirus	Presence	Copies/L
CA (Pond)	Winter	-	-	-	-	+	-	+	+	(+)[b]	1.7×10^2	-	+	+	+	1.3×10^3
	Spring	-	-	-	-	-	-	+	+	-	N/A[c]	-	+	-	+	8.4×10^2
	Summer	-	-	-	-	+	-	-	+	+	4.0×10^5	-	+	-	+	2.3×10^5
	Fall	+	-	+	-	-	-	+	+	(+)	1.2×10^8	-	+	-	-	N/A
FL (Effluent)	Winter	+	-	-	-	-	+	+	-	+	1.3×10^3	-	+	+	-	N/A
	Spring	-	-	-	-	+	-	-	+	(+)	2.4×10^6	-	+	-	+	1.4×10^6
	Summer	-	-	-	ND[d]	+	+	+	+	-	N/A	-	+	-	-	N/A
	Fall	-	-	-	ND	+	+	+	+	+	1.8×10^5	-	+	-	+	2.3×10^2
MA (Effluent)	Winter	-	-	-	-	-	-	-	-	-	N/A	-	+	-	-	N/A
	Spring	-	-	-	-	-	-	+	+	-	N/A	-	+	-	-	N/A
	Summer	-	-	-	-	-	-	+	+	-	N/A	-	+	-	-	N/A
	Fall	-	-	-	-	-	-	+	-	+	3.8×10^6	-	+	-	+	1.6×10^2
NY (Tank)	Winter	-	-	-	-	-	-	+	-	-	N/A	-	+	-	-	N/A
	Spring	-	-	-	-	-	-	-	+	-	N/A	-	+	-	-	N/A
	Summer	-	-	+	-	-	+	+	+	-	N/A	-	+	-	-	N/A
	Fall	-	-	-	-	-	-	+	+	-	N/A	-	+	-	-	N/A

[a]Abbreviations: Ent = Enterococci; Crytpo = *Cryptosporidium* spp.; MS = male-specific.
[b](+) = sample was positive only after reamplification of the RT-PCR products (namely, "nested PCR").
[c]N/A = not applicable.
[d]ND = not determined.

The bacterial indicators did not successfully reflect the quality of reclaimed water effluents with regard to protozoan parasites either. More specifically, plant effluent samples were positive for *Giardia* spp. (for FL) or *Cryptosporidium* spp. (for CA) even though no indicator organisms were detected. The FL plant effluent sample in spring was also positive for *Cryptosporidium* spp. despite being negative for coliforms, fecal coliforms, and *E. coli*. Similar trends were observed at this facility in summer and fall when the treated water contained both *Giardia* spp. and *Cryptosporidium* spp. without any coliforms or *E. coli*. Similarly, the NY MBR plant contained *Giardia* spp. in summer despite being negative for coliforms. No parasites were detected in the MA MBR throughout the sampling period indicating a more robust treatment regimen at this facility with regard to protozoan cysts.and oocysts. *Giardia* cysts and *Cryptosporidium* oocysts are environmentally resistant protozoa that are resistant to disinfectants at levels that would readily inactivate enteric bacteria (LeChevallier and Au, 2004). The immunofluorescence method used in this study does not determine the viability or infectivity of the *Giardia* and *Cryptosporidium* spp. detected in this study.

Bacteriophage have also been widely used as indicators of microbial contamination, and they have been suggested as an alternative indicator for enteric viruses as their morphology and survival characteristics are similar to those of enteric viruses (Armon and Kott, 1993). One or both types of coliphage assayed (namely, male-specific and somatic phage) were present in the treated effluent in a majority of cases, except in CA and MA in winter and in CA during the fall sampling event. The presence of these viruses was registered even in water that was negative for coliforms and *E. coli* (Table 8.9). Bacteriophage are reported to be persistent in water as they are not removed as well as bacteria and other viruses by treatment processes (Rose et al., 1996; also see Figure 7.16). However, the relationship between phage and other viruses may not be universally applicable to all types of water (Havelaar et al., 1993).

In the present study, bacteriophage were present even in the absence of enterovirus, rotavirus, and NV in all of the NY MBR-generated reclaimed water and in summer for FL and MA (Table 8.9). NVs are one of the most common causes of acute nonbacterial gastroenteritis in humans (Menton et al., 2007). It is highly contagious and can be transmitted as an aerosol, via the oral route, or through direct contact, causing serious outbreaks in settings such as hospitals, schools, nursing homes, and hotels where people are in close contact. In a couple of instances, enterovirus detection was hampered in the initial RT-PCR but was later detected by use of nested PCR. The number of enterovirus and NV genome copies was calculated from the formula:

$$\text{Copies/L} = \frac{(\textit{Copies per uL of RT} - \textit{PCR} \times 0.8 \times \textit{mL of concentrate after eluting in beef extract})}{\textit{Liters of reclaimed water sampled through the virus filter}} \times 1000$$

where the 0.8 constant is the concentration factor of 125 µl equivalent to 100 µl of final RNA eluted. Where detected in the water, enteroviral genome copies ranged between 10^2/L and 10^8/L. The quantification was computed based on the amount of cDNA produced by the RT-PCR (or nested PCR) using a calibration of the DNA from a probe control kit (Roche Diagnostics, Indianapolis, IN). It should be noted that the number of genome copies includes both the genes and noncoding sequences of the viral RNA, from which the cDNA was derived. By comparison, Rose et al. (2006) detected between 94 and 730 HAV genomes/L and between 2×10^3 and 1.6×10^3 enterovirus genomes/L in beach waters. Thus, the numbers of viral genome copies detected in the reclaimed water in our systems were several orders of magnitude higher than those reported by Rose et al. (2006) in beach waters. The concept of

genome copy number has not been previously used in assessing the viral content of reclaimed water. It is important that PCRs do not determine virus infectivity but indicate only the presence of viral RNA. Therefore, these data cannot be used to evaluate risk with a high level of confidence but only to show the potential for treatment breakthrough. For perspective, Harwood et al. (2005), using three cell lines (namely, buffalo green monkey, rhabdosarcoma, and MA-104), detected infectious enteroviruses in 31% of the disinfected reclaimed water effluents they tested.

A typical reaction output is shown in Figure 8.18. HAV was absent in the reclaimed water from all of the four sites tested throughout the year as confirmed by the absence of HAV-specific primer amplifications even after a nested PCR. As part of the quality control process, most of the HAV spiked in the reclaimed water samples was recovered (Figure 8.19). The set of primers for detecting HAV was initially used by Costa-Mattioli et al. (2002) and specifically targets the most constant genomic region (namely, the 5' noncoding region) of HAV identified by Cohen et al. (1987) and Jansen et al. (1988). The primers used were compared with an existing database to confirm that they are functional with all known human HAV sequences. The primers were specific for HAV and nonfunctional with enteroviruses as represented by a negative reaction with RNA from poliovirus. Rotavirus and NVs were detected in several samples, most of which were from the conventional treatment processes (Table 8.9). It is most noticeable that viruses were generally absent in the MBR effluent, except in the Fall MA sample. That plant underwent a significant upgrade during the course of this study, a process that involved switching the Zenon membranes to Torray flat plate membranes.

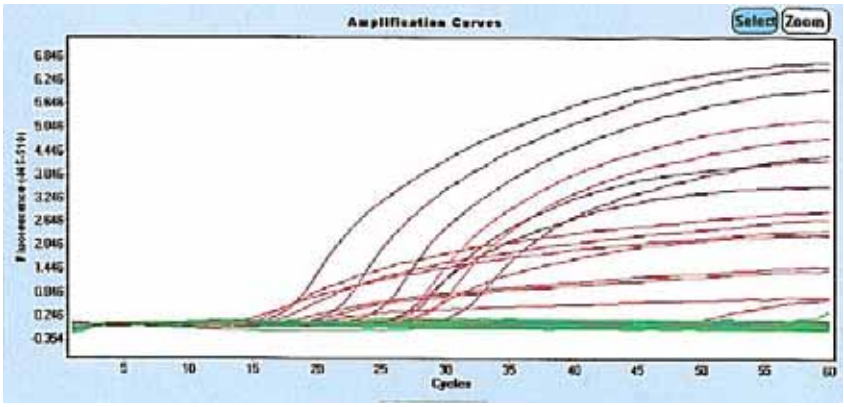


Figure 8.18. RT-PCR for enterovirus samples and the associated output.

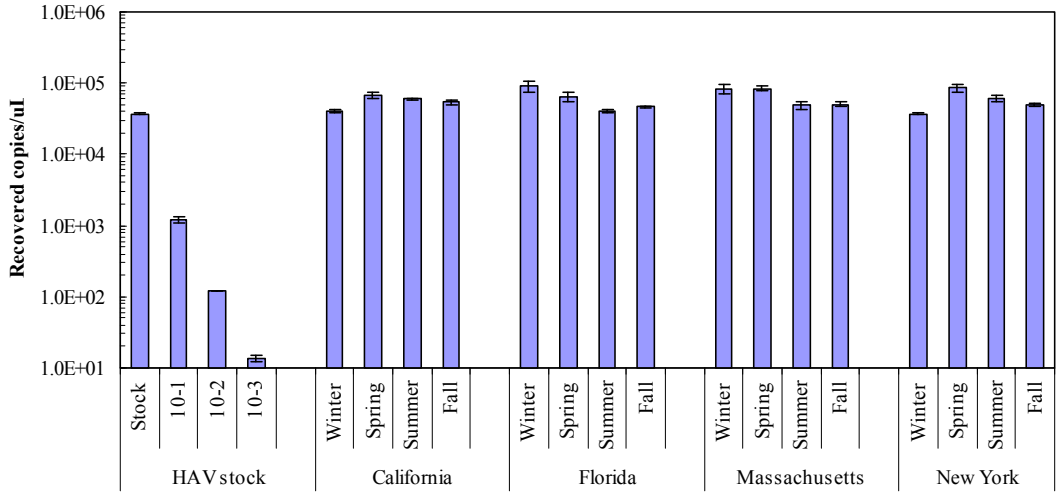


Figure 8.19. Recovery of HAV in the spiked reclaimed water samples compared to the stock. Error bars are standard deviation from duplicates.

By comparison, enterovirus recoveries from the reclaimed water were comparatively lower, ranging between 18% and 57% (Figure 8.20). The reason for the recoveries for enteroviruses being comparatively lower than for HAV are unclear but could be linked to the presence of some specific inhibitors. No enteroviruses in the spiked CA sample obtained in the fall were recovered, signaling a serious presence of PCR inhibitors in the concentrate. This observation agrees with the fact that enterovirus in the nonspiked sample was detected only after a nested PCR (Table 8.9). In general, percent recoveries of enteroviruses appear to be slightly higher in the two MBR-treated effluents.

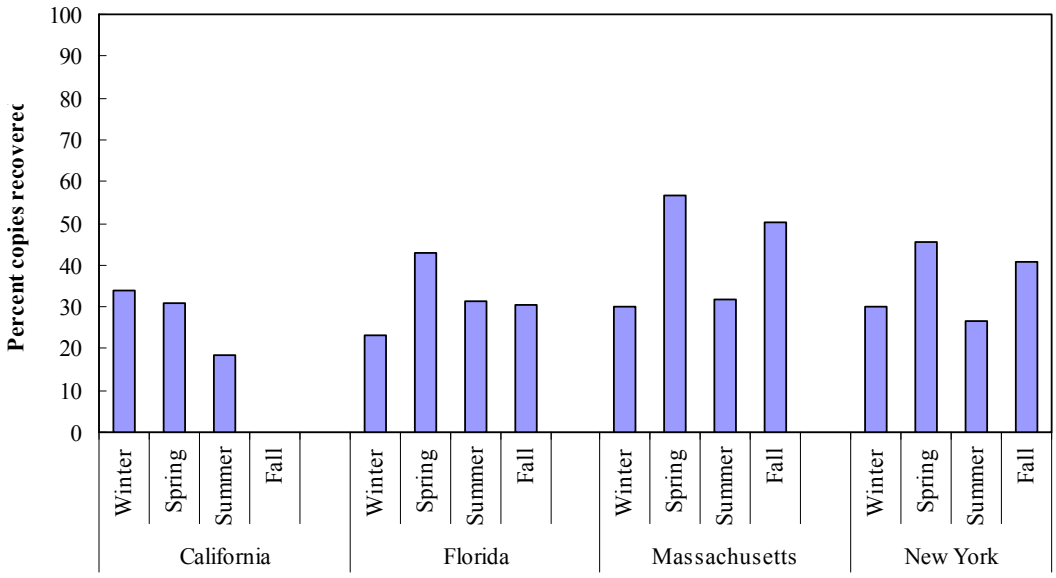


Figure 8.20. Percent number of enterovirus copies recovered from spiked reclaimed water.

Both *Giardia* spp. and *Cryptosporidium* spp. are important pathogens in water and wastewater. As they only increase in abundance in the presence of their host, their presence is justifiably less frequently tested for in environmental samples (see Section 5.2). Table 8.9 summarizes the frequency with which each of these organisms was encountered in the reclaimed water at all four locations. Quantitative analysis shows that the FL facility had the most abundant *Giardia* spp. and *Cryptosporidium* spp. (Table 8.10).

Both *Giardia* and *Cryptosporidium* were jointly detected in the FL facility during the summer (June 2007) and fall (September 2007) sampling events. In all of the other instances of detection, only one and not the other was present. No protozoan parasites were detected in the MBR-generated reclaimed water at the facility in MA, whereas only 1 *Giardia* cyst/100 L was detected in the NY facility reclaimed water. By comparison, a total of 54 *Cryptosporidium* oocysts/100 L and 27 *Giardia* cysts/100 L were detected in the reclaimed water at the FL facility. The CA system where open pond storage is also practiced did not register any *Giardia* but harbored 2 *Cryptosporidium* oocysts/100 L. Thus, both parasites were most abundant in the FL system, which uses a conventional treatment process. The absence of these parasites in the MA reclaimed water system, which uses MBR, is not entirely surprising as most membranes have a pore size of ≤4 μm (Wagner, 2001; Jjemba, 2008), a size that can exclude these parasites. Whereas bacteria and viruses are typically 0.2 to 10 μm and ≤0.1 μm, respectively (Jjemba, 2004), both *Giardia* spp. and *Cryptosporidium* spp. are larger, although their (oo)cysts can range between 0.001 and 0.15 mm (namely, 0.1 to 15 μm [Mara and Horan, 2003]). *C. parvum* oocysts are spherical with a diameter of 3 to 7 μm (USEPA, 1995). However, the NY MBR system registered a low level of *Giardia* spp. (namely, 1 cyst/100 L) possibly because of a compromised membrane and/or the presence of atypically small *Giardia* cysts that were able to flow through the membrane pores. These parasites and their (oo)cysts are resistant to some of the most widely utilized disinfectants such as chlorine, and MBRs thus separate them from the effluent.

Table 8.10. Occurrence of Protozoan Parasites in Reclaimed Water over a 1-Year Period

Location	No. of Times Sampled	No. Positive		Total Volume Collected (L)	Total/100 L	
		Cryptosporidium	*Giardia*		*Cryptosporidium* Oocysts	*Giardia* Cysts
CA	4	2	0	151.5	2	0
FL	4	3	3	121.25	54	27
MA	4	0	0	96.0	0	0
NY	4	0	1	200.5	0	1

It should be emphasized that both *Giardia* spp. and *Cryptosporidium* spp. in this study were not identified to species level. There are five established *Giardia* species and more than eight *Cryptosporidium* spp. known, of which *C. parvum* is the one that is of most concern to human infections. Furthermore, the viability of these (oo)cysts was not determined, as viability determination was beyond the scope of this study. However, in a report by York et al. (2003)

13 facilities in FL that reported detected levels of *Giardia* found 10 to 90% (average of 61%) of those *Giardia* organisms viable, whereas of the three plants that detected *Cryptosporidium*, 70 to 90% (average, 77%) of the *Cryptosporidium* organisms were viable. Viability was in each instance determined based on microscopic examination and vital staining. Ideally, even more informative would be the infectivity of the parasites. The infectivity of *Cryptosporidium* spp. from these plants, together with findings for a number of other plants, is currently being researched under a different project (WRF-06-003). Infection studies showed a clear relationship between the dose ingested and the probability of infection with a lowest dose of 30 *C. parvum* oocysts tested. That dose carried a 20% probability of infection in healthy human volunteers (DuPont et al., 1995). Higher doses of *C. parvum* induced the symptoms sooner, and the symptoms lasted for a longer period. At the laboratory level, infectivity can be determined by using cell culture infectivity assays (LeChevallier et al., 2003; Aboytes et al., 2004).

8.6. IN SYSTEMS THAT EMPLOYED OPEN STORAGE, THE OCCURRENCE OF ALGAL GROWTH HAD A SIGNIFICANT EFFECT ON ORGANIC CARBON AND INCREASED AOC AND BDOC IN THE DISTRIBUTION SYSTEM

If the water reservoir is an open system, the quality of the water can be impacted by UV light (see Section 5.7). Open storage reservoirs are vulnerable to algal growth, as seen in the results for the CA and FL conventional treatment facilities. Chlorophyll measurements were taken to track the presence of algal material in distribution system samples. Increases in algal levels occurred to a greater extent during the warmer months, intensifying in the summer and reaching maximum concentrations in the fall (Figure 8.21; Table 8.11).

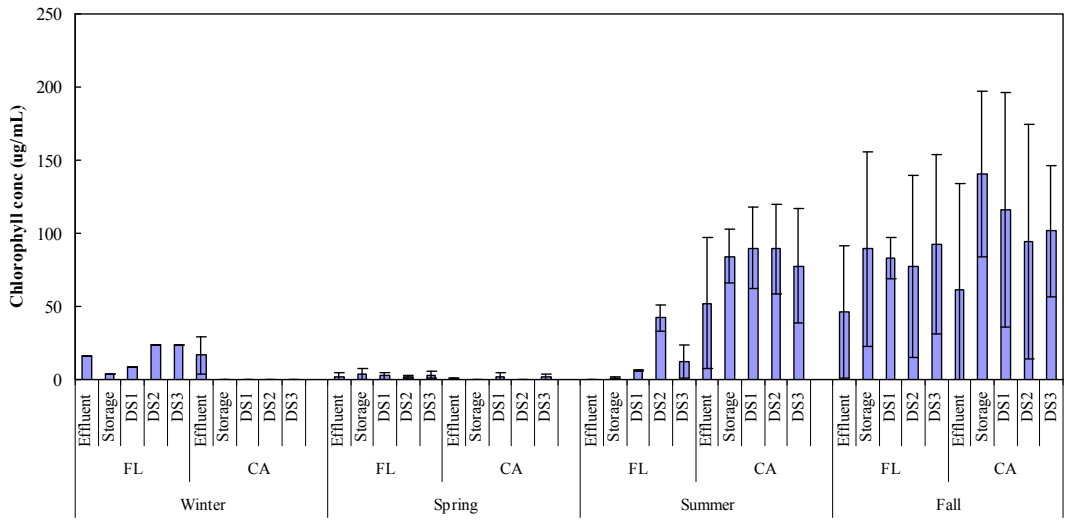


Figure 8.21. Chlorophyll concentrations in conventional utilities with open storage ponds. Each bar represents the mean concentration (±SD) over four consecutive days of sampling, with the exception of FL in winter, when data from only 1 day were obtained (see text for explanation).

It was important that algal cells persisted throughout the entire distribution system and were not confined to the storage pond (Figure 8.21). The accumulation of algal cells in the distribution system would have significant impacts on water quality, and the decay of the cells would release organic carbon and increase the disinfectant demand. Both of these factors would stimulate bacterial growth, resulting in a loss of oxygen and creating anoxic conditions. Anoxic conditions would favor the growth of anaerobic bacteria, which could result in foul smells (hydrogen sulfide; see Section 8.11) and black water (iron sulfides). The accumulation of algal cells can be controlled by regular flushing of the reclaimed water systems or removal of the cells after storage through the use of fine-mesh screens. Because the large open reservoirs serving reclaimed water systems are essentially unfiltered source waters, the impact of the reservoir was far more important than the efficacy of the wastewater treatment plant.

