LONG-TERM LAB SCALE STUDIES OF SIMULATED RECLAIMED WATER DISTRIBUTION: EFFECTS OF DISINFECTANTS, BIOFILTRATION, TEMPERATURE AND RIG DESIGN

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ABSTRACT

As demand for alternative water sources intensifies, increased use of reclaimed water is important to help achieve water sustainability. In addition to treatment, the manner in which reclaimed water is distributed is a key consideration as it governs the water quality at the point of use.

In this work, simulated reclaimed water distribution systems (SRWDSs) were operated for more than two years to examine the role of system design, biofiltration, residual disinfectant type (i.e., chlorine, chloramine, no residual) and temperature on important aspects of chemistry and microbial regrowth under laboratory-controlled conditions. Turbidity decreased to 0.78 NTU after biofiltration and chlorinated treatments from 10.0-12.6 NTU for conditions with chloramine and no residuals. SRWDSs were susceptible to sediment accumulation, which occupied 0.83-3.2% of the volume of the first pipe segment (1 day of hydraulic residence time), compared to 0.32-0.45% volume in the corresponding chlorinated SRWDSs. The mass of accumulated sediment positively correlated ($R^2 = 0.82$) with influent turbidity.

Contrary to experiences with potable water systems, chlorine was found to be more persistent and better at maintaining biological stability in the SRWDSs than chloramine, especially at the higher temperatures >22°C common to many water scarce regions. The severe nitrification at the warmer temperatures rapidly depleted chloramine residuals, decreased dissolved oxygen, and caused elevated levels of nitrifiers and heterotrophic cell counts. A metagenomic taxonomic survey revealed high levels of gene markers of nitrifiers in the biofilm samples at 22°C for the chloraminated system. Non-metric multidimensional scaling analysis confirmed distinct taxonomic and functional microbial profiles between the chlorine and chloramine SRWDSs.

Reflecting on multiyear experiences operating two different SRWDSs reactor designs, including thin tubes (0.32-cm diameter) and pipe reactors (10.2-cm), illustrated strengths and weaknesses of both approaches in recreating key aspects of biochemical changes in reclaimed water distribution systems. It is clear that approaches deemed successful with drinking water distribution systems may not always directly transfer to simulating reclaimed distribution systems, or to proactively managing full-scale reclaimed systems that have long periods of stagnation and where minimally-treated wastewater with high levels of nutrients and turbidity are used.

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GENERAL AUDIENCE ABSTRACT

Increasing water scarcity is creating an impetus for creating more sustainable water supplies. Wastewater effluent is increasingly viewed as in important resource that can reduce both water and energy demand. Reclaiming moderately- to minimally-treated secondary wastewater effluent for non-potable reuse (NPR) applications; such as agricultural irrigation, landscaping, and toilet flushing, helps reduce demand for higher quality potable water sources. NPR presently accounts for more than 50% of total reuse and is projected to become increasingly important.

While NPR is attractive, important knowledge gaps remain in terms of managing water quality and safety as it is transported through distribution pipes to the point of use. A comprehensive literature review revealed that NPR distribution systems are distinct from conventional drinking water distribution systems (DWDSs) and that it is doubtful if our current understanding of DWDSs would directly transfer to NPR systems. Unlike drinking water systems, NPR systems are currently unregulated at the national level and corresponding state-to-state regulations vary widely. The levels of water treatment can vary from simply distributing untreated effluent from wastewater treatment plants, to very high level treatment with membranes that produces water of equal or even higher quality than many existing tap waters.

A common treatment train for minimally-treated NPR involves biologically activated carbon (BAC) filtration and use of disinfectants (e.g., chlorine or chloramine) to control microbial water quality to the point of use. Prior studies from DWDSs have demonstrated water quality degradation in terms of disinfectant loss, bacterial growth, and aesthetic problems, with settling of trace particulate matter producing sediment within pipe distribution systems. In particular, accumulated sediment can become a hotspot for water quality deterioration. Considering that minimally-treated reclaimed water can have much higher levels of particulate matter and nutrients than drinking water, it was predicted that NPR distribution systems could suffer from faster water quality degradation than corresponding drinking water systems, especially at the warmer temperatures common in water scarce regions. This work was the first multi-year attempt to examine the effects of disinfectant (i.e. free chlorine, chloramine, no residual), BAC filtration versus no filtration, water age (up to 5-d versus 28-min), and temperature (14°C, 22°C, 30°C) in different types of lab-scale reactors.

Two simulated reclaimed water distribution systems (SRWDSs) including 4-in. diameter Pipe SRWDSs versus 1/8-in. diameter Tube SRWDSs, were designed to study

key aspects of full-scale NPR systems and were operated for more than two years to study chemical and microbial changes as distributed water traveled through the two systems. The Pipe SRWDSs were designed to assess the impacts on final water quality after long-term operation that allowed sediment to slowly accumulate, whereas the complementary Tube SRWDS design did not allow sediment to accumulate and only held the water for 28 minutes. Water was sampled regularly to track the trends of key water quality parameters, including disinfectant residuals, dissolved oxygen, nitrogen compounds involved in nitrification reactions, and various types of bacteria of interest. Sequencing of the biological genetic materials on selected samples was conducted to understand the types of bacteria present and their functions under the different circumstances.

High levels of sediment were found to accumulate near the beginning of the Pipe SRWDSs, which caused loss of oxygen and disinfectants at the bottom of the pipes. Chlorine was more persistent and better at preventing bacteria growth as water traveled through the distribution system. In contrast, a type of bacteria that used ammonia as a nutrient (i.e., nitrifying bacteria) were observed in the pipes with chloramine (i.e., ammonia plus chlorine) as the disinfectant. The nitrifying bacteria caused rapid depletion of chloramine residuals, especially at temperatures above 22°C. At 30°C both chlorine and chloramine were almost immediately consumed in the pipe reactors. Nitrification is known to trigger water quality problems in chloraminated DWDSs, and we expect that chloraminated RWDSs would be even more susceptible to nitrification and associated water quality degradation issues in

Compare the Tube SRWDSs to the Pipe SRWDSs, aside from heavy accumulations of sediment in the pipes versus no sediment in the thin tubes, the tubes clogged repeatedly from formation of thick biofoulants in the systems treated with no disinfectant and chloramine, whereas they remained relatively free of biofoulants and clogging in the tubes with chlorine. Even in just 28 minutes, it took water to move from the start to the end of the tube, both chlorine and chloramine were almost completely consumed in the tubes, due to the unrealistically high pipe surface area to the small flow volume inherent to this reactor design.

As NPR becomes increasingly common to help achieve water sustainability, it will be important to deploy laboratory simulations, that are capable of testing and revealing key chemical and microbial processes that affect operation of these systems and water safety at the point of use. The insights from this first long-term efforts of simulating RWDSs highlight some unique characteristics and challenges of RWDSs, and reveals key concepts to help guide future research.

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CHAPTER 1: INTRODUCTION

As climate change and rising population pressure our existing water sources, identifying alternative sources is important to meeting sustainability goals and projected future demand (Council and Press, 2012). Use of reclaimed wastewater effluent as a source is an important approach to reduce demands on conventional potable water supplies. The NRC estimated that around 12 billion gallons per day of municipal wastewater effluent was directly discharged into oceans or estuaries nationwide. Reusing this amount of coastal discharge would potentially argument up to 6% of total water use and 27% of municipal water use in the U.S. (Council and Press, 2012; Kenny et al., 2009). In fact, *de facto* water reuse, the unintentional reuse of wastewater effluent either as a source water for downstream drinking water treatment plants or to be directly used for non-potable purposes such as for agricultural irrigation, has already been a common practice for many water-stressed regions (Angelakis et al., 2018).

In recent years, as the *One Water* concept becomes more recognized and accepted by the public (US Water Alliance, 2016), there has been a shifting paradigm that views wastewater effluent as an important resource to be recovered and reused. Planned reuse of treated wastewater effluent for beneficial application has been rising rapidly (Bixio et al., 2008; ISSUE, 2014). For example, the Inland Empire Utilities Agency in Southern California currently recycles half of the 60000 acre-feet of total wastewater produced every year and is expected to increase the recycled volume to 50000 acre-feet by 2025 (ISSUE, 2014). Along the spectrum of water reuse applications, non-potable reuse (NPR) for agricultural irrigation, toilet flushing, landscaping, accounts for over half of the total reuse volume (Okun, 2000, Parsons et al., 2010). However, since NPR is not intended for direct consumption, there are no regulations on water reuse at the federal level and the State regulations can vary (US Environmental Protection Agency, 2012). Furthermore, most of the current regulations heavily rely on the existing standards established for drinking water treatment.

The most relevant EPA guideline on water reuse recommends the overarching fit-for-purpose principal to match treatment level for specific reuse applications (US Environmental Protection Agency, 2012). The level of treatment can range from reusing raw secondary treatment effluent, to highly treated water quality on par with drinking water. The highest levels of treatment are intended for surface injection and direct potable reuse, whereas reduced levels of treatment are considered acceptable for NPR systems. A key risking concern, is the possible growth of pathogenic bacteria with antibiotic resistance as the water is transported through the pipe distribution system to the point of use. Over the last few decades the analogous health threat from drinking water distribution systems (DWDSs) as manifested by Legionnaires' disease and non-tuberculosis mycobacterial infections have been recognized, researched and at least partly addressed through policies, regulation, enhanced treatments and monitoring. The pipe system is now viewed as a chemical and biological reactor, which changes the health risk as water is transported to the point of use (NRC, 2006).

In the case of minimally treated NPR systems, bacterial cells and particulate matter at relatively high concentration are present in the influent to the pipe system, which can serve as a possible source of nutrients that settle as loose deposits and which

can be remobilized during flow disturbances. A few recent studies have even identified settled biofilm and loose deposits as potential hotspots for water quality problems in DWDSs (Gauthier et al., 1999; Poças et al., 2015; Torvinen et al., 2004), despite very stringent monitoring and multiple barriers to ensure very low levels of particles in water that flows into the distribution system. This is an example of a difference between relatively well-understood DWDSs and minimally treated NPR systems that could alter the persistence of disinfectants and microbial regrowth.

RESEARCH OBJECTIVES

Recognizing the poor existing understanding of water quality degradation in RWDSs, this dissertation seeks to study the dynamics of chemistry and microbiology during water transport through experiments using lab-scale simulated RWDSs. A head-to-head parallel experimental design allowed direct evaluation of key factors of interest including temperature, disinfectant types, biofiltration, water age, flow pattern. A particular focus was the mechanistic processes occurring in these systems, as highlighted by differences between the experimental conditions, that cannot be readily elucidated by sampling of full-scale RWDSs. Specifically, the dissertation aims to address the following objectives:

1. To document the distinct characteristics of NPR systems relative to experiences with drinking water, that could potentially contribute to water quality degradation in the distribution systems;
2. To examine the interplay between water chemistry, water retention time and reactor design, that might shape the microbial communities that inhabit RWDSs;
3. To consider the limitations and strengths of various approaches to studying full-scale RWDSs, through use of simulated lab-scale systems.

DISSERTATION OUTLINE AND ATTRIBUTIONS

Planning and executing this complex project over its more than 3 years of operation, involved more than a dozen graduate and undergraduate students. The results of this dissertation are organized into four chapters, and key intellectual contributions of individuals are explicitly described below.

Chapter 2: A Human Exposome Framework for Guiding Risk Management and Holistic Assessment of Recycled Water Quality

This initial effort to systematically anticipate differences between DWDSs and RWDSs, proposed a holistic “human exposome” risk assessment framework to guide future research, management and treatment strategies for RWDSs. The work highlights the unique characteristics of reclaimed water systems that could make them more susceptible to chemical and microbial water quality degradation during distribution, and calls for

controlled research studies to better understand the factors and processes that could impact public health at the point of use.
This manuscript has been published:

Garner, E., Zhu, N., Strom, L., Edwards, M., and Pruden, A. (2016). A human exposome framework for guiding risk management and holistic assessment of recycled water quality. *Environ. Sci. Water Res. Technol. 2.*

Attributions: Ni Zhu (author of this dissertation) shared co-first authorship with Emily Garner on this manuscript and made an equal intellectual contribution to the work. Garner led authorship of the sections on "ARGs, OPs, and other emerging microbial concerns," whereas I led sections on "Important chemical differences anticipated between recycled water and potable water distribution systems." Co-author Laurel Strom contributed to the discussion on free-living amoebae, review and proofreading. Amy Pruden and Marc Edwards guided the overall planning and structure, and provided extensive revisions and review of the research.

Chapter 3: Sediment and Biofilm Affect Disinfectant Decay Rates During Long-term Operation of Simulated Reclaimed Water Distribution Systems

Chapter 3 reports novel insights gained from operating and sampling the simulated RWDSs for more than 3 years, thereby providing some preliminary answers to questions raised in the landmark literature review in Chapter 2. This work demonstrated that sediment and/or biofoulant accumulations are an important feature of RWDSs, which can control the interplay between chemistry and microbiology. The discovery of the important role of sediment accumulation in RWDSs and the differences between the origins of disinfectant demand for chloramine versus chlorine, reveals a new dimension to our conceptualization of water quality degradation in RWDSs.

This manuscript has been submitted to *Environ. Sci.: Water Res. Technol,* for review.

Attributions: As the first author of this paper, I led the execution of the experimental work reported in the paper throughout the entire duration of the experiment. I was responsible for all the data analysis and visualization, and led the writing of this work. Co-authors of this chapter include Kris Mapili, Haniyyah Majeed, Amy Pruden and Marc A. Edwards. Kris Mapili and Haniyyah Majeed helped with operation, sampling and maintenance of the experimental reactors, and provided input to review of the manuscript. Amy Pruden and Marc A. Edwards provided the original experimental design, assisted with the data interpretation, revision and review of the manuscript.

Chapter 4: Metagenomic Analysis of BAC-filtration, Disinfection and Temperature Effects on Water Quality in Simulated Reclaimed Water Distribution Systems.

This work reports on the insights gained from operating the simulated RWDSs over a designated cycle of temperature phases to investigate seasonal effects. Head-to-head comparison of six controlled simulated RWDSs pipe systems, allowed the individual

impacts of each experimental factor to be assessed including the types of disinfectants, water age, biofiltration, and temperature. The comparison between the simulated chlorine and chloramine RWDSs reveals distinct metagenomic profiles of each system. Simulated chloramine RWDSs was found to be susceptible to severe nitrification and chloramine depletion, especially at elevated temperatures. Chlorine was generally more persistent and better controlled regrowth of heterotrophic bacteria.

This manuscript has been submitted to *Water Research*.

Attributions: I was responsible for executing the experiment and coordinating sampling for the 3^{+} year duration of the work. I coordinated all samples submitted for sequencing, and conducted all data analysis on the water chemistry datasets and metagenomics data analysis. Other co-authors of this manuscript include Sudeshna Ghosh, Laurel Strom, Amy Pruden and Marc A. Edwards. Sudeshna Ghosh coordinated the analysis and initial draft of the metagenomic functional genes analysis, as well as the planning, revision and review of the manuscript. Laurel Strom contributed with experimental operation and sampling, and revised and reviewed the manuscript. Amy Pruden and Marc A. Edwards provided intellectual input on the experimental design and operation, interpretation of the results, revision and review of the work.

Chapter 5: Considering the Role of Scale and Similitude in Laboratory studies of Reclaimed Water Distribution Systems for Non-Potable Reuse

Building upon the experience of conceiving, operating and analyzing results from this multi-year experiment with lab-scale simulated RWDSs, this chapter summarizes the advantages and limitations of four different simulated RWDS design scenarios. While it is not possible to ever achieve complete similitude to full-scale RWDSs, selecting an appropriate simulation can nonetheless isolate key variables and biological/chemical processes, or examine extremes that affect full-scale RWDSs. For instance, the use of both thin tubes with very short detention times, prevents sediment from accumulating while exacerbating the impact of biofilm, whereas the converse is true for larger diameter pipe systems. This analysis provides a useful starting point for conceiving future experiments that delve into phenomena occurring in full-scale RWDSs.

This manuscript is in preparation and will be submitted to *Water Research*.

Attributions: I led the operation of the two experiments, sample analysis, and prepared the initial draft of the manuscript. Amy Pruden and Marc A. Edwards assisted with the conceptual development of the manuscript, revision and review of the work.

Chapter 6: Conclusion and Recommendations for Future Work

This chapter provides a summary of key conclusions for each chapter and proposes some additional future research as an outgrowth of this dissertation.

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CHAPTER 2: A HUMAN EXPOSOME FRAMEWORK FOR GUIDING RISK MANAGEMENT AND HOLISTIC ASSESSMENT OF RECYCLED WATER QUALITY

Emily Garner[†], Ni Zhu[†], Laurel Strom, Marc Edwards, Amy Pruden

[†]These authors contributed equally to this work

ABSTRACT

Challenges associated with water scarcity and increasing water demand are leading many cities around the globe to consider water reuse as a step towards water sustainability. Recycled water may be used in a spectrum of applications, from irrigation or industrial use to direct potable reuse, and thus presents a challenge to regulators as not all applications require the same level of treatment. We propose that traditional drinking water standards identifying "safe" water quality are insufficient for recycled water and that using the "human exposome" as a framework to guide development of a risk management strategy offers a holistic means by which to base decisions impacting water quality. A successful and comprehensive plan for water reuse must consider 1) health impacts associated with both acute and chronic exposures, 2) all routes of exposure by which individuals may encounter recycled water, and 3) water quality at the true point of use after storage and transport through pipe networks, rather than at the point of treatment. Based on these principles we explore key chemical differences between recycled and traditional potable water, implications for distribution systems with respect to design and operation, occurrence of chronic contaminants, and the presence of emerging and often underappreciated microbial contaminants. The unique nature of recycled water has the potential to provide rapid regrowth conditions for certain microbial contaminants in these systems, which must be considered to achieve safe water quality at the point of use.

2.1 INTRODUCTION

Water reuse is essential for satisfying domestic and industrial water demand worldwide and achieving water sustainability (Anderson, 2003). Domestic wastewater can be treated to the necessary level of quality and reused to reduce loss of treated effluent via discharge, relieve pressures on depleting groundwater aquifers, and minimize extraction of water from fragile environments such as drought-stricken surface waters. Treatment of wastewater for reuse is also cost effective compared to alternative approaches, such as obtaining freshwater from desalination (Goodwin et al., 2015). Particularly when wastewater is treated for direct or indirect potable reuse, a "multi-barrier" treatment framework is typically used to ensure that multiple means of removing pathogens or harmful chemicals will protect public health in the case of a process failure or other unexpected event that could compromise water quality (Gerrity et al., 2013).

However, while this approach is logical for controlling acute health threats associated with water as it leaves the treatment facility, it does not address concerns with respect to low-level chronic exposures or changes in water quality during distribution to the point of use (Jjemba et al., 2014).

A major concern for water reuse in general is the lack of federal regulations (DiGiano and Aitken, 2010). Further, the few nascent recycled water quality regulations and guidelines available, typically at the state and local level, have been narrowly focused on fecal indicator bacteria (i.e., total and fecal coliforms) (Lahnsteiner and Lempert, 2007; National Water Quality Management, 2006; US Environmental Protection Agency, 2012a). This approach addresses traditional concerns regarding fecal-associated pathogens, but does not necessarily provide insight into safeguarding microbial water quality during distribution (Agulló-Barceló et al., 2013; Harwood et al., 2005). In particular, microbial contaminants that are of concern due to their ability to grow within distribution systems, such as opportunistic pathogens (OP), antibiotic resistant bacteria and their associated antibiotic resistance genes (ARG), and free-living amoebae (FLA), have little or no relationship with fecal indicator bacteria based standards. Alternative frameworks for assessing and managing recycled water quality more holistically are emerging. For example, adaptations of the Hazard Analysis and Critical Control Point (HACCP) (Swierc et al., 2005) paradigm, which originated in the food safety industry, and the World Health Organization's (WHO) Water Safety Plan (WSP) (Goodwin et al., 2015; WHO and IWA, 2009) have been proposed for use as risk management frameworks for recycled water. Application of these adaptable frameworks could have the advantage of drawing attention to the entire treatment process, rather than focusing solely on absence of indicator organisms as a proxy for safe water. Still, we identify three key elements that should be taken into account for a truly comprehensive consideration of public health concerns: 1) evaluation of health impacts associated with both acute and chronic exposures; 2) accounting for all routes of exposure by which individuals may encounter contaminants in recycled water; and 3) consideration of water quality at the true point of use after storage and transport through pipe networks, rather than at the point of treatment.

A more holistic approach to characterizing the physical, chemical, and microbial characteristics of recycled water, as well as the routes by which humans are exposed, can be derived from the emerging concept of the "human exposome." The exposome has been defined as "the cumulative measure of environmental influences and associated biological responses throughout the lifespan, including exposures from the environment, diet, behavior, and endogenous processes." (Miller and Jones, 2014). The exposome includes general (e.g., climate, urban environment), specific (e.g., water, food, air), and internal (e.g., metabolism, gut/lung microbes) factors and their role in disease (Wild, 2005). Clearly, water, and its corresponding chemical and microbial properties is a fundamental component of the exposome. Water is fundamental to human health, survival, and hygiene and is an integral part of daily life, including direct contact (i.e., drinking, cooking, and showering) and indirect exposures via bioaerosols (i.e., cooling water, flush toilets, or lawn irrigation). We propose that adopting the exposome paradigm as a model for recycled water quality assessment can be used to guide development of an HACCP, WSP, or other comprehensive risk management strategy that more accurately

reflects the true risks and exposures associated with water reuse and can strengthen implementation of existing risk management strategies.

In terms of safeguarding recycled water from the point of treatment to the point of use, we note that there are important distinctions between potable and recycled water that should not be ignored, particularly with respect to design, operation, and maintenance of recycled water distribution systems (RWDS). In this critical review, we note key chemical differences between recycled and potable waters, with a particular emphasis on organic matter, and explore implications for RWDSs with respect to design, operation, and intended application. We also discuss how these key differences impact the presence of chronic contaminants and emerging concerns about the presence of OPs, ARGs, and other microbial contaminants (**Error! Not a valid bookmark self-reference.**) . Our goal is to proactively address plausible public health risks associated with practical realities of recycled water use.

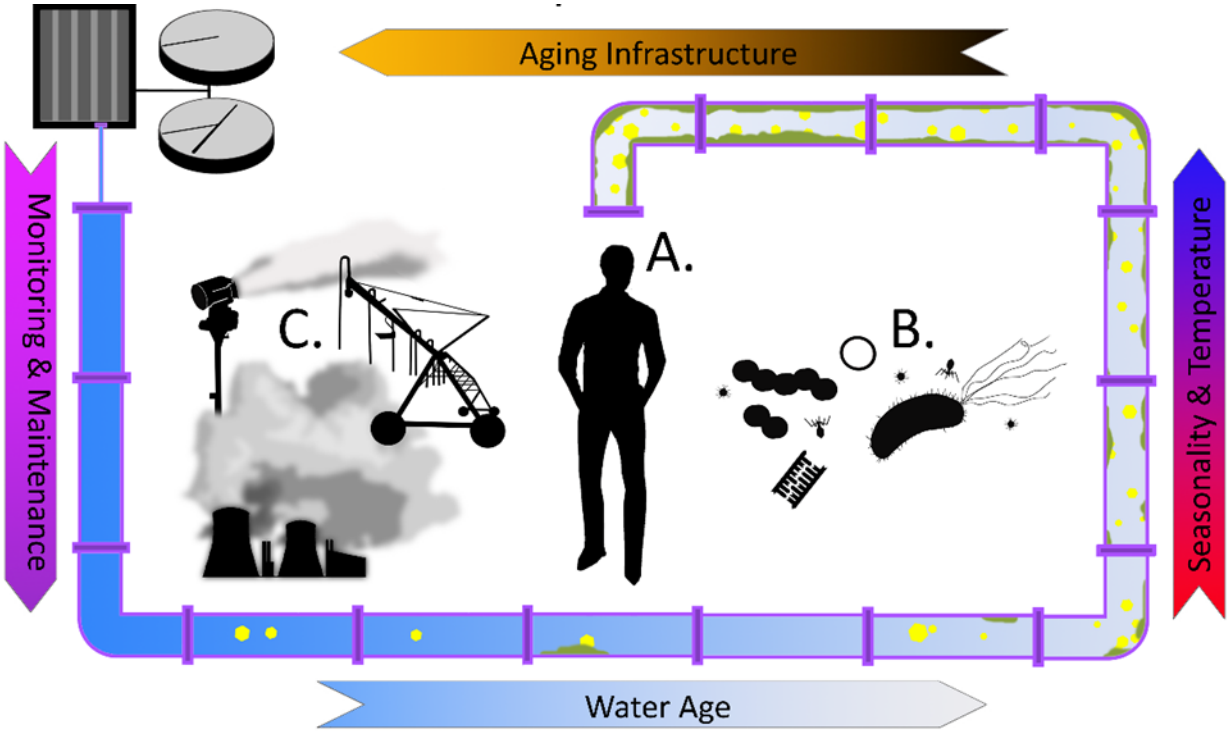

Figure 2-1 Key aspects of the exposome paradigm for managing RWDS emphasize holistic consideration of potential exposures to recycled water, including A) chemical distinctions of recycled vs traditional potable water, such as enriched organic matter/nutrients, disinfectant decay, critical reactive zones and chronic contaminants; B) emerging conerns about ARGs, OPs and other microbial contaminants; C) nontraditional routes of exposure, including inhalation, dermal contact.

2.2 UNIQUE ASPECT OF RWDS DESIGN, OPERATION, AND WATER USE

There is a broad continuum of applications for water reuse, ranging from unintended *de facto* reuse to direct potable reuse (DPR) produced by advanced treatment

processes. *De facto* reuse refers to a situation where reuse of treated wastewater occurs but is not planned, for example, when a drinking water treatment plant intake is located downstream from a wastewater discharge (US Environmental Protection Agency, 2012b). In the U.S., *de facto* reuse is widespread and becoming increasingly common in recent decades. Rice *et al.* found that of the top 25 drinking water treatment plants most impacted by upstream wastewater treatment plant (WWTP) discharges in the United States, the fraction of their water source comprised of WWTP discharges increased from between 2 to 16% in 1980 to an average of 68% under typical streamflow conditions in 2008 (Rice et al., 2013). Some treatment plants received as much as 100% WWTP effluent under low flow conditions. Indirect potable reuse refers to the use of treated wastewater to augment other potable source waters following retention in an environmental buffer (US Environmental Protection Agency, 2012b). Common environmental buffers include groundwater aquifer recharge and subsequent withdrawal prior to drinking water treatment or intentional discharge of wastewater effluent upstream or into a reservoir from which water is withdrawn for drinking water treatment. DPR consists of treating wastewater for direct use as a source water for drinking water treatment. DPR is currently limited in full scale application, with Windhoek, Namibia (Lafforgue and Lenouvel, 2015) and Big Spring, Texas, USA (Sutherland et al., 2015) serving as prime examples, but there is growing interest in expanding DPR infrastructure in the U.S.

Wastewater may also be treated for non-potable reuse when it can offset water demand associated with landscape or recreation area irrigation, agricultural and food crop irrigation, snowmaking, industrial use such as in cooling towers or in natural gas production, or to augment environmental waters such as in groundwater aquifer recharge, river or stream flow augmentation, or in wetlands (US Environmental Protection Agency, 2012b). Though treatment requirements may reasonably be lower in these cases, all of these non-potable reuse scenarios have relevant human exposures that should be taken into consideration.

Table 2-1illustrates that there is a wide range of observed recycled water quality characteristics as a function of increasing levels of treatment. Unlike drinking water, where consistent regulations are applied, recycled water is used for a wide spectrum of applications with required treatment varying based on intended use. For example, the most stringent requirements are applied to DPR, whereas residual nutrients can be viewed as a beneficial fertilizer in non-potable reuse scenarios. Hence, efficient reuse treatments ideally match the intended purpose of the recycled water. A number of U.S. states pioneering water reuse have recognized this concept of “fit-for-purpose,” tailoring recycled water treatment regulatory guidelines based on end uses. Determining the ideal configuration of treatment processes for different reuse scenarios would greatly benefit from research integrating water treatment outcomes with the exposome paradigm for more comprehensively considering chemical and microbial risks.

Table 2-1 Water quality as a function of treatment processes

Water quality parameter (units)	**Treatment processes**								
	CAS effluent	CAS with filtration	CAS with BNR	CAS with BNR and filtration	MBR	Filtration with chlorine	CAS with filtration and chlorine	MBR with chlorine	MBR UV/ ozone
Turbidity (NTU)	2-15	0.5-4	2-8	0.3-2	≤1	1.5-8.7	3.6-6.3	1.7-4.3	0.2-0.5
Total suspended solids (mg/L)	5-25	2-8	5-20	1-4	<2				
TOC (mg-C/L)	10-40	8-30	8-20	1-5	0.5-5	12-16	6-8	3-5	2-3
BDOC (mg-C/L)						5-7	1-2	1-2	<1
AOC (mg-C/L)					0.2-1.4	1-2	1-2	1-2	<1
Total nitrogen (mg-N/L)	15-35	15-35	3-8	2-5	<10	5-10	<1	1-3	5-6
Total phosphorus (mg-P/L)	4-10	4-8	1-2	≤2	<0.3-5	3-5	1-2	5-9	<1
Volatile organic compounds (µg/L)	10-40	10-40	10-20	10-20	10-20				
Total coliforms (CFU/100 mL)	10^4-10^5	10^3-10^5	10^4-10^5	10^4-10^5	<100	<1	1-10	1-10	1-10
Protozoa and cysts (CFU/100 mL)	10-10^2	0-10	0-10	0-1	0-1	1-10	10-100	<1	≤1
Viruses (CFU/100 mL)	10-10^3	10-10^3	10^1-10^3	10-10^3	1-10^3	present	present	present	negative
Source (Asano, 2016; Jjemba et al., 2010a)									
CAS – conventional activated sludge; BNR – biological nutrient removal; MBR – membrane bioreactor									

2.2.1 Routes of Exposure

Stemming from the continuum of recycled water uses described above, there is also a range of relevant exposure scenarios (Table 2-1). Use of a traditional water quality paradigm based on monitoring of fecal indicator bacteria as a sole benchmark for establishing safe water quality neglects risk associated with non-fecal pathogens and routes of exposure other than ingestion. Though ingestion and aspiration are potential modes of exposure associated with DPR, inhalation and dermal contact are important, yet often overlooked, exposure routes for potable water and even more so for recycled water. Recycled water is commonly used for purposes that generate inhalable aerosols, including use in cooling towers (Addiss et al., 1989), spray irrigation (Brissaud et al., 2008; Rosenberg Goldstein et al., 2014a), fire-fighting (Borboudaki et al., 2005), toilet flushing (Gerba et al., 1975), as well as for aesthetic purposes, such as in decorative fountains (Haupt et al., 2012; Hlady et al., 1993; Palmore et al., 2009). Importantly, relevant exposure zones may be vast, with Legionnaire's disease infection associated with inhalation of aerosols from cooling towers located more than a mile away (Addiss et al., 1989). There is also a strong likelihood of dermal contact when recycled water is used for irrigation in public recreation areas. This may be particularly relevant in irrigated parks, athletic fields, and snowmaking for recreational purposes. Dermal abrasions or other lacerations that may be pre-existing or occur during use of these facilities create an additional route of exposure for infection. When recycled water is used for food crop irrigation, chemical and microbial constituents may also be transmitted to humans on or within crops via ingestion (Rose and Clark, 1986). When recycled water is used for DPR, a number of often overlooked routes of exposure should also be considered, for example, including use in humidifiers, ice machines, and decorative water features. A more holistic characterization of human exposures to recycled water constituents via such non-conventional routes is important for accurate assessment of health risk associated with use of recycled water.

2.2.2 Physical and Operational Issues

Water and wastewater infrastructure degradation has become one of the leading threats to public health and water security (ASCE, 2013). Aging and poorly managed pipes can lead to a drastic decrease in water quality of the transported water, arising from complex interactions among chemical, physical, and microbial constituents. New design considerations are needed to ensure sustainable management of recycled water infrastructure, considerate of their distinct physical and operational characteristics relative to potable water systems.

In a recent survey of 71 recycled water systems in the US and Australia, Jjemba *et al.* (2014) identified infrastructure issues as the most prevalent problem associated with managing and maintaining water quality in RWDSs. Over 20% of recycled water facilities listed infrastructure integrity as a water quality concern. The extensive list of infrastructure challenges revealed by the survey includes infrastructure deterioration from high chlorine residual, maintenance of desired pressure and flow during low and inconsistent usage, lack of redundant design and storage, complicated branched distribution systems designed to supply multiple recycled applications from a centralized treatment plant, high corrosivity of water damaging metal pipes, and effective monitoring

of the chemical and microbial quality (Jjemba et al., 2014). For example, to control microbial activity resulting from nutrient-rich recycled water, up to 40 mg/L chlorine has been used in some systems, which can potentially result in widespread damage to water infrastructure (Wang et al., 2012a). Use of reservoirs as a way to satisfy on-demand recycled water applications has also been observed to be a challenge, with impaired water quality resulting from long stagnation time and proliferation of algae and aquatic vegetation (Jjemba et al., 2010a). Experience from potable water systems has also shown that interaction of iron pipes with water containing high organic content and oxygen tends to promote iron release, producing unacceptable discolored water following stagnation.(Sarin et al., 2004) It is important to bear in mind that a shift in water chemistry can have disastrous unintended consequences for corrosion (MACTOR, 2013; Mlive, 2016.), and given the unique chemistry of recycled water (Table 2-2), it will be especially critical to bear this in mind. For example, previous studies have documented cases where switching potable water pipes to DPR pipes resulted in destabilization of the of existing corrosion scales and biofilms and an undesirable degradation of water quality at the point of use (Yang et al., 2014). Despite the unresolved challenges associated with transporting recycled water, this alternative type of water also presents a creative opportunity for solving the challenging issue of leaking pipes. Tang *et al*. have successfully demonstrated the autogenous repair phenomenon in copper and iron pipes in drinking water distribution systems (DWDS) via beneficial corrosion deposition (Tang et al., 2015). Optimistically, with a more diverse water chemistry profile, recycled water may be an even better candidate for protecting aging pipes.

Table 2-2 Overview of non-traditional routes of exposure for recycled water and putative risk of infection or exposure

Route of Exposure	**Recycled Water Application (in addition to other potable uses)**	**Putative Hazard**	**Infectious Dose**	**Documented Concentrations in Recycled Water**	**Documented Concentrations in Drinking Water**
				[Percent Samples positive (positive concentration range)]	
Dermal Contact	Snowmaking, Irrigation of athletic and recreation facilities, Toilet flushing (Gerba et al., 1975)	<u>Opportunistic pathogen infection of wounds</u>			
		Staphylococcusaureus (Lowy, 1998)		17% (Rosenberg Goldstein et al., 2012)	6.25% (LeChevallier and Seidler, 1980)
		Pseudomonasaeruginosa (Bodey et al., 1983)	10^8 - 10^9 colony forming units (CFU)[a;] (Rusin et al., 1997)	88% (1±1 - 9±10 CFU/100 mL)[b;] (Jjemba et al., 2010a)	5.6% (up to 700.3±158.7 gene copies / mL) (Wang et al., 2012a)
		Acanthamoeba spp. (Martinez and Visvesvara,	10^4 trophozoi-tes[c;]		Not detected (Wang et al., 2012a)

		1997)	(Xianmin et al., 2015)		
		Cyanobacteria toxicity (Codd et al., 1999)		up to ~140 µg/mL chlorophyll (Jjemba et al., 2010b)	
		Antibiotic resistant infections (Macmillian, 2012)		8% for MRSA of 17 % for susceptible *S. aureus*[d;] (Rosenberg Goldstein et al., 2012)	
Inhalation and Aspiration	Cooling towers, Spray irrigation, Toilet flushing (Gerba et al., 1975), Fire suppress-ion (Borboudaki et al., 2005), Car washing (Wu et al., 2013)	Opportunistic pathogen infection of lungs			
		Legionellapneum ophila[e ;](Brissaud et al., 2008)	10^3 - 10^6 CFU (Armstrong and Haas, 2007)	81% ($0.4x10^3$±0. $2x10^3$ - $3.5x10^3$± $16x10^3$CFU /100 mL)[f;](Jjemba et al., 2010a)	5.6% (up to 219.4±23.8 gene copies / mL) (Wang et al., 2012a)
		Mycobacterium spp. (Primm et al., 2004)	10^4 - 10^7 CFU[ag;] (Rusin et al., 1997)		98.1% (2.1±104 - 4.2±103 gene copies / mL) (Wang et al., 2012a)
		Pseudomonas aeruginosa (Williams et al., 2010)	10^8 - 10^9 CFU[a;] (Rusin et al., 1997)	88% (1±1 - 9±10 CFU/100 mL)[b;](Jjemba et al., 2010a)	5.6% (up to 700.3±158.7 gene copies / mL) (Wang et al., 2012a)
		Staphylococcus aureus (Rosenberg Goldstein et al., 2014a)		17% (Rosenberg Goldstein et al., 2012)	6.25% (LeChevallier and Seidler, 1980)
		Naegleria fowleri (Bartrand et al., 2014; Martinez and Visvesvara, 1997)	10^3 -10^5 trophozoi-tes (Bartrand et al., 2014; LEE et al.,		8-27% (Bartrand et al., 2014)

		2011)			
		Cyanobacteria toxicity (Codd et al., 1999)		up to ~140 µg/mL chlorophyll (Jjemba et al., 2010b)	
		Antibiotic resistant infections (Rosenberg Goldstein et al., 2014a)		8% for MRSA of 17 % for susceptible *S. aureus*[d] (Rosenberg Goldstein et al., 2012)	
		Fecal pathogens			
		Disinfection byproducts (Wang et al., 2013)		9.70 - 399.37 µg TTHM[h]/L (Wang et al., 2013))	
Nasal Aspiration	Recreation, Potable reuse (sinus irrigation)	Opportunistic pathogen infection			
		Naegleria fowleri (Bartrand et al., 2014)			8-27% (Bartrand et al., 2014)
Eye and Ear Contact	Recreation, Direct potable reuse, Indirect potable reuse	Opportunistic pathogen infection			
		Acanthamoeba keratitis (Martinez and Visvesvara, 1997)	10^4 trophozoi-tes (Xianmin et al., 2015)		
		Pseudomonas aeruginosa (Bodey et al., 1983)	10^8 - 10^9 CFU[a;] (Rusin et al., 1997)	88% (1±1 - 9±10 CFU/100 mL)[b;](Jjemba et al., 2010a)	5.6% (up to 700.3±158.7 gene copies/ mL) (Wang et al., 2012a)
Colonization and Delayed Infection	Various	Antibiotic resistant infections (Bogaert et al., 2004; Davis et al., 2004; von Eiff et al., 2001)		8% for MRSA of 17 % for susceptible *S. aureus*[d] (Rosenberg Goldstein et al., 2012)	
		Opportunistic pathogen infection			
		Pseudomonas aeruginosa	10^8 - 10^9 CFU[a;]	88% (1±1 - 9±10	

	(Bodey et al., 1983)	(Rusin et al., 1997)	CFU/100 mL)[b] (Jjemba et al., 2010a)	
	Staphylococcus aureus (Lowy, 1998)		17% (Rosenberg Goldstein et al., 2012)	6.25% (LeChevallier and Seidler, 1980)

[a]Oral route of infection
[b]Based on *Pseudomonas spp.*
[c]Based on *Acanthamoeba keratitis*
[d]MRSA = Methicillin resistant *Staphylococcus aureus*
[e]Putative hazards consider both *Legionella pneumophila* and other pathogenic *Legionella*
[f]Based on detection of *Legionella* spp.
[g]Based on *Mycobacterium avium*
[h]TTHM = Total trihalomethanes

Temperature, as an overarching parameter, is another critical factor that could have profound implications in designing and monitoring water reuse systems (Storey and Kaucner, 2009). Not only is temperature directly related to microbial activity, disinfectant residual decay, corrosion rate, and dissolved oxygen levels, it is also indirectly linked to consumption patterns, flow patterns and velocity, and bulk water and biofilm interactions. Elevated recycled water temperatures may stem from extended stagnation times, particularly during the day in cases where irrigation is conducted at night to limit evaporation, as well as from use of above-ground pipelines, which facilitate transport of recycled water over long distances.

