Influence of residence time of reclaimed water within distribution systems on water quality

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ABSTRACT

The influence of residence time of reclaimed water within water distribution systems on microbial water quality was evaluated in two wastewater reclamation facilities in southern Arizona over a 15-month period. These utilities differed in age, geographic location, means of treatment, and disinfection (i.e. UV versus chlorine). At both facilities, samples were collected from the point of compliance (POC) directly after disinfection, and at discrete locations with increasing distance from the POC. Following entry into reclaimed water distribution systems, overall microbial water quality decreased rapidly due to microbial regrowth. However, following such regrowth, microbial concentrations remained relatively constant. Water-based opportunistic pathogens (Legionella, Mycobacterium, and Aeromonas) were frequently detected in both reclaimed water systems. In contrast, waterborne indicators such as Escherichia coli and Enterococcus were rarely detected, and only at low concentrations. These dates suggest the need for new indicators of water-based pathogens to be developed. Rechlorination in one of the distribution systems only reduced the concentration of bacteria temporarily due to rapid dissipation of chlorine, and subsequent regrowth of both water-based pathogens and indicators. Amoebic activity was detected in approximately one-third of all samples tested from both utilities, but was not correlated with either water-based pathogens or indicators.

Key words | reclaimed water quality, residence time

INTRODUCTION

Population increases in water-scarce arid regions and an inadequate supply of new water resources has led to increased use of reclaimed water for non-potable purposes including irrigation. Reclaimed water is municipal wastewater that has undergone a series of treatments to reduce the level of organic matter and improve water quality (Harwood et al. 2005). Specifically, it has been defined as effluent that has undergone a combination of physical, chemical, and biological treatment technologies to remove suspended solids, dissolved solids, organic matter, nutrients, metals and pathogens (Jjemba et al. 2010). ‘Water reclamation’ involves treatment of wastewater to make it reusable. ‘Water reuse’ is the beneficial use of treated wastewater. Despite treatment and disinfection, it is hypothesized that a wide variety of pathogens may remain in reclaimed water distribution systems including waterborne and water-based pathogens. Waterborne pathogens are found in warm-blooded animal feces, and are responsible for disease via the fecal–oral route or ingestion of contaminated water. Examples include enteropathogenic Escherichia coli, Salmonella and Cryptosporidium. In contrast, water-based pathogens are organisms that spend part, of all, of their life cycle in water and are capable of growth, metabolism and reproduction in water. Here, examples include Mycobacterium, Legionella and Acanthamoeba. Currently, utilities routinely monitor for waterborne pathogens using indicator organisms such as
coliforms, fecal coliforms or E. coli. No such monitoring is conducted for water-based pathogens because to date there are no indicators for water-based pathogens.

Currently there are no nationwide federal regulations governing the use and quality of non-potable reclaimed water in the United States. However, the United States Environmental Protection Agency (USEPA) has published Guidelines for Water Reuse (USEPA 2004), and the World Health Organization (WHO 2006) also has guidelines for reclaimed water use in irrigated agriculture. In addition, 25 states in the USA currently have statewide regulations for water reuse, and 16 states have guidelines. In Australia, extensive guidelines for water recycling have been issued on various aspects including human health aspects. Specifically, the Australians have used human health-based targets for pathogens and chemicals, and exposure routes, as part of a risk-based approach to evaluating potential adverse health effects.

The chemical and microbial water quality of reclaimed water at the point of entry is routinely monitored as it leaves the treatment plant, since this is frequently the point of compliance (POC). However, due to the fact that water must travel extensive distances through the distribution system to the point of use, there is potential for the quality of water to deteriorate within the distribution system, with residence time or water age being a key factor. Residence time will normally be a function of water demand as well as system operation and design. As residence time increases, both chemical and microbial water quality can be diminished (Narasimhan et al. 2005; Weinrich et al. 2010). Frequently, such deterioration of microbial water quality with increased water age is due to chlorine dissipation as it interacts with organic matter, allowing for bacterial regrowth (Ryu et al. 2005).

In this present study, the influence of the residence time of reclaimed water within distribution systems on microbial water quality was evaluated. Specifically, two wastewater reclamation facilities (WWRF) which utilized different methods of treatment and disinfection were monitored for an extended period of time. An additional objective was to document the incidence of both indicator bacteria and water-based pathogens in reclaimed water distribution systems.

