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Viral and Bacterial Removal Efficiencies in Different Wastewater Treatment Processes for Various Types of Reclamation Purposes

THESIS

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for the degree of

MASTER OF SCIENCE
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by

Zheng Zhao

Thesis Committee:
Professor Sunny Jiang, Ph.D, Chair
Professor Soroosh Sorooshian, Ph.D
Professor Amir AghaKouchak, Ph.D

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ABSTRACT OF THE THESIS

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By

Zheng Zhao

Master of Science in Engineering

With a Concentration in Environmental Engineering

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Professor Sunny Jiang, Chair

Water reclamation has been used as an alternative to supplement the traditional water resource to meet the increasing demand owing to population growth and economic growth. However viruses and bacteria in wastewater can be a potential safety hazard for the public health in water reuse practices if are not effectively removed during water reclamation process. This research utilized a rapid flow cytometry (FCM) method to detect total viruses and total bacteria in each unit process of four different treatment process trains for water reclamation purposes, aiming at understanding their viral and bacterial removal efficiencies, which could provide a reference for the application of various treatment processes into water reclamation in the aspect of protecting public health. Multiple samplings was taken at four treatment processes trains over a half-year period, including activated sludge process (ASP) and membrane bioreactor (MBR) process of Michelson Water Reclamation Plant (MWRP), ASP of Chiquita Water Recycling Plant (CWRP), and microfiltration-reverse osmosis (MF-RO) process of Edward C. Little Water Recycling

Facility (ECLWRF). The results showed viral removal in the ASP of MWRP was 4.6$\log_{10}$, with 4.4$\log_{10}$ at chlorine disinfection tank (CD); total bacterial removal in the ASP was 5.9$\log_{10}$, with 1.7$\log_{10}$ achieved at secondary clarifier (SC), 0.2$\log_{10}$ at DMF, and 3.8$\log_{10}$ at CD respectively; in the MBR train, viruses and bacteria were reduced by 4.3$\log_{10}$ and 5.9$\log_{10}$ respectively in MBR. In the ASP train of CWRP, only 1.4$\log_{10}$ reduction of bacteria achieved through out the whole process train at SC, which indicate undesired removal towards viruses and bacteria. This result is due partially to a rapid conversion of chlorine to chloramine in the presence of high ammonia concentration in the influent water. Many gram-positive bacteria and viruses are more resistant to chloramine disinfection than indicator *E. coli*. In the MF-RO train of ECLWRF, viruses and bacteria were reduced by 4.5$\log_{10}$ and 5.1$\log_{10}$ as a consequence, with mainly reduction achieved at MF. This result indicates high efficiency of microbial removal capability of the advanced treatment train. However, the viral reduction rate by MF can vary significantly, the removal efficiency may be improved as the MF is clogged with reduced physical pore-size. This study contributes to our understanding of current wastewater treatment technology for virus and bacteria removal and offer insight to the policy decision for water reuse.

CHAPTER 1. INTRODUCTION

With the intensification of water scarcity in large urban cities around the world, the reuse of treated domestic wastewater has become a new practice to supplement the dwindling natural water resources. In the process of water reuse, human may get in contact with toxins, harmful chemicals and pathogens through various routes (Zhou et al., 2015). Thus, it is necessary for municipal wastewater to be treated to removal hazards prior to applications. One of the most essential considerations in water reclamation is the waterborne pathogens that could cause the spread of infectious diseases (Toze, 2006). The objective of this study is to investigate viral and bacterial removal efficiencies in each unit of wastewater treatment processes by using a rapid flow cytometry (FCM) method. The viral and bacterial removal efficiencies can be used as a reference on efficiency of various treatment processes in pathogen removal and the types of reclaimed water complying with their quality requirement in order to reduce health risk related to the exposure to pathogenic organisms. In the follow chapters of the thesis, different types of water reuse and quality of water treated for various reuse purposes are introduced. The health concern of water reuse application is discussed and the method for rapid monitoring microbial removal is presented. After describing the study sites and methods used in this study, the results obtained through the course of the investigation are presented. The discussion section further analyzes the results in the context of the water treatment variability and offers suggestion for the further improving water quality. Following is a roadmap of the

thesis structure to help the reader to navigate through the thesis.

Chapter 2 presents a literature review explaining the demand and the categories of water reclamation. The major waterborne pathogens related to health risks in reclaimed water are introduced. Different types of wastewater treatment processes and their mechanisms in treatment process are explained. Lastly previous research studies making use of FCM in the environmental field are reviewed.

The next chapter focuses on the sampling process and experiment methodology contributing to determining viral and bacterial removal efficienc.

In chapter 4, the viral and bacterial removal efficiencies of various treatment processes are reported, which is followed by the chapter explains the reasons of different viral and bacterial removal efficiencies that take place in the studied processes.

Finally a summary of the research and future consideration is presented in chapter 6.

CHAPTER 2. LITERATURE REVIEW

2.1 Alternative water resources

Water is a vital natural resource for humans. Due to the increasing water demand of growing population, and unequally distributed water resources as well as its poor quality, global water stress is becoming more severe (Wintgens et al., 2008). Traditional water resources are no longer able to provide enough water to the public; therefore alternative water resources should be explored and used to mitigate water scarcity, for instance, wastewater reclamation is one of the most effective ways to provide alternative water resources. By applying reclaimed water for various end-use purposes (i.e. irrigation, commercial, industrial activities, drinking aquifer recharge) depending on the level of treatment required, demands on freshwater resources can be alleviated, and water extracted from the environment can be reduced.

2.2 Categories of water reuse

Water reuse includes six categories: irrigation reuse, industrial reuse, recreational and environmental uses, non-potable urban uses, potable uses, and aquifer recharge (Beekman, 1998). Water reuse can be classified as direct and indirect reuse based on whether the reclaimed water will directly go into the distribution system or go to the water bodies to be retrieved in the future; it can also be classified as direct, indirect and non-potable reuse based on different water qualities to serve potable use (Table 1.) (Levine and Asano, 2004).

Table 1. Water reuse categories and definition

Term	Definition
Direct reuse	Applications include agricultural and landscape irrigation, cooling water and other industrial uses, urban applications, and dual water systems.
Indirect reuse	Mixing, dilution, and dispersion of treated wastewater by discharge into an impoundment, receiving water, or groundwater aquifer prior to reuse, such as in aquifer recharge.
Direct potable reuse	Incorporation of reclaimed water into a potable water supply system, without relinquishing control over the resource.
Indirect potable reuse	Incorporation of reclaimed water into a potable water supply by including an intermediate step in which reclaimed water is mixed with surface or groundwater sources upstream of intakes for drinking water treatment facilities.
Non-potable reuse	Includes all water reuse applications other than direct or indirect use for drinking water supplies.

2.2.1 Irrigation reuse

Irrigation reuse is a type of direct use of reclaimed water applying to agricultural crops or landscaped areas. Reclaimed water for irrigation conveys risks for health and environment depending on the water quality, of which pathogen content is one of the main quality factors that determines the suitability of recycled water for irrigation purpose (Lazarova and Bahri, 2004).

2.2.2 Industrial reuse

As a result of water stress, industries have not only focused on internal water recycling to minimize the dependence on the additional freshwater, but also on purchasing of reclaimed

water as external resources by working closely with the municipal wastewater recycling plants, which adapt new treatment technologies to produce high quality effluent to meet the industrial requirement (Shuval, 2012). In this reuse approach, aerosols containing toxic volatile, organic compounds and biological pathogens may induce illness of workers, which are parts of the quality concerns that related to human health; other quality concerns include scaling, corrosion and fouling caused by the presence of nutrients and metals water (Asano, 1998).

2.2.3 Environmental and recreational uses

Since reclaimed water can be applied to creating artificial wetlands, enhancing natural wetlands and recreation lakes (Friedler et al., 2006; Chu et al., 2004). Human may get infection when exposed to the pathogens exist in the environment and recreation water.