For on-demand non-potable water reuse applications, such as agricultural irrigation, landscaping, and toilet flushing, many studies have observed distinct consumption variations in daily and seasonal demand patterns (Rufenacht and Guibentif, 1997; Willis et al., 2011). For example, on a daily scale, the generation of wastewater effluent usually peaks in the daytime when people are active, but the demand of irrigation water usually occurs at night with an offset time of approximately 12 hours. Discrepancies in user patterns makes water stagnation and storage, along with associated water quality deterioration, a prominent concern in design and maintenance of recycled systems. Multiple studies have also documented water quality deterioration during winter or high rainfall periods in systems largely used for irrigation, due to low user frequency (Nawrocki et al., 2010).

True water age may differ substantially from the designed hydraulic residence time of the recycled water systems based on the actual end-use applications. Emerging work in premise (i.e., building) plumbing systems, i.e. the water pipe networks within homes and buildings, has highlighted unique systematic features in terms of longer stagnation time, elevated temperature, and loss of disinfectant residual, which serve to stimulate microbial proliferation precisely at the point of use, thus amplifying any potential exposure risk to end users (Ji et al., 2015a; Rhoads et al., 2016). Similar investigations are needed to quantify the risk of exposure associated with user-driven demand patterns in non-potable reuse systems.

2.3 IMPORTANT CHEMICAL DIFFERENCES ANTICIPATED BETWEEN RECYCLED AND POTABLE WATER DISTRIBUTION SYSTEMS

2.3.1 Organic Matter

One of the most distinctive characteristics of recycled water is the nature of dissolved organic matter (DOM) and its occurrence at elevated levels. The organic matter in typical potable waters consists of natural organic matter (NOM) derived mostly from oil, planktonic and vegetative matter, and decay by-products in natural water sources. However, it is important to note that DOM present in recycled waters may be quite distinct from that of potable water due to different sources and treatment processes. In a recent review comparing organic matter data published in the last 15 years for drinking water and recycled water systems, Hu *et al*. identified four distinct classes of NOM in recycled water: recalcitrant DOM, soluble microbial products from biological wastewater treatment units, transformation products from advanced treatment, and emerging contaminants associated with anthropogenic activities (Hu et al., 2016). It was concluded that DOM composition differed significantly between recycled water and drinking water evaluated against five critical chemical indicators: dissolved organic carbon (DOC), dissolved organic nitrogen, assimilable organic carbon (AOC), estrogenic activity, and disinfection byproduct (DBP) formation potential (Figure 2-2). DOC in drinking water ranged from 1.5-11.2 mg/L with a median of 3.9 mg/L while that in recycled water ranged from 3.6-14.6 mg/L with a median of 7.5 mg/L, indicating recycled water as a much more nutrient rich environment for microbial regrowth and byproduct formation. The heightened levels of biotoxicity, in terms of estrogenic levels, is also widely reported in studies examining effluent organic matter compositions, suggesting a potential health risk when used for recycling applications (Shon et al., 2006)

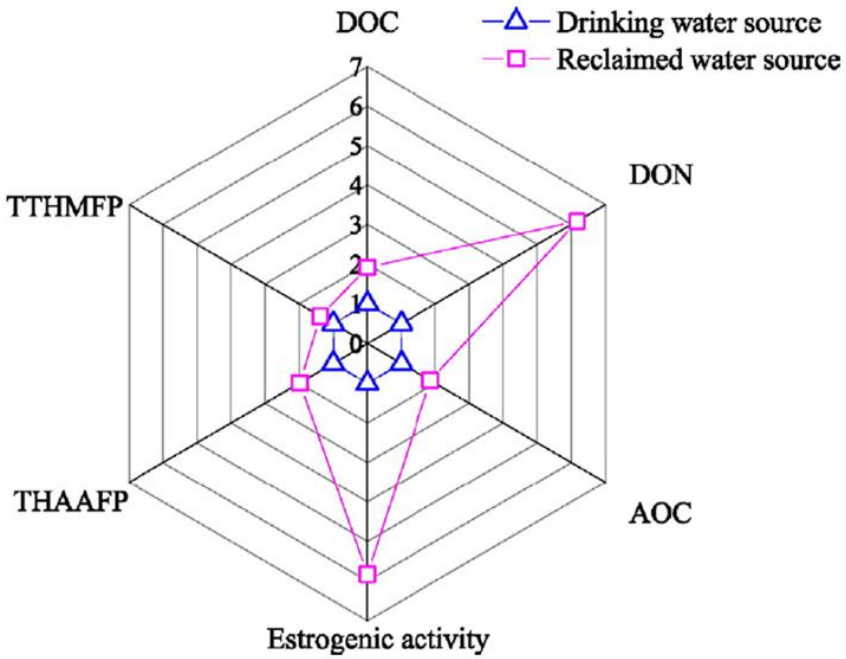

Figure 2-2 Overview of typical normalized composition and potential magnitude of dissolved organic matter (DOM) in drinking water sources compared to recycled water sources, presented in terms of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), assimilable organic carbon (AOC), estrogenic activity, total haloacetic acid formation potential (THAAFP), and total trihalomethane formation potential (TTHMFP). (Reprinted from *Science of The Total Environment*, 551-552, Hong-Ying Hu, Ye Du, Qian-Yuan Wu, Xin Zhao, Xin Tang, Zhuo Chen, Differences in dissolved organic matter between reclaimed water source and drinking water source, page 133-142, 2016, with permission from Elsevier.)

Biological stability, i.e., the ability of drinking water to suppress microbial growth in the absence of disinfectants (Rittman and Snoeyink, 1984), is especially of concern for safe transport and storage of treated water. Ideally, low nutrient water will limit growth in the distribution system, a strategy applied successfully in some European countries for eliminating the need for secondary disinfectant in DWDSs (van der Wielen and van der Kooij, 2010). The proportion of DOC that facilitates bacterial regrowth is typically measured by either biodegradable dissolved organic carbon (BDOC) or AOC assays. An array of methods has been used to best evaluate the bacterial growth potential of various types of water samples, with established approaches generally being to measure the decrease in measured DOC over time or an increase in indicator bacteria counts as a proxy for biologically available DOC. To date, there is no widely accepted standardized method to quantify biostability. Reported threshold BDOC and AOC values to achieve biostability in drinking water systems using different methods are documented in Table 2-3. Existing surveys of recycled water systems have indicated orders of magnitude higher levels of organic carbon than in typical U.S. drinking water systems (Thayanukul et al., 2013; Weinrich et al., 2010). In particular, biodegradable organic matter has been

observed to be four or five times higher than that of drinking water (Jjemba et al., 2010a), while AOC can range from 505 to 918 µg/L in moderately treated recycled water (Karim and LeChevallier, 2005), compared to 18 to 189 µg/L in drinking water (LeChevallier et al., 1996)

Table 2-3 Proposed threshold values to achieve biostability in drinking water distribution systems

Carbon source	Threshold values	Criteria	Reference
BDOC	≤ 0.15 mg-C/L	Stable BDOC values	(Volk et al., 1994; Servais et al., 1995; Laurent et al., 1999)
	≤ 0.25 mg-C/L		(Niquette et al., 2001)
	≤ 0.30 mg-C/L at 15°C		(Volk et al., 1994)
	≤ 0.15 mg-C/L	No coliform growth	(Volk and LeChevallier, 2000)
AOC	10 µg-C/L	No heterotrophic plate count growth	(van der Kooij et al., 1982)
	50 µg-C /L	No coliform growth	(LeChevallier et al., 1991)
	50 µg-C /L	No *V. cholerae* growth	(Vital et al., 2007)
	100 µg-C /L	No *E. Coli* growth	(LeChevallier et al., 1996)

Table 2-4 Comparing water quality of typical drinking water versus different recycled water applications

Parameter		Drinking Water	Recycled water applications				Implications for Distribution
			Private, urban and irrigation	Direct Environ-mental reuse	Indirect potable recharge	Industrial applications	
pH		6.5-8.5	6-9	6-9	7-9	7-8.5	
Carbon source	**Total dissolved solids (mg/L)**	500					-Provides the most limiting nutrient source for bacterial regrowth in distribution systems; -Consumption of carbons in the distribution system is observed to relate with increased bacterial activity (Jjemba et al., 2010a; Ryu et al., 2005)
	Chemical oxygen demand (mg/L)		100	70-100	70-100	70	
Biochemical oxygen demand (mg/L)			10-20	10-20		10	
DO (mg/L)		Near saturation	>0.5	>3	>8	>3	
Total suspended solids (mg/L)		N/A	10	10		10	
Chlorine residual (mg/L)		<4	0.2-1.0	0.05		0.05	-Control bacterial growth in the distribution system; -Excess chlorine can cause carbon fragmentation and DBPs formation -Chlorine may exacerbate antibiotic resistance (Huang et al., 2013a; Shi et al., 2013a)
Total Kjeldahl N (mg/L)		<10	15-20	10-20		10	-Concerns for nitrification and denitrification
Ammonia-N (mg/L)		< 0.2 [a]	2-20	1.5	0.2	1.5	-Concern for nitrification
Total phosphorus (mg/L)			2-5	0.2		0.2	-Eutrophication and degradation of

						water quality
Iron (mg/L)	0.3	2	2			-Caused “red water” -Promote growth of corrosion bacteria and damage pipe integrity -May select for antibiotic resistant bacteria (Knapp et al., 2011)
Copper (mg/L)	1.0	0.2-1.0	0.2-1.0			-promote growth of certain corrosion bacteria -toxic to certain bacterial and aquatic species at elevated levels -May select for antibiotic resistant bacteria (Baker-Austin et al., 2006; Mohammad Rusan et al., 2007)
Zinc (mg/L)	5	0.5-2	0.5-2			-May select for antibiotic resistant bacteria (Baker-Austin et al., 2006; Mohammad Rusan et al., 2007)
Pesticide (mg/L)		0.05	0.05			-May select for antibiotic resistant bacteria (Kurenbach et al., 2015)
Fecal coliforms (CFU/100 mL)	Zero	Zero	< 200[b]	Zero[c]	<200[a]	Indicator bacteria for pathogenicity of water

(Source: (Edzwald, 2011; Salgot et al., 2006; US Environmental Protection Agency, 2012b))

[a]WHO guidelines for drinking water quality

[b]Based on 7-day median with none > 800 per 100 mL

[c]Based on 7-day median with non > 14 per 100 mL

The abundance and type of biodegradable carbon in recycled water calls into question the extent to which the science of potable water delivery is directly translatable to recycled water distribution. In the only available study of its kind, Jjemba *et al*. examined four RWDSs in the U.S. and observed a trend of AOC and BDOC consumption with increasing residence time, with an average reduction of 475 µg/L AOC and 370 µg/L BDOC from the distribution system point of entry to the point of use (Jjemba et al., 2010a). They concluded that the change in AOC and BDOC was due to enhanced microbial activity, indicating significant changes in both the quantity and the quality of the available carbon in the RWDSs. In parallel simulated RWDS loop studies, high organic carbon was also observed to result in rapid consumption of disinfectant residuals in the distribution systems (Jjemba et al., 2010a). Up to 6 mg/L of chlorine was completely consumed within minutes in all systems, leaving the remainder of the distribution system vulnerable to bacterial growth (Jjemba et al., 2010a).

Given the unique nature of organic matter and microbial composition of recycled water, existing assays such as those for AOC or BDOC, may not be suitable. Only one study could be found specifically aimed at adapting the AOC assay to recycled water (Zhao et al., 2013). By including test strains that are more ecologically representative of the sample waters, Zhao *et al*. concluded that the standard P17 and NOX strains applied in the AOC assay largely underestimate levels in recycled water (Zhao et al., 2013). Khan *et al.* (1998) have similarly highlighted the need to optimize the BDOC method for recycled water with their modified protocol improving repeatability and precision of results as verified by independent biochemical oxygen demand and chemical oxygen demand measurements.

Another negative consequence of NOM in distributed water is that it can accelerate biocorrosion of pipes, which in turn can further stimulate AOC generation (Edwards and Sprague, 2001; McNeill et al., 2001; Nawrocki et al., 2010). BDOC is also believed to play an important role in microbiologically induced corrosion (Camper, 2004a; LeChevallier et al., 1993). Recycled water, as an abundant source of sulfate and nitrogen species, is likely to provide a nutrient-rich environment for iron-oxidizing/reducing bacteria (Wang et al., 2014; Haibo Wang et al., 2012a; Zhu et al., 2014), and sulfate-reducing bacteria (Yang et al., 2014) to thrive in the DS, further raising concerns about the potential for recycled water to accelerate damage to pipe networks.

2.3.2 Redox zones and degradation of water quality

The distribution system can be thought of as a complex reactor with interrelated chemical and biological reactions occurring spatially and temporally as the water passes through the pipes (Camper and Dircks, 1996). The chemistry of treated potable water changes significantly during transport, with deteriorating DWDS water quality documented since the early 1920s (Berry et al., 2006; Buswell, 1938; Hall et al., 2007; Howard, 1940; Piriou et al., 2005; Powell, 1921). Masters *et al*. (2015b) illustrated the water distribution system reaction phenomenon by demonstrating the formation of sequential redox zones as a function of water age in simulated DWDS. Given greater physiochemical and microbial complexity in RWDS, we speculate that they would foster development of even more dramatic reactive zones, as a function of key physical and hydraulic design parameters such as residence time, flow pattern, hydraulic surface to volume ratio, and pipe layout. Consistent with this expectation, studies in lab and field-scale RWDS recently demonstrated elevated microbial activity as indicated by rapid AOC/BDOC consumption, even at the earliest water age, and attenuated organic carbon at higher water ages (Jjemba et al., 2010a). Similarly, in a 15-month monitoring study of RWDSs, the general pattern observed was

an initial reactive zone where rapid microbial regrowth and chemical reactions occurred followed by relatively constant microbial and chemical reactivity along the length of the pipes (Ajibode et al., 2013). Recognizing the reality of reactive zones in distribution systems and more deliberately monitoring them may provide valuable insight into predicting and preempting potential problems resulting from issues related to water chemistry.

2.3.3 Disinfectant residual

The intricate relationship between chlorine-based disinfectants and microbial and chemical stability has been intensely studied in DWDSs. Due to its strong oxidizing power, chlorination is generally the disinfectant of choice for microbial control in drinking water treatment. Chlorination can greatly reduce general bacterial counts and help satisfy drinking water microbial regulations. However, as a strong oxidizing agent, it is also known to interact with reductive species, metals, organic matter and pipe materials and, as a result, significantly impact the downstream water chemistry (Ramseier et al., 2011). The most widely noted issue with disinfectants is the fragmentation of complex carbon compounds, thus increasing the fraction of biologically available carbon when high concentrations of chlorine are used (Jjemba et al., 2010a; Thayanukul et al., 2013; Weinrich et al., 2010). Given the tendency to use fecal indicators as a benchmark for assessing recycled water quality, it can be tempting for utilities to dose high concentrations of chlorine. Due to a higher chlorine demand typical of recycled water, disinfectant residual may be rapidly lost, leaving the rest of the RWDS vulnerable to microbial instability (Ajibode et al., 2013). Also important to note is that there is growing concern regarding the efficacy of chlorine-based disinfectants against emerging resistant pathogens, which might be more abundant in recycled water than traditional potable water (Dupuy et al., 2011; Marchesi et al., 2011; Steed and Falkinham, 2006; Taylor et al., 2000). The potential for indiscriminate use of disinfectants to inadvertently select disinfectant-resistant bacteria in the RWDS is worthy of exploring in future research. Further, the ability of bacteria to repair and recover in the distribution system following the shock of ultraviolet irradiation (UV), chlorine, or other disinfectant should be considered, as exemplified by recovery of viable but non-culturable bacteria (Guo et al., 2011; Huang et al., 2011; Süss et al., 2009).

Another issue worthy of consideration is the potential for enhanced DBP formation in recycled waters (Clark and Sivaganesan, 1998; Krasner, 2009). In a study comparing DBP formation between wastewater effluent and surface water, Sirivedhin and Gray found that effluent-derived organic matter stimulated formation of higher proportions of brominated DBPs (Sirivedhin and Gray, 2005). Nurizzo *et al*. (2005) evaluated the DBP formation potential with various disinfection agents and concluded that hypochlorite yielded the greatest total trihalomethanes, exceeding the Italian regulation for agricultural reuse, even when starting with high quality recycled water. While DBPs tend to be ignored in recycled waters, particularly for non-potable applications, it is important to recognize that inhalation is also a relevant exposure route to consider, with one model characterizing the inhalation exposure to trihalomethanes of irrigation workers using recycled water suggesting that there was a 13% risk of exceeding acceptable exposure levels for cancer risk (Wang et al., 2013). The DBP issue illustrates that there can be tradeoffs between microbial control and chemical risks and that clearer guidance and alternative approaches are needed for recycled water to avoid negative consequences of blindly over-chlorinating.

2.3.4 Chronic Contaminants

The exposome highlights the importance of considering exposures over the course of one's lifetime, and thus, chronic contaminants are an important hazard worthy of consideration during risk assessment of recycled water. WWTPs are generally not designed with the intention of removing micro-constituents, such as pharmaceuticals and personal care products, recalcitrant organic compounds, heavy metals, nanomaterials, and industrial agricultural additives (Coetsier et al., 2009; Gros et al., 2010; Huerta-Fontela et al., 2011; Jelic et al., 2011; Mompelat et al., 2009). Jelic *et al*. (2011) was able to detect 29 pharmaceutical products in the final effluent of one WWTP, versus 32 in the influent. Even when discharged at micro-concentrations, up to hundreds of nanograms per liter of targeted micropollutants can still be consistently detected in receiving water bodies and levels can accumulate (Coetsier et al., 2009). In a study that monitored 15 different WWTPs generating recycled water for groundwater recharge, detectable levels of all 20 most commonly used antibiotics were still found at elevated concentrations of 212-4,035 ng/L in recycled water and 19-1,270 ng/L in groundwater (Ma et al., 2015). Several studies have also observed seasonal patterns of higher discharge of pharmaceuticals and personal care products in wastewater effluent during low flow and less during high flow periods (Kolpin et al., 2004; Loraine and Pettigrove, 2006).

While increasing research attention and regulatory efforts have been devoted to understanding prevalence of non-conventional chemical constituents in WWTPs and in receiving environments (Peterson et al., 2011), studies specifically focusing on characterization and risk assessment of emerging chemical constituents of concern in the context of recycled water applications are limited (Busetti et al., 2015; Khan, 2011; Weber et al., 2006). Advanced oxidation processes are particularly promising for removal of these pharmaceuticals and other organic compounds (Table 2-5) (Huber et al., 2003; Kim et al., 2009). Negative ecological effects of chemical constituents on the aquatic environment have received much attention (Santos et al., 2010; Tyler et al., 2008). Although a multi-barrier approach consisting of sequential treatment processes has promise, questions remain regarding the ideal treatment for various contaminant types and reasonable end point concentrations that are protective of human and ecological health. Given the diverse applications of recycled water, relevant, accurate and comprehensive risk models are needed considerate of the various environmental spheres of influence. Wastewater effluent discharged to surface water has resulted in detection of emerging pharmaceutical products in 80% of surface water samples (Kolpin et al., 2002). Thus, the science and practice of distributing recycled water should proceed with a comprehensive approach to understanding of the fate and impacts of these emerging contaminants in relevant environments.

Table 2-5 Case studies of existing application of advanced treatment processes for intended reuse purposes. Treatment trains rely on use of biological activated carbon (BAC), reverse osmosis (RO), ultrafiltration, and UV.

Advanced treatment processes	Intended reuse	Key Results	Reference
Ozone/H_2O_2 + BAC	Piloted indirect potable reuse	·H_2O_2/ozone process demonstrated higher than 90% average removal rate in 21 of 31 targeted trace organic contaminants and hormonal products ·BAC unit achieved higher than 95% removal for all targeted contaminant except benzophenone ·High degree microbial inactivation ·Raised concerns on elevated AOCs and microbial regrowth potential after H_2O_2/ozone treatment and ·Fluorescence excitation-emission matrix showed distinctively transformed organic matter footprints after treatment	(Gerrity et al., 2011)
Standalone BAC	DOC and nitrogen removal	·Diminishing DOC removal rate after breakthrough is reached ·More than 50% of total nitrogen removal rate	(Kalkan et al., 2011)
Ozone/peroxide + RO	General reuse applications	·Ozone and ozone/peroxide showed similar trace organic contaminant removal performance, likely due to inherently high hydroxyl radicals in wastewater effluent. ·Formation of up to 48ng/L NDMA is observed in wastewater effluent ozone systems, raising concern for future reuse applications	(Pisarenko et al., 2012)
Ultrafiltration+RO+UV	Groundwater recharge	·13 out of 291 targeted compounds are detected in post-UV and post-RO water ·Calculated risk quotient for detected chemicals indicates safe reuse	(Busetti et al., 2015)

2.4 ARGS, OPS, AND OTHER EMERGING MICROBIAL CONCERNS

It is important to recognize the complexity of RWDSs as an ecological habitat and that microbial concerns reach beyond traditional indicator organism paradigms. Here we consider these emerging microbial concerns within a comprehensive microbiome/exposome framework. Several recent studies have utilized DNA sequencing to provide new insight into the composition of the drinking water microbiome, but few have attempted to characterize the recycled water microbiome. Recycled water, and even potable water, both represent surprisingly complex microbial niches, housing a vast array of microbial species about which little is known. Normal fecal indicator bacterial monitoring fails to provide information about the broader microbiome, particularly with respect to oligotrophic organisms residing in distribution systems. Thus, a more holistic approach for characterizing water quality is needed to accurately describe the water

quality at the point of use. Here we elaborate on microbial aspects of the exposome that are generally unrecognized in the regulatory landscape and are particularly relevant to RWDSs. While occurrence of fecal-associated pathogens is also of importance in recycled water systems, we have limited the scope of this review to emerging microbial concerns.

Epidemiological studies examining associations between recycled water exposure and disease have been limited and are crucial to identifying potential for disease transmission, determining suitability for public use, and informing effective risk mitigation strategies. For example, Durand and Schwebach (1989) did not find an association of gastrointestinal illness when irrigating public parks with non-potable recycled water versus potable water (6% versus 7% of park users reporting symptoms associated with recycled wastewater irrigation versus potable water irrigation, respectively), though wet grass conditions during park usage were associated with an increased rate of illness. A study of food crop irrigation with recycled water over a five-year period found no undesirable consequences to the quality of vegetables or soil, thus exposure restrictions for farm workers were not deemed necessary (Sheikh et al., 1990). In one study conducted in the U.S., even irrigation using trickling filter effluent wastewater was not associated with an increased rate of infection of rotavirus for residents of surrounding areas (Ward et al., 1989). A study that examined occurrence of methicillin resistant *Staphylococcus aureus* (MRSA) in spray irrigation workers using recycled water did not find the presence of the resistant organism in nasal swabs from any workers tested, though the odds of carrying a non-resistant strain of the organism were slightly higher among spray irrigation workers than among office workers (Goldstein et al., 2014b). While isolated reports of disease stemming from exposure to recycled water are helpful, rigorous, long-term epidemiological studies are needed to more precisely determine sources of disease and accurately characterize risk and to address emerging concerns.

Of rising interest is the influence of the distinct physiochemical nature of recycled water on the regrowth or attenuation of emerging pathogens and contaminants, particularly considering exposures relevant to non-conventional water reuse applications. Especially when organic carbon is no longer a growth-limiting resource, conventional fecal bacterial indicators are likely to be even less relevant to shifts in microbial ecology during distribution and the associated health risk. Efforts are underway to recognize the importance of microbial ecological interactions in distribution systems and the potential to harness them to foster a distribution system that favors the growth of non-pathogenic bacteria over pathogenic ones (Berry et al., 2006). For example, Egli (2010) has identified the survival and growth strategies of various microbes in low-nutrient and stressed environments and competition between pathogens and the indigenous microbiota. A strategy of capitalizing upon specific ecological interactions, such as nutritional competition, antagonist growth, and symbiotic relationships for improved water quality and human health has been previously proposed for drinking water (Wang et al., 2013). This presents a potentially transformative and highly relevant approach for guiding RWDS management.

2.4.1 Opportunistic Pathogens

RWDSs offer several unique characteristics that make them particularly well-suited for supporting regrowth of OPs. OPs in DWDSs are thought to be the primary source of waterborne disease in developed countries, including the U.S. (Brunkard et al., 2008; Pruden et al., 2013). Unlike most fecal pathogens, OPs do not typically impact the gastrointestinal system but rather they infect via alternative routes. To name a few, *Legionella pneumophila*, *Acinetobacter baumanni*, *Mycobacterium avium*, *Burkholderia pseudomallei*, *Stenotrophomonas maltophilia*,

and *Aspergillus fumigatus* can infect hosts' lungs via inhalation (Fraser et al., 1989; Horsburgh, 1991; Peleg et al., 2008); *S. aureus* infects via broken skin or mucus membrane (Lowy, 1998); *Pseudomonas aeruginosa* can infect hosts via the bloodstream, eyes, ears, skin, or lungs (Bodey et al., 1983); and *Acanthamoeba* spp. can cause infection of the eyes or central nervous system when inhaled or upon penetration of skin lesions (Marciano-Cabral and Cabral, 2003). These alternative routes of infection make OPs of particular interest for recycled water, where exposure routes other than simple ingestion are more relevant. Inhalation of aerosols from cooling towers or spray irrigation and dermal contact with irrigated surfaces, are important routes of exposure that should be accounted for when considering risks associated with OPs in recycled water.

OPs possess several distinct properties that make them particularly well suited for growth in RWDSs (Figure 2-3). OPs tend to be resistant to disinfection, ranging from 21-658 times as resistant to chlorine as *Escherichia coli*, as in the cases of *P. aeruginosa* and *A. baumanii*, respectively (Falkinham, 2015). Many OPs are also resistant to phagocytosis by amoebae, becoming enclosed within an amoebic cyst, where they can be further protected from disinfectants and other harsh environmental conditions. Biofilms, where OPs tend to reside, offer protection from similar environmental assaults, in addition to acidic and alkaline conditions and shear force from high flow velocities (Falkinham, 2015; Wang et al., 2013). OPs also tend to grow at low organic carbon concentrations, which is pertinent to both DWDSs and RWDSs (Falkinham, 2015). Stagnation is a notorious risk factor for OP outbreak, and is common in RWDSs due to intermittent demand and seasonal shutdown (Wang et al., 2012a).

2.4.2 Antibiotic Resistance Genes

Antibiotic resistance among human pathogens is a major public health concern. In the U.S., the Centers for Disease Control has estimated that antibiotic resistant bacteria cause at least two million infections and 23,000 deaths each year (US Centers for Disease Control and Prevention, 2013). ARGs are now well-known to be elevated in human-contaminated surface waters (Amos et al., 2014; Graham et al., 2011; Marti et al., 2013; Pruden et al., 2012), however with respect to human pathogens, specifically, there is reasonable evidence that they can gain ARGs from environmental bacteria via horizontal gene transfer (**Error! Reference source not found.**) (Benveniste and Davies, 1973; Forsberg et al., 2012). Therefore, all members of the microbiome carrying ARGs are potentially of concern, particularly those that are common in human pathogens. In addition to the possibility of infection by antibiotic resistant bacteria upon exposure, human hosts may also become colonized and infected later (Bogaert et al., 2004; Davis et al., 2004; von Eiff et al., 2001). Similarly, it is possible that horizontal gene transfer may occur from colonized non-pathogenic bacteria to pathogenic ones, leading to antibiotic resistant infection. Thus, infection by antibiotic resistant bacteria may occur at a time and place separate from that of the initial exposure, which complicates traditional dose-response risk assumptions. ARGs or bacteria expressing ARGs corresponding to resistance to aminoglycoside, beta-lactam, chloramphenicol, fluoroquinolone, lincomycide, linezolid, lipopeptide, macrolide, sulfonamide, tetracyclines, and vancomycin antibiotics have been previously identified in recycled water or environments directly impacted by irrigation, infiltration, or groundwater recharge using recycled water (Barker-Reid et al., 2010; Bockelmann et al., 2009; Fahrenfeld et al., 2013; Goldstein et al., 2014; McLain and Williams, 2014; Wang et al., 2014; Wang et al., 2014). Since antibiotic resistance is a natural phenomenon inherent among many bacteria, studies that compare these abundances to relevant control environments, such as corresponding potable water or environments unimpacted by recycled water are of particular value. While the nature of

reusing human wastewater means that prior to treatment, human pathogens or other bacteria carrying ARGs will be enriched compared to other source waters, multiple studies have demonstrated that ARGs are often not removed during treatment, and in some cases, are even amplified (Munir et al., 2011; Rizzo et al., 2013; Schlüter et al., 2007; Zhang et al., 2009). Additionally, a study by Fahrenfeld *et al*. (2013) found that ARGs may also increase during distribution of recycled water as a broader range of monitored ARGs were present in point of use samples than in samples leaving the treatment plant.

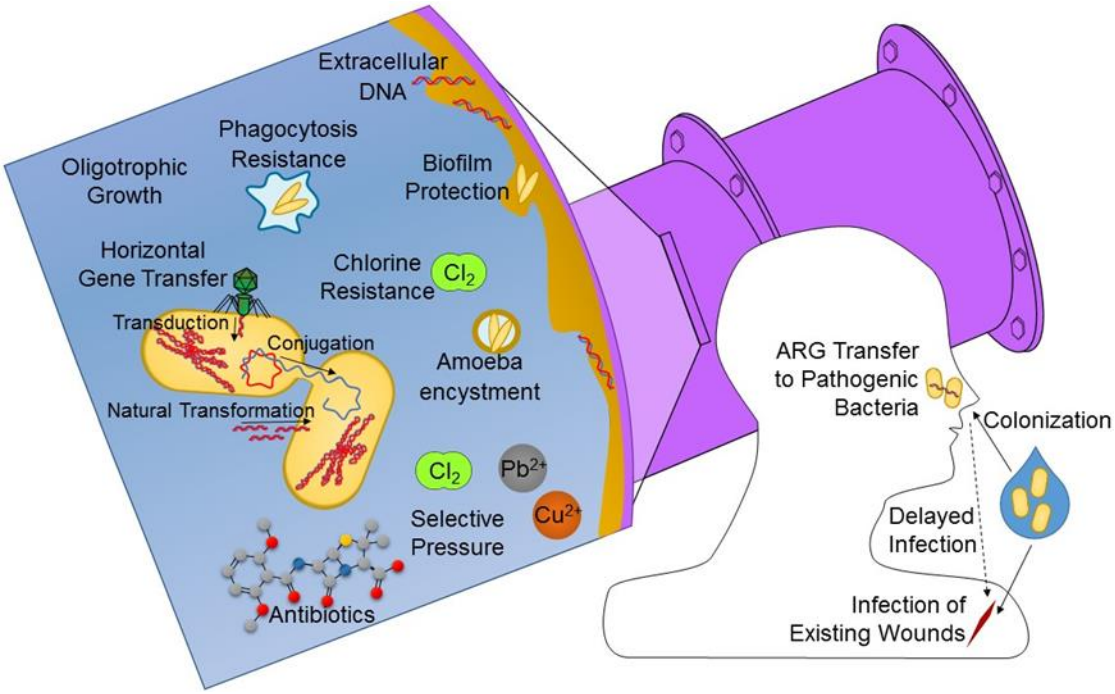

Figure 2-3 Processes by which antibiotic resistant bacteria and opportunistic pathogens (OPs) can re-grow in RWDSs and relevant exposure routes.

Various features of recycled water potentially make it a prime medium for the growth of antibiotic resistant bacteria and propagation of their associated ARGs during distribution. In particular, residual antibiotics that escape removal during treatment can exert selective pressure and encourage persistence of ARG-carrying bacteria. Though antibiotics will likely be found in recycled water at sub-lethal concentrations, this low level exposure has actually been shown to encourage the persistence of bacteria that carry ARGs via several mechanisms (Andersson and Hughes, 2014; Liu et al., 2011; McVicker et al., 2014). Gullberg *et al.* (2014) found that bacteria maintained plasmids carrying beta-lactam resistance genes even at concentrations of antibiotics and heavy metals nearly 140 times below the compound's minimum inhibitory concentration. Other studies have also demonstrated that sublethal antibiotics can stimulate propagation of ARGs by activating horizontal gene transfer (Beaber et al., 2004; Prudhomme et al., 2006; Song et al., 2009; Úbeda et al., 2005; Xia et al., 2008; Zhang et al., 2013). Prudhomme *et al.* (2006) demonstrated that intermediate concentrations of streptomycin induced genetic transformation in *Streptococcus pneumoniae*. Beaber *et al*. (2004) demonstrated that 'SOS response' among *Vibrio cholerae* induced by the presence of ciprofloxacin enhances transfer of resistance genes via

conjugation. Low levels of antibiotics or other selective agents also act to encourage adaptive evolution including development of resistance mutants (Andersson and Hughes, 2014).

Antibiotics are not the only antimicrobials with potential to select for ARGs in recycled water systems. Heavy metals, such as copper and iron (which are commonly used in distribution systems), have long been suspected to select for ARGs in a variety of environments (Baker-Austin et al., 2006). Metal-driven selection of ARGs is also of concern due to the presence of various heavy metals capable of ARG selection common in many wastewaters, such as copper, zinc, nickel, mercury, and even nanosilver (Brar et al., 2010; Sörme and Lagerkvist, 2002). Disinfectants have also been known to select for ARGs (Huang et al., 2013b; Karumathil et al., 2014; Shi et al., 2013b). Following chlorination, *E. coli* carrying the *tet*A tetracycline resistance gene were found to be even more tolerant to tetracycline than non-chlorinated *E. coli* (Huang et al., 2013a). Chlorination has also been reported to concentrate a variety of ARGs in potable water (Shi et al., 2013a).

In addition to increasing ARGs via mutations, natural selection, and horizontal gene transfer, presence of residual antibiotics can enhance biofilm formation (Boehm et al., 2009). Studies of *Staphylococcus aureus*, *E. coli*, and *P. aeruginosa* have all indicated that sub-inhibitory concentrations of various antibiotics induce biofilm formation (Blickwede et al., 2005; Hoffman et al., 2005; Li et al., 2005). Extensive biofilm formation provides a fertile environment for the transfer of ARGs via horizontal gene transfer. Dense microbial communities existing in biofilms with extensive cell to cell contact facilitate transfer via conjugation (Hausner and Wuertz, 1999; Molin and Tolker-Nielsen, 2003). Notably, a key component of biofilms, extracellular polymeric substances, is partially comprised of DNA expelled from cells (Das et al., 2013). This may provide a reservoir of free DNA-based ARGs, which have been shown to be available for uptake into cells via transformation (Hannan et al., 2010; Li et al., 2001). Biofilms themselves offer protection from antibiotics or other antimicrobial agents via the principle of collective resistance, where cells are physically shielded from exposure to the antimicrobial (Vega and Gore, 2014).