MATERIALS AND METHODS

Water quality monitoring: site description and sampling strategy

The distribution systems of two WWRF located in southern Arizona were sampled monthly for microbial water quality over a 15-month period from November 2009 to February 2011. At both facilities, samples were collected from the POC at the plant directly after disinfection, and at discrete points along the distribution system with increasing distance from the POC. Distance from the POC was used as a surrogate for residence time, since residence time itself within the distribution system is unknown. The distance between sampling locations for each reclamation facility varied due to the differences in the length and design of each distribution system. For Utility A, 25 miles of pipeline were monitored in this study. For Utility B, 8 miles of pipeline were monitored. For both utilities, reclaimed water was utilized for irrigation of turfgrass areas including parks, golf courses and schools.

The two reclamation facilities studied differed by age, geographic location, means of treatment, disinfection and the quality of water produced. The Arizona Department of Environmental Quality (ADEQ) has established five classes of reclaimed water based on water quality and its intended use. In this study, Class A and Class A+ water were produced at the two utilities. The major difference in reclaimed water quality class between Class A+ and Class A reclaimed water is the level of nitrogen content and regulation. ADEQ requires that Class A+ has a total nitrogen concentration of less than 10 mg/L; this type of water may be used for any type of direct reuse other than potable reuse. In contrast, Class A has no such nitrogen limitation, and may be used for any type of direct reuse except those that require Class A+. Examples of Class A uses include irrigation of food crops, residential, school ground and open access landscape irrigation, and toilet and urinal flushing.

Utility A produces Class A water and is an older facility of approximately 25 years of age and utilizes chlorine as a means of disinfection. This utility is a 10 million gallon per day (mgd) tertiary filtration plant consisting of an effluent booster station, chlorine contact chamber and a
reclaimed chlorine booster station. At the POC, average chlorine concentrations were 5.9 mg/L. Utility B produces Class A+ reclaimed water, is about 6 years of age, and uses ultraviolet (UV) light as a primary means of disinfection. The UV system uses 10 low pressure/high output UV modules each equipped with 40 individual light bulbs (400 bulbs total). At nominal flow rates the UV dosage is >100,000 μW sec/cm². Overall, with a contact time of 1.2 min @ 2,800 gpm, the system can treat 4 mgd.

Sample collection

At monthly intervals, multiple samples were collected at discrete locations along the distribution system and analyzed for physico-chemical characteristics such as pH, temperature, and total chlorine, as well as microbial water quality. Samples for bacterial analyses were collected in sterile 1 L polypropylene wide-mouth Nalgene bottles (Nalge Nunc Corporation, Rochester, NY). One milliliter of sterile 10% sodium thiosulfate was added to the polypropylene bottles prior to sampling to neutralize any residual chlorine. The bottles were completely filled, precluding headspace according to Standard Method 9060A (APHA 2011). Samples were transported on ice in a cooler to the laboratory within 12 h for analysis, with the exception of samples for amoebic activity that were transported at room temperature to prevent the amoebae from encysting.

Bacterial analyses

Samples were analyzed for Aeromonas spp., heterotrophic plate count (HPC) bacteria, total coliforms, Enterococcus spp., E. coli, Legionella spp., and Mycobacterium spp. Total coliforms, E. coli, and Enterococcus were identified using defined substrate technology, the Colilert and Enterolert systems (IDEXX laboratories, Westbrook ME). The quanti-tray (a 51-well tray designed for bacterial enumeration) is a colometric method for the detection and enumeration of coliforms and E. coli. The number of positive wells (bright yellow wells for coliforms, fluorescent blue for E. coli and Enterococcus, when exposed to UV light) were counted and utilized to obtain a Most Probable Number (MPN) per 100 mL. For E. coli, only fluorescent wells using Colilert substrate after exposure to 365 nm UV light with prior incubation at 35 ± 0.5 °C for 25 ± 2 h were considered positive. For the Enterolert system, fluorescent wells were only counted as positive for enterococci after incubation at 41 ± 0.2 °C for 18–24 h and exposure to UV light at 365 nm.