2.2.4 Aquifer recharge

Aquifer recharge (AR) is the enhancement of natural groundwater supplies implemented by rapid infiltration basins and injection wells (Dispersal Series Wastewater Reuse). One of the types of AR is aquifer storage and recovery (ASR) that can be used to store any types of water, e.g. potable water, reclaimed wastewater, surface water, storm water (Almulla et al., 2005). Those types of water are potentially infectious to humans if retrieved in the future.

2.2.5 Urban non-potable and potable uses

Reclaimed water used for non-potable purposes, as fire fighting and toilet flushing, possesses some constrains regarding public health, which includes pathogen transmission via sprays and the risk of connection with the potable water supply system (Beekman, 1998); for potable reuse, existence of human pathogen is one of the most fundamental considerations of human health (Usepa, 2004).

2.3 Microbial pathogens in wastewater

In order to minimize public health risks associated with reclaimed water exposure, human microbial pathogens is a kind of important consideration besides nutrients, toxins and harmful chemicals, which are enteric in origin, including viruses, bacteria, pathogenic protozoa and pathogenic helminths (Toze, 1999; Costan-Longares et al., 2008).

2.3.1 Virus

Viruses, in comparison with other pathogens that transmitted through water, present more challenge in removal. Exposure to human viruses contaminated water might cause hepatitis, gastroenteritis and respiratory diseases, for example, hepatitis A virus, enterovirus, and adenovirus are commonly found in human sewage (Toze, 1999; Rosario et al., 2009). Commonly 10^8 VLP/mL of virus like particles exist in the reclaimed water (Rosario et al., 2009). These pathogenic viruses are more infectious due to their low-dose infectivity (10 viral particles or less) and more resistant to treatment processes compared to most other pathogen types (Khan, 2012; Asano, 2005).

2.3.2 Bacteria

Bacterial pathogens are the most common and numerous microbial pathogens in reclaimed water, e.g., *E. coli, Salmonella* sp*, Enterococcus* sp (Toze, 1999). The majority of pathogenic enteric bacteria require a high dose of ingestion to cause infection (>10^6 cells) (Toze, 2006).

2.4 Wastewater treatment processes

Municipal wastewater treatment consists a combination of physical, chemical, and biological processes of removing solids, organic matters, nutrients, metal and pathogens. The treatment levels including preliminary, primary, secondary, tertiary, and advanced treatment.

Wastewater treatment plants are usually designed to efficiently remove biological oxygen demand compounds and nutrients, but seldom have they been planned specifically to remove pathogenic viruses and bacteria. Reductions in treatment processes can vary extensively according to the treatment process type, hydraulic retention time, pH, temperature and the efficiency in removing suspended solids (Koivunen et al., 2003). The following content included an overview of different treatment levels and their capability of viral and bacterial removal.

2.4.1 Preliminary and primary treatment

In conventional wastewater treatment facilities, preliminary is installed to reduce gross solids as large objects and grit that may cause maintenance and operational problems. Primary treatment including screening, grit removal and sedimentation can remove about

50% of suspended solids and 25 to 50% of BOD_5 from the untreated wastewater (Metcalf, 2003).

2.4.2 Secondary treatment

Secondary treatment systems refer to an array of biological treatment processes coupled with solid/liquid separation. The microbial processes including suspended growth and fixed-film processes are engineered to provide effective microbiological metabolism of organic substrates dissolved or suspended in the wastewater. Suspended growth processes include activated sludge, lagoon, etc.; attached growth processes include trickling filter, rotating biological contactor, and other biofilm bioreactors (Asano, 1998).

2.4.3 Tertiary treatment

Tertiary treatment refers to removal of residual suspended solid by chemical coagulation and granular medium filtration, reduction of inorganic compounds including nitrogen and phosphorus by nutrient removal processes, as well as inactivation of pathogens by disinfection (Asano and Levine, 1996; Hijnen et al., 2006; Okoh et al., 2007).

2.4.3.1 Chemical coagulation and flocculation

Coagulation or flocculation processes involve the addition of chemicals to wastewater to promote aggregation of particles for improved solid/liquid separation by sedimentation and filtration. Inorganic coagulant chemicals are metallic salts include alum (aluminum sulfate) etc., which can react with partible surfaces resulting in particle destabilization (Asano, 1998). Eventually combined with filtration process, coagulation can assistant

reducing viruses and bacteria associated with particles in the water(Koivunen et al., 2003). Nevertheless, removal of bacteria is far less effective than chlorination, and viral removal is less significant at pH 8 than at 6 or 7, and less efficient in large-scale plants than in bench scale plants (Shirasaki et al., 2014; Nieuwstad et al., 1988).

2.4.3.2 Granular-medium filtration

Granular-medium filtration is a solid/liquid separation process that is effective of removing suspended particles larger than 3μm and reducing pathogens associated with particles in the water, which can be installed downstream of primary or secondary sedimentation and upstream of disinfection unit to improve the efficacy of disinfection when more efficient elimination of microorganisms is needed (Asano, 1998; Koivunen et al., 2003).

2.4.3.3 Biological nutrient removal

Biological nutrient removal (BNR) systems is a biochemical operation that can be designed in the wastewater treatment to reduce biological nutrients including nitrogen and phosphorus that can foster the growth of algae in reservoirs, streams and storage facilities if not being treated properly (Asano, 1998; Grady Jr et al., 2012).

Biological nitrogen-removal processes include an aerobic zone in which biological nitrification occurs, and an anoxic zone to provide denitrification to complete the objective of total nitrogen removal by NH_4-N oxidation as well as NO_2-N and NO_3-N reduction to nitrogen gas (Metcalf, 2003). Biological phosphorus removal is accomplished by chemical precipitation that converts soluble phosphorus to particulate phosphorus that can be

removed by sedimentation and filtration; or by operating a biological phosphorus removal process with anaerobic and aerobic conditions that promote uptake phosphorus by microorganism (Metcalf, 2003).

2.4.3.4 Disinfection

Municipal wastewater has been identified as one of the main sources of pathogenic organisms and the potential vector of diseases. Hence, disinfection as the essential treatment component for almost all wastewater reclamation applications is mandatory to destroy the pathogenic organisms, which can be achieved by chemical disinfection processed based on addition of oxidizing chemicals including chlorine, ozone, and hydrogen peroxide, or by ultraviolet (UV) radiation as the alternative to chemical disinfection (Asano, 1998; Bustos et al., 2014).

2.4.3.4.1 Chlorination

Chlorination is the most common type of disinfection systems due to its high efficiency and low cost, at typical dosages ranging from 5 to 20 mg/L with a maximum of two hours of contact time (Asano, 1998; Guo et al., 2009). However, chlorination is not the ideal choice due to its potential to react with organic matters present in municipal effluents, which results in the formation of disinfection by-products (DBP) that are animal carcinogens and suspected human carcinogens, e.g., trihalomethanes (THMs) and haloacetic acids (HAAs) (Bustos et al., 2014; Hua and Reckhow, 2007; Singer, 1994; Liberti et al., 2003).

2.4.3.4.2 Ozonation

Ozone is an efficient disinfection agent that has been used in water applications, able not only to destroy pathogens rapidly, but also to oxidize organic constituents even at low concentrations (1-5 mg/L) and for short exposure times (1-5 min) (Okoh et al., 2007; Drury et al., 2006). Moreover, it has higher antimicrobial activity than chlorine; and cannot produce a persistent disinfectant residual due to its higher instability compared to chlorine as a result of no DBP and residue problem (Singer, 1994; Khadre et al., 2001).

2.4.3.4.3 Ultraviolet radiation

Ultraviolet (UV) radiation is a physical procedure for water and wastewater disinfection that typically eliminates enteric bacteria, viruses, bacterial spores and parasite cysts with high efficiency without producing DBPs or other chemical residues (Hijnen et al., 2006; Lazarova et al., 1998). The number of facilities using UV disinfection has augmented over the last 20 years and is expected to increase in the next years as an essential alternative to conventional chlorination for municipal wastewater disinfection (Bustos et al., 2014; Davila et al., 2008; Koivunen and Heinonen-Tanski, 2005).