While transmission of antibiotic resistant bacteria is an acute public health threat, the possibility that water reuse may exacerbate the overall spread of antibiotic resistance has been suggested (Pruden, 2014). The water cycle as a whole has recently been subject to scrutiny as a potentially important, yet understudied, route for the spread of antibiotic resistance (Allen et al., 2010; Ashbolt et al., 2013; Wellington et al., 2013). Given the gravity of the antibiotic resistance problem and several lines of reasoning that water reuse can contribute to its spread, additional research is urgently needed to determine whether consideration of antibiotic resistance should be of central concern to comprehensive long-term risk management strategies.

2.4.3 Viruses

Though removal of viruses in recycled water is of great importance, the presence of viruses in recycled water is rarely monitored. Treatment goals and regulations regarding virus removal are typically presented in the form of expected log-removal achieved through treatment such as disinfection, largely due to the analytical difficulty of direct virus detection (US Environmental Protection Agency, 2012b). Low recovery rates, complex and time-consuming laboratory culture procedures, slow turn-around time for culture results, and inability of molecular techniques to differentiate viable from non-viable viruses are major challenges. Problems with the indirect monitoring paradigm may arise, however, because viruses may be

resistant to some modes of disinfection. For example, adenoviruses are known to resist ultraviolet irradiation (US Environmental Protection Agency, 2012b).

A recent study of the viral metagenome (i.e., total DNA extracted from viral component) revealed approximately 10^8 virus-like particles (VLP) per mL in non-potable recycled water, 1,000 times more than that measured in potable water (Rosario et al., 2009). Further, genetic markers corresponding to viruses targeting eukaryotes were non-detectable in potable water, while two percent of the viruses in recycled water corresponded to eukaryotic hosts. This is logical, indicating that recycled water is more susceptible to carrying viruses associated with humans than traditional potable water.

Bacteriophages, which represent the vast majority of viruses in both potable and recycled waters, have their own relevance to human health as they act as agents of transfer for ARGs among bacteria via transduction. Bacteriophages have been largely neglected as constituents in potable and recycled water, though they have been found to be highly abundant in both raw wastewater (10^8 VLP/mL) and in potable water (10^5-10^6 VLP/mL) (Rosario et al., 2009). Though transduction is generally considered a rare transfer event, occurring only once in every 10^7-10^9 phage infections (Muniesa et al., 2013), the shear abundance of bacteriophages documented in wastewater and potable water suggests that it is likely a significant phenomenon in recycled water.

2.4.4 Amoebae

FLA are of growing concern in drinking water plumbing. Many FLA, such as *Acanthamoeba* spp. and *Vermameoba* spp., graze on bacterial biofilms, and in doing so, can serve as an important vector for amplifying and disseminating OPs (Thomas et al., 2011). For example, *Legionella*, *Mycobacterium*, and *Pseudomonas* spp. can amplify within FLA when grazed upon, which enhances their dissemination and virulence (Thomas et al., 2011).

FLA themselves can sometimes be pathogenic, as is the case with keratitis or primary amoebic meningoencephalitis (PAM) (Thomas et al., 2011). Similar to other OPs, non-ingestion routes of exposure are important for pathogenic FLA. PAM is contracted when *N. fowleri* is forced into the nasal cavity and migrates to the brain, while keratitis occurs when pathogenic *Acanthamoeba* spp. infect the eye (US Centers for Disease Control and Prevention) Such exposures have been documented both in recreational and drinking water (Bartrand et al., 2014; Blair et al., 2008; Marciano-Cabral and Cabral, 2003). However, relevant to recycled water, inhalation is under investigation as a primary transmission method (Bartrand et al., 2014; Martinez and Visvesvara, 1997).

The design of RWDSs can instigate the growth of biofilm, providing a reservoir for FLA and an environment to promote interactions with amoeba-resisting bacteria. Recycled waters have complex microbial communities and high availability of nutrients and other organic matter, creating optimal conditions for biofilm establishment (Liang et al., 2015). Recent studies have also shown increased chlorine resistance of FLA in the presence of naturally established biofilm (Miller et al., 2015a; Thomas et al., 2008) and even non-biofilm *Vermamoeba* spp. have been observed to resist chlorination (Thomas and Ashbolt, 2011).

The relationship between *L. pneumophila* and FLA has been the most closely studied. Resistance to amoebae can provide protection from disinfection, competition, environmental stress and predation for *L. pneumophila* (García et al., 2007; Jjemba et al., 2015; Lu et al., 2014). Additionally, different FLA have been shown to survive a wide range of temperatures, from 10-45°C, with some cases indicating survival near 0°C, potentially allowing for protection of

Legionella spp. and other amoeba-resisting bacteria during winter, while amoeba are encysted (Buse and Ashbolt, 2011; Griffin, 1972; Söderberg et al., 2008; Thomas et al., 2011). Little is still known about the diversity and abundance of amoebae and their interactions with amoeba-resisting bacteria in drinking water, let alone recycled water. Gaining a better understanding of the interactions between these microorganisms and the ways in which this may aid the growth of pathogenic bacteria is essential for better understanding the exposome associated with recycled water.

2.4.5 Algae

Algae are a common nuisance in recycled water systems. Though algal growth frequently occurs in systems that use open storage rather than in distribution system pipes, algal cells can persist throughout distribution systems where they have been found to correlate with AOC and BDOC (Jjemba et al., 2010a). Decaying algal cells can even be a source of BDOC, contributing to the regrowth of other microbial constituents. Increased regrowth resulting from organic carbon made available from decaying algal cells has also been linked with a loss of oxygen and dissipation of chlorine residual (Jjemba et al., 2010a). Elevated concentrations of algae may carry potential for the production of harmful algal toxins. Cyanobacteria toxins have been linked to liver damage, neurotoxicity, gastroenteritis, pneumonia, and even death (Hitzfeld et al., 2000). Though these symptoms primarily arise from ingestion of the toxins, skin irritations and allergic reactions have been noted following dermal contact with cyanobacteria toxins and respiratory disease has been documented following suspected inhalation of the toxins (Codd et al., 1999). In cases where non-potable reuse occurs, these problems may be particularly challenging to identify as taste and odor complaints from consumers will be unlikely.

2.5 CONCLUSION

Given the increasing trend of water reuse across the globe, it is important that all aspects, including end-users, treatment plant management and operation, regulation, and public health protection are taken into consideration in the planning and implementation of water reuse risk management strategies. In this paper, we summarized the inherently different biochemistry of recycled water in the distribution system as a function of various usage and operational factors. We also discussed acute and long-term risks from the chemical and microbial contaminants that may result from multi-dimensional usage routes and the associated exposure risks associated with various end use of the recycled waters.

Increased awareness of traditionally underappreciated routes of exposure is key to the safe use of recycled water. The history of drinking water epidemiology provides numerous examples of infection via atypical routes of exposure. For example, in 2015 an outbreak of Legionnaire's disease that killed 12 people in New York City was linked to infection via aerosolized bacteria from cooling towers sourced from potable drinking water (Mueller, 2015). *N. fowleri* infection from drinking water has occurred from use for nasal irrigation and in children via bathing or playing on an outdoor water slide (Bartrand et al., 2014). Prevention of infection as a result of unintended exposures with recycled water requires proactive action when planning for treatment and distribution of recycled water. One key "critical control point" that must be considered in this planning is the distribution system to avoid degradation of water quality during distribution. Treating recycled water to remove nutrients and achieve biostability is one promising approach to help ensure safe water at the point of use, but additional treatment

at the point of use may also be necessary in some cases, as both the physiochemical and microbial water quality change significantly during distribution and in premise plumbing systems. A key research gap exists regarding the most effective approaches for achieving biostability of recycled water during distribution. Specific and cost-effective engineering controls for nutrient recycling and limiting regrowth during distribution must be identified for respective intended uses. Identification of emerging chronic contaminants and microbial contaminants is also important in minimizing potentially harmful exposures. Rigorous studies that examine the health implications of non-traditional routes of exposure are quite limited and are challenging to design given the lack of available knowledge about infectious doses (particularly based on non-oral routes of exposure), magnitude of exposure via non-traditional routes, and concentrations of emerging contaminants that are typical of recycled water. In addition, virulence and individual susceptibility varies widely for many of the microbial constituents discussed, making it important to consider exposure of immunocompromised populations when assessing risk. In addition to these research gaps, development of quantitative microbiological risk assessments (QMRA) would be extremely valuable for assessing the risk associated with the presence OPs, ARGs, FLA, and viruses in recycled water. Epidemiological studies are also critical for linking actual human illness and associated microbial sources with recycled water. Finally, research is needed to tailor treatment processes to serve specific intended end uses (e.g. Table 2-1), along with addressing emerging concerns identified here, while also developing best management practices for distribution systems and premise plumbing for preventing re-growth and deterioration of water quality in RWDSs.

While overarching regulations that consider the comprehensive implications and scope of water recycling are currently lacking in many places, there are also practical lessons we can learn from international leaders on adopting a comprehensive risk management approach towards water reuse, notably the Australian Guidelines for Water Recycling, the WHO's framework of WSP, and the HACCP paradigm. Complementing these strategies with a holistic approach focused on the human exposome creates a framework in which consideration of user exposures drives establishment of water quality standards. Though a regulatory framework that addresses the exposure risks and potential for regrowth associated with use of recycled water is an ideal long term goal, an interim approach of more basic best management practices, as suggested by Jjemba *et al*. (2012a), are a reasonable starting place to enable municipalities utilizing recycled water to proactively act to limit bacterial regrowth and preserve water quality at the point of use. Best management practices should continually be revised as knowledge gaps are addressed, to ensure that the most meaningful water quality indicators are targeted.

Adoption of the human exposome paradigm aims to ensure comprehensive understanding of the risks and uncertainties regarding alternative recycled water sources. Enhanced knowledge could provide critical guidance on safe management and inform much-needed regulations as the use of recycled water expands. However, while this exposome approach highlights the multi-dimensional risks and uncertainties regarding use of recycled water, it also must be recognized that water reuse plays an integral role in addressing the grand challenge of water scarcity. It is estimated that a third of the world's population is currently living with moderate to high levels of water stress (Hatt et al., 2006) and approximately 50% will suffer water shortages by 2025 (National Health and Medical Research Council, 2004). Implementing water reuse projects is imperative to meet water needs in drought-stricken areas, despite potential risks and concerns. As estimated by Brown, current groundwater sources, serving more than half of the world's population, are largely over drafted (Brown, 2012). Lack of new alternative water supplies,

compounded with increasing water demand, would further intensify water scarcity stress. Schoreder *et al*. (2012) has estimated that the potential benefits of reuse could offset water supply for a community of 1 million people by 75 million gallons per day. In cases where water scarcity lends to the likelihood of *de facto* reuse, then it is better to have intentional reuse guided by best management practices to minimize risks.

Equally important to the exposure risk from recycled water is lack of access to traditional potable water sources and poor water quality due to degraded source water. Globally, there are over five million deaths associated with poor water quality every year (Adapa S, Bhullar N, 2016). Achieving an environmentally sustainable and socially beneficially water demand management plan requires proactive evaluation of the highest priority needs and identification of the key drivers and barriers to the implementation of water recycling projects. Positive associations between information availability and the acceptance of water reuse have been noted (Baumann, 1983; Lohman and Milliken, 1985; Dolnicar and Hurliman, 2010). As end users become more educated about this alternative water source, their willingness to use recycled water increases (Tsagarakis et al., 2013). Nonetheless, comprehensively addressing all possible public health concerns will be an essential pillar to advancing water sustainability.

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CHAPTER 3: SEDIMENT AND BIOFILM AFFECT DISINFECTANT DECAY RATES DURING LONG-TERM OPERATION OF SIMULATED RECLAIMED WATER DISTRIBUTION SYSTEMS

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ABSTRACT

Changing chemistry and microbiology of treated wastewater as it is conveyed via reclaimed water distribution systems (RWDSs) can influence water quality at the point of use. Two simulated RWDSs configurations comprised of 0.32-cm tubes and 10-cm diameter pipes were designed to investigate how extremes in water age (28-min versus 5-d) and surface area to volume (SAV) ratio (6.25 cm^{-1} versus 0.20 cm^{-1}) affect the potential for sediment accumulation and disinfectant decay rates. Effluent from a conventional municipal wastewater treatment plant was breakpoint chlorinated and tested with or without biological filtration pre-treatment, followed by three different disinfectant scenarios: no disinfectant, 4 mg/L chlorine or 4 mg/L chloramine. After three years of operation, sediment accumulations occupied 0.32-3.2% of the total volume of the 10-cm diameter Pipe rig segment. The unfiltered free chlorine condition had about 10 times less influent turbidity and sediment accumulation than conditions with no disinfectant or chloramine, resulting in lower levels of biological activity, loss of dissolved oxygen and disinfectant. The sediment in the chloramine Pipe rigs was highly biologically active (high VS/TS, high levels of nitrifiers) and created a high disinfectant demand. In contrast, the Tube RWDSs had no visible sediment accumulations, but accumulated very thick bio-foulant in conditions with chloramine and no disinfectant. Chloramine had greater stability than free chlorine in Tube rigs, whereas the opposite trend was observed in Pipe rigs. An improved holistic understanding of RWDSs chemistry, biology and operation will help achieve desired water qualities at the point of use.

3.1 INTRODUCTION

Population growth, climate change, and increased water scarcity necessitate sustainable water management that includes reuse of moderate to highly treated wastewater effluent (US Environmental Protection Agency, 2012a). For instance, the Inland Empire Utilities Agency in San Bernardino County currently reuses 50% of its wastewater and is targeting 80% reuse by 2025 (ISSUE 2014). The level of water reuse treatment should be tailored for its intended use, ranging from conventional secondary wastewater treatment plant (WWTP) effluent with disinfection that delivers beneficial nutrients for crop irrigation, to technologies that can produce water far exceeding potable water standards (US Environmental Protection Agency, 2012a).

More than 50% of reuse in the United States is directed towards non-potable applications, such as agricultural, urban, and industrial reuse (Bryk et al., 2011). Such applications do not

generally target high removal of suspended solids or pathogens beyond conventional disinfection. Most state regulations for distributing reclaimed water allow up to 5 mg/L total suspended solids, which roughly translates to 5-10 NTU (Becker, 2010), whereas drinking water turbidity is often rigidly controlled to less than 0.03 NTU (US Environmental Protection Agency 2012a). This creates a much greater potential for sediment accumulation in reclaimed water distribution systems (RWDSs) versus drinking water distribution systems (DWDSs).

According to a WateReuse Research Foundation survey of US reclaimed water system operators, even though they do not usually expect non-turbid or aesthetically pleasing water, 12% cite "biofilm growth," 5% report "sediment accumulation," and 25% note frequent problems with "clogging of sprinkler heads" as issues of concern in reclaimed water (Jjemba et al., 2010). The effective removal of particulates is a primary goal of drinking water treatment using conventional sedimentation and filtration, but even so, recent research demonstrated that accumulated loose deposits in highly treated water distributed through a polyvinyl chloride (PVC) pipe network in the Netherlands served as reservoirs for microorganisms that included opportunistic pathogens (Liu et al., 2014; Mussared et al., 2018). A nation-wide survey of drinking water storage tanks in the U.S, also revealed high sediment accumulation and growth of opportunistic pathogens with sediment (Struewing et al., 2015). All of this work suggests that stagnant or low flow portions of RWDSs, would be highly susceptible to sediment accumulation and microbial growth.

Understanding of key processes controlling the chemistry and microbiology of potable water distribution to ensure high quality water at the point-of-use has advanced substantially in recent decades. A knowledge base has been established about relative rates of decay for different disinfectants (Zhang and Edwards, 2009; Li et al., 2019), importance of nutrients (Liu et al., 2002; Escobar et al., 2001; Hijnen et al., 2018), and formation of distinct redox zones as a function of water age in DWDSs (Masters et al., 2015; Wang et al., 2012). In recent years, understanding of water quality problems in DWDSs has been extended to a few studies of RWDSs, where impacts of high levels of particulates, organic matter, nutrients, and bacteria on the final water quality of distributed water are likely to be magnified (Weinrich et al., 2010; Jjemba et al., 2014; Garner et al., 2018; Garner et al., 2016). Intermittent flow is another common characteristic of RWDSs, allowing for sedimentation of particulates and causing depletion of secondary disinfectant residuals during stagnation events (Jjemba et al., 2010; Jjemba et al., 2014), potentially leaving systems vulnerable to water quality degradation (Erickson et al., 2017; Speight 2008).

There are currently no specific federal regulations controlling water reuse or distribution. Given the wide variation of influent water quality, treatment processes, water use patterns and intended end uses comparing DWDSs and RWDSs, improved fundamental understanding and management practices are needed. Here we employ two types of controlled, simulated RWDSs to better understand the role of sediments and biofilms in controlling water chemistry and overall microbial regrowth potential. The specific objectives were to determine: 1) how different treatments, including biologically activated carbon (BAC)-filtration and disinfection, affect sediment composition and accumulation; 2) the role of different design of simulated RWDSs; and 3) the interplay between treatments and design that affect chemical redox zones and levels of secondary disinfectant residuals levels.

3.2 MATERIALS AND METHODS

3.2.1 Simulated RWDS Influents

Final wastewater effluent from a conventional municipal WWTP was collected twice a week and stored at 4 °C before being subjected to various treatment scenarios. Three secondary disinfectant conditions (chlorine, chloramine and no residual), with and without prior BAC-filtration, were applied towards creating six distinct RWDSs influent reservoirs (Table 3-1). The unfiltered chlorine condition was prepared with a two-step chlorination process (superchlorination) to allow a stable chlorine residual to be maintained in the reservoir. The unfiltered water was first chlorinated to 8 mg/L Cl_2 to maintain a residual > 0.5 mg/L for over 24 hours and then boosted to a final target residual of 4 mg/L as Cl_2 to be used as the influent reservoir. In the BAC-filtered chlorine reservoir and in the chloramine reservoirs with and without BAC-filtration, the disinfectant residuals were relatively stable at the targeted 4 mg/L without superchlorination. In the conditions with no residual, breakpoint chlorination was performed after which the residual remained < 0.02 mg/L. Each influent reservoir was stored at 4 °C as the water was pumped into the simulated RWDSs. All reservoirs were changed every 30 hours.

Table 3-1 Summary of key water quality parameters of the two feed water conditions, before and after BAC-filtration, and the six influent conditions representing different BAC-filtration and disinfection strategies.

	Reservoir Conditions					
	Unfiltered			BAC-Filtered		
TOC (mg/L)[a]	6-8			<2		
Soluble AOC[b] (ppb)	48.6-160			11.9-29.3		
Total AOC[b] (ppb)	399-610			292-660		
Total cell counts (TCC) (cells/µL)	6624 ± 2190			2672 ± 1136		
	Chlorine	Chloramine	No Residual	Chlorine	Chloramine	No Residual
Targeted residual (mg Cl_2/L)	4 as chlorine	4 as chloramine	No residual	4 as chlorine	4 as chloramine	No residual
Mean residual[d] (mg Cl_2/L)	3.81	3.92	0. 01	3.69	3.87	0.02
Total NH3 (mg/L)	No	0.98	No	No	0.97	No
Breakpoint chlorination	Yes	Yes	Yes	Yes	Yes	Yes
Superchlorination	Yes	No	No	No	No	No
BAC-filtration	No	No	No	Yes	Yes	Yes
Turbidity[e] (NTU)	1.20	10.0	12.6	0.78	2.43	3.00
Sediment volume (mL)	27.0	50.0*	190.0	19.00	65.0	60.0
Sediment/Pipe volume ratio (%)	0.45	0.83	3.2	0.32	1.1	1.0
Volatile Solids (VS) in Sediment (g/L)	10.0	22.7	24.9	3.63	21.5	19.5
Total Solids (TS) in Sediment (g/L)	21.0	27.4	32.5	7.13	26.3	25.6
Mass of VS (mg)	121	793	4366	14.5	1074	879
Mass of TS (mg)	252	957	5679	28.5	1313	1150
VS/TS (%)	48.1	82.8	76.9	50.9	79.1	76.4

* Sediment collected in the unfiltered chloramine Pipe only corresponded to Sep 2016 – Sep 2018 due to pipe replacement.

a The range of TOC values were collected over the 3-year operation.

b Soluble and total AOC range were from 2 independent measurements at the beginning of the final sampling event.

c Total cell counts measurements were averaged over 6 sampling events throughout the 3-year operation.

d The mean disinfectant residuals in the influent reservoirs were averaged over 245 measurements collected at each water change over the 3-year operation.

e Turbidity was measured as a one-time event at the final sampling event.

The water age, or the time to travel from treatment plant to point of use, can vary from 0 days to more than 24 days in real water systems and can influence water quality at the point of use (US Environmental Protection Agency, 2002). Water pipes can also have inner diameters ranging from a few millimeters in buildings up to a few meters in water mains (Gur and Spuhler, 2019), and flow can range from completely stagnant to turbulent. Two stimulated RWDS rig designs – the "Pipe" and "Tube", were employed to represent extremes in terms of water age, flow patterns, SAV ratio, and likelihood of sediment accumulation (Figure 3-1).

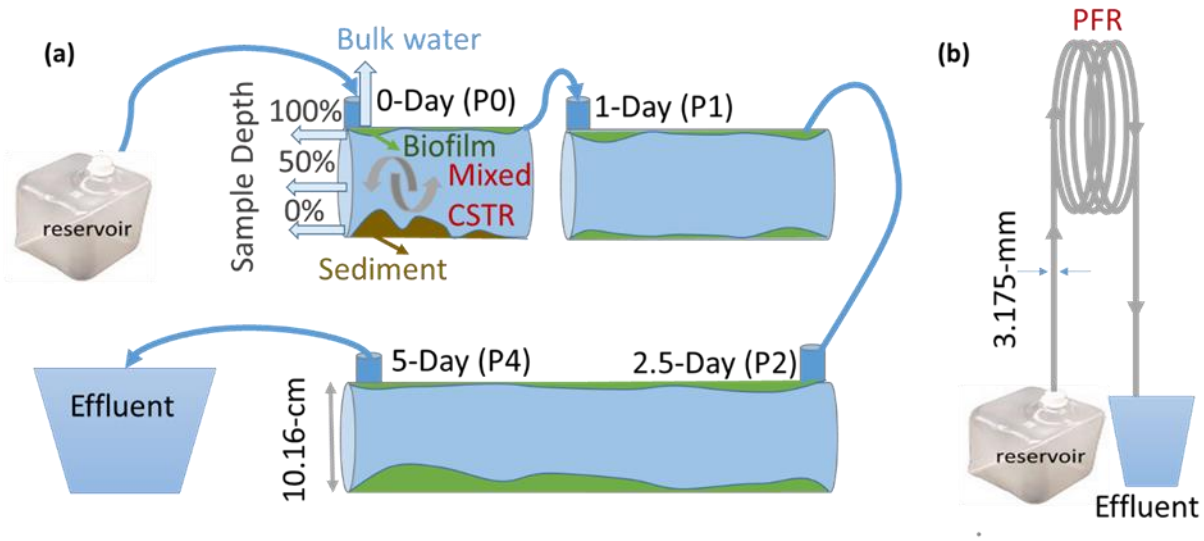


Figure 3-1(a) Simulated 10.16-cm (4-in) Pipe RWDS rigs with sampling ports at the point of entry (P0), and at calculated water ages of 1-Day(P1), 2.5-Day (P2), and 5-Day (P3). Samples were also taken at the bottom (0%), mid-point (50%), and the top (100%) of the vertical depth of the pipe. (b) 3.175-mm (1/8-in) Tube RWDS rigs with total length of 15.24-m (50-ft) and were sampled every 3.05-m (10-ft) Duplicate Tube rigs were set up in parallel for each disinfectant condition to compare reproducibility.

The wide "Pipe" RWDSs were designed to achieve a realistic simulation of both water age and surface area to volume ratio, albeit at an unrealistically low flow velocity. The complementary "Tube" RWDSs were designed to test a narrow pipe diameter that prevented sediment from accumulating and a higher internal flow velocity, but with an extremely low water age and high surface area to volume ratio. The Pipe rigs were comprised of three 10-cm (4-in.) diameter PVC pipe segments connected in series with short PVC tubing in between (Figure 3-1a). The calculated water age (volume/flow rate) of the three pipe segments corresponded to 1 d, 2.5 d and 5 d. The narrow "Tube" simulated RWDS rigs consisted of six 15.24-meter (50-ft) 0.32-cm (1/8-in.) inner diameter PVC tubing with a calculated water age of 32-min (Figure 3-1b). The surface area to volume ratio was 6.25 cm^{-1} and 0.20 cm^{-1} for the tube and pipe RWDS's, respectively. Three disinfectant conditions (no disinfectant, chlorine, chloramine) applied to unfiltered water were operated in duplicate for the Tube rigs. The flow rate for the Pipe and Tube rigs was identical at 4.2 ml/min.

Prior to the experiment described herein, all Pipe rigs had been operating under the targeted influent conditions for 39 months (June 2015 – May 2017) to allow a mature biofilm to establish. There was a one-time repair and replacement of a leaking first segment of the unfiltered chloramine Pipe rig in Sep 2016. The repaired Pipe remained in continuous operation over the subsequent 24 months. The Tube rigs were first operated from April 2017 – August 2017 (5 months), left stagnant for one year to simulate an extreme non-use event, and then reconditioned April 2018 – August 2018 (6 months). To ensure a valid head-to-head comparison between the two rigs, all rigs were operated under the same ambient temperature (~22 °C) and influent water conditions from April – August 2018. The same batch of effluent water was used to prepare the influent reservoirs during the sampling events to eliminate any background variation in influent water quality.

3.2.2 Tracer Study

A tracer study was conducted to define the hydraulic flow patterns of the rigs. Potassium chloride (KCl was injected to the influent of one representative Pipe (at 50,000 mg/L KCl) and Tube rigs for each of the three disinfectant conditions (at 30,000 mg/L), with all effluent collected in aliquots until the potassium level returned to approximately the initial background levels. A clean rig without any sediment was tested as a control.

3.2.3 Sample Collection and Water Quality Analysis

The Pipe rigs were sampled both along the horizontal flow direction at 0%, 20%, 50% and 100% of the pipe length and along the vertical pipe profile at 0% (top), 50% (middle), and 100% (bottom) of the depth of the 10-cm pipes to explore variations in water chemistry in proximity to the sediment (Figure 3-1). To reduce effects of disturbances on sample results, measurements were made in reverse order from highest to lowest water age and from top to bottom to reduce potential for disturbances. Routine samples of Tube rigs were from the effluent, whereas end of study profile samples were collected by cutting the Tubes at sequential 10-foot (3.05 meter) intervals and collecting the effluent at 40, 30, 20 and 10 feet.

After the final sampling event, all water and accumulated sediment from the first pipe segment were transferred into separate buckets. The water and sediment mixture was settled and carefully decanted to avoid disturbance of settled sediment. The remaining 500 mL of water and sediment mixture was poured into a graduated cylinder. After allowing for 30 min of settling, the supernatant bulk water was carefully pipetted out without disturbing the settled sediment and the final settled volume was determined. The resulting sediment was analyzed for VS and TS according to EPA Method 1684 (US Environmental Protection Agency, 2001). All containers employed in sediment harvesting and quantification were sterilized either by ethanol or autoclave.

Water chemistry was tracked in all simulated RWDS rigs, including chlorine and chloramine residuals (HACH DR 2700 Spectrophotometer Method 8021), DO (Orion Star A 326 DO meter), disinfection by-products (DBPs) (EPA Method 502.2), pH (Oakton pH 110), ammonia (Hach Method 8155), nitrite and nitrate (Dionex® DX-120 Ion Chromatograph), and total organic carbon (TOC) (Sievers 5310C Laboratory TOC Analyzer).

Microbial characterization included nitrifiers (HACH N-BART™ Test), denitrifiers (HACH DN-BART™ Test), and total cell counts using a BD Accuri C6® flow cytometer and previously reported methods (Vital et al., 2012). Heterotrophic plate counts (HPCs) were used as

an indicator of general microbial activity in both bulk water and in collected sediment (Reasoner, 2002). Assimilable organic carbon (AOC) was measured using the method described by Hammes and Egli (Hammes and Egli, 2005). One mL of wastewater effluent without any on-site laboratory treatment was used as the indigenous bacterial consortium inoculum to seed 50 mL for AOC analysis. The bacterial growth stage was carried out at 35°C for 5 days. The net increase in biomass over the 5-day incubation was measured by flow cytometry (BD Accuri C6®) and converted to equivalent AOC. Soluble AOC was measured after filtering the water through a 0.22 μm filter (Millipore® Millex® sterile syringe filters, Durapore® PVDF membrane). Total AOC was measured by replacing filtration with pasteurization at 70°C for 30 min, as previously described (Weinrich et al., 2009).

3.2.4 Disinfectant Demand Characterization

The relative contribution of disinfectant demand from bulk water, biofilm, and sediment in the filtered and unfiltered chlorine and chloramine Pipes was measured in a flask test (Figure 3-2). Flasks (250 mL) included bulk water as the control, bulk water with 10 cm^2 swab of biofilm from a chlorine/chloramine Pipe wall added (the cotton swabs were verified not to incur additional chlorine demand in a side test), and bulk water with 10 mL sediment collected from the chlorine/chloramine Pipe added. All flasks were initiated with 4 mg/L of either free chlorine or chloramine and were monitored for up to 5 days for free/total chlorine residuals to establish decay curves for each condition.

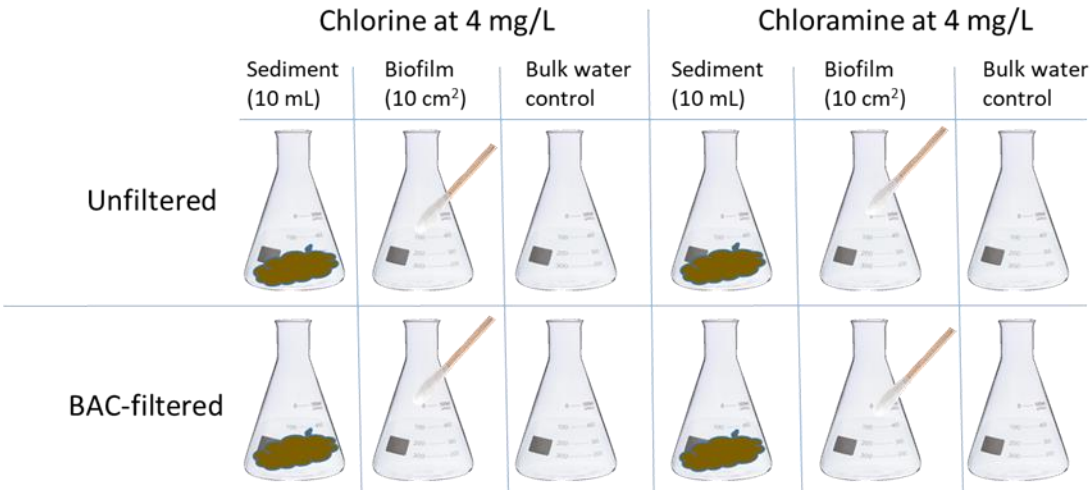


Figure 3-2 Experimental design of flask test aimed at quantifying disinfectant demand associated with bulk water, biofilm, and sediment collected from Pipe RWDS. Unfiltered and BAC-filtered influent water corresponding to that from the Pipe rigs were used in the t the test. Initial chlorine or chloramine levels for the flask tests were targeted at 4 mg/L to simulate the rig reservoir conditions. 10 cm2 of swabbed biofilm and 10 mL of sediment samples collected from the corresponding Pipe rig conditions were added to each flask representing those conditions. All flasks were incubated at room temperature (25 °C) during the decay tests.

3.2.5 Data analysis and statistics

Pearson's correlation test between influent turbidity and accumulated sediment volume was conducted with the ggpubr package in R and plots were produced with the ggplot2 package in R. First order decay model with a fixed initial disinfectant level of 4 mg/L was used to fit the disinfectant decay data in the flask decay study.

3.3 RESULTS AND DISCUSSION

3.3.1 Characteristics of sediment in the Pipe Rigs and Tube Rigs

Sediment accumulation in the Pipe Rigs

After three years of operation, substantial quantities of sediment accumulated in the bottom of the first pipe segment of the Pipe RWDSs. The accumulated sediment appeared loose, flocculent, and dark (Figure 3-3), with a total volume ranging from 19 to 190 mL (Table 3-1). The greatest sediment volume was observed in the condition with no filtration or disinfectant residual, occupying 3.2% of the 6 L total volume of this segment, whereas sediment in the filtered chlorine condition occupied just 0.32% of the total volume. The VS/TS ratio of the sediment was independent of whether or not the water had been biologically filtered, but could be ranked according to disinfectant condition: chloramine (82.9% and 79.1%) > no disinfectant residual (76.9% and 76.4%) > chlorine (48.1% and 50.9%) (Table 3-1). The high VS/TS ratio in the chloramine and no disinfectant residual conditions indicates a higher fraction of viable biomass (Bullock et al., 1996), relative to the 60% VS/TS ratio reported for DWDS sediment (Vreeburg et al., 2008) or the corresponding condition with free chlorine reported herein. Suspended solids in secondary reclaimed water effluent are known to contain high levels of organic particulates that produce biologically-active sediments in lakes (Wurzbarcher et al., 2016; Yoshimura et al., 2010). Such effects were observed for all RWDS conditions without free chlorine tested herein.

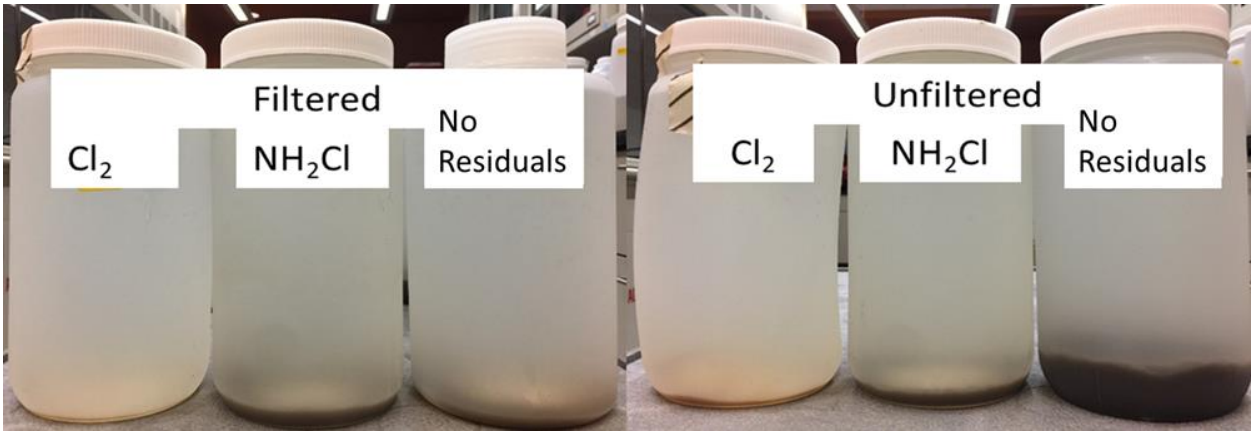


Figure 3-3 Settled sediment removed from the bottom of the first pipe segment of the six Pipe RWDS conditions (2-L containers). Sediment was collected after three years of operation, except for the unfiltered chloramine condition, which was collected after two years of operation due to a pipe failure and replacement mid-experiment.

The two most likely sources of sediment accumulation in the Pipes were physical settling of suspended influent particulate matter or sloughed biofilm. The representative measured influent turbidity varied from 0.78 – 12.6 NTU (Table 3-1), where expectations that the BAC-filtered influent would consistently have lower turbidity than the corresponding disinfectant conditions without filtration was confirmed. However, the high levels of free chlorine residual destroyed turbidity, presumably via direct reactions with natural organic matter and particulate organics, producing influent with low turbidity of 0.78 – 1.20 NTU in water with or without filtration. There was a strong positive correlation between influent turbidity and sediment mass and total sediment volume, supporting the notion that the sediment was largely derived from settling of influent particulates (Pearson's correlation, r = 0.91 and 0.89, Figure 3-4). The accumulation of sediment in the filtered chloramine Pipe was above the trend line, suggesting sloughing of biofilm might have been a more substantial contributor for this condition.