Chlorophyll measurements in the distribution system were correlated with increased concentrations of AOC and BDOC (Figures 8.22 and 8.23). The correlations suggested that algal growth from the storage reservoir contributed to increases in biodegradable carbon in the distribution system. Research has shown that algal cellular fractions are a significant source of biodegradable carbon, especially in the presence of an oxidant such as chlorine (Hammes et al., 2007, Bouteleux et al., 2005, Schmidt et al., 1998), and specific increases of BDOC have been observed after an algal bloom (Servais et al., 1995). Data from the present survey suggest that an increase of AOC and BDOC in systems using open storage was a result of the release of these biodegradable carbon sources from algal growth. As a result carbon was introduced into these distribution systems from the storage location, thereby increasing the potential for microbial growth.

Because of the influence of algal cells from open reservoirs, AOC trends in these reclaimed systems were complicated by both the generation of carbon (from decaying algal cells) and the consumption of carbon owing to bacterial growth. In the FL and CA systems, average AOC concentrations in the distribution system were higher than in the effluent (Figures 8.24 and 8.25) because of AOC loading from the open storage reservoir. AOC levels from the storage area in CA ranged from 343 to 2891 µg/L and averaged 1850 ± 720 µg/L and in FL ranged from 710 to 1910 µg/L and averaged 1020 ± 341 µg/L. AOC (Figure 8.26) and to a lesser extent BDOC (Figure 8.27) showed significant variability as water moved from the storage area into the distribution system.

Table 8.11. Average Temperatures (°C ± SD) of 5 Distribution Points over 4 Consecutive Days

Site	Mo.	Season	Avg. Temp (^{o}C)
NY[a]	January	Winter	28 ± 2.9
	April	Spring	28 ± 1.1
	June	Summer	29 ± 2.0
	September	Fall	26 ± 3.1
MA	February	Winter	13 ± 7.0
	April	Spring	18 ± 6.1
	July	Summer	26 ± 2.6
	October	Fall	22 ± 2.9
FL	December	Winter	22 ± 1.2
	March	Spring	23 ± 0.7
	June	Summer	28 ± 0.9
	September	Fall	29 ± 0.7
CA	March	Winter	17 ± 2.5
	May	Spring	19 ± 2.0
	August	Summer	21 ± 2.1
	October	Fall	19 ± 2.7

[a]The NY temperatures were more stable and even higher than those of FL because NY is an indoor system that is kept warm even in the winter.

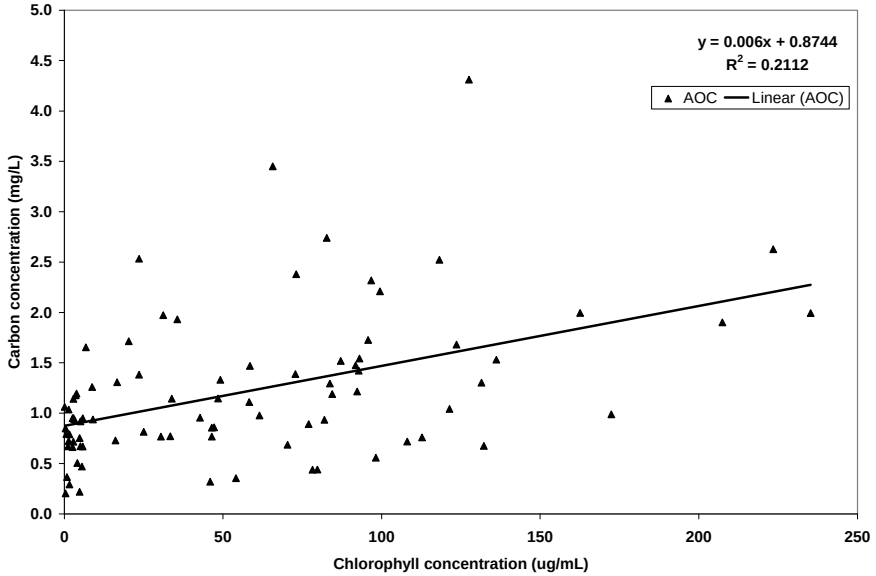


Figure 8.22. Linear relationship between chlorophyll and AOC (R^2 = 0.2112).

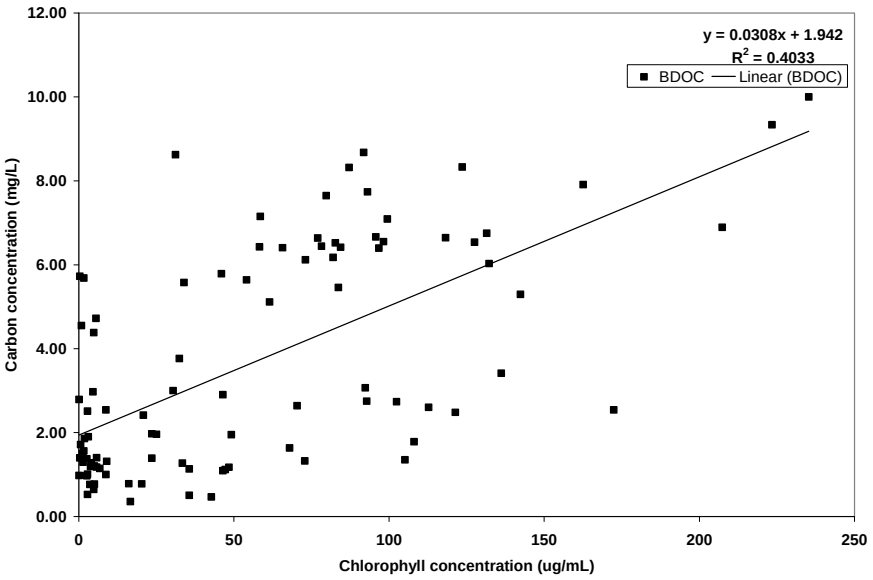


Figure 8.23. Linear relationship between chlorophyll and BDOC (R^2 = 0.4033).

In contrast, MBR systems (MA and NY) had covered storage and lower carbon levels in the distribution system than in the effluent. In these systems, AOC levels declined because of consumption by bacterial growth (see Section 8.10), but there was no increase from algal cells. Evidence of decreasing AOC concentrations in both drinking and reclaimed water with increasing distance in water distribution systems has previously been reported (LeChevallier et al., 1987; Ryu et al., 2005).

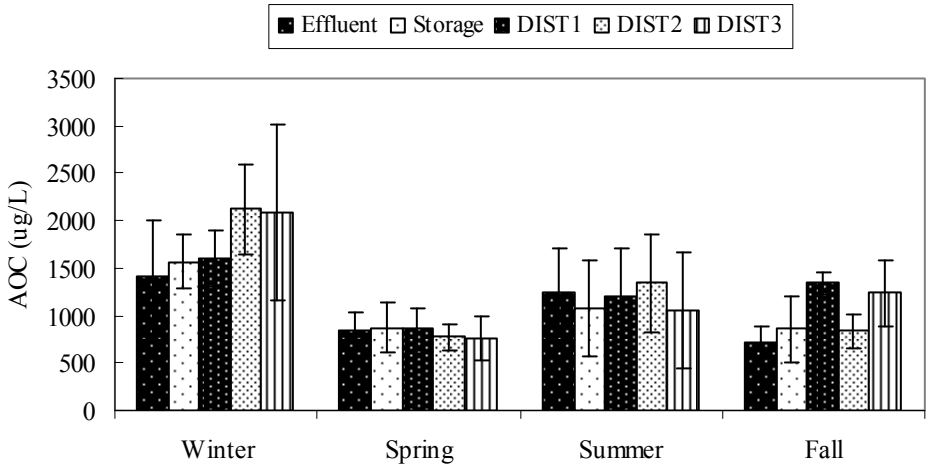


Figure 8.24. FL AOC trends in conventional plant indicating carbon lability. Each bar represents the mean concentration (±SD) over four consecutive days of sampling.

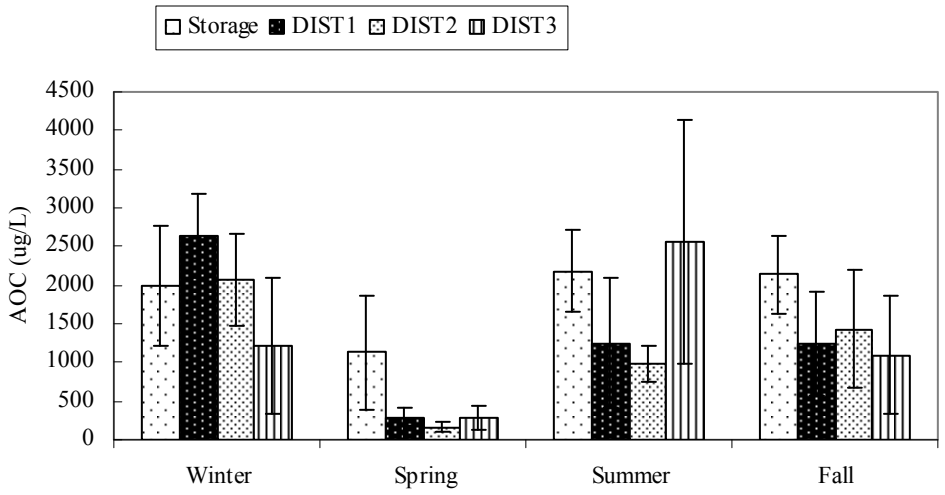


Figure 8.25. CA AOC trends in conventional plant indicating carbon lability. Each bar represents the mean concentration (±SD) over four consecutive days of sampling.

The variability in AOC levels in reclaimed water was also influenced by seasonal variations in algal cells. In FL (Figure 8.22), a systemwide trend of increasing AOC concentrations occurred in all seasons except spring. During the spring, FL AOC levels were stable. This finding was significant because spring was the only season when chlorophyll concentrations were the lowest (Figure 8.21) and when the system was continuously operated (for example, no odd/even rationing of water). During the other seasons, the system was shut down on Mondays (for maintenance) and the flow of water alternated between two separate zones for the remainder of the week. This disruption in flow affected carbon stability (Figure 8.24) as well as biological stability and suggests that the consistency of system operation is a critical factor in the microbial quality of the reclaimed water. In contrast, the CA system also had an open finished water reservoir with algal growth but was operated continuously and AOC levels in the distribution system (Figure 8.25) showed increases during the summer.

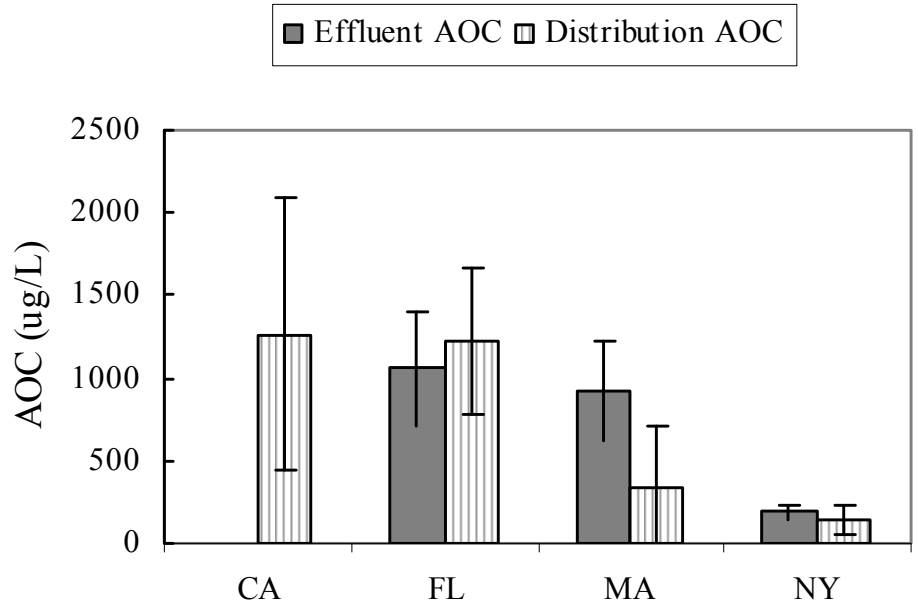


Figure 8.26. MBR and conventional treatment impacts on AOC (CA effluent not measured because of inhibition).

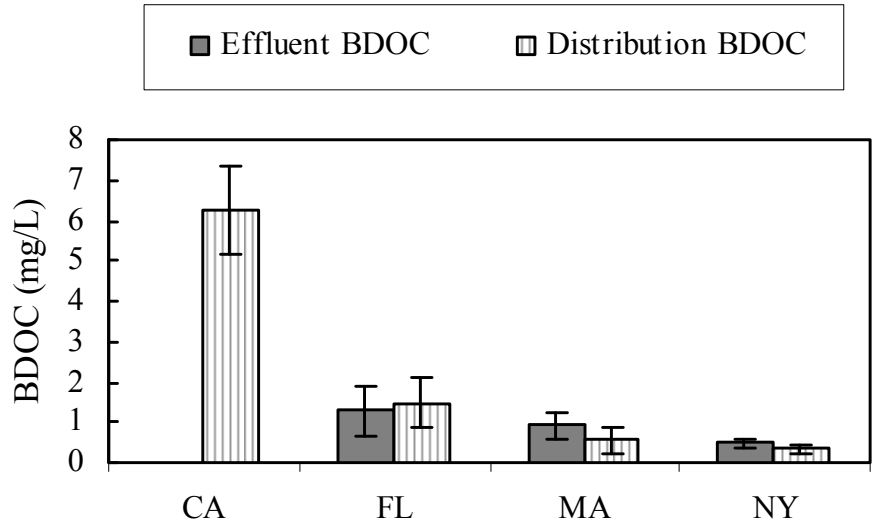


Figure 8.27. MBR and conventional treatment impacts on BDOC (CA effluent not measured because of inhibition).

Controlling algal growth in the storage reservoir can be another alternative to improve the quality of reclaimed water in such systems. Warmer seasons lead to a greater potential for the occurrence of algal growth and its associated impacts within the distribution system.

Reducing algal growth through limiting the introduction of nitrogen or phosphorus in the reclaimed water plant effluents, or through active management of the reservoir (destratification, aeration, or chemical treatment), could be another way to reduce organic carbon loading into the distribution system, thereby reducing the potential for regrowth. Future research should explore algal cell management both in the reservoirs and in the distribution systems as a means of improving reclaimed water quality.

8.7. TURBIDITY LEVELS WERE HIGHEST IN THE TWO PLANTS THAT EMPLOYED OPEN RESERVOIR STORAGE, AND TURBIDITY WAS ASSOCIATED WITH FREQUENT MICROBIAL OCCURRENCES.

Following the previous discussion of algal cells, it logically follows that turbidity would also be a problem in open finished water reservoirs. Water turbidity is a robust means of assessing the quality of water from an aesthetic perspective. In reclaimed water, high turbidity is attributable to finely divided organic matter, sediments, corrosion products, and microscopic organisms. It represents an optical property that causes light to be scattered rather than be transmitted without change in direction through the sample. In general, the turbidity of the water increased as the water travelled through the reclaimed distribution system (Figure 8.28), indicating an accumulation of sediments in the system. Treated plant effluent water was typically less than 5 NTU in all of the systems studied and less than 1 NTU for most of the time. The requirement for unrestricted and restricted urban uses for many states (CA, AZ, and WA) specifies plant effluent turbidity of less than 5 NTU with an average of 2 NTU (see Tables 4.2, 4.5, 4.11, and 4.12 and Appendix I).

However, turbidity typically exceeded 5 NTU in the reclaimed water distribution systems, with maximum values approaching five times this limit. The increase in distribution system turbidity levels was most prominent in the facilities with open reservoir storage, and the magnitude of increase the highest as the seasons advanced from winter through fall. Some of this change in turbidity was attributed to the growth of algae in the reservoir water, and both the FL and CA systems had clear evidence that algal growth was a major contributor to turbidity. Most prominent was the increase in turbidity of the CA water in fall, which coincided with a significant algal growth and tremendously reduced water levels in the pond because of increased demand for irrigation of the landscape. However, the NY and MA systems also experienced increases in distribution system turbidity that could be associated with precipitation of colloids and the formation and subsequent sloughing off of biofilms. Furthermore, they experienced sediments in distribution system piping as well as corrosion of the plumbing materials. Routine flushing of the systems should be practiced to prevent the accumulation of these materials. Some regulators do not permit flushing of reclaimed water distribution systems (see Chapter 4). This restriction might compromise the maintainance of the reclaimed water sysem as it prevents the removal of accumulated algae, debris, and biofilms.

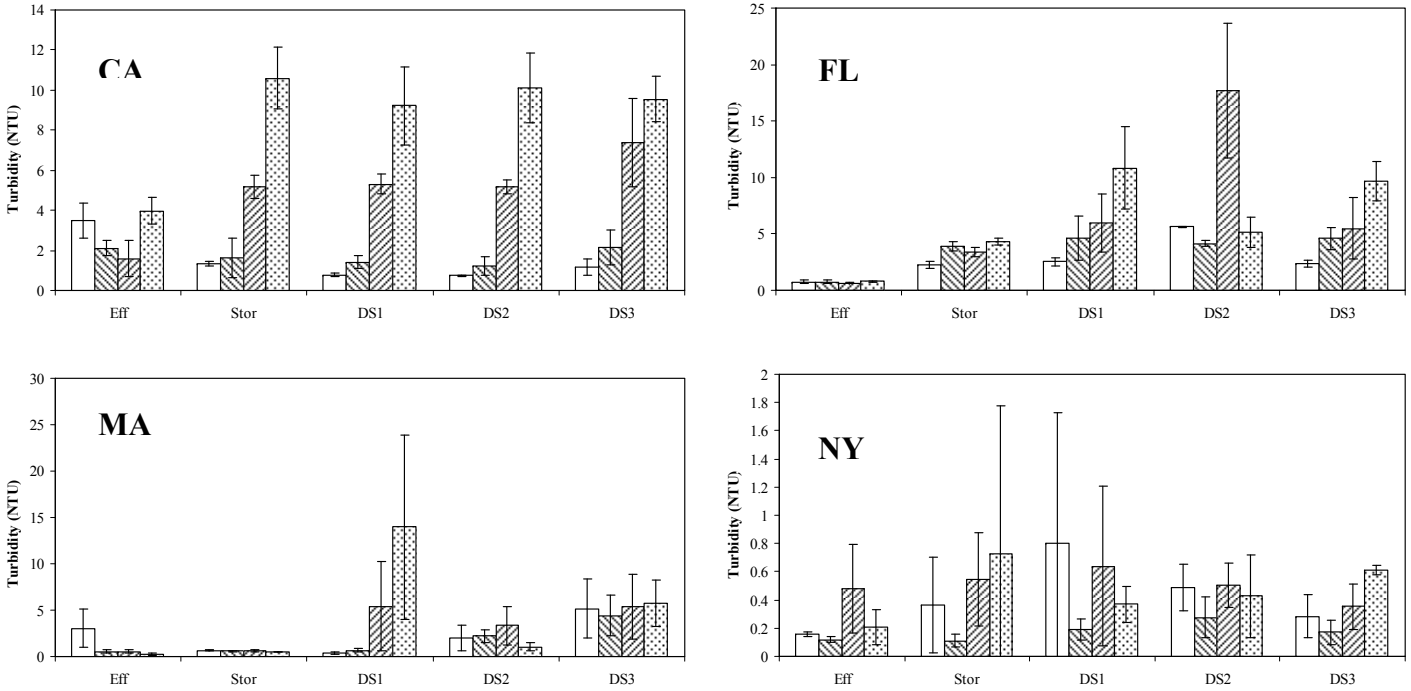


Figure 8.28. Turbidity of distribution system reclaimed water in winter (□), spring (▨), summer (▨), and fall (▨). Each bar is a mean (SD) of four consecutive days.