2.4.3.5 Pressure-driven membrane processes

Pressure-driven membrane processes including microfiltration, ultrafiltration, nanofiltration and reverse osmosis can be defined as splitting a feed stream by membrane into a concentrated retentate and a purified permeate fraction (Van der Bruggen et al., 2003a; Van der Bruggen et al., 2003b). Different types of membrane processes and their characteristics are illustrated in Table 2.. During the transportation of the solvent through

the membrane caused by pressure difference between the feed and permeate side, particles and dissolved components are retained based on properties such as size, shape, and charge (Van der Bruggen et al., 2003a).

Table 2. Overview of pressure-driven membrane processes and their characteristics.

	Microfiltration	**Ultrafiltration**	**Nanofiltration**	**Reverse Osmosis**
Permeability ($1/h.m^2.bar$)	> 1000	10-1000	1.5-30	0.05-1.5
Pressure (bar)	< 5	2-8	5-15	15-100
Pore size (nm)	100-2000	2-100	0.5-2	< 0.5
Mechanism	Sieving	Sieving	Sieving Charge effects	Solution-diffusion
Application	Clarification; pretreatment; removal of bacteria	Removal of macromolecules, bacteria, viruses	Removal of ions and relatively small organics	Ultrapure water; desalination

2.5 Flow cytometry

Flow cytometry (FCM) is a well-established technique exercised in the field of clinical microbiology and environmental microbiology for rapid analysis and sorting individual cells and other biological or non-biological particles with selected physical and chemical characteristics through the measuring apparatus, a flow cytometer, in a fluid stream (Amann et al., 1990; Shapiro, 2005; Vives-Rego et al., 2000).

In the field of environmental application, FCM has been applied for accurate and rapid enumeration of fluorescently stained bacteria in saliva (Sahar et al., 1983), milk (Donnelly and Baigent, 1986), activated sludge (Völsch et al., 1990; Wallner et al., 1995), and marine

environment (Chen et al., 2001). Likewise, FCM has become one of the choices besides plaque assays (PAs), most-probable number assays (MPNs), transmission electron microscopy (TEM), and epifluorescence microscopy (EfM) for quantifying viruses in aquatic samples, after stained with nucleic acids stain including SYBR Green I, SYBR II, or SYBR Gold, which is more sensitive than SYBR Green I and II (Chen et al., 2001; Brussaard et al., 2010; Marie et al., 1999; Suttle, 2007).

CHAPTER 3. MATERIALS AND METHODS

3.1 WWTPs description and sampling

Three southern California WWTPs that include four different treatment processes for production of recycled water were chosen to evaluate the viruses and bacteria removal efficiency in this study. They are Michelson Water Recycling Plant (MWRP), Chiquita Water Reclamation Plant (CWRP), and Edward C. Little Water Recycling Facility (ECLWRF).

3.1.1 Michelson Water Recycling Plant

MWRP is one of the two recycling plants managed by Irvine Ranch Water District (IRWD). In 1967 MWRP began delivering approximately 2 million gallons per day (MGD) of tertiary standard recycled water (California Title 22), which were used in the community for state-approved non-drinking water purposes such as irrigation and toilet flushing. By 2008, MWRP's capacity had grown to 18MGD. By early 2013, plant production increased to 28MGD by the newly constructed MBR facility with a capacity of 10MGD (Abi-Samra et al., 2008).

MWRP has two treatment trains. Train 1 uses activated sludge process (ASP) with the Modified Ludzak-Ettinger process to achieve nitrification and denitrification, followed by seven dual-media (sand and anthracite) gravity filters to remove suspended solids with an average flow of 18MGD. Alum is added in the secondary clarifier effluent prior flowing into the dual-media filters to realize coagulation. Size of anthracite in dual-media filters is 1.05-1.15mm with a 1.4 uniformity coefficient.

Train 2 uses membrane bioreactor process (MBR) that combines biological treatment and filtration with an average flow of 10MGD. MBR facility provides high-quality recycled water capable of meeting the effluent requirements of California Title 22. The membrane possesses a pore size of 0.1-0.4μm.

Effluents from both trains are combined before flowing into chlorine contact tank. Approximately 110 gallons of 12.5% sodium hypochlorite per every 1 million gallon is added in the combined flow prior chlorination. Sodium hypochlorite concentration is approximately 17 mg L^{-1} in the combined influent, and 6 mg L^{-1} in the effluent. Free chlorine (the combination of hypochlorous acid and hypochlorite ions) was also measured in the end of the contact tank with a concentration of 7 mg L^{-1} on average. Fig. 1 illustrates the schematic layout of both treatment process trains at MWRP and the locations where samples were collected for determining the viral and bacterial removal efficiencies throughout the processes.

Approximately 250 mL sample was aseptically collected from each of the sampling points, which included the effluent of primary sedimentation tanks (PS), secondary high-rate clarifiers (SC), dual-media filters (DMF), chlorine disinfection tanks (CD) and MBR. Samples were transported in sterile Whirl Pak bags on ice to UC Irvine lab and processed for biological measurements within 2 hours of collection. Samples were taken on a biweekly basis, from June 2014 until January 2015.

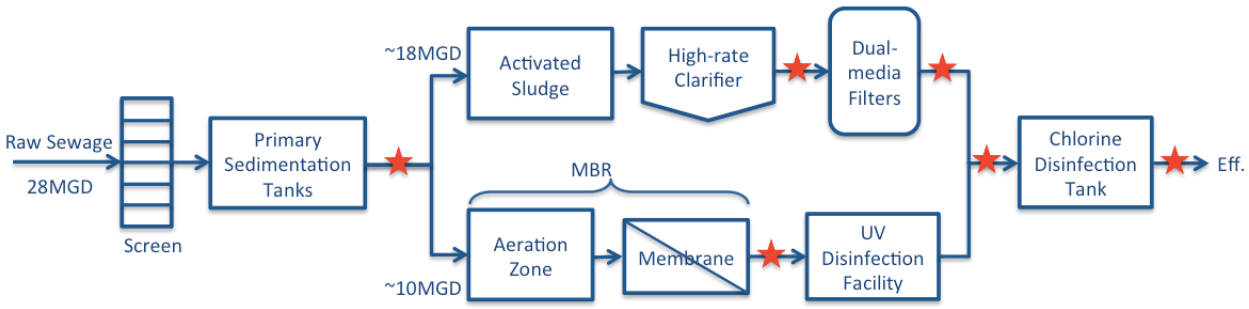

Fig. 1. Schematic of wastewater treatment processes at MWRP. Stars indicate sampling locations of this study.

3.1.2 Chiquita Water Reclamation Plant

CWRP is one of the two reclamation plants managed by Santa Margarita Water District located in the city of San Juan Capistrano. It produces approximately 5MGD of reclaimed water by using ASP and chlorine disinfection (Fig. 2) for irrigation purposes throughout the district's 62,674 acre service area (Reati et al., 1996).

Mainly nitrification occurred in the AS tank process of CWRP. Approximately 100 gallons of sodium hypochlorite per every 1 million gallons was added in the influent of chlorine contact tanks. Total chlorine residual consisted with free and combined chlorine residual was measured in the beginning and the end of the contact tank, with concentrations of 12 mg L^{-1} and 9 mg L^{-1} respectively.

From August 2014 to January 2015, water samples were aseptically collected from the effluent of PS, SC and CD in sterile Whirl Pak bags on a biweekly basis. 10 mL/L of 10% w/w Sodium Thiosulfate (Science Company) was added on site in the water sample collected after CD phase to neutralize the residual chlorine and promote the survival of viruses and bacteria in the effluent of CD. Fig. 2 shows the schematic layout of CWRP

treatment process and the sampling locations.

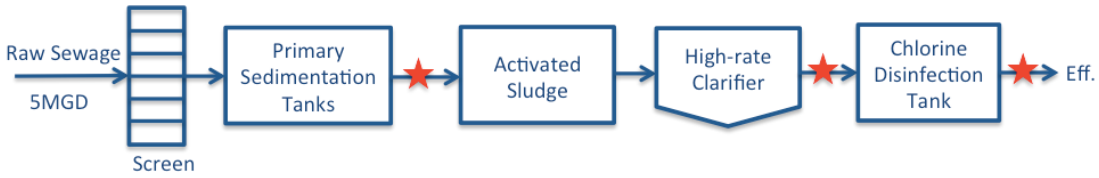

Fig. 2. Schematic of wastewater treatment processes at CWRP. Stars indicate sampling locations of this study.

