DISINFECTION OF WASTEWATER FOR REUSE USING MICROCHANNEL PLASMA OZONE GENERATOR

BY

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DISSERTATION

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ABSTRACT

Waster reuse is increasing which alleviates pressure on water resources. However, successful reuse demands that the finished water be disinfected to prevent the spread of pathogens. One of the pathogens of potential public health concerns is *Legionella pneumophila*. This thesis investigated the performance of an energy efficient and compact microchannel plasma-based ozone generator to inactivate *L. pneumophila* for safer water reuse.

Inactivation kinetics study quantified the inactivation. More than four Log_{10} of *L. pneumophila* inactivation was achieved within 1 minute at low energy consumption (< 0.022 kWh) by the microplasma ozonator, using only ambient air as the feedstock gas at a driving voltage of 120V. Contrary to previous studies, the CT (product of available disinfectant concentration and exposure duration) concept for wastewater disinfection using ozone appeared to be valid. A framework to derive wastewater-specific CT equations was therefore developed to predict ozone inactivation of *L. pneumophila*. Temperature was found to affect *L. pneumophila* inactivation only in the absence of wastewater organic matter (WOM). In the presence of WOM, inactivation was temperature-independent and controlled by the disinfection contact time, initial ozone concentration and initial WOM loading.

An integrated life cycle assessment (LCA) and quantitative microbial risk assessment (QMRA) was used to compare microplasma ozonation versus chlorination to disinfect wastewater for landscape irrigational reuse. *L. pneumophila*, *Giardia*, and *Cryptosporidium parvum* were selected as the pathogens. Microplasma-based ozonation was significantly more competitive in environmental performance compared to chlorination for five of six impact categories, due to its low energy consumption and the high susceptibility of the pathogens to ozone. Across different electricity fuel sources in Florida, California, and Texas, the

microplasma ozonation system consistently offset more disability adjusted life years values to provide greater human health protection as compared to the chlorination disinfection system. As such, from the point of view of reducing human health impact, the emerging microplasma ozonation technology is superior to chlorination (with dechlorination) for wastewater reuse disinfection. To reduce the overall human health impact, future design efforts should focus on a long hydraulic residence time (HRT) with low chlorine doses for the chlorination system, and a moderately high transferred ozone dose with long HRT for the microplasma ozonation system.

In vitro mammalian cell cytotoxicity was evaluated before and after disinfection of two sources of wastewaters, using ozonation or chlorination. The swine farm wastewater was approximately 2000× more cytotoxic than the secondary effluent. Ozonation consistently reduced the mammalian cell cytotoxicity of the wastewaters by as much as 10×. Chlorination lowered the cytotoxicity only when followed by dechlorination. Based on mammalian cell cytotoxicity, secondary effluent is preferred for agricultural reuse over swine wastewater regardless of the disinfectants. Importantly, ozonation may hold the most promise in reducing the overall cytotoxicity of wastewater and this method may prove useful in agricultural reuse of wastewaters. The only significant correlation was observed between total haloacetonitriles and cytotoxicity in secondary effluent. Despite that the connection between reduced cytotoxicity and modification or reduction of certain compound(s) is not clear, regulated DBPs may not be the leading forcing agents.

To summarize, the results from these studies demonstrated the promise of using the microchannel plasma-based ozonation technology as a sustainable and effective means for water reuse disinfection.

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CHAPTER 1

INTRODUCTION

1.1 Background

Fresh water scarcity is a worldwide issue - approximately one-third of the world's population lives in countries with moderate to high water stress [1]. To alleviate this problem, water reclamation is a promising step [2]. Water reclamation conserves precious fresh water resources by recycling wastewater and treating it to quality sufficient for environmental and public health protection. Reclaimed water can thus be used for downstream applications such as irrigation [3]. Currently wastewater reuse is gaining popularity in the United States, especially in states with water scarcity such as California and Texas [4]. In 2010, the total reclaimed water reuse in the United States was estimated to have increased from 1,690 million gallons per day (MGD) in 2004 [4] to 2,400 MGD [5]. However, the likelihood of human contact with the reclaimed water during both the treatment and downstream application stages raises concerns for human health safety, owing to the presence of pathogens in the reclaimed water [6]. Therefore, to safely reuse water, it is required that the finished water be disinfected to prevent the spread of pathogens and outbreak of diseases.

Among the pathogens, one that is of specific concern is *Legionella pneumophila*. *L. pneumophila* is an opportunistic bacterium that target the lungs, and is typically found in various systems including engineered, human impacted, and natural systems [7]. Despite various treatment processes at water reclamation facilities, *L. pneumophila* has been found not only during all stages of wastewater treatment[8, 9], but also in water that has already been reclaimed [10, 11]. *L. pneumophila* is usually spread in the form of small droplets that people can breathe in, which can cause the infamous Legionnaires' disease, or Pontiac fever as a milder symptom [12]. Two recent

incidents to demonstrate the potential effect of this pathogen are an outbreak in New York City in the summer of 2015 during which more than 10 people died and more than 100 were affected [13], and an outbreak in Flint, MI that caused more than 10 deaths [14]. Each year, approximately 45% of total hospitalization cost to treat waterborne pathogens is attributed to Legionnaire's disease, which is significantly more than that for the treatment of Giardiasis (4%) or Cryptosporidiosis (5%) [15].

Ozone is the most powerful commercially available water disinfectant for a number of critical microorganisms such as the Norwalk virus [16] and poliovirus [17]. Several pathogens that are known to be resistant to chlorine, including *Giardia*, are readily inactivated by ozone [18-22]. *L. pneumophila* has been shown to be susceptible to ozone disinfection [23, 24], however, past studies were limited to drinking water systems [23, 25, 26] and therefore the performance of ozone to inactivate *L. pneumophila* in reclaimed water therefore remains unclear. In a reclaimed water system, wastewater organic matter (WOM) loading has been shown to have a significant contribution to the ozone demand of the reclaimed water [27]. In previous studies, inactivation of bacteria in wastewater was observed while dissolved ozone was below detection limit [27-29]. This puts the validity of the CT concept (product of available disinfectant concentration and exposure duration) in question [28], which hinders the application of ozonation for pathogen inactivation in reclaimed waters. One unique aspect of this study is the method to produce ozone. Currently ozone used in the water/wastewater treatment industry is based almost entirely on the dielectric barrier discharge structure [30]. The cost, complexity, and low efficiency of this structure greatly hinder the application of ozone technology for municipal water/wastewater treatment [31]. Microchannel plasma generation of ozone is a new technology that can yield generators 1-2 orders of magnitude smaller as compared to dielectric barrier discharge reactors of the same

ozone production capacity [31]. Production efficiencies above 120 g/kWh using oxygen as the feedstock gas have been achieved owing to the combination of a much smaller plasma impedance (~41 kΩ) and a much lower driving voltage [31]. This is more than double the efficiencies of conventional dielectric barrier discharge reactors, which typically have a maximum efficiency of 50 g/kWh [32]. As a result, effort was also devoted to evaluating the performance of the novel ozone production technology to inactivate *L. pneumophila* in secondary wastewater, regarding both the inactivation performance and the energy consumption.

To use ozone for water reclamation disinfection, the goal is undoubtedly to reduce pathogen concentration in reclaimed waters to protect human health. However, any technology that consumes electricity and other resources can result in environmental emissions that can in turn negatively affect human health [33-41], conflicting the direct benefits (reduction in pathogen exposure) with negative health impacts originating from the indirect release of pollutants (e.g., SO_2 released from coal fire power plants) [42-46]. Therefore it is of great interests to investigate the overall human health impact that roots from both the operation of ozonation for disinfection, as well as pathogen inactivation, from the point of view of disinfection system design and operational decisions. To do so, a previously developed hybridized Life Cycle Assessment (LCA) and Quantitative Microbial Risk Assessment (QMRA) methodology can be utilized, using the common unit of Disability Adjusted Life Years (DALYs) [47].

Another issue with disinfection processes is the potential to produce water with elevated toxicity due to disinfection byproducts (DBPs) that are formed from reactions between disinfectants and the organic matter, bromide, and iodide in wastewater [48-51]. Ozonation is no exception [50, 52]. Disinfection for water reclamation represents a more complex system than its drinking water counterpart, due to the potential to form a more diverse range of DBPs as the

abundance and variety of organic matter and other inorganic constituents are in general higher in concentration [53], which could function as DBP precursors. Previous research studied the DBPs separately and tried to connect the toxic responses with corresponding chemical analyses [49, 54-56]. However, these attempts are limited in the types of actual toxins in wastewater and therefore cannot represent or be extrapolated to reveal the overall toxicity of the wastewaters that most often contain multiple toxins. Thereof, it is of interests to study the response of mammalian cells to environmental samples of wastewaters that are of different characteristics, before and after ozonation.

1.2 Research Objectives

The overall objective of this work was to investigate the microplasma ozonation technology for water reclamation disinfection. This work allows for the prediction of ozone inactivation of *L. pneumophila* in secondary effluent. It also helps to compare the microplasma ozonation technology against the traditional chlorination in terms of human health protection as well as to evaluate the potential change in cytotoxicity on mammalian cells. The specific objectives are:

1. To evaluate the validity of the CT concept for ozone inactivation of *L. pneumophila* in secondary effluent and to evaluate the inactivation and energy consumption performance of the microplasma ozone production technology.

2. To compare microplasma ozonation technology to chlorination for the disinfection of wastewater for landscape irrigational reuse, focusing on the protection of human health.

3. To compare the overall mammalian cell cytotoxicity of two sources of wastewaters that have gone through different levels of treatment, before and after two disinfection methods, microplasma ozonation or chlorination.

1.3 Experimental Approach

All of the disinfection experiments were conducted in a semi-batch reactor inside a biosafety level 2 certified biosafety cabinet. Ambient air served as the feedstock gas for the microplasma ozone generator. Ozone gas concentration was adjusted with a transformer connected to the generator. Semi-batch reactors were submerged in a water bath for temperature control (Figure 1). Mixing in the reactors was accomplished by a magnetic stirrer. Through a ceramic diffuser, the generated ozone gas was directed into the reactor, where compositions of solutions were adjusted to reflect various experimental conditions. Samples were collected from the reactor at specific time points. The off-gas was quenched and eventually vented to a chemical fume hood. We used OriginLab for model fitting that yielded ozone concentration profiles as a function of time under various conditions.

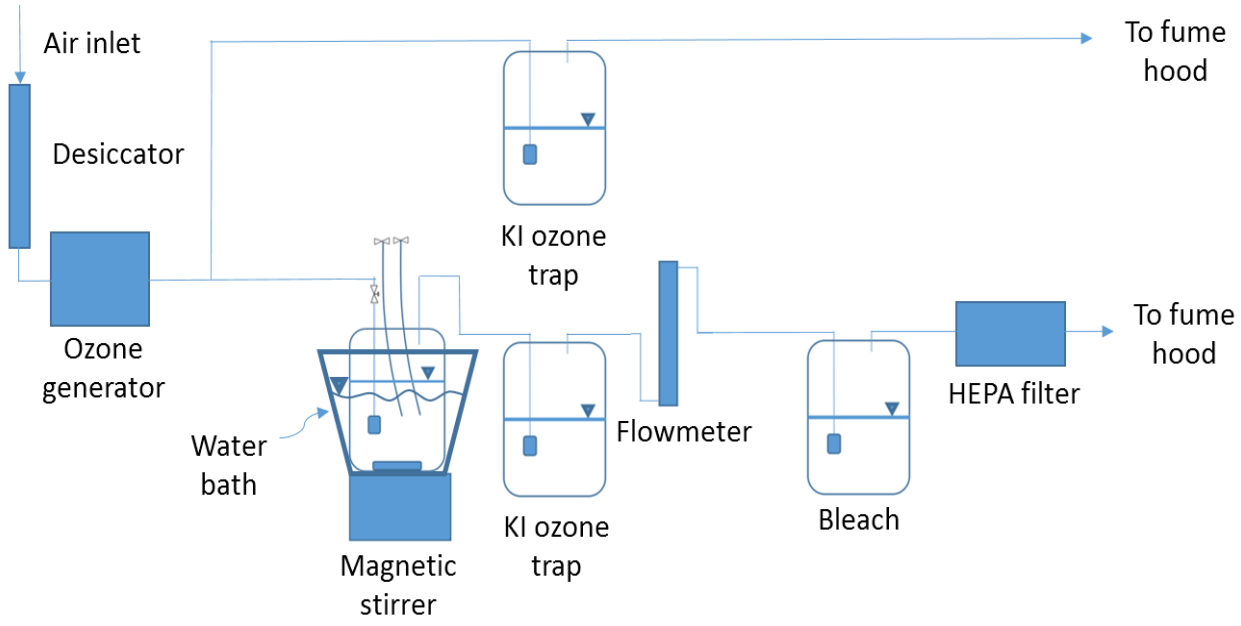

Figure 1.1 Simplified system setup for the disinfection experiment.

LCA and QMRA were conducted using the Microsoft Excel 2013 software with macro enabled. Design of the ozonation and chlorination treatment trains followed design manuals. Data from manufactures, literature, and experiments (done in the first part of the study) were also used during the design. Emission data were obtained from ecoinvent database V3. ReCiPe method in SimaPro was used to convert the emission data to human health impact.

For mammalian cell cytotoxicity experiments, the original wastewaters were first used for all experiments. Further concentration using the XAD 2 and XAD 8 resins was done when no significant decrease in cell density was observed. 96-well cell culture microplates were used to allow for a series of concentrations of test samples to be evaluated. LC_{50} values were subsequently generated and further converted to cytotoxicity index values for comparisons.

1.4 Dissertation Organization

In Chapter 2, titled "Inactivation of *Legionella pneumophila* in wastewater for reuse using ozone produced by a microchannel plasma ozone generator", *L. pneumophila* inactivation experiments were conducted in secondary effluent using ozone produced by a microchannel plasma ozonator. A mass balance model was established first to predict the dissolved ozone concentration as a function of time given other environmental and operational parameters, such as the WOM loadings. Then inactivation kinetics of *L. pneumophila* was studied, and combined with the residual ozone prediction model, an attempt was made to validate the Chick-Watson's CT model under the tested conditions. Finally, the energy consumption per $\log_{10}$ inactivation of *L. pneumophila* was evaluated at various ozone generator operating voltages and at various initial ozone concentrations.

The results indicated that under the tested conditions the CT model appeared to be valid. Temperature was found to be none-influential for *L. pneumophila* inactivation in the presence of

WOM. These findings were directly implemented in the design of the disinfection treatment trains in Chapter 3. The modeling framework on dissolved ozone concentration was also used in Chapter 4 to deliver a desired transferred ozone dose.

In Chapter 3, titled “Human Health Trade-offs in the Disinfection of Wastewater for Landscape Irrigation: Microplasma Ozonation versus Chlorination”, full scale disinfection trains using microplasma ozonation, and chlorination were designed and compared against each other regarding the human health impact. Both the human health impact caused by emissions from operating the treatment train, as well as the human health protection brought forth by the inactivation of three pathogens were taken into account. The variation in the consumed energy profile by source from three states within the United States that have the heaviest reliance on reclaimed water was also evaluated.

The results suggested that the microplasma ozonation technology is superior to chlorination for wastewater reuse disinfection in terms of the overall human health protection under the investigated conditions. Recommendations were also given to both the microplasma ozonation technology and the chlorination technology to reduce the overall human health impact, by adjusting the disinfection treatment operating condition.

In Chapter 4, titled “Comparative Mammalian Cell Cytotoxicity of Wastewaters for Agricultural Reuse after Ozonation or Chlorination”, the overall cytotoxicity on mammalian cells before and after disinfection of two types of wastewaters: secondary effluent of municipal wastewater, and minimally treated wastewater from a swine farm was evaluated. Ozonation and chlorination were used as two alternative disinfection methods.

The results revealed that ozonation more consistently reduced the cytotoxicity to mammalian cells as compared to chlorination. Secondary effluent was found to be less toxic to mammalian cells as compared to the swine farm wastewater.

The main findings, contributions, as well as future work of this thesis are summarized in the last chapter, Chapter 5.

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CHAPTER 2

INACTIVATION OF *LEGIONELLA PNEUMOPHILA* IN WASTEWATER FOR REUSE USING OZONE PRODUCED BY A MICROCHANNEL PLASMA OZONE GENERATOR

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Inactivation of *Legionella pneumophila* in wastewater for reuse using ozone produced by a microchannel plasma ozone generator

2.1 Abstract

This study evaluated the performance of a novel microchannel plasma-based ozone generator to inactivate a pathogen, *Legionella pneumophila*, in secondary wastewater for safer water reuse. Several operating and environmental parameters were considered for the evaluation, and an inactivation kinetics study was conducted to quantify the inactivation. More than four $\log_{10}$ of *L. pneumophila* inactivation was achieved within 1 minute at low energy consumption (< 0.022 kWh), using only ambient air as the feedstock gas at a driving voltage of 120V. Contrary to previous studies, the CT (product of available disinfectant concentration and exposure duration) concept for wastewater disinfection using ozone appeared to be valid. A framework to derive wastewater-specific CT equations was therefore developed to predict ozone inactivation of *L. pneumophila* to facilitate implementation of the technology. Temperature was found to affect *L. pneumophila* inactivation only in the absence of wastewater organic matter (WOM). In the presence of WOM, inactivation was temperature-independent and controlled by the disinfection contact time, initial ozone concentration and initial WOM loading. These results

will aid the implementation of the compact microchannel plasma ozonator to inactivate *L. pneumophila* for water reuse, ranging from point-of-use systems to wastewater reclamation utilities.

2.2 Introduction

Water reclamation is on the rise for more efficient water usage [1]. However, concerns have been raised due to the presence of pathogens in the reclaimed water [2]. One pathogen that has been found in treated wastewater [3], and therefore is of specific concern is *Legionella pneumophila*, which caused a fatal outbreak of Legionellosis in New York City in summer 2015 [4]. Traditional disinfectants such as chlorine are subject to debate for reasons such as producing a high level of disinfection byproducts in the finished water, which may result in high risks of certain cancers [5]. Therefore, alternative technologies that aim to provide safe and pathogen-free reclaimed water need to be evaluated.