Table 8.12. Frequency of Occurrence of Different Microorganisms at Different Average Turbidity Levels

Location and Season	Avg. Turbidity (NTU)	Percent Occurrence												
		HPC	Total Coliform	Fecal Coliform	*E. coli*	MS Phage	Somatic	*Aeromonas*	Enterococci	*Pseudomonas*	Sulfur Bacteria	Iron Bacteria	*Mycobacterium*	*Legionella*
Fall-CA	8.7 ± 2.8	100	37.5	0	35.7	42.9	28.6	40	0	37.5	20	80	92.9	71.4
Summer-FL	6.3 ± 6.0	100	80	80	76.9	100	100	80	40	75	0	100	92.3	61.5
Winter-FL	5.9 ± 10.7	90.9	92.3	69.2	69.2	92.3	46.2	80	100	71.4	60	0	76.9	76.9
Fall-FL	5.8 ± 4.1	100	20	20	30.8	76.9	53.8	80	60	62.5	40	80	100	53.8
Summer-CA	4.9 ± 2.2	92.9	12.5	0	7.1	57.1	50	80	20	87.5	20	80	92.9	71.4
Fall-MA	4.3 ± 6.7	100	12.5	0	0	43.9	14.3	40	0	62.5	20	100	100	64.3
Spring-FL	3.6 ± 1.7	92.9	62.5	75	64.3	50	71.4	100	40	62.5	0	80	50	92.9
Summer-MA	3.1 ± 3.3	100	12.5	0	14.3	85.7	78.6	60	0	62.5	20	100	100	78.6
Winter-MA	2.3 ± 2.4	92.9	25	0	7.1	35.7	0	60	0	12.5	40	0	92.9	71.4
Spring-CA	1.7 ± 0.7	100	12.5	12.5	0	85.7	78.6	100	0	12.5	100	60	78.6	50
Spring-MA	1.7 ± 1.8	100	12.5	0	0	71.4	64.3	80	0	25	80	100	92.9	28.6
Winter-CA	1.5 ± 1.1	78.6	37.5	0	21.4	64.3	64.3	80	0	62.5	60	0	64.3	42.9
Summer-NY	0.5 ± 0.3	100	12.5	12.5	28.6	92.9	92.9	100	20	12.5	0	100	100	64.3
Fall-NY	0.5 ± 0.5	78.6	0	0	0	35.7	42.9	0	0	25	20	60	100	92.9
Winter-NY	0.4 ± 0.5	100	25	12.5	7.7	69.2	46.2	80	0	37.5	40	0	92.9	42.9
Spring-NY	0.2 ± 0.1	100	0	0	0	35.7	50	100	0	25	40	60	100	78.6

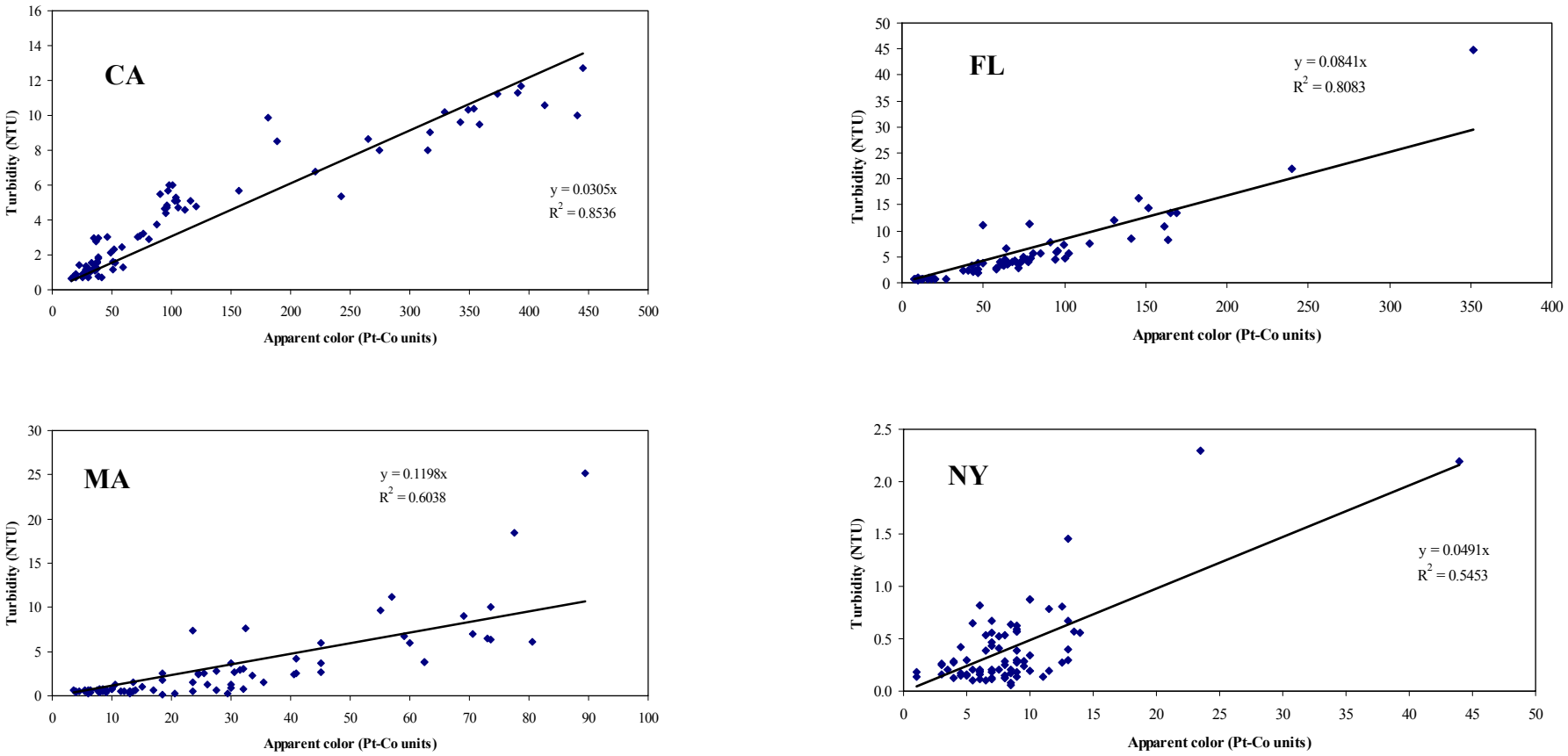


Figure 8.29. Relationship between turbidity and color in reclaimed water systems. Notice the differences in turbidity and color scales among the systems.

Water turbidity can also contribute to the color of the water. Water color is measured in platinum-cobalt units (Pt-Co units). Apparent color indicates the color of the water prior to removal of dissolved particulate material. The correlation between turbidity and apparent color was stronger in the two systems with open pond storage ($R^2 \geq 0.8$; Figure 8.29) than in the MBR systems ($R^2 \leq 0.8$). In contrast, true color reflects the color of the water after all particulates have been filtered out. True color did not have a significant correlation with turbidity (data not shown). Increases in turbidity and color could pose a problem for customer acceptance of the quality of the reclaimed water (Rowe and Abdel-Magid, 1995).

Increases in the turbidity of the water within the distribution system were also associated with a higher frequency at which total coliforms, fecal coliforms, *E. coli*, and enterococci were detected (Table 8.12). By contrast, the detection frequency for *Mycobacterium*, HPC, bacteriophage, *Aeromonas*, sulfur bacteria, iron bacteria, and *Legionella* was consistently stable across the spectrum of turbidity values.

8.8. CHLORINE DISINFECTION INCREASED AOC IN TREATED WATERS

Operators who add disinfectants to treated water do so with the intent of inactivating microorganisms. It may come as a surprise therefore, that this practice can also stimulate the growth of these organisms in biofilms within the distribution system. Studies have shown that AOC concentrations can increase in water samples treated with chlorine, ozone, or other oxidants (LeChevallier et al., 1992; Huck, 1990; van der Kooij, 1987; see discussion in Section 6.2). The AOC is actually measured by using two microbes, *P. fluorescens* strain P17 and *Spirillum* strain NOX (designated AOC_{P17} and AOC_{NOX}). AOC_{P17} is influenced by substrates like amino acids and carbohydrates, whereas AOC_{NOX} grows primarily on carboxylic acids and oxalate (van der Kooij, 1990; van der Kooij and Hijnen, 1984; van der Kooij, 1979). Often higher concentrations of the AOC_{NOX} fraction are associated with the transformation of organic matter as a result of oxidation by a strong disinfectant, like ozone or chlorine.

Table 8.13. Increase of AOC Fractions Resulting from Disinfection in Pipe Loop Inlet

Site	Treatment	AOC_{NOX} (µg/L)	AOC_{P17} (µg/L)	Change in AOC Level Compared to Control (i.e., Nondisinfected) Loop Inlet	
				% NOX Increase	% P17 Increase
CA	Control	240	29		
	Chlorine	**942**	**766**	293	96
	Chloramine	264	19	10	55
FL	Control	823	*[a]		
	Chlorine	**1373**	**1353**	N/A	N/A
	Chloramine	477	1370	N/A	N/A
MA	Control	363	105		
	Chlorine	**1183**	**217**	226	52
	Chloramine	444	198	22	47
NY	Control	79	75		
	Chlorine	**357**	**229**	350	67
	Chloramine	207	356	62	79

[a]Analytical error. The chlorine AOC_{NOX} and AOC_{P17} are boldfaced.

Reclaimed water frequently showed a large increase in AOC following disinfection. In this study, three loops were tested over the course of 2 days in which reclaimed water effluent (control) was amended with a disinfectant to provide either a free chlorine residual (chlorine loop) or a total chlorine residual (as a monochloramine-chloramine loop). Comparisons of the AOC between the chlorinated inlet and control inlet (Table 8.13) showed an average AOC_{NOX} increase of 290% and an average AOC_{P17} increase of 72% (excluding the FL samples, which were contaminated). These results confirm that disinfection with free chlorine increased AOC, the largest increases being specifically attributed to the AOC measured with AOC_{NOX}. In the chloraminated loops, average AOC_{NOX} concentrations increased 31% compared to the control. The oxidation potential of monochloramine is less than that of chlorine, and therefore the oxidation reactions to transform the organic matter into AOC did not occur to the same extent. Increases in AOC in drinking water after disinfection with ozone or chlorine have also been documented in potable water (see discussion in Section 6.2).

BDOC levels increased an average 25% following free chlorination (Table 8.14). Although the trend toward increased BDOC levels after disinfection was similar to the trend reported for AOC, the compounds that comprise BDOC are a mixture of both low- and higher-molecular-weight organic matter that may not undergo the same transformations to readily biodegradable organic matter. Often, an increase in BDOC suggests an increase in chlorine demand (or DBP formation in drinking water) whereas an increase in AOC suggests an increase in bacterial growth potential. Maintaining a low chlorine demand in the distribution system is a critical factor for stabilizing water quality. Therefore, disinfecting the water to meet effluent quality standards may also increase the regrowth potential of the water (AOC) as well as decrease stability in the presence of that disinfectant (BDOC).

Table 8.14. Increase of BDOC Resulting from Disinfection in Pipe Loop Inlet

Site	Treatment	BDOC (mg/L)	Percent Change in BDOC Level Compared to Control
CA	Control	5.00	
	Chlorine	**6.90**	38
	Chloramine	4.01	−20
FL	Control	*[a]	
	Chlorine	**1.78**	N/A
	Chloramine	2.59	N/A
MA	Control	0.98	
	Chlorine	**1.26**	29
	Chloramine	0.77	−22
NY	Control	0.77	
	Chlorine	**0.83**	8
	Chloramine	0.84	9

[a]Analytical error.

The ability of a disinfectant to increase the level of BDOC is an important parameter to consider. The purpose of the pipe loop study was to investigate disinfectant strategies on microbial populations and their regrowth control. In this study, results showed high AOC levels were the by-products of disinfection and could be associated with the potential for microbial regrowth. Because of the controlled conditions of the pipe loop study, it was possible to observe the impact of disinfection separate from the other treatment processes. The increased BDOC levels in the pipe loop system were 4 to 40 times greater than average AOC levels observed in the full-scale studies. The practical application of these results for reclaimed system operators who practice chlorine (or ozone) disinfection, where AOC levels would be increased, is to use a biologically active filter on disinfected effluents to reduce the AOC (LeChevallier et al., 1996). Future research should carefully examine reclaimed water operations to optimize the treatment for control of AOC and BDOC levels. LeChevallier et al. (1996) proposed reducing biofouling by filtering the disinfected water through a biologically active medium such as GAC (see discussion in Section 6.2). Such a practice could greatly limit the amount of AOC generated after disinfection that is able to enter the distribution system. This approach is currently being used by a few reclaimed water plants: for example, Gwinnett County (Schimmoller and Macpherson, 2008), although its efficacy has not yet been critically studied.

8.9. CARBON, INCLUDING BDOC AND AOC, WAS EFFECTIVELY REMOVED IN MBR SYSTEMS

As outlined in Section 6.7.1, NOM is present at variable concentrations in all water and wastewater systems. MBR technology has been used successfully for the removal of organic matter in pilot scale applications (Williams and Pirbazari, 2007) and for removal of high TOC in landfill leachates (Pirbazari et al., 1996). This study confirmed the benefits of the MBR

treatment process, demonstrating that organic carbon levels were typically much lower than those attained in conventional plants. While the scope of this work did not encompass characterizing the wastewater influent, the effluent and storage samples afforded a strong set of data for making a comparison between the conventional and MBR plants. Mean carbon concentrations over the yearlong sampling campaign illustrated the superiority of MBR treatment in MA and NY, compared to the conventional treatment in CA and FL (Table 8.15). This pattern was consistent for all three carbon parameters (TOC, BDOC, and AOC) and indicated that the MBR plants had comparatively lower carbon values, based on the average of all five system samples (Figure 8.30).

Table 8.15. Mean and Quartile Data for Carbon Parameters including TOC, BDOC, and AOC

Site	*N*	*N* > DL	Mean	Stdev	Min	25%ile	50%ile	75%ile	90%ile	95%ile	Max	DL
TOC (mg/L)												
CA	80	80	12.8	1.9	9.5	11.3	12.4	14.1	15.5	16.3	18.2	0.1
FL	71	71	7.2	0.8	5.8	6.6	7.2	7.8	8.1	8.5	9.5	0.1
MA	80	80	3.8	1.2	1.0	2.8	3.6	4.8	5.6	5.7	6.1	0.1
NY	80	80	2.7	0.5	1.6	2.5	2.8	2.9	3.2	3.4	3.6	0.1
BDOC (mg/L)												
CA	64	64	6.2	1.4	2.0	5.4	6.4	6.9	7.9	8.6	10.0	0.1
FL	71	71	1.4	0.8	0.3	1.0	1.2	1.7	2.6	3.0	3.4	0.1
MA	80	80	0.6	0.4	0.1	0.3	0.5	0.9	1.3	1.4	1.6	0.1
NY	75	72	0.4	0.2	0	0.2	0.4	0.5	0.6	0.7	0.9	0.1
AOC (µg/L)												
CA	63	63	1407	983	66	445	1447	2015	2607	2887	4312	25
FL	71	71	1141	503	443	769	979	1386	1837	1951	3128	25
MA	80	79	459	467	21	120	263	714	1296	1467	1939	25
NY	80	77	149	109	18	72	109	210	331	377	478	25

Research has correlated threshold levels of organic carbon above which there were excessive levels of microbial growth in drinking water systems. Escobar et al. (2001) cite various sources that determined that biological stability in drinking water distribution systems occurred at threshold BDOC values ranging from 0.10 to 0.30 mg/L, and work by Dukan et al. (1996) reported a threshold of around 0.25 mg of BDOC/L. Page and Dillon (2007) presented a compilation of additional sources indicating threshold BDOC values ranging from 0.15 to 0.30 mg/L. Taken together, the available data demonstrate that BDOC levels above 0.3 mg/L can be a strong indicator for biological instability in distribution systems. It might then come as a surprise to reclaimed water system operators that the median (50th percentile; Table 8.15) BDOC concentration in the systems studied was 1.5 to 21 times the levels recommended for biological stability.

Biologically unstable AOC levels in drinking water without a disinfectant residual are >10 µg/L, and those in the presence of a disinfectant are >100 µg/L (Volk and LeChevallier, 2000). By comparison, AOC standards have not been set for reclaimed water, but in the

present study, all of the reclaimed water systems exceeded the AOC guidelines for drinking water, with AOC levels varying 240-fold from a minimum of 18 µg/L to a maximum of 4312 µg/L (Table 8.15). In general, AOC levels in the MBR systems (MA and NY) were about 10 times lower than in the conventional (CA and FL) systems.

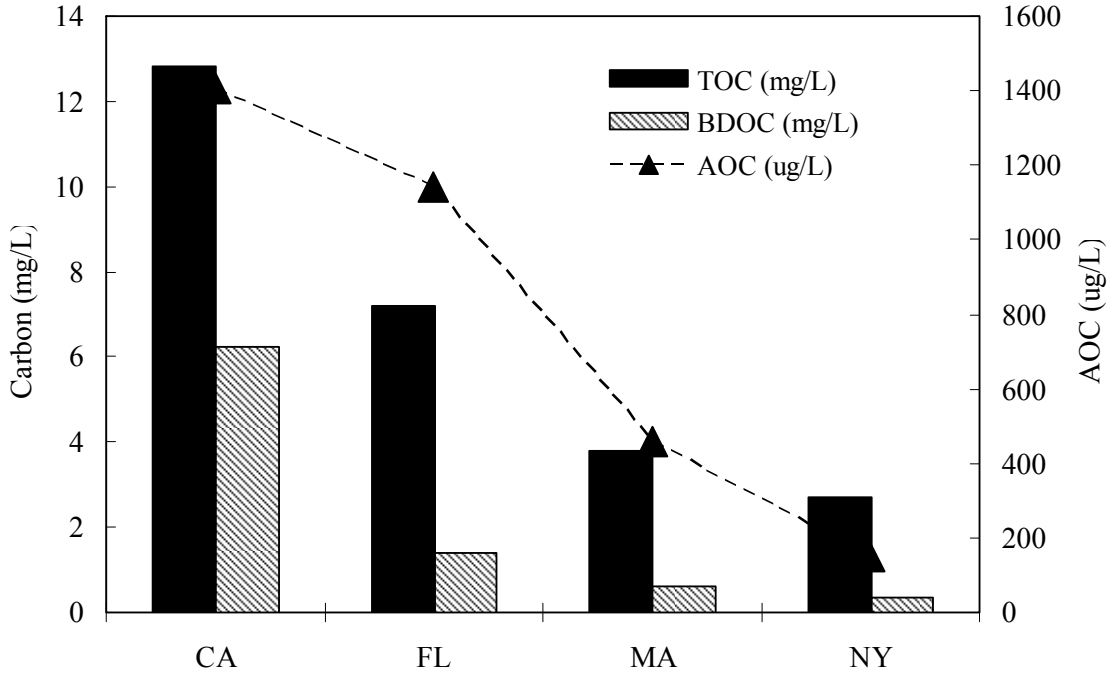


Figure 8.30. Decreased carbon (namely, TOC, BDOC, and AOC) in the MBR-generated reclaimed water compared to that from conventional treatment plants. Note: CA and FL were conventional, whereas MA and NY use MBR treatment. Each data set is an average of five distribution points over four sampling seasons.

The organic carbon data (TOC, BDOC, and AOC) at the point of entry into the distribution system were further investigated and provided important information about the carbon-removal efficiency of the treatment process. Average effluent and storage organic carbon values (Table 8.16) and the differences in the conventional and MBR facilities (Table 8.17) demonstrate that the conventional treatment plants consistently had high levels of organic carbon. MBR facilities produced lower levels of organic carbon (TOC, BDOC, and AOC) possibly because these systems operate with long bacterial retention times. The performance of MBRs in the removal of BDOC is consistent with prior studies (Williams and Pirbazari, 2007) that focused on removal of ozonation by-products, specifically aldehydes and AOC. Given the importance of organic carbon in reclaimed water systems, future research should look at optimizing both conventional and MBR systems for removal of organic carbon, specifically the removal of BDOC. Increasing the mixed liquor concentration and the solid retention time or employing biologically active GAC filters could be possible strategies for improving the removal of AOC and BDOC in treated waters.