3.1.3 Edward C. Little Water Recycling Facility

ECLWRF, managed by West Basin Municipal Water District in Los Angeles, is one of the largest water recycling facilities of its kind in the United States. ECLWRF produces five different qualities of custom-made recycled water that meet the unique needs including cooling towers, industrial boilers, landscape irrigation, and recharge of groundwater (Hering et al., 2013). These five types are tertiary water (Title 22 water), nitrified water, softened reverse osmosis water, pure reverse osmosis water, and ultra-pure reverse osmosis water. Among these five types of water, softened reverse osmosis water was chosen as the research target, which is designated for groundwater recharge for indirect potable reuse purpose.

ECLWRF uses 15% of the sewage treated to the secondary treatment level by Hyperion Treatment Plant (HTP) as influent for advanced treatment with the capacity of approximately 40 MGD. Three parameters of Hyperion treatment plant on a year average base are shown in the following table.

Table 3. Hyperion treatment plant wastewater parameters

Particles	TSS	NH_3-N	TOC
Concentration (mg L^{-1})	17±4.8	36±5.9	13±3.8

The process of obtaining softened reverse osmosis water from tertiary sewage effluent of HTP using ozonation, microfiltration, reverse osmosis, UV disinfection and chlorination is illustrated in Fig. 3. The ozone dosage exploited in the ozone reactor ranged from 10 to 20 mg L^{-1} associated with the amount of the flow; the pore size of microfiltration ranged from 0.1-0.2 µm; UV intensity was 115mJ/cm^2.

Samples were aseptically collected every two weeks from the effluent of ozone reactor (OR), microfiltration facility (MF), reverse osmosis facility (RO), ultraviolet radiated facility (UV) and CD in sterile Whirl Pak bags from August 2014 until January 2015.

Fig. 3. Schematic of wastewater treatment processes at ECLWRF. Stars indicate sampling locations of this study.

3.2 Sample analyses

Upon returning to the lab, 1 mL of each water sample was fixed with 50% electron microscopy (EM)-grade glutaraldehyde solution (SIGMA®) to the final concentration of 2%. All the mixtures were placed on the ice at 4°C and away from light exposure for 15 minutes during fixation.

Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilution) were made for all fixed sample using sterile

1× TE buffer (Life Technologies). All the diluted samples were stained with 50×SYBR® Gold Nucleic Acid Gel Stain (Life Technologies) at a final concentration of 2% and were allowed 15 minutes for staining after being vortexed.

Analyses of total viruses and bacteria in diluted stained samples were performed on a BD Accuri™ C6 Flow Cytometer equipped with a blue and a red laser, two light scatter detectors, and four fluorescence detectors following the protocols developed by Huang et al. (2015). In brief, 20 μL of samples were loaded for each test after thoroughly flushing the system with deionized (DI) water using fast flow fluidics setting for 2min. For sample testing, fluidics was set to medium flow and a threshold on channel Fluorescent 1 (FL1) was set to 500 to eliminate debris and background electronic noise. A single-parameter histogram was used to detect different particle peaks, with FL1 as x-axis and counts as the y-axis (Fig. 4a). A two-parameter plot was used to identify different populations of particles, with FL1 as its x-axis, and channel Fluorescent 3 (FL3) as y-axis. Distinct signal clusters for each population were gated based on visual inspection of the two-parameter histogram to exclude the background and debris noise, and to maximize the count number within each population (Fig. 4b). A gate is a numerical and graphical boundary that is used to define the characteristics of particles for further analysis. As shown in Fig. 4a, marker M1 illustrates peak of viral particles, marker M2 illustrates peak of bacterial particles; in Fig. b, the gated region R1 is defined as the cluster of viral particles and the region R2 is defined as the cluster of bacterial particles in the FL1 vs. FL3 plot.

The current setting ensures the events collected are greater than 5000 counts for each sample.

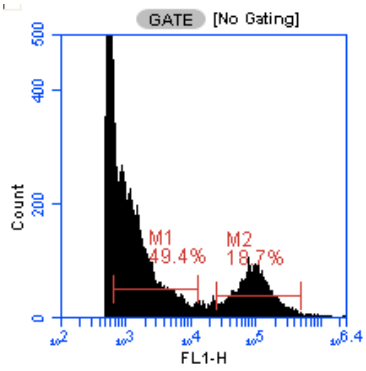

Fig. 4a. Single-parameter histogram of flow cytometry data output

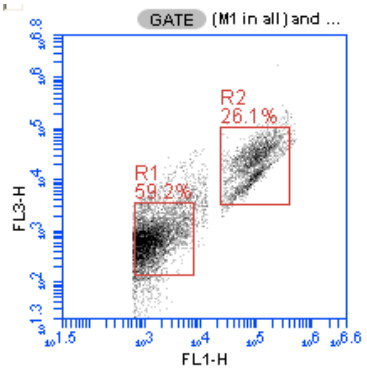

Fig. 4b. Two-parameter histogram of flow cytometry data output

3.3 Statistical analyses

3.3.1 Total viral and bacterial concentration

The viral and bacterial concentration in the original samples were calculated using the following equations:

$C\left(\frac{\text{Counts}}{\text{mL}}\right) = \frac{\text{Count}}{\text{V}} \times \frac{1000\mu\text{L}}{1\text{mL}} \times$Dilution Factor, where C (counts/mL) is either viral or bacterial concentration; Count is number of events in R1 region (for viruses) or R2 region (for bacteria); and V is the test volume (μL).

3.3.2 Removal of total viruses and bacteria

For each treatment process, viral and bacterial removal was calculated on the base of count collected by FCM. The count data was first transformed to a logarithmic ($\log_{10}$) scale. Afterwards, the removal efficiency was computed by subtraction of effluent concentration from the influent concentration for each unit treatment.

For MWRP AS treatment train, the $\log_{10}$ removal of viruses and bacteria was calculated for ASP using $\text{Log Removal}_{\text{AS}} = \text{LogC}_{\text{PSE}} - \text{LogC}_{\text{SCE}}$; for DMF process using $\text{Log Removal}_{\text{DMF}} = \text{LogC}_{\text{SCE}} - \text{LogC}_{\text{DMFE}}$; for the combination process prior to chlorination using $\text{Log Removal}_{\text{CDI}} = \text{LogC}_{\text{DMFE}} - \text{LogC}_{\text{CDI}}$; for combined disinfection process using $\text{Log Removal}_{\text{CD}} = \text{LogC}_{\text{CDI}} - \text{LogC}_{\text{CDE}}$.

For MWRP MBR treatment train, the removal efficiency for MBR was calculated using $\text{Log Removal}_{\text{MBR}} = \text{LogC}_{\text{PSE}} - \text{LogC}_{\text{MBRE}}$, for combined disinfection using $\text{Log Removal}_{\text{CD}} = \text{LogC}_{\text{CDI}} - \text{LogC}_{\text{CDE}}$.

For CWRP ASP treatment train, the removal efficiency was calculated for ASP using $\text{Log Removal}_{\text{AS}} = \text{LogC}_{\text{PSE}} - \text{LogC}_{\text{SCE}}$; for chlorine disinfection process using $\text{Log Removal}_{\text{CD}} = \text{LogC}_{\text{SCE}} - \text{LogC}_{\text{CDE}}$

For ECLWRF, the removal efficiency was calculated for ozone reactor (OR) using $\text{Log Removal}_{\text{OR}} = \text{LogC}_{\text{RI}} - \text{LogC}_{\text{ORE}}$; for MF facility using $\text{Log Removal}_{\text{MF}} = \text{LogC}_{\text{ORE}} - \text{LogC}_{\text{MFE}}$; for RO facility, using $\text{Log Removal}_{\text{RO}} = \text{LogC}_{\text{MFE}} - \text{LogC}_{\text{ROE}}$; for UV facility, using $\text{Log Removal}_{\text{UV}} = \text{LogC}_{\text{ROE}} - \text{LogC}_{\text{UVE}}$; for CD, using $\text{Log Removal}_{\text{CD}} = \text{LogC}_{\text{CDE}} - \text{LogC}_{\text{UVE}}$.