Heterotrophic bacteria were enumerated using the spread plate method according to the Standard Method 9215C (APHA 2011). Specifically, 0.1 mL was spread on pre-dried R2A agar plates (BD Diagnostics, Sparks, MD) and incubated for 7 days at 27 °C. Colonies were enumerated after 7 days and recorded as colony forming units (CFU)/100 mL.

Aeromonas analysis was both qualitative and quantitative using ampicillin dextrin agar (Havelaar et al. 1987). Serial volumes of 100, 10, 1 and 0.1 mL were filtered through separate 0.45 μm pore size filters (Millipore Corporation, Bedford, MA) as outlined in EPA Method 1605 (USEPA 2001). The filters were then aseptically placed onto dextrin agar plates. The genera of the resulting colonies were identified using API 20NE (Biomerieux, Durham, NC) as described in Penna et al. (2002). The interpretation of the reactions were made according to the reading table identification software containing the API 2ONE data base.

Samples for Legionella were analyzed according to Standard Method 9260J (APHA 2011). Briefly, a portion of the sample was treated with acid, incubated for 15 min, neutralized, and then spread plated onto buffered charcoal yeast extract (BCYE) agar with Legionella supplements (cysteine) (BD Diagnostics, Sparks, MD). The plates were incubated for up to 7 days and colonies enumerated. Presumptive Legionella colonies were subcultured using streaking for isolation on BCYE agar without cysteine and growth indicated a positive Legionella colony (NHS Identification 2007). Colonies were also confirmed by polymerase chain reaction (PCR) (Rock et al. 2013).

Samples for mycobacteria were analyzed according to Standard Method 9260M (APHA 2011). Briefly, 250 mL samples were treated with 10 mL of 0.04% cetylpyridinium chloride (CPC), and incubated for 24 h at room temperature. Subsequently, 10, 1 or 0.1 mL samples were filtered using a 0.45 μm membrane filter, and grown on Middlebrook 7H10 agar plates containing OADC enrichment, glycerol, sodium propionate and 10 mL of an antibiotic suspension containing 5 mL of antibiotic cocktail comprised of 0.25 g
nalidixic acid, 250,000 units of penicillin and 500,000 units of nystatin. The antibiotics were mixed and filter sterilized prior to use to reduce fungal growth. Presumptive colonies that were smooth and opaque or transparent were transferred to 3 mL of 7H9 broth as described in Standard Method 9260M and confirmed by acid-fast staining as described in Seely et al. (1991).

Prior to the start of each experiment, an Erlenmeyer flask containing 1 L of tryptic soy broth (TSB; Difco, Sparks, MD) was inoculated with E. coli (ATCC #15597) and incubated with agitation at 37 °C overnight. After incubation, the E. coli were pelleted via centrifugation (9,800 g, 15 min, 25 °C). The pelleted cells were washed by re-suspension in 100 mL of sterile phosphate buffered saline (PBS; pH 7.4; Sigma-Aldrich, St Louis, MO) followed by two rounds of centrifugation/re-suspension as described previously. The final pellet was resuspended in Ringers Solution (1.69 g NaCl, 0.022 g KCl, 0.028 g CaCl2, and 0.057 g NaHCO3 per L).

A volume of 500 mL of each sample was seeded with 3 mL of the pre-prepared E. coli. The seeded sample was then concentrated by centrifugation (9,800 g, 20 min, 25 °C). All but 10 mL of the supernatant was discarded. This volume was then collected and inoculated onto non-nutrient agar (Difco, Sparks, MD) using the spread plate method. The plates were then incubated for 3 days at 44 °C with daily observation using bright field light microscopy to detect amoebic growth as evidenced by a plaque or clearing in the bacterial lawn.

Statistical analyses

To determine if there were any differences between the levels of microorganisms detected between populations (e.g. between samples collected in the winter and summer), analysis of variance (ANOVA) statistical tests were conducted (Microsoft Excel version 14.0.6112.5000). The numbers of microorganisms were log-transformed to ensure that the data were normally distributed prior to the analysis. The differences were considered to be statistically significant if the P value was ≤0.05.

Pearson’s correlation were conducted (Microsoft Excel version 14.0.6112.5000) to determine if there were any correlations between the presence and levels of waterborne or water-based pathogens and any of the other measured water quality parameters. Values between 0.6 and 1.0 (positive correlations) and also between −0.6 and −1.0 (negative correlations) were considered significant.