Ozone is a powerful disinfectant for a number of critical microorganisms such as the Norwalk virus [6], poliovirus [7], and *Escherichia coli* [8, 9]. Indeed, several pathogens that are known to be resistant to chlorine, including *Cryptosporidium parvum*, are readily deactivated by ozone [10]. Currently ozone used in the water/wastewater treatment industry is based almost entirely on the macroscopic dielectric barrier discharge structure [11]. The cost, complexity, and low efficiency of this structure greatly hinder the application of ozone technology for municipal water/wastewater treatment [12]. Microchannel plasma generation of ozone is a new technology that yields generators 1-2 orders of magnitude smaller in size when compared to conventional dielectric barrier discharge reactors of the same ozone production capacity [12]. Because the building block of microplasma generators, known as a “chip”, produces 2-3 grams of ozone per hour, virtually any ozone production rate can be generated by stacking individual chips.

Production efficiencies above 120 g/kWh using oxygen as the feedstock gas have been realized owing to the combination of a much smaller plasma impedance (~41 kΩ) and a much lower driving voltage [12]. This is more than double the efficiencies of conventional dielectric barrier discharge reactors, which typically have a maximum efficiency of 50 g/kWh [13]. Additionally, for a conventional tube type dielectric barrier discharge reactor, water cooling is necessary to avoid overheating and subsequent ozone decomposition, whereas for the microchannel plasma ozonator, owing to its large surface area to volume ratio and a low temperature nature of the plasmas [14], a mechanical fan is sufficient for effective heat dissipation, reducing complexity and size of the system without compromising functionality.

To evaluate and implement the ozone technology for *L. pneumophila* deactivation, the inactivation kinetics of *L. pneumophila* must be known. However, previous studies on *L. pneumophila* inactivation were all limited to drinking water systems [15, 16], and the inactivation kinetics in secondary wastewater remains unclear. In wastewater systems, ozone concentration could not be instrumentally measured at low dosages [17, 18], and the concept based on the product of available concentration of disinfectant and exposure duration, i.e. CT concept, was suggested to be not applicable to wastewater treatment [17]. The CT approach has been used by the U.S. EPA to regulate water disinfection [19] by estimating the dose of the disinfectant required to inactivate a certain fraction of specific microorganisms under specified conditions. This approach provides an effective way to not only estimate the disinfection requirements, but also evaluate the extent of a disinfection process. Therefore, a lack of validation of the CT concept for ozone inactivation of bacteria poses a barrier for the application of the technology for *L. pneumophila* inactivation in wastewater.

In this study, in order to evaluate the novel ozone production technology and the validity of the CT concept, we designed a study to inactivate *L. pneumophila* in secondary wastewater, under the influence of the following operating and environmental parameters: disinfection contact time, wastewater organic matter (WOM) loading, initial ozone concentration, and temperature. Two kinds of experiments were conducted to determine *L. pneumophila* inactivation kinetics as part of the performance evaluation, using solutions with and without WOM at two different temperatures. A mass balance model was applied to predict low levels of dissolved ozone concentration (<281 μg/L). The modeled ozone concentrations were used together with the measured *L. pneumophila* inactivation kinetics to check the validity of the CT concept, which could function as a framework to predict *L. pneumophila* inactivation in wastewater. An energy performance evaluation was also conducted to facilitate implementation of the new technology.

2.3 Materials and methods

Bacteria cultivation, processing, and enumeration

Once every four weeks, *L. pneumophila* strain ATCC 33152 were restreaked onto charcoal-containing CYE agar plates from -80^{o}C stocks to guarantee consistent *L. pneumophila* characteristics. The freshly grown *L. pneumophila* cells were inspected under a microscope to check for contamination. Propagation was accomplished following the ATCC instructions. A sterilized CYE solution at pH 6.9 ± 0.05 was inoculated with *L. pneumophila* that were previously grown on an agar plate made of the same medium, with additional charcoal added. *L. pneumophila* cells were harvested after 48 hours of incubation at 37 ^{o}C by centrifugation at 5000 rpm for 10 minutes at 20 ^{o}C. The pellets were washed three times with sterilized 0.01M phosphate buffer at pH 6.8 by alternating centrifugation at 5000 rpm, and resuspended with the

same phosphate buffer. The *L. pneumophila* cells were enumerated by serial dilutions, which were done with the same phosphate buffer and followed by plating onto charcoal-containing CYE agar plates. Colony forming units were counted after 72 hours of incubation at 37 ^{o}C in an atmosphere containing 5% CO_2.

Setup configuration

The experimental setup was established inside a certified biosafety cabinet (Figure 1.1). Ozone gas was produced by a prototype microchannel plasma ozone generator manufactured by EP Purification Inc. Ambient air served as the feedstock gas for the generator, and was drawn through a silica gel desiccator to remove moisture prior to entering the ozone generator. Part of the gas that exited the ozone generator was bypassed directly to a potassium iodide trap, allowing for better control over the mass transfer rate of ozone into the reactor. Ozone gas concentration was adjusted with a transformer connected to the generator so as to achieve a gas phase ozone concentration in the range of 1.3 to 4.9 g/Nm^3. At this range of operation, the electricity consumption of the system varied from 0.012 to 0.022 kWh. Semi-batch reactors containing a final volume of 700 mL solution were submerged in a water bath for temperature control. Mixing in the reactors was accomplished by a magnetic stirrer operating at 700 rpm. The generated ozone gas was directed into the reactor through a ceramic diffuser and the flow rate of ozone was controlled by a Swagelok® valve, and monitored by an in-line flow tube. The off-gas was collected, passed through a 0.25% potassium iodide solution, and finally through a 10% (v/v) bleach solution and a HEPA-CAP filter to prevent the release of any aerosol containing *L. pneumophila* into the atmosphere. The treated off-gas was eventually vented through a chemical fume hood. Luerlok syringes were used to take samples via PTFE tubing attached through the cap of the reactor. The volume inside the PTFE tubing was taken into consideration during

sampling. The dissolved ozone concentration was determined by the Indigo colorimetric method [20] read by a portable spectrophotometer (HACH, model DR2800, Loveland, CO).

Disinfection experiment

Two types of experiments to determine inactivation performance were conducted using solutions with and without WOM. The organic matter-free solution was buffered by phosphate at pH 6.8-7.0, because previous research has identified the insignificance of pH on ozone disinfection kinetics of *L. pneumophila* [21]. The solutions containing organic matter were a mixture of phosphate buffer and secondary wastewater filtered through a 0.22 μm filter. Wastewater was collected from the effluent of a secondary clarifier at the Urbana (Illinois, USA) Northeast Wastewater Treatment Plant and kept at 4°C in dark until used.

Prior to the inactivation experiments, the ozone generator was warmed up for 5 minutes with ozone passed directly into the ozone destruction units. Subsequently, ozone was pumped through a solution of 0.01 M phosphate buffer at a high flow rate (40 mL/min) for a period of 20 seconds to 3 minutes, until the desired initial dissolved ozone concentration was achieved. The flow rate was then reduced to a level that maintained an initial dissolved ozone concentration of 15 to 25 μg/L for the organic-free solution experiments, and 33 to 281 μg/L for the experiments with WOM. This required an adjustment of the gas phase ozone concentration by changing the voltage applied to the ozone generator (50 V to deliver an initial dissolved ozone concentration of 33 μg/L, and 120 V to deliver any concentrations beyond). For these experiments with WOM, a mixture of undiluted filtered wastewater and *L. pneumophila* cells was injected into the reactor through a PTFE tube, while only *L. pneumophila* in buffered solution was injected for experiments with organic-free solutions. The temperature of the reactor was maintained by a water bath (Thermo Fisher Scientific Inc., Waltham, MA) at 7 or 22 ^{o}C. A final *L. pneumophila*

cell concentration of 10^5-10^6 cells/mL in the reactor was used for all experiments. Samples were subsequently obtained at different times through additional PTFE tubing with Luerlok syringes, and immediately transferred into a 0.1% sterilized sodium thiosulfate solution. This solution was then subjected to serial dilutions and plating on charcoal-containing CYE agar plates. At least three separate experiments using different batches of *L. pneumophila* were conducted. No significant *L. pneumophila* inactivation occurred in control experiments without the presence of ozone.

Although currently no CT requirements have yet been established for *L. pneumophila* inactivation, CT value is a crucial parameter for the design and evaluation of the *L. pneumophila* inactivation processes. To determine the obtained ozone CT value, the dissolved ozone concentration has to be known. However, residual ozone at low applied ozone dosage during wastewater treatment can be difficult to measure due to turbidity [22] and ozone consumption by WOM [23]. These issues were noted in not only prior research [17, 24], but also present during this study. It has been observed previously (<100 µg/L) and in this study (0-33 µg/L) that at low levels of dissolved ozone below the detection limit in wastewater, bacteria were inactivated [18, 25], which in combination with the added benefit of reduced bromate formation potential and less wastage of ozone and conserved energy for a required level of treatment [26], makes disinfection of *L. pneumophila* at low levels of dissolved ozone an attractive option to investigate. Because this low level of ozone was very difficult to measure, we developed a model to predict the dissolved ozone concentration based on a separate set of experiments that determined the ozone decomposition and transfer rate in solutions with and without WOM. The measured dissolved ozone concentrations were fitted to a series of mass balance equations to develop a model to predict the dissolved ozone concentration, which was subsequently used to calculate the obtained

ozone CT value for both the evaluation and prediction of *L. pneumophila* inactivation. The details of this effort are provided in SI.

2.4 Results and Discussion

Ozone production performance

Between the driving voltages of interest, i.e. 50 and 120 V, the gas phase ozone concentration increased linearly with voltage, with power consumption increasing following a similar trend. The highest gas phase ozone concentration was obtained at a driving voltage of 120 V, with a corresponding power consumption of 0.022 kWh (Figure 2.1 a)). To visualize the transfer of gas phase ozone into water, ozone gas produced at different driving voltages was passed through a stone diffuser at the same flow rate (40 ml/min) into buffered solution at pH 7 (Figure 2.1 b)). It can be seen that at 22 ^{o}C, the attainable steady state dissolved ozone concentration was 2.5 fold higher when driven at 120V than that driven at 50V.

Inactivation performance of microchannel plasma ozone generator for *L. pneumophila* inactivation under the influence of operating and environmental conditions

- The influence of disinfection contact time

Disinfection contact time was found to influence *L. pneumophila* inactivation, which contradicts a previous finding that it has no impact on bacterial inactivation in wastewater [17]. At high WOM loading (2.4 and 3.1 mg C/L) and low initial ozone dosage (30 μg/L), the inactivation of *L. pneumophila* appeared to be independent of time (Figure 2.2). However, as the WOM loading decreased (down to 0.8 mg C/L) or the initial ozone dosage increased (up to 281 μg/L), a positive correlation could be observed between the contact time and *L. pneumophila* inactivation (Figure 2.2 and 2.3 a)). This observation is in agreement with a previous study on

ozone inactivation of *Cryptosporidium* oocysts in river water [10]. At a high WOM loading (3.1 mg C/L) and an initial ozone dosage above 165 μg/L (up to 281 μg/L), the dependence of *L. pneumophila* inactivation on contact time weakened with time. This can be quantified by an increase in the slope of *L. pneumophila* inactivation data. For example, at an initial WOM of 3.1 mg C/L and initial ozone concentration of 281 μg/L, the slope of the inactivation kinetics data increased from − 15 min^{-1} during the first 0.2 minutes to − 1.9 min^{-1} for the following 0.8 minutes, suggesting slower inactivation as time progressed (Figure 2.3 a)). The variation in the time dependence of *L. pneumophila* inactivation with contact time could be attributed to the availability of ozone to inactivate *L. pneumophila* while being consumed by WOM, as indicated by the predicted dissolved ozone concentration profile (Figure 2.3 b)). This explanation agrees with the results of Hunt and Mariñas (1999), which showed that availability of ozone impacted the rate of bacteria inactivation.

- The influence of temperature and WOM

Under organic-free water environments, the *L. pneumophila* inactivation performance depended strongly on temperature. At 22 ^{o}C, *L. pneumophila* concentration was reduced by 3.5 log_{10} within half a minute for a CT value of 13 μg/L min. However, at 7 ^{o}C less than 0.5 log_{10} of *L. pneumophila* were inactivated at the same CT value. Even when the maximum CT value was tripled to 35 μg/L min, less than 1.5 log_{10} of *L. pneumophila* inactivation was observed at 7 ^{o}C (Figure 2.4). In addition, the second order inactivation rate constant at 22 ^{o}C of 0.68 ± 0.04 L/ (μg min) was found to be significantly larger than that at 7 ^{o}C (0.09 ± 0.001 L/ (μg min)). At a lower temperature, though ozone is more readily dissolved and more stable in water, chemical reactions between ozone and *L. pneumophila* were meanwhile expected to be slower. The observations indicated that, in the absence of WOM, slowed reactions were a more dominant

factor than higher concentration of available ozone for longer periods of time, so that synergistically *L. pneumophila* inactivation was slower at a lower temperature. While this result contradicts previous work on ozone inactivation of *L. pneumophila*, which concluded that ozone was either behaving similarly at temperature from 25 to 45 ^{o}C [21], or that ozone was more effective at 5 compared to 15 ^{o}C [27], our findings are in agreement with previous research on ozone inactivation of *E. coli* [8]. We therefore conclude based on our results that in the absence of WOM, higher CT values are required for lower temperature.

In contrast to the findings without WOM, the role of temperature became negligible in the presence of WOM. Based on the good fitting of the *L. pneumophila* inactivation in the presence of WOM (Figure 2.6), the same CT value is required to obtain the same $\log_{10}$ inactivation of *L. pneumophila* at different temperatures. In other words, at lower temperature, the combined effect of slower decay of dissolved ozone and slower reactions made the disinfection continue for a longer time, whereas at higher temperature, dissolved ozone was less stable and soluble, and combining with faster reactions, disinfection happened faster. The similarity in the overall effect at different temperatures in the presence of WOM could be due to the fact that consumption reactions of dissolved ozone with organics outweigh the change in reactivity of dissolved ozone incurred by the variation in temperature.

-The influence of WOM and initial ozone concentration

As *L. pneumophila* inactivation was found to be insensitive to temperature variations in the presence of WOM, subsequent discussions will only refer to those conducted at 22 ^{o}C. Specifically, the *L. pneumophila* inactivation performance was evaluated for water containing different concentrations of WOM (Figure 2.2). The volumetric percentage of wastewater over the final solution volume in the reactors ranged from 3.6 to 14.3%, with TOC varying from 0.8 to

3.1 mg C/L. Within the range of wastewater concentrations tested, higher TOC levels led to slower *L. pneumophila* inactivation, given the same initial ozone concentration and contact time. At an initial dissolved ozone concentration of 33 µg/L, higher initial WOM loadings slowed the inactivation of *L. pneumophila*. For an initial TOC loading of 0.8 mg C/L, for example, more than 3 $\log_{10}$ *L. pneumophila* inactivation was observed within 15 minutes while the dissolved ozone concentration decreased from 33 µg/L to below 1 µg/L. However, as the loadings of WOM increased to 2.4 and 3.1 mg C/L, the inactivation efficiency decreased, with less than 1 $\log_{10}$ of *L. pneumophila* inactivated in 15 minutes. This was accompanied by a decrease of ozone concentration from 33 µg/L to less than 1 µg/L. At the highest WOM loadings of 2.4 and 3.1 mg C/L, the inactivation kinetics did not significantly differ from each other ($p = 0.11$). The decreased *L. pneumophila* inactivation efficiency could be attributed to the lack of dissolved ozone in the solutions containing WOM, due to the consumption of ozone by WOM. Figure 2.5 demonstrates the change in dissolved ozone concentration as a function of time at various initial WOM loadings. All three curves show a two-stage profile, which is composed of a fast initial drop, followed by a more gradual decrease in ozone concentration. It is clear that the higher the initial WOM loadings, the steeper the slopes during the fast initial decrease stage suggesting that dissolved ozone consumption is faster at higher initial WOM concentrations. At the initial TOC of 3.1 mg C/L, the residual ozone significantly reduced from 33 to 1 µg/L within 3 minutes (Figure 2.5). Lower inactivation rate constants were therefore obtained for solutions containing higher WOM.

At the same initial WOM loading, faster inactivation of *L. pneumophila* was observed as the initial dissolved ozone concentration increased (Figure 2.3). At an initial dissolved ozone concentration of 165 µg/L, for example, 1.5 $\log_{10}$ of *L. pneumophila* were inactivated within 1

minute. For 281 µg/L of initial dissolved ozone, however, more than 4 $\log_{10}$ of *L. pneumophila* were inactivated for the same contact time (1 minute). The slower decay of *L. pneumophila* for small values of dissolved ozone is explained by the availability of dissolved ozone in the reactor. The predicted ozone concentration in the experiment with higher initial ozone loading was consistently higher than the experiment with a lower initial ozone concentration (Figure 2.3). This is in agreement with the observation that *L. pneumophila* were inactivated faster at a higher initial dissolved ozone concentration, given the same initial WOM loading. As the dissolved ozone concentration increased, more ozone was available to inactivate *L. pneumophila* despite the presence of organic matter, which competed with *L. pneumophila* to react with ozone.

CT equation for the prediction of L. pneumophila inactivation in wastewater

CT equations express the relationship between the inactivation ratios with the obtained cumulative CT value. Therefore they are very useful to help to determine the required cumulative CT value to eliminate a given fraction of microorganisms. As an example to deploy the CT equation to predict *L. pneumophila* inactivation in wastewater, $\log(\frac{N}{N_0})$ from all experiments using Urbana wastewater was plotted versus $\int_0^t C_L\, dt$, where C_L is the instantaneous dissolved ozone concentration at time t as predicted by the modelling approach as depicted in Figure 2.6 and described in the SI. The fitting equation and coefficient of determination were $\log\left(\frac{N}{N_0}\right) = -0.063Ct$, and 0.94, respectively. Within the TOC range of 0.8 to 3.1 mg C/L and at both 7 and 22 ^oC, a good fitting was observed for *L. pneumophila* inactivation, following the Chick-Watson model (not shown, but plotted as $\mathrm{Ln}(\frac{N}{N_0})$ versus $\int_0^t C_L dt$). To predict the required ozone CT value to inactivate *L. pneumophila* in wastewater similar to the Urbana wastewater, one can simply read off the graph to obtain the CT value corresponding to a certain inactivation

ratio. The goodness of the Chick-Watson model fitting of experimental data obtained over a range of wastewater containing 0.8 to 3.1 mg C/L WOM indicates the validity of the dissolved ozone concentration predicted by the established mass balance model.