CHAPTER 4. RESULTS

4.1 MWRP

The concentrations of viruses and bacteria for ASP treatment train throughout the treatment process of MWRP were presented in Fig. 5. The viral concentration of 14 samples collected at sampling points of PSE, SCE, DMFE and CDI of MWRP over the eight-month period was $2.3\pm1.0\times10^{8}$ mL^{-1}, $4.7\pm2.0\times10^{8}$ mL^{-1}, $4.4\pm2.7\times10^{8}$ mL^{-1}, $2.8\pm1.7\times10^{8}$ mL^{-1} respectively. The bacterial concentrations at sampling points of PSE, SCE, DMFE and CDI were $8.5\pm3.4\times10^{7}$cells mL^{-1}, $1.8\pm1.2\times10^{6}$ cells mL^{-1}, $1.1\pm0.8\times10^{6}$ cells mL^{-1}, $9.7\pm5.1\times10^{5}$ cells mL^{-1}. In the CDI, concentrations of viruses and bacteria were decreased slightly from that of DMF by the dilution of MBRE. In the CDE, viral and bacterial concentrations were below detection limit in CDE. The viral concentration was lower than 10^{4} mL^{-1}, and bacterial concentration was lower than 10^{2} cells mL^{-1} due to the detection limits of FCM for viruses and bacteria, which were 10^{4} mL^{-1} and 10^{2} cells mL^{-1} respectively. The viral and bacterial concentrations determined by the detection limits were used for computation of their removal efficiencies.

For all the units in the AS train, viruses and bacteria had no significant variation detected over the eight-month period sampling activities, indicating consistent influent concentration of bacteria and viruses in the raw sewage.

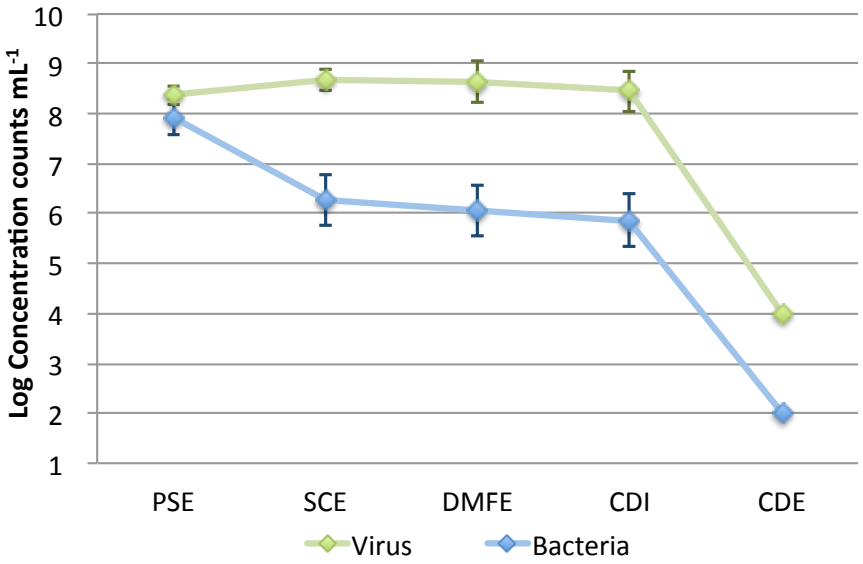

Fig. 5. Viral and bacterial log concentration in ASP train of MWRP (n=14).

Through out the treatment processes in MWRP ASP treatment train, there was 0.19log_{10} of viral reduction in the CDI due to the mixing of effluent from DMF and MBR; at chlorination process, a greater than 4.42log_{10} of removal was achieved. Bacteria were reduced by 1.65log_{10} at SC, 0.23log_{10} at DMF, 0.22log_{10} at CDI, and greater than 3.82log_{10} at CD. As a result, totally 4.64log_{10} of viral and 5.92log_{10} of bacterial removal was achieved in the ASP train of MWRP (Table 4).

Table 4. Summary of log_{10} removal efficiencies (mean ± standard deviation) of viruses and bacteria in ASP train of MWRP (n=14)

Particles	SC	DMF	CDI	CD	Total Removal
Virus	0	0	0.19±0.01	4.42±0.39	4.64±0.24
Bacteria	1.65±0.39	0.23±0.15	0.22±0.14	3.82±0.49	5.92±0.31

For MBR train, in PSE viral concentration of 14 samples was 2.4±1.0×10^8 mL^{-1}, bacterial concentration was 9.2±3.4×10^7cells mL^{-1} (Fig. 6). In MBR effluent (MBRE), viral and

bacterial concentration was below the detection limit of flow cytometer, thus viral concentration was lower than 10^4 mL^{-1}, bacterial concentration was lower than 10^2 cells mL^{-1}. In CDI, viral and bacterial concentrations increased dramatically due to the combination with the effluent from the DMF of the ASP train, which were $2.8\pm1.7\times10^8$ mL^{-1} and $9.7\pm5.1\times10^5$ cells mL^{-1}. In CDE, viral and bacterial concentrations were below 10^4 mL^{-1} and 10^2 cells mL^{-1} separately. Fig.6 shows viral and bacterial concentration of 14 samples collected in the MBR train.

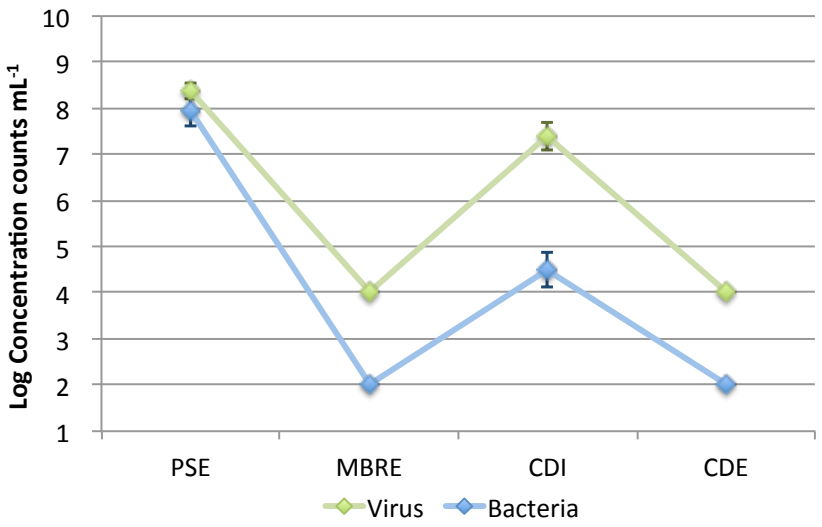

Fig. 6. Viral and bacterial log concentration in MBR process train of MWRP (n=14).

Through out the treatment processes in MWRP MBR treatment train, total viruses and bacteria were reduced by $4.3\log_{10}$ and $5.9\log_{10}$ at MBR. When combining with the effluent form ASP train, the concentrations of viruses and bacteria increased, then decreased after chlorination to the concentration of 10^4 mL^{-1} and 10^2 cells mL^{-1} respectively as a consequence. Totally $4.3\log_{10}$ of viral and $5.9\log_{10}$ of bacterial removal was achieved in the MBR treatment train of MWRP (Table 5).

Table 5. Summary of log_{10} removal efficiencies (mean ± standard deviation) of viruses and bacteria in MBR train of MWRP (n=14)

Particles	MBR	Total Removal
Virus	4.34±0.17	4.34±0.17
Bacteria	5.92±0.31	5.92±0.31

4.2 CWRP

The concentrations of viruses and bacteria for ASP treatment train through the treatment process of CWRP were illustrated in Fig. 7. The viral concentration of 10 samples collected at PSE sampling point at CWRP over the seven-month period was $3.9±1.4×10^8$ mL^{-1}; bacterial concentration of 10 samples was $8.4±3.1×10^7$ cells mL^{-1}. In SCE, viral concentration slightly rose to $5.5±2.2×10^8$ mL^{-1}, bacterial concentration dropped to $3.6±0.6×10^6$ cells mL^{-1}. In CDE, mean viral concentration was $4.8±2.1×10^8$ mL^{-1}, bacterial concentration was $4.0±1.3×10^6$ cells mL^{-1}. There was no seasonal variability detected over the seven-month period, indicting consistent influent concentration of bacteria and viruses in the raw sewage of CWRP. Fig.6 shows viral and bacterial concentration in ASP train based on 10 sampling events.