RESULTS

Physico-chemical parameters

Data on the water quality from each utility are shown in Tables 1 and 2. Most chemicals and nutrients were found in both utility waters at similar concentrations except for alkalinity and total dissolved solids, which were found in higher concentrations at Utility B than at Utility A. Turbidity levels of reclaimed water averaged over the four seasons were typically less than two nephelometric turbidity units (NTU). The maximum mean value was 2.4 NTU from the POC in Utility A, and 3.2 NTU in Utility B. Water temperatures varied between 21 and 26 °C at Utility A and 21 and 28 °C at Utility B.

Chlorine stability

In Utility A, total chlorine levels dissipated rapidly with increased distance from the POC starting with an average of 3.9 mg/L at the source, to less than 0.1 mg/L at the furthest end of the distribution system. This decrease in chlorine occurred despite a chlorine booster was located within the distribution system 11.7 miles away from the plant, which temporarily resulted in increased concentrations of the disinfectant at that location ranging from 0.1 to 0.7 mg/L total chlorine. Thus, the chlorine concentration was not consistently maintained throughout the distribution system. This decrease in chlorine occurred more rapidly during the summer. Utility B did not utilize chlorine as a means of disinfection, relying instead on UV.

Microbial water quality

The microbial water quality was evaluated in terms of: (i) frequency of detection; (ii) seasonal effects; and (iii) residence time effects. With respect to frequency of detection, HPC bacteria were essentially always found in both
### Table 1 | Chemical reclaimed water quality, Utility A

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SDb (mg/L)</th>
<th>Maximum value</th>
<th>Minimum value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkalinity as bicarbonate</td>
<td>180.0 ± 4.1</td>
<td>270.0</td>
<td>102.0</td>
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<tr>
<td>Nitrate – N</td>
<td>5.1 ± 1.7</td>
<td>11.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Nitrite – N</td>
<td>1.3 ± 1.1</td>
<td>4.2</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen</td>
<td>5.2 ± 4.2</td>
<td>15.0</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Orthophosphate – P</td>
<td>1.7 ± 0.7</td>
<td>3.9</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Total phosphate as P</td>
<td>1.9 ± 1.0</td>
<td>8.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Total organic carbon</td>
<td>5.3 ± 1.6</td>
<td>9.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Total organic carbon, dissolved</td>
<td>5.1 ± 1.6</td>
<td>9.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Total dissolved solids</td>
<td>685.0 ± 64.8</td>
<td>845.0</td>
<td>547.0</td>
</tr>
<tr>
<td>Total suspended solids</td>
<td>3.4 ± 4.1</td>
<td>18.9</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>Chloride</td>
<td>131.9 ± 16.8</td>
<td>241.0</td>
<td>92.0</td>
</tr>
<tr>
<td>Sulfate</td>
<td>153.4 ± 11.1</td>
<td>194.0</td>
<td>116.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>76.3 ± 9.4</td>
<td>99.0</td>
<td>54.0</td>
</tr>
<tr>
<td>Magnesium</td>
<td>12.5 ± 2.7</td>
<td>16.0</td>
<td>7.1</td>
</tr>
<tr>
<td>Sodium</td>
<td>130.1 ± 19.4</td>
<td>210.0</td>
<td>89.0</td>
</tr>
</tbody>
</table>