The good fit to the Chick-Watson model suggests that the inactivation ratio of *L. pneumophila* in wastewater can be explained by the CT concept, in agreement with previous study on *E. coli* inactivation in drinking water [29]. This observation contradicts previous research on wastewater disinfection by ozone, which proposed that the standard CT approach should not be applied to ozone disinfection of wastewater because bacteria participated in the immediate ozone demand of the wastewater, and thus consumed the ozone to such an extent that its level is below detection during disinfection [17]. The proposed incompatibility of CT concept proposed by Xu et al., 2002 might have been due to low ozone concentration that was difficult to detect. This obstacle could be overcome with the mass balance model used in this study, the result of which was validated by the close fit to the Chick-Watson model. Future improvement of the model fitting could involve the reaction between ozone and other components of wastewater. For instance, carbonate and bicarbonate species could react with hydroxyl radicals, which are important intermediates during the cycle of ozone decomposition, thus quenching the decomposition process [31].

Potential implications for treatment processes

The approach to develop the CT equation as presented in this study can be used to develop scenario specific CT equations to predict the required CT value for a given level of *L. pneumophila* inactivation in specific wastewater, regardless of the operating temperatures, as shown in Figure5. The observed inactivation of *L. pneumophila* up to five $\log_{10}$ at low ozone concentrations also suggests that CT credit might be issued even if the dissolved ozone is

undetectable by instruments, which allows for a reduction in the applied ozone dosage and thus a reduction in cost and bromate formation potential.

To comply with the selected CT value, the dissolved ozone concentration in wastewater has to be monitored. The modeling approach used in this study provides an alternative means to measurement, as WOM and turbidity in wastewater often result in fast consumption of ozone to concentrations below detection limits, and interference with the Indigo colorimetric method. Before applying the model framework presented in this work, parameters in the developed model including the ozone self-decay rate constant k, the mass transfer coefficient K_La, and the reaction rate constant between WOM and ozone k_2 must be fitted to wastewater of specific sources, as discussed in SI of this manuscript to account for variations in wastewater's characteristics. To apply the model for dissolved ozone prediction, information including the initial ozone concentration C_0, initial TOC loading TOC_0, Henry's law constant m, and gas phase ozone concentration C_{gi} will be needed as inputs based on specific operating conditions.

The operating dissolved ozone concentration could be determined based on a high influent WOM loading to the plant. This is because higher WOM levels were shown in this study to result in slower *L. pneumophila* inactivation and faster ozone consumption. Additionally, the rate of *L. pneumophila* inactivation was also shown to increase with the initial ozone concentration, given the same WOM loading. By selecting the operating ozone concentration as proposed, the rate of *L. pneumophila* inactivation can be maximized while reducing the contact time and thus the contact tank volume. As an example, at a WOM loading of 3.1 mg C/L, a 1.7 fold increase in operating ozone dosage from 165 to 281 μg/L could reduce the time required to achieve 2 $\log_{10}$ inactivation of *L. pneumophila* by 10 fold. This translates to a reduction in reactor volume by a factor of 10. If higher *L. pneumophila* inactivation is desired, the positive

correlation between contact time and *L. pneumophila* inactivation in wastewater suggests that besides a higher operating dissolved ozone concentration, longer contact times could be a viable option.

While it is recognized that the ozone CT requirements for other more persistent pathogens, such as the *Cryptosporidium* oocysts, are much higher than that for the same $\log_{10}$ inactivation of *L. pneumophila* even in a drinking water environment [19], the study also provides valuable information for utilities that aim specifically to polish the partially treated wastewater to reduce the effluent *L. pneumophila* concentration. For instance, a water reclamation plant targeting to provide reclaimed wastewater for downstream irrigation and landscaping, during which *L. pneumophila* in aerosols may pose a health hazard to the public.

To evaluate the energy performance of the microchannel plasma ozone generator so as to provide operational suggestions, the energy consumption per $\log_{10}$ inactivation of *L. pneumophila* was investigated based on the disinfection contact time (Figure 2.7). Previous discussion concluded that the operating dissolved ozone concentration should be determined based on a high influent WOM loading to the plant, the analysis was therefore conducted using the highest WOM loading tested. The energy consumption per $\log_{10}$ inactivation increased for all conditions over time. Operating at 120V, the higher the initial dissolved ozone concentration was, the smaller the increase was over time, which signified less energy consumption per $\log_{10}$ inactivation of *L. pneumophila*. The inactivation was also faster at higher initial ozone concentration, as can be seen marked by the vertical line at 9 minute as an example (Figure 2.7). At 50V of driving voltage, although the energy consumption per $\log_{10}$ inactivation was lower than at 120V the entire contact time, the slower and insignificant $\log_{10}$ inactivation (0.3 $\log_{10}$ inactivation of *L. pneumophila* in 9 minutes) still made 50V a less desirable driving

voltage. Additionally, as shown in Figure 2.1 b) achieving a high steady state dissolved ozone concentration was more difficult when driving at 50V. As a result, the microplasma ozone generator should preferably be driven at 120V to achieve an ideal balance between *L. pneumophila* inactivation performance and energy consumption.

2.5 Conclusions

The performance of the microchannel plasma ozone generator to inactivate *L. pneumophila* under the impact of several operating and environmental parameters, and the kinetics of *L. pneumophila* inactivation were investigated in this study. When operated at 120 V of driving voltage, up to five $\log_{10}$ of *L. pneumophila* inactivation in wastewater was achieved within 1 minute using only ambient air as the feedstock gas and at low power consumption of less than 0.023 kWh. Contrary to previous findings, the concept of CT appeared to be valid. A framework to develop CT equations was subsequently developed to predict the *L. pneumophila* inactivation by ozone in specific wastewater conditions. The inactivation kinetics of *L. pneumophila* in organic-free solution was found to depend strongly on the temperature and was less effective at lower temperature, which is contrary to previous findings. However, the inactivation kinetics in wastewater was independent of temperature. High loadings of WOM was found to require an increase in initial dissolved ozone concentration to achieve the same level of *L. pneumophila* inactivation. Contrary to a previous finding, contact time may have a positive correlation with *L. pneumophila* inactivation in wastewater. TOC also appeared to be a good indicator of the ozone consumption for the studied wastewater. These parametrical findings, together with the bench-scale tested microchannel plasma ozone generator, is the first step toward the application of this promising technology for wastewater reclamation.

2.6 List of Figures and Tables

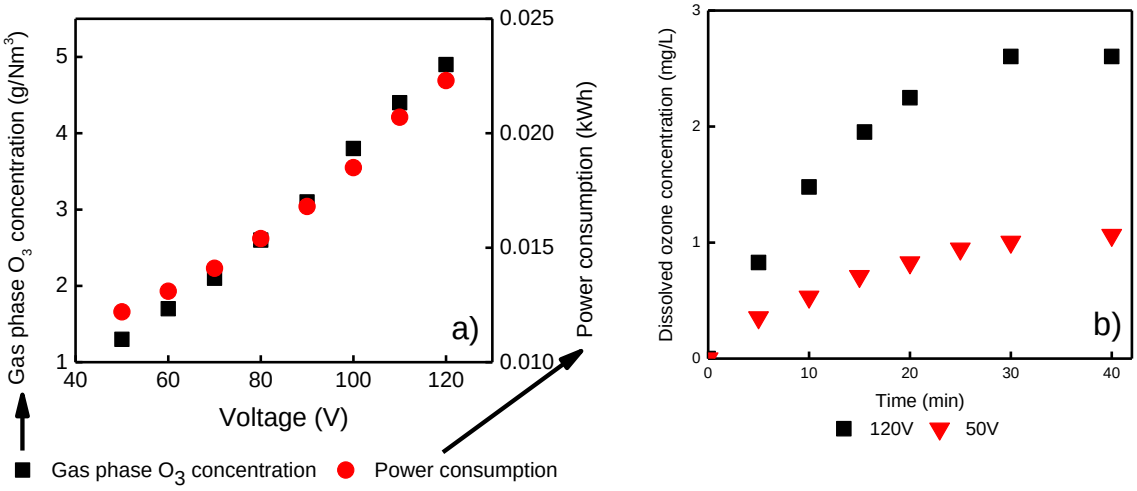


Figure 2.1 a) Power consumption and the produced gas phase ozone concentration at various driving voltages of the microchannel plasma ozone generator. b) The profile of the increase in dissolved ozone concentration as a result of purging ozone gas produced at two different voltages. C_{gi} at 120 and 50V of driving voltages are 4.9 and 1.3 g/Nm^3, respectively.

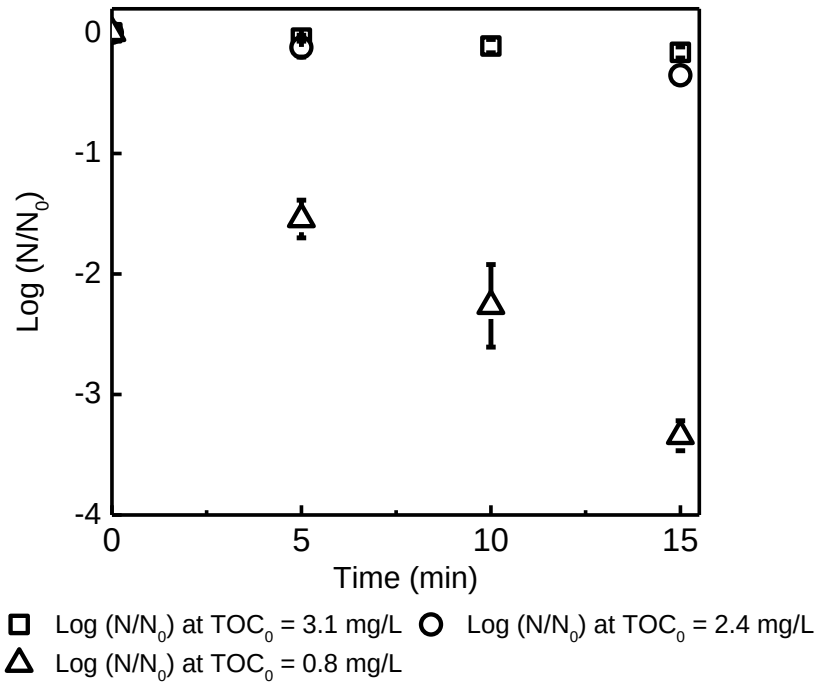


Figure 2.2 At 22 ^{o}C, the effect of initial WOM loadings on *L. pneumophila* inactivation kinetics, for an initial dissolved ozone concentration of 33 µg/L as a function of time at various initial WOM loadings. Data plotted are average values and standard deviations of three replicates.

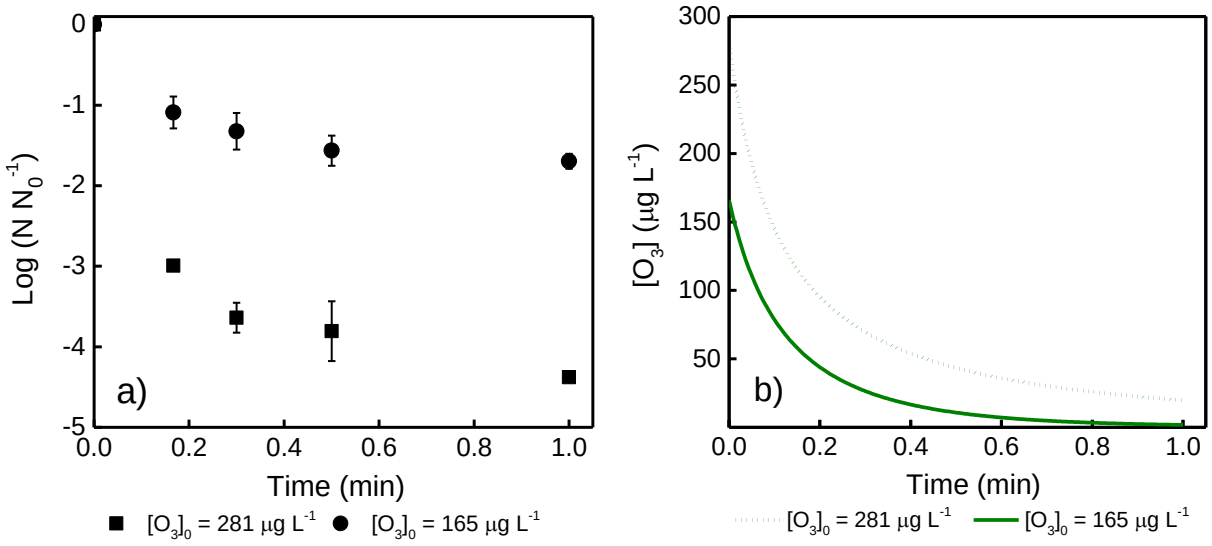


Figure 2.3 At 22 ^{o}C, the effect of initial dissolved ozone concentration on *L. pneumophila* inactivation kinetics, for an initial WOM loading of 3.1 mg C/L a), combined with the modeled ozone decomposition profile as a function of time at various initial dissolved ozone concentrations b). The data plotted are average values and standard deviations of three replicates. The dashed and solid curves are the results of calculations.

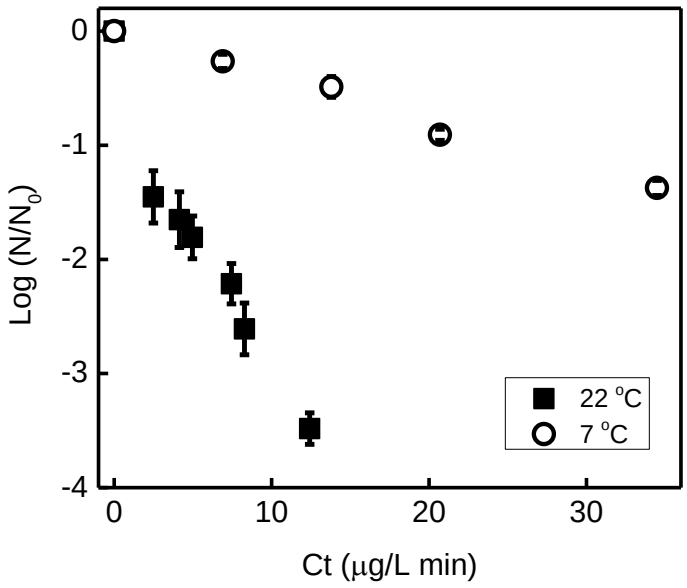


Figure 2.4 *L. pneumophila* inactivation without WOM at 7 and 22 °C. Data plotted are average values and standard deviations of three replicates.

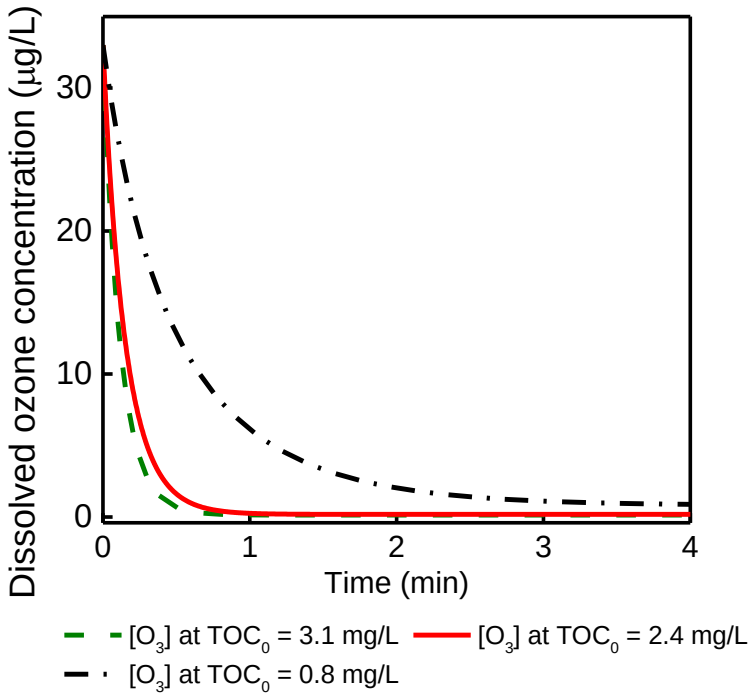
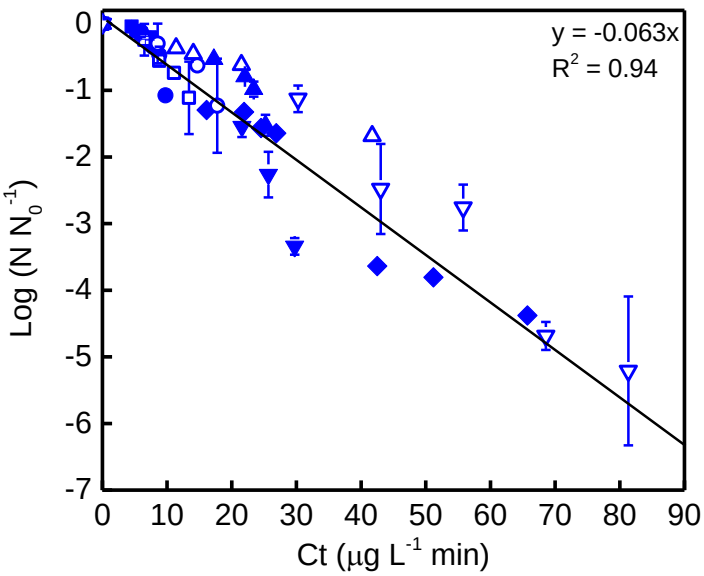


Figure 2.5 At 22 ^{o}C, the effect of initial WOM loadings on ozone decay. Data plotted are average values and standard deviations of three replicates. The dashed and solid curves are the results of calculations.



Legend	TOC (mg L^{-1})	Temperature (^{o}C)	$[O_3]_{gas}$ (mg L^{-1})
■	3.1	22	1.3
●	2.4	22	1.3
▲	1.6	22	1.3
▼	0.8	22	1.3
◆	3.1	22	4.9
□	3.1	7	1.3
○	2.4	7	1.3
△	1.6	7	1.3
▽	0.8	7	1.3

Figure 2.6 Summary of *L. pneumophila* inactivation in Urbana secondary wastewater at 7 and 22 ^{o}C with various initial TOC loadings, and initial ozone concentrations generated when the ozone generator was driven at 50 V (C_{gi} = 1.3 g/m^3) and 120 V (C_{gi} = 4.9 g/m^3). CT concept appears to be valid, and the inactivation trend could be used to predict *L. pneumophila* disinfection performance. Data plotted are average values and standard deviations of at least three replicates.