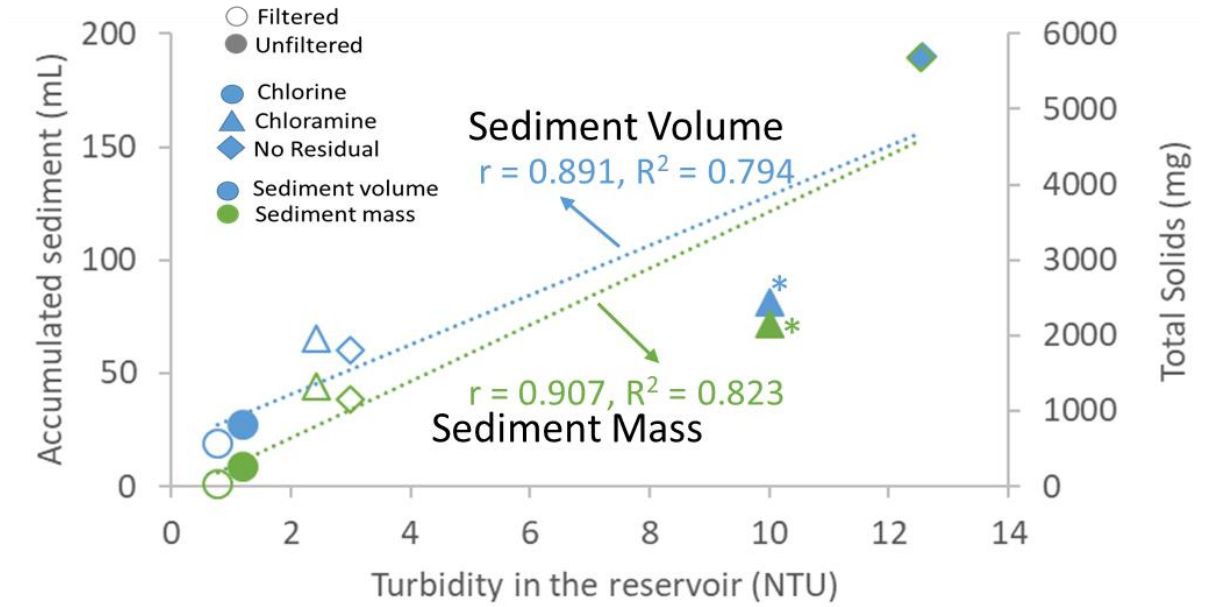


Figure 3-4 Correlation between the turbidity of the influent reservoir and accumulated sediment in the first pipe segment of the Pipe RWDS after 39 months of operation, as measured by sediment volume (blue symbols, primary y-axis) and total solids mass (green symbols, secondary y-axis). The solid symbols in the legend indicate unfiltered influent and the empty symbols indicate BAC filtered influent. * Due to a pipe replacement, 39 months accumulation was linearly extrapolated based on measurements after 24 months of operation.

Bio-foulant accumulation in the Tube rigs

While sediment deposits were not observed at the bottom of the Tube rigs at the end of operation, "bio-foulant", which herein refers to symmetrical accumulation of biological and non-biological buildup on the Tube walls, was prevalent and varied among the disinfectant conditions (Figure 3-5). The bio-foulant thickness was very thin in the chlorine condition, consistent with observations that this condition never clogged at any point of the study. In contrast, the chloramine and no disinfectant residual conditions clogged repeatedly after two months of operation and had to be cleared by brief (10 seconds) reversal of the pump flow direction on a weekly basis. The bio-foulant in the chlorine Tubes was also lighter in color than in the chloramine or no residual condition, possibly due to bleaching (Mayer and Ofial, 2018). The trends in bio-foulant accumulation in the Tube rigs due to disinfectant type was similar to what was observed for sediment accumulation in the Pipe rigs. However, the design of the Tubes rigs achieved prevention of deposit accumulation at the downward orientation as observed in the Pipe rigs.

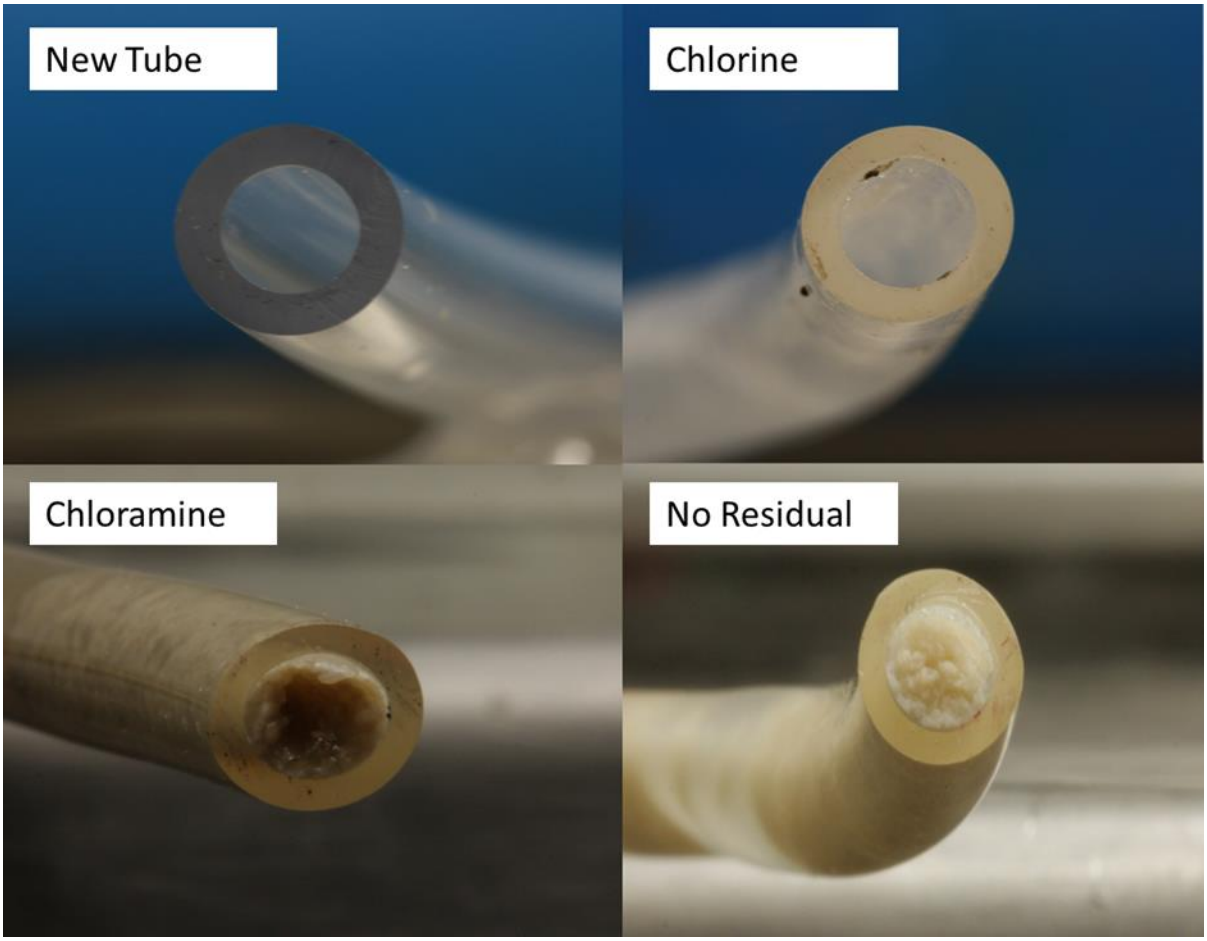


Figure 3-5 Cross-section photographs of the Tube rigs revealed that the extent and morphology of sediments/biofilms were distinct across the three disinfection conditions. Photos were taken at cross sections 40-ft from influent. Only unfiltered feed water was used to prepare the three disinfectant conditions in the Tube Rigs.

3.3.2 *Assessing the effects of rig design, sediment, and biofilm on tracer curves.*

Pipe RWDS

The observed tracer curves varied depending on the accumulation of sediment and biofilm in the Pipe rigs. The tracer curve for a new Pipe section without any accumulated sediment and biofilm peaked at a residence time of 20 hours, with a mean tracer residence time of 33 hours. This was in the range of the calculated 26-hour hydraulic residence time for a plug-flow reactor (PFR). In contrast, the tracer curve from the first pipe segment with accumulated sediment and biofilm illustrated a curve with stronger CSTR than PFR characteristic (Figure 3-6). In the Pipe rigs with sediment accumulations (i.e., the filtered chloramine Pipe), the tracer peaked at 14 hours instead of 20 hours for a clean pipe, but had a mean tracer residence time of 73.3 hours.

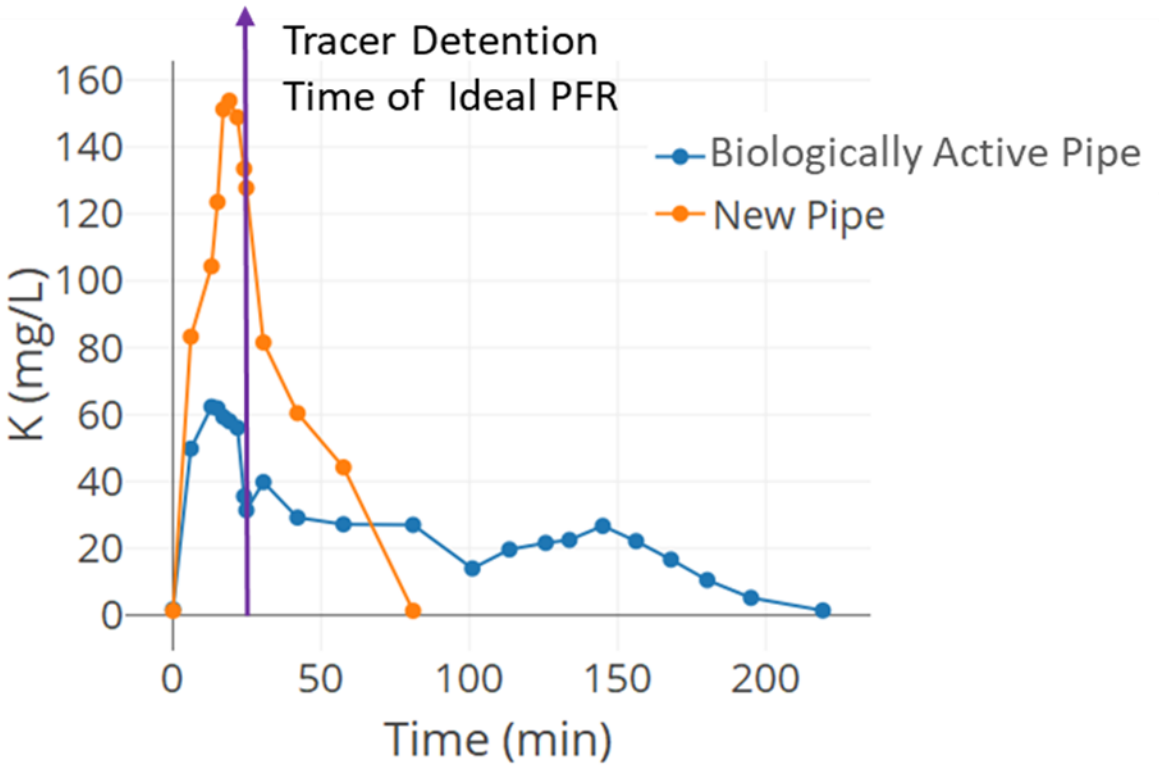


Figure 3-6 Tracer study in the first pipe segment of new versus biologically-active (i.e., operated ~3 years) Pipe Rigs. Potassium (K) was injected at 50,000 mg/L potassium chloride. The ideal plug flow reactor (PFR) wash out curve is shown for comparison.

Clearly, the potassium chloride tracer, which is often considered conservative and relatively non-reactive in DWDS applications (Levenspiel, 2012), was significantly retarded in the established Pipe RWDSs, especially considering that the accumulated sediment was expected to create dead space that caused the tracer to elute more quickly than in the new condition. After repeating this result several times and observing it applied to the chloride counter ion as well, we conclude that the potassium chloride was not acting as an idealized conservative tracer, due to either biological uptake or sorptive ion exchange with biofilms, retarding its transport (Freeman et al., 1995; Steyl and Marais, 2014).

Tube RWDS

Potassium tracer studies confirmed a strong PFR flow character for a new Tube rig or an established rig with relatively little bio-foulant (chlorinated reclaimed water), with peaks eluting near the calculated hydraulic residence time (Figure 3-7). However, in the corresponding rigs with visibly thick bio-foulant levels, the tracer took nearly twice as long to elute (Figure 3-5), consistent with the same counterintuitive result obtained in the Pipe RWDS rigs. This further supports the conclusion that the potassium chloride transport was retarded by the bio-foulant.

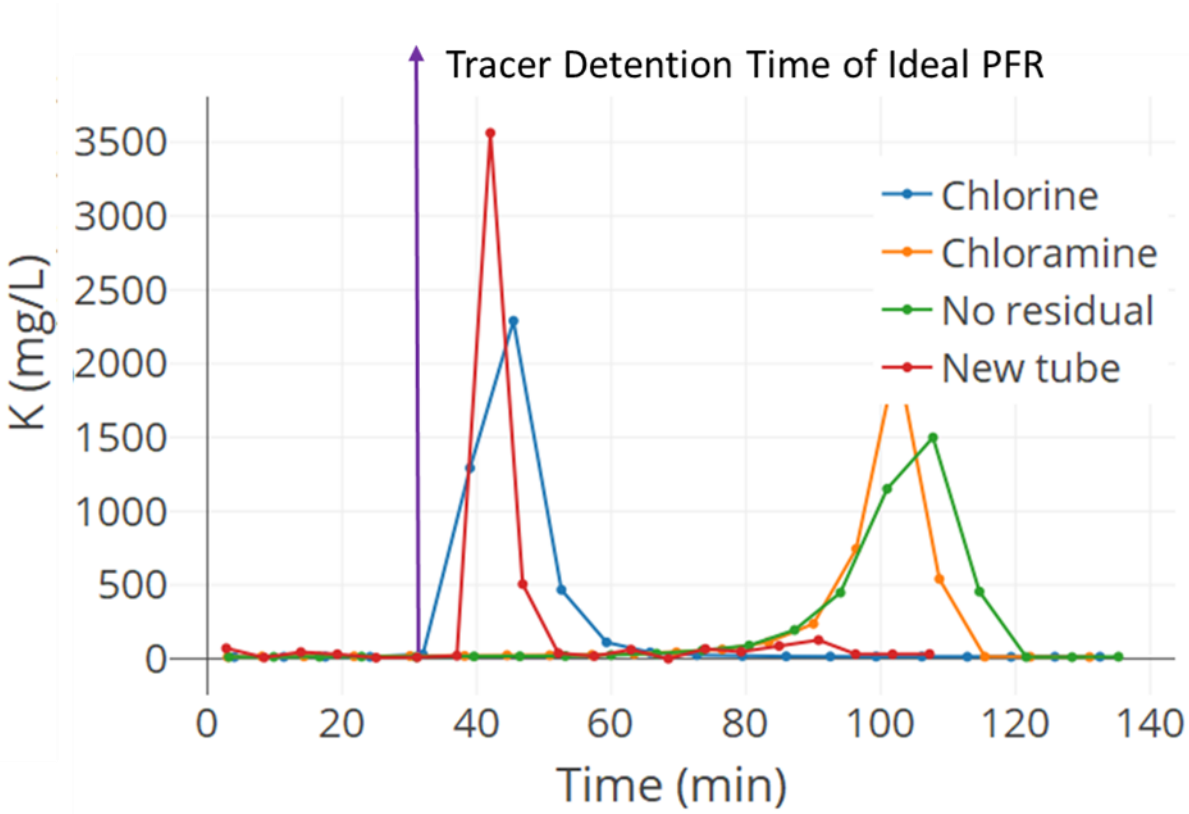


Figure 3-7 Potassium tracer studies in the Tube rigs demonstrated PFR flow but with a delayed retention time for conditions with heavy biofilms. Potassium (K) was injected at 30,000 mg/L KCl.

3.3.3 *Sediment can create distinct redox zones in simulated RWDS*

Disinfectant decay trends

The disinfectant residuals in the simulated RWDS rigs differed from what has been previously observed in DWDSs. As water passes through a DWDS, the level of disinfectant residual decays at a characteristic rate (US Environmental Protection Agency, 2002). Here, both chlorine and chloramine residuals were completely depleted after the 5-day calculated hydraulic residence time for all conditions (Figure 3-8a). Contrary to expectations based on experiences in full-scale and some smaller-scale DWDSs (Norton and LeChevallier, 1997), chlorine residual was observed to be more persistent than chloramine residual in this simulated RWDS design. For example, ~1 mg/L chlorine residual still remained after 1 day for both the unfiltered and BAC-filtered waters in this testing at 22°C, whereas chloramine was between non-detectable to 0.06 mg/L.

A distinct disinfectant decay gradient along the vertical axis of the Pipes was observed within the first pipe segment, where nearly all of the disinfectant decay occurred. Specifically, comparing sample aliquots collected from the top versus bottom of the Pipe rigs at the point of entry (P0), 2.61 mg/L of chloramine residual and 1.90 mg/L chlorine residual were lost in the unfiltered influent conditions as the sample approached the sediment at the bottom of the Pipe rigs (Figure 3-8b). The corresponding BAC-filtered conditions indicated similar disinfectant

losses from top to bottom with 1.31 mg/L and 2.06 mg/L loss for chorine and chloramine, respectively. Sediment accumulation in the chloramine Pipes, which also had a much higher mass and VS/TS ratio than the corresponding sediment in chlorine Pipes, likely contributed to this faster rate of chloramine decay for both BAC-filtered and unfiltered water.

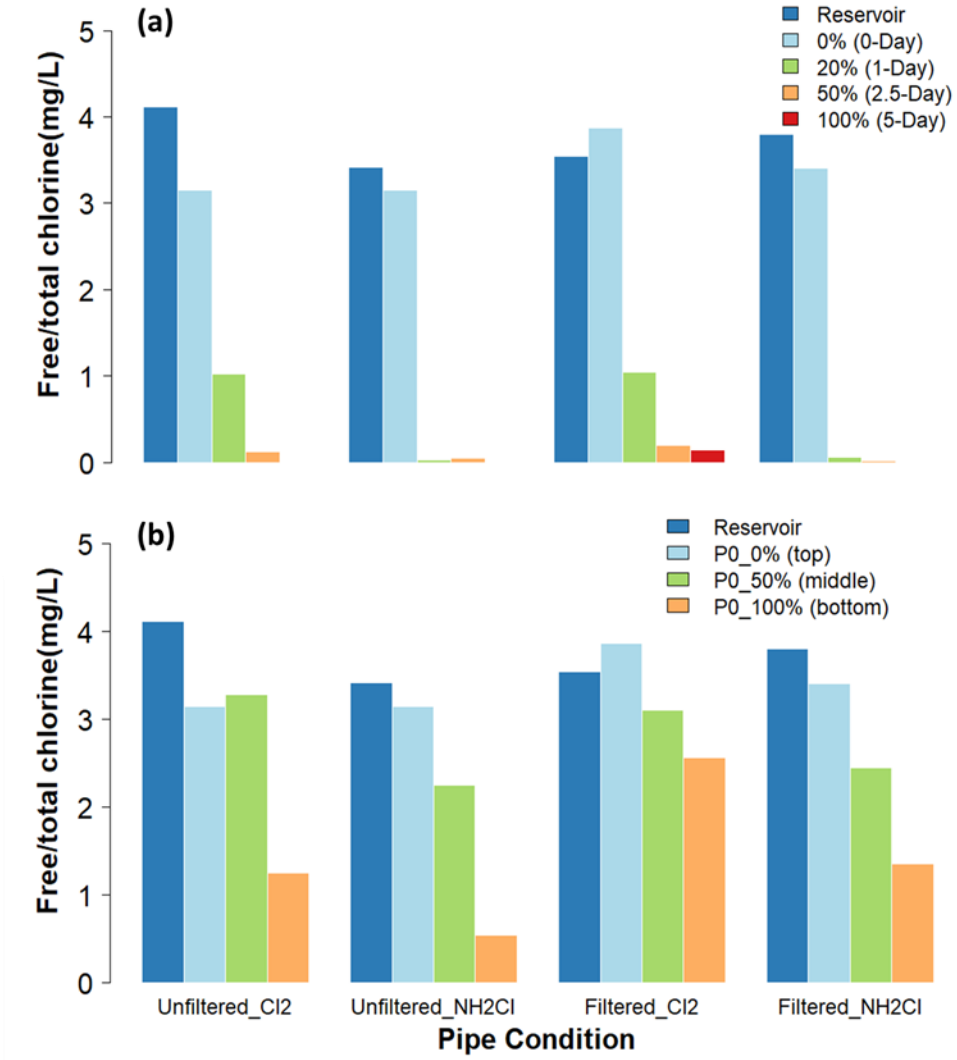


Figure 3-8 Depletion of disinfectant residuals (a) along the horizontal pipe flow direction and (b) with increasing depth at the point of entry (P0) of the Pipe RWDS. The a and b sampling events were conducted 1 week apart at an operational temperature of 22 ºC. The legend in (a) indicates calculated hydraulic residence times (i.e., assuming plug-flow for an empty Pipe) at the sampling ports and in (b) percent distance from the top of the Pipe. Measurement in (a) were sampled in reverse order from the last sampling point (P3) to the first sampling point and measurements in (b) from the top to bottom water level to minimize disturbance of subsequent sample. Filtered = BAC filtered influent; Unfiltered = unfiltered influent; Cl2 = chlorine condition; NH2Cl = chloramine condition.

DO consumption in the Pipe RWDSs

Low DO has been considered a limiting factor in irrigation water, contributing to suboptimal crop growth, low yields, and plant diseases (Bhattarai et al., 2008). In the present study, DO generally decreased as water flowed horizontally through the biologically active Pipe rigs (Figure 3-10a), with a few slight exceptions where DO increased because the system was not designed to be completely impermeable to gases. Differences in DO losses were notable, with 3.22 or 5.41 mg/L DO lost for the BAC-filtered and unfiltered chloramine conditions, respectively, while only 0.38 – 1.14 mg/L was lost in the corresponding free chlorine conditions after 5 days.

As was the case for the disinfectant, DO decreased markedly moving vertically from the top of the Pipe towards the sediment in the first pipe segment for both the chloramine and no residual conditions (Figure 3-10b). However, this trend was not observed in the chlorine condition, presumably due to the much reduced accumulation of sediment and less biological activity. The DO drop from the top to the bottom of the first pipe segment ranged from 3.01 – 5.89 mg/L among the unfiltered or filtered influent conditions with chloramine or no disinfectant residual, compared to a drop of just 0.89 – 1.81 mg/L in the corresponding conditions with free chlorine.

Clearly, the type of disinfectant played a more important role than BAC-filtration in controlling the consumption of DO in this study. Possible factors for higher DO in the presence of free chlorine include production of DO up to 0.95 mg DO from 4 mg free Cl_2 auto-decomposition (Adam and Gordon, 1999; Lister, 1956; Sandin et al., 2015) and cytotoxicity from DBPs produced by free chlorine reactions with organic matter inhibiting biological activity (Sobczak et al., 2002). The lower levels of turbidity and sediment in the chlorine rigs may also translate to less microbial activity in the sediment, while nitrification in the presence of chloramine also consumes oxygen.

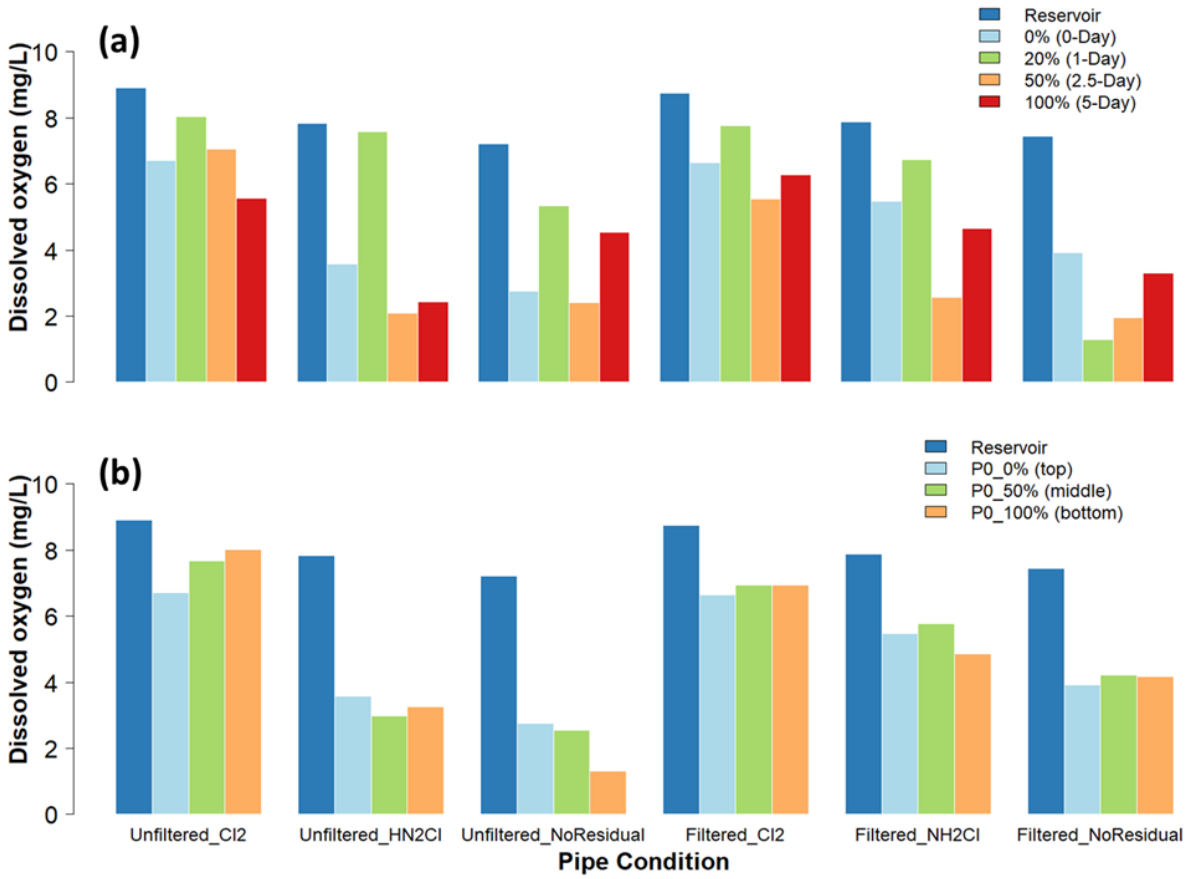


Figure 3-9 Dissolved oxygen level (a) along the horizontal pipe flow direction and (b) with increasing depth at the point of entry (P0) of the Pipe RWDS. The legend in (a) indicates calculated hydraulic residence times (i.e., assuming plug-flow for an empty Pipe) at the sampling ports and in (b) percent distance from the top of the Pipe. Measurements in (a) were sampled in reverse order from the last sampling point (P3) to the first sampling point and measurements in (b) from the top to bottom water level to minimize disturbance of subsequent samples.

Rapid disinfectant and DO loss in Tube RWDSs

Despite the dramatic difference in reactor characteristics and sediment accumulation, there was also very rapid loss of disinfectant and DO in the Tube rigs. Specifically, both chlorine and chloramine were almost depleted by the end of the 15.24-meter (50-ft) tubes after only a 32-min hydraulic residence time. In contrast to the Pipe RWDS rigs, where chloramine was depleted faster than chlorine, chloramine was more persistent than chlorine in the Tube rigs. In samples collected at the end of the study, about 50% of the chlorine residual was lost at the 3.05-meter (10-ft) tubing interval, compared to just 3% of chloramine. At the end of the Tube rigs, chlorine was non-detectable, whereas chloramine was still measureable at 0.24 mg/L (**Error! Reference source not found.**a).

The DO level after just 32-min residence time in the Tube rigs was roughly equivalent to that observed at 1,440-min (1-day water age) in the Pipe rigs. This difference factor of roughly

45 (1440/32) likely reflects the greater SAV ratio for the Tube versus Pipe RWDS (32 × greater), enhancing the influence of bio-foulant growth on bulk water chemistry. The DO trends in the Tube rigs were similar to those observed for the Pipe rigs with respect to the type of disinfectant. The DO drop decreased on the order of 6.22 – 8.92 mg/L, > 3.73 – 4.17 mg/L, and > 0.95 – 1.21 mg/L for the chloramine, no disinfectant, and free chlorine conditions, respectively (**Error! Reference source not found.**b). Disinfectant decay and DO trends were consistent in the duplicate Tube rigs (**Error! Reference source not found.** and **Error! Reference source not found.**).

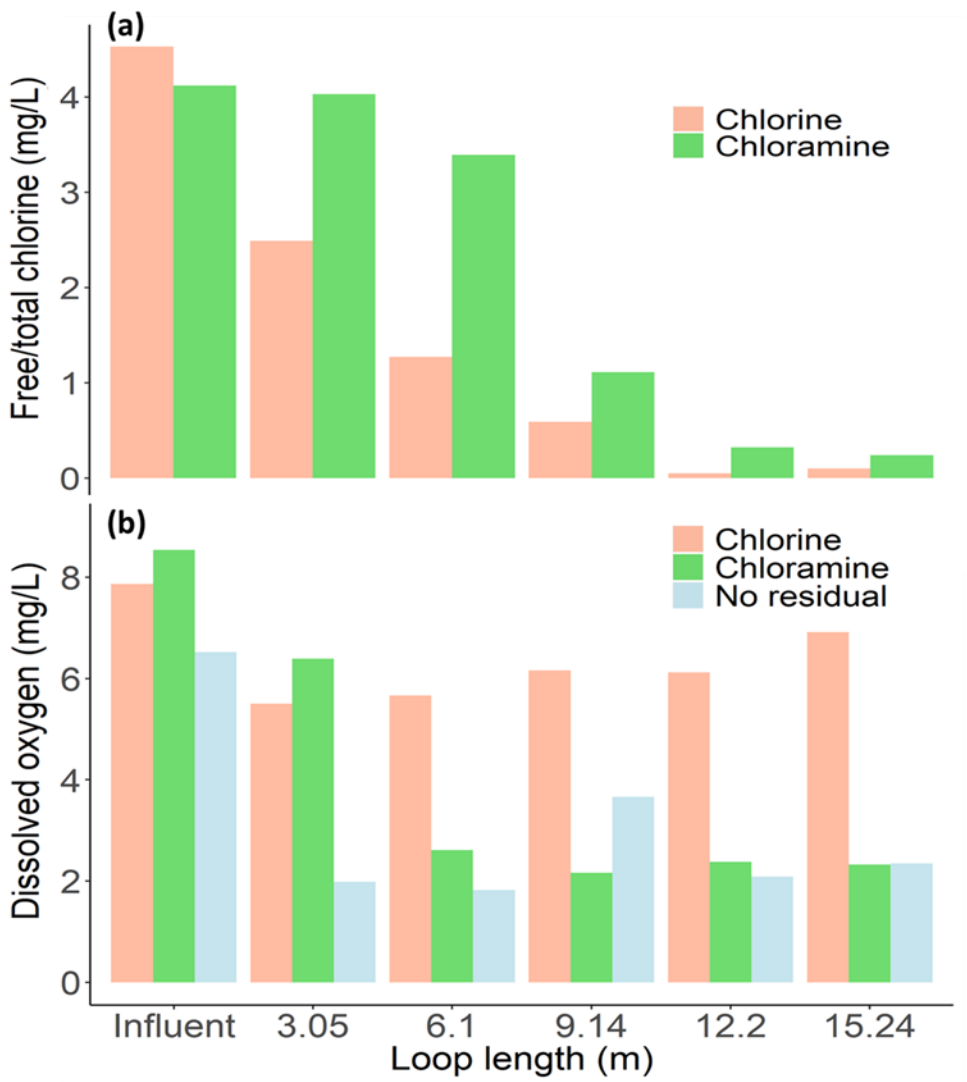


Figure 3-10 (a) Chlorine and chloramine measured at the end of the 15.24-m (50-ft) Tube rigs (31-min calculated residence time) and corresponding (b) dissolved oxygen measurements at 3.05-m (10-ft) intervals of the Tube rigs, measured by cutting and sacrificing the tubing at the end of the experiment.

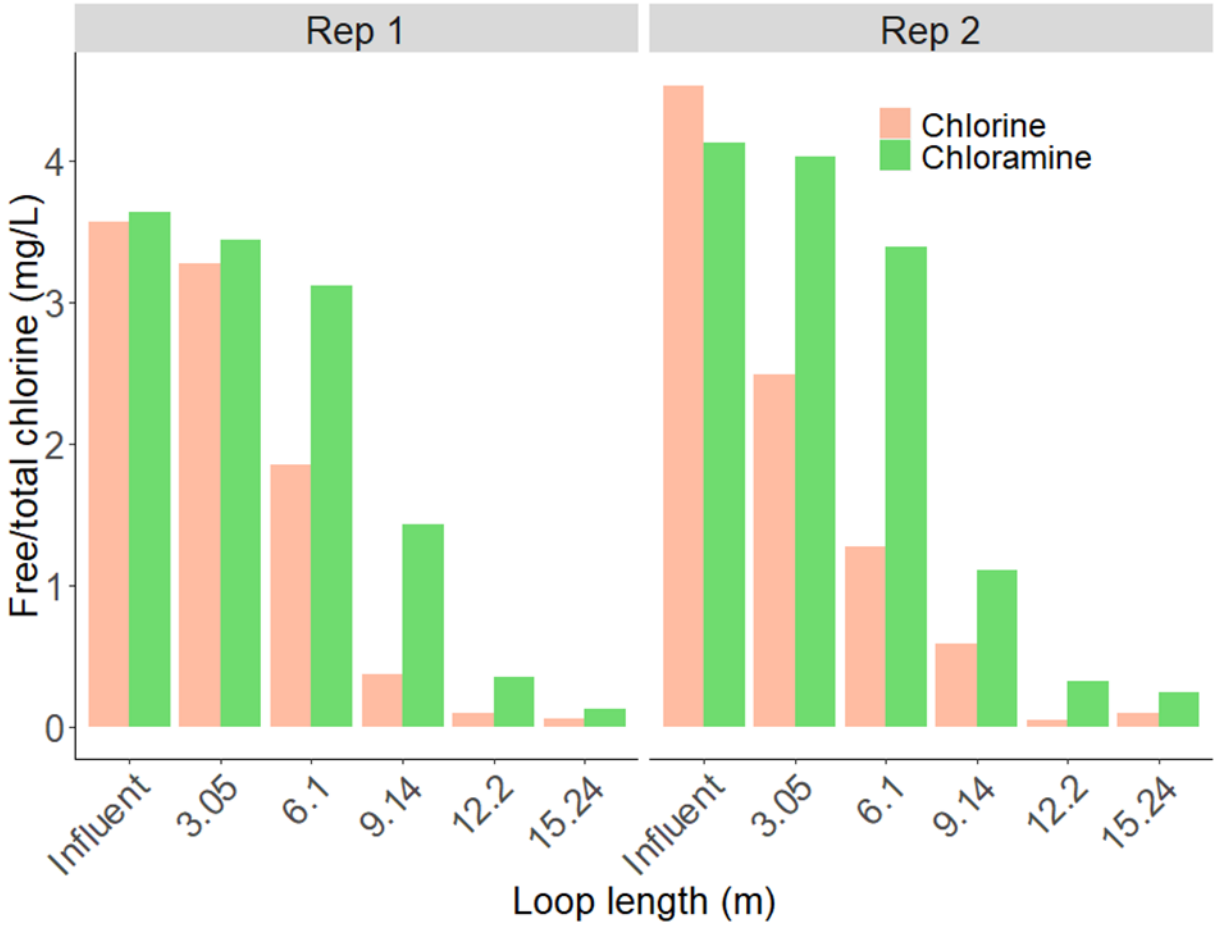


Figure 3-11 Comparison of chlorine and chloramine measurements in Tube RWDS duplicates (Rep 1, Rep2) operated in parallel displayed comparable trends of disinfectant depletion.