Table 8.16. Organic Carbon Concentrations (± SD) in Effluent and Storage Locations over the Yearlong Monitoring

	Concn of:					
	TOC (mg/L)		BDOC (mg/L)		AOC (µg/L)	
Site	Effluent	Storage	Effluent	Storage	Effluent	Storage
CA	13.88 ± 2.6	13.27 ± 1.37	Inhibition	6.39 ± 1.34	Inhibition	1862 ± 500
FL	7.08 ± 0.67	7.24 ± 1.05	1.28 ± 0.62	1.45 ± 0.55	1056 ± 330	1094 ± 330
MA	4.25 ± 0.94	4.16 ± 0.99	0.93 ± 0.32	0.99 ± 0.34	920 ± 560	800 ± 450
NY	2.85 ± 0.24	2.73 ± 0.22	0.47 ± 0.08	0.37 ± 0.14	190 ± 123	194 ± 130

Table 8.17. Difference in Organic Carbon Levels between Conventional and MBR Facilities

	% Level for:							
	Effluent				Storage			
Parameter	CA and MA	CA and NY	FL and MA	Fl and NY	CA and MA	CA and NY	FL and MA	Fl and NY
TOC	327%	486%	167%	248%	319%	487%	174%	266%
BDOC			138%	270%	646%	1738%	146%	393%
AOC			115%	557%	233%	960%	137%	564%

8.10. BDOC WAS CONSUMED IN RECLAIMED DISTRIBUTION SYSTEMS, CHANGING THE CONCENTRATION AND NATURE OF NUTRIENTS AVAILABLE FOR BACTERIAL GROWTH

The bioluminescence method used for AOC analyses was useful because it not only generated data on the concentration of AOC but also indicated how quickly the carbon was utilized. The more labile the carbon, the more quickly it was utilized, corresponding with a maximum level of the achieved bacterial growth. Peak growth occurs when the organisms have completely utilized the substrate, reflecting maximum biomass and resulting in maximum, or peak, luminescence. This peak growth occurred at different times postinoculation, depending on how easily biodegradable the AOC in the sample was. Typically, AOC levels revealed peak growth by day 3 in the assay (Figure 8.31), indicating that the AOC was energy-intensive and was likely to be a mixture of carbohydrates and amino acids that were not removed during treatment. However, AOC at the point of entry (treatment plant effluent) often peaked by day 2 compared to AOC levels analyzed after the storage reservoir or at points in the distribution system, which typically peaked at least 1 day later. Therefore, the data shown in Figure 8.31 illustrate that not only the levels of AOC changed as the water flowed through the reclaimed distribution systems but that the nature of the organic carbon changes, with the most rapidly biodegradable carbon being utilized first. The more slowly biodegradable carbon remained at the ends of the system. The results also

suggest that the greatest microbial activity would be at points near the beginning of the system (even in the storage reservoir) rather than at the ends of the system.

As AOC and BDOC sources flow through a distribution system, consumption by microbial populations present either in the biofilm or in the water will reduce the concentrations, ultimately diminishing the lability of the organic carbon. Evidence of decreasing AOC concentrations with increasing distance in water distribution systems has previously been reported (LeChevallier et al., 1987). MBR utilities in NY and MA clearly exhibited this trend with consumption of AOC and BDOC in the distribution system (Figures 8.32 to 8.35). The average difference (between the plan effluent and the end of the distribution system [DS 3]) in AOC concentration was 833 ± 537 µg/L in the MA system and 118 ± 137 µg/L for the NY system. The average difference in BDOC levels between the plan effluent and the end of the distribution system (DS 3) was 0.61 ± 0.43 mg/L for the MA system and 0.13 ± 0.23 mg/L for the NY system.

As previously stated (Section 8.6), the accumulation of algal cells from the open reservoirs in the conventionally treated systems (CA and FL) provided another source of organic carbon, particularly as the cells slowly decayed in distribution system sediments.

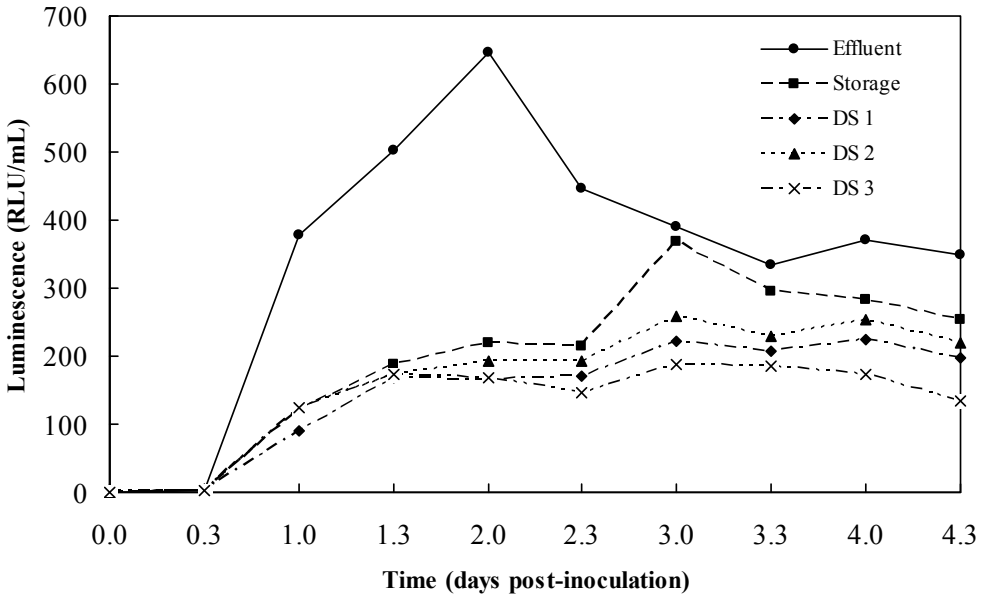


Figure 8.31. Measurement of AOC levels using the bioluminescence method. The point of maximum growth is noted for the different water samples.

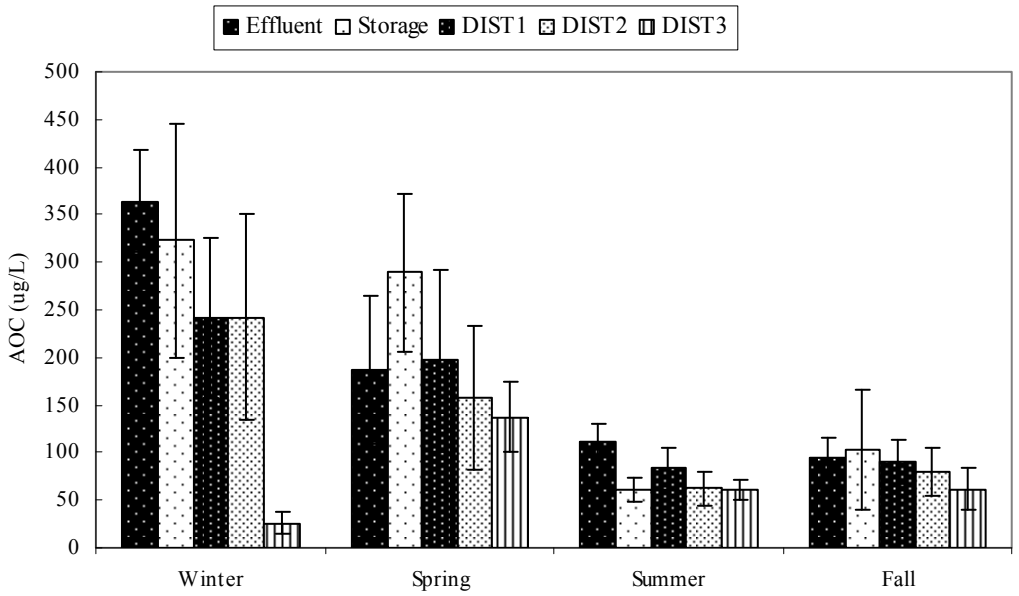


Figure 8.32. NY AOC trends indicating consumption of carbon in the distribution system.

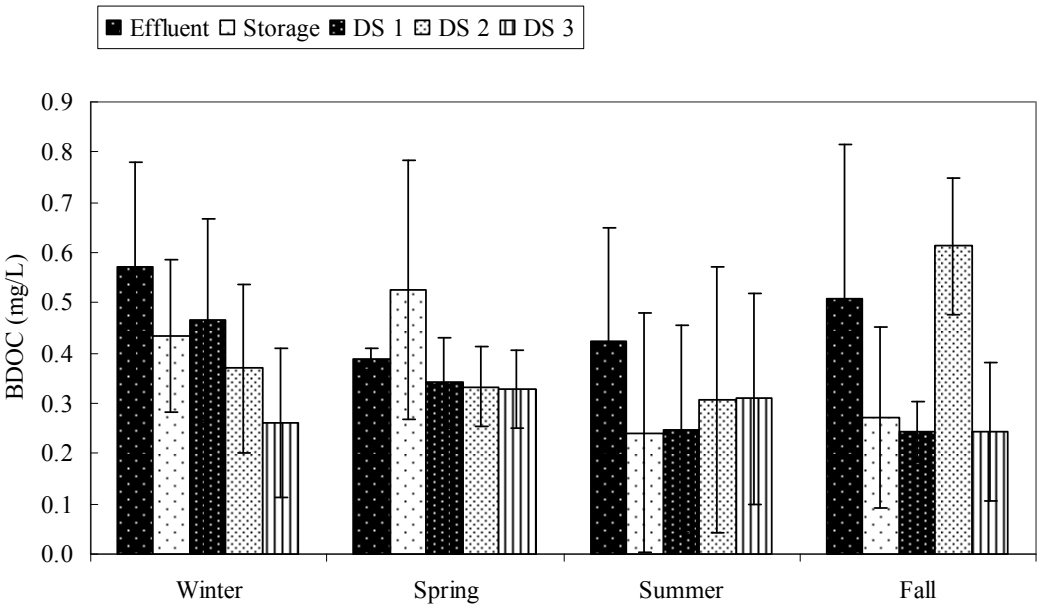


Figure 8.33. NY BDOC trends indicating consumption of carbon in the distribution system.

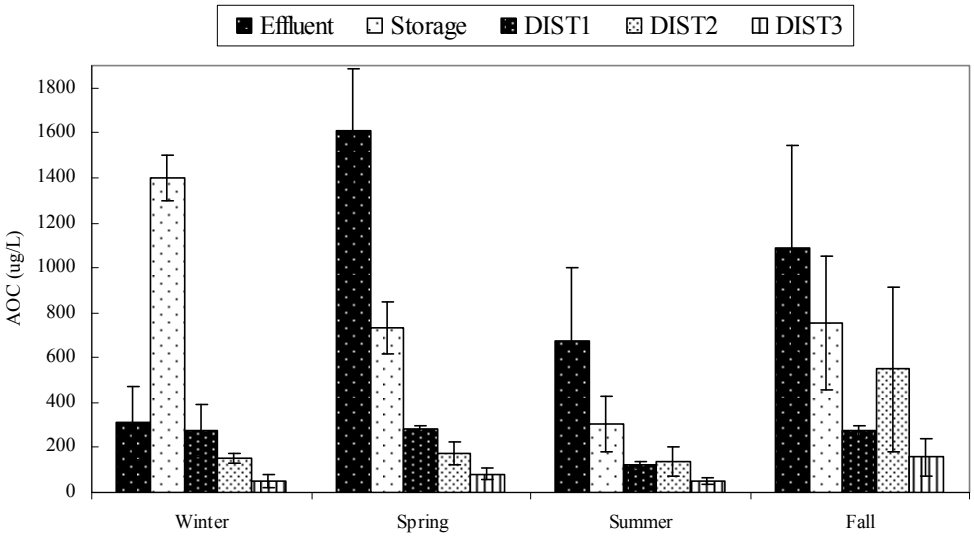


Figure 8.34. MA AOC trends indicating consumption of carbon in the distribution system.

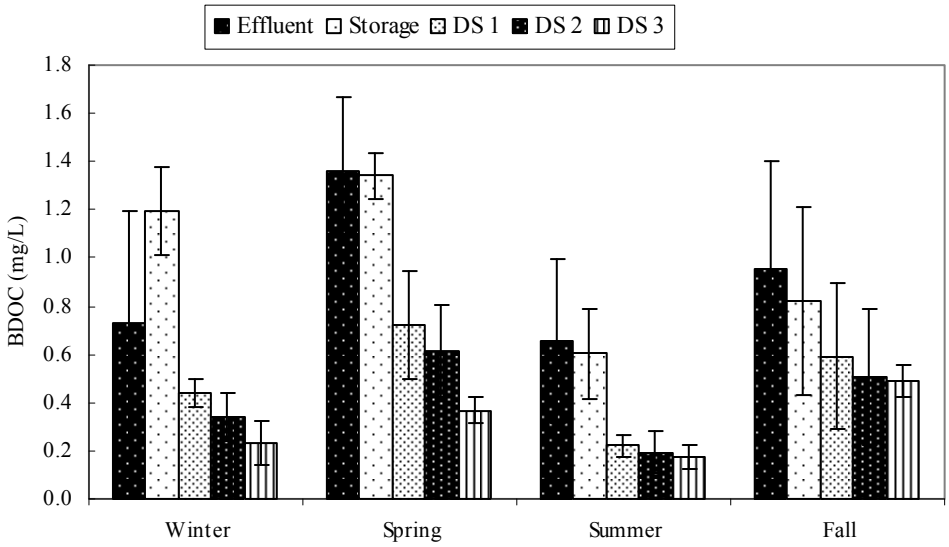


Figure 8.35. MA BDOC trends indicating consumption of carbon in the distribution system.

The pipe loop experiments also allowed us to examine the changes in AOC and BDOC levels under controlled conditions as the water flowed from the inlet through the 150-ft model (Table 8.18). The trend of decreasing organic carbon concentration was observed primarily for the chlorine-treated loop, where the consumption of AOC ranged from 107 to 545 µg/L and the consumption of BDOC ranged from 0.2 to 0.9 mg/L. We previously discussed how

oxidation of organic carbon by chlorine resulted in increased AOC and BDOC levels. The pipe loop model demonstrated how this increase in BDOC impacts the distribution system.

In the control loops, there was little consumption of AOC but significant decreases in BDOC, particularly in the systems with very high (>5 mg/L) levels of BDOC. It is possible that complex transformations were occurring in these systems where AOC was both being consumed and also released as microbial metabolic by-products. Likewise in the chloraminated loops, there was little consumption of BDOC and, in fact, some evidence of increases in AOC and BDOC levels. These increases could be attributed either to desorption of organic matter from the biofilms or to the continued reaction of the chloramines with organic carbon to slowly produce AOC as a DBP. However, the stability of the AOC and BDOC levels in chloraminated waters suggests that disinfection with chloramine is likely to maintain the carbon stability in distribution system waters by controlling microbial consumption in biofilms.

Table 8.18. Pipe Loop Data for AOC and BDOC at the Inlet and Farthest Collected Sample at 150 ft[a]

		Control			Chlorine			Chloramine		
Analyte	Site	Inlet	At 150 ft	Difference	Inlet	At 150 ft	Difference	Inlet	At 150 ft	Difference
AOC (µg/L)										
	CA	268	284	16	1707	1162	−545	283	337	54
	FL	*[b]	*		2726	2612	−114	1846	1765	−81
	MA	468	468	0	1400	1293	−107	641	619	−22
	NY	154	86	-68	586	366	−220	563	767	205
BDOC (mg/L)										
	CA	5.0	3.9	−1.1	6.9	7.8	0.9	4.0	3.9	−0.1
	FL	*	*		1.8	2.5	0.7	2.6	3.1	0.5
	MA	1.0	1.0	0	1.3	1.5	0.2	0.8	1.1	0.3
	NY	0.8	0.8	0	0.8	1.1	0.2	0.8	0.7	−0.1

[a]The change in carbon was calculated as the difference between carbon in the inlet and at the end of the loop (namely, at 150 ft).
[b]Analytical error as the sample was contaminated.

8.11. INORGANIC PARAMETERS VARIED BETWEEN THE DIFFERENT SYSTEMS STUDIED BUT DID NOT APPEAR TO HAVE A MAJOR ROLE IN INFLUENCING THE MICROBIAL QUALITY OF RECLAIMED WATER

In this study we examined a variety of inorganic parameters that could be related to changes in microbial quality in reclaimed distribution systems. These data have been summarized in Tables 8.19a through g. The study sites were selected so that the impact of specific inorganic parameters could be examined. For example, the NY system has no requirement for nitrate removal, and as a result the level of nitrate in this system was much higher than in the other three. Similarly, phosphate levels in the MA system were more than twice as high as in the other systems. However, as previously indicated, the microbial quality of both of these MBR facilities was superior to that of the conventional systems, suggesting that nitrate and phosphate were not critical parameters influencing microbial water quality in these reclaimed water systems.

The inorganic requirements of wastewater plants are determined based on their discharge permits, and the requirements can vary according to the specific location. Excessive nitrogen and phosphorus are associated with eutrophication, which is exemplified by excessive algal blooms and a vigorous growth of aquatic plants (Helen et al., 2006). However, when the water is reclaimed and tapped for reuse, the requirement to reduce nutrients can greatly vary, based on the intended end use of the water. For the MA system, the reclaimed water recirculated between the urinals/toilets and the treatment plant. Because the water in such a system is closed without the effluents being discharged into the environment, the high phosphate levels would not have any environmental impact but can, with time, possibly build up in the system. High phosphate levels may be beneficial in protecting the system from corrosion (Demaree et al., 1992; DeBlois, 2002). Corrosion involves both chemical and microbiological processes (Jjemba, 2004), and in the studies conducted by Demaree et al. (1992), the presence of phosphates displayed a strong effect on the anodic current density of the samples, reducing the passive current density by more than 2 orders of magnitude and completely eliminating the prominent active peak.

Nitrogen is transformed into various species, notably nitrate-N, nitrite-N, and ammonium-N. The predominance of a particular species of nitrogen gives an idea of what is happening in that system. Most of the nitrogen in untreated wastewater typically contains ammonium-N. Ammonium-N was predominant in only the CA system. Ammonium-N can be converted to nitrate-N through nitrification. Through denitrification, nitrogen (N_2) and nitrogen oxides (namely, N_2O and NO) are generated and released into the atmosphere. Nitrate-N was more than threefold higher in the anoxic NY MBR system, which routinely practices nitrification/denitrification. The major use for reclaimed water at the NY facility is flushing toilets and cooling towers, and the ultimate disposal is to the sewer. So it was uneconomical for the NY plant to practice enhanced denitrification, a process that would have required energy-intensive aeration.

For the systems in which the reclaimed water is intended for landscaping, as is the case with CA, FL, and to some extent NY, the nutrient levels, particularly with regard to nitrates, have to be high enough to meet the demand for the target plants (namely, the lawn) but not so excessive as to leach into the groundwater. A nitrate leaching index has been developed as an indicator of the potential for nitrates to reach groundwater (Van Es and Delgado, 2006). Just like nitrates, soil colloids are negatively charged, enabling the nitrates to leach easily through the soil profile. Thus, if the reclaimed water is to primarily be used for irrigation purposes,

operators have to be mindful of nutrient levels that, if excessive, increase the possibility of contaminating the groundwater.

Because the CA system did not practice breakpoint chlorination, the process resulted in excess ammonia-nitrogen (average, 7.8 mg/L). This high level of ammonia could stimulate the growth of nitrifying bacteria, which in turn could produce nitrite through incomplete denitrification. The relatively high levels of nitrite in the CA system could have been the result of this process. Both of these processes could contribute to the instability of the disinfectant residual and directly or indirectly contribute to the microbial quality of the reclaimed water.

Sulfide levels in both the CA and FL systems suggest the presence of anoxic conditions favoring the growth of sulfate-reducing bacteria. Water systems with as little as 1 µg of sulfide/L are corrosive (Miller and Mancl, 1997), and hydrogen sulfide can give the water a "rotten egg" odor. The loss of disinfectant residuals, the high levels of organic carbon, accumulation of algal cells, and increased turbidity from distribution system sediments all point to conditions where such anaerobic bacteria could grow. Hydrogen sulfide in the reclaimed water was several magnitudes higher in the two conventional plants than in the MBRs (Figure 8.36). It is also notable that the hydrogen sulfide concentration increased at points that are farther away from the effluent. Foul odors from reclaimed water are a common complaint from end users (ACCB, 2006) that is mainly attributable to hydrogen sulfide. Humans are particularly sensitive to the odor of hydrogen sulfide, and the presence of even trace levels of the compound can generate customer complaints.