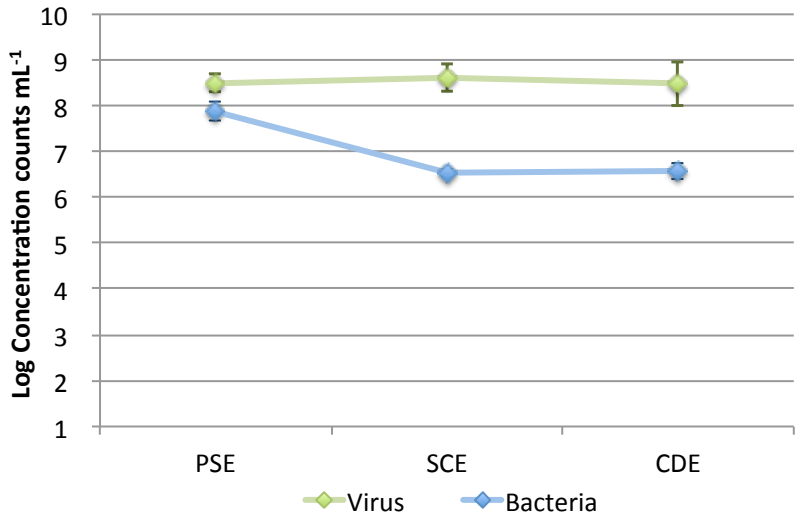

Fig. 7. Viral and bacterial log concentration in ASP train of CWRP (n=9).

Through out the treatment processes in CWRP ASP treatment train, there was no reduction of total viruses at SC, CD. Bacteria reduced by 1.4$\log_{10}$ at SC, none at CD. Therefore, totally none of viral and 1.4$\log_{10}$ of bacterial reduction was achieved throughout the ASP treatment train of CWRP as a result (Table 6).

Table 6. Summary of $\log_{10}$ removal efficiencies (mean ± standard deviation) of viruses and bacteria in ASP train of CWRP (n=9)

Particles	SC	CD	Total Removal
Virus	0	0	0
Bacteria	1.36±0.17	0	1.36±0.17

4.3 ECLWRF

The concentrations of viruses and bacteria for treatment train throughout the process of ECLWRF were illustrated in Fig. 7. The viral concentration of 8 samples collected at RI sampling point at ECLWRF over the six-month period was $3.5\pm0.4\times10^{8}$ mL^{-1}; bacterial concentration was $1.5\pm0.6\times10^{7}$ cells mL^{-1}. In ORE, viral concentration was $1.9\pm0.7\times10^{8}$

mL^{-1}; bacterial concentration was $1.7\pm0.7\times10^{7}$ cells mL^{-1}. In MFE, ROE, UVE and CDE, concentrations of viruses and bacteria were below detection limits of FCM. Thus viral concentration was below 10^{4} mL^{-1}, bacterial concentration was below 10^{2} cells mL^{-1}. There was no seasonal variability detected over the six-month period, indicting consistent concentration of viruses and bacteria in the raw tertiary influent. Fig.8 shows viral and bacterial concentration throughout the processes of ECLWRF based on 8 events.

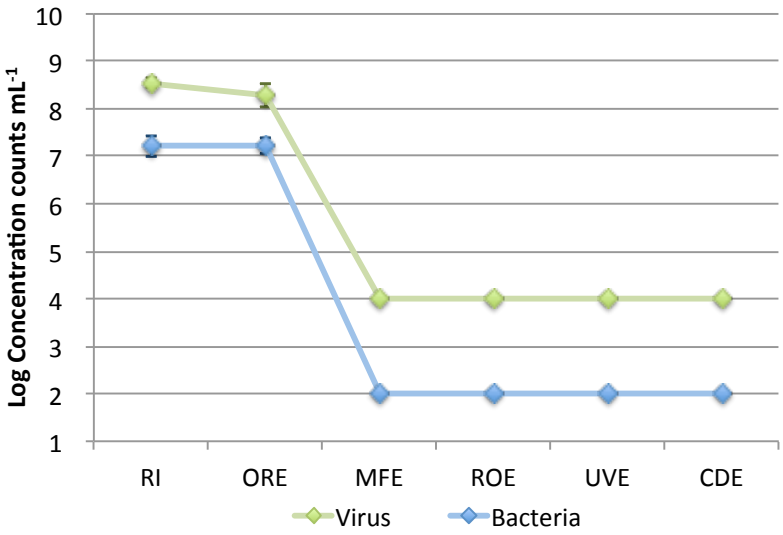

Fig. 8. Viral and bacterial log concentration in treatment train of ECLWRF (n=8).

Throughout the treatment processes in ECLWRF, viruses were retained by $0.29\log_{10}$ at OR, $4.26\log_{10}$ at MF, none at RO, UV and CD. Bacteria had none reduction at OR, $5.11\log_{10}$ reduction at MF, none reduction at RO, UV and CD. As a result, totally $4.54\log_{10}$ of viral and $5.11\log_{10}$ of bacterial removal were achieved throughout the treatment train of ECLWRF (Table 7).

Table 7. Summary of $\log_{10}$ removal efficiencies (mean ± standard deviation) of viruses and bacteria in ECLWRF (n=8)

Particles	OR	MF	RO	UV	CD	Total Removal
Virus	0.29±0.16	4.26±0.18	0	0	0	4.54±0.05
Bacteria	0	5.11±0.10	0	0	0	5.11±0.10

CHAPTER 5. DISCUSSION

5.1 MWRP

A possible explanation of the increasing of viral concentration at secondary clarifier effluent from 2.4×10^8 to 5×10^8 mL^{-1} in the ASP train is that the secondary aeration tank encourages the rapid replication and activities of aerobic bacteria, which also supports the replication of bacteriophage due to the enrichment of bacteria (Ewert and Paynter, 1980). Viruses, due to their small size, are not settled in the high rate clarifier. Since the secondary high-rate clarifier functions to remove the activated sludge from the aeration tank, which is composed of flocs of bacteria and other organisms, a portion of bacteria can be removed during the settlement of the activated sludge but not viruses.

There was little viral and bacterial removal in dual-media filters. A possible reason is that after being treated through ASP, water passed by gravity through dual media filters containing an anthracite coal layer followed by a sand layer with addition of alum prior to the filters, which was efficient at reducing particles greater than the filtration pore size of 1.05-1.15mm, but not effective at removing much smaller microorganisms including viruses and bacteria (Shirasaki et al., 2014; Nieuwstad et al., 1988; Cornwell et al., 2003). Viruses and bacteria achieved greater than $4\log_{10}$ removals in MBR process, because MBR process is the combination of activated sludge treatment together with a separation of the biological sludge by microfiltration membranes with a pore size of 0.1-0.4μm, which is able to remove a wide range of microorganisms by size exclusion in far fewer steps than the

conventional ASP (Zhang and Farahbakhsh, 2007). In this sense, most of the bacteria (>0.5 μm wide and >2 μm long) could be removed. Although human viruses are smaller than the pore dimensions of the membrane, most of them could be retained by the membranes, due either to the biofilm forming on the membrane or to the viruses' adsorption to the biomass (da Silva et al., 2007; Wong et al., 2009).

5.2 CWRP

CWRP employed the ASP, similar to one of the trains in MWRP, to produce reclaimed water. Throughout the entire process train, however, there was little reduction of total viral and bacterial concentration at SC and CD. In spite of the similar process used at MWRP and CWRP, some differences exist between the two processes, which could contribute to the different removal rates. MWRP includes tertiary treatment and consists of a different chlorine disinfection process in comparison with CWRP.