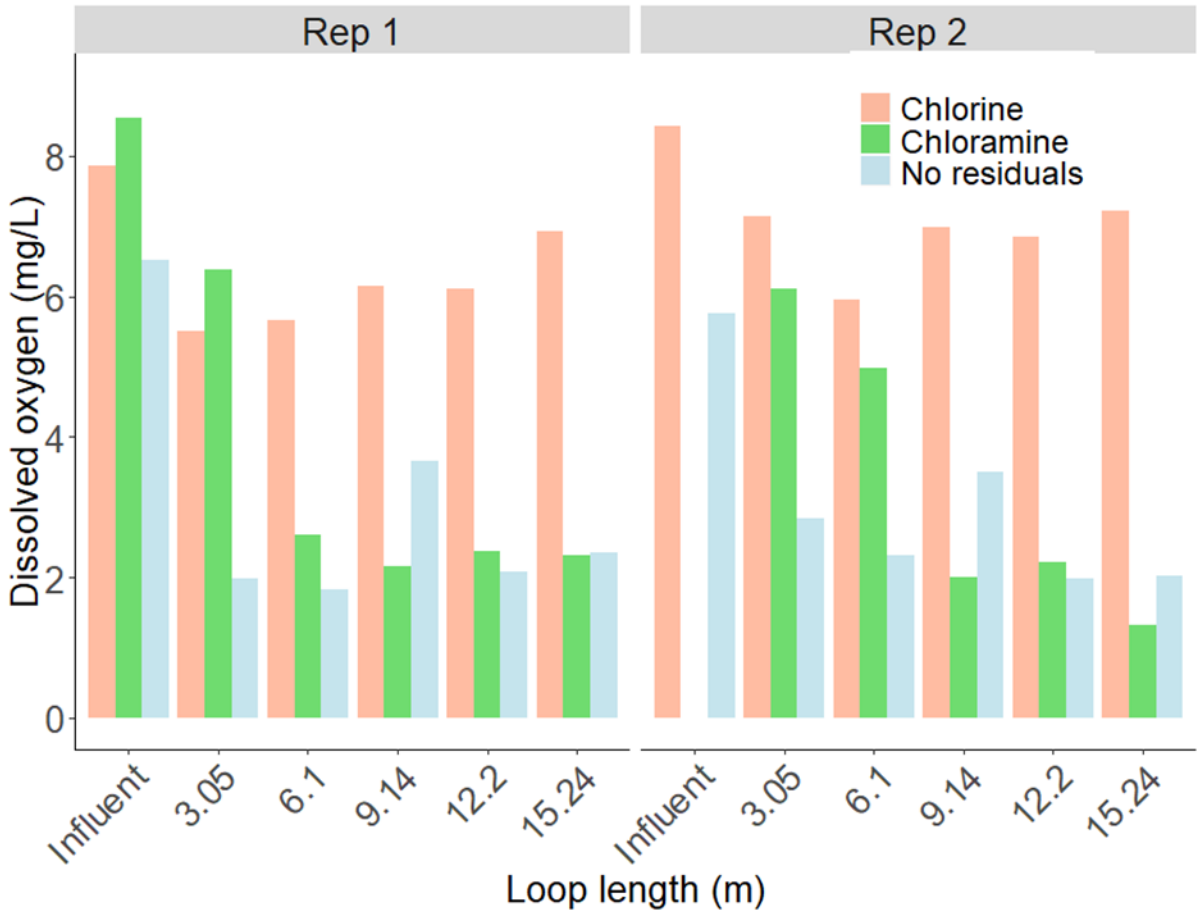


Figure 3-12 Comparison of dissolved oxygen profiles in duplicate Tube rigs (Rep 1, Rep2). Dissolved oxygen was consumed in the chloramine and no disinfectant residual Tube rigs, but remained high in the chlorine Tube RWDSs

3.3.4 Rapid chloramine decay and nitrification

In the present study, BART testing of the filtered and unfiltered conditions revealed very high levels of nitrifiers in the bulk water and sediment of the first pipe segment in the chloramine condition (Table 3-2). The semi-quantitative BART results were all over range in all four (filtered/unfiltered sediment and water) chloramine samples (>10^5 cfu/ml), which helps explain the observed rapid chloramine loss in this segment. This is consistent with an analogous study of simulated DWDSs using similar Pipe rigs, where higher than expected chloramine loss rates were attributed to nitrification (Masters et al., 2015). Another study also reported "super nitrification" and high levels of ammonia-oxidizing and nitrite-oxidizing bacteria near the entry point of a simulated RWDS (Wang et al., 2016). Bal Krishna et al. (2012) further showed that the presence of soluble microbial products, which were likely to be abundant in the water and sediment in the chloraminated condition in this study, can further accelerate the rate of nitrification. There were much lower levels of nitrifiers (10^3 cfu/mL) in the unfiltered no disinfectant residual Pipes. Even though the influent water underwent breakpoint chlorination to

remove ammonia, ammonia can be generated during microbial degradation of organic matter, e.g., as observed in lake sediment (Jones et al., 1982; Tatrai, 1986). No other conditions had detectable nitrifiers (**Error! Reference source not found.**).

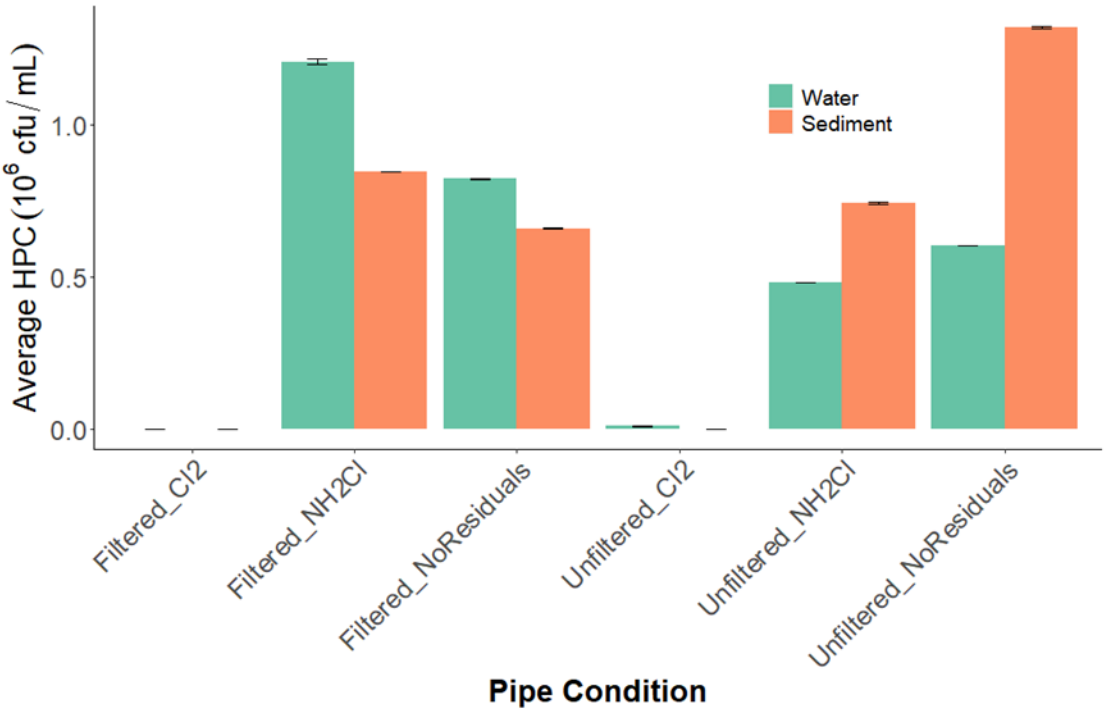
Figure 3-13 BART Nitrifier results indicated the presence of high levels of nitrifiers in the bulk water and sediment of the chloramine conditions. Development of pink color indicates the presence of nitrifiers. The intensity of the pink color is proportional to the potential nitrifiers population and is compared to a colorimetric chart to estimate the nitrifier abundance. The highest pink intensity approximates to 100,000 cfu/mL.

Table 3-2 Nitrifiers BART results comparing the BAC-filtered and unfiltered waters and three disinfectant conditions in the first pipe segment.

Approximate Nitrifiers Population (log_{10} cfu/mL)	**Unfiltered**		**BAC-Filtered**	
	Water	Sediment	Water	Sediment
Chlorine	*bd*	*bd*	*bd*	*bd*
Chloramine	5	5	5	5
No Residual	3	3	*bd*	*bd*

Note1. *bd* – below detection if there was no color change
Note 2. 7.5mL of water/sediment sample as per the product manual was used in each vial to semi-quantitatively evaluate the nitrifiers population. The upper detection limit is 10^5 cfu/mL.

HPCs measured in sediment and bulk water samples were very high in the first pipe segments of the chloramine and no residual Pipes, ranging from 5×10^5 to 1×10^6 cfu/mL. This is consistent with other indications of high biological activity in these conditions. Remarkably, no HPCs were detected in either chlorine bulk water or sediment, even on plates inoculated with undiluted samples. This confirmed that the superchlorination pretreatment without BAC-filtration, or a BAC-filtration with 4 mg/L chlorine residual, dramatically reduced biological regrowth. This was consistent with the low sediment VS/TS ratio (igure 3-14).



igure 3-14 HPCs in the bulk water and sediment of the Pipe Rigs enumerated after 5 days of incubation according to Standard Methods 9215. Error bars indicate standard deviation of triplicate plates.

3.3.5 Flask test comparing disinfectant demand for Pipe RWDS sediment versus biofilm

The disinfectant decay trends observed within the Pipe and Tube RWDSs represent a composite of reactions occurring in the bulk water, biofilm, and sediment under defined flow regimes. A batch flask test was designed to elucidate the relative contributions of each component to the total observed disinfectant demand. Chlorine decay in all test conditions generally fit first-order decay kinetics, with moderate to high R^2 values ranging from 0.79 to 0.98 (Table 3-3). In the filtered conditions with chlorine, the reactions in bulk water alone without sediment or biofilm resulted in about 50% residual loss in 24 hours. However, addition of swabbed biofilm achieved a 50% chlorine loss 4 – 9 times faster (i.e., 2.5 – 6 hours) indicating high free chlorine demand from biofilms (Figure 3-15). Addition of sediment from this system did not appreciably increase the rate of chlorine loss beyond that observed in the bulk water alone. Sediment in the chlorine pipes versus chloramine pipes contained 16.7-44.1% less VS (3.6-10 g/L vs 21.5-22.7 g/L) and a lower ratio of VS/TS (48-51% vs 79-83%) (Table 3-1), indicating less biologically active sediment on an absolute and relative basis. In contrast to the rapid chlorine loss, chloramine was extremely stable in the BAC-filtered bulk water condition, with more than 6 days required to achieve 50% residual loss. However, in the presence of biofilm or sediment, chloramine loss was 6.8 – 11 times faster, achieving 50% loss in 5.5 hours with sediment addition and in 20 hours with biofilm addition. For the corresponding unfiltered chloramine conditions, 10 – 10.5 hours was required to achieve a 50% residual loss in the bulk water or biofilm condition, but with sediment chloramine loss was about twice as fast, achieving 50% loss in only 5.5 hours. This is consistent with several indications of high biological activity in the sediments from the chloramine rigs and the observation that it was rich in nitrifiers that can cause a high chloramine demand. The above results also help explain the difference in relative stability of chloramine versus chlorine in the Tube versus Pipe rigs. The Tube rig design prevented accumulation of the sediment that was the major source of chloramine demand in the Pipe rig, but the high SAV ratio enhanced the effect of biofilm that was a major source of chlorine demand. The net result is that in the Tube rigs, chloramine was more stable than chlorine (**Error! Reference source not found.**a), whereas in the Pipe rigs the opposite trend was observed (Figure 3-8a).

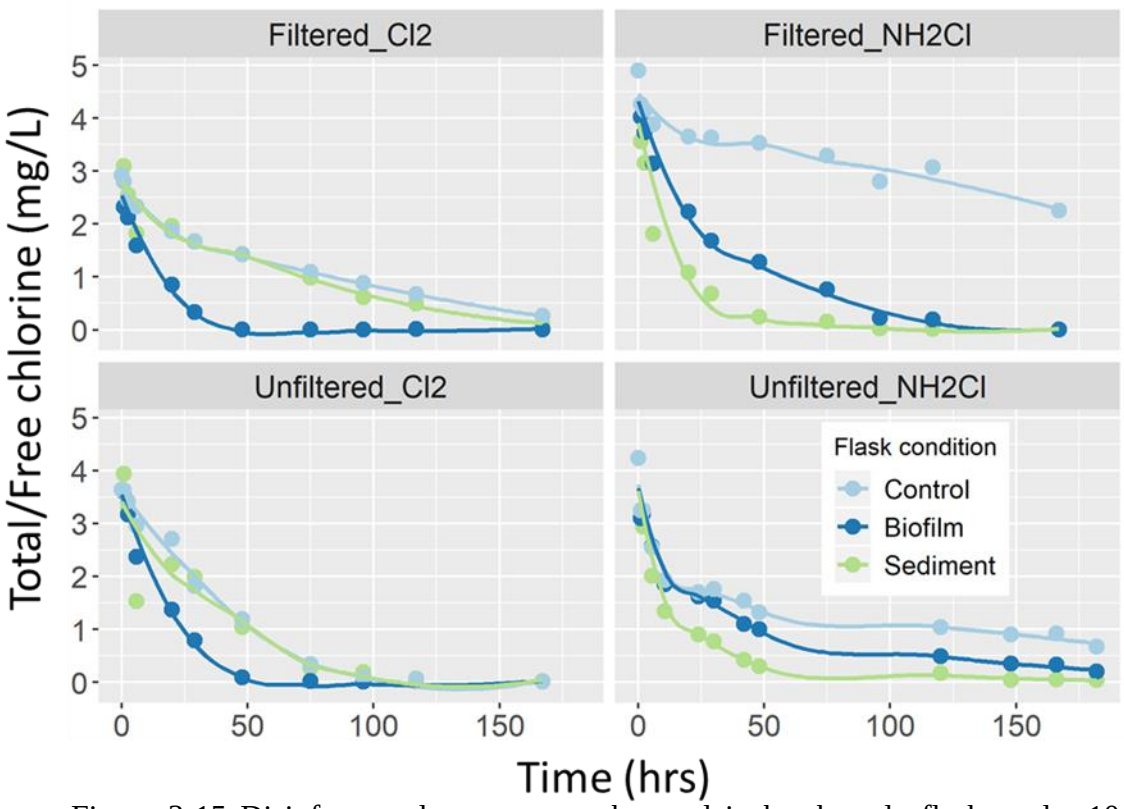


Figure 3-15 Disinfectant decay curves observed in batch-scale flask study. 10 mL sediment or a biofilm swab of 10 cm2 from the indicated Pipe RWDS conditions was added to 250 mL flasks containing a targeted initial chlorine or chloramine residual of 4 mg/L added to biofilm/sediments collected from the corresponding rig from which it was collected. Cl_2 = chlorine; NH_2Cl = chloramine; filtered = BAC-filtered influent; unfiltered = no BAC pre-treatment.

Table 3-3 First-order decay constants derived from the flask tests containing 10 mL sediment or 10 cm2 biofilm swabs of corresponding disinfectant conditions from the Pipe RWDS. The color from red to green corresponds to decreasing decay constants and slower decay rate.

First order decay constant (hr^{-1})	Chlorine			Chloramine		
	Sediment	Biofilm	Control	Sediment	Biofilm	Control
Unfiltered	-0.032 (R^2 = 0.96)	-0.054 (R^2 = 0.92)	-0.037 (R^2 = 0.97)	-0.024 (R^2 = 0.92)	-0.014 (R^2 = 0.95)	-0.007 (R^2 = 0.82)
Filtered	-0.014 (R^2 = 0.96)	-0.053 (R^2 = 0.79)	-0.012 (R^2 = 0.98)	-0.045 (R^2 = 0.97)	-0.027 (R^2 = 0.98)	-0.004 (R^2 = 0.89)

The only other lab-scale simulated RWDS study comparing chlorine versus chloramine disinfectant residuals used a Tube rig design with the same diameter tubing (0.318-cm) and reported very rapid free chlorine loss of 0.04 – 3.6 mg/L (average 1.23 mg/L) with just a 4-min hydraulic residence time (Liu et al., 2014). In that study, the chloramine loss rates were lower, at 0.09 – 1.23 mg/L (average 0.54 mg/L), in agreement with the results presented herein (Jjemba et al., 2010).

3.3.6 Formation of DBPs in the Pipe Rigs

DBP formation in chlorinated and chloraminated distribution systems is a topic of ongoing research in DWDSs (Sirivedhin and Gray, 2005). Although there are no DBP regulations for reclaimed water (Snawar et al., 2017), regulated drinking water DBPs were examined in the six Pipe RWDS reservoirs to assess the maximum DBP-generating potential in each condition After superchlorination in the case of unfiltered chlorine and breakpoint chlorination for all other conditions, and then 30 minutes reaction time with the targeted residual type and dose, all DBPs exceeded the 80 ppb total trihalomethane maximum contaminant level drinking water standard (US Environmental Protection Agency, 2012). The highest levels of DBPs were measured in the two Pipes with chlorine residuals, with chloroform dominating. Levels of regulated DBPs in the chloramine Pipes and in the no disinfectant residual Pipes were comparable. Chloraminated DBPs were not measured in this study.

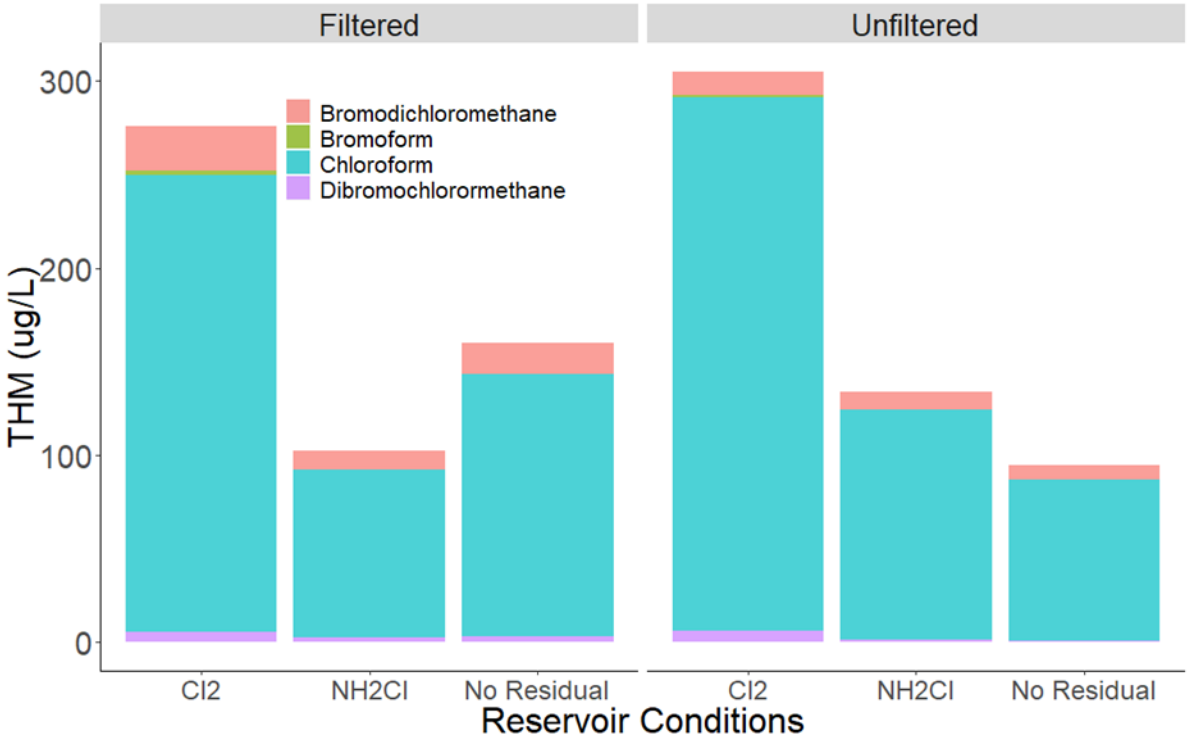


Figure 3-16 Illustrative trihalomethane (THM) levels in the six reservoirs, 30 minutes after adding chlorine or chloramine. As there were no detectable THMs in the treatment plant effluent, THMs in the condition with no residual reflect prior

3.4 CONCLUSION

Pipe versus Tube RWDS rigs were employed to represent extremes in hydraulic regimes and potential to accumulate sediment. Pre-treatments, disinfectant types, and hydraulic design controlled the amount and nature of sediments that accumulated. Sediment deposits in systems with chloramine or no disinfectant residual were highly biologically reactive compared to those in systems with chlorine, as indicated by VS/TS ratio, HPCs, disinfectant and DO decay.

The relative importance of sediment versus biofilm in Pipe versus Tube RWDS rigs and dominance of biofilm surface area determined the relative stability of chlorine versus chloramine residual. Specifically, chloramine was less stable than chlorine in systems with more sediment and less biofilm per unit volume, whereas the converse was true in the Tube systems, which had relatively little sediment and very high bio-foulant per unit volume of water.

While it is not possible to precisely replicate a real-world RWDSs in the lab, the designs implemented in this study can provide insight into expectations representing extremes encountered in real-world systems. Findings here can aid in identifying management strategies that minimize potential for biofouling and sediment accumulation in RWDSs, and improve understanding of how distribution of reclaimed water could alter its suitability at the point of use.

ACKNOWLEDGEMENTS

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CHAPTER 4: METAGENOMIC ANALYSIS OF BAC-FILTRATION, DISINFECTION, AND TEMPERATURE EFFECTS ON WATER QUALITY IN SIMULATED RECLAIMED WATER DISTRIBUTION SYSTEMS

Ni Zhu, Sudeshna Ghosh, Laurel Strom, Amy Pruden, Marc A. Edwards

ABSTRACT

The distinct characteristics of reclaimed water versus potable water has important implications for design and operation of reclaimed water distribution systems (RWDSs). Here we operated six simulated RWDSs in parallel under controlled conditions to determine the effects of feed water treatment (with and without biologically-active carbon filtration) and residual disinfectants (chlorine, chloramines, or no residual) on the distributed water quality. Following a six-month acclimation period, the experimental conditions were implemented over 2 years of operation, during which time temperatures of 14°C→22°C→30°C→22°C→14°C were imposed to simulate seasonal variation. Water and biofilms were sampled for complementary physiochemical and metagenomic profiling after operating at each temperature for a period of 3-4 months, over a range of water ages (0-d, 1-d, 2.5-d and 5-d). When temperature was elevated to 22°C and above, RWDSs became vulnerable to nitrifier growth, observed in terms of accelerated chloramine decay and increased in nitrifier taxonomic markers and functional genes, Contrary to the conventional experience in potable water applications, chlorine generally became more persistent than chloramine from the first 22°C phase onwards. Better persistence of chlorine was accompanied by increased biological stability as measured by lower cell counts and reduced dissolved oxygen demand. BAC-filtration initially improved water quality, but benefits of biofiltration were generally over the years of operation. Taxonomic and functional metagenomic profiles served as an indicator of relative effects of operating conditions on microbial communities, revealing general similarities between chloramine and the condition with no residuals, and distinctions of the condition with chlorine. The long-term operation strategy employed here enabled evaluation of individual management practices, and how they interacted and changed as the distributions systems experienced temperature shifts and aged.

4.1 INTRODUCTION

Climate change, extreme weather patterns and urbanization are spurring use of alternative and more sustainable water resources, including reclamation of treated wastewater effluent (Wada and Bierkens, 2014). Reclaimed water can have distinct physiochemical and microbial water quality properties compared to conventional drinking water, including higher levels of nutrients, disinfectant demand, and elevated levels of microbes and biomass (Garner et al., 2016; Jjemba et al., 2010a). Reclaimed water distribution systems (RWDSs), i.e., systems designed to carry non-potable water, are not typically operated in the same manner as drinking water

distribution systems (DWDSs), as intermittent demand and extremely prolonged stagnation are much more common, creating further challenges to achieving water quality targets at the point of use. Despite these known distinctions, there is a lack of long-term studies that systematically examine the effects of typical treatments on water quality in RWDSs.

Non-potable reuse of reclaimed water, including irrigation, toilet flushing, landscaping and industrial cooling, accounts for more than 50% of total reuse in the United States (Bryk et al., 2011). The California State Water Resources Control Board requires reclaimed water to be "oxidized" and "disinfected" for most agricultural reuse purposes, with an additional filtration step recommended for broader surface reuse applications (California State Water Resources Control Board, 2018). Granular/biological activated carbon (GAC/BAC) filtration and disinfection are amongst the most commonly applied final treatments for non-potable reuse. GAC removes some organic compounds via adsorption (Sbardella et al., 2018) and still others via biodegradation (Jin et al., 2013; Reungoat et al., 2012).

RWDSs face many of the same issues as DWDSs, including deterioration of water quality along the pipes due to depletion of disinfectant residuals and biological regrowth (Garner et al., 2018a, 2016; Potgieter et al., 2018; Prest et al., 2016). In DWDSs, two most common disinfectants, chlorine and chloramine, each have relative strengths and weaknesses, dependent on temperature, source water quality, disinfection by-products, distribution system materials, water use patterns and other factors [236–](Zhang and Edwards, 2009). While chlorine is a stronger oxidant than chloramine (Deborde and von Gunten, 2008; Fisher et al., 2017a), the residual is usually lost more quickly and forms more regulated disinfection-byproducts (Li et al., 2019; Plewa et al., 2017). In contrast, chloramine is less reactive, but forms fewer regulated disinfection-byproducts and tends to be more persistent during distribution, especially in systems with unlined iron pipes (Li et al., 2019; Norton and LeChevallier, 1997; Zhang and Edwards, 2009). However, in circumstances with rapid nitrification in potable water systems, chloramine decay rates can sometimes equal or exceed those of free chlorine (Lieu et al., 1993; Sathasivan et al., 2009; Zhang and Edwards, 2009). It is critical that the relative advantages and disadvantages of these two common disinfectants are rigorously evaluated in terms of their application in RWDSs, especially for higher temperatures encountered in hot climates that tend to be early-adopters of sustainable water strategies (Garner et al., 2016).

Only a few short-term (< 12 months) studies have examined the effects of different reclaimed water treatment options on finished water quality (Thayanukul et al., 2013; Weinrich et al., 2010) and relatively little is known about how water quality changes in the RWDS and at the point of use as may impact human health (Garner et al., 2016). Recent field-scale studies have revealed several concerns with respect to water quality delivered by RWDSs (Acharya et al., 2016; Garner et al., 2018a; Jjemba et al., 2015, 2010a; Thayanukul et al., 2013); however, it is impossible to unambiguously identify the key controlling variables in field-scale studies.

The goal of this study was to advance understanding of the treatment processes and operational factors that shape the chemical and biological water quality delivered by RWDSs through controlled studies of laboratory-scale RWDSs. This was achieved through parallel operation of simulated RWDSs comparing effects of BAC filtration and disinfectant residual conditions (chlorine, chloramines, or no residual) as well as seasonal temperature variation. Concomitant water chemistry and metagenomic analysis of bulk water and biofilm under these combinations of conditions and at different water ages provided insight into the interplay between microbial and physicochemical aspects of reclaimed water quality at the point of use.

4.2 METHODS

4.2.1 Design and operation of simulated RWDSs

Final effluent before UV-disinfection, collected from a local municipal wastewater treatment plant (Virginia, USA), was used as the source water to simulate reclaimed water before pretreatment. The source water was collected twice per week and stored at 4 °C for a maximum of 3 days before treatment to minimize changes in the source water quality. Two levels of treatment were sequentially performed on the source water: BAC filtration and chlorine-based disinfection. BAC-filtration was conducted by re-circulating the source water through two filters (Culligan D-20A Drinking Water Filter) connected in series for 60 hours. Three disinfectant conditions: free chlorine, chloramine and breakpoint chlorination (no residual), were subsequently applied to both the filtered and unfiltered source water to achieve a stable disinfectant residual target of 4 mg/L free chlorine for the free chlorine conditions, 4 mg/L total chlorine for the chloramine conditions, and less than 0.2 mg/L free chlorine residuals for the breakpoint chlorination conditions. To achieve a stable 4 mg/L free chlorine residual in the unfiltered water, the source water was dosed with 8 mg/L free chlorine and allowed to sit for 30 hours to destroy the high chlorine demand. The pre-chlorinated water was readjusted to 4 mg/L after 30 hours. The six pretreated feed water reservoirs; including chlorine, chloramine and no residual (3 levels of disinfection), with and without BAC filtration (2 levels of filtration) for each, were maintained at 4°C before the water was fed to the simulated RWDSs. Influent feed reservoirs were changed every 30 hours. A detailed characterization of the influent water quality including impacts of BAC are reported in a prior study (Zhu et al. submitted).

The RWDSs were constructed from three consecutive 4-in. diameter PVC pipe segments connected with 3/8-in. diameter PVC tubing. The pipe diameter was selected to achieve realistic pipe surface area to volume ratio and the pipe length was selected to achieve the targeted hydraulic residence times. The smaller diameter tubing connecting each larger pipe segment created zones with slightly higher flow velocity (8.66×10^{-6} m/s in the main pipe sections and 9.85×10^{-4} m/s in the biofilm tube sections) and also facilitated easy sampling of biofilm by snipping segments as desired during operation without disrupting biofilm in the larger pipe segments. Four sample ports were located on each pipe corresponding to calculated water ages of 0-d (P0), 1-d (P1), 2.5-d (P2) and 5-d (P5) (**Error! Reference source not found.**a). All six RWDSs were initially acclimated with unfiltered source water without disinfectants for six months at room temperature (25°C) to allow the establishment of a consistent and mature biofilm, as previously suggested by Zhou et al. (2016) and Fish et al. (2015). Afterward, each RWDS was fed water from the designated reservoir and operated for 4-5 months at each target temperature to acclimate the RWDSs prior to sampling. During the second 22°C phase, the first segment of unfiltered chloramine RWDS had to be replaced due to leaking and was operated for 2 months before sampling in Nov 2016 to allow acclimation (**Error! Reference source not found.**b). The temperature cycle (14°C→22°C→30°C→22°C→14°C) was designed to simulate a range of relevant ambient temperatures.

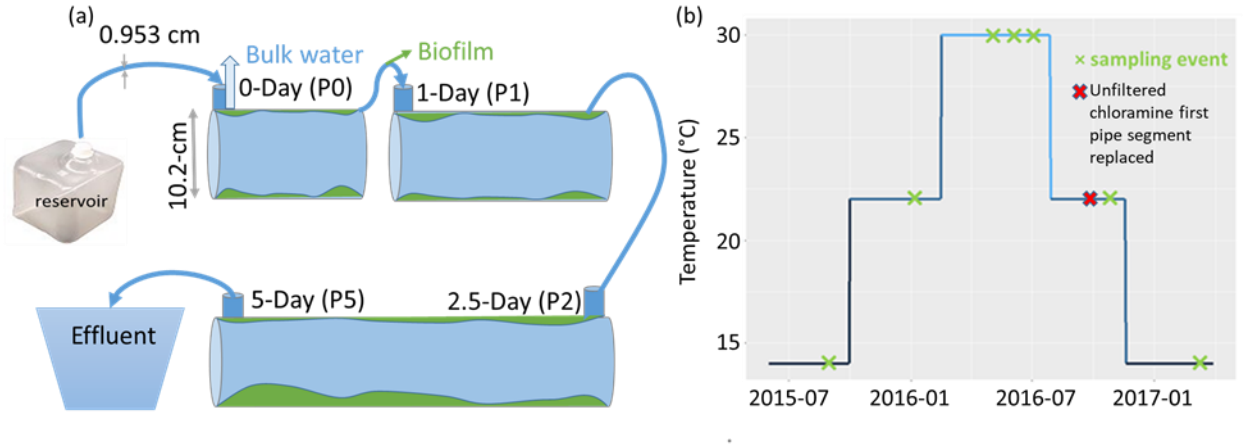

Figure 4-1 Simulated RWDS rig design, temperature phases, and operational timeline. (a) Each rig consisted of three 10.2 cm (4 in.) diameter pipe segments connected with 0.953 cm (3/8 in.) in diameter PVC tubes and was fed influent from a reservoir with a designated pretreated feed. Four sample ports, P0, P1, P2 and P5, were located along the length of the rigs. (b) The rigs underwent five temperature phases 14°C→22°C→30°C→22°C→14°C by adjusting ambient temperature. Prior to operating the rigs at the designated conditions, all rigs were acclimated at 25 °C for six months by running untreated wastewater effluent

4.2.2 Water chemistry and microbial sampling and analysis

At the end of each temperature phase, paired comprehensive water chemistry and biological sampling were conducted, with three independent water chemistry and biological sampling events at the end of the 30°C phase to assess reproducibility of the measurements (**Error! Reference source not found.**b).

Monitored water chemistry parameters included free chlorine and total chlorine residuals (HACH DR 2700 Spectrophotometer Method 8021), dissolved oxygen (DO) (Orion Star A 326 DO meter), pH (Oakton pH 110), ammonia (Hach Method 8155), nitrite and nitrate (Dionex® DX-120 Ion Chromatograph), and total organic carbon (TOC) (Sievers 5310C Laboratory TOC Analyzer). Total cell counts (TCC) were measured with a BD Accuri C6® flow cytometer (Vital et al., 2012) starting with the first 22°C phase.

For microbial analysis, 500 mL of bulk water was collected from each sample port and 3-cm lengths of the 3/8-in. diameter PVC connecting tubing (external surfaces sterilized with ethanol) were clipped in duplicate for biofilm analysis. Bulk water was collected in sterile bottles from each sample port and disinfectant residual was immediately quenched with sodium thiosulfate prior to splitting into duplicate 250 mL samples and filtering through a sterile 0.22-µm mixed cellulose ester filter (Millipore, Billerica, MA). DNA was extracted using the FastDNA® SPIN Kit (MP Biomedical, Inc., Solon, OH) and FastPrep® Instrument (MP Biomedical, Inc., Solon, OH) and samples were stored at -80°C. A total of 84 DNA extracts were selected for shotgun whole-genome metagenomic sequencing, in three separate sequencing runs carried out by the Biocomplexity Institute of Virginia Tech (Blacksburg, VA). The first two runs were sequenced with the Illumina HiSeq 2500 using the rapid run mode with 2×100 bp pair-ended reads. The last run was sequenced with the Illumina NextSeq with 100 bp pair-ended reads. Change in sequencing platform was unavoidable due to evolving next-generation DNA

sequencing technology and appropriate normalization approaches were applied to account for potential differences. Sequencing libraries for all samples were prepared using the Illumina Nextera XT DNA library Prep Kit (San Diego, CA). The sample matrix of the selected sequencing samples (detailed in Table 4-1) included two levels of water age – P1 or P2 samples for low water age and P5 for high water age and three temperature events - the first 14°C and 22°C unfiltered at all three disinfectant conditions and three independent sampling events at 30°C at all three disinfectant conditions, both unfiltered and filtered.

4.2.3 Data analysis and statistical tools

Raw metagenomic forward and reverse reads were merged with FLASH (Magoc and Salzberg, 2011). Merged reads were trimmed and filtered by TRIMMOMATIC to remove low quality reads according to default parameter (Bolger et al., 2014). Taxonomy annotation of the merged reads were performed via the MetaStorm platform (Arango-Argoty et al., 2016) using the MetaPhlAn2 database with best-hit-alignment and identity > 90% for taxonomic mapping (Truong et al., 2015). Taxonomic annotations were performed at the genus-level and normalized using the Reads per Kilobase per Million Mapped Reads (RPKM) to account for variations in sequencing depth. Functional profiling of the metagenomic reads was performed using the HUMAnN2 pipeline (Franzosa et al., 2018). Forward and reverse reads were trimmed using TRIMMOMATIC and mapped to a universal protein reference database, UniRef50 (Suzek et al., 2007), using a translated search alignment method with >50% sequence identity over 90% of the translated query sequence and 50% of the translated subject sequence. Functional gene levels were expressed in copies per million and included an additional step to remove low abundance functional gene families to reduce variations between HiSeq and NextSeq sequencing platforms (**Error! Reference source not found.**).

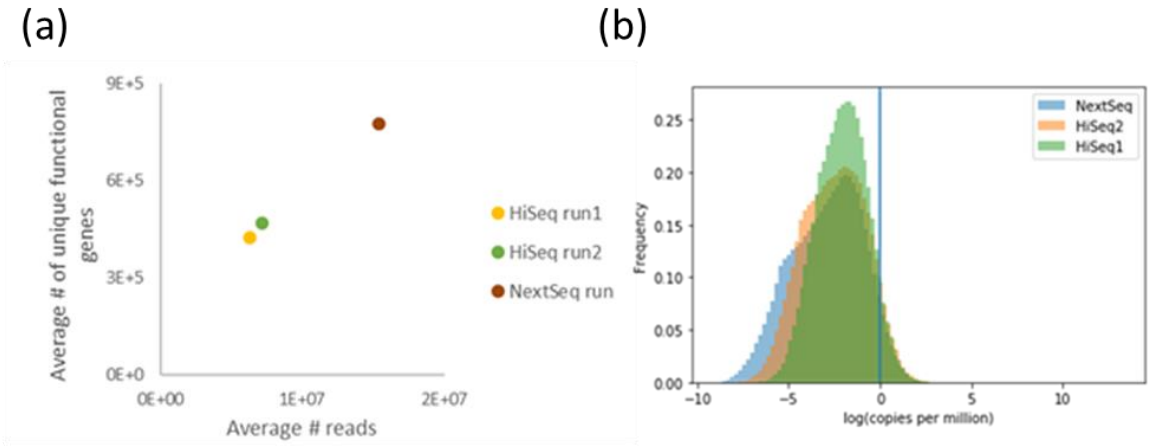

Figure 4-2 (a) Plot of average number of unique UniRef50 functional gene families identified vs. the average the number of reads in the two HiSeq and one NextSeq runs. (b) Histograms of log of mean abundance of each unique functional gene family in copies per million. The platforms diverged below a cutoff value (vertical blue line). For comparison of functional gene families across sequencing platforms. Only functional genes with abundances above the cutoff value were considered.

Water chemistry profiles were plotted in JMP Pro 14.0 and an effect test in standard least squares method was applied to the water chemistry matrix to identify significance of tested parameters. Wilcoxon rank sum and Kruskal-Wallis tests were used to determine differences in nitrifier genus marker gene profiles across water chemistry conditions. One-way and crossed two-way analysis of similarities (ANOSIM) from Bray-Curtis resemblance matrix was conducted in PRIMER_E (Version 6.1.13) to determine dissimilarity in microbial communities across experimental conditions. Non-metric multidimensional scaling (NMDS) plots of metagenomic taxonomy annotations were produced in R (Version 3.4.1) using the Vegan package (Oksanen et al., 2019). Differences in metagenomic functional gene family composition were analyzed by performing an unsupervised transformation of the functional gene dataset to a high-dimensional sparse representation using Random Trees Embedding (number of trees = 500, maximum depth of each tree = 5, minimum number of samples required to split an internal node = 5, minimum number of samples required to be at a leaf node = 5) followed by Truncated SVD (Halko et al., 2011; Geurts et al., 2006) using scikit-learn v0.21.3 in Python v3.7.3 (Pedregosa et al., 2012).

4.3 RESULTS & DISCUSSION

4.3.1 Study Overview: Unique Insights Gained from Simulated RWDSs

This study employed a unique simulated RWDS design that enabled sampling over a range of relevant water ages (0-d, 1-d, 2.5-d and 5-d) and sacrificial analysis of biofilms formed under higher flow velocity conditions in connecting tubes, along with the ability to control ambient temperature to simulate seasonal variation. This made it possible to compare effects of feed water treatment (without and with BAC filtration) and residual disinfectant (chlorine, chloramines, or no residual) in parallel (i.e., six RWDSs). Testing two of the temperatures (14°C and 22°C) at the beginning and near the end of the experiment, allowed comparison of ambient temperature effects versus aging of the biofilm and pipe network. Comprehensive physicochemical analysis was performed at all time points, along with metagenomic analysis over a cross-section of key conditions (Table 4-1), providing insights into the interplay of water chemistry and microbial activity in determining the quality of water delivered by RWDSs.