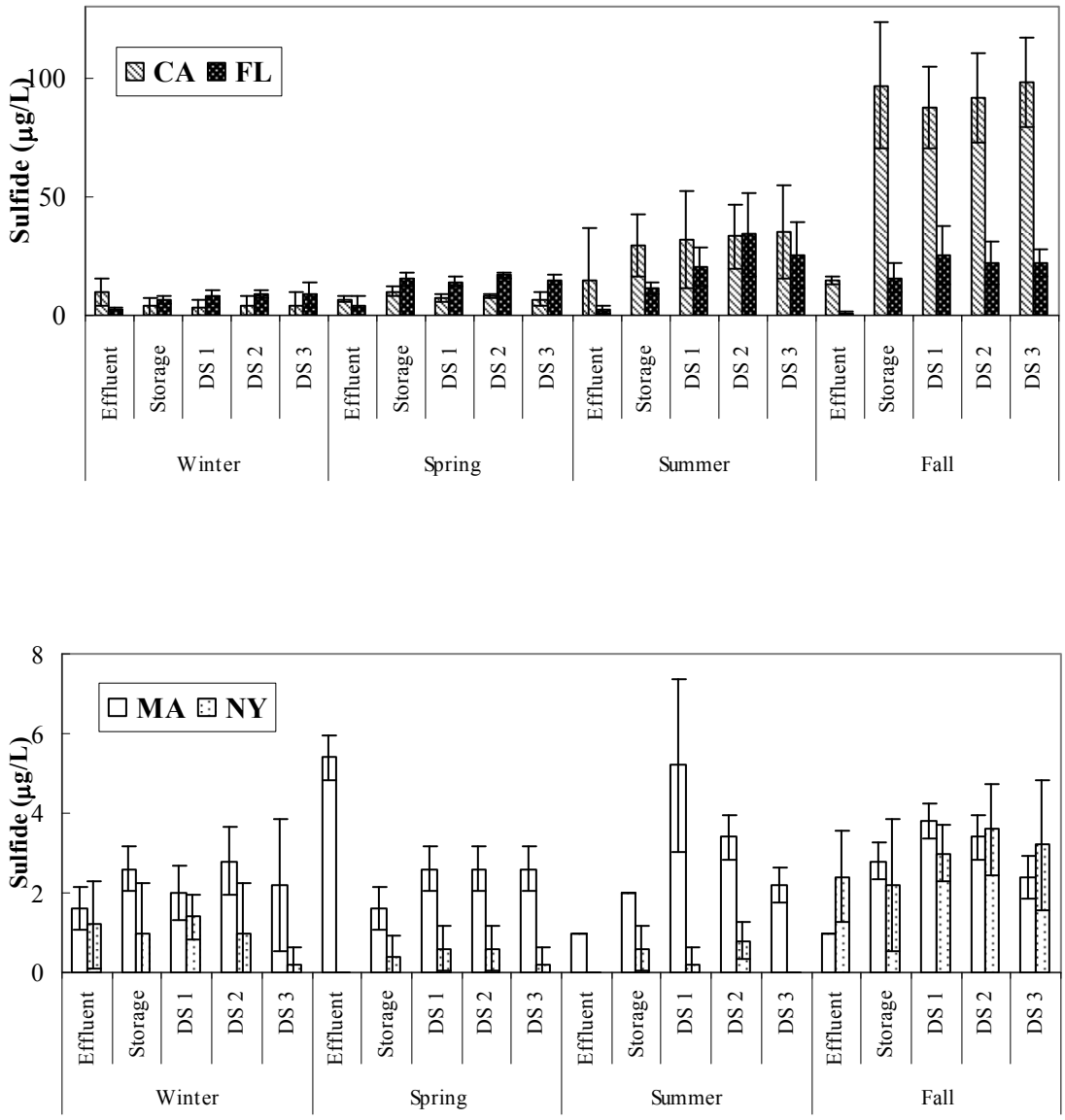


Figure 8.36. Hydrogen sulfide in four reclaimed water distribution systems. Each bar is the mean (±SD) of four measurements taken on consecutive days. Note the difference in the *y* axis scale between the conventional (namely, CA and FL) and MBR (namely, MA and NY) plants.

Table 8.19a. Occurrence of Nitrate (mg/L) in Reclaimed Water

Location	N	*N* > DL	Mean	STD	Min	25%ile	50%ile	75%ile	90%ile	95%ile	Max	DL
CA	100	100	6.2	10	0.2	0.9	2.3	3.9	22.8	30.6	54.6	0.1
FL	91	70	0.7	1	0	0.2	0.5	1.0	1.6	1.8	2.4	0.1
MA	100	100	2.5	1	0.4	1.7	2.3	3.1	4.4	4.9	6.5	0.1
NY	100	100	24.5	10	0.3	20.6	27.2	31.5	33.4	34.6	40.0	0.1

Table 8.19b. Occurrence of Nitrite (mg/L) in Reclaimed Water

Location	*N*	*N* > DL	Mean	STD	Min	25%ile	50%ile	75%ile	90%ile	95%ile	Max	DL
CA	100	89	3	1	0	2	3	4	5	5	8	1
FL	91	27	1	1	0	1	1	1	3	3	4	1
MA	100	14	1	0	0	1	1	1	1	1	2	1
NY	100	45	1	1	0	1	1	2	2	2	3	1

Table 8.19c. Occurrence of Ammonia (mg/L) in Reclaimed Water

Location	*N*	*N* > DL	Mean	STD	Min	25%ile	50%ile	75%ile	90%ile	95%ile	Max	DL
CA	94	94	7.80	10	0.4	1.45	4.85	10.00	16.70	23.35	54.00	0.01
FL	91	67	0.10	0.2	0	0.1	0.02	0.04	0.33	0.55	0.93	0.01
MA	100	45	0.04	0.2	0	0	0.01	0.04	0.07	0.09	2.00	0.01
NY	100	21	0.01	0	0	0	0.01	0.01	0.02	0.03	0.07	0.01

Table 8.19d. Occurrence of Phosphate (mg/L) in Reclaimed Water

Location	*N*	*N* > DL	Mean	STD	Min	25%ile	50%ile	75%ile	90%ile	95%ile	Max	DL
CA	100	100	11	3	5	8	10	13	15	16	18	0.3
FL	91	91	4	2	1	3	4	6	7	7	8	0.3
MA	100	100	22	12	1	18	23	29	35	38	41	0.3
NY	100	99	2	0.4	0.2	1	3	2	2	2	3	0.3

Table 8.19e. Occurrence of Sulfate (mg/L) in Reclaimed Water

Location	*N*	*N* > DL	Mean	STD	Min	25%ile	50%ile	75%ile	90%ile	95%ile	Max	DL
CA	100	100	102	18	40	94	102	111	122	126	140	2
FL	91	91	60	14	42	47	64	73	79	81	83	2
MA	100	100	32	5	10	29	31	32	37	39	47	2
NY	100	100	26	7	5	26	29	30	31	31	37	2

Table 8.19f. Occurrence of Sulfide (µg/L) in Reclaimed Water

Location	*N*	*N* > DL	Mean	STD	Min	25%ile	50%ile	75%ile	90%ile	95%ile	Max	DL
CA	100	79	11	36	0	6	11	47	89	108	131	5
FL	91	70	13	10	0	6	12	18	28	32	60	5
MA	100	3	3	1	1	2	2	3	4	5	9	5
NY	100	1	3	2	1	2	3	3	5	6	9	5

Table 8.19g. Occurrence of Alkalinity (mg of $CaCO_3$/L) in Reclaimed Water

Location	*N*	*N* > DL	Mean	STD	Min	25%ile	50%ile	75%ile	90%ile	95%ile	Max	DL
CA	80	80	236	28	180	218	234	248	270	295	312	20
FL	71	71	205	9	175	198	207	211	216	218	224	20
MA	80	80	145	16	89	135	142	157	165	167	168	20
NY	80	65	33	10	14	24	35	40	46	46	49	20

Reclaimed water that is used for irrigation also has to be treated to minimize sodicity and salinity, both of which can occur if the water contains high levels of sodium bicarbonates (Wu et al., 2008). In soils, salinity means high levels of salts (namely, sodium, potassium, and magnesium) whereas sodic soils specifically contain high concentrations of sodium ions. Saline soils display a good structure, whereas sodic soils have a poor structure and a high pH (namely, pH > 9 [McBride, 1994]). Despite the good structure, saline soils have a high electrical conductivity (namely, >4 mS/cm). Both salinity and sodicity can negatively affect vegetation, with the former lowering the free energy of water in the soil matrix and reducing the ability of the plant roots to extract moisture from the soil owing to the osmotic pressure generated by the electrical conductivity.

The conductivity of potable water within the United States ranges between 50 and 1500 µS/cm (Eaton et al., 2005). The conductivity of the reclaimed water in the four systems studied ranged between 390 and 1800 µS/cm (Figure 8.37). In most instances, conductivity does not change within the distribution system. It was consistently highest in the reclaimed water from the CA system. High conductivity levels may have implications for the vegetation that the reclaimed water irrigates, unless tolerant plant varieties are identified (Anonymous, 2006). A relatively simple parameter to measure, conductivity is a general indicator of the quality of water as a function of the amount of dissolved salts.

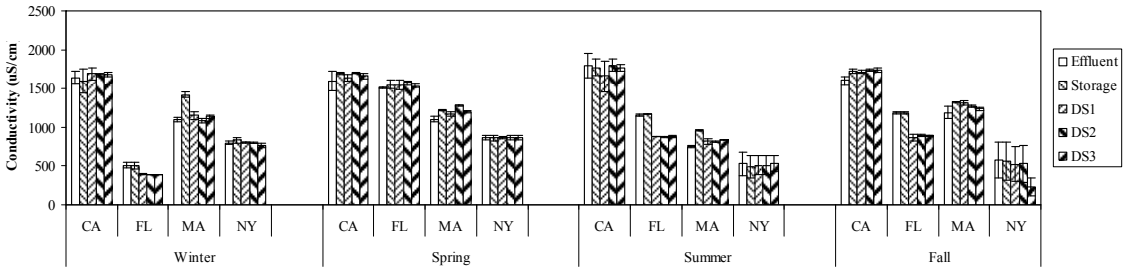


Figure 8.37. Conductivity of the reclaimed water at four different plants over a 1-year duration.

8.12. WATER TEMPERATURE AFFECTED THE MICROBIOLOGY OF RECLAIMED WATER, BUT SEASONAL CHANGES WERE APPARENT ONLY IN SOME SYSTEMS

Seasonal effects primarily because of changes in temperature were prominent only in the MA system, ideally providing an opportunity to investigate the biostability of reclaimed water at low temperatures. At this location, temperatures at the lowest extremes were 2 ^{o}C in the storage tank in winter but increased to 28.5 ^{o}C in the summer (Figure 8.38). By comparison, temperatures at the CA site ranged between 15 and 25 ^{o}C, whereas those in the FL system were 20 to 30 ^{o}C. Because the NY facility was indoors where temperature changes were insignificant, data from this location are not included in the subsequent discussion of seasonality.

Microorganisms display a great ability to exist in a wide range of temperatures compared to other living organisms. That said, however, each microbial species has minimum, optimum, and maximum temperatures that affect growth. The maximum optimal temperature is determined by the sensitivity of enzymes essential for the cells to replicate. Above the optimum temperature, chemical reactions and growth rapidly decline. Based on the

temperature range that they can tolerate, microorganisms are classified as psychrophiles (cold-loving), psychrotrophs, mesophiles (moderate-temperature loving), thermophiles (heat-loving), or extreme thermophiles. Changes in temperature cause physiological changes in biological membranes. Functioning membranes tend to have a fluid liquid-crystalline interior. Chilling temperatures change these membranes to a gel phase, which impairs function, whereas high temperatures tend to disrupt the integrity of the membranes. The temperature at which the membrane phase occurs is related to the nature of fatty acids that compose the membrane. Membranes that are rich in unsaturated or branched-chain fatty acids have lower transition temperatures than do membranes that are rich in saturated straight-chain fatty acids.

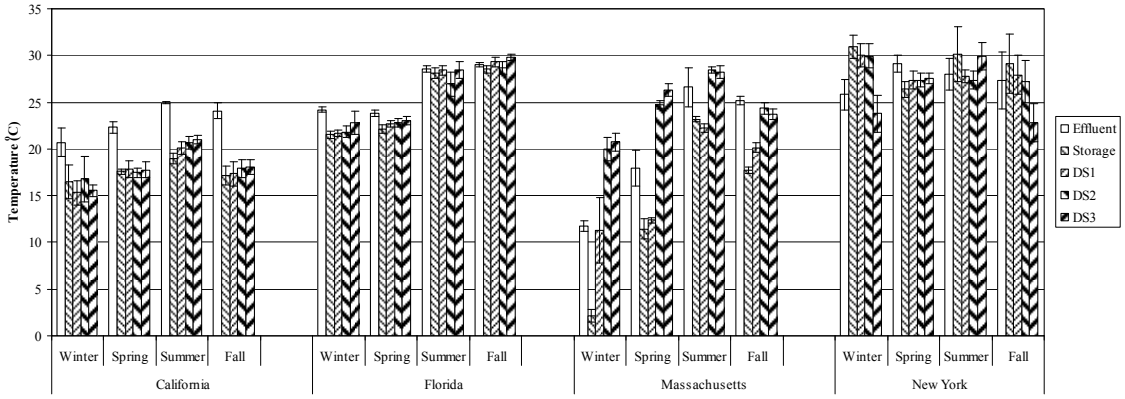


Figure 8.38. Temperature profile of the water in the four distribution systems throughout the year. Error bars are the standard deviation among four consecutive days of sampling from each location.

A summary of the relationship between various types of microorganisms and temperature (data not presented) shows that bacterial heterotrophs were present at all temperatures in the water but were greatly diminished in the MA system, when water temperatures were less than 10 ^{o}C. Thus, most of the bacteria associated with the reclaimed water in the systems tested appear to be mesophilic (see Section 6.1), although some psychrophiles were present at low temperature extremes. Geldreich (1996) reported an increase in coliform-positive samples in potable water distribution systems as temperatures rose in the spring. However, in the present study, coliforms did not seem to be directly affected by temperature and *E. coli*, *Aeromonas* spp., *Legionella* spp., enterococci, and *Pseudomonas* spp. were also not greatly affected by temperature differences across the seasons. *Legionella* spp. are known to withstand temperatures as high as those encountered in domestic hot-water heaters (Borella et al., 2004). Paszko-Kolva et al. (1993) found that both environmental and clinical *Legionella* spp. survived well at low water temperatures (namely, 4 ^{o}C) in which the metabolic activity of their protozoan predators was significantly reduced. There was some limited evidence of increasing temperatures being associated with higher densities of *Mycobacterium* spp. (Figure 8.39).

As presented in Section 8.7, the two facilities at which the reclaimed water is stored in an open reservoir had some significant growth of algae. The abundance of algae, as reflected by chlorophyll content, exponentially increased with increasing temperatures at the FL facility. In the CA system, however, 15 to 20 ^{o}C was more favorable to algal growth, with higher temperatures having only a minimal effect on the algae.

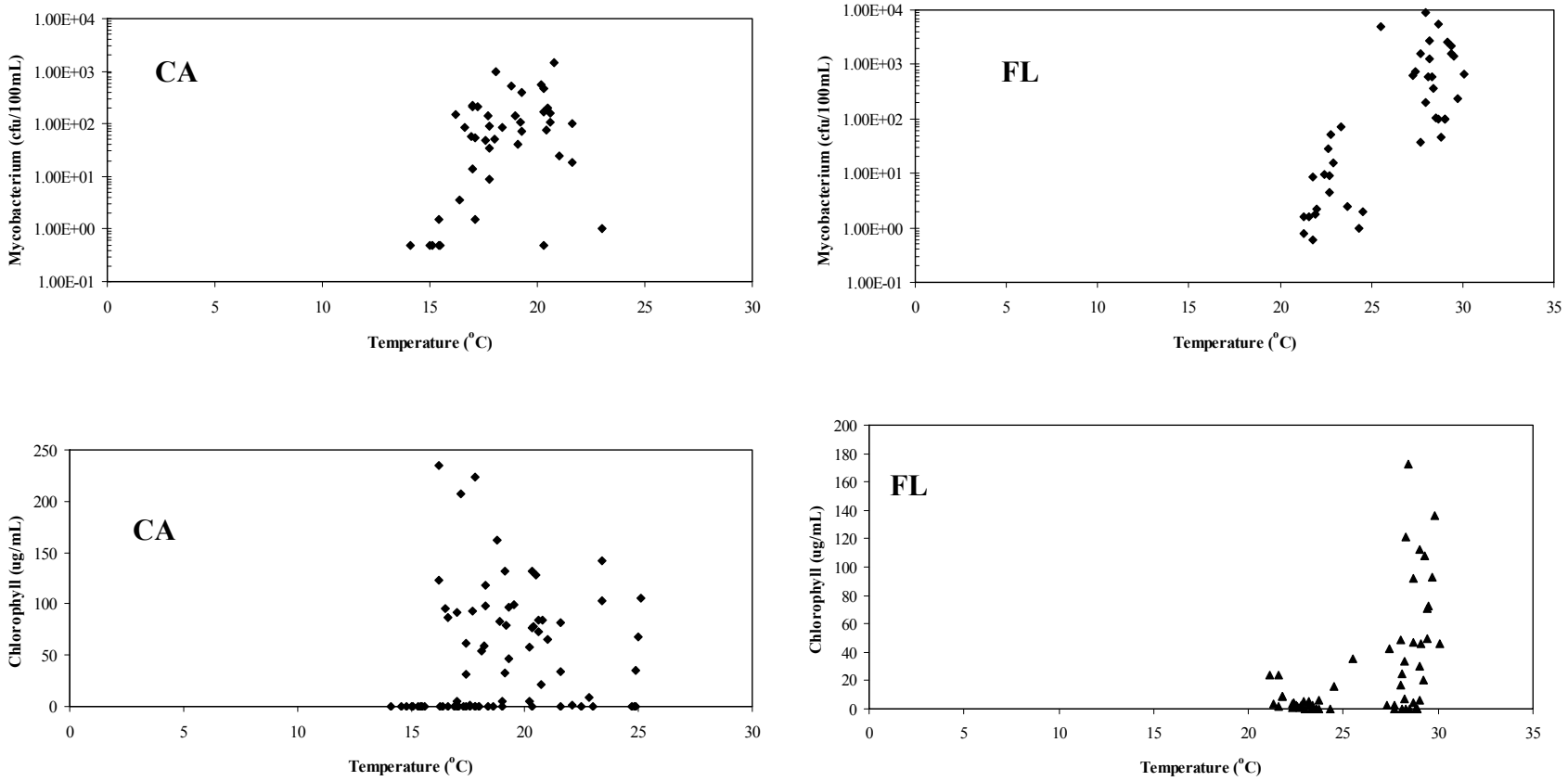


Figure 8.39. Relationship between increasing abundance of *Mycobacterium* spp. and algae with temperature in the CA and FL systems.

A key question in this aspect is how seasonality affects reclaimed water operations. In a number of instances, the water is reclaimed for irrigation purposes. Thus, its demand can be largely impacted by the prevailing season. A critical example of the importance of seasons in the present study was displayed by the increased need for water in winter, summer, and fall at the FL facility, leading to some form of rationing so as to meet client demand. A proper understanding of this scenario requires additional data including rainfall and evapotranspiration, which were beyond the scope of the present study. In practical terms, issues that relate to meeting reclaimed water quantity needs with seasonality by the respective facilities have to be front and center in the production process. As will be detailed in Section 8.14, these changes in demand triggered a practice of intermittent distribution patterns that may have led to stagnation of the water within the system as well as to backflow incidents, ultimately diminishing the microbial quality of the water. In the two instances where the water was stored in ponds, seasonality also provided for more growth of algae, which was not confined to the storage pond but also was transported to the end user.