5.2.1 Differences in selection of tertiary treatments

Following ASP, MWRP uses alum to facilitate coagulation of flocs, followed by dual-media filtration to reduce suspended solids, particulates and micro-organisms in the ASC effluent (Koivunen et al., 2003). In these processes, $0.23\log_{10}$ of bacterial reduction were achieved. The absence of coagulation and dual-media filtration processes in CWRP has rendered the relative inferior water quality of the ASP effluent in the CWRP. Furthermore, the MWRP ASP effluent is combined with the high-quality MBR effluent from the parallel train, which improves the water quality by dilution before it enters into the disinfection tank.

5.2.2 Differences in chlorine disinfection tanks

Different factors in chlorine disinfection tanks play an essential role in determining viral and bacterial removal efficiencies, including types and concentration of disinfectant, contact time, temperature, pH, and concentration of organic matter (Cromeans et al., 2010). The contact time in the chlorine disinfection tanks of CWRP and MWRP were the same, which were both approximately 90 minutes; pH were both 7.2 on average. However the concentrations of ammonia (NH_3) were different in the influent of the two chlorine disinfection tanks, which influenced the availability of free chlorine concentrations. Although there is no direct measurement of total organics in the influent water to the disinfection tank at either plant, it is suspected that higher organics in the influent water of CWRP due to the absence of coagulation and dual-media filtration processes.

In the chlorine disinfection tanks of MWRP, free chlorine residual at the end of the chlorine contact tank is 7 mg L^{-1} on average based on the daily data over the year of 2014. Free chlorine is much more effective disinfectant for viruses and bacteria in comparison with chloramines. Thus both the concentration of viruses and bacteria were below the detection limit in the effluent of chlorine disinfection tank of MWRP.

For CWRP, free chlorine was not measured in the disinfection tank. Total chlorine was measured in the beginning and at the end of the contact tank with concentrations of 12 mg L^{-1} and 9 mg L^{-1} respectively. The influent water to chlorine disinfection tank in CWRP contained a high concentration of ammonia, with annual average of 38.88 mg L^{-1} in

comparison with 0.39 mg L^{-1} in the MWRP (Table 8). Free chlorine would react with the ammonia present in the water to form chloramine (mono-, di-, tri- chloramines) (Eqs. (1)-(3)), predominantly in the form of monochloramine. The combined chlorine is weak at killing protozoa, bacteria, and non-effective at killing viruses due to its low oxidation potential, resulting in lower disinfection efficacy than chlorine (Cromeans et al., 2010; White, 2005; Donnermair and Blatchley, 2003; Shaibu-Imodagbe, 2013; Deborde and von Gunten, 2008). Thus, by using equations below, the presence of free chlorine in the chlorination process can be determined.

$$HOCl + HN_3 \rightarrow NH_2Cl \text{ (monochloramine)} + H_2O \quad (1)$$

$$HOCl + NH_2Cl \rightarrow NHCl_2 \text{ (dichloramine)} + H_2O \quad (2)$$

$$HOCl + NHCl_2 \rightarrow NCl_3 \text{ (trichloramine)} + H_2O \quad (3)$$

Table 8. Ammonia, nitrite and nitrate concentration in CDI of CWRP and MWRP in 2014

WWTP	NH_3 (mg L^{-1})	NO_2^- (mg L^{-1})	NO_3^- (mg L^{-1})
CWRP	38.88±5.48	1.71±0.99	1.85±1.04
MWRP	0.39±1.10	0.15±0.10	10.90±1.55

In CWRP, the initial dose of chlorine ($HOCl+OCl^-$) in the disinfection tank was 12mg L^{-1}, which was equivalent to 2.29×10^{-4} moles L^{-1}; NH_3 was initially 2.35×10^{-3} moles L^{-1}. Based on Jafvert and Vlentine (1992), the breakpoint for the chlorination were greater than approximately 1.6 (Cl_2/NH_3, molar ratio) (Jafvert and Valentine, 1992). Since the ratio of Cl_2/NH_3 in CWRP was 0.1, which was much below the breakpoint concentration of chlorine. Thus there was no free chlorine residual in the disinfection tank, and chloramination took place in this circumstance. Studies have shown chloramine compounds could reduce

coliforms to levels below the concentration recommended by the Environment Protection Agency (EPA) to meet the compliant requirement of coliform concentration tested in the final effluent of CWRP (Metcalf, 2003).

The relatively large amount of ammonia and organic nitrogen in the CWRP ASP effluent was due to traditional ASP employed in CWRP, which promoted nitrification with little denitrification; whereas, MWRP used the Modified Ludzak-Ettinger (MLE) process (Fig. 9) that was a nitrification-denitrification nutrient removal process, which could achieve a better organic nitrogen removal.

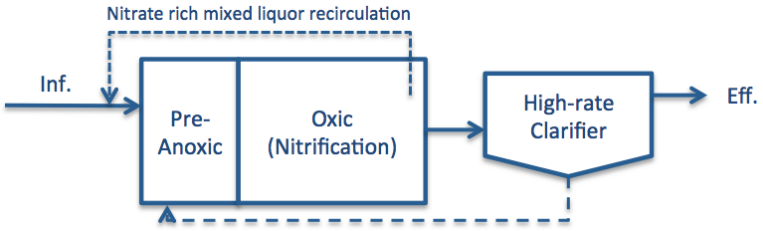

Fig. 9. Modified Ludzak-Ettinger (MLE) process

5.3 ECLWRF

Ozonation was applied to degrade macromolecule organic matters that may cause fouling problem of microfiltration (MF) (Song et al., 2010; Van Geluwe et al., 2011) in ECLWRF. Ozone was also a disinfectant that can inactivate the microorganisms, resulting to 0.3$\log_{10}$ of viral removal at the ozone reactor with ozone dose at 10-20 mg L^{-1}. The influent of the ECLWRF's treatment process is a secondary effluent from Hyperion Treatment Plant (HTP) that contains high concentration of organic matters (Table 3.), which could weaken the ozonation efficacy of inactivating microorganisms. Ozone is extremely labile that even a

small amount of organic matter may causes its rapid dissipation, therefore the contact time should be extended or the concentration of ozone increased in the ozonation process, in order to promote the higher inactivation efficiency (Burleson et al., 1975; Katzenelson et al., 1974). Since the dose added in the ozone reactor was 10-20 mg L^{-1}, and the ozone residual in the effluent was 0 mg L^{-1}, which indicated additional ozone was required to reduce the remained organic matters, and the current concentration were not effective to remove viruses and bacteria. Furthermore, the very short contact time in the ozone reactor was another explanation of the less efficient inactivation; with the flow immediately passing through the reactor, ozone did not have enough time to contact with and reduce the microorganisms.

After being treated by ozone, water was treated through MF with a pore size of 0.1-0.2 μm, during which process greater than $5\log_{10}$ bacterial removal was achieved due to its dimensions (>0.5 μm wide and >2 μm long). Viruses achieved more than $4\log_{10}$ reduction, which was beyond expectation, because the sizes of viruses are much smaller than the pore dimensions of MF (Matsushita et al., 2013). However, the viral retention rate by MF can vary significantly. Some recent studies show MF membrane appear to be capable of high viral removal efficiencies, and the pore size along are not adequate to describe the ability of removing particulates from solutions (McGahey and Olivieri, 1993). The removal efficiency may be improved due to the attachment of the negative viruses to the charged membrane surface (Van Voorthuizen et al., 2001), or to the retention by the membrane fouling caused

by the accumulation of the organic matter on the membrane surface, ect. (Huang et al., 2012; Jolis et al., 1999).

CHAPTER 6. CONCLUSION AND FUTURE CONSIDERATIONS

For MWRP, MBR process was more efficient at removing viruses and bacteria than conventional activated sludge process (ASP), which achieved greater than $4\log_{10}$ of viral and $5\log_{10}$ bacterial removal. Chlorination effectively removed viruses and bacteria to the concentrations below 10^4 mL^{-1} and 10^2 cells mL^{-1} respectively.