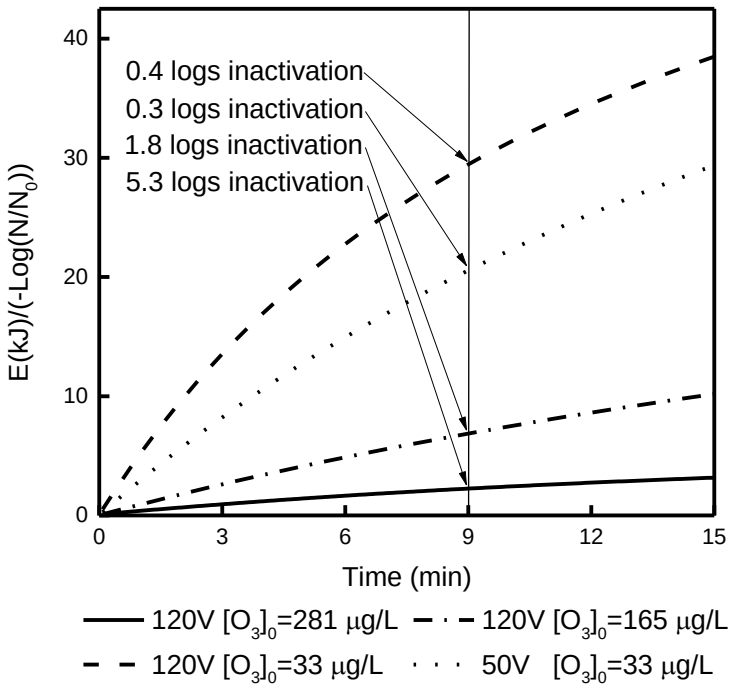


Figure 2.7 Microchannel plasma ozone generator energy consumption per $\log_{10}$ inactivation of *L. pneumophila* in secondary wastewater at initial TOC loading of 3.1 mg C/L, at various initial ozone concentrations and driving voltages.

2.7 Supplementary Information

Determination of ozone concentration in wastewater

Dissolved ozone concentration is a crucial parameter to evaluating the disinfection performance of the microchannel plasma ozone generator. However, low levels of dissolved ozone in wastewater are usually difficult to measure due to rapid reaction of wastewater organic matter with ozone, and the presence of turbidity as interference. For these reasons, a mass balance model was constructed to predict the ozone concentration as a function of disinfection time.

At both 7 and 22 ^{o}C, the decomposition kinetics of dissolved ozone in solutions containing various concentrations of initial WOM loadings and initial dissolved ozone concentrations were individually assessed. This eliminated the interference of turbidity caused by bacteria biomass on the Indigo colorimetric method for ozone detection, particularly during disinfection at low ozone concentration. The transfer rate of ozone into water at both 7 and 22 ^{o}C was determined by purging ozone into organic-free buffered water at different ozonator driving voltages, during which the increase in dissolved ozone concentration was recorded as a function of time. The dissolved ozone concentrations obtained were then fitted to a series of mass balance equations to develop a model using Origin Pro 9.1 (Northampton, MA) that predicts the dissolved ozone concentration. The final function predicts the dissolved ozone concentration based on input parameters such as the initial ozone concentration and Henry's law constant at different temperatures. The predicted ozone concentration was subsequently used to calculate the obtained ozone CT value.

In detail, the measured dissolved ozone concentrations (C_L) at different times were fitted to the solution to the mass balance equation for the batch reactor:

$$\frac{dC_L}{dt} = -kC_L \qquad \text{S (2.1)}$$

$$Ln\left(\frac{C_L}{C_0}\right) = -kt \qquad \text{S (2.2)}$$

where the fitting parameter is the first order rate constant (k) for ozone decomposition in organic-free solutions. The fitted k values were 54 (±1.8) × $10^{-4}$$min^{-1}$ ($R^2 \geq 0.99$) for 22 °C and 18 (±0.48) × $10^{-4}$$min^{-1}$ ($R^2 \geq 0.99$) for 7 °C. The results are comparable to those published previously [32]. The solution to S (2.1) is provided as S (2.2). To determine the volumetric liquid-phase mass transfer coefficient (K_La) [33], ozone was added continuously to the buffered organic-free solution, and C_L was measured at different times . The mass balance for ozone mass transfer and decay in this experiment is given as:

$$\frac{dC_L}{dt} = K_La\left(\frac{C_{gi}}{m} - C_L\right) - kC_L \qquad \text{S (2.3)}$$

$$C_L = \frac{\exp(t(K_La+k))K_La\frac{C_{gi}}{m} - K_La\frac{C_{gi}}{m}}{\exp(t(K_La+k))(K_La+k)} \qquad \text{S (2.4)}$$

where C_{gi} is the ozone concentration at the interphase, and m is the Henry's law constant at different temperatures. The value of K_La was then determined by fitting the measured C_L to S (2.4), to be 3.1 (±1.3) × $10^{-3}$$min^{-1}$ ($R^2 \geq 0.88$) for 22 °C and 9.3 (±5.8) × $10^{-3}$$min^{-1}$ ($R^2 \geq 0.95$) for 7 °C. The decomposition of ozone by WOM (represented by TOC) was thus determined by monitoring the change in C_L in wastewater for a given initial dissolved ozone concentration C_0. The mass balance relation for this set of experiments is:

$$\frac{dC_L}{dt} = -k_2C_Lx - kC_L \qquad \text{S (2.5)}$$

$$C_L = \frac{x_0 k_2 C_0 - k_2 C_0^2 + C_0 k}{(k_2 x_0 + k)\exp((k_2 x_0 - k_2 C_0 + k)t) - k_2 C_0} \quad \text{S (2.6)}$$

where x is the reactive portion of the TOC. It is assumed that $C_0 - C_L = x_0 - x$, and that the initial value for x is x_0, where $x_0 = \alpha TOC_0$ [29]. Also, α is the fraction of organic carbon that exerts ozone demand. The fraction of reactive WOM (α) and the reaction rate constant (k_2 in unit of L/(μg min)) were determined by fitting the measured C_L to S (2.6). The fitted parameters are $\alpha = 0.1 \pm 0$ mg O_3/mg C for both 22 ($R^2 \geq 0.72$) and 7 °C ($R^2 \geq 0.58$), and k_2 = 0.029 ± 0.037 L/ (μg min) ($R^2 \geq 0.76$) for 22 °C and k_2 = 0.026 ± 0.019 L/ (μg min) ($R^2 \geq 0.83$) for 7 °C. All fitting parameters were average values from at least four biological replicates. The values for the second order reaction rate constant are similar to the ones reported in the literature [17]. S (2.5) can be solved by substituting x and x_0 into the equation. The overall mass balance for ozone is thus expressed as:

$$\frac{dC_L}{dt} = K_L a\left(\frac{C_{gi}}{m} - C_L\right) - k_1 C_L y - k_2 C_L(x_0 - C_0 + C_L) - kC_L \quad \text{S (2.7)}$$

where the term $-k_1 C_L y$ represents the consumption of ozone by bacteria. Experiments examining *L. pneumophila* inactivation in the organic-free solution showed statistically insignificant difference of ozone decay rate with and without the presence of *L. pneumophila* ($p > 0.05$), therefore the term considering bacterial consumption of ozone is negligible in this study, and the final mass balance equation used for parameter fitting is:

$$\frac{dC_L}{dt} = K_L a\left(\frac{C_{gi}}{m} - C_L\right) - k_2 C_L(x_0 - C_0 + C_L) - kC_L \quad \text{S (2.8)}$$

Terms in the above equation that were measured in the experiments are the dissolved ozone concentration as a function of time (C_L), the initial total organic carbon loading (TOC_0), the initial dissolved ozone concentration (C_0), and the ozone concentration at the interphase (C_{gi}),

which is assumed to be equal to the gas phase ozone concentration due to negligible mass transfer resistance in the ozone gas film. A finite difference method was used to solve S (2.8) on a time scale as large as 15 minutes, with a time interval of 0.2 or 5 seconds, depending on the time scale of interest. The solution to S (2.8) was used to predict the dissolved ozone concentration under a variety of experimental conditions. These calculated profiles of the dissolved ozone were then used to evaluate the *L. pneumophila* inactivation performance by the microchannel plasma ozone generator, and the development of the CT equation for *L. pneumophila* inactivation prediction for the Urbana wastewater.

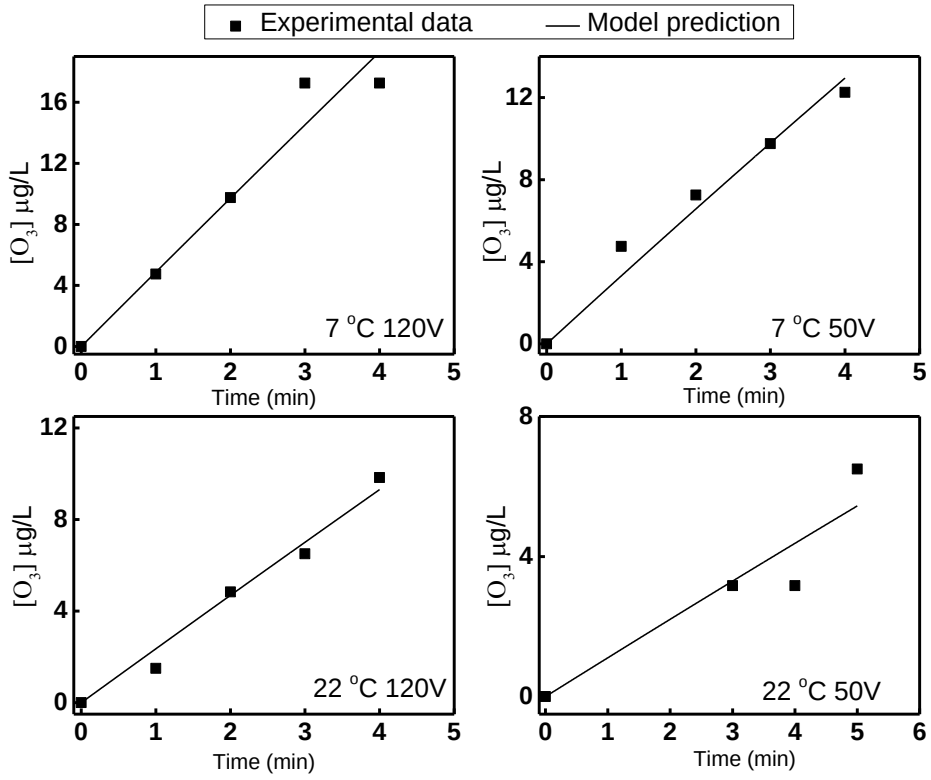


Figure S 2.1. Examples of profile of ozone concentration during constant flow rate purging into buffer solution at pH 6.8 – 7, and at 7 and 22 °C.

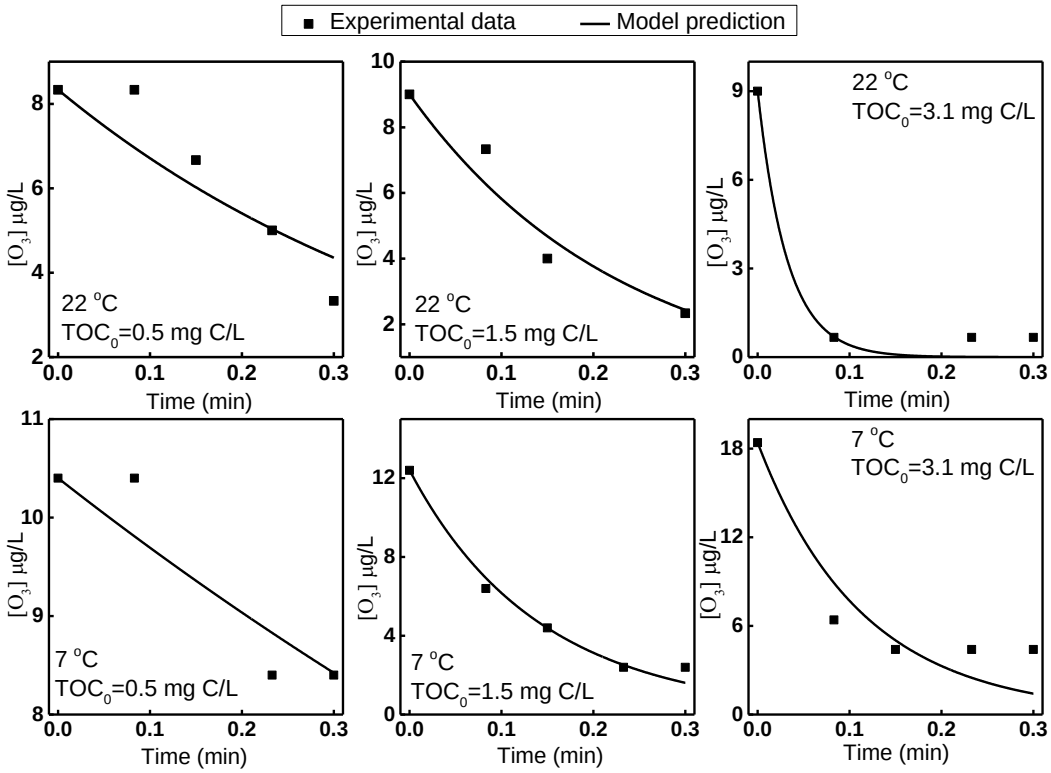


Figure S 2.2. Examples of profile of ozone concentration during ozone reaction with WOM at various initial TOC loadings at pH 6.8 – 7, and at 7 and 22 °C. Due to the complexity of the wastewater matrix and difficulties encountered to precisely capture the ozone decay profiles, uncertainties were involved during the fitting of the reaction rate constant k_2, which was subsequently used in the ozonation system design (see SI, section 2.3). These uncertainties were accounted for using the Pedigree matrix approach in the uncertainty analysis.

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CHAPTER 3

HUMAN HEALTH TRADE-OFFS IN THE DISINFECTION OF WASTEWATER FOR LANDSCAPE IRRIGATION: MICROPLASMA OZONATION VERSUS CHLORINATION

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Shengkun Dong, Jun Li, Min-Hwan Kim, Sung-Jin Park, J. Gary Eden, Jeremy S. Guest, Thanh H. Nguyen, Human Health Trade-offs in the Disinfection of Wastewater for Landscape Irrigation: Microplasma Ozonation versus Chlorination

3.1 Abstract

Wastewater reuse is becoming increasingly common, and there is a need for decentralized and small-scale systems to support the safe recovery of water resources. In this study, an integrated life cycle assessment (LCA) and quantitative microbial risk assessment (QMRA) were used to compare microplasma ozonation (an emerging technology) to chlorination (an established technology) for the disinfection of wastewater for landscape irrigational reuse. Three waterborne pathogens *Legionella pneumophila*, *Giardia*, and *Cryptosporidium parvum* were selected to include bacteria and protozoans covering the transmission routes of inhalation and ingestion. Inactivation data from literature were coupled with bench scale experiments (to establish inactivation parameters for *L. pneumophila* by ozone in wastewater) for the design and simulation of disinfection processes. Microplasma-based ozonation reduced more life cycle human health impacts as compared to chlorination for five of six impact categories, because of the high susceptibility of the pathogens to ozone and the lower impacts stemming from electricity (required in ozonation) vs. chemical production (required in chlorination). These

results were consistent across all U.S. state electricity fuel mixes. These results indicate that from the point of view of reducing human health impact, the emerging microplasma ozonation technology is superior to chlorination for wastewater reuse disinfection. To reduce the overall human health impact, future design efforts should focus on reducing process consumables (i.e., chemical and electricity consumption) through longer hydraulic residence times (HRTs), while maintaining adequate disinfectant dosing to maintain reliable disinfection efficacy despite influent variability in compounds that may quench or interfere with the disinfectant.

3.2 Introduction

Wastewater reuse alleviates pressure on freshwater resources. In 2010, the total water reuse in the United States was estimated to be 2,400 million gallons per day (MGD),[1] which increased by 42% compared to that in 2004.[2] However, successful reuse requires that the finished water be disinfected to prevent the spread of pathogens, especially for reuse applications – such as landscape irrigation – that could result in human contact. Currently, chlorination using sodium hypochlorite is a well-established disinfection method for wastewater. Pathogens are inactivated mainly through free chlorine or chloramines (depending on the ammonia content of the treated wastewater), which are effective against a wide range of waterborne pathogens. Due to its efficacy and affordability, chlorination is the most widely used technique for wastewater disinfection.[3] However, when dechlorination is required (which is typical, especially if reclaimed water is used for irrigation), the economic benefits of chlorination over alternative disinfection techniques is not always guaranteed.[4] Beyond cost, chlorination may also encourage the formation of harmful disinfection byproducts (DBPs) [5-7] and is ineffective at low doses against a sub-set of pathogens of concern such as *Cryptosporidium parvum*.[8]

Ozone is a powerful disinfectant for a number of critical microorganisms such as the Norwalk virus,[9] poliovirus,[10] and *Escherichia coli*.[11, 12] Indeed, several pathogens that are known to be resistant to chlorine, including *Cryptosporidium parvum,* are readily inactivated by ozone.[13-17] Recently, an emerging method to produce ozone – microplasma technology – was invented and brought to market.[18] Because the building block of microplasma generators (known as a "chip") is modular and produces 2-3 grams of ozone per hour, virtually any ozone production rate can be generated by stacking individual chips. As such, ozone may become a more feasible disinfection alternative for small-scale treatment systems. In addition to increased modularity, microplasma generators using oxygen as the feedstock gas have achieved energy efficiencies more than twice that of conventional dielectric barrier discharge reactors (120 g kWh^{-1} [18] vs. 50 g kWh^{-1} [19]), stemming from the combination of a much smaller plasma impedance (~41 kΩ) and a much lower driving voltage.[18] Finally, microplasma ozone generators do not need water cooling units, owing to their large surface area to volume ratio and the low temperature of the plasmas.[20] This additional benefit further reduces complexity and size of the system without compromising functionality.

Both chlorination and ozonation technologies have the shared goal of reducing pathogen concentrations in reclaimed water to protect human health. However, technologies that consume energy, chemicals, and other resources can result in a variety of environmental emissions across space and time that can be detrimental to human health, creating a tension between direct benefits (reduced pathogen exposure) and negative health consequences stemming from the indirect release of environmental stressors (e.g., $PM_{2.5}$ released during the transport of materials to the treatment plant via truck). Although direct human health risks from pathogen exposure can be evaluated by Quantitative Microbial Risk Assessment (QMRA, which assesses the risks

associated with exposure to specific pathogens of concern[21]), the implications of indirect impacts of a given technology are better assessed via Life Cycle Assessment (LCA).[22] To better understand the health implications of disinfection system design and operational decisions, a hybridized LCA and QMRA methodology can be established by leveraging the common unit of Disability Adjusted Life Years (DALYs).[23] This approach was recently used to quantify the human health impacts of different flow regimes at several water reclamation plants,[23] and could offer insights in the selection and development of disinfection technologies for water reuse.