8.13. THE OCCURRENCE AND GROWTH OF *LEGIONELLA* SPP. AND *MYCOBACTERIUM* SPP. IN RECLAIMED WATER SYSTEMS COULD HAVE PUBLIC HEALTH SIGNIFICANCE

Both *Legionella* spp. and *Mycobacterium* spp. were present in the effluent and storage reservoir and throughout the reclaimed distribution system during the year (Table 8.20). In most instances, the density of *Mycobacterium* spp. was highest in the reclaimed water at the ends of the distribution system. *Legionella* spp. were also at least 10-fold denser in the distribution system than in the point of entry (or disinfection point). The only exception to this generalization was the NY system, which used UV/ozone disinfection and had consistent levels (approximately 8×10^2 CFU/100 mL) throughout the system (Table 8.20). In a model system designed by Muraca et al. (1987), each of these disinfection methods (chlorine, UV, and ozone) was deemed effective for reducing *L. pneumophila* in potable water. Eldestein et al. (1982) estimated a residual concentration of 0.36 mg of ozone/L as sufficient to inactivate *Legionella* spp. The efficacy of a combination of both UV and ozone used simultaneously on *Legionella* spp. has not been tested, but work by Muraca et al. (1987) showed that ozone residuals typically dissipate quite rapidly. Despite the somewhat ineffective performance of UV/ozone against *Legionella* spp., *Legionella* in the MA and NY distribution systems did not increase in density, possibly because of the much lower AOC concentrations of the reclaimed water in both of these MBR systems. Growth of *Legionella* in the CA and FL systems was largely limited to a 1- to 2-$\log_{10}$ increase in the system.

The densities of both *Legionella* spp. and *Mycobacterium* spp. in reclaimed distribution systems were co-correlated (Figure 8.40). When transformed to $\log_{10}$, the equation of the relationship was

$$y = 0.407x + 2.6642 \qquad R^2 = 0.1642$$

where $y = \log_{10}$ density of *Legionella* spp. and $x = \log_{10}$ density of *Mycobacterium* spp.

This co-occurrence was not entirely surprising as the organisms have similar ecologies, typically associating with ciliated protozoa and amoebae (Adékambi et al., 2004 and 2006; La Scola et al., 2004). It has been hypothesized that their intracellular survival provides a training ground for enhancing their pathogenicity to humans (Cirillo et al., 1994 and 1999; Molmeret et al., 2005). In the case of *Mycobacterium* spp., the possession of an impermeable lipid cell wall also provides protection against disinfectants (Taylor et al., 2000).

Table 8.20. Geometric Mean Densities of *Mycobacterium* spp. and *Legionella* spp. in Reclaimed Water at 4 Plants[a]

Site and Species	Effluent	Storage	DS1[b]	DS2	DS3
Mycobacterium spp. (CFU/100 mL)					
CA	1 ± 1	5 ± 17	22 ± 15	35 ± 46	30 ± 120
FL	11 ± 20	65 ± 220	55 ± 390	73 ± 600	107 ± 800
MA	170 ± 190	2 ± 1[c]	57 ± 25	320 ± 130	120 ± 80
NY	6 ± 15	50 ± 80	42 ± 110	16 ± 14	31 ± 29
Legionella spp. (10^3 CFU/100 mL)					
CA	<0.3	2.2 ± 4.1	2.3 ± 7.1	0.9 ± 1.5	1.9 ± 1.6
FL	<0.3	3.0 ± 70	2.7 ± 13	3.5 ± 16	8 ± 52
MA	0.4 ± 0.1	<0.3[c]	1.6 ± 2.8	0.9 ± 2.0	0.6 ± 0.6
NY	0.6 ± 2.1	0.7 ± 0.6	0.5 ± 0.6	0.6 ± 0.6	0.6 ± 0.4

[a]Values are geometric means (±SE) based on aggregate densities over the yearlong monitoring.
[b]DS = distribution system.
[c]Disinfection point is at the storage tank for this location.

During the present study, *Legionella* spp. were identified only to the genus and not to the species level. Currently, there are 48 known *Legionella* spp., 20 of which have been associated with human disease. *L. pneumophila* is the species most frequently associated with human infections, notably Legionnaires' disease and Pontiac fever (CDC, 2007; Söderberg et al., 2008), and is implicated in 90% of the reported cases (Che et al., 2003; O'Loughlin et al., 2007). Symptoms of Legionnaires' disease are nonspecific, including fever of >39 °C, chills, muscle aches, coughing, and diarrhea. However, the disease is thought to be underdiagnosed. No person-to-person transmission exists, making transmission from the environment the only plausible explanation for Legionnaires' disease outbreaks. A preliminary study using PCR amplification demonstrated that *Legionella* spp. were present in reclaimed water at all of the five locations tested, with three of those five locations registering *L. pneumophila* in 11 to 40% of the *Legionella*-positive samples (Palmer et al., 1995). Subsequent testing at a wider range of sites also detected *Legionella* spp. using PCR and direct fluorescent antibody staining, with 5 of the 16 sites specifically containing *L. pneumophila*. The presence of *L. pneumophila* can pose a hazard, particularly in instances where the water is used via sprinklers to irrigate lawns, as the aerosols generated can expose the general public to this pathogen. Inhalation of airborne droplets or of drop nuclei that contain *Legionella* spp. is believed to be the most common mode of transmission (Armstrong and Haas, 2007a). Such droplets can also be generated from cooling towers or decorative water fountains where reclaimed water is used. *Legionella* spp. were isolated from soil, indicating that where *Legionella*-contaminated water was used for irrigation (including irrigation of lawns), the soil (namely, dust) can serve as a conduit for infection (Steele et al., 1990).

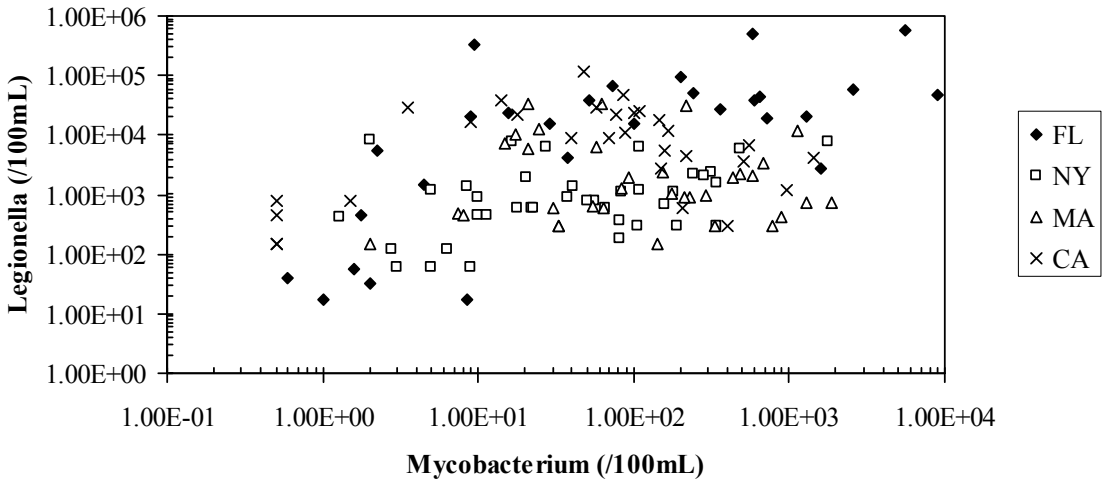


Figure 8.40. Evidence of co-occurrence of *Mycobacterium* spp. and *Legionella* spp. in reclaimed water.

An accurate assessment of the incidence of Legionnaires' disease is hard to come by as most cases are not reported. For example, an average of only 356 cases were reported to the US Centers for Disease Control and Prevention (CDC) between 1980 and 1998 out of an estimated 8000 to 18,000 cases each year (Fields et al., 2002). Similar instances of Legionnaires' disease underreporting have been reported in other countries that mandate notification of health authorities of any case that is handled by clinics (Che et al., 2003). Part of the shortfall is the fact that many clinicians do not test for Legionnaires' disease, which manifests itself as pneumonia whose etiology is rarely determined before treatment regimens are adopted, unless the patient is hospitalized. The proportion of pneumonia cases that are attributed to *Legionella* ranges between 1 and 16% of the cases (Che at al., 2003). Most cases of Legionnaires' disease are sporadic and community acquired, leaving the exact source of infection unidentified. Testing for *Legionella* spp. as the causative agent in such patients would include both culture and serological analyses with direct fluorescent antibody staining and/or *Legionella* urinary antigen tests. Thus, processes that rely on reclaimed water, particularly those that apply such water in a way that generates aerosols, can expose the general population to *Legionella* spp. This organism can be disseminated over long distances (namely, miles) (Addiss et al., 1989; Pastoris et al., 1997) where it can still have an impact on human health.

Similar to *Legionella* spp., *Mycobacterium* spp. were frequently encountered in the reclaimed water but not identified to the species level. *M. gordonae*, *M. kansasii*, and *M. xenopi* were the predominant species recovered by Thomas et al. (2006) in a third of all the hospital water network samples examined. Other species typically encountered in aquatic environments include *M. avium* and *M. intracellulare* (Falkinham et al., 2004). *M. avium* complex is currently on the USEPA Candidate Contaminant List. The resistance of *Mycobacterium* spp. to ozone and chlorine-based disinfectants as used at the facilities that were surveyed has also been documented by Taylor et al. (2000) in potable water. Similar to *Legionella* spp., *Mycobacterium* spp. can also be transported through aerosols and water droplets during processes such as spraying the reclaimed water on lawns. The extent of aerosolization is even

more pronounced in the more hydrophobic species of mycobacterium, such as *M. avium* and *M. intracellulare* (Parker et al., 1983; Falkinham, 2004). Some strains of *Mycobacterium* are suspected of causing Crohn's disease, a disease that more than 800,000 people in North America currently suffer from (Nacy and Buckley, 2008).

To better understand the public health impact of *Legionella* and *Mycobacterium* spp. in reclaimed water distribution systems, epidemiological studies would need to target individuals who routinely have contact with the water. To determine exposure, such studies could assay for the presence/absence of antibodies against *Legionella* spp. and *Mycobacterium* spp. The latency of Legionnaires' disease is 2 to 10 days, but the antibodies can remain detectable for a much longer duration. This line of research could be coupled with access to medical records of individuals in areas where reclaimed water is used. Some design lessons can be obtained from studies such as those by Che et al. (2003), who, by considering various ecological aspects, identified new sources of sporadic cases of Legionnaires' disease, developing likelihood ratios, relative risks under different settings, and exposure categories.

The kinds of analysis for the future epidemiological work highlighted above are beyond the scope of the current study but do provide important direction for the reuse industry. Equally important in future studies is the determination of strain virulence for opportunistic *Mycobacterium* and *Legionella* spp. isolates. The virulence of clinical *Legionella* spp. grown in culture has led to the identification of a range of low-molecular-weight cytotoxins (Belyi, 1999). The use of similar approaches on environmental *Legionella* spp. can provide a rapid screening assay to identify strains that could be of public health concern. A quantitative risk assessment of human exposure to *Legionella* through aerosols has been conducted by Armstrong and Haas (2007a and 2007b) using data reconstructed from outbreaks of Legionnaires' disease. Although their results cannot be directly extrapolated to reclaimed water systems (because of the unknown status of *Legionella* virulence), the approach can serve as a useful guide for developing risk models under various reuse situations. Because of increased attention to *Legionella* in water-related Legionnaires'disease outbreaks by public health authorities, as presented in the recent surveillance summaries of the 2005–2006 outbreaks in the United States (Yoder et al., 2008), addressing concerns about potential risks from the abundance of this organism in reclaimed water is prudent.

Several methods for disinfection of *Legionella* spp. have been suggested. These include thermal eradication (heat and flush), hyperchlorination, and copper-silver ionization. For example, maintaining the temperature in hot water tanks at 50 to 60 °C has been recommended for hospital distribution systems (Muraca et al., 1987). Currently, none of these control techniques are implemented by reclaimed water facilities. The practicality of some of these approaches along with AOC control should be investigated to better mitigate *Legionella* risks in reclaimed water.

8.14. RECLAIMED WATER SYSTEM OPERATIONS IMPACTED THE OCCURRENCE OF MICROORGANISMS IN THE DISTRIBUTION SYSTEM

Because of high demand for reclaimed water, the plant in FL could provide service only to half of its customers during the peak demand seasons. As a result, it shut off the flow to half of its distribution system on alternating days and initiated a complete shutdown on Mondays for maintenance. This alternating schedule was implemented in three (winter, summer, and fall) of the four quarters examined. This practice appeared to have a significant impact on the microbial quality of reclaimed water, because the occurrence of common indicator bacteria

and opportunistic microbes in the distribution system was so much higher than in the other three systems (Tables 8.6 and 8.20). Another way to demonstrate the effects of this distribution practice was to compare the occurrence of bacteria in the system during the spring (when the system was operated continuously) to occurrence during the other quarters, when there were intermittent operations. Data shown in Figure 8.41 illustrate the low levels of HPC bacteria during continuous operation (spring) compared to the levels of the other seasons. The benefits of continuous operation were most apparent in the initial sections of the distribution and on Tuesday (after the system was completely shut down the day before). It is theorized that the higher bacterial levels and sediments at the ends of the system may mask some of the effect of the periodic operation of the system.

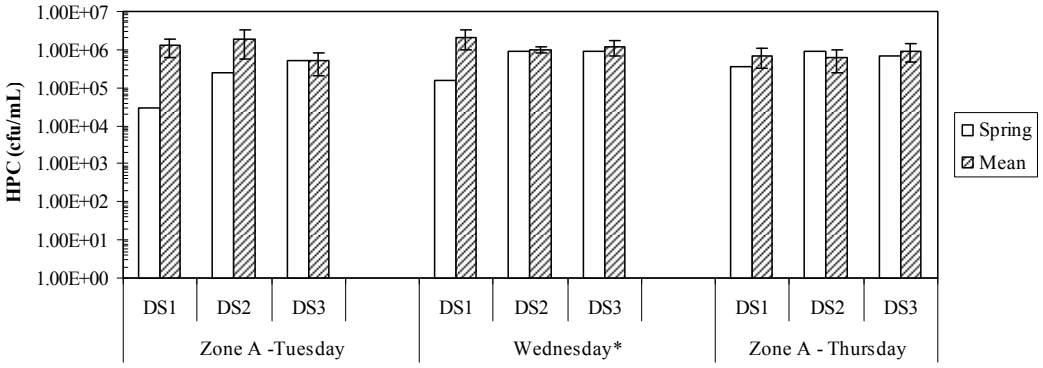


Figure 8.41. The density of heterotrophic bacteria in spring compared to its mean density in winter, summer, and fall with the FL distribution system. The mean densities on Wednesday (as marked by an asterisk) were determined in zone B when the zone A distribution system was closed.

The intermittent operation of a water supply system is associated with loss of water flow and pressure in the system. These frequent pressure differentials can lead to cracks and leaks, resulting in intrusion of backflow into the system. The periods of stagnation could also promote bacterial growth owing to loss of the disinfectant residual. Furthermore, the stagnant water left after the shutoff could result in oxygen depletion, causing offensive odors. Andey and Kelkar (2007) found a moderately positive correlation ($R^2 = 0.60$) between the duration (namely, length) of supply of water in a distribution system and the percent occurrence of fecal coliforms in four potable water distribution system in India. Thus, short hours of intermittent distribution (namely, 3 to 5 h) were less liable to have fecal coliforms than were intermittent supplies of more than 10 h. The FL plant staff observed a 24-h supply break between operation of its two zones and a total shutdown of the distribution system once a week.

Figure 8.42. A portion of the storage pond at the FL facility with a swarm of birds in the background. In the foreground is the recovery of a carcass that had been trapped by the system to minimize bird landing.

The operation of the open reservoirs has previously been noted as a source of algal cells, but the impacts of these facilities were more than just a result of the accumulation of organic carbon. The open reservoirs could also be a source of fecal contamination from ducks and other migratory birds that visit the pond. Indeed, many birds were seen around the pond during the sampling episodes (Figure 8.42). Data presented in Table 8.21 show that the treated plant effluent typically had no or low levels of total and fecal coliform bacteria; however, levels after the open storage were much higher and this bacterial loading had a negative impact on the microbiology of the reclaimed water network. Therefore, it is likely that operations to better minimize bird populations could improve reclaimed water quality in systems with open pond storage. Alternatively, the birds can be deterred by using sound repellers, solar repellers, or spikes (see www.bird-b-gone.com; www.nixalite.com; www.bird-x.com, and related sites).

Table 8.21. Total Coliform Changes with Intermittent versus Continuous Supply in the FL System[a]

		CFU (per 100 mL)				
Season	**Organism**	**Effluent**	**Storage**	**DS1**	**DS2**	**DS3**
Winter	Total coliform	0.3 ± 0.3	4 ± 27	10 ± 27	290 ± 65	36 ± 67
Spring		<0.3	13	<0.3	7 ± 12	5 ± 15
Summer		<0.3	12 ± 7	1	5	7
Fall		<0.3	25 ± 10	<0.3	<0.3	<0.3
Winter	Fecal coliform	0.3 ± 0.1	2 ± 30	1 ± 8	9 ± 17	2 ± 18
Spring		<0.3	13	0.5 ± 1	9 ± 4	7 ± 13
Summer		<0.3	4 ± 2	0.5	1	6
Fall		<0.3	44 ± 5	<0.3	<0.3	<0.3
Winter	*E. coli*	0.3 ± 0.1	2 ± 30	1 ± 8	7 ± 16	1 ± 10
Spring		<0.3	9	<0.3	3 ± 6	7 ± 3
Summer		<0.3	11 ± 16	0.6 ± 2	1 ± 0	2 ± 1
Fall		<0.3	1 ± 4	1 ± 5	<0.3	1 ± 9
Winter	MS phage	10 ± 2	1 ± 0	23 ± 1	9 ± 9	18 ± 25
Spring		<1	12	2 ± 7	6 ± 12	11 ± 9
Summer		15 ± 8	14 ± 12	24 ± 12	27 ± 3	25 ± 3
Fall		22 ± 7	20 ± 4	3 ± 6	3 ± 3	10 ± 15
Winter	Somatic	1 ± 1	1 ± 1	2 ± 16	5 ± 13	4 ± 7
Spring		1	<1	11 ± 9	8 ± 8	3 ± 10
Summer		134 ± 6	126 ± 69	50 ± 33	67 ± 5	128 ± 29
Fall		33 ± 5	26 ± 17	7 ± 25	12 ± 24	1 ± 0

[a]The supply was intermittent during winter, summer, and fall but continuous in spring. All values with a ±SE are geometric means of daily samples obtained over two to four consecutive days.

8.15. THE ABILITY OF A WASTEWATER SYSTEM TO REMOVE CARBON WAS NOT NECESSARILY DEPENDENT ON THE TREATMENT TECHNOLOGY ITSELF BUT ON THE MANAGEMENT OF THE OPERATIONS

One of the primary goals of this project was to better understand the key parameters that influenced changes in microbial quality in reclaimed distribution systems. Toward that end, we extensively studied four systems to develop a sufficient database from which to draw these conclusions. Data presented in this report provide strong evidence that BDOC is a critical parameter influencing reclaimed water quality.

However, the evidence for AOC and BDOC was from only a limited number of systems, making it important to understand how other treatment processes could affect these influential parameters. A wastewater survey was therefore conducted to examine the impact of various wastewater processes on the final effluent quality and to assess the suitability of the effluents for reclamation applications. Examined wastewater sources included the following categories, the number in parentheses corresponding to the number of utilities surveyed:

- domestic and municipal sources (7),
- domestic (6),
- domestic and industrial (5),
- municipal (2), and
- municipal and industrial (1).

The wastewater treatment technologies surveyed included activated sludge, RBCs, sequencing batch reactors, and MBRs. Additional process details and disinfection practices are presented in Table 8.22.