Table 4-1Summary of samples from the simulated RWDSs rigs selected for metagenomic sequencing. There were three sequencing events. The first two sequencing events focused on the unfiltered samples and the third sequencing event included the filtered samples. Sampling ports (P1-P5) refer to those defined in Figure 4-1. W= bulk water sample; B= biofilm sample.

	First sequencing		Second sequencing		Third sequencing		
Condition	**14 °C-1**		**22 °C-1**	**30 °C-1**	**30 °C-2**		**30 °C-3**
Unfiltered Chlorine	P2_W P5_W P2_B P5_B	P2_W P5_W P2_B P5_B	P2_W P5_W P2_B P5_B	P2_W P5_W P2_B P5_B	P2_W P5_W P2_B P5_B		P2_W P5_W P2_B P5_B
Unfiltered Chloramine	P1_W P5_W P1_B P5_B		P2_W P5_W P2_B P5_B		P2_W P5_W P2_B P5_B	P2_W P5_W P2_B P5_B	P2_W P5_W P2_B P5_B
Unfiltered No Residuals	P1_W P5_W P1_B P5_B		P2_W P5_W P2_B P5_B		P2_W P5_W P2_B P5_B	P2_W P5_W P2_B P5_B	P2_W P5_W P2_B P5_B
Filtered Chlorine							P2_W P5_W P2_B P5_B
Filtered Chloramine					P2_W P5_W P2_B P5_B		P2_W P5_W P2_B P5_B
Filtered No Residuals					P2_W P5_W P2_B P5_B		P2_W P5_W P2_B P5_B

Disinfectant decay in RWDSs

Chlorine profiles were significantly affected by temperature ($p<0.0001$), water age ($p<0.0001$), and BAC-filtration ($p=0.0086$) based on the standard least squares effect test, while temperature ($p<0.0001$) and water age ($p<0.0001$) significantly affected chloramine profiles. In the following subsections, we first focus on the three RWDSs receiving unfiltered water, describing how the disinfectants behaved as a function of water age under the different temperature regimes. The effects of BAC-filtration of the influent are subsequently examined.

RWDSs receiving unfiltered water

30°C – 30°C was by far the worst case scenario among the three temperature conditions in terms of loss of disinfectants. At this higher temperature, chloramine decayed faster than chlorine (Figure 4-3). Triplicate sampling events at 30°C indicated consistent disinfectant

residual profiles. Chloramine levels dropped to 0.05 ± 0.03 mg/L versus 0.35 ± 0.49 mg/L for chlorine at the end of the first pipe segment (P1). At the end of the second pipe segment (P2), chloramine levels averaged 0.05 ± 0.02 mg/L while corresponding chlorine levels were still slightly higher at 0.17 ± 0.16 mg/L. Both disinfectants were nondetectable (< 0.02 mg/L) at the end of the third pipe segment (P5).

14°C – Both chlorine and chloramine residuals had greatest persistence at the lowest temperature tested. During the first 14°C phase, higher levels of chloramine than chlorine were observed at both P2 and P5. There was 1.5 mg/L of chloramine versus 0.14 mg/L of chlorine remaining at P2, whereas at P5, chloramine dropped to 0.11 mg/L and chlorine to 0.06 mg/L (Figure 4-3). During the second 14°C phase, while chloramine levels were still higher than corresponding chlorine levels, differences were less striking, with 1.16 mg/L of chloramine and 0.72 mg/L of chlorine remaining at P2 and 0.47 and 0.41 mg/L of chloramine and chlorine remaining at P5.

22°C - Chloramine was also more persistent than chlorine during the first 22°C phase, with 0.82 mg/L chloramine and 0.04 mg/L of chlorine remaining at P5. But during the second 22°C phase, persistence of chlorine and chloramine were reversed, with a chlorine residual of 0.44 mg/L and chloramine residual of 0.08 mg/L at P5. This was a key indicator of changes to the RWDSs as they aged in a manner that impacted water quality and relative disinfectant stability.

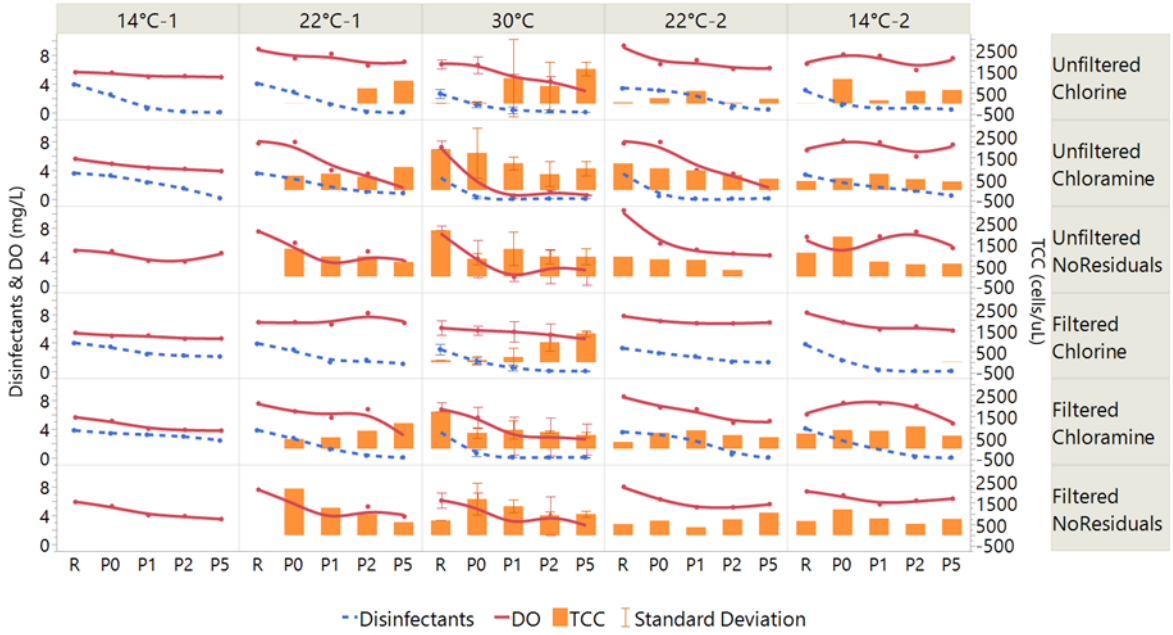

Figure 4-3 Disinfectant residuals, DO, and total cell counts (TCC) measured along the length of the six RWDSs at the end of each temperature phase. The x-axes represent the feed reservoir and the four sampling ports (**Error! Reference source not found.**). 30°C data are mean and standard deviation of three sequential sampling events, other measurements are single measurements. Disinfectant residuals data represent free chlorine in the two chlorinated RWDSs and total chlorine in the two chloraminated RWDSs. TCC measurements were initiated at the first 22°C phase and therefore are not available for the first 14°C phase.

RWDSs receiving BAC-filtered water

BAC-filtration is a means of improving biostability (Terry and Summers, 2018) and potentially reducing disinfectant losses. During the 30°C temperature phase, disinfectant losses were only marginally less in RWDSs receiving filtered versus unfiltered water (Kruskal Wallis test, $p = 0.26$) (Figure 4-3). The benefits of BAC filtration were most pronounced during the first 14°C phase. High chloramine residuals persisted throughout the systems, with 3.21 mg/L of chloramine at P1 and 2.39 mg/L at P5. The corresponding chlorine levels were 2.39 mg/L at P1 and 2.08 mg/L at P5. However, during the second 14°C phase, much lower disinfectant residuals were measured and, remarkably, residual levels were lower in filtered versus unfiltered conditions. Specifically, 0.03 mg/L free chlorine and 0.01 mg/L chloramine were present at P5 in the RWDSs under filtered conditions, compared to 0.41 mg/L free chlorine and 0.47 mg/L chloramine under unfiltered conditions. During the 22°C temperature phases, chlorinated systems still benefited from BAC-filtration, as observed by lower residual losses in filtered systems compared to unfiltered chlorinated systems. However, benefits of BAC-filtration were lost in chloraminated systems even during the first 22°C phase, with 1.03 mg/L of free chlorine at P5 in the RWDS fed filtered chlorinated water compared to only 0.03 mg/L of chloramine at P5 in the RWDS fed filtered chloraminated water. During the second 22°C phase, chloramine levels remained low and dropped to 0.03 mg/L by P5, while 1.27 mg/L of free chlorine was measured at P5 in the filtered condition.

4.3.2 Biological Stability in the RWDSs

Four key indicators of biological stability were quantified: (a) TOC - a measure of organic carbon used by heterotrophic microbes; (b) DO loss - a measure of aerobic microbial respiration (here "DO loss" is defined as difference from the Reservoir to P5); (c) TCC - a direct measure of microbial biomass in bulk water; and (d) shifts in ammonia, nitrite, and nitrate driven by nitrifiers and/or denitrifiers. Comparison of the chlorine and chloramine conditions to the no residual conditions provided a means to distinguish relative effects of disinfectants in suppressing microbial activity.

(a) TOC: TOC levels of 6.2 ± 0.80 mg/L were comparable to an average of 7.0 mg/L reported in our prior field study of full-scale reclaimed water systems (Garner et al., 2018). TOC levels in all three RWDSs receiving unfiltered water remained relatively uniform across water age and temperature, with a mean of 5.6 ± 1.0 mg/L (Figure 4-4) BAC-filtration was effective at reducing influent TOC to 1.3 ± 0.76 mg/L in the reservoir. In the three RWDSs receiving BAC-filtered water, TOC was stable across all water ages along the systems, but the influent level did vary throughout the study due to seasonality 41–43 and slight changes in performance of BAC filtration in removal of organic matter 40 (Figure 4-4).

(b) DO loss: DO profiles in the simulated RWDSs exhibited distinct trends across the six RWDSs (Figure 4-3). Overall, chlorinating the feed source and BAC-filtration reduced DO loss. DO losses were highest at 30°C, with greater loss in RWDSs receiving unfiltered versus BAC-filtered water. In terms of effects of disinfectant, at 30°C DO loss was greatest in RWDSs with chloraminated feed, followed by no residual and chlorinated systems. DO loss in unfiltered and filtered chlorinated conditions averaged 3.82 ± 1.30 mg/L and 1.56 ± 1.62 mg/L, respectively. Corresponding losses were 6.43 ± 0.81mg/L

and 4.16 ± 2.54 mg/L in the chloraminated RWDSs receiving unfiltered and filtered water and 5.26 ± 1.71 mg/L and 3.19 ± 1.22 mg/L in the no residual RWDSs receiving unfiltered and filtered water. DO losses were lowest at 14°C in all six RWDSs, with a maximum loss of 2.54 mg/L. At 22°C, DO losses were still low in RWDSs fed chlorinated, unfiltered and filtered, water with 0.085 - 3.13 mg/L loss. In contrast, both chloraminated and no residual systems experienced greater overall losses of 4.24 – 6.3 mg/L in unfiltered and 2.42 - 4.87 mg/L in BAC-filtered systems.

(c) TCC: Chlorine was effective in maintaining low/undetectable TCC at 14°C and 22°C, but at 30°C there was a consistent trend of increasing TCC from the reservoir to P5 in both filtered and unfiltered chlorinated systems. TCC levels in the 30°C chlorinated systems were higher than those measured in the chloramine and no residual RWDSs at P5 ($p=0.0425$) (Figure 4-3). In contrast, RWDSs receiving feed that was chloraminated or that contained no residual consistently yielded detectable levels of TCC at all temperatures. Aggregating measurements across temperature conditions, TCC in the chlorinated RWDSs were found to be significantly lower than in the chloraminated (Kruskal Wallis test, $p=0.00011$) and no residual (Kruskal Wallis test, $p=1.1\times105$) RWDSs, while chloramine and no residual TCC were comparable (Kruskal Wallis test, $p=0.438$). BAC-filtration only appeared to be effective at keeping TCC levels low in chlorinated systems at 14°C and 22°C, with no detectable TCC in the filtered condition.

(d) Ammonia, nitrite, and nitrate levels: Nitrification is a well-documented problem in DWDSs, as the nitrite formation removes chloramine, especially in the warmer summer months. In the RWDSs, ammonia loss was found to be consistent with the loss of chloramine, with a 4:1 chloramine to ammonia mass ratio as of N at each sample port (Figure 3). Ammonia loss increased as temperature increased and was most pronounced at 30°C. Nitrite, which reacts directly with any chloramine that is present, only accumulated between the reservoir and P5 during the first 22°C phase in both BAC-filtered and unfiltered conditions (Kruskal Wallis $p = 0.00213$, compared to all other temperature phases). For all other temperature phases, nitrite was low/non-detectable and did not vary along the length of the distribution systems. Final nitrate levels, measured in all six RWDSs, averaged at 9.17 mg/L ± 2.51 mg/L across all RWDSs and was not strongly affected by experimental conditions (Figure 4-4).

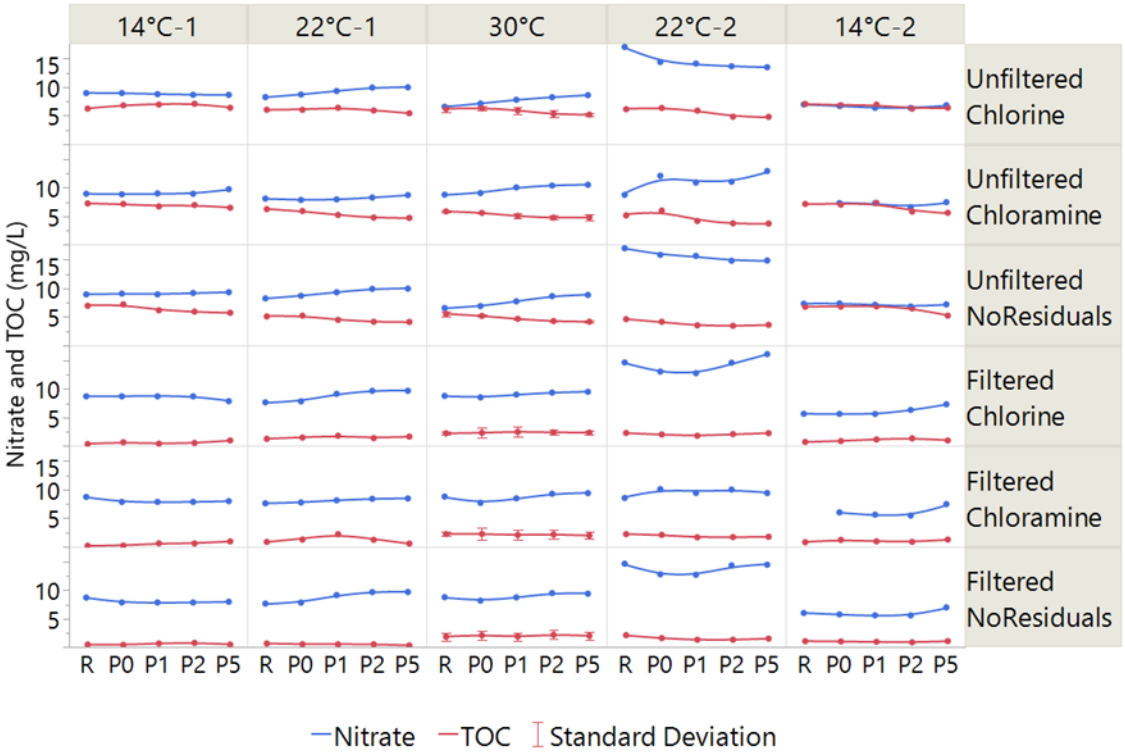

Figure 4-4 Levels of nitrate and TOC in the simulated RWDSs at each temperature phase. TOC at 30°C are mean and standard deviations of the three independent water chemistry sampling measurements.

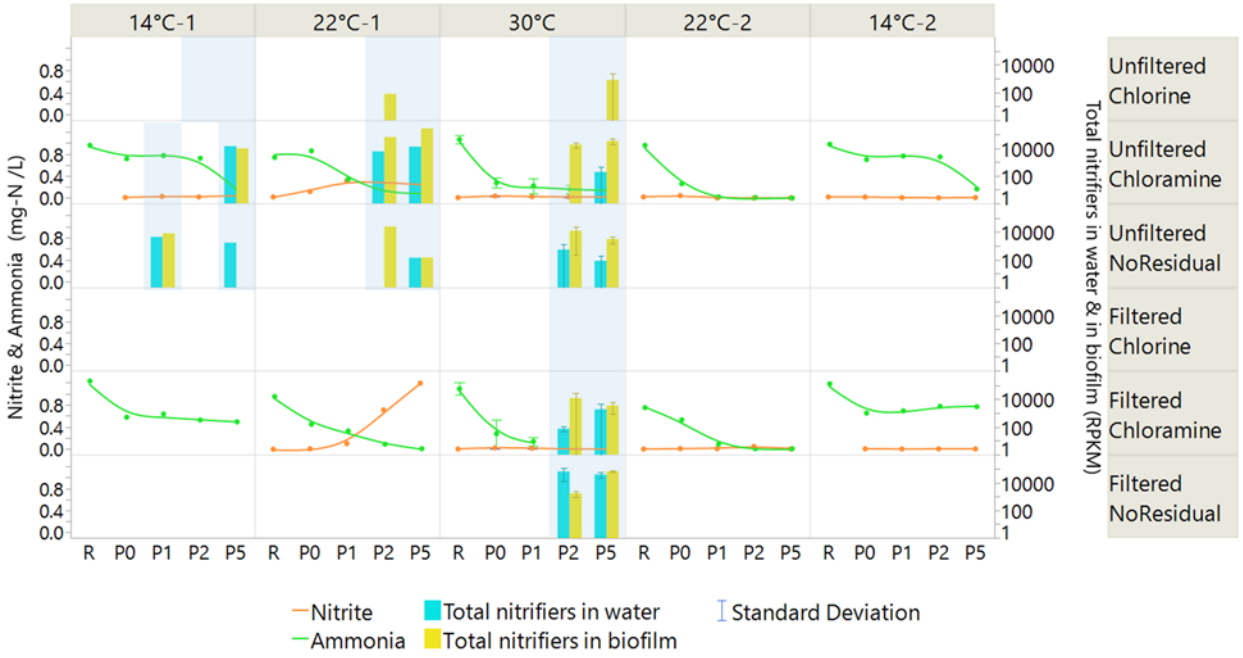

Figure 4-5 Ammonia and nitrite measurements in the two chloramine RWDSs for each temperature phase and corresponding number of reads annotated as nitrifiers in bulk water and biofilm in the six RWDSs. Sampling locations and time points where metagenomic data were available are shaded. The x-axes represent the reservoir (R) and the four sample ports (P0, P1, P2, P5) (Figure 4-1). Nitrite and ammonia data at 30°C are mean and standard deviation of three sequential sampling events, other measurements are single measurements. Colored bars indicate the relative abundance of reads (Reads Per Kilobase of transcript per Million mapped reads (RPKM)) taxonomically-annotated as known nitrifier genera using the MetaPhlAn2 database (Truong et al., 2015). Nitrifier abundance data at 30°C are mean and standard deviation of three independently sequenced samples. Details of samples included in each of the three sequencing runs is reported in Table 4-1.

4.3.3 Taxonomic and functional gene-based indicators of nitrification and denitrification

Shotgun metagenomic sequencing served to comprehensively profile taxonomic and functional gene indicators of nitrifiers. At the taxonomic level, five known nitrifier genus marker genes curated in the MetaPhlAn2 database were screened (Figure 4-6). Concomitant with the nitrification trends observed based on increase in nitrite and a decrease in ammonia during the first 22°C phase, total nitrifier genera abundance also spiked during the first 22°C phase and were an order of magnitude higher than nitrifier genera abundances during the first 14°C phase (Figure 4-5). At 30°C, which is closer to the optimum nitrifier growth temperature range between 25 – 35°C, both ammonia and nitrite levels dropped faster along the RWDSs and were near the detection limit at P1, P2 and P5. Correspondingly, total nitrifier genera levels were lower at P2 and P5 at 30°C than at 22°C.

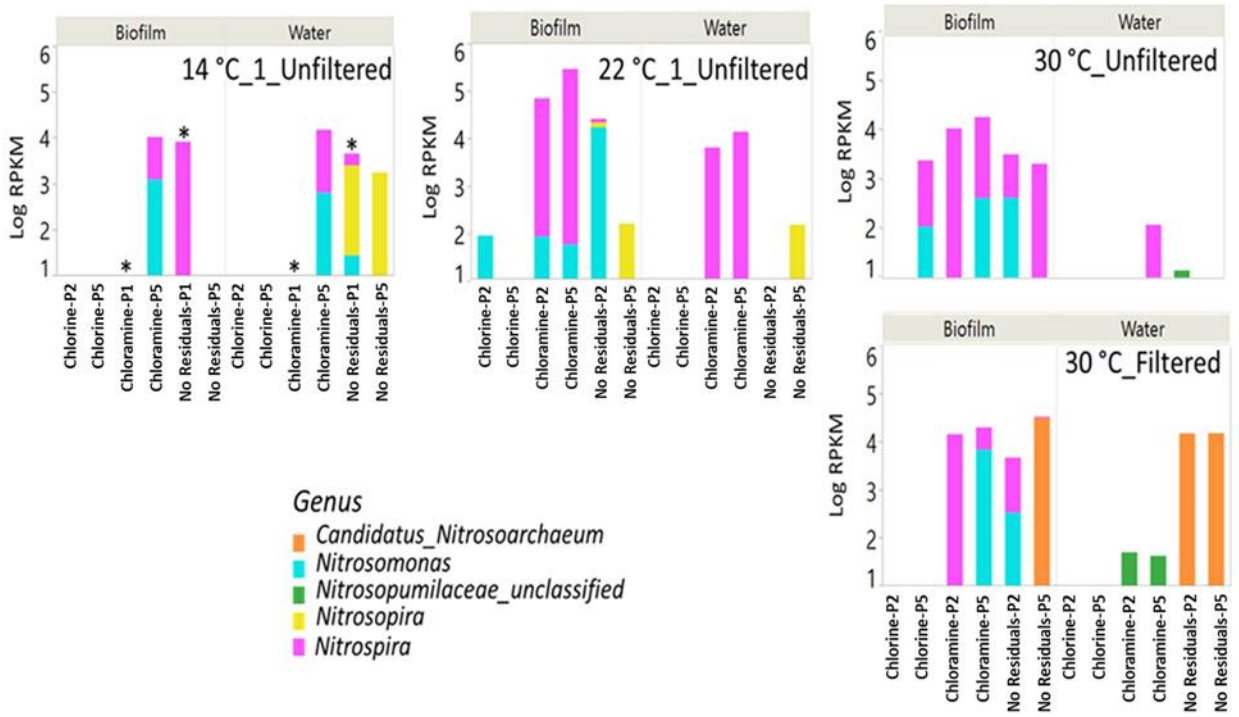

Figure 4-6 Relative abundance of reads taxonomically-annotated to known nitrifier genera in the RWDSs for the unfiltered feed water condition and all three temperature phases. Comparison of filtered versus unfiltered feed water is available for 30°C. Chloramine and No Residual samples at 14 °C_1 (indicated by *) were sampled at P1 instead of P2.

The genera of nitrifiers detected also varied across the disinfection and between water and biofilm (Kruskal-Wallis, $p_{disinfection}= 5.68\times10^{-4}$ and $p_{biofilm}= 0.0198$). Nitrifiers were never detected in the bulk water of chlorinated RWDSs, but *Nitrosmonas* and *Nitrospira* were detected in the biofilm at 22°C and 30°C of systems fed unfiltered chlorinated water (Figure 4-6). Nitrifiers detected in the bulk water of chloraminated systems were similar to those found in the biofilm, except for the BAC-filtered condition at 30°C, which harbored an unclassified genus of *Nitrosopumilaceae*. Distinct nitrifiers were detected in the no residuals RWDSs, including *Nitrosospira* and *Candidatus_Nitrosoarchaeum* (an Archaeae), indicating that the disinfectants incurred a selection pressure on the types of nitrifiers encountered compared to what was naturally present in the wastewater effluent.

Functional gene profiling provides a direct measure of potential for bacteria to express functions of interest. Here we examined 2 genes involved in nitrification: ammonia monooxygenase (*amo*A) and nitrite oxidoreductase (*nxr*). We also looked at 2 genes involved in denitrification, which could potentially be occurring as nitrate builds up as a by-product of nitrification: the copper-containing nitrite reductase (*nir*K) and Cd1-type nitrite reductase (*nir*S). Based on UniRef50 gene functional family annotation of all metagenomic samples, six different *amo*A, three different *nir*K and one each of *nxr* and *nir*S were detected (Figure 4-7). Temperature affected the relative abundances of *amo*A, *nxr* and *nir*K, with an increase in abundance with increasing temperature. Notably, chlorination suppressed levels of these 3 genes

at all the 3 temperatures, but chloramination did not. In fact, a pronounced spike in *amo*A and *nir*K were noted in chloraminated RWDS biofilms at 22°C (Figure 4-7). Also remarkable were the increased levels of *amo*A and *nir*K in chloramine and no residual filtered RWDSs at 30°C, compared to unfiltered RWDSs.

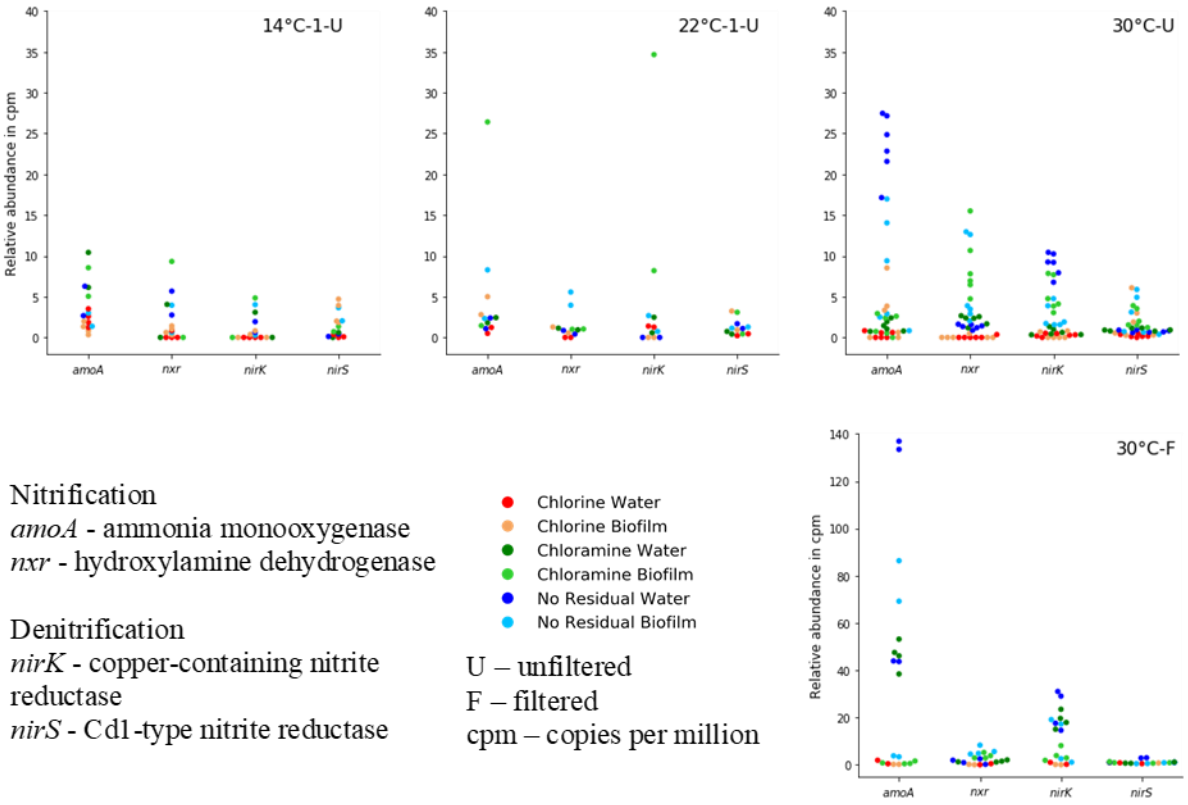

Figure 4-7 Relative abundances of genes involved in nitrification and denitrification detected through functional annotation of metagenomic data during the first 14°C and 22°C phases and 30°C. Metagenomic reads were mapped to the UniRef50 database for functional gene family annotations using the HUMAnN2 pipeline (Franzosa et al., 2018). Note the spikes in *amoA* and *nirK* in chloramine biofilm at 22°C-1-U. y-axis scales are different for unfiltered and BAC-filtered conditions.

4.3.4 Reflection of RWDS Experimental Conditions in Microbial Community Profiles

The broader microbial community composition, beyond nitrifiers, was profiled via metagenomic sequencing to gain deeper insight into the effects of the operational conditions on RWDS water quality. Diversity was significantly higher in biofilm than in bulk water based on Shannon and Simpson (Kruskal-Wallis p = 0.00470 and p = 0.00124, respectively) indices. Shannon and Simpson diversity indices were also significantly (p = 0.0219) higher in water samples from the chlorine and chloramine compared to the no residual conditions, while diversity in biofilm was comparable across the three disinfection conditions (Figure 4-8). No significant differences were observed based on water age, filtration or temperature.

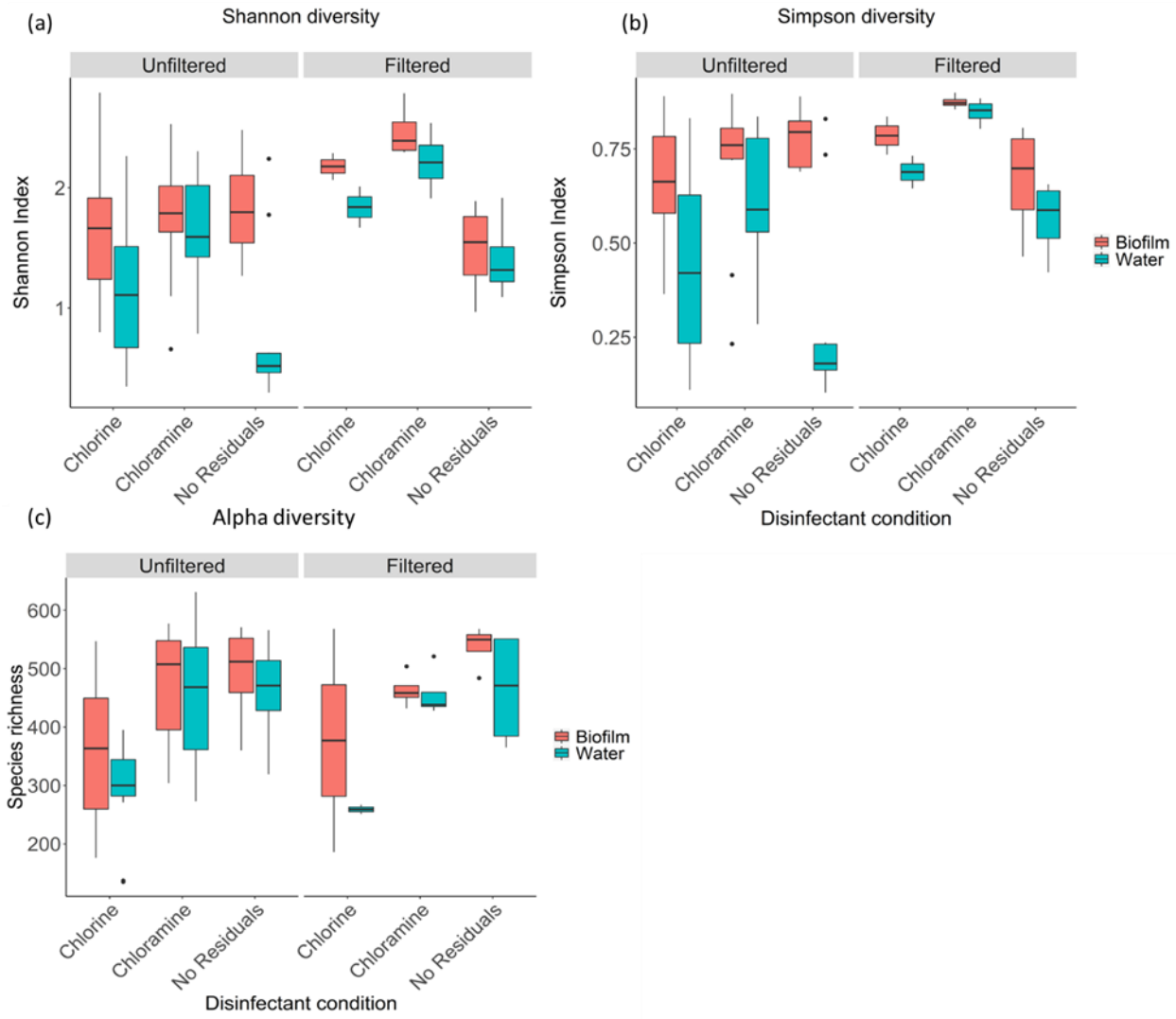

Figure 4-8 Distribution of microbial diversity indices (a) Shannon diversity (b) Simpson diversity and (c) Alpha diversity across three disinfectant conditions in water and biofilm samples.

NMDS analysis revealed that the microbial community taxonomic composition diverged as the experiments progressed from 14°C, to 22°C, and to 30°C (Figure 4-9). At 30°C, microbial community composition was distinct in the chlorine versus chloramine (ANOSIM R = 0.511, p = 0.0001) and no residual (ANOSIM R = 0.51, p= 0.0001) conditions, while composition in chloramine and no residual conditions were more similar to each other (ANOSIM R = 0.298, p = 0.0001). Filtration (ANOSIM R = 0.203, p = 0.001) and water age (ANOSIM R = -0.015, p = 0.561) had no distinguishable impacts on shaping microbial community taxonomic composition. Distinct composition of water versus biofilm was noted mainly at 14°C (ANOSIM R= 0.398, p = 0.001), while generally the selective effect of disinfectant conditions was more apparent in the bulk water, than in the biofilm, according to a two-way crossed ANOSIM of disinfectants across water/biofilm (ANOSIM R = 0.456, p = 0.0001). A complete summary of all ANOSIM test results at 14°C_1, 22°C_1 and 30°C were described in Table 4-2, Table 4-3 and **Error! Reference source not found.**.

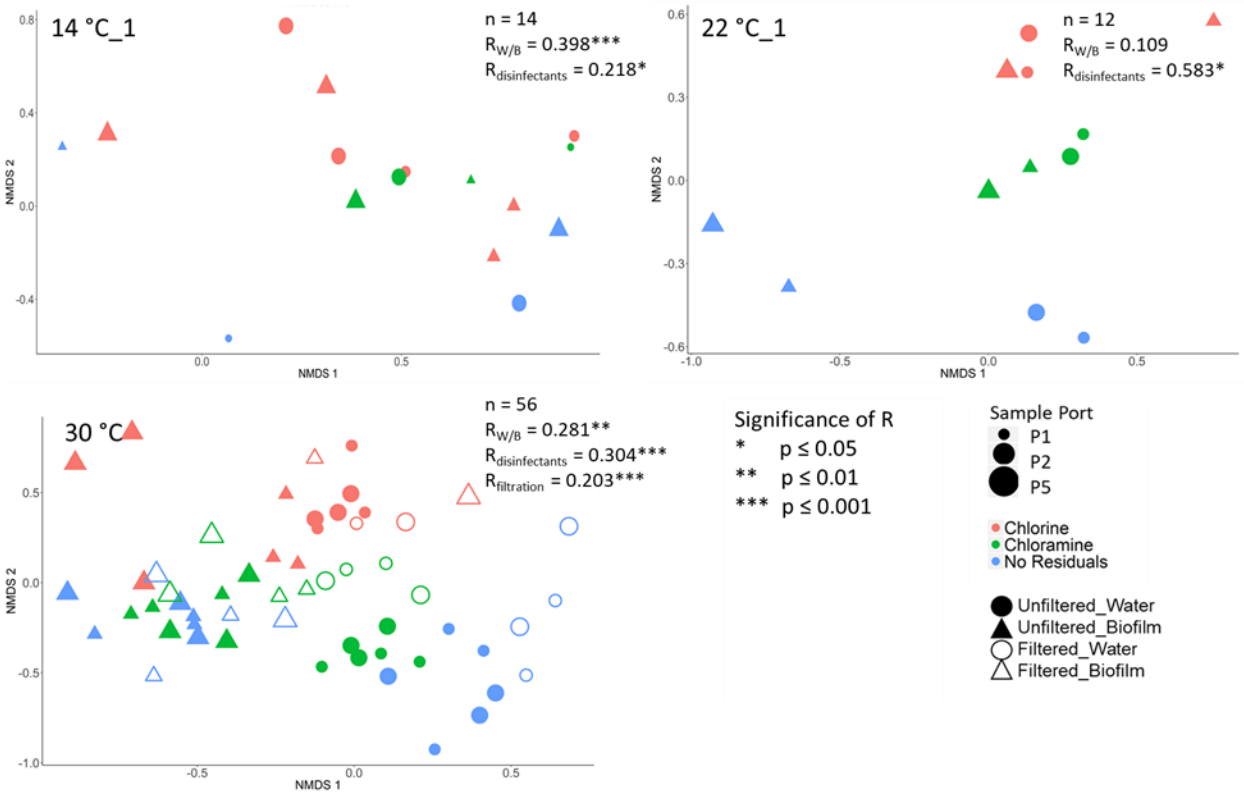

Figure 4-9 NMDS plots of microbial community genus- level taxonomic composition in the RWDSs. Metagenomic sequencing was performed for unfiltered RWDS samples (solid symbols) at 14°C-1 and 22°C-1 and for both filtered (solid symbols) and unfiltered (open symbols) conditions at 30°C. The number of samples subject to metagenomics sequencing (n) is indicated for each temperature phase. ANOSIM was conducted for each experimental condition and significant R values are noted in each figure panel. Metagenomic reads were taxonomically-annotated using the MetaPhlAn2 database.