However for CWRP, ASP did not achieve notable viruses and bacteria removal, mainly owing to less efficient inactivation of the chloramine produced in the disinfection tank due to the reaction of free chlorine with the high concentration of ammonia, which was not reduced effectively in the secondary treatment process. Approximately no viruses and 1.5Log_{10} of bacteria were removed in the whole process train as a consequence. Moreover, even though coliform concentration was below the level recommended by EPA due to the inactivation capacity of chloramines, the level of viruses and bacteria in the final effluent was potentially harmful to humans. Another conclusion was that tertiary treatment including dual-media filtration, chlorination and other disinfection processes were important processes of removing viruses and bacteria.

For ECLWRF, a conclusion can be made that MF was very effective at removing bacteria, and at removing viruses due to the membrane fouling; totally 5Log_{10} viral and 4Log_{10} bacterial removals were achieved.

For future research, more data could be collected in a long range of time to promote the reliability of the results, and other types of methods with higher sensitivity could be

introduced to detect viruses and bacteria besides the rapid FCM method, which detection limit is a constraint in precisely determining viral and bacterial concentrations especially for tertiary and advanced treatment processes including chlorination, reverse osmosis, and UV disinfection. In addition, since this research is mainly focused on the overall performance of the processes trains, less attentions are paid on each single unit process, many other factors are still needed to be considered particularly with collection of detailed parameters and with necessary experiments. Lastly, testing the removal efficiencies of specific types of viral and bacterial pathogens should be incooperated with the removal rate of total viruses and bacteria to provide more specific information on water safety for various water reclamation applications.

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APPENDIX A. Viral and Bacterial Log Concentration Data

Table 9. The Viral and Bacterial concentration in ASP train of MWRP.

Date	PSE	SCE	DMFE	CDI	CDE
Virus (log_{10})					
Jun.13	8.26	8.52	7.57	7.38	4.00
Jun.27	8.26	8.67	8.66	8.47	4.00
Jul.10	8.29	8.88	8.87	8.68	4.00
Jul.25	8.41	8.81	8.91	8.71	4.00
Aug.04	8.42	8.73	8.67	8.48	4.00
Aug.19	8.16	8.29	8.01	7.82	4.00
Oct.02	8.35	8.79	8.72	8.53	4.00
Oct.24	8.02	8.22	8.11	7.92	4.00
Nov.07	8.66	8.75	8.78	8.58	4.00
Nov.21	8.56	8.79	8.81	8.62	4.00
Dec.04	8.31	8.76	8.74	8.55	4.00
Dec.19	8.28	8.35	8.41	8.22	4.00
Jan.15	8.14	8.77	8.25	8.08	4.00
Jan.29	8.23	8.64	8.18	8.20	4.00
AVERAGE	8.34	8.68	8.61	8.42	4.00
STDEV	0.16	0.20	0.39	0.38	0.00
Bacteria (log_{10})					
Jun.13	7.85	5.18	5.18	4.51	2.00
Jun.27	7.86	6.19	6.09	5.90	2.00
Jul.10	8.01	6.49	6.46	6.27	2.00
Jul.25	8.03	6.20	5.93	5.74	2.00
Aug.04	8.05	6.38	6.05	5.86	2.00
Aug.19	7.70	6.55	6.25	6.05	2.00
Oct.02	7.94	6.48	6.31	6.12	2.00
Oct.24	8.05	6.48	6.19	6.00	2.00
Nov.07	7.92	6.23	5.91	5.72	2.00

Nov.21	8.09	5.79	5.33	5.14	2.00
Dec.04	6.84	5.99	5.94	5.75	2.00
Dec.19	7.99	5.76	5.22	5.03	2.00
Jan.15	7.78	5.65	5.59	5.24	2.00
Jan.29	7.93	6.37	6.04	5.61	2.00
AVERAGE	7.92	6.25	6.03	5.82	2.00
STDEV	0.31	0.40	0.41	0.49	0.00

Table 10. The Viral and Bacterial concentration in MBR train of MWRP.

Date	**PSE**	**MBRE**	**CDE**
	Virus (log_{10})		
Jun.13	8.26	4.00	4.00
Jun.27	8.26	4.00	4.00
Jul.10	8.29	4.00	4.00
Jul.25	8.41	4.00	4.00
Aug.04	8.42	4.00	4.00
Aug.19	8.16	4.00	4.00
Oct.02	8.35	4.00	4.00
Oct.24	8.02	4.00	4.00
Nov.07	8.66	4.00	4.00
Nov.21	8.56	4.00	4.00
Dec.04	8.31	4.00	4.00
Dec.19	8.28	4.00	4.00
Jan.15	8.14	4.00	4.00
Jan.29	8.23	4.00	4.00
AVERAGE	8.34	4.00	4.00
STDEV	0.16	0.00	0.00
	Bacteria (log_{10})		
Jun.13	7.85	2.00	2.00

Jun.27	7.86	2.00	2.00
Jul.10	8.01	2.00	2.00
Jul.25	8.03	2.00	2.00
Aug.04	8.05	2.00	2.00
Aug.19	7.70	2.00	2.00
Oct.02	7.94	2.00	2.00
Oct.24	8.05	2.00	2.00
Nov.07	7.92	2.00	2.00
Nov.21	8.09	2.00	2.00
Dec.04	6.84	2.00	2.00
Dec.19	7.99	2.00	2.00
Jan.15	7.78	2.00	2.00
Jan.29	7.93	2.00	2.00
AVERAGE	7.92	2.00	2.00
STDEV	0.31	0.00	0.00

Table 11. The Viral and Bacterial concentration in ASP train of CWRP.

Date	**PSE**	**SCE**	**CDE**
	Virus (log_{10})		
Aug.20	8.66	8.80	8.90
Aug.29	8.09	8.00	7.38
Sep.23	8.52	8.74	8.73
Oct.02	8.53	8.67	8.63
Oct.21	8.52	8.56	8.61
Nov.13	8.65	8.90	8.66
Nov.26	8.75	8.90	8.78
Dec.11	8.69	8.83	8.76
Jan.16	8.48	8.57	8.44
AVERAGE	8.58	8.72	8.63
STDEV.	0.19	0.28	1.13

Bacteria (log_{10})			
Aug.20	7.96	6.63	6.35
Aug.29	7.49	6.55	6.36
Sep.23	8.11	6.53	6.74
Oct.02	7.91	6.55	6.55
Oct.21	7.80	6.41	6.69
Nov.13	8.00	6.54	6.71
Nov.26	7.77	6.57	6.75
Dec.11	8.09	6.67	6.49
Jan.16	7.83	6.50	6.51
AVERAGE	7.92	6.56	6.60
STDEV.	0.19	0.07	0.16

Table 12. The Viral and Bacterial concentration in the process train of ECLWRF.

Date	RI	ORE	MFE	ROE	UVE	CDE
Virus						
Aug.13	8.52	7.97	4.00	4.00	4.00	4.00
Aug.28	8.56	8.20	4.00	4.00	4.00	4.00
Sep.23	8.63	8.43	4.00	4.00	4.00	4.00
Oct.3	8.52	8.38	4.00	4.00	4.00	4.00
Oct.30	8.48	8.11	4.00	4.00	4.00	4.00
Nov.18	8.53	8.36	4.00	4.00	4.00	4.00
Dec.16	8.58	8.21	4.00	4.00	4.00	4.00
Feb.5	8.60	8.36	4.00	4.00	4.00	4.00
AVERAGE	8.55	8.27	4.00	4.00	4.00	4.00
STDEV	0.05	0.16	0.00	0.00	0.00	0.00
Bacteria						
Aug.13	7.18	7.07	2.00	2.00	2.00	2.00
Aug.28	7.31	7.12	2.00	2.00	2.00	2.00

Sep.23	6.90	6.98	2.00	2.00	2.00	2.00
Oct.3	7.33	7.29	2.00	2.00	2.00	2.00
Oct.30	7.06	7.11	2.00	2.00	2.00	2.00
Nov.18	7.16	7.13	2.00	2.00	2.00	2.00
Dec.16	7.11	6.99	2.00	2.00	2.00	2.00
Feb.5	7.30	7.11	2.00	2.00	2.00	2.00
AVERAGE	7.19	7.11	2.00	2.00	2.00	2.00
STDEV	0.15	0.10	0.00	0.00	0.00	0.00