A total of 21 samples were collected from full-scale wastewater treatment facilities and analyzed for a range of compounds with special emphasis on the organic carbon fraction in the treated effluents. Throughout the project, results have shown that organic carbon, specifically the AOC and BDOC fractions, present in the reclaimed water was directly related to microbial regrowth in the distribution system. Regrowth has been especially prevalent in high-AOC systems that lack a disinfectant residual. High AOC concentrations were prevalent in the conventional activated sludge-treated effluents, and it was of interest to examine other treatment processes to determine the impact of treatment on the organic carbon fractions.

The organic carbon content of the surveyed plants is shown in Figure 8.43, with the data sorted from highest to lowest AOC concentrations. TOC concentrations averaged 5.5 ± 2.4 mg/L, and BDOC averaged 1.3 ± 0.9 mg/L. The BDOC median value was 1.3 mg/L. AOC ranged from 45 to 3200 µg/L, with a median value of 452 µg/L. TOC and BDOC concentrations showed agreement with the decreasing trend in AOC; the correlation coefficient (R^2) for TOC versus AOC was 0.38 and for BDOC versus AOC $R^2 = 0.42$.

Owing to their advanced treatment methodology, MBR systems were capable of producing a high-quality effluent. In fact, all three of the MBR utilities surveyed had AOC and BDOC levels below the respective median (Figure 8.44). Error bars indicate the variability of the results across the 21 utilities. For example, the variability suggests that some activated sludge utilities produced effluent quality similar to those produced by MBRs based on BDOC measurements.

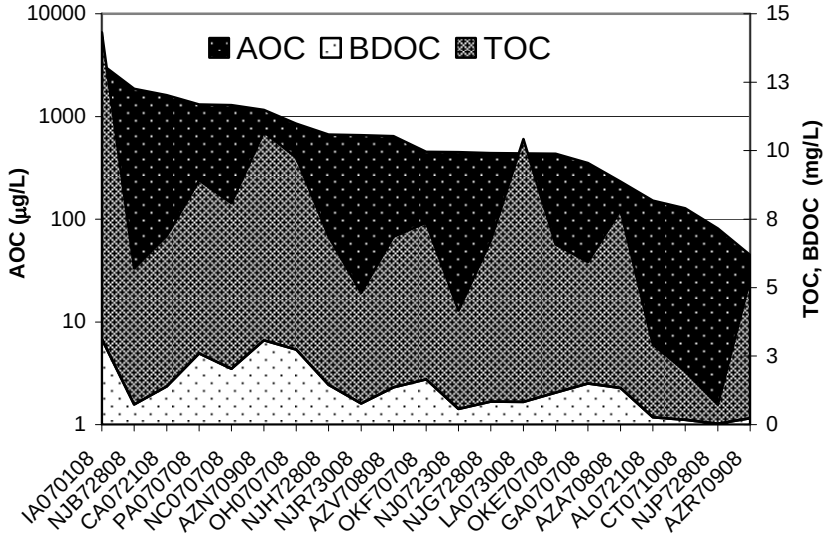


Figure 8.43. Wastewater survey results tracking AOC levels.

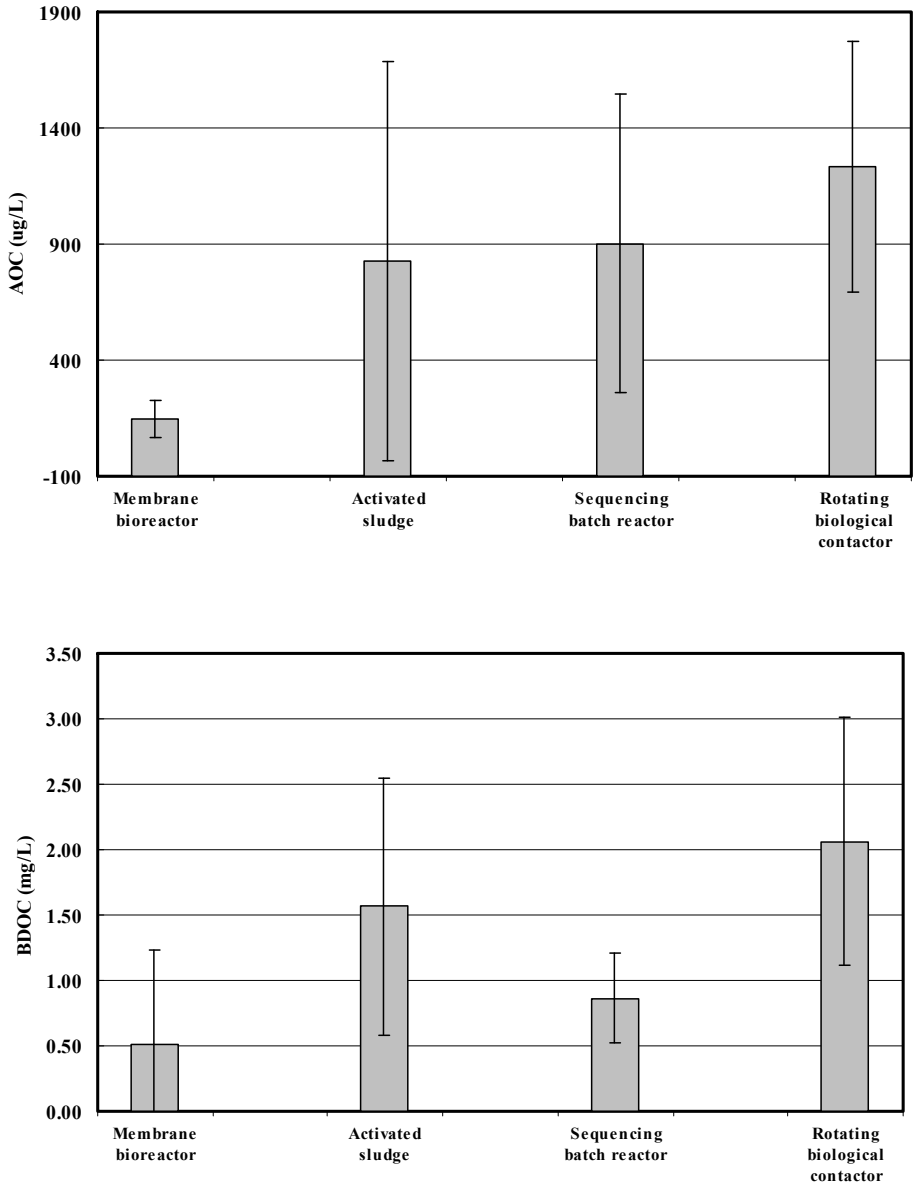


Figure 8.44. Mean AOC and BDOC in effluents generated by plants that use different treatment systems.

The data suggest that system operation, not necessarily the treatment process, influenced the ability of a system to achieve a low-AOC-containing effluent. Because these utilities represented a range of collection systems, the main driver for producing high-quality effluent, in terms of biological stability, was not necessarily the wastewater source or even the type of treatment. While few of the utilities surveyed had plant effluents with BDOC of <0.25 mg/L and AOC levels of <100 µg/L (14% and 10%, respectively), the ability to achieve low organic carbon levels was not exclusively produced by MBR facilities. Although in general MBR systems did produce high-quality water, the broader survey showed that other treatment

configurations (activated sludge and sequencing batch reactors) were also capable of producing effluents with low levels of BDOC. Specifically, 58% of the utilities employing activated sludge treatment technology and 25% of those using sequencing batch reactors had effluents with below-median AOC.

Based on these results, a variety of the wastewater technologies can be suitable for producing biologically stable reclaimed water, provided that they were operated in a manner to optimize BDOC removal. Further research is necessary to determine the specific operations and optimization configurations for organic carbon reduction. These options could include adjustment of hydraulic retention time and mixed liquor suspended solids, in order to increase effluent water quality and obtain biologically stable treated water.

Table 8.22. Wastewater Survey Sites, Process Details, and Carbon Concentrations[a]

Coded Facility	Source	Process	Secondary Treatment	Disinfection	Tertiary Filtration	Nutrient Removal	TOC (mg/L)	BDOC (mg/L)	AOC (µg/L)	AOC ≤ Median
IA070108	Municipal/Industrial	AS	Extended Aeration	Chlorination	None	None	11.20	3.12	3203	NO
NC070708	Domestic/Industrial	AS	Oxidation Ditch	Chlorination/ Dechlorination	None	N and P Removal	6.06	2.03	1292	NO
PA070708	Domestic/Industrial	Attached Growth	Trickling Filter and AS	UV	None	N and P Removal	6.32	2.60	1313	NO
GA070708	Domestic/Industrial	AS	A2O	UV	Yes	N and P Removal	4.43	1.50	351	YES
OH070708	Domestic/Industrial	Attached Growth	RBC	Chlorination/ Dechlorination	Yes	P Removal	7.01	2.73	853	NO
OKF70708	Domestic/Municipal	AS	Extended Aeration	Chlorination	Yes	None	5.73	1.65	452	YES
OKE70708	Domestic/Municipal	AS	Extended Aeration	UV, Chlorination Dechlorination	Yes	None	5.41	1.17	432	YES
AZA70808	Domestic	AS	MBR	UV	Yes[b]	N Removal	6.49	1.34	232	YES
AZV70808	Domestic	AS	SBR	Chlorination	None	N Removal	5.49	1.36	645	NO
AZN70908	Domestic/Municipal	AS	MLE	Chlorination/ Dechlorination	Yes	N Removal	7.62	3.07	1160	NO
AZR70908	Domestic	AS	Extended Aeration	Chlorination/ Dechlorination		N Removal	5.07	0.24	45	YES
CT071008	Municipal	AS	MBR	UV	Yes[b]	N Removal	1.78	0.17	128	YES
CA072108	Domestic/Municipal	Attached Growth	RBC	Chlorination	Yes	None	5.50	1.39	1615	NO
AL072108	Domestic/Municipal	AS	Extended Aeration			None	2.66	0.27	152	YES
NJ072308	Domestic	AS	SBR	UV	Yes	N Removal	3.59	0.58	450	YES
NJG72808	Domestic	AS	MLE	UV	Yes	N Removal	5.88	0.84	438	YES
NJP72808	Municipal	AS	MBR	UV	Yes[b]	N Removal	0.70	0.03	81	YES
NJB72808	Domestic/Municipal	AS	SBR	UV	Yes	N Removal	4.96	0.74	1859	NO
NJH72808	Domestic/Municipal	AS	Extended Aeration	Chlorination/ Dechlorination		N Removal	5.42	1.45	664	NO
NJR73008	Domestic	AS	SBR	UV	Yes	N removal	4.03	0.77	656	NO
LA073008	Domestic/Industrial	AS	Aeration	Chlorination	None	None	9.60	0.83	438	YES

[a]Median AOC = 452 µg/L. AS = activated sludge; SBR = sequencing batch reactor.
[b]Plant treatment process is equivalent to tertiary filtration.

CHAPTER 9

UTILITY APPLICATIONS

The results of this study have a number of practical applications for reclaimed water utilities to help improve treatment processes and better manage reclaimed distribution system water quality. The following recommendations are based on the data collected in this study, and the seven guidelines appear in descending order of importance to reclaimed water systems.

1. In the FL system, different zones of the reclaimed system were operated on alternate days, and the entire system was shut down on Mondays. The occurrence of indicator bacteria including *E. coli* and of opportunistic pathogens was highest in this system, suggesting that the stagnation of the water and depressurization of the pipeline had a negative impact on microbial quality. **To the extent possible, reclaimed water systems should maintain constant flow in the pipe networks, preventing water stagnation and intrusion of untreated groundwater during periods of depressurization.**

2. The presence of open finished water storage reservoirs also negatively impacted microbial quality in the distribution system. The open reservoirs promoted algal growth, increased BDOC levels, dissipated disinfectant residuals, and contributed to increased bacterial loading of the distribution system, possibly because of birds roosting on the reservoir. Therefore, **where open storage is practiced, attention should be paid to algal control through reservoir destratification, nutrient limitation (phosphorus and nitrogen), chemical treatment, or installation of fine-mesh screens to control entry of the algae and cyanobacteria into the distribution system.** Microstrainers with apertures ranging from 15 to 45 μm have been demonstrated to remove 10 to 100% algae besides reducing the turbidity of the water (Mouchet and Bonnelye, 1998). Some natural coagulants have also been shown to be effective in controlling algae (see Section 5.4). Algal growth can be controlled by reducing the detention time of the reclaimed water in the reservoir, particularly in the warmer periods where temperature and solar intensity increase the growth rate of the algae (USEPA, 2004).

3. Accumulation of algal cells and particulate material in the distribution system resulted in increases in chlorophyll and turbidity at dead-end locations. The biodegradation of the algal cells likely resulted in increases in AOC and BDOC in the systems. This slow biodegradation provided an endogenous source of nutrients and could contribute to the generation of anoxic conditions in the system. The accumulation of particulates and debris from the open reservoirs resulted in accumulation of sediments and turbidity in the system, which could provide habitats for bacterial growth. **It is particularly important, especially for systems with open reservoirs, to have an effective, routine flushing program that achieves scouring velocities capable of mobilizing and removing the sediments. It is recommended that a unidirectional flushing program be developed to realize the maximum benefits from these efforts.**

4. High levels of biodegradable organic matter had a clear impact on the microbial quality of reclaimed water. BDOC levels averaged between 0.4 and 6.2 mg/L, and average AOC levels ranged between 150 and 1400 μg/L. In general, these levels were about 10 times higher than those found in drinking water systems and more than sufficient to account for the microbial growth observed in the reclaimed systems studied. The growth of

heterotrophic bacteria could result in an anoxic environment that would produce hydrogen sulfide odors, nitrification, and corrosive conditions. **Reclaimed water system staff should evaluate treatment strategies that could improve removal of BDOC, including operation at longer solid retention times, implementation of biologically activated carbon filtration, application of membrane filtration, or other innovative techniques.** Because low-molecular-weight BDOC compounds also cause a disinfectant demand, their removal can improve the disinfectant stability in reclaimed water systems.

5. The analysis of 21 wastewater treatment plants showed plant effluent BDOC levels were generally lower in systems that used UV radiation for postdisinfection. This finding was particularly true for systems that also employed MBRs. UV technology, at the doses typically used for disinfection, does not lead to an increase in AOC or BDOC levels. **Reclaimed water systems that require a high level of water quality in the distribution system should consider coupling MBR technology with UV disinfection because this combination typically produced some of the lowest levels of biodegradable organic matter.**

6. Regrowth of microorganisms was especially prevalent in high-AOC systems that lacked a disinfectant residual. High levels of organic carbon, combined with open finished water reservoirs, resulted in rapid depletion of residual disinfectants. **When a disinfectant residual was maintained in the distribution system, it was effective in controlling microbial occurrence.** In the pipe loop studies, free chlorine was more effective than chloramines for HPC and *Legionella* control under the conditions studied. However, the short duration of the exposure (3 days) was probably insufficient to observe the long-term impacts of the disinfectant. **Because chloramines are more stable and likely to persist longer in reclaimed distribution systems, utilities could consider maintaining a monochloramine residual, but they need to be careful to minimize any remaining free ammonia that could cause nitrification.**

7. **Consider installing disinfectant booster stations to maintain a residual disinfectant at all points within the distribution system. A free chlorine residual of 0.2 mg/L is typically deemed protective against bacterial growth.** It would be especially important to disinfect after open storage reservoirs since residuals are dissipated in these open ponds.

8. Disinfection of reclaimed waters requires a delicate balance: one needs to inactivate pathogenic microbes, yet the oxidants will break down higher-molecular-weight organic molecules into smaller, more biodegradable organic matter. Therefore, **reclaimed water utilities should evaluate this balance through the monitoring of AOC and BDOC in treated effluents.** The bioluminescence AOC test used in this study would be suitable for this purpose because the assay was rapid, accurate, and easy to use. The bioluminescence AOC test permitted insights into changes in the nature of the BDOC as it travelled through the reclaimed systems.

9. The conventional and MBR wastewater treatment systems were generally effective in removing/inactivating microbial pathogens in treated effluents, but regrowth occurred in the distribution systems. Increased levels of occurrence of nearly all of the microbes monitored (HPC, total coliforms, *E. coli*, *Pseudomonas*, *Aeromonas*, *Enterococci*, *Legionella*, and *Mycobacterium*) were noted. Water temperatures affected the microbiology of reclaimed water, but seasonal changes were apparent only in some systems. *E. coli* O157:H7 was detected only two times in the plant effluent of one

conventional system but never showed evidence of regrowth in the distribution system. The absence of common indicator bacteria (total coliform and *E. coli*), however, did not preclude the presence of potentially pathogenic organisms. *Legionella* spp. and *Mycobacterium* spp. were commonly detected in reclaimed water systems and could have public health significance. **It is recommended, therefore, that reclaimed water systems monitor beyond just the basic indicator bacteria but also begin to measure and control opportunistic organisms like *Legionella* and *Mycobacterium*.**

CHAPTER 10

FUTURE RESEARCH NEEDS

The present study does not answer all the questions pertaining to maintenance of biologically stable reclaimed water, nor does it address some important issues regarding the risks of opportunistic and frank pathogens in treated and distributed effluents. Some suggestions for future research are given underneath:

(i) It is necessary to examine how to optimize conventional wastewater treatment for improved removal of BDOC and develop design and operational criteria when various treatment processes are used to produce reclaimed water.

(ii) Additional research is needed to examine the potential risks from *Legionella* and *Mycobacterium* spp. in reclaimed water. Future studies should evaluate the specific species and serotypes prevalent in reclaimed water. Where possible, virulence determinants should be examined, as well as the interaction of these organisms with amoebae that could increase their public health significance.

(iii) The infectivity of *Giardia* cysts, *Cryptosporidium* oocysts, and enteric viruses should be addressed. Ongoing studies are examining the infectivity of cysts and oocysts in reclaimed water, but additional attention should be directed toward risks of enteric viruses.

(iv) This study did not examine the hydraulics of reclaimed distribution systems (it wasn't needed to observe the changes in water quality). However, future studies should examine the impact of system hydraulics on degradation in water quality.

(v) Because improved operation of reclaimed distribution systems would be the fastest, lowest-cost, mechanism to improve water quality, a project to develop best operating practices for reclaimed water distribution system management should be initiated.