The objective of this study part of the thesis is to characterize the human health implications of wastewater disinfection for landscape irrigational reuse, with a focus on microplasma ozonation (an emerging technology) and chlorination (an established technology) and the factors governing their performance and uncertainty. To date, LCA studies have compared ozonation or chlorination to other treatment alternatives,[24-26] but no study has considered microplasma ozonation for water disinfection. In this study, two full-scale disinfection treatment trains were designed in parallel: (i) chlorination followed by dechlorination, and (ii) microplasma ozonation. Pathogens explicitly modeled included *Giardia* and *C. parvum* because they are two of the most frequently documented waterborne enteric pathogens in the United States.[2] Additionally, *Legionella pneumophila* was also included in this analysis due to the high health care cost of over $33,000 per episode of sickness due to infection,[27] as well as the recent outbreak of Legionellosis in New York City (U.S.A.) in the summer of 2015.[28] This choice of pathogens encompasses protozoans and bacteria covering the transmission routes of both ingestion and inhalation that are likely to occur in landscape irrigation.[29] Both data from literature (for *Giardia* and *C. parvum* inactivation) and data from bench scale experiments conducted in this study (for *L. pneumophila* inactivation) were used to

design the treatment trains and perform the subsequent LCA-QMRA analysis, using DALYs as a common metric. Given the anticipated importance of electricity consumption to indirect health impacts,[23, 30] the comparative assessment was conducted across all 50 U.S. states with a focus on three states with the largest reclaimed water use (Florida, California, and Texas),[2] using their respective fuel mixes for the consumed electricity and the quantification of environmental impacts. Finally, the LCA-QMRA modeling tool was integrated in a Monte Carlo framework and sensitivity analyses were conducted to determine the relative sensitivity of results to input parameters as well as design and operational decisions.

3.3 Materials and Methods

Pathogen Inactivation via Chlorination or Ozonation

- *Giardia* and *C. parvum* Inactivation Parameters

Literature data were used to design both the chlorination and ozonation disinfection systems for *Giardia* and *C. parvum* inactivation. The inactivation kinetics were assumed to follow the Chick-Watson inactivation model with inactivation rate constants of 2.5×10^{-2} (at 18 ^{o}C) and 8.36×10^{-4} (at 20 ^{o}C) L mg^{-1} min^{-1} for *Giardia* and *C. parvum* inactivation by chlorine, respectively.[31, 32] The inactivation rate constants for *Giardia* and *C. parvum* using ozone were 27.1 (at 25 ^{o}C) and 0.8 (at 20 ^{o}C) L mg^{-1} min^{-1}, respectively.[31, 33] The effect of temperature on the inactivation rate constants was addressed using the Arrhenius equation. The impact of the water matrix on the residual disinfectant concentration was accounted for using mathematical simulation. Detailed equations, values, and steps are provided in the Supplementary Information (SI).

- Determination of *L. pneumophila* Inactivation Parameters

The chlorination inactivation parameters for *L. pneumophila* were obtained from literature, with a second-order inactivation rate constant at 30 ^{o}C of 0.307 L mg^{-1} min^{-1}.[34] To obtain a representative inactivation rate constant for *L. pneumophila* by ozone in secondary wastewater, a set of bench-scale experiments were performed in chapter 2 of this thesis.

Life Cycle Assessment (LCA)

- System Description

Two alternative disinfection technologies for secondary effluent were considered in this study: (i) chlorination followed by dechlorination, and (ii) microplasma ozonation. The former was used as a benchmark because it is a well-established and widely accepted technology for water reclamation disinfection. Both systems were designed using design manuals and specifications provided by manufacturers. Design details can be found in the SI.

The chlorination-based disinfection system consists primarily of a sodium hypochlorite storage tank and dosing system, a contact tank, a sodium bisulfite storage tank and dosing system, and two inline static mixers (one for each chemical; Figure S3.3). Secondary effluent is mixed with sodium hypochlorite at the entrance of the serpentine contact tank. The inactivation kinetic data were obtained from literature. The chlorine contact tank was designed to have a large length to width ratio (L:W = 40:1) to minimize dispersion. The chlorinated wastewater continues flowing towards the end of the contact tank where it is then mixed with sodium bisulfite for dechlorination. The dose of sodium bisulfite was designed to dynamically match the hypochlorite residual. The final discharge is subsequently used for immediate downstream landscape irrigation.

The microplasma ozonation disinfection system consists of a microplasma ozone generator, a venturi ozone injection nozzle, an ozone contact tank, and an ozone destruction unit (Figure S3.3). Air is compressed into the microplasma ozone generator, which then generates plasmas to produce ozone. Ozone gas is injected into the secondary effluent using a venturi injector. The ozonated wastewater flows into an ozone contact tank, which also has a large length to width ratio (L:W = 40:1) to minimize dispersion. The inactivation in the ozone contact tank was modeled based on kinetic data obtained from this study as well as literature.[31, 33] The escaped ozone from the ozonated wastewater is collected at the top of the contact tank and passed through a thermal catalytic ozone destruction device. The final discharge is used for immediate downstream landscape irrigation.

- Goal and System Boundary

The goal of this LCA was to compare the environmental impacts on human health stemming from two alternative disinfection technologies. The system boundary included the construction and operation of both systems, and did not consider processes that were common to both technologies (e.g., primary and secondary treatment) (Figure S3.3). The functional unit for this study was the disinfection (more than 1 $\log_{10}$ inactivation) of 4 MGD of secondary effluent (with pathogen distributions defined in Table S3.6) with a project lifetime of ten years.

- Inventory Analysis

The quantity of materials and energy consumption for the construction and operation of both the chlorination and ozonation systems were generated from the detailed design (see the SI) and assumptions (Table S3.1). The ozone generator, in particular, was inventoried using design documents for a microplasma ozone generator (2 g h^{-1} production per "chip"; E.P. Purification,

Inc.). To account for differences in environmental impacts stemming from locality specific electricity fuel sources, fuel mixes for three separate states in the U.S. – the states with the highest total annual quantity of reclaimed water usage (Florida, California, and Texas) – were each individually used to quantify the sensitivity of LCA results to the location of the disinfection system. To explore the generalizability of the data, results from the three states were also compared against the rest of the U.S. states in terms of the human health intensity consumed energy. Data on the annual state net electricity consumption profile by fuel source were obtained from the US Energy Information Administration for 2013 and 2014 (Table S3.3).[35] Inventory data were obtained from the ecoinvent database (v3, accessed through SimaPro v8.0.5.13) encompassing raw material extraction, processing, manufacturing, and transportation. A detailed summary of the design equations (see the SI), a breakdown list of materials and their quantities, and the inventory materials/processes from ecoinvent 3 are provided in the SI.

- Impact Assessment

Because the impact on human health is the basis of comparison for this study, six out of seventeen impact categories in the ReCiPe method relevant to human health were evaluated, which included climate change, ozone depletion, human toxicity, photochemical oxidant formation, particulate matter formation, and ionizing radiation. The impact assessment was conducted using the ReCiPe endpoint method to express results in DALYs. Although endpoint methods introduce greater uncertainty to the system due to additional embedded assumptions, the use of endpoint impact categories is often more accessible and relevant for stakeholders.[36] The hierarchist cultural perspective was set as the base case cultural perspective, which was required for the ReCiPe method. A similar approach was used in a previous study using DALYs as a

measurement of the human health effects.[23] The final results were normalized by functional unit and expressed in DALYs per year per $\log_{10}$ pathogen inactivation.

- Uncertainty and Sensitivity Analysis

Uncertainty analysis of the human health impacts of both systems were performed using Monte Carlo with 1,500 simulations in Microsoft Excel 2013. Input variables were assigned uniform distributions unless compelling evidence suggested otherwise. Temperature followed a normal distribution [37] and the input parameter k – which is the identical survival probability for each organism – was assigned a log-normal distribution for each pathogen according to literature.[38-40] The uncertainty surrounding input parameters with limited data (i.e., point estimations) in the literature were addressed using the Pedigree matrix approach.[41, 42] Briefly, a matrix consisting of five data quality indicators – reliability, completeness, temporal correlation, geographical correlation, and further technological correlation – were used to select indicator scores corresponding to each input parameter and data quality indicator combination. The indicator scores for these input parameter were then calculated to yield a standard deviation that log-normally distributed data would follow. These standard deviation values were converted and are reported as coefficients of variation provided together with the inventory data (see the SI).

A sensitivity analysis was performed by adjusting each input value individually from its median to the 10^{th} and 90^{th} percentiles. The corresponding change of the output metric (human health impact) was recorded to characterize the relative importance of individual sources of uncertainty. Additionally, the percentage change in the output metric was also normalized to the percentage change in the input value to offer additional insight to the system through a relative response (Figure S3.2 a and b, the larger the ratio, the more sensitive human health impacts were to relative changes in a given input value).

Quantitative Microbial Risk Assessment

- Hazard Identification

Giardia, *C. parvum*, and *L. pneumophila* were identified as the pathogens of concern that could be exposed to people during landscape irrigation using reclaimed water. *Giardia* and *C. parvum* represent the chlorine resistant pathogens that the state of Florida regulates as one of the reclaimed water quality monitoring parameters.[1] Accidental ingestion during landscape irrigation makes these pathogens public health concerns.[43] *L. pneumophila* was selected because it was identified in reclaimed wastewater [44, 45] and it poses a potential hazard to human health when such water is aerosolized (e.g., during landscape irrigation).[46]

- Exposure Assessment

The most common landscape irrigation system is the sprinkler irrigation system,[47] which creates aerosols consisting of large water droplets as well as fine mist that could be accidentally ingested and/or inhaled by people. As discussed above, the primary route of exposure was identified as ingestion for both *Giardia* and *C. parvum*, and inhalation for *L. pneumophila*. To calculate the final dose, a population size of 40,000 was assumed along with 100 gallons of wastewater produced per capita per day.[48] It was assumed that the reclaimed water would be used to irrigate public landscape, and that the full population would share access to this space. Additionally, a park visit frequency of once a week per person was assumed. For ingestion, an estimated 1 mL ingestion of municipal irrigation water per person per visit to the public lawn was used.[43] For inhalation, 10^{-2} CFU m air^{-3} (CFU mL $water^{-1})^{-1}$ of partitioning coefficient,[49] 0.72 m^3 h^{-1} inhalation rate,[49] 0.5 h of exposure duration per visit, and 50% retention fraction of *L. pneumophila* in lungs were used as the default case.[40]

- Dose Response Assessment

Dose response data for the three chosen pathogens were all obtained from literature. The exponential model (eqn (3.1)) was the best fit across dose response data for all three pathogens.

$$\text{Risk probability} = 1 - e^{-k \times \text{dose}} \quad (3.1)$$

For *Giardia*, a dose response model using adult males was used, with infection being the response measured and ingestion being the exposure route.[39] The log-normally distributed maximum likelihood estimate (MLE) k value was 1.99×10^{-2} with a 95% percentile of 2.92×10^{-2}.[39] For *C. parvum*, a dose response model using human volunteers as hosts and infected with Iowa strain was used, with infection being the response measured. The log-normally distributed MLE k value was 4.19×10^{-3} with a 95% percentile value of 7.52×10^{-3}.[38] A dose response model for *L. pneumophila* using guinea pigs as hosts was used, with infection being the response measured and inhalation being the exposure route. The log-normally distributed MLE k value was 5.99×10^{-2} with a 95% percentile value of 0.111.[40]

- Risk Calculation in DALYs

The probabilities of infection calculated above were converted to DALYs incurred per year by multiplying the probability value (in the unit of symptomatic cases per year) by the severity factor (also known as the characterization factor in DALYs per symptomatic case for each pathogen). The severity factors for both *Giardia* and *C. parvum* were taken from literature (1.6×10^{-3} and 1.47×10^{-3} DALYs per symptomatic case for *Giardia* and *C. parvum*, respectively).[50, 51] The severity factor for *L. pneumophila* was estimated to follow a uniform distribution between 1.05×10^{-3} and 4.37×10^{-2} DALYs per symptomatic case due to a lack of direct estimate from literature. Detailed calculations for this estimation is provided in the SI. The

final results were normalized by functional unit and expressed in DALYs per year per $\log_{10}$ pathogens inactivated.

3.4 Results and Discussion

Determination of parameters for *L. pneumophila* inactivation by ozone

LCA and QMRA requires the knowledge of *L. pneumophila* inactivation kinetics and related disinfection parameters. For this reason, we conducted inactivation experiments and analyzed the data based on a mass balance model that predicted the ozone concentration as a function of disinfection time. Details of the mass balance are presented in the SI. Based on this mass balance model, the calculated profiles of the dissolved ozone were subsequently used in the Chick-Watson equation to calculate the inactivation rate constant for *L. pneumophila* in wastewater. Following the $\log_{10}$ scale version of the Chick-Watson law, $\log(\frac{N}{N_0})$ values ($\log_{10}$ of the *L. pneumophila* survival ratio) from all experiments were plotted versus $\int_0^t C_L\, dt$, where C_L is the instantaneous dissolved ozone concentration at time t. Although a temperature dependence of *L. pneumophila* inactivation by ozone would be expected,[12, 33, 52] the complex wastewater matrix used for parameter calibration did not result in significantly different inactivation rate constants at 7 and 22 ^oC at a confidence level of 95% ($p > 0.05$). We therefore assumed an identical *L. pneumophila* inactivation rate constant using ozone, with the fitting equation and coefficient of determination being $\log\left(\frac{N}{N_0}\right) = -0.063Ct$ and 0.94, respectively (Figure 2.6). Within the TOC range of 0.8 to 3.1 mg C L^{-1} and at both 7 and 22 ^oC, a good fit ($R^2 = 0.94$) was observed for *L. pneumophila* inactivation. The goodness of the Chick-Watson model fitting of experimental data obtained over a range of wastewater containing 0.8 to 3.1 mg C L^{-1} WOM indicates the validity of the dissolved ozone concentration predicted by the established mass balance model. The

obtained inactivation rate constant for *L. pneumophila* inactivation, together with the modeling framework for the ozonation decay kinetics in wastewater, were subsequently used for *L. pneumophila* inactivation predictions in both the LCA and QMRA analysis.

Life Cycle Human Health Impacts of Disinfection Systems

Both disinfection technologies are capable of reducing the overall human health implications of landscape irrigation with secondary effluent, as reflected by net negative total DALY values in all but one scenario (chlorination with egalitarian weighting; Figure 3.1). Regardless of the assumed cultural perspective (individualists, hierarchists, and egalitarians), the DALYs incurred by the treatment are consistently lower for microplasma ozonation than chlorination, and the DALYs avoided because of pathogen inactivation are consistently greater for microplasma ozonation than chlorination. As a result, microplasma ozonation resulted in lesser human health impacts than chlorination when providing the same level of pathogen inactivation. The averted human health impact was in general more substantial than the life cycle impacts incurred by the construction and operation of the treatment system for both technologies across cultural approaches, exclusive of the egalitarian approach for chlorination (Figure 3.1). The microplasma ozonation resulted in net negative DALYs values across all cultural perspectives, and no statistical difference was observed between the human health impact obtained using the hierarchist or individualist approach ($p > 0.05$).

Sensitivity to scenario and technology assumptions

The microplasma ozonation incurred less human health impacts than chlorination across the majority of the impact categories, such as climate change, human toxicity, and particulate matter emissions (Figure 3.2). The impact categories of ozone depletion (OD), photochemical

oxidant formation (PO), and ionizing radiation (IR) are much less significant than the other impact categories, being responsible for less than 0.5% of human health impacts for both technologies. For climate change (CC), in particular, chlorination caused more than double the DALYs to achieve the same level of disinfection. The main contributor to the impacts of chlorination were consistently consumables and their associated transportation (Figure 3.2), accounting for 67 - 89% and 10 - 22% of the total incurred DALYs, respectively. The largest sources of impact for the microplasma ozonation were predominantly from electricity consumption (70 - 99 % across all impact categories), with the required materials for the ozone generator and piping assembly being responsible for roughly 1 - 20% and 1 - 13% of the total incurred DALYs, respectively. In short, microplasma ozonation provided more human health protection than chlorination across the most impactful environmental categories under the designed conditions, and simultaneously incurred lesser human health impacts. These results also point out several directions to reduce the disinfection technology-induced human health impact. Specifically, the greatest reductions may be achieved through reductions in consumables (chemicals and energy), a finding that is consistent with centralized drinking water treatment systems as well.[53]

Given the importance of electricity to the ozonation system, the sensitivity of these results to the electricity fuel mix was also evaluated (Figure 3.3). Despite nearly an order-of-magnitude variability in the human health impact intensity of states' non-petroleum energy consumption profiles (9.8×10^{-8} to 1.4×10^{-6} DALYs kWh^{-1}; used as a surrogate for fuel sources for consumed electricity in a given state), the human health impacts incurred by ozonation were consistently less than chlorination across all U.S. states. This holds true for the three states with the largest usage of reclaimed wastewater: Florida, California, and Texas (Figure 3.3, Figure

S3.1, Table S3.3). Additionally, both chlorination and microplasma ozonation technologies produced negative net DALYs values across all states, suggesting that these technologies are capable of reducing the human health impacts of secondary wastewater effluent reuse for landscape irrigation.

For both chlorination and ozonation the inactivation rate constant for *L. pneumophila* strongly influenced the net human health impact, underscoring the importance of inactivating such bacteria in reclaimed water for landscape irrigation (Figure 3.4a and b). Although *C. parvum* and *Giardia* are more resistant to chlorination and ozonation as compared to *L. pneumophila*, the contribution of the *L. pneumophila* inactivation rate constants to the human health impact was consistently higher than those of the either *C. parvum* or *Giardia* (Figure 3.4a and b), due to the higher likelihood of infection caused by the higher identical survival probability for *L. pneumophila* (MLE estimate of 6×10^{-2}, compared to 4×10^{-3} for *C. parvum*, and 2×10^{-2} for *Giardia*). For the chlorination system, the human health impact is most sensitive to the applied sodium hypochlorite dose and the hydraulic residence time, followed by *L. pneumophila* inactivation rate constant and water temperature (Figure 3.4a). Future improvements to chlorination, therefore, should focus on navigating trade-offs between chlorine dose and HRT. For the microplasma ozonation system, the reaction of ozone with wastewater organic matter (WOM) plays the most important role as reflected in sensitivity of human health impacts to COD concentration, to the fraction of ozone demand per carbon of WOM, to the transferred ozone dose, and to the ozone reaction rate constant with WOM (Figure 3.4b). Although the need for pre-treatment (e.g., WOM removal) will be locality specific (dependent on the wastewater, preceding processes, etc.), significant reductions in indirect health impacts from microplasma

ozonation can also be achieved by increasing the ozone mass transfer efficiency, which increases the transferred ozone dose given a certain applied ozone dose.