*aAll analyses conducted by Standard Methods (APHA 2011).*

*bSD – standard deviation of three replicate analyses from a single sample.*

### Table 2 | Chemical reclaimed water quality, Utility B

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SDb (mg/L)</th>
<th>Maximum value</th>
<th>Minimum value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkalinity as CaCO₃</td>
<td>315.4 ± 28.8</td>
<td>360.0</td>
<td>260.0</td>
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<tr>
<td>Alkalinity as bicarbonate</td>
<td>313.4 ± 27.0</td>
<td>370.0</td>
<td>260.0</td>
</tr>
<tr>
<td>Ammonia – N</td>
<td>0.6 ± 0.1</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Nitrate – N</td>
<td>4.5 ± 4.5</td>
<td>18.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Nitrite – N</td>
<td>0.8 ± 1.1</td>
<td>5.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen</td>
<td>1.4 ± 0.4</td>
<td>2.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Orthophosphate – P</td>
<td>3.4 ± 0.9</td>
<td>18.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Total phosphate as P</td>
<td>3.6 ± 1.2</td>
<td>5.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Total dissolved solids</td>
<td>1,438.1 ± 139.6</td>
<td>1,800.0</td>
<td>1,100.0</td>
</tr>
<tr>
<td>Total organic carbon, dissolved</td>
<td>5.1 ± 1.2</td>
<td>10.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Chloride</td>
<td>409.7 ± 68.9</td>
<td>480.0</td>
<td>220.0</td>
</tr>
<tr>
<td>Sulfate</td>
<td>285.6 ± 29.9</td>
<td>350.0</td>
<td>260.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>87.4 ± 14.5</td>
<td>120.0</td>
<td>71.0</td>
</tr>
<tr>
<td>Magnesium</td>
<td>476.1 ± 48.3</td>
<td>560.0</td>
<td>400.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>61.8 ± 6.8</td>
<td>70.0</td>
<td>48.0</td>
</tr>
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</table>

*aAll analyses conducted by Standard Methods (APHA 2011).*

*bSD – standard deviation of three replicate analyses from a single sample.*
reclaimed waters at high concentrations (≃100%) (Figures 1 and 2). *Aeromonas* and total coliforms were also found at high frequencies in both utilities. At Utility B, *Enterococci* was found within ≃80% of all samples compared to <30% of samples within Utility A. The water-based pathogen, *Legionella* was found within ≃40% of all samples from both utilities. *Mycobacterium* was found within 40% of all Utility A samples, and 56% of all Utility B samples. In contrast, the waterborne *E. coli* was found at 40% incidence in Utility B, and 3% incidence in Utility A. Amoebic activity was found with similar frequency at both utilities (28%). In contrast, both somatic coliphage and male-specific phage were found at higher frequency within Utility A (52 and 28% respectively) than within Utility B (8 and 3% respectively).

For seasonal effects, the samples were classified as either summer (June to August), fall (September to November), winter (December to February), or spring (March to May). For the residence time, as the distance from the POC increased, water age was also assumed to increase.

The seasonal occurrence of microorganisms in reclaimed water at both utilities are shown in Figures 3 and 4. Data show the average seasonal distribution of microorganisms. For both utilities, HPC bacteria were present at greater concentrations than other bacteria; concentrations of $10^7$–$10^8$ CFU/100 mL were consistently observed with no apparent
statistically significant seasonal effects. At Utility A, *E. coli* was rarely detected during the study and enterococci were only detected at very low concentrations (<1 log$_{10}$/100 mL) in the summer and fall. In contrast, the water-based opportunistic pathogens *Aeromonas*, *Legionella* and *Mycobacterium* were found at higher concentrations of up to 4 log$_{10}$/100 mL, but seasonal differences were not significant. Similar trends were seen at Utility B; however, the indicator organisms (*E. coli* and *Enterococcus*) were found at higher concentrations than at Utility A. These concentrations correspond to the frequency of detection of *E. coli* and *Enterococcus*, which was also higher in Utility B than Utility A.

The indicator organisms *E. coli* and *Enterococcus* were detected at low frequency at both utilities relative to the incidence of water-based pathogens such as *Legionella*, *Mycobacterium*, and *Aeromonas* (Figures 1 and 2).

The influence of residence time on the concentrations of water-based pathogens, HPC and indicator organisms at both utilities are presented in Figures 5 and 6 for Utility A, and in Figures 7 and 8 for Utility B. For HPC and water-based pathogens, the influence of residence time was consistent and significant. At both utilities, concentrations of these organisms increased significantly once the reclaimed water was within the distribution system. However, following this rapid initial increase, the concentrations of all the organisms remained relatively constant, exhibiting minor fluctuations that might be expected under field conditions. The only exception to this occurred at the chlorine booster station at Utility A
Figure 5 | Influence of distribution system residence time on the concentrations of water-based pathogens in chlorinated recycled water (Utility A).

Figure 6 | Influence of distribution system residence time on microbial indicators in chlorinated recycled water (Utility A).

Figure 7 | Influence of distribution system residence time on concentrations of water-based pathogens in UV disinfected recycled water (Utility B).
(approximately 11 miles from the POC). Here, following additional inputs of chlorine, HPC concentrations decreased by two orders of magnitude; water-based pathogen levels also decreased, and indicator organisms were not detected. Nevertheless, the effect of chlorine was only temporary; three miles distant from the booster station, microbial concentrations were similar to prechlorinated levels. At Utility A, *E. coli* and *Enterococcus* were only present at low concentrations and were not influenced by water age. At Utility B, *E. coli* and *Enterococcus* did increase with increased residence time.