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APPENDIX I

GUIDELINES FOR USING RECLAIMED WATER IN VARIOUS STATES IN RELATION TO THOSE PUBLISHED BY THE USEPA

Guidelines for Using Reclaimed Water in Various States in Relation to Those Published by the USEPA[a]

Jurisdiction	Uses	Treatment				pH	BOD (mg/L)	Turbidity (NTU)	TSS (mg/L)	Fecal Coliform/100 mL	Chlorine Residual (mg/L)	Other Remarks
		Secondary	Filtration	Disinfection	Advanced							
USEPA	Urban, crops eaten as raw, recreational impoundments	√	√	√		6-9	<10	≤2	NS	Nondetectable (based on 7-day median w/ none >14 per 100 mL)	≥1 (w/min. contact t = 30 min)	
	Restricted access area irrigation, processed food crops, nonfood crops, aesthetic impoundments, cooling (recirculating)	√		√		6-9	≤30		≤30	<200 (based on 7-day median w/ none >800 per 100 mL)	≥1 (w/min. contact t = 30 min)	
	Groundwater recharge of potable aquifers by injection	√	√	√	√	6-9		≤2		Nondetectable (based on 7-day median w/ none >14 per 100 mL)	≥1 (w/min. contact t = 30 min)	Meet drinking water standards
	Groundwater recharge of potable aquifers by spreading	√		√								Meet drinking water standards
AZ	**Class A** (namely, food crop (inc. vineyards) and open-access landscape irrigation, fire protection, flushing, recreational impoundments, school landscaping, car and equipment washing (exc. self-serve), A/C systems (closed), and snow-making	√	√	√				≤2; not to exceed 5 at any time		<23 in a single sample		

Jurisdiction	Uses	Treatment				pH	BOD (mg/L)	Turbidity (NTU)	TSS (mg/L)	Fecal Coliform/100 mL	Chlorine Residual (mg/L)	Other Remarks
		Secondary	Filtration	Disinfection	Advanced							
	Class B (Surface irrigation (orchards and vineyards), golf courses, dust control, dairy pasture and watering, concrete mixing, landscape impoundments, restricted access landscape irrigation, street cleaning, washing and sieving materials	√		√						<200 in 4 out of 7 samples; max. in a single sample <800		
	Class C (Pasture and watering nondairy, irrigating sod farms, as well as fiber, seed, forage, and silviculture crops)	√ (in series of ponds)								<200 in 4 out of 7 samples; max. in a single sample <800		Ponds should include aeration
CA	Irrigating fodder, fiber, seed crops, orchards, vineyards, processed food crops, non-food-bearing trees, ornamentals, sod and flushing	√										

Jurisdiction	Uses	Treatment				pH	BOD (mg/L)	Turbidity (NTU)	TSS (mg/L)	Fecal Coliform/100 mL	Chlorine Residual (mg/L)	Other Remarks
		Secondary	Filtration	Disinfection	Advanced							
	Dairy irrigation, landscaping, landscape impoundments, cooling towers, firefighting, dust control, road cleaning, industrial boiler feed, soil compaction, sod and ornamental nurseries w/ restricted access	√		√						≤23; ≤240 in a single sample in any 30-day period		
	Irrigation for food crops (no contact with edible part), aquaculture, and restricted recreational impoundments	√		√						≤2.2; ≤23 in a single sample in any 30-day period; 240 is the allowable max.		
	Irrigation for food crops (contact with edible part), flushing, fountains, car washes, laundries, snow-making, firefighting, cooling	√	√	√						≤2.2; ≤23 in a single sample in any 30-day period		Coagulation required if turbidity continuously exceeds 5 NTU
	Nonrestricted recreational impoundments	√	√	√						≤2.2; ≤23 in a single sample in any 30-day period		Coagulation required if turbidity continuously exceeds 5 NTU, and clarification is required if enteric viruses, *Giardia* and *Cryptospridium* are not monitored

Jurisdiction	Uses	Treatment				pH	BOD (mg/L)	Turbidity (NTU)	TSS (mg/L)	Fecal Coliform/100 mL	Chlorine Residual (mg/L)	Other Remarks
		Secondary	Filtration	Disinfection	Advanced							
Colorado	Category 1 (Not allowed for unrestricted access landscape irrigation or nonresidential fire protection)	√		√					30 mg/L as a daily maximum	≤126 *E. coli* bacteria/100 mL monthly geometric mean. ≤235/100 mL for single sample in any calendar		Also referred to as "Restricted access". Must satisfy both TSS and *E. coli* limits. Application should be within plant needs to minimize runoff.
	Category 2 (Usable for cooling towers, concrete mixing, dust control, soil compaction, street cleaning, zoo operations, nonresidential fire protection and both restricted and unrestricted access landscape irrigation)			√				≤3 as a monthly average. Not to exceed 5 in >5% of samples during calendar month		≤126 *E. coli* bacteria/100 mL monthly geometric mean. ≤235/100 mL for single sample in any calendar month		Also referred to as "Unrestricted access". Should undergo oxidation with disinfection at a minimum
FL	Low-rate land application systems – Restricted public access	√		√					<10 mg/L			Treatment must be met before discharging to storage ponds or reuse systems

Jurisdiction	Uses	Treatment				pH	BOD (mg/L)	Turbidity (NTU)	TSS (mg/L)	Fecal Coliform/100 mL	Chlorine Residual (mg/L)	Other Remarks
		Secondary	Filtration	Disinfection	Advanced							
	Low-rate land application systems – Public access areas (for example, golf courses, cemeteries, parks, highway medians, residential lawns), Residential irrigation (fire protection, aesthetic, dust control) and irrigating edible crops	√	√	√	√ (Chemical feed for coagulants required)				<5 mg/L before applying the disinfectant			Removal of TSS before disinfecting ensures increased inactivation of viruses. Filtration removes protozoa pathogens (namely, *Cryptosporidium, Giardia*, etc.). Adding coagulants also increases pathogen removal. ≤12 mg NO_3-N/L in reclaimed H_2O or ≤10 mg NO_3-N/L in receiving groundwater, whichever is lower
Missouri	Land application, e.g., golf courses.									<200/100 mL		Treatment evaluated on a case-by-case basis.
Nevada	Category A: Irrigation to pasture or other agricultural purposes except growing crops for human consumption	√								No limit		800 ft of maximum buffer zone
	Category A(1):	√								≤200/100 mL as a 30-day geometric mean and daily maximum of 400/100 mL		400 ft of maximum buffer zone

Jurisdiction	Uses	Treatment				pH	BOD (mg/L)	Turbidity (NTU)	TSS (mg/L)	Fecal Coliform/100 mL	Chlorine Residual (mg/L)	Other Remarks
		Secondary	Filtration	Disinfection	Advanced							
	Category B: Irrigation of golf courses, cemetery, or greenbelt with controlled access	√								≤23/100 mL as a 30-day geometric mean and daily maximum of 240/100 mL		100 ft of maximum buffer zone
	Category C: Irrigating cemeteries, highway median, greenbelt, park, playground, residential or commercial lawn with controlled access	√								≤2.2/100 mL as a 30-day geometric mean and daily maximum of 23/100 mL		0 ft of maximum buffer zone
New Mexico	Landscape irrigation – unrestricted access	√	√	√		6–9 (or up to 10 for lagoon	≤10 µg/L	≤2 NTU	≤5	≤2.2 (No single exceeding 23/100 mL for 7 consecutive days)	≥0.2	Excessive pooling of effluent should be avoided; 50" to potable water
	Landscape irrigation – restricted access	√		√		6–9 (or up to 10 for lagoon	≤30 µg/L		≤30	≤200 (No single exceeding 800/100 mL for 7 consecutive days)	≥0.2	300" to potable water supply; 100" to publicly accessible areas
	Commercially processed food crops	√		√		6–9 (or up to 10 for lagoon	≤30 µg/L		≤30	≤200 (No single exceeding 800/100 mL for 7 consecutive days)	≥0.2	300" to potable water supply; 100" to publicly accessible areas
	Food crops that are not commercially processed	√	√	√		6–9 (or up to 10 for lagoon	≤30 µg/L		≤30	≤200 (No single exceeding 800/100 mL for 7 consecutive days)	≥0.2	50" to potable water supply wells
	Nonfood crops	√		√		6–9 (or up to 10 for lagoon	≤30 µg/L		≤30	≤200 (No single exceeding 800/100 mL for 7 consecutive days)	≥0.2	300' to potable water supply wells; 100' to publicly accessible areas

Jurisdiction	Uses	Treatment				pH	BOD (mg/L)	Turbidity (NTU)	TSS (mg/L)	Fecal Coliform/100 mL	Chlorine Residual (mg/L)	Other Remarks
		Secondary	Filtration	Disinfection	Advanced							
	Pasture for milking cows	√	√	√		6–9 (or up to 10 for lagoon	≤30 µg/L		≤30	≤23 (No single exceeding 92/100 mL for 7 consecutive days)	≥0.2	300' to potable water supply wells; 100' to publicly accessible areas
Texas	Type I use: Irrigation of residences, landscaping, public parks, golf courses, school yards, athletic fields; fire protection; dairy pastures; flushing; recreational impoundments						5			20 (geometric mean) or ≤75 (single grab)		Need sampling at least twice a week
	Type II use: Irrigation of sod, silviculture; highways; food crops that are pasteurized; nondairy pastures; dust control, cooling towers; construction						20 (or 30 for ponding system)			200 (geometric mean) or ≤800 (single grab)		Need sampling at least twice a week

Jurisdiction	Uses	Treatment				pH	BOD (mg/L)	Turbidity (NTU)	TSS (mg/L)	Fecal Coliform/100 mL	Chlorine Residual (mg/L)	Other Remarks
		Secondary	Filtration	Disinfection	Advanced							
WA	Class A: Irrigation of nonfood crops (trees, fodder, fiber, seed crops, sod, ornamentals, pasture including dairy), spray irrigated food crops, surface irrigated where water does not contact edible parts, root crops, orchards, vineyards, foods that undergo chemical/physical treatment, landscaping at restricted- access sites (for example, cemeteries, freeways) and open-access sites (for example, school yards, golf courses, parks, residentials), fish hatcheries, flushing, decorative, recreational impoundments, street cleaning, dust control, construction, firefighting, ship ballast, making concrete, boiler feeds, cooling towers, industrial process, direct recharge in potable ground water	√	√	√						Median ≤2.2/100 mL for 7 days and none of the determinations exceeding 23 CFU/100 mL		Undergoes coagulation

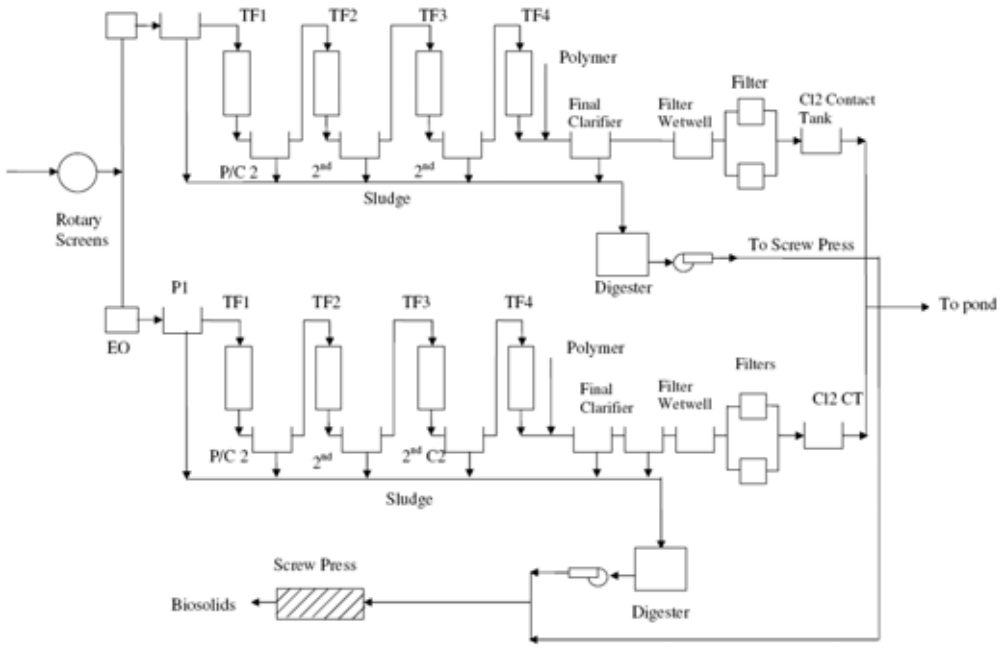
Jurisdiction	Uses	Treatment				pH	BOD (mg/L)	Turbidity (NTU)	TSS (mg/L)	Fecal Coliform/100 mL	Chlorine Residual (mg/L)	Other Remarks
		Secondary	Filtration	Disinfection	Advanced							
	Class B: Irrigation of nonfood crops (trees, fodder, fiber, seed crops, sod, ornamentals, pasture including dairy), spray irrigated food crops, surface irrigated where water does not contact edible parts, orchards, vineyards, foods that undergo chemical/physical treatment, landscaping at restricted- access sites (for example, cemeteries, freeways), fish hatcheries, flushing, street cleaning (except washing), dust control, construction, firefighting (except indoor hydrants and sprinklers), ship ballast, making concrete, boiler feeds, cooling towers (except where aerosols are generated), industrial process (except where workers are exposed)	√		√						Median ≤2.2/100 mL for 7 days and none of the determinations exceeding 23 CFU/100 mL		No coagulation required

Jurisdiction	Uses	Treatment				pH	BOD (mg/L)	Turbidity (NTU)	TSS (mg/L)	Fecal Coliform/100 mL	Chlorine Residual (mg/L)	Other Remarks
		Secondary	Filtration	Disinfection	Advanced							
	Class C: Irrigation of nonfood crops (trees, fodder, fiber, seed crops, sod, ornamentals, pasture including dairy), spray irrigated food crops where physical/chemical treatment destroys pathogens (no root crops) and water does not contact edible parts, landscaping at restricted-access sites (for example, cemeteries, freeways), flushing, street cleaning (except street, lot and sidewalk washing), dust control, construction, firefighting (except indoor hydrants and sprinklers), ship ballast, making concrete, boiler feeds, cooling towers (except where aerosols are generated), industrial process (except where workers are exposed)	√		√						Median ≤23/100 mL for 7 days and none of the determinations exceeding 240 CFU/100 mL		

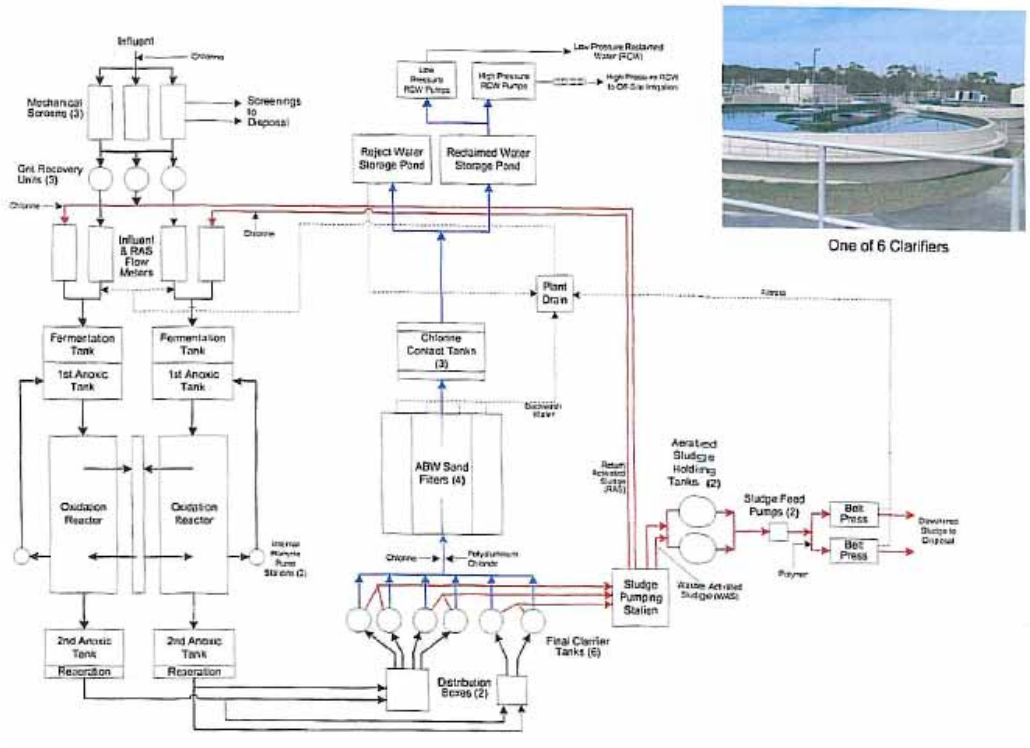
Jurisdiction	Uses	Treatment				pH	BOD (mg/L)	Turbidity (NTU)	TSS (mg/L)	Fecal Coliform/100 mL	Chlorine Residual (mg/L)	Other Remarks
		Secondary	Filtration	Disinfection	Advanced							
	Class D: Irrigation of nonfood crops (trees, fodder, fiber, seed crops, sod, ornamentals, pasture including dairy), spray irrigated food crops where physical/chemical treatment destroys pathogens (no root crops) and water does not contact edible parts, flushing	√		√						Median ≤240/100 mL for 7 days		

[a]Table compiled from Narasimhan et al. (2005)

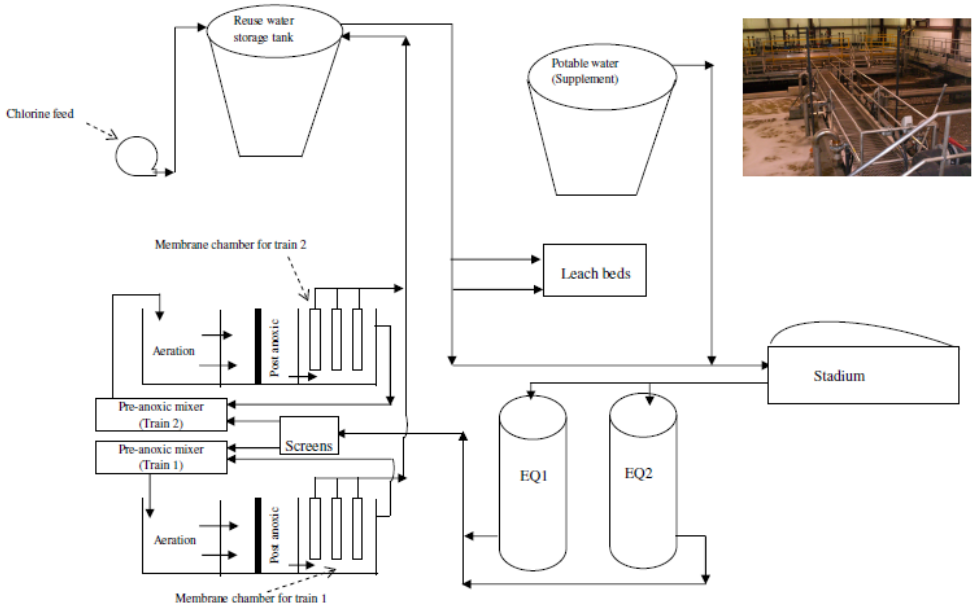
APPENDIX II



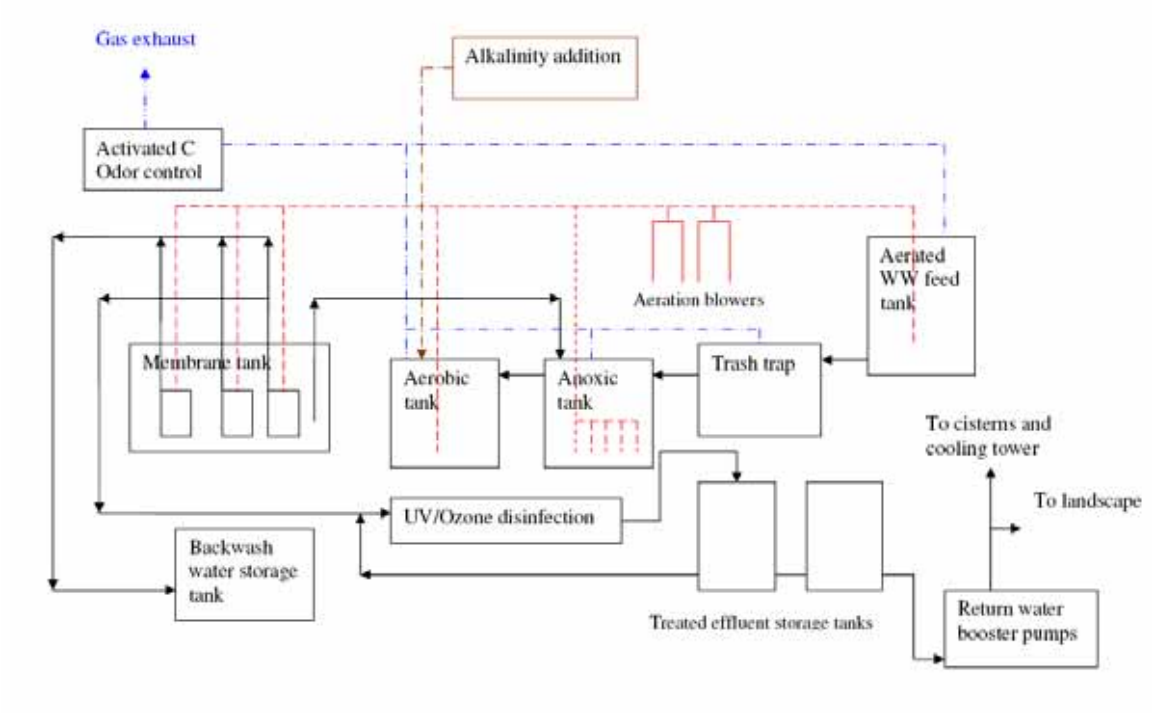
Process flow chart for the CA plant. TF = Trickling filter



Process flow for the FL plant



Process flow for the MA plant. EQ1 and EQ2 are equilibration tanks.



Process flow for the NY plant.

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