Implications for disinfection technologies

To better understand how individual design decisions impact technology performance, a sub-set of parameters that strongly influenced results (initial sodium hypochlorite concentration ,$C_0|_{Cl2}$; the transferred ozone dose, $C_0|_{O3}$; hydraulic residence time, HRT) were further analyzed. For chlorination, the life cycle human health impacts were more sensitive to chlorine consumption than reactor sizing (Figure 3.2 and Figure 3.4a). As a result, for a fixed level of inactivation, increasing HRT at a given $C_0|_{Cl2}$ (which can achieve the same $\log_{10}$ inactivation as a combination of lower HRT and a higher $C_0|_{Cl2}$) decreases the total DALYs incurred (Figure 3.5a and b). To achieve a much higher inactivation level, a higher $C_0|_{Cl2}$ and HRT combination must be used, leading to an increase in the resulted total DALYs normalized by the level of treatment. In other words, the additional human health impact incurred by the excess levels of treatment cannot be overcome by the additional health impact averted from more thorough disinfection. For chlorination, therefore, the limited flexibility to reduce life cycle environmental impacts will stem from increasing HRT to reduce chlorine consumption. As an example, given the identical level of treatment performance, the change in total net DALYs could be more than 0.2 DALYs per year of operation per $\log_{10}$ inactivation, between operating at $C_0|_{Cl2}$ = 6 mg L^{-1} and HRT = 20 min, versus $C_0|_{Cl2}$ = 3 mg L^{-1} and HRT = 40 min (Figure 3.5a and b).

For microplasma ozonation, the transferred ozone dose and HRT are critical for life cycle human health impacts. During operation, the level of inactivation by ozone depends on the value of transferred ozone dose $C_0|_{O3}$ and HRT. When the transferred ozone dose $C_0|_{O3}$ is too small, O_3

is quickly consumed, and the inactivation would appear independent of HRT as represented by a horizontal trend in Figure 3.5c at lower $C_0|_{O3}$. This observation is consistent with previous findings that transferred ozone dose rather than HRT could sometimes be the determining factor in controlling inactivation.[11] At this low level of transferred ozone dose, for a given level of inactivation, the normalized DALYs values did not increase significantly with HRT since contact tank materials were not a significant driver for life cycle environmental impacts (as identified in Figure 3.2).

To increase the pathogen inactivation, the transferred ozone dose must increase. At a higher $C_0|_{O3}$, an increase in HRT will also increase inactivation. This influence of HRT on total pathogen inactivation is only observable at a higher $C_0|_{O3}$. This result is consistent with the results from the *L. pneumophila* inactivation by microplasma ozonation (Figure 2.3). At a dissolved ozone concentration of 50 μg L^{-1} at 0.2 minutes (Figure 2.3b), the $\log_{10}$ inactivation of *L. pneumophila* was approximately 3 (Figure 2.3a), which was increasing as the dissolved ozone was consumed gradually as the contact time increased. In other words, so long as the residual ozone was available, the HRT would have an effect on the overall inactivation. Therefore, the transferred ozone dose must be greater than the wastewater-specific thresholds to achieve residual ozone and increase disinfection efficacy with increasing HRT. A further increase in $C_0|_{O3}$ will enhance the inactivation, but the DALYs incurred because of additional life cycle impacts will be larger than the local DALYs averted due to reduced pathogen exposure locally (Figure 3.5c and d).

For the same mass of ozone produced, given the linear relationship between the incurred DALY values and the electricity consumption, less dependence on electricity directly corresponds to less incurred DALYs. Microchannel plasma ozone generator requires less

electricity (90 g kWh^{-1} if fed with air, more than 120 g kWh^{-1} if fed with oxygen) than a conventional dielectric barrier discharge reactor (50 g kWh^{-1}) per mass of ozone produced. Therefore, from the point of view of lowering the life cycle human health impact, one aspect that the future ozone technology development could focus on is reducing the energy consumption per mass of ozone produced.

Limitations of the study

Before applying the model framework presented in this work to predict dissolved ozone concentration, parameters in the developed model including the ozone self-decay rate constant k, the mass transfer coefficient K_La, and the reaction rate constant between WOM and ozone k_2 must be fitted to wastewater of specific sources to account for variations in wastewater characteristics. Additionally, since the actual reactions involved between ozone and various components in the wastewater are likely to be very complex and numerous, models incorporating more details in specific reactions (e.g., additional ozone consuming reactions, such as between ozone and nitrite ions) will likely improve the modeling results. To factor in the effect of temperature, Arrheinous law was employed for all reaction rate constants, except for ozone inactivation of *L. pneumophila* since the wastewater matrix led to an insignificant difference in inactivation rate constants at 7 and 22 ^oC ($p > 0.05$). Therefore, assumed an identical *L. pneumophila* inactivation rate constant using ozone throughout the simulation. Future efforts should validate such observation in wastewater matrix that are equally complex as, if not more complex than, the secondary effluent used in this study.

Additionally, more robust modeling of direct human health implications (both from pathogens and chemicals in reclaimed water) would also improve model accuracy. For example, DBPs generated during disinfection may pose risks to human health, but were not addressed in

this study due to the lack of characterization factors. Although more data related to pathogens of concern would also improve modeling accuracy and enable real-time optimization of disinfection systems, a more routinely monitored microbial parameter to evaluate wastewater disinfection is the total coliform concentration (which is regulated based on different permissible levels depending on the downstream purposes of the wastewater[1]). If future management strategies incorporate the studied pathogens for routine monitoring, the inclusion of pathogens that are prevalent in wastewater, such as noroviruses, may further enhance the comprehensiveness of the current results, as reflected by the sensitivity of the results to pathogen selection (Figure 3.4a and b). Moreover, although uncertainties of various sources were taken into account throughout the study, the lack of information at numerous occasions, such as the influent concentration of pathogens, may have caused unwanted uncertainties in the results.

3.5 Conclusions

We determined the inactivation parameters for ozone inactivation of *L. pneumophila* in secondary wastewater, and compared two alternative technologies – chlorination followed by dechlorination, and microplasma ozonation – for wastewater reuse disinfection based on the human health impacts. LCA results revealed that the operation of the microplasma ozonation system had lower impact on human health than the chlorination system in five out of six impact categories. These results were robust, and were consistent across U.S. electricity fuel mixes (including Florida, California, and Texas), as well as the three cultural perspectives used in impact assessment – in all cases, the overall human health impact caused by the microplasma ozonation was consistently lower than that of the chlorination approach.

For the chlorination system, life cycle DALYs were most sensitive to chlorine consumption, enabling minor reductions in impacts by increasing HRT and reducing chemical

dosing to achieve the same level of disinfection. For the microplasma ozonation system, depending on the desired level of pathogen inactivation, design criteria such as using a higher transferred ozone dose (just enough to produce residual ozone throughout the ideal part of HRT) combined with a longer HRT should be considered to optimize the system's human health impact performance for a given inactivation goal. Nevertheless, other important factors such as costs also need to be considered to produce a feasible design plan

3.6 List of Figures and Tables

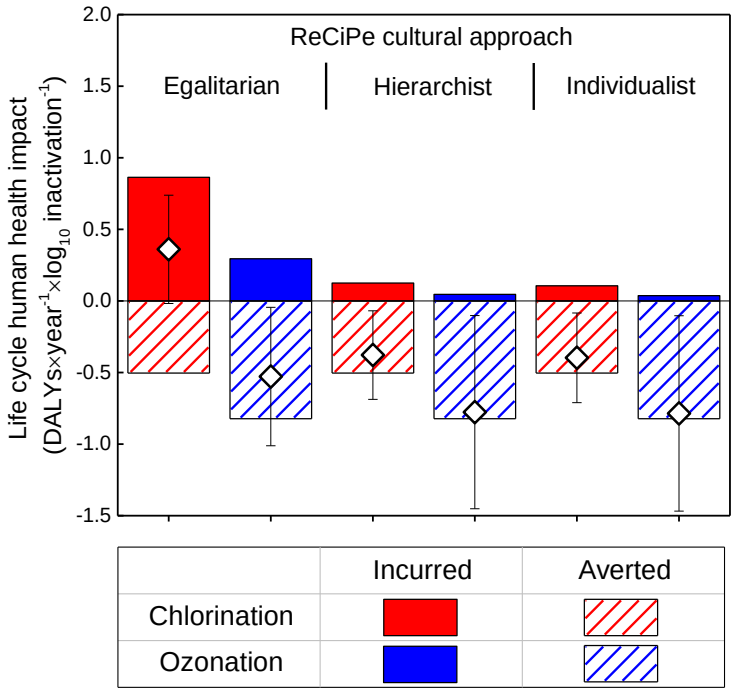

Figure 3.1. The impact of cultural perspectives of egalitarians, hierarchists, and individualists on DALYs caused, averted by chlorination and ozonation, together with the total net DALYs.

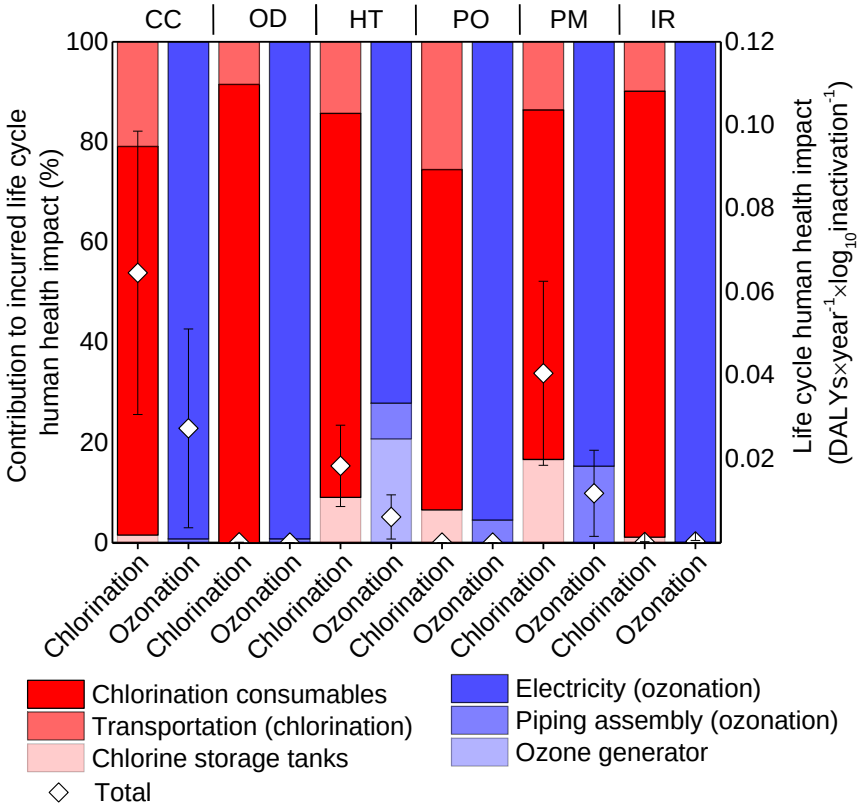

Figure 3.2. DALYs incurred by treatment stages and disinfection strategy options (chlorine vs. ozone). White diamonds represent the total normalized DALYs values to be read off the secondary y axis to the right. This figure excludes averted impacts stemming from reduced human exposure to pathogens.

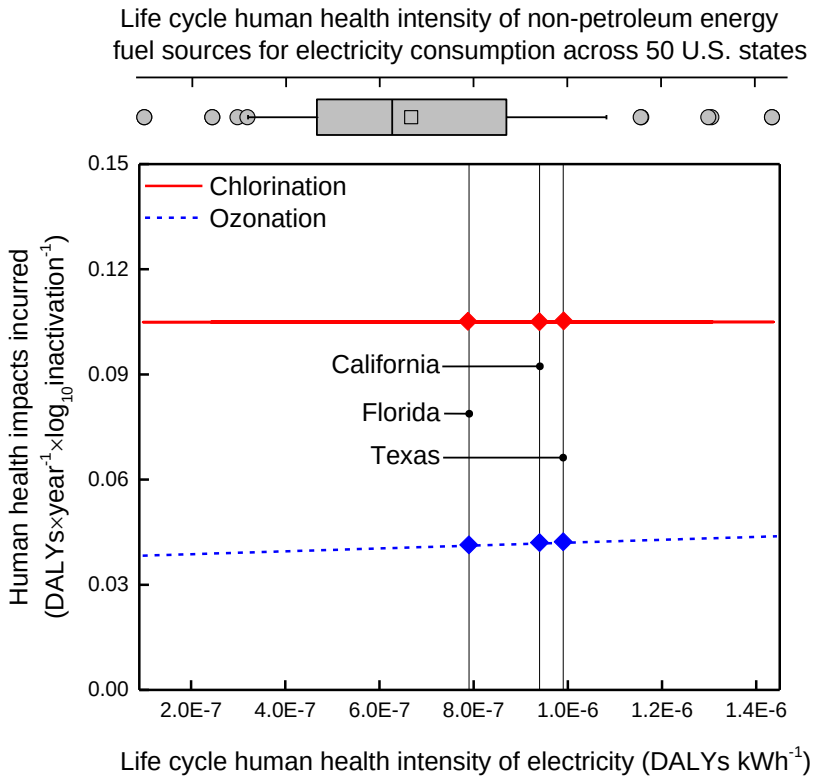

Figure 3.3. Relationship between the incurred human health impact and the life cycle human health intensity (in the unit of DALYs kWh^{-1}) of non-petroleum energy fuel sources for the consumed electricity across 50 U.S. states. Three states with the heaviest reliance on reclaimed water, California, Florida, and Texas are marked.

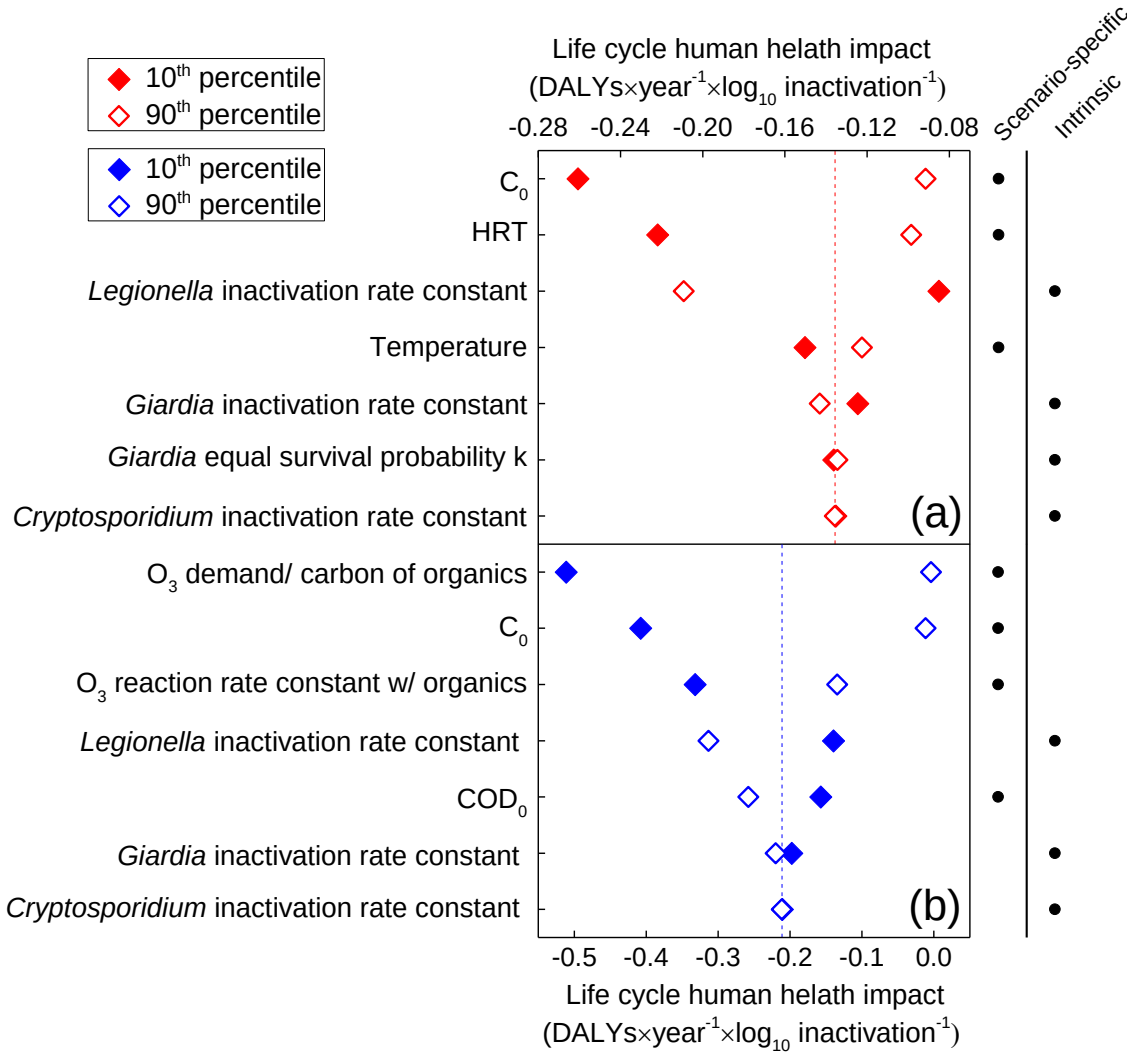

Figure 3.4. Top seven input parameters to which human health impact is most sensitive, for chlorination (a) and microplasma ozonation (b), expressed in DALYs × year^{-1} × $\log_{10}$ pathogen inactivated^{-1}. Sensitivity analysis for the rest of the parameters can be found in the SI.