Shifts in microbial community functional gene composition paralleled shifts in taxonomy, except for a noticeable filtration effect on bulk water (Figure 4-10). The effect of disinfection became more pronounced as temperature increased. While during the first 14°C, there was no clear separation based on disinfection, at 22°C all the bulk water samples separated based on disinfection condition. At 30°C, water and biofilm samples from the unfiltered chloramine and no residual RWDSs clustered together, while the unfiltered chlorinated condition RWDS samples formed a separate cluster (Figure 4-10 and **Error! Reference source not found.**). Filtration increased separation between the no residual and chloramine water samples and shifted the filtered chloraminated water samples closer to the filtered chlorinated water samples.

Table 4-2 Summary of ANOSIM R values of the metagenomic taxonomy profiles for all experimental condition factors at 14 °C_1.

14 °C_1							
One-factor							
Disinfectants		0.218*					
Water/Biofilm		0.398**					
Water age		0.037					
Two-factor crossed							
		Across					
		Disinfectants	Water/Biofilm	Water age			
Factor	Disinfectants		0.319*	0.238*			
	Water/Biofilm	0.565**		0.25			
	Water Age	0.098	-0.089				
Crossed between disinfectants							
			Water/Biofilm			Water age	
		Chlorine	Chloramine	No Residual	Chlorine	Chloramine	No Residual
Disinfectants	Chlorine						
	Chloramine	0.268			0.107		
	No Residual	0.321	0.25		0.321	0.625	

Table 4-3 Summary of ANOSIM R values of the metagenomic taxonomy profiles for each experimental condition factor 22 °C_1.

22 °C_2							
One-factor							
Disinfectants		0.583**					
Water/Biofilm		0.109					
Water age		-0.15					
Two-factor crossed							
		Across					
		Disinfectants	Water/Biofilm	Water age			
Factor	Disinfectants		0.972**	0.5**			
	Water/Biofilm	0.667*		-0.13			
	Water Age	-0.333	-0.426				
Two-factor crossed between disinfectants							
			Water/Biofilm			Water age	
		Chlorine	Chloramine	No Residual	Chlorine	Chloramine	No Residual
Disinfectants	Chlorine						
	Chloramine	1			0.5		
	No Residual	1	0.875		0.875	0.25	

Table 4-4 Summary of ANOSIM R of the metagenomic taxonomy profiles for each experimental condition factor at 30°C.

30 °C							
One-factor							
Disinfectants		0.304***					
Water/Biofilm		0.281**					
Water age		-0.015					
Filtration		0.203***					
Two-factor crossed							
Across							
		Disinfectants		Water/Biofilm		Water Age	Filtration
Factor	Disinfectants			0.456***		0.272***	0.41***
	Water/Biofilm	0.459***				0.299***	0.407***
	Water Age	-0.046		-0.006			-0.025
	Filtration	0.375***		0.307***		0.148**	
Two-factor crossed between disinfectants							
		Water/Biofilm		Water age		Filtration	
		Chlorine	Chloramine	Chlorine	Chloramine	Chlorine	Chloramine
Disinfectants	Chlorine						
	Chloramine	0.511***		0.33***		0.473***	
	No Residual	0.51***	0.298***	0.421***	0.097*	0.588***	0.173*

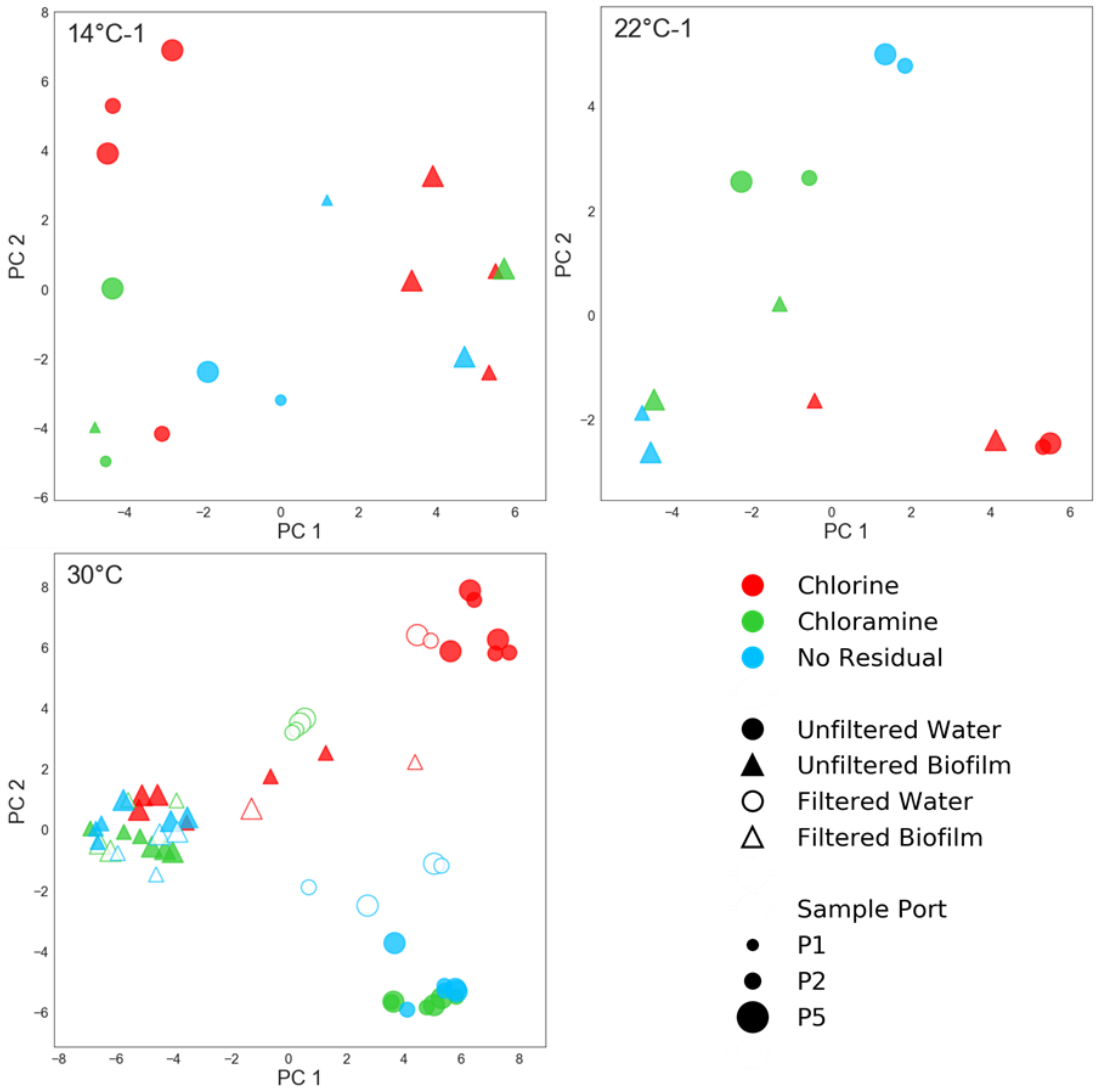

Figure 4-10 Comparison of RWDS water and biofilm microbiomes based on composition of functional gene family UniRef50 annotations. Metagenomic sequencing was performed for unfiltered RWDS microbiome samples (solid symbols) at 14°C-1 and 22°C-1 and for both filtered (solid symbols) and unfiltered (open symbols) conditions at 30°C. The plots indicate that the largest two principal components, PC1 and PC2. 14°C-1: PC1 and PC2 explain 41% of variance among samples, 22°C-1: PC1 and PC2 explain 50% of variance among samples, and 30°C: PC1 and PC2 explain 30% of variance among samples. Metagenomic reads were mapped to the UniRef50 database for functional gene family annotations using the HUMAnN2 pipeline.

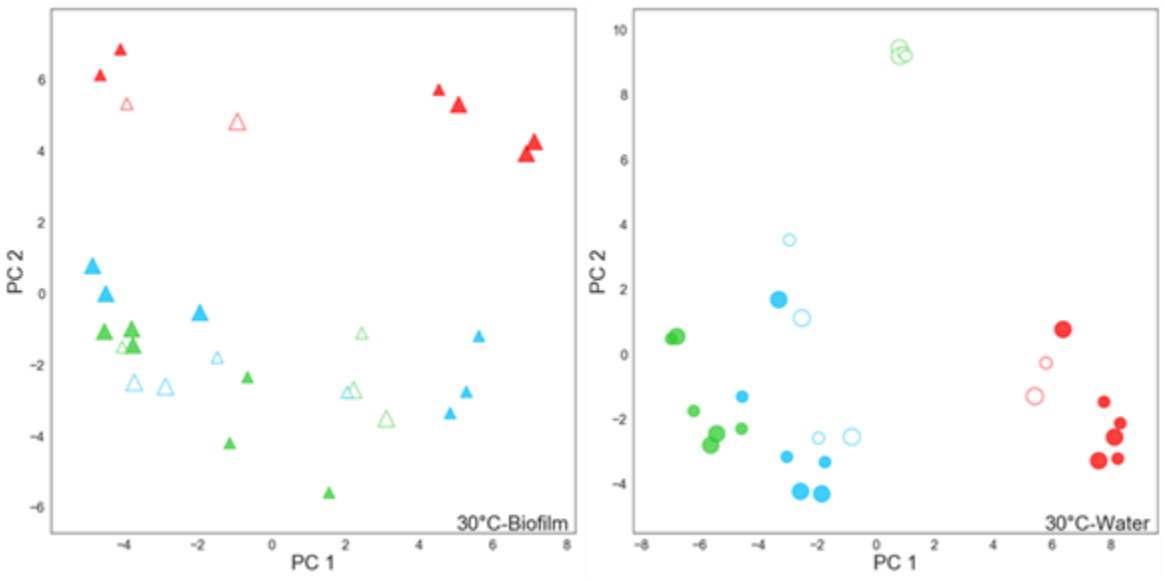

Figure 4-11 Comparison of RWDS microbiomes based on composition of functional gene family UniRef50 annotations at 30°C. The plots show the largest two principal components, PC1 and PC2. 30°C-Biofilm: PC1 and PC2 explain 31% of variance among samples, 30°C-Water: PC1 and PC2 explain 36% of variance among samples. Metagenomic reads were mapped to the UniRef50 database for functional gene family annotations using the HUMAnN2 pipeline.

4.3.5 Management Implications for RWDSs

In contrast to prior field surveys, this study provided a controlled, direct comparison of multiple factors impacting RWDSs over a period of years to help inform improved management strategies. Here we observed that RWDSs are particularly vulnerable to disinfectant loss, especially at the higher temperatures that are common in water stressed areas. Chloramine decayed rapidly due to nitrification and chlorine performed better as a disinfectant. Interestingly, the chloramine condition was often indistinguishable from that of the no disinfectant residual control condition in terms of DO loss, TCC and microbial community composition. Based on a parallel study, after decommissioning and analysis of these pipe rigs, extensive amounts of sediment and biofilm accumulated in this system with time, especially in the chloramine and no residual conditions (Zhu et al., submitted). Jar tests revealed that the biofilm and sediment exerted high disinfectant demand, which can explain why the ability to maintain residual worsened with time in the current study. Sediment and biofilm are also known to be problematic in DWDSs, and this and the parallel study both indicate that they are likely to cause even worse problems in RWDSs (Zhu et al. submitted).

Choice of disinfectant for RWDSs: chlorine vs. chloramine

A critical decision for RWDS operators is choice of disinfectant. This study emphasizes how ambient temperatures may affect this choice. Although higher temperature generally increases microbial and chemical reaction rates affecting disinfectant decay, herein, chloramine

was more strongly affected by temperature than chlorine. Specifically, in the simulated RWDSs, chloramine was more persistent than chlorine during the initial low temperature phase, but the trend was reversed as temperatures increased. Chloramine decay remained high even as temperatures decreased, showing that, once nitrifiers were established in a system, nitrification can still persist and cause chloramine loss at lower temperatures. On the other hand, chlorine was found to be a superior disinfectant in these lab-scale simulated RWDSs and was able to better maintain consistent residual levels over a range of conditions, while also maintaining high DO levels and reducing biological activity. Nonetheless, at 30°C, chlorine also decayed extensively and was frequently below 0.2 mg/L at higher water ages. Thus, at very warm temperatures, such as 30°C, it will be very difficult to manage water quality of RWDSs, as even chlorine residual was lost at this temperature. The reduced relative persistence of chloramine versus chlorine in RWDS, may illustrate how experiences with some RWDS may be expected to diverge from those obtained in potable water distribution systems.

Influence of BAC-filtration on feed water quality

Use of BAC-filtration markedly reduced TOC levels and DO losses in the RWDSs when compared to unfiltered water, but it did not consistently enhance disinfectant stability. BAC filtration did lower disinfectant losses during the initial low temperature phase as generally expected (Jin et al., 2013; Reungoat et al., 2012), but benefits of BAC-filtration were less apparent as temperature rose and time progressed. Surprisingly, during the final 14°C phase, disinfectant losses in the RWDSs fed BAC-filtered water exceeded that of those fed unfiltered water, which may be a result of altered nitrifier composition in case of the chloramine RWDSs. Ammonia-oxidizing archaea genetic markers, i.e., *Candidatus_Nitrosoarchaeum* and *Nitrosopumilaceae*, were detected at higher levels in the BAC-filtered condition and higher levels of *amoA* and *nirK* genes were detected in chloramine and no residual RWDSs fed BAC-filtered water compared to the unfiltered conditions.

These results reveal that benefits of BAC-filtration can become overshadowed by other RWDS operational challenges, which includes buildup of biofilm and sediment. In particular, BAC-filtration was not protective against nitrification and the biofilter itself might have supported the growth and release of nitrifying bacteria, with RWDS in filtered condition carrying a different variety than those found in the RWDSs receiving unfiltered water. Similar observations of the high ammonia-oxidizing archaea abundances associated with biofilters and bioreactors have been well-documented (Bagchi et al., 2014; Bartelme et al., 2017; Park et al., 2006) and it is important to understand the underlying ecology that selects for the ammonia-oxidizing archaea. Furthermore, the performance of BAC filters has been shown to change as the microbial communities on the filters mature, resulting in less capacity to absorb and degrade organic compounds, increased bacterial counts in the filtered effluent due to biofilm sloughing and general release of bacteria (Sbardella et al., 2018; Terry and Summers, 2018), and altered disinfectant demand in BAC-filtered source water.

Managing Nitrification in RWDSs

At higher temperatures, nitrifiers were abundant in both chloramine and no residual RWDSs, with and with BAC-filtration, while chlorine suppressed nitrifiers. The dominant nitrifier group in the unfiltered condition was the *Nitrospira* genus. *Nitrospira* spp. harbor comammox members capable of converting ammonia to nitrite and nitrate to nitrate (Daims et al., 2016; van Kessel et al., 2015), which have now been observed in a variety of environmental

systems (Chao et al., 2016; Daims et al., 2015; Gruber-Dorninger et al., 2015). Detection of comammox-containing *Nitrospira* in the simulated RWDSs as the most abundant nitrifier genus could help explain why nitrite was not a particularly accurate indicator of nitrification in this study, as it is commonly applied in DWDSs. Also interesting were the detection of ammonia-oxidizing archaea and high disinfectant losses in BAC-filtered systems.

Managing Biofilm in RWDSs

The relative impact of biofilm on water quality is likely to be exacerbated by operational features inherent to many RWDSs (Garner et al., 2016; J. Liu et al., 2017). Biofilm accumulation with time was likely a major driver for the increase in overall disinfectant decay rates, as evidenced by greater disinfectant loss and poorer water quality during the second 14°C and 22°C phases compared to the first 14°C and 22°C phases. Biofilm formed a more microbially-diverse environment than bulk water and served as a niche for nitrifiers, as evidenced by the spike in nitrifier taxonomic and functional genes in biofilm samples at 22°C. Biofilm continued to be the preferred environment of nitrifiers at 30°C, particularly in the unfiltered chloramine condition. Since disinfectants are known to be limited in their capability to penetrate biofilms (Chen and Stewart, 1996; Lau and Ashbolt, 2009; Miller et al., 2015b), once nitrifiers are established in the biofilm, RWDSs could be permanently susceptible to nitrification.

4.4 CONCLUSION

This multiyear study, with parallel analysis of water chemistry and metagenomic profiles, revealed distinct operational challenges for RWDSs. Maintenance of disinfectant residuals, both chlorine and chloramine, was challenging at higher temperatures and as the distribution systems aged. Chlorine was a superior disinfectant relative to chloramine under most conditions tested and resulted in better water quality with higher DO levels and lower microbial growth, while performance with chloramine was comparable to the no disinfect residual conditions. Nitrification was an important contributing factor in poorer outcome with chloramine. BAC-filtration led to minimal improvements and did not prevent chloramine loss over the course of the long-term operation.

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CHAPTER 5: CONSIDERING THE ROLE OF SCALE AND SIMILITUDE IN LABORATORY STUDIES OF RECLAIMED WATER DISTRIBUTION SYSTEMS FOR NON-POTABLE REUSE

Ni Zhu, Amy Pruden, Marc A. Edwards

ABSTRACT

The increased use of reclaimed water will require an improved understanding of the water chemistry and microbial changes associated with its transport through pipes to the point of use. Prior work using lab-scale simulated drinking water distribution systems (SDWDSs) reproduced a classic hierarchy of redox zones associated with distance (water age) along the simulated distribution systems (SDSs), which shaped the microbial safety and aesthetics at consumer homes. However, simulating and studying similar reactions in minimally treated reclaimed water distribution systems (RWDSs) is a challenge due to the higher levels of turbidity, nutrients, temperatures and interruptions to flow associated with full-scale RWDSs compared to corresponding DWDSs. Here we consider the relative advantages of four lab-scale SDWDS designs in achieving similitude to key aspects of full-scale RWDS operation. Each of the four SDWDS design can match one or two dimensions of a full-scale RWDS system such as surface area to volume, flow velocity, water age, and ability to collect sediment. Our years of practical experience operating lab-scale simulated RWDSs demonstrate that very thick biofoulants and the high levels of sediment accumulated in the reactors, pose major challenges to operating experimental SRWDSs that were not as obvious in corresponding simulation of DWDSs.

5.1 MOTIVATION

An improved fundamental understanding of water quality degradation in reclaimed water distribution systems (RWDSs) is needed to develop effective pretreatment, monitoring and management strategies that will meet all water quality goals at the designated point of use. Over the last a few decades, intensive laboratory and field work in drinking water distribution systems (DWDSs), has demonstrated how distinct zones of redox, disinfectant decay and flow regime (i.e., continuous, laminar, turbulent) can shape the microbial and aesthetic quality of water that consumers are exposed to at the point of use (Nescerecka et al., 2014; Srinivasan et al., 2008; Srinivasan and Harrington, 2007; US EPA, 2002). Complementary testing in batch-, bench-, pilot-scale simulated distribution systems (SDSs) and at full-scale, has been key to unraveling problems at the point of use including taste and odors (Lu et al., 1999; Camper, 2004; Prest et al., 2016), elevated lead and copper (Nguyen et al., 2011; Sarin et al., 2004; Sun et al., 2014), nitrification (Liu et al., 2005; Yang et al., 2007; Zhang et al., 2009), scaling and pipe clogging (Richards et al., 2018) and opportunistic pathogen growth (van der Lugt et al., 2017; Wang et al., 2012).

In comparison to distribution of drinking water from conventional water treatment plants, reclaimed water for non-potable reuse (NPR) will often have much higher levels of turbidity, carbon, phosphorus, nitrogen and other nutrients, making distributed reclaimed water much more susceptible to microbial growth, disinfectant loss, clogging, odors and sediment accumulation (Garner et al., 2016; Gauthier et al., 1999; Liu et al., 2017; Mussared et al., 2018). The high temperatures in regions of the country that tend to first adopt reclaimed water is another important exacerbating factor (Norton et al., 2004). Some of these issues have been illustrated in recent pioneering efforts to examine effects of pre-treatments, disinfectant type and residuals, water age, biofilms, antibiotic resistance and pathogen re-growth during reclaimed water distribution in both field studies and reactor simulations, in order to assess possible public health risks and develop improved operational strategies (Christou et al. 2017; Phungsai et al. 2016; Chhipi-Shrestha et al 2017; Garner et al. 2018, 2016).

In DWDS research over the last few decades, practical scientific advances were derived from complementary research work using field surveillance of full-scale systems and simulated drinking water distribution systems (SDWDSs) in the laboratory. The field studies reveal ambient trends and conditions in full-scale systems that affect water quality and resulting human health risks, whereas lab-scale SDWDSs are necessary to obtain results under controlled test conditions with statistical rigor and without endangering human health.

A wide range of SDS designs have been used to study important aspects of water distribution systems including batch-scale beakers (Lu et al. 1999), well-aged pipe coupons in batch reactors (Wang et al. 2012), and various small-scale SDSs with real pipes (Lee et al. 2015; Jjemba et al. 2010). Each of these approaches has their place, dependent on the biological or chemical reactions of interest and experimental goals. A key concept is that of "similitude", which refers to recreating the essential design conditions in scaled down model distribution systems that represent the chemical reactions or microbial regrowth in full-scale systems. This is a common concept in various engineering fields including harbor design, tsunami's and airplane wings (Casaburo et al., 2019; Dobre et al., 2007; Zhu et al., 2017).

Extending scaled down concepts to systems involving chemical and microbial reactions that occur in the bulk water, sediment or pipe surfaces of full-scale water distribution systems is sometimes straightforward, but in other situations it is not. While it is obvious that the only perfect simulation of a full-scale system, impractically involves creating and operating identical replicate full-scale systems, lab studies that target certain dimensions of similitude in terms of water volume, pipe lengths, pipe surface area to volume ratio (SAV), flow velocities (V), and hydraulic shear stress are achievable. Unfortunately, each of these factors might influence various aspects of biological reactions such as biofilm formation and pathogen growth to different extents. Hence, if only chemical reactions in the bulk water were the subject of concern, lab studies conducted in batch-scale glass reactors can simulate the reactions occurring in bulk water of a full-scale distribution system with plug-flow reactor (PFR) character, and sampling the glass batch reactors at the corresponding to the "water age" of the actual pipe network reveals realistic and accurate results (Korshin et al., 2005; Wang et al., 2016; Young et al., 2018). For example, Korshin et al (2005) exposed lead-containing coupons in batch reactors to synthetic water, and monitored changes in the bulk water chemistry at designated time points.

Likewise, if chemical reactions occurring at pipe surfaces are of interest, a scaled down pipe reactor with the same SAV is most appropriate at simulating surface mediated reactions in full-scale water distribution systems in many circumstances, even if the scaled down horizontal flow velocity and flow volumes are orders of magnitude lower (Masters et al., 2015; Zhang and

Edwards, 2009). Such compromises are also common for SDWDSs at bench- and pilot-scale that study corrosion control of iron, cement, lead and copper pipes (Masters et al., 2015; Wang et al., 2012), disinfectant decay in cold or hot water premise plumbing (DiGiano and Zhang, 2005), and even biofilm and pathogen growth (Ji et al., 2015; McLellan and Roguet, 2019).

The selection of a scaled down SDS design must be considerate of these key controlling variables, as well as the space, funding and even the logistics of obtaining sufficient volumes of water required for the study. For instance, the experimental design attempting to simulate a RWDS described herein, required collecting 255 liters of wastewater treatment plant effluent 2-3 times weekly for 3.5 years, to simply match a full-scale SAV ratio and realistic 5 days of water retention time (i.e., water age) with 3.9 meters of 4" pipe segments incubated in a Biosafety Level (BSL) II rated laboratory—the resulting compromise in pipe velocities were 5-6 orders of magnitude lower than a full-scale system.

The objective of this paper is to: 1) review critical engineering considerations that are important in recreating aspects of chemical and biological similitude between an SDS and a full-scale RWDS; 2) explore the strengths and limitations of each possible SDS design to achieve similitude; and 3) compare and contrast the applicability of two SDS designs used for DWDSs in preliminary studies of RWDSs. This work was motivated by past successes in creating effective simulations of key aspects of DWDSs to advance scientific understanding, and the practical limitations we encountered in attempting to apply those concepts to a multi-year complementary studies of both full-scale and lab-scale SRWDSs. The goal is to help future researchers better design, execute and interpret future lab-scale RWDS experiments that will be necessary to improve operation and design of full-scale water distribution systems.

5.2 CONCEPT DEVELOPMENT

5.2.1 Typical SDS Design Scenarios

In terms of the hydraulic, chemical and biological parameters known to govern RWDS operation, the critical factors include SAV, flow velocity (V), shear stress, water age and reactor flow pattern. In prior studies simulating reactions that occur predominantly in the bulk water (i.e., relatively weak influence from pipe wall or mixing), it has been possible to reasonably simulate disinfection by-product formation, chlorine or chloramine decay in drinking water pipes with inert surfaces, or radioactive decay using very simple glass beaker batch reactors or a simple practical PRF design sampled at different reaction times (e.g., Dobre et al. 2007; Ravindran et al. 1999). If the dominant reactions of interest are occurring pipe surfaces in a PFR situation, such as biofilm development and detachment, dissolution of iron or copper pipe materials to water, then matching the reactive SAV ratio has often been successful (Crittenden et al., 1991). Selecting one or two of these factors as the governing design criteria leads to four types of common SDS design used in prior research of drinking water distribution systems (Table 5-1).

In conceptual Design Scenario 1, SAV and water age are used as the target similitude factors, by using actual 4-in. distribution system pipe segments and targeting a maximum water age of 5 days achieving a flow velocity 5-6 orders of magnitude lower than the typical full-scale flow velocity (≈ 1 m/sec). The extremely slow forward flow velocity makes the radial diffusive flux and internal convective mixing important by comparison, creating a situation in which each segment behaves more like a complete mix reactor (CMR) than a classic practical PFR (Biswas et al., 1993; See Chapter 3 Section 3.1). Obviously, this design is necessary for studies in which

the effects of water age and SAV are dominant, but is unsuitable for researching phenomenon in which effects of flow velocity are important (Benchaita et al., 1983; Heitz, 1991; Li et al., 2017). For instance, the processes of re-suspending sediment or pipe corrosion in a distribution system where the likelihood of the events increases with V^n, where n could range from 1-3 (Benson et al., 2012; Ryan et al., 2008).

In hypothetical Design Scenario 2, the goal is to achieve a more realistic flow velocity in the design by employing a very thin 0.32 cm tubing. But the resulting SAV ratio is 32 times higher than normal, and the length of thin-wall tubing would create back pressures on the order of 10^5 psi if biofilm start to form and constrict flow.

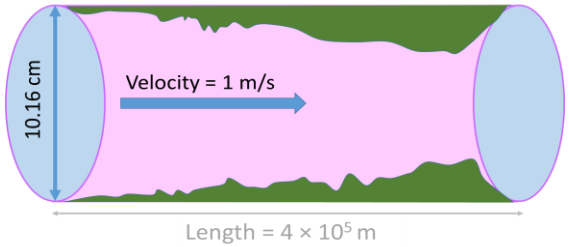
Design Scenario 3 attempts to overcome these limitations, and achieve a realistic simulation of SAV, flow velocity and shear stress by using real pipe sections equipped with internal impellers to create turbulence and fast flow conditions at the pipe wall (Westbook and DiGiano 2009; DiGiano and Zhang 2005). But practical attempts to connect two of these pipe reactors in series, failed to operate due to creation of high backpressure. Furthermore, such a design is effectively two CMRs in series and does not create a true PFR pattern that occurs in a full-scale distribution system (Vikesland et al., 2006). The energy input from the motors needed to mix this type of system also raises the temperature 20° C above ambient room temperature, which would add complexity in terms of cooling if precise temperature control is necessary.

Design Scenario 4 focuses on providing a pipe surface area for biofilm development by immersing pipe coupons into flowing water with a well-controlled water chemistry. Flow rate and conditions within the reactor, are controlled to some extent by pumping influent adjusted to target water matrix conditions. Mixing and some effects of moderate flow velocity can be simulated with the use of stir plates and shake tables, but similitude in terms of SAV and flow pattern is sacrificed, and the reactor essentially operates as a CMR, and many such reactors would have to be connected in series to simulate the hierarchy of chemical and biological profiles with a water system that occur naturally during distribution. A classic SDS example of Scenario 4 is the design of the CDC Biofilm Reactors®, which hold immersed coupons in a designated water chemistry and mixing rates (US EPA, 2013).

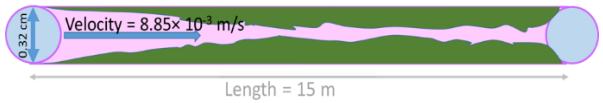
Table 5-1A comparison of the five typical SDS scenarios used to simulate water distribution systems in the laboratory.

SDS Scenario	SAV (m^{-1})	V (m/s)	Water (days)	ageLength (m)	R#	Flow (L/day)	Flow pattern
Full-scale	39	1.0	5	4× 10^5	10^5	≈10^6	PFR
1. Match SAV and water age	39	≈10^{-7}	5	4	1	32	Mix@
2. Match a higher velocity	1260	≈10^{-3}	32 mins	15	32	6	PFR
3. Scenario 1/internal mixer	39	1.0*	5	0.5	10^5	4.05	Mix@
4. CDC Biofilm Reactor	0.7-10	Internal Mix	5	N.A.	N.A.	0.35	CMR

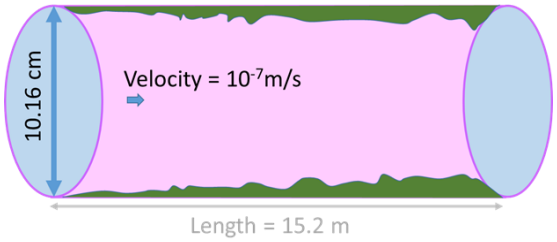
#= Reynolds number, * = at wall, @ = CMR's in series



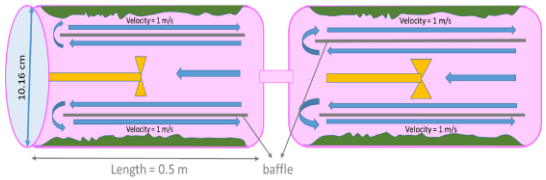
Illustrative full-scale system.



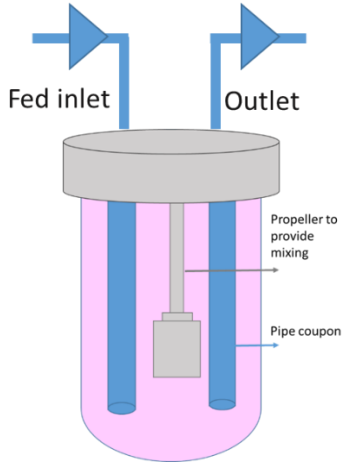
Scenario 2. Tube SDSs with thin tube to achieve a higher velocity.



Scenario 1. Pipe SDS using pipe segments matching diameter, SAV and detention time of real system



Scenario 3. Modified Scenario 2 with internal mixers to match velocity



Scenario 4. CDC Biofilm Reactor.

While each of these designs has obvious limitations, they all played a role in deciphering one or two important aspects of chemical and microbial reactions in the laboratory, as they occur in the full-scale water distribution systems. One of the main advantages of the lab-scale SDSs is to enable mechanistic study of the processes of interest under carefully controlled experimental conditions, which cannot be achieved in field studies of the complex full-scale distribution systems. However, as the SDSs only accommodate one or two similitude factors, the observations derived from laboratory observations will not always directly translate to the corresponding full-scale systems. Understanding the implications and limitations of each SDS design would be critical to: 1) selecting the most scientifically-sound and efficient experiment design that addresses a specific research question; 2) to understand deviation of each SDS design compared to the full-scale system; 3) accurately interpreting the scientific findings of each SDS design.

5.2.2 Applying Experiences of DWDSs to RWDSs

There are logical reasons to believe that the compromises in the lab-scale designs that manage to successfully simulated chemical and microbial reactions in DWDSs will not be as successful when applied to RWDSs. Conventional drinking water has a greatly reduced propensity to form biofilms due to relatively low levels of assimilable organic carbon (Hu et al., 2016), stable disinfectant residuals that are closely monitored by regular monitoring of DWDSs (Deininger, 2000), much lower levels of particles and pathogens (Christou et al., 2017; Moreno, 2004), and relatively stable flow with well-established operational protocols to minimize stagnation and water age, in comparison to operation of NPR RWDSs (Table 2). Each of these issues will be addressed in the following section.

Table 5-2 Comparison between potable water and reclaimed water systems.

	Potable Water Systems	**Reclaimed Water Systems**
Source Water	Surface water; groundwater	Wastewater effluent
	Source water often protected and may be selected for high quality	Relatively high levels of anthropogenic chemicals, dissolved nutrients, suspended biomass and particulates
Treatment process	Well established treatment processes, with multiple barriers to pathogens and particulates	Final disinfection is the main barrier to pathogens, allowing relative high levels of suspended solids
Distribution system	Maintenance of disinfectant residuals with required monitoring throughout main distribution system, design and operation to avoid stagnation	No regulations for residual monitoring, intermittent flow with stagnation periods of weeks to months, much higher sediment potential

1) Role of biofilm accumulation in constricting and clogging flow.

Prior research in drinking water systems that tends to have less nutrients, regular flow and stable disinfectant residuals reports characteristic biofilm thicknesses up to 1 mm (dos Santos and Livingston, 1995; Lazarova and Manew, 1995; Shieh and Keenan, 1986). Considering hypothetical scenarios of having 1 mm thick biofilm in the 10.16-cm diameter Pipe

SDS, would only have a slight (< 3%) impact on the velocity, SAV and retention time compared to the clean Pipe SDS without biofilm (Figure 5-1).

However, our operation of a Tube SDS (Scenario 2 with 0.32 cm diameter and 15.2 m length) using chloraminated and non-disinfected reclaimed water, resulted in complete clogging of the entire cross-section of the tube reactors with biofoulants in approximately two weeks, whereas the chlorinated tube SDS had almost negligible biofilm thickness by comparison (Figure 3-5). This rapid clogging in the tubes with no disinfectant and chloramine, occurred even after removing larger particles with mesh filter screens (Mesh Liquid Filter Bags PEMU300P1P, 300 Micron) and with biofiltration pre-treatments. The calculated impacts of having a 1 mm thick biofilm or biofoulants on the Tube SDS (Figure 5-1b) increase the SAV by 2.64 times, velocity 7 times, and reduce the retention time by 7 times compared to reactors before formation of biofilm.

Thus, in practice, using a tube reactor for chlorinated water with relatively low biofilm thickness compared to chloraminated water with a 1 mm or greater thickness, produces unequal comparisons in terms of SAV, flow velocity, and retention time even when the reactor design is initially identical. This complicates drawing scientifically sound conclusions regarding the relative performance, compared to the full-scale systems where biofilm thickness has relatively minor impacts. In reality, reviewing of field RWDSs reveal that clogging of relatively small orifices is a major problem in NPR applications (Han et al., 2019; K. Miyamoto et al., 2013), and thus observations such as rapid clogging in one system versus another have major practical relevance as observed herein.

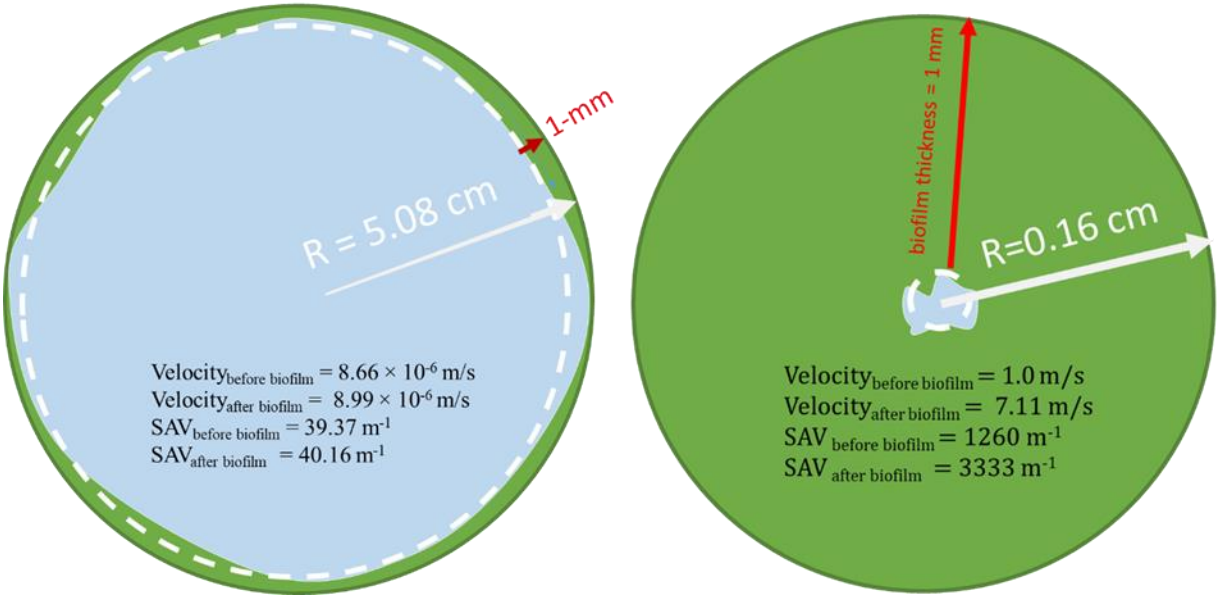


Figure 5-1 (a) Illustrative change in velocity and SAV due to growth of a 1 mm of biofilm in a 10.16-cm. diameter wide Pipe SDS (left); (b) Illustrative change in velocity and SAV in a narrow 0.32-cm. dimeter narrow tube SDS due to formation of a 1 mm thick biofilm or biofoulant (right). The white dotted line demarcates an effective hydraulic diameter of the pipe after biofilm formation. In practice the 0.32 cm diameter tube reactors clogged completely after 2 weeks of operation.