In addition to bacteria, amoebic activity was monitored. For Utility A, 42 of 143 samples tested positive for amoebic activity (29.4%). For Utility B, 39 of 135 samples were positive (28.8%).

In Utility A, fairly strong correlations were observed, primarily between some of the bacterial species. A positive correlation between two variables indicates that when one variable increases or decreases, the other also increases or decreases in a similar manner. Conversely, a negative correlation indicates that when one increases, the other decreases. For example, there was a negative correlation (−0.8) between the level of total chlorine measured in Utility A in the winter and the numbers of HPC bacteria. Interestingly, the water-based pathogens, *Mycobacterium* and *Legionella* exhibited a negative relationship (−0.8) in the fall. There was a positive correlation between the levels of HPC bacteria and *Legionella* (0.8) in the summer in Utility A.

In Utility B, strong positive correlations existed between bacterial species over various seasons. For example, positive correlations were found between *E. coli* and *Enterococcus* and HPC in the summer (0.9 and 0.7, respectively); *E. coli* and *Legionella* in the fall (0.8); *E. coli* and *Aeromonas* in the fall (0.8); *E. coli* and *Mycobacterium* in the fall (0.7); *Aeromonas* and HPC in the fall; winter and spring (0.8, 0.8, and 0.9, respectively); *Aeromonas* and *Legionella* in the fall; winter, and spring (0.9 for each); *Aeromonas* and *Mycobacterium* in the summer only (0.7); *Legionella* and HPCs in the fall, winter and spring (0.8, 0.9, and 0.9; respectively), and *Legionella* and *Mycobacterium* in the summer only (0.8). The presence of amoebic activity could not be correlated with the presence of any of the bacteria in either utility at any time.

**DISCUSSION**

Federal guidelines require an average turbidity of 2 NTU (USEPA 2004). The samples collected at varying distances along the distribution system met this criterion in both utilities. The required pH levels for Class A and Class A+ reclaimed water is within the pH range of 6–9. In Utility A, the reclaimed water remained close to neutral throughout the study, while in Utility B, reclaimed water was slightly alkaline, but still met the stipulated requirements.

The loss of chlorine disinfectant in Utility A was accompanied by a rapid increase in the level of HPC
bacteria, however HPC per se are not necessarily a human health threat.

Rapid disinfectant loss has been reported in part to be due to the presence of organic carbon within the distribution system (Jjemba et al. 2010). Interestingly, following regrowth, HPC levels remained fairly consistent, indicating that the population attained a pseudo-equilibrium level perhaps regulated by the amount of bioavailable or assimilable carbon present within the reclaimed water. There was no statistical difference between the levels of any microorganisms found within different seasons at either utility. However, microbial concentrations were generally lower in Utility A than Utility B. This could in part be attributed to the effect of residual chlorine present within the Utility A distribution system. A significant negative correlation was observed between total chlorine and HPC bacteria (−0.8) and positive correlations between HPC and many of the water-based pathogens, suggesting that total control of disinfectant concentration will successfully impact the growth of other microbial organisms.

Traditional indicator organism(s), such as *E. coli*, were rarely detected or detected at very low concentrations in both systems. Furthermore, water-based or opportunistic pathogens were detected during occasions where currently used indicators of microbial water quality were not detected, suggesting that indicators such as *E. coli* and total coliforms traditionally used for compliance monitoring may not accurately represent microbial water quality from a risk assessment/public safety perspective. These data are supported by other studies that have found similar trends (Jjemba et al. 2010).

Total coliform indicator organisms were detected at lower concentrations and frequencies in the chlorinated system (Utility A) than the UV system (Utility B). *E. coli* was only found at low concentrations and frequency in both systems. In the UV disinfected system, enterococci were found at levels one order of magnitude greater than the chlorinated system. For the chlorinated system, all mean indicator concentrations were less than 100 CFU per 100 mL, while all values were generally ≤1,000 CFU per 100 mL for the UV system. The UV system at Utility B is designed to produce water with <2 coliforms/100 mL. However, it is difficult to determine if the indicator concentrations within the distribution system are due to repair or to regrowth due to the lack of residual chlorine in the distribution system.