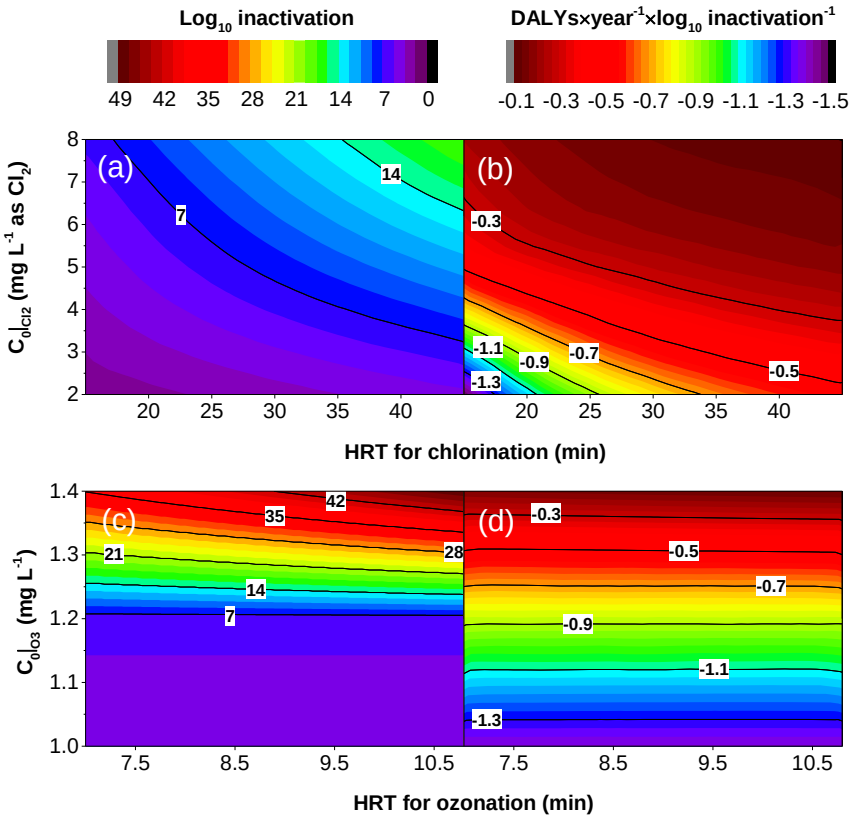

Figure 3.5. The relationship between $C_{0|Cl2}$, HRT, and the net DALYs for different levels of total pathogen inactivation by chlorination (a and b), and the relationship between $C_{0|O3}$, HRT, and the net DALYs for different levels of total pathogen inactivation by microplasma ozonation (c and d). The left panel (a and c) demonstrates the relationship between $\log_{10}$ pathogens inactivation versus HRT at various levels of disinfectants; the right panel (b and d) demonstrates the relationship between the normalized net DALYs values and HRT at various levels of disinfectants. Total pathogen inactivation represents the summation of $\log_{10}$ inactivation of three pathogens. For instance, at 12.7 ^{o}C, a 27 $\log_{10}$ total inactivation corresponds to approximately 5.2, 0.3, and 21.5 $\log_{10}$ inactivation of *Giardia*, *Cryptosporidium*, and *L. pneumophila*.

3.7 Supplementary Information

Obtaining *L. pneumophila* inactivation parameters

The effort to obtain the *L. pneumophila* inactivation kinetics can be found in the SI of chapter 2.

General assumptions

Assumptions were made during the design process when detailed information was not available. The summary of major assumptions is provided in Table S3.1.

Table S3.1. Summary of major assumptions for the life cycle assessment.

Assumptions
Fuel sources for electricity in California, Florida, and Texas are based on the US 2015, 2013, and 2013 energy consumption data, respectively
The flow inside the chlorine contact tank follows an ideal plug-flow pattern
The chemical storage tanks and injectors do not need to be replaced in 10 years
Thickness of individual layers of materials of the chemical storage tanks is assumed (later shown to be insensitive to the final results), with the total thickness fixed according to design handbook
Only consumables for treatment operation are included
The flow inside the ozone contact tank follows an ideal plug-flow pattern
Consumption of ozone is entirely incurred by the organics in wastewater
The microplasma ozone generator does not need to be replaced in 10 years
No post ozonation filtration is implemented

Chlorine disinfection system design

The design of chlorination/dechlorination system in general followed the EPA design manual for the municipal wastewater disinfection [54]. The system boundary included chemical storage tanks, pumps, inline static mixers, chlorine contact tank, and consumables (including

energy and the production and transport of chemicals to the site). The system was designed to handle 4 Million Gallons per Day (MGD) of secondary effluent, with a HRT of 15 to 45 minutes and a sodium hypochlorite dose of 2 to 8 mg L^{-1}. The designed annual operation temperature range was from 10 to 21.1 ^{o}C with 15.6 ^{o}C as the medium value, which is typical for municipal wastewater [55].

The decay of sodium hypochlorite was modelled with a parallel decay of two components of total chlorine residual for secondary effluents [56]:

$$C = C_0 e^{-k_1 t} + C_0(1 - x)e^{-k_2 t} \quad \text{S(3.1)}$$

For a variety of wastewater sources and water conditions, values of $x = 0.3$, $k_1 = 1.67 \times 10^{-2}\ s^{-1}$, and $k_2 = 5 \times 10^{-5}\ s^{-1}$ yielded satisfactory results [54]. Since a hydraulic residence time (HRT) of 15 to 45 minutes is typical for wastewater chlorination disinfection, which is also the design HRT range for the current study, for HRT > 1 minute, eqn S(3.1) can thus be re-written as[54]:

$$C = 0.7C_0 e^{-0.003t} \quad \text{S(3.2)}$$

Combine with Chick-Watson inactivation model (eqn S(3.8)) and integrate, we arrive at:

$$\frac{N}{N_0} = e^{[-0.7kC_0(333 - 333e^{-0.003t})]} \quad \text{S(3.3)}$$

where k is the inactivation rate constant for pathogens, and C_0 is the applied chlorine dose. The inactivation rate constants for *Giardia*, *Cryptosporidium parvum*, and *Legionella pneumophila* are 2.5×10^{-2} (at 18 ^{o}C), 8.36×10^{-4} (at 20 ^{o}C), and 0.307 (at 30 ^{o}C) L mg^{-1} min^{-1}, respectively [57-59]. The temperature effect was considered by incorporating the Arrhenius correction:

$$k_{T_2} = k_{T_1} e^{\frac{E_a}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)} \quad \text{S(3.4)}$$

where E_a is the activation energy in J mol^{-1}, R is 8.314 J mol^{-1} K^{-1}. T_1 and T_2 are temperatures before and after temperature correction. Whenever not directly available, E_a values were fitted from published experimental data. The E_a values for *Giardia*, *C. parvum*, and *L. pneumophila* used in this study are 45259, 75570, and 38293 J mol^{-1}, respectively. The activation energy for *L. pneumophila* was estimated based on monochloramine inactivation data of eight species of bacteria [60].

The chlorine contactor was designed following a length to width ratio of 40 to 1, to minimize axial diffusion [54, 55]. The depth of the contactor was empirically chosen based on the EPA design manual as 2 meters, with an effective depth of 1.8 meters. The horizontal velocity of wastewater inside the contact tank ranged from 2 – 4 m min^{-1} [55]. Since the influent flow rate was known, given the horizontal velocity the cross sectional area of the tank and thus width of the tank were calculated. Length was calculated given the width and the length to width ratio. The final volume of materials was determined by applying the density of the concrete.

The storage, injection, and mixing of the sodium hypochlorite and sodium bisulfite shared similar configuration as the equipment is usually interchangeable. Exceptions were the materials for the storage tanks and the wetted parts of the feed pumps, as sodium hypochlorite is highly corrosive. Information on the storage tanks were obtained from the design handbook [3]. The masses of components and materials of the proportioning feed pumps were obtained from manufacture's specification sheets, which were scaled up or down according to the pump's horsepower. Mixing of both the sodium hypochlorite and sodium bisulfite were done with static

inline mixers. Since the dechlorination reactions happen instantaneously, contact chambers for dechlorination was not required [55].

The usage of sodium hypochlorite and sodium bisulfite was calculated based on the reactions:

$$NH_2Cl + HSO_3^- + H_2O \rightarrow Cl^- + SO_4^{2-} + NH_4^+ + H^+ \quad \text{REACTION S3.1}$$

$$HOCl + HSO_3^- \rightarrow Cl^- + SO_4^{2-} + 2H^+ \quad \text{REACTION S3.2}$$

The final results calculated from stoichiometry was further combined with 12% empirical safety factors to yield a ratio of 1.63 mg L^{-1} $NaHSO_3$ per 1 mg L^{-1} residual chlorine as Cl_2 [55].

The requirements for the sodium hypochlorite and sodium bisulfite were calculated following the design manual [54]. In brief, the mass rate requirements were calculated using the stoichiometry, the influent flow rate, and the applied chemical dose. The volume of chemicals to be stored onsite was calculated following the EPA recommendation that utilities should have the volume of chemicals that equal the amount of chemicals needed for a time equal to the shipping time from the vendor plus fifteen days. A 4-day shipping time was assumed as the base value for the uncertainty analysis.

Microchannel plasma ozonation disinfection system design

The design of microplasma ozonation system was based on a combination of both scaled-up results from bench-scale studies, manufacture's data and design criteria from the design manuals and previous publications. The system boundary included the ozone generator and injection device, ozone contact tank, and the ozone destruction device. Identical with the chlorination system, the microplasma disinfection system was designed to handle 4 MGD of

secondary effluent, with a HRT of 7 to 11 minutes and a transferred ozone dose of 1 to 2 mg L^{-1}. The operational temperature range is identical with the chlorination system, from 10 to 21.1 ^{o}C.

The decay of ozone caused by various components of the secondary effluent was addressed as follows. First of all, it was assumed that Total Organic Carbon (TOC) is a good representation of the components of wastewater that could exert ozone demand [61]. Since the representative TOC concentration was not readily available for secondary effluent, a correlation between TOC and Chemical Oxygen Demand (COD) was used following a prior publication:

$$\mathrm{COD} = 7.25 + 2.99\mathrm{TOC} \qquad \text{S(3.5)}$$

which was developed for the final effluent [62]. The designed COD concentration for the microplasma ozonation unit's receiving stream was 30 to 35 mg L^{-1}, which is typical for secondary effluent [55]. The decomposition of ozone, which includes its self-decomposition and the reaction with organic matter in wastewater (represented by TOC) was thus modeled for a batch system as:

$$\frac{dC_L}{dt} = -k_2 C_L x - kC_L \qquad \text{S(3.6)}$$

where C_L is the transferred ozone dose in mg/L, and x is the reactive portion of the TOC. It is assumed that $C_0 - C_L = x_0 - x$, and that the initial value for x is x_0, where $x_0 = \alpha \mathrm{TOC}_0$. Also, α is the fraction of organic carbon that exerts ozone demand. k_2 is the second order reaction rate constant between ozone and TOC, and k is the first order reaction rate constant for ozone self-decomposition. The values for k_2 and k were obtained from the bench-scale experiments conducted in this study. To correct for temperature variations, eqn S(3.4) was applied and the activation energy was 5005 and 50350 J mol^{-1} for k_2 and k, respectively. After integration, the solution to eqn S(3.6) is:

$$C_L = \frac{x_0 k_2 C_0 - k_2 {C_0}^2 + C_0 k}{(x_0 k_2 + k) e^{t(x_0 k_2 - k_2 C_0 + k)} - k_2 C_0} \quad \text{S(3.7)}$$

Combine with Chick-Watson equation (eqn S(3.8)), integrate, one can arrive at the equation to predict level of inactivation with ozone produced from the microplasma ozone generator (eqn S(3.9)):

$$\frac{dN}{dt} = -k^* C_L N \quad \text{S(3.8)}$$

$$\text{Ln}\frac{N}{N_0} = \frac{k^*}{k_2}\left\{t[k_2(x_0 - C_0) + k] - \text{Ln}\frac{(k_2 x_0 + k)e^{t(k_2 x_0 - k_2 C_0 + k)} - k_2 C_0}{k_2 x_0 + k - k_2 C_0}\right\} \quad \text{S(3.9)}$$

where N and N_0 are the concentration of pathogens in wastewater at time t and at time 0, respectively. The ozone inactivation rate constants for *Giardia*, *C. parvum*, and *L. pneumophila* are 27.1 (at 25 ^oC), 0.8 (at 20 ^oC), and 145 (across 7 to 22 ^oC) L mg^{-1} min^{-1}, respectively [57, 63]. To correct the rate constants for temperature variations, the Arrhenius rule (eqn S(3.4)) was applied, where the activation energy E_a was 39201 and 82500 J mol^{-1} for *Giardia* and *C. parvum*, respectively [57, 63]. The only exception for the temperature correction for rate constants is the inactivation rate constant for *L. pneumophila*, which was demonstrated to be not significantly sensitive to temperature variation in the presence of wastewater organic matter, based on the good fitting to the Chick-Watson equation at different temperatures (Figure. 2.6).

Ozone generated from the microplasma ozonator was designed to be injected into the wastewater through a venturi injector. To obtain the number of the microplasma chips (n), which are the very components that control the total ozone mass production rate of the microplasma ozonator, as well as the factors that directly determine the material requirement of the ozone generator, eqn S(3.10) was applied, which was derived based on the venturi injector manufacture's design document on the relationship between MTE and ozone demand for the

87% MTE venturi injector that the current study uses (eqn S(3.11)). Ozone demand was empirically predicted using an equation that was fitted from data provided in the EPA design manual (eqn S(3.12)):

$$n = (100C_0 - 13 \times \text{Ozone demand}) \frac{Q_{Lin}}{87 \times O_{3|\text{per piece}}} \quad \text{S(3.10)}$$

$$\text{MTE}(\%) = (13 \frac{\text{ozone demand}}{\text{applied ozone dose}} + 87) \quad \text{S(3.11)}$$

$$\text{Ozone demand} = 0.0844 \times \text{COD} - 1.3698 \quad \text{S(3.12)}$$

where the applied ozone dose is defined in eqn S(3.13):

$$\text{Applied ozone dose} = \frac{Q_{gin} C_{gin}}{Q_{Lin}} \quad \text{S(3.13)}$$

The ozone contact tank shared a similar configuration with the chlorine contact tank [3], with the addition of a concrete cover to collect and pass the off-gas to the thermal catalytic ozone destruction unit. The thermal catalytic ozone destruction unit contains MnO_2 and CuO as catalysts and operates at 300 to 350 ^oC to remove approximately 99% ozone.

Calculating DALYs per symptomatic case for *L. pneumophila*

Legionellosis is a common syndrome of pneumonia caused by *Legionella* [64], and the bacterium *L. pneumophila* is responsible for most cases of Legionellosis [65]. 1- 40% of hospital acquired pneumonia can be attributed to Legionellosis [66], and 95.4 % of Legionellosis is caused by *L. pneumophila* [67]. Therefore, the same weighting was assumed to be reflective of the proportion of pneumonia DALYs attributable to Legionellosis.

Based on RIVM, The Netherland (Table S3.2), the total amount of people affected by pneumonia nationwide in a year was 645584. Therefore DALYs per symptomatic case can be determined as:

$$\frac{73900}{6984+638600} = 0.1145 \text{ DALYs per symptomatic case} \qquad \text{S(3.14)}$$

As discussed 1- 40% of hospital acquired pneumonia is attributed to Legionellosis, and 95.4 % of Legionellosis is caused by *L. pneumophila*, it can therefore be assumed that the DALYs/symptomatic case for *L. pneumophila* infection is within the range of 1.05×10^{-3} to 4.37×10^{-2}. This source of uncertainty was incorporated into the uncertainty analysis.

Table S3.2. Yearly population affected by pneumonia in The Netherland.

	# Deaths	**Years of life lost (YLL)**	**# Disease**	**Severity**	**Years of Life with Disability (YLD)**	**DALYs**
Pneumonia	6984	49448	638600	0.04	24500	73900

Table S3.3. Electricity consumption source profiles in percentage (%) for three states within the United States with the highest reclaimed water usage. Distillate fuel oil was represented with light fuel oil. Motor gasoline was represented with unleaded petrol. Other petroleum was represented with 15% (v/v) ETBE added petroleum. Other renewable energy sources were represented with biofuel.

Electricity fuel sources based on consumption	**Ecoinvent 3 database items**	**Florida**	**California**	**Texas**
Natural gas	Electricity, natural gas, at power plant/US	31	61	32
Nuclear	Electricity, nuclear, at power plant/US	7	10	3
Hydroelectric	Electricity from hydroelectric power plant, AC, production mix, at power plant, < 1kV RER S	0	5	0
Coal	Electricity, bituminous coal, at power plant/US	12	0	12
Liquefied petroleum gas	Liquefied petroleum gas [29]| market for | Alloc Def, U	0	0	15
Distillate fuel oil	Light fuel oil {RoW}| market for | Alloc Def, U	7	0	7
Motor gasoline	Petrol, unleaded {RoW}| market for | Alloc Def, U	23	0	11
Other Petroleum	Petrol, 15% ETBE additive by volume, with ethanol from biomass {GLO}| market for | Alloc Def, U	1	0	10
Other renewable	Electricity, biomass, at power plant/US	8	22	0

Table S3.4. Summary of input materials for the chlorination system.

Summary of input materials for chlorination system															
Materials	**Processes**	Storage tanks		Piping assembly				Contact tank	Pump		Consumables (annual requirements)		Electricity (annual requirements)		
		For NaOCl storage (kg)	For $NaHSO_3$ storage (kg)	Injection pipe for NaOCl (kg)	Injection pipe for $NaHSO_3$ (kg)	Inline static mixing pipe for NaOCl (kg)	Inline static mixing pipe for $NaHSO_3$ (kg)	Contact tank (m^3)	For NaOCl (kg)	For $NaHSO_3$ (kg)	NaOCl (kg)	$NaHSO_3$ (kg)	NaOCl feed pump (kWh)	$NaHSO_3$ feed pump (kWh)	Coefficient of variation
Fiberglass		1353.6													0.23
PVC lining		1052.8													0.23
316 SS			6044.5		46.1	171.8	171.8		27.6	27.6					0.10
PTFE									0.3	0.3					0.11
Copper wire									0.6	0.6					0.23
Steel									5.8	5.8					0.23
Schedule 80 PVC				8.1											0.36
Slab concrete								42.0							0.23
$NaHSO_3$ powder												22981.6			0.03
15% NaOCl solution											177719.4				0.03
Electricity, low voltage													952.9	411.5	0.04
	Blow moulding	1052.8													0.36

Table S3.5. Summary of input materials for the microplasma ozonation system.

Summary of input materials for microplasma ozonation system									
	Ozone generator	Piping assembly			Contact tank	Ozone destruction unit	Electricity		
Materials	Ozone generator (kg)	Injection pipe (kg)	Venturi (kg)	Equalization pipe (kg)	Contact tank (m^3)	Ozone destruction unit (kg)	Ozone generator electricity (kWh)	Ozone destruction unit electricity (kWh)	Coefficient of variation
316 SS	14.7	46.1	151.1	414.9		0.05			0.10
Copper wire	9.8								0.23
Slab concrete					23.6				0.23
Aluminum Foil	163.3								0.01
Titanium dioxide	1.0								0.21
Silicon	0.1								0.01
Polycarbonate	22.5								0.01
PVC for Fan Blades	2.9								0.03
Printed Circuit board-Pb containing	0.1								0.01
Printed Circuit board-No Pb	0.1								0.01
MnO_2						0.01			0.04
CuO						0.01			0.04
Electricity, low voltage							94224.8	2.2	0.04

Table S3.6. Input parameters for the life cycle analysis and quantitative microbial risk assessments.