2) Hypothesizing Trends in Biofilm Growth Under Different Scenarios.

In conventional DWDS a practical PFR flow pattern assumption is often used as a basis for creating one-dimensional mass transport models of substrates, nutrients, disinfectants and microorganisms along the direction of the flow (Biswas et al., 1993). However, in SDS scenario 1, 3 and 4, or even in full-scale distribution systems where flow is either intermittent or very slow, dispersive axial transport, convective backflow or imposed complete mixing, can be of similar or greater magnitude than the net forward velocity. This can dramatically alter the delivery of disinfectants and nutrients from the bulk water to biofilm on the pipe surface. The implications of these effects are considered below for representative scenarios in full-scale and simulated systems below.

1. Full-scale turbulent continuous flow scenario

Typical flow in a pressurized high velocity distribution system (the full-scale systems) is highly turbulent with an actual Reynold's number (Re) of $\approx 10^5$ versus the threshold of > 4000 for turbulent flow. This effectively maintains a PFR character of a pipe reactor and delivers high levels of nutrients and disinfectants from the bulk water to the biofilm formed on the pipe surfaces (Armbruster et al., 2012; Hallam et al., 2001; Munavalli and Kumar, 2003). In the complete absence of disinfectant residuals, there is no delivery of disinfectants to biofilm, but there is a high flux of nutrients (*e.g.,* organic matter and oxygen) into the biofilm causing the most rapid biofilm growth at the earliest water ages of the systems. The biofilm growth rates would decrease if nutrients delivered by bulk water flow are depleted at higher water (Figure 5-2a upper condition) (Jjemba et al., 2014; Volk and LeChevallier, 2000; Weinrich et al., 2010). However, if high levels of disinfectant residuals are present, the trend in biofilm thickness as a function of water age would likely be reversed, as the rapid mass transport of the disinfectant from the bulk water to the biofilm could be controlling at the point of entry, whereas biofilm growth is more rapid at higher water ages after the disinfectants are depleted (Figure 5-2a lower condition).

2. Laminar flow condition in SDSs

In the low velocity scenario 1, the Re is 0.98, which is far below the demarcating laminar flow threshold of Re < 2300. The PFR character of the pipe flow is drastically reduced, along with the corresponding flux of disinfectants or nutrients from the bulk water to the pipe surfaces, and reduced transport of potentially toxic microbial reaction products away from the biofilm surface. While biofilm growth would be highest at the point of entry when there are no disinfectants, the growth rate is expected to be much lower than in turbulent flow scenario (Figure 5-2b upper condition). In the presence of high levels of disinfectant residuals, biofilm growth at the point of entry might reduce the rate of growth somewhat, but as these disinfectant residuals are consumed at higher water age zones, more biofilm would start to accumulate (Figure 5-2b lower condition).

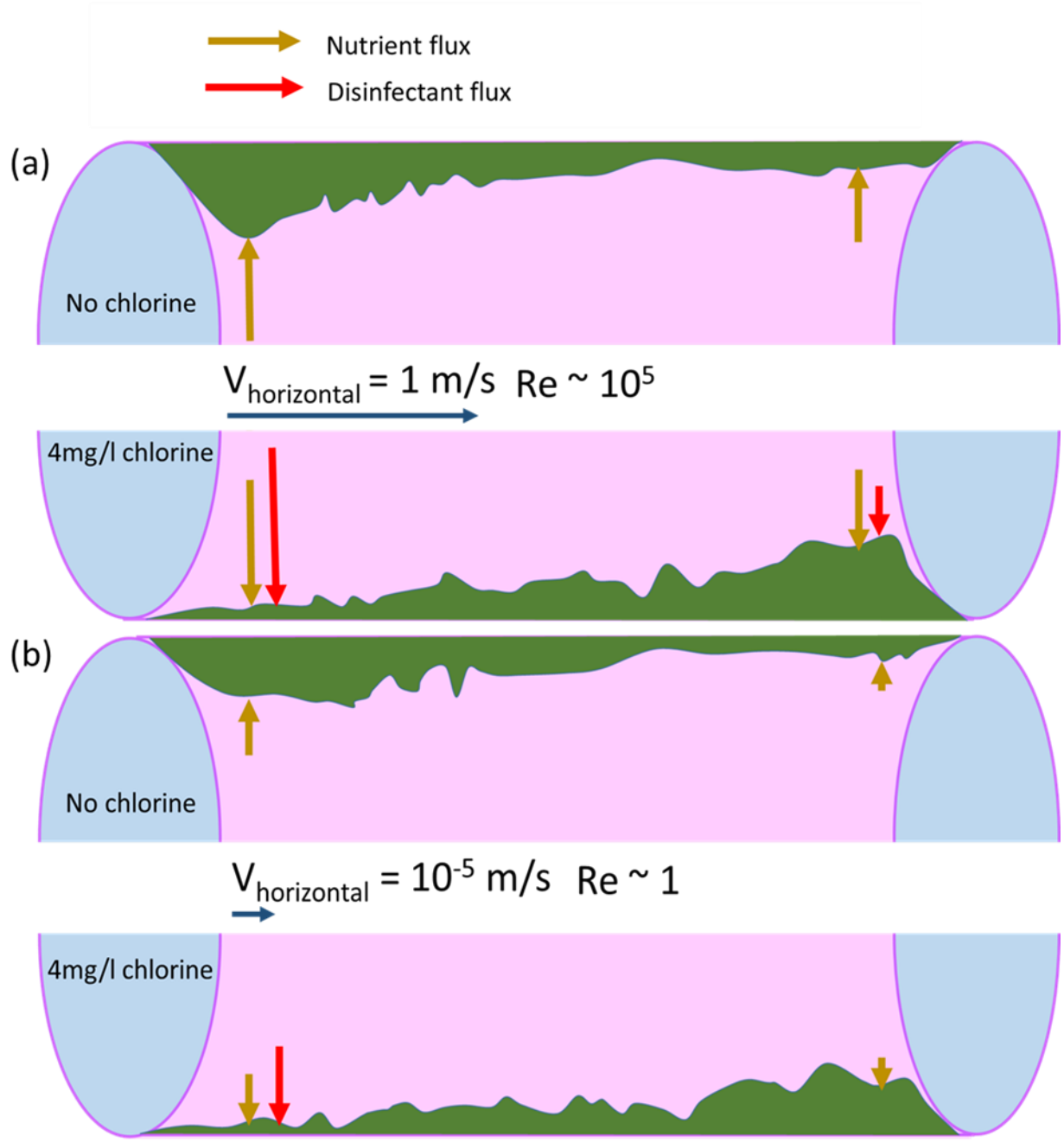


Figure 5-2 (a) In a fast flowing PFR SDS or full-scale RWDS there is rapid radial transport of disinfectants and nutrients to biofilm at the pipe wall. Without disinfectants biofilm growth would be the most rapid at the point of entry and may decrease with distance, but the opposite scenario would tend occur in the presence of disinfectants that gradually decay with high water age. (b) When flow velocity is low in the SDS or stagnant, transport of nutrients from the bulk water to the pipe wall is very slow in the resulting laminar flow, allowing the biofilm to flourish and locally buildup reactive metabolites such as nitrite that can further destroy disinfectant and protect biofilms.

3) Role of Sediment in Disinfectant Decay.

Existing literature on disinfectant decay in well maintained full-scale DWDSs with a regular flow has indicated a first-order decay model often applies with respect to free chlorine and chloramine residuals in the bulk water as a function of water age (Li et al., 2003). In such cases the key reactions can easily be simulated using glass batch reactors (Fisher et al., 2017). Even so, recent studies in full-scale DWDSs reported that loose deposits accumulating in such systems, had disproportionate impacts on water quality deterioration and microbial growth (Gauthier et al., 2001; Mussared et al., 2018; Pocas, 2014). In our multi-year study of operating SRWDSs, typical turbidity in our simulated non-potable RWDSs was as high as 12.4 NTU (Zhu et al. submitted), which is more than 10 times higher than existing US EPA National Secondary Drinking Water Standards for drinking water. Given the orders of magnitude higher particulate matter in distributed reclaimed water and frequent stagnation events in the distribution systems, it is reasonable to believe that the issue of sediment deposition would play an even greater role in RWDSs than in DWDSs. In terms of simulating the sediment accumulation phenomena in an SDS, accumulation of sediment deposits need be maximized at very low laminar velocity or during non-use stagnation events (i.e., Scenario 1). In contrast, the tube reactor design (Scenario 2) would not allow significant sediment deposits to accumulate, because the reactor would either be completely clogged or the sediment would be flushed out of the tube.

5.2.3 Illustrative Knowledge Gained from three simulated SDSs

In this section, we summarize practical experiences in operating SDSs to simulate DWDSs and RWDSs in the laboratory over the last decade. Three case studies consisted of one DWDS Pipe SDS and two complementary scales of RWDS SDSs that were aimed to evaluate the extreme simulation conditions.

a) <u>The DWDS Pipe SDS</u>

The DWDS Pipe SDS Design and Operation. Recent work on designing and operating a continuous flow SDWDS connected to premise plumbing systems that were frequently stagnant was described by Masters et al. (2015) and Wang et al. (2012). The SDS consisted of 18 3.8-cm (equivalent to a SAV ratio of 0.12 cm^{-1}) diameter pipes lined with coupons representative SAV of unlined cast iron, PVC or cement mains. Three levels of disinfection including chlorine, chloramine and no disinfectants were tested. The SDS operated as continuously fed PFRs at room temperature with a flow rate of 0.40 ± 0.0-5 mL/min (Re = 2520) and a total water age of 10.2 days with sampling ports along the pipes at 1, 2.3, 3.6, 5.7 and 10.2 days. To represent microbial growth in stagnant consumer water heaters that drew from the DWDS at each water age, aliquots were collected from the sampling ports, and placed into glass containers incubated at a target temperature using a dump and fill protocol in a BSL II laboratory due to hypothesized high likelihood of pathogen growth (Proctor et al., 2017). Synthesized drinking water was made up in the laboratory using tap water available in the laboratory (36 liters per week).

Observations. The DWDS Pipe SDS successfully replicated reactive redox zones developed in full-scale pipes as a function of water age. A wide range of water chemistry parameters, such as chlorine and chloramine residuals, dissolved oxygen (DO), nitrogen species (i.e. ammonia, nitrite, and nitrate) involved in nitrification processes, generally changed as expected with

increasing water age up to 10.2 days of retention time. These changes in water chemistry parameters were found to inform biological processes in the SDSs, including the observation that chlorinated systems with unlined iron would experience loss of disinfectant that could trigger Legionella growth in consumer water heaters. This exact scenario was then discovered to have been important in the 2014-2015 Flint, MI Legionella outbreak (Rhoads et al., 2017; Schwake et al., 2016).

b) The RWDS Pipe SDS – Scenario 1

The RWDS Pipe SDS Design and Operation. Informed by the observations from the nearly identically designed SDWDSs successfully described above, a SRWDS focused on achieving a representative water age and a similitude to pipe dimension and SAV (Scenario 1) was operated for more than 2 years herein. Reactors with 10.16-cm (4-in.) in diameter by 3.9 m (12.75-ft) long PVC pipe segments were constructed in a temperature controlled room to simulate seasonality in the field. Even with collection of 67 gallons of secondary wastewater 2-3 times weekly, an extremely slow flow velocity (8.66×10^{-6} m/s) was necessary to achieve the targeted total water age of 5-d with intermediate sample ports at 0-d, 1-d, 2.5-d to capture the water chemistry and microbial profiles throughout the pipes.

Six designated influent conditions – three disinfectant conditions (4 mg/L of free chlorine, 4 mg/L of chloramine and breakpoint with no residuals) and two filtration conditions (unfiltered and filtered with biologically activated filters) were prepared with wastewater effluent collected from a local wastewater treatment plant. Fresh influent to the RWDS Pipe SDSs was prepared and fed to the SDSs every 30 hours. Water chemistry profiling and replicated biological samplings of bulk water, biofilm and sediment were conducted at designated temperature phases (14°C→22°C→30°C→22°C→14°C). The experiment was conducted in a BSL II laboratory due to the hypothesized high likelihood of pathogen growth in the SRWDS with high nutrients and warm temperature.

Observations. The RWDS Pipe SDSs achieved key objectives of generating distinct water chemistry, microbiological profiles and very high levels of Legionella growth (unpublished data), trends that were similar to our complementary study of full-scale RWDSs (Garner et al., 2018b), the multi-year operation also demonstrated several practical limitations compared to prior experiences with SDWDSs including:

1) Chronic leaks at sampling ports and joints from built-up back pressure.
2) High financial and labor costs due to the massive volumes of source water needed to be transported from the wastewater treatment plant to the lab.
3) The extremely low flow velocity in the 4-in. pipe reactors resulted in relatively high rates of convection and diffusion along the radial axis of the pipes versus the longitudinal net forward velocity, causing the flow pattern in the RWDS Pipe SDSs to deviate markedly from the known practical PFR at full-scale, to a CMR in series flow pattern.
4) High levels of particulate matter in the reclaimed water influent resulted in large accumulation of sediment at the bottom of the pipes near the feed water entry point, which was found to cause DO and disinfectants decay gradients with depth, which was analogous to what was observed near sediment of river beds (Aku and Tonn, 1999; Hawley et al., 2006).

5) Very rapid depletion of disinfectants residuals immediately after entering the pipes, created an unrealistic situation in which the vast majority of the distribution system had no disinfectant residuals.
6) Despite the long water age designed, virtually all of the chemical and microbial reactions of interest were completed in the first pipe segment, between 0-d and 1-d sampling points (Figure 5-3), including growth of key microbial contaminants of interests. Likewise, the abundance of targeted microbial constituents, such as16S rRNA, *Legionella* spp., and *Mycobacterium spp.* increased remarkably within the first 1-d pipe segment and did not increase much more in the remaining 80% of the distribution system as indicated by results from the 1-d to 5-d sampling ports (Figure 5-3). Despite successfully achieving similitude in the capacity to capture sediment, SAV and water age relative to the full-scale RWDS, the usually rapid depletion of disinfectants in the beginning of the SDS, did not recreate realistic trends observed with water ages in our companion study of full-scale RWDSs, as illustrated below.

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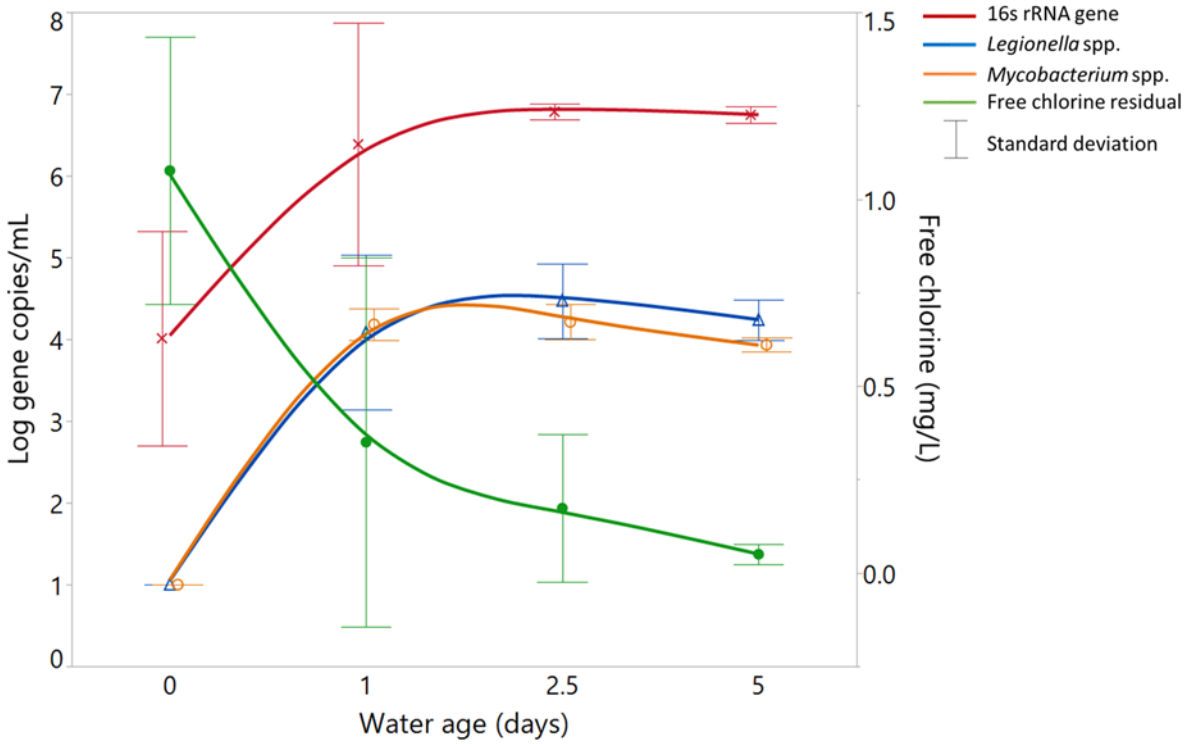

Figure 5-3 The interplay between disinfectant decay and microbial regrowth of 16s rRNA gene, *Legionella* spp., and *Mycobacterium* spp. in the low flow velocity RWDS Pipe SDS at 30°C. The most intense regrowth occurred in the first 1-d chlorine pipe segment and was inversely related to the level of chlorine residuals.

Benchmarking the disinfectant decay trends in our RWDS Pipe SDSs to our field survey result of two RWDSs (Garner et al., 2018b) further illustrates the certain aspects of deviation in this design. The chloramine residuals in our two Pipe SDSs were depleted near the first sample, whereas chloramine residuals in the full-scale field systems remained relatively stable throughout both full-scale systems and were able to persist for the full length of the RWDSs (Figure 5-4). The deviation in the disinfectant trends between our Pipe SDSs and the field survey

results reflected the differences in disinfectant flux trends as previously discussed in Figure 5-2, between a fast flow PRF pipe represented by the full-scale field systems (Figure 3a lower section) and a slow flow pipe represented by our Pipe SDSs (Figure 5-2b lower section). While the Pipe SDSs focused on simulating the conditions for sediment accumulation by adopting a slow flow velocity, the capacity to achieve similitude to a realistic flow pattern and a disinfectant decay profile that is representative of a full-scale RWDS was lost.

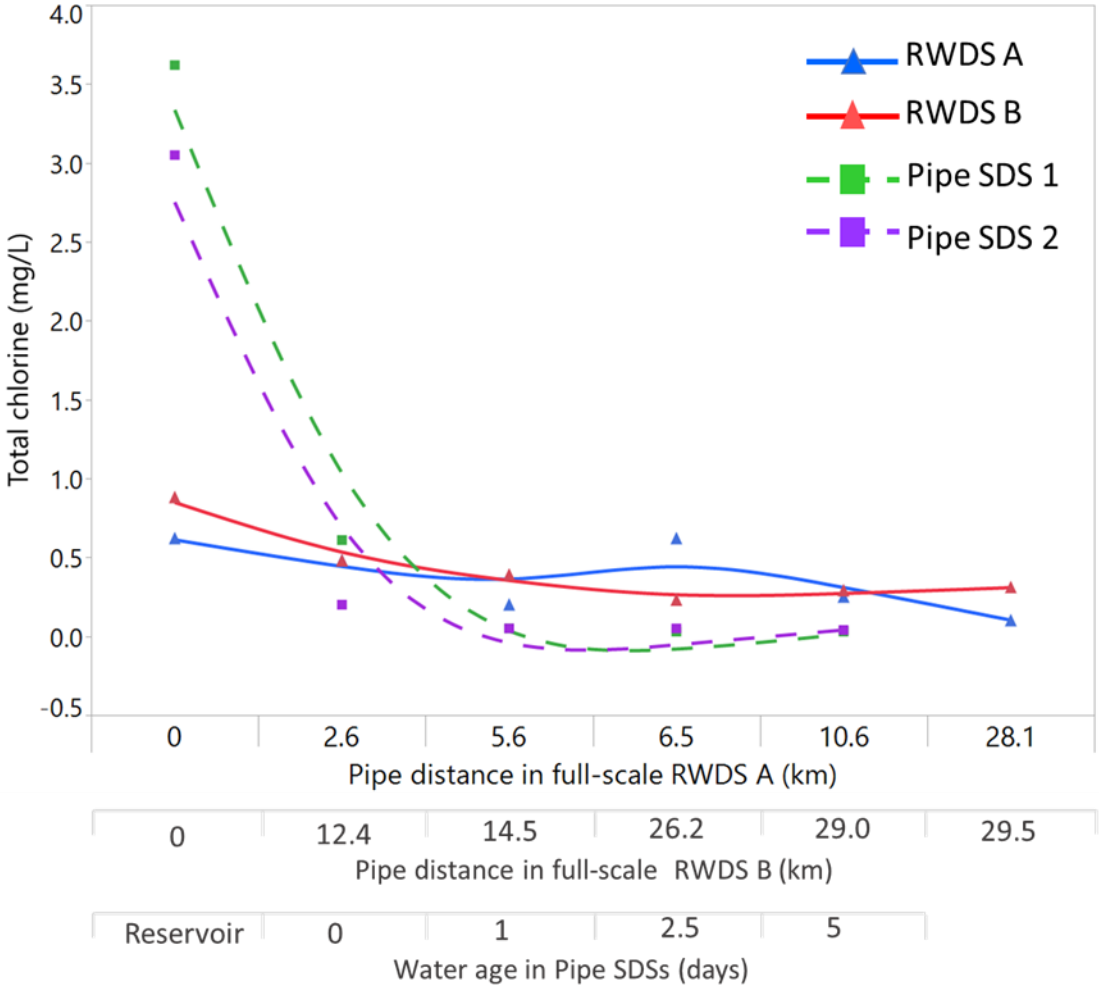

Figure 5-4 Distinct disinfectant decay trends were observed between our Pipe SDSs and the full-scale RWDSs. Much more stable disinfectant profiles were observed in the two field systems compared to the simulation results from our Pipe SDSs. The temperature range of the surveyed RWDSs was comparable to the lab-scale temperature at 30 °C, ranging from 29 - 33°C. Data for this figure was obtained from a published work by Garner et al (2018b). The two x-axis labeled in terms of pipe distances (km) were for two full-scale RWDSs. The x-axis labeled with water age (days) was for the two Pipe SDSs. The simulated feed reclaimed water was BAC-filtered WWTP effluent with 4 mg/L of chloramine residual for Pipe SDS 1 and unfiltered wastewater effluent with 4 mg/L of chloramine for Pipe SDS 2.

c) The RWDS Tube SDS – Scenario 2

The RWDS Tube SDS Design and Operation. Comparison testing of the SRWDS Tube SDSs was designed to complement the SRWDS Pipe SDSs by trying to better approximate the target flow velocity as the similitude factor at the expense of a more realistic SAV and water age (Scenario 2). The Tube SDSs consisted of six 15.2-m (50-ft) 0.32-cm (1/8-in.) inner diameter PVC tubing with a clean reactor calculated water age of 28-min and a flow velocity of 8.85×10^{-3} m/s. The exact same source water and influent conditions were applied to both the Pipe SDSs and the Tube SDSs for a period of 2.5 months to create a uniform baseline biofilm condition for controlled comparison between these two differently scaled SDSs. The small scale of the Tube SDSs had the added advantage of including biological replicates for each condition to assess the reproducibility of the observations. To profile the water chemistry and microbial trends along the tube length, at the end of the conditioning period, bulk water and biofilm samples were collected at every 10-ft interval by clipped away the tubes. The destructive nature of the sampling method did not allow replicate sampling of the Tube SDS at different times points to track temporal changes in the reactors, nor repurposing of the reactors for new experimental conditions such as the temperature cycling that occurred over a period of years in the pipe reactor.

Observations. Despite the extremely short water retention time of 28-minutes calculated based on new tubes without biofilm, the Tube SDSs displayed a practical PFR pattern as confirmed by our tracer studies, and reproduced a full redox profile and biological regrowth profiles. Distinct redox zones were observed in the chlorine and chloramine SDSs. In the chloramine SDSs, a slight increase in cell counts was observed within the first 20-ft as chloramine residuals decreased from 3.88 to 3.26 mg/L, and the cell counts stayed elevated afterwards. Chloramine has been known as a weaker disinfectant compared to chlorine due to a lower oxidizing strength, making it less effective at controlling regrowth (Hoff and Geldreich, 1981). In comparison, chlorine quickly dropped to below 0.2 mg/L at the 30-ft sampling point. Correspondingly, heterotrophic cell counts spiked up, showing a realistic relationship between disinfectant decay and biological regrowth that was typical of full-scale distribution systems. Chlorine and chloramine were almost depleted at the end of the 15.2-m length tube (Figure 5-5).

Despite the heavy growth of biofilm/biofoulants along the tubes, chloramine levels stayed consistently higher than in the chlorine tubes. As shown in the SDS Scenario 2, formation of 1-mm biofilm in the Tube SDS could have effectively reduce the actual retention time by 7 times, resulting in a much lower practical hydraulic residence time in the tubes with chloramine and no disinfectant versus the tubes with free chlorine, thus the greater persistence of chloramine versus chlorine in this condition might be due to the much lower effective residence time. The resulting heterotrophic cell counts in the chloramine tubes were also much higher than in the chlorine tubes, despite the very short hypothesized residence time in these tubes that were frequently clogging. The key point is that it is difficult to conduct a head-to-head comparison between the chlorine and the chloramine tubes in reality due to the different extents of biofoulant formation in each condition and the consequential impacts on the SDS design characteristics, even when the SDSs initially designed to be identical reactors.

In the only other prior lab-scale simulated study on microbial regrowth in RWDSs conducted at a wastewater treatment plant, Jjemba et al.(2010) used a simple 150-ft coiled loop of 1/8-in. inner diameter PVC loop to simulate reactions occurring in a RWDS over 6-10 weeks

of experiment, to test the impacts of different disinfection strategies on microbial water quality intended for NPR. No severe clogging or biofilm/biofoulant formation in the loops was reported in that study, likely due to three orders of magnitude higher flow velocity (V = 0.177 m/s) compared to that in our Tube SDSs (V = 8.85×10^{-3} m/s), and a short residence time of 4 minutes (calculated based on an assumption of clean loops). Interestingly, despite these factors, Jjemba et al. (2010), still reported rapid losses of up to 65% of the free chlorine residuals and 99% of the chloramine residuals between the inlet and the end of the 150-ft loop (3.56 mg/L of chlorine losses and 1.23 mg/L of chloramine losses). High bacterial regrowth (1-3 logs increase in heterotrophic plate counts) was observed with the losses of disinfectants in the simulated loops, as was observed in our Tube and Pipe SDSs.

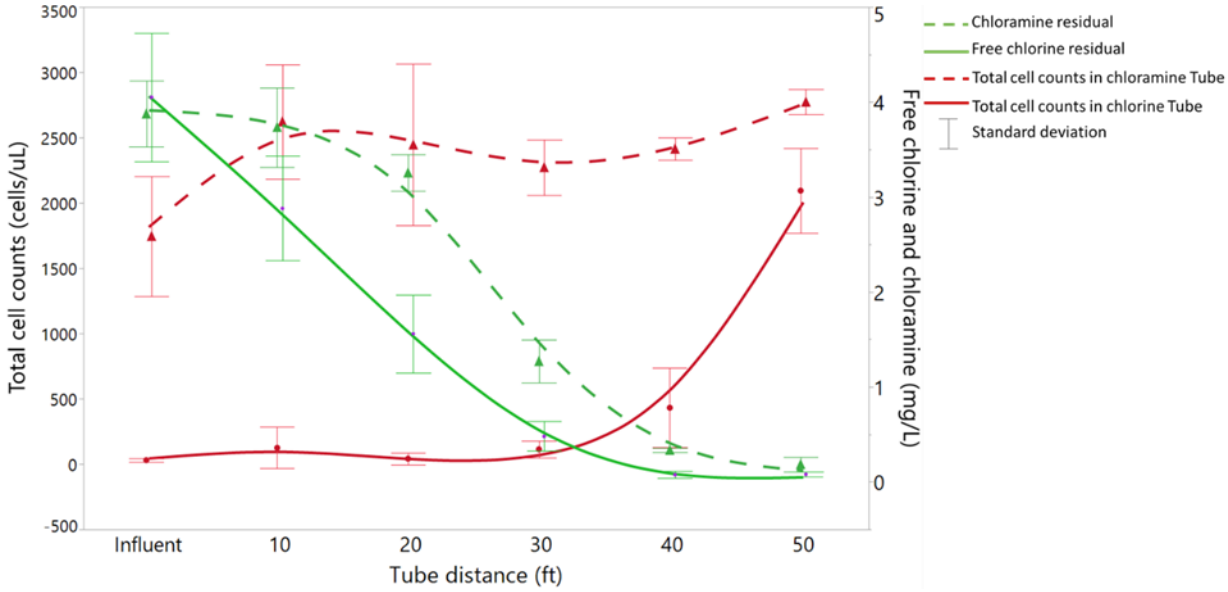

Figure 5-5 The RWDS Tube SDS was able to reproduce the interplay between disinfectant decay and microbial regrowth within a short 28-min retention time.

5.3 CONCLUSION

The strengths and weaknesses of various approaches to SRWDS design and operation are described, along with challenges to recreating key phenomenon as they are thought to occur in full-scale RWDSs. The high levels of nutrients, sediment, and the needs for BSL II safety and realistic wastewater effluent creates challenges to attaining a comprehensive simulation of all the key aspects affecting chemistry and biological regrowth of minimally treated reclaimed water systems in a laboratory setting.

While it is not possible to achieve similitude for all parameters, it is still possible to recreate ecological niches of chemical and biological interest, including bulk water, biofilm and sediment accumulations. For instance, in this work, two complementary designs did recreate extremes, that replicated heavy sediment accumulations in a pipe reactor, and which did not allow sediment accumulations to occur in a tube reactor. The tube reactor did recreate a realistic redox profile for water flowing through the reactor, albeit at a length 4 orders of magnitude

lower and water retention times 2 orders of magnitude lower than in a corresponding full-scale system.

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CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Establishing a sound scientific basis for operating and managing non-potable reuse (NPR) reclaimed water distribution systems (RWDSs) to meet water quality and safety goals at the point of use will be important to expanded use of this emerging water source. RWDSs pose challenges in terms of controlling water chemistry, suppressing microbial regrowth, dealing with inherently long-stagnation times and a wider range of influent water quality relative to highly treated and regulated drinking water distribution systems (DWDSs). This dissertation conducted long-term experiments using well controlled studies with simulated RWDSs (SRWDSs) in the laboratory. The work documented extensive water chemistry and microbial water quality trends in our SRWDSs, with respect to a suite of key experimental variables, including temperature, types of disinfectants, biological filtration, water age and pipe age. This work provided unique insights on NPR RWDSs to help understand emerging issues in full-scale RWDSs and ultimately improving future practices.

Chapter 2 provided a literature review foundation for this dissertation by outlining the fundamental differences between DWDSs and RWDSs and emerging public health concerns. It was argued that effective and safe management of reclaimed water systems would require comprehensive consideration of the differential water quality characteristics, distinct water reuse patterns and multiple routes of human exposure. A "human exposome" framework was proposed as a holistic risk assessment model for RWDSs, which identified three novel considerations 1) both acute and chronic exposure risks, 2) all potential routes of exposure to the end users, 3) final water quality after distribution at the end of point of use instead of at the point of treatment. An increased awareness of the boarder public health implications of reclaimed water prompted thorough study of water quality changes during distribution of reclaimed water and the effectiveness of selected engineering control strategies including biofiltration and disinfection, to prevent water quality degradation in RWDSs.

Chapter 3 summarized novel findings from a long-term study of two complementary SRWDSs, the wide Pipe RWDSs and the narrow Tube RWDSs, designed to maximize differences in flow patterns and propensity to accumulate sediment. Water chemistry and microbial water quality were profiled long the two SRWDSs to investigate the impacts of reactor design, biological filtration and disinfection on the resulting water quality profiles. The SRWDSs fed with chloramine or no residuals had orders of magnitude higher levels of turbidity than typical drinking water. Elevated levels of particular matter in the reclaimed water at the very low flow velocities, led to accumulation of sediment at the bottom of the wide Pipe RWDSs, resulting in rapid loss of disinfectant residuals and dissolved oxygen with depth near the entry point. Biologically active sediment was the main source of chloramine disinfectant demand and a hotspot for nitrification. Severe biofouling was observed in the narrow Tube RWDSs with chloramine and no residuals. Much less sediment and biofouling were observed in the chlorinated Pipe and Tube RWDSs, where biofilm was the main source of disinfectant demand. The use of chlorine also predictably resulted in much higher levels of regulated disinfection byproducts in our SRWDSs compared to conditions without disinfectant or using chloramine. This controlled head-to-head profiling of the two SRWDSs called for attention to the importance of sediment accumulation and its implications for water quality degradation in RWDSs.

Chapter 4 described a multi-year study of water chemistry and biostability in the Pipe RWDSs over a range of temperature from 14°C→22°C→30°C→22°C→14°C to simulate the a seasonal effect. Comprehensive water chemistry and microbiology sampling was conducted at the end of each temperature phase to characterize the systems. Temperature was observed to be the overarching factor controlling disinfectant decay, dissolved oxygen trends, heterotrophic cell growth and microbial community. At 30°C, both chlorine and chloramine were rapidly depleted upon entering the pipe SRWDs. Severe nitrification was triggered in the chloramine systems at 22 °C and persisted afterwards. Biofilm was found to a preferred habitat for nitrifiers especially in stressed ambient conditions. Chlorine was more persistent than chloramine and better at controlling biological regrowth along the RWDSs. Metagenomic profiles revealed that the use of chlorine had the most dominant effect in shaping the microbial taxonomic and functional compositions while chloramine and no residuals samples had more similar metagenomic profiles.

Chapter 5 explored a spectrum of alternative SRWDSs design in terms of achieving similitude to the full scale RWDSs. The advantages and limitations of each SRWDS design were discussed in relation to the unique characteristics of full-scale RWDSs. Identifying and selecting the most appropriate SRWDS design to scientifically study important distribution system issues, will required special consideration of the unique circumstances giving rise to each problem of concern based on parameters including the effects of influent water quality, active surface areas to volume ratio, flow velocity, water age and actual water flow pattern.

The overall dissertation provided a long-term, well-controlled systematic investigation of water quality issues in NPR RWDSs. We established that RWDSs are subject to greater complexity in terms of hydraulic, chemical and microbiological processes. As reclaimed water becomes more popular, it is likely that research on such systems will also increase, and the documented experiences and trends herein can help guide that work.

FUTURE WORK

Multi-year operation of these SRWDSs created an archive of comprehensive data on water chemistry including disinfectant condition, biological filtration, water age, temporal effects, flow pattern and microbiological niches (i.e. water, biofilm or sediment). Although only select biological samples from a carefully designed matrix of experimental conditions were preserved and sent for metagenomic sequencing in this work, samples have been archived to allow future exploration of the interplay between water chemistry and microbial constituents of interest, including opportunistic pathogens (OPs) and antibiotic resistance genes (ARGs) which are critical emerging microbial contaminants in water systems. OPs and ARGs have unconventional routes of exposure and have both acute and chronic health impacts, making them of intense current interest for treatment and regulation. Prior field studies on RWDSs have shown that disinfectants and biological filtration are critical factors in influencing the dissemination or destruction of OPs and ARGs. Future research on the mechanisms of OPs and ARGs attenuation/proliferation using samples collected in this work, would offer insights to enhance our understanding on controlling these emerging microbial constituents in RWDSs.

Recognizing the distinct nature of reclaimed water systems will prompt a broader consideration of tools applied to complementary studies of both full and lab scale systems. One prominent example is the nature and composition of biologically available organic carbon in reclaimed water distribution systems. In conventional DWDSs, assimilable organic carbon (AOC) is typically used as an indicator to assess the level of biological available organic carbon

and biostability. The standard AOC protocol established from drinking water requires filter sterilization with 0.22 μm membrane filters, and the results are based on the assumption that virtually all AOC is soluble and can pass through the filters. However, reclaimed water, which is derived from wastewater effluent, is likely to contain a much higher proportion of biomass and biomass-derived cellular components in a biologically available organic carbon form, that could also stimulate biological regrowth. The conventional AOC method would fail to capture the full spectrum of biological available organic carbon in reclaimed water.

Meeting future research challenges associated with reclaimed water systems would benefit from a similar mechanistic evaluation of similarities and difference between non-potable reclaimed water quality and potable distribution, including a critical evaluation of the techniques that directly transfer and those which must be modified to develop improved mechanistic understanding.