The water-based pathogens, *Aeromonas*, *Legionella* and *Mycobacterium* spp. were routinely found in samples collected from both systems. In the chlorinated system, *Legionella* was found in the highest concentrations. Approximately 5 miles from the POC, *Legionella* concentrations were ≈100 CFU/100 mL. Some *Legionella* isolates were confirmed by PCR analysis to be *Legionella pneumophila* type 1. This increased to ≈10⁴ CFU/100 mL after 7 miles, and remained at that level throughout the remainder of the distribution system. A similar initial increase was found for *Aeromonas* with values reaching ≈1,000 CFU/100 mL. *Mycobacterium* concentrations were found at fairly constant concentrations, albeit at lower levels than the other water-based pathogens. Overall, it can be noted that there were numerous instances where water-based pathogens were found at relatively high concentrations and in the absence of *E. coli* and enterococci. This is not wholly unexpected, given that these indicators used for monitoring by utilities only indicate fecal waterborne contaminants, and numerous studies have found no relation between such indicators and water-based pathogens (Carter et al. 1987; Baggi et al. 2001; Jjemba et al. 2010). Interestingly, the injection of chlorine at the 11.7 mile booster station within Utility A had only a slight effect on indicator or pathogen concentrations. This may suggest an adaptation to the environmental conditions including resistance to chlorine, i.e. only resistant species survived.

Regardless of the organism monitored, all water-based pathogen concentrations increased fairly rapidly following entry into the distribution system of either utility. This in turn was followed by reasonably constant concentrations of all organisms despite further increases in residence time. This is in contrast to the conventional view that microbial water quality decreases consistently as the age of water increases. The maintenance level of organisms could be due to assimilable carbon concentrations becoming constant as some organisms continued to grow, while others died and were lysed. Falkingham et al. (2001) found a strong positive correlation between *Mycobacterium* levels and assimilable organic carbon within potable water systems.

Data from this study demonstrate that following entry into reclaimed water distribution systems, overall microbial
water quality decreases rapidly due to microbial regrowth. However, following such regrowth, microbial concentrations remained relatively constant. Of interest is the fact that water-based opportunistic pathogens were detected in both reclaimed water systems, and all species exhibited regrowth. In contrast, waterborne indicators such as *E. coli* were rarely detected and only at low concentrations, with no evidence of regrowth. In addition, fecal indicators were at times not correlated with water-based pathogen incidence or concentration. This suggests the need for new indicators of water-based pathogens.

Rechlorination of the distribution system at a booster station in Utility A only reduced the concentrations of bacteria temporarily, resulting in the survival of some chlorine resistant organisms. Although some die-off occurred following rechlorination, rapid dissipation of chlorine allowed for growth and re-growth of bacterial pathogens.

Amoebic activity was detected in approximately one-third of all samples from the systems tested, but showed no correlation with any of the other microorganisms detected. Amoebas have also been detected in other studies involving distribution systems (Jjemba et al. 2010; Thomas & Ashbolt 2011). In fact, a close association between *Legionella* spp. and amoebae such as *Acanthamoeba* has been reported, and even replication of *Legionella* within *Acanthamoeba* has been observed (Declerck et al. 2009). However, no correlations were found between amoebic activity and the presence of any of the indicators, or between amoebic activity and *Legionella*, *Mycobacterium* or *Aeromonas*, suggesting that water conditions promoting the growth of amoebae may differ from those promoting pathogen and indicator regrowth.

**CONCLUSIONS**

Microbial reclaimed water quality within both utility distribution systems initially decreased with increased residence time due to microbial regrowth. However, following this rapid initial increase in microbial numbers, microbial water quality remained relatively constant despite prolonged residence time within the distribution systems. Water-based opportunistic pathogens were detected with greater frequency and at higher concentrations than waterborne indicators, suggesting the need for new indicators of water-based pathogens. Rechlorination in one of the distribution systems temporarily reduced bacterial concentrations, but due to rapid dissipation of chlorine, subsequent regrowth of water-based pathogens and indicators occurred. Amoebic activity was found in approximately one-third of all samples tested from both utilities, but was not correlated with either wastewater pathogens or indicators.

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