								Distribution		
Parameter	Unit	Minimum	Maximum	Mean for log normal distribution	Standard deviation for log normal distribution	Mean	Standard deviation	Uniform	Log normal	Normal
Temperature	^{o}C			-	-	15.6	1.4			•
Influent COD	mg L^{-1}	30	35	-	-	-	-	•		
Cryptosporidium parvum concentration in influent	oocysts mL^{-1}	0.000013	0.00013	-	-	-	-	•		
Legionella pneumophila concentration in influent	cells mL^{-1}	900	1000	-	-	-	-	•		
Giardia concentration in influent	cysts mL^{-1}	0.000007	0.00018	-	-	-	-	•		
Initial chlorine dose	mg L^{-1} as Cl_2	2	8	-	-	-	-	•		
Hydraulic residence time in chlorine contact tank	min	15	45	-	-	-	-	•		
Horizontal velocity in chlorine contact tank	m min^{-1}	2	4	-	-	-	-	•		
Transferred ozone dose	mg L^{-1}	1	2	-	-	-	-	•		
Hydraulic residence time in ozone contact tank	min	7	11	-	-	-	-	•		
Horizontal velocity in ozone contact tank	m min^{-1}	2	4	-	-	-	-	•		
Identical survival probability for *Cryptosporidium parvum*	-	-	-	-5.475054545	0.362099799	-	-		•	
Identical survival probability for *Legionella pneumophila*	-	-	-	-2.815078774	0.399245315	-	-		•	
Identical survival probability for *Giardia*	-	-	-	-3.917035547	0.256506595	-	-		•	
DALYs/symptomatic case for *Legionella pneumophila*	DALYs per symptomatic case	0.00105	0.0437	-	-	-	-	•		

Table S3.6. (cont.)

Chlorine inactivation rate constant for *Cryptosporidium parvum*	L mg^{-1} min^{-1}	-	-	-7.086881945	0.406	-	-		•	
Chlorine inactivation rate constant for *Legionella pneumophila*	L mg^{-1} min^{-1}	-	-	-1.180907531	0.406	-	-		•	
Chlorine inactivation rate constant for *Giardia*	L mg^{-1} min^{-1}	-	-	-3.688879454	0.406	-	-		•	
NaOCl shipping time	day	-	-	1.386294361	0.458257569	-	-		•	
Thickness of chlorine storage tank and contactor	m	-	-	-2.574393816	0.458257569	-	-		•	
$NaHSO_3$ shipping time	day	-	-	1.386294361	0.458257569	-	-		•	
Specific energy consumption for chlorine feeding pump	kWh m^{-3}	-	-	1.93441577	0.037416574	-	-		•	
Specific energy consumption for $NaHSO_3$ feeding pump	kWh m^{-3}	-	-	1.93441577	0.037416574	-	-		•	
Ozone self-decay rate constant	min^{-1}	-	-	-5.221356325	0.352987252	-	-		•	
Ozone reaction rate constant with organic matter	L mg^{-1} min^{-1}	-	-	3.36729583	0.352987252	-	-		•	
Ozone inactivation rate constant for *Cryptosporidium parvum*	L mg^{-1} min^{-1}	-	-	-0.223143551	0.405709256	-	-		•	
Ozone inactivation rate constant for *Legionella pneumophila*	L mg^{-1} min^{-1}	-	-	4.976733742	0.352987252	-	-		•	
Ozone inactivation rate constant for *Giardia*	L mg^{-1} min^{-1}	-	-	3.299533728	0.405709256	-	-		•	
Fraction of ozone demand per carbon of organic matter	-	-	-	-1.560647748	0.352987252	-	-		•	
Ozone destruction unit specific energy consumption	Wh m^{-3}	-	-	1.549687908	0.043588989	-	-		•	
Pipe thickness (percent of diameter)	-	-	-	-2.525728644	0.458257569	-	-		•	

Table S3.6. (cont.)

Cryptosporidium parvum and *Giardia* ingestion amount per exposure	mL	-	-	0	0.096953597	-	-		•	
Exposure frequency for all three pathogens	events $year^{-1}$	-	-	3.951243719	0.096953597	-	-		•	
Exposure duration per event for *Legionella pneumophila* calculation	hr	-	-	-0.693147181	0.458257569	-	-		•	
Wastewater generated per person per day	Gallons $person^{-1}$ day^{-1}	-	-	4.605170186	0.127279221	-	-		•	
Inhalation rate	m^3 hr^{-1}	-	-	-0.328504067	0.091241438	-	-		•	
Retention fraction of *Legionella pneumophila* in lungs	-	-	-	-0.693147181	0.21937411	-	-		•	
Consumables' transportation distance	km	-	-	3.912023005	0.458257569	-	-		•	

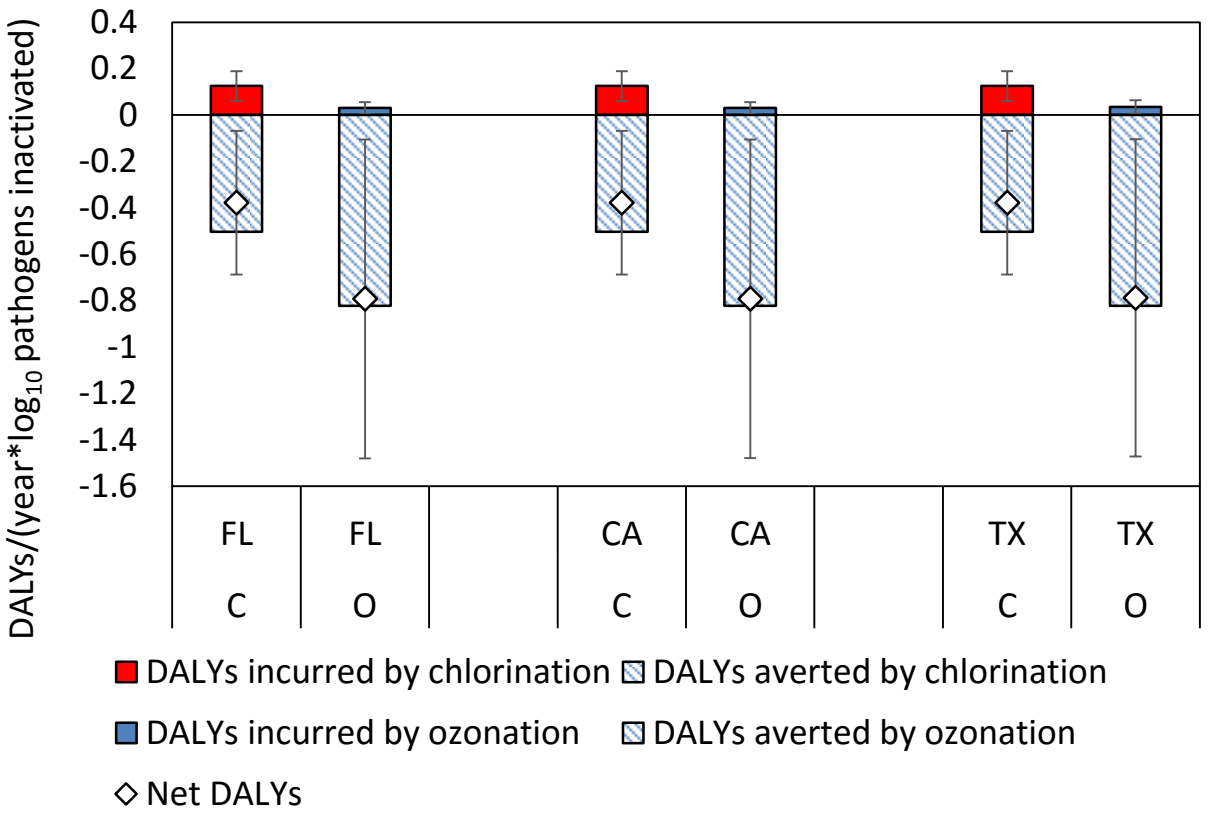

Figure S3.1. The impact of energy source fuel mix for consumed electricity, expressed in DALYs caused and averted by the disinfection systems, together with the total net DALYs , for the states of Florida, California, and Texas. Abbreviations: FL = Florida, CA = California, TX = Texas, C = Chlorination (combined with dechlorination using sodium bisulfite), O = Microplasma ozonation.

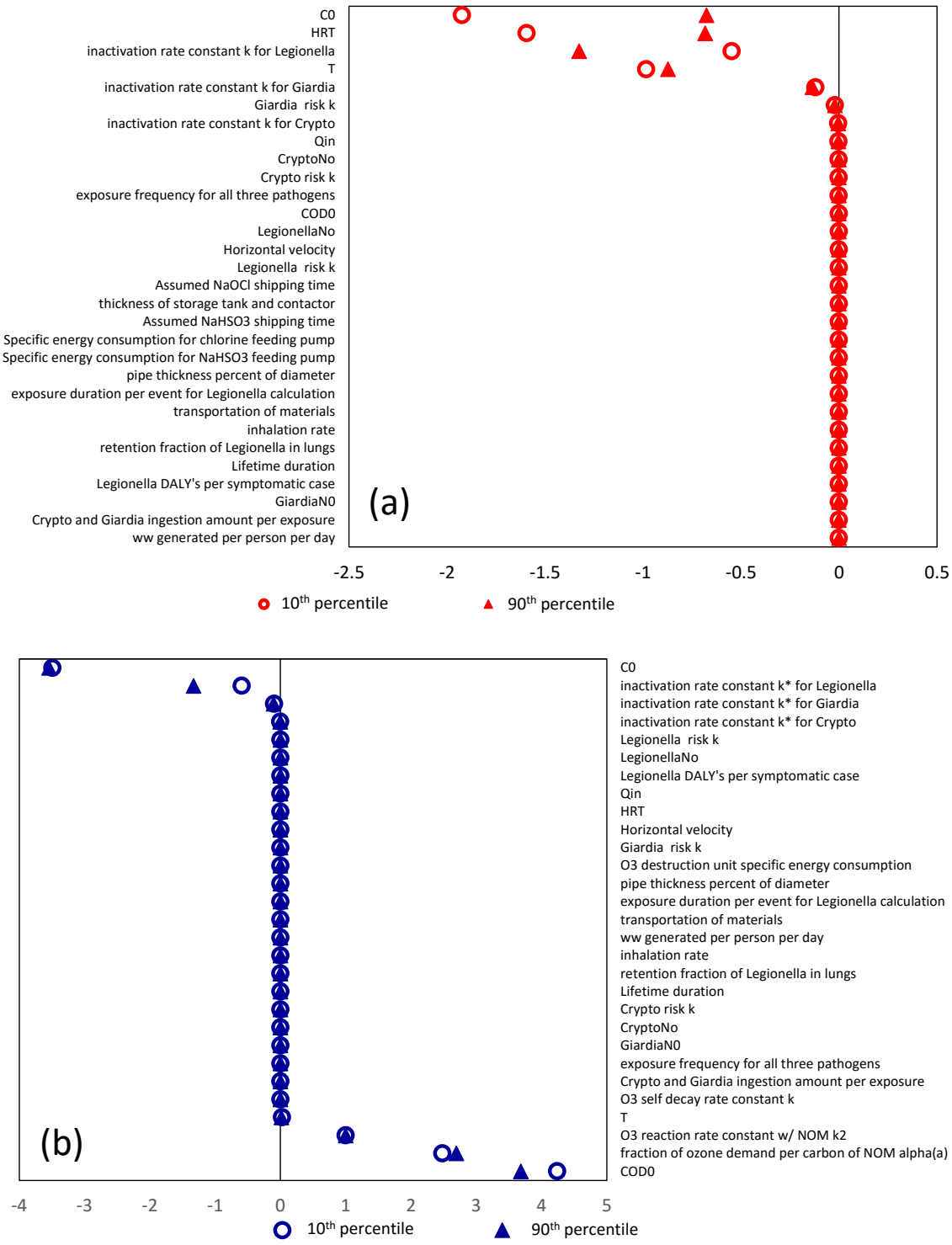

Figure S3.2. Sensitivity of human health impact to input parameters for chlorination (a) and microplasma ozonation (b) expressed in relative response. Negative values indicate direct correlation between the input values and the output values, meaning that an increase in these input parameters will result in an increase in the overall normalized net DALYs values (less human health protection).

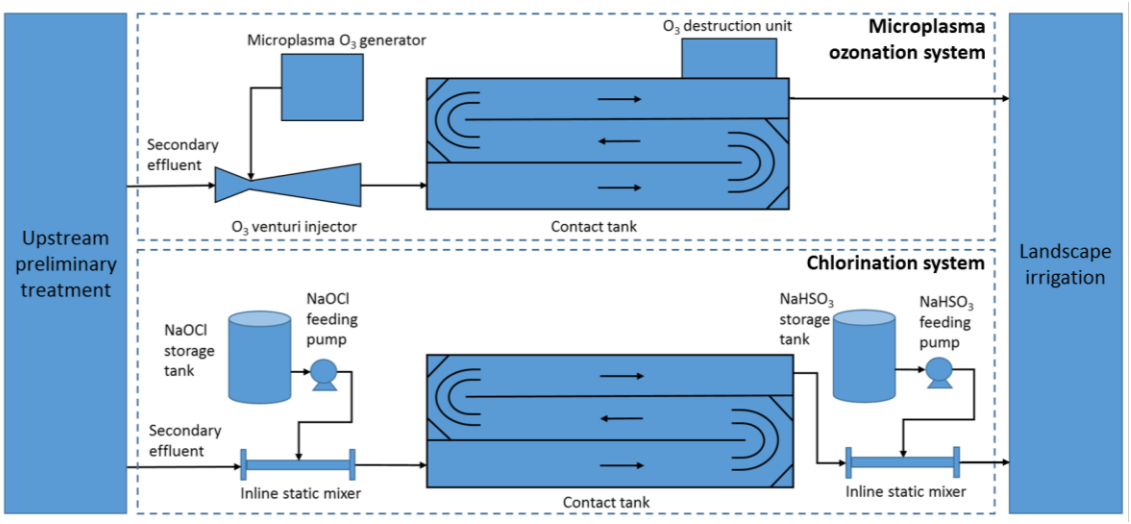

Figure S3.3. Disinfection system components and LCA system boundaries (dashed lines) of the (top) chlorination system and (bottom) microplasma ozonation system. The construction and operation of the systems were included in the analysis.

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CHAPTER 4

COMPARATIVE MAMMALIAN CELL CYTOTOXICITY OF WASTEWATERS FOR AGRICULTURAL REUSE AFTER OZONATION OR CHLORINATION

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Shengkun Dong, Jinfeng Lu, Michael J. Plewa, Thanh H. Nguyen, Comparative Mammalian Cell Cytotoxicity of Wastewaters for Agricultural Reuse after Ozonation or Chlorination

4.1 Abstract

Reusing wastewater in agriculture alleviates the pressure on freshwater resources. Wastewater disinfection is necessary to ensure safety and to prevent the spread of pathogens. Disinfectants can react with wastewater constituents to form disinfection byproducts (DBPs), many of which are toxic and restrict the goal of safe reuse. Our objective was to compare the induction of mammalian cell cytotoxicity before and after disinfection for two types of wastewaters: secondary effluent of municipal wastewater and minimally treated wastewater from a swine farm. Chlorination and ozonation were employed as two alternative disinfection methods. The swine farm wastewater was approximately 2000× more cytotoxic than the secondary effluent. Ozonation consistently reduced the cytotoxicity of the wastewaters by as much as ten times. Chlorination lowered the cytotoxicity only when followed by dechlorination. The results indicate that secondary effluent is preferred for agricultural reuse over swine wastewater regardless of disinfectant use. Ozonation disinfection reduced the cytotoxicity of wastewater and may prove useful in agricultural reuse of wastewaters. For secondary wastewater, the only significant correlation was observed between total haloacetonitriles and

cytotoxicity. While the association of reduced toxicity with the modification or reduction of specific compound(s) is unclear regulated DBPs may not be the primary forcing agents.

4.2 Introduction

Wastewater reuse can alleviate the pressure on freshwater resources. Currently wastewater reuse is gaining popularity in the United States, especially in states with water scarcity such as California and Texas.[1] In 2010, the total reclaimed water reuse in the United States was estimated to have increased by 42% from 1,690 million gallons per day (MGD) in 2004 [1] to 2,400 MGD.[2] Agricultural reuse of wastewater is preferred since agriculture consumes approximately 70% and 90% of worldwide and fast-growing economies' freshwater withdrawals, respectively.[3] Examples of agricultural wastewater reuse include both irrigation and livestock raising operations. Safe wastewater reuse requires that the finished water be disinfected to prevent the spread of pathogens and outbreak of diseases. Chlorination using sodium hypochlorite is a well-established disinfection method for wastewater disinfection. Pathogens are inactivated mainly with free chlorine or chloramines (depending on the ammonia content of the treated wastewater), which are effective against a wide range of waterborne pathogens. Due to its efficacy and affordability, chlorination is the most widely used technique for wastewater disinfection. [4] Another powerful alternative disinfectant is ozone, which is effective even against several pathogens that are known to be resistant to chlorine, such as *Cryptosporidium parvum*. [5-8] Nevertheless, disinfectants could generate adverse health impacts associated with disinfection byproducts (DBPs), which are formed from reactions between disinfectants and the organic matter, bromide, and iodide in wastewater. [9-13] Although close to 700 disinfection byproducts have been identified, only a small number of these have undergone systematic, quantitative, comparative toxicological analyses.[12, 14, 15] Disinfected wastewater for irrigational reuse is

complex and may form a more diverse range of DBPs due to the elevated abundance and variety of organic matter [16] and other inorganic constituents, which could function as DBP precursors. The nitrogen rich organic matter in wastewater could lead to the formation of nitrogenous DBPs (N-DBPs). [17] Other DBPs, such as organic halogens that are commonly associated with chlorination, [12, 18] and bromate and aldehydes that are associated with ozonation, [16, 19, 20] could all elevate the cytotoxicity of disinfected wastewater. The environmental and health implications of *in vitro* adverse biological analyses are closely related to the variables in the environment. Most studies address these factors separately and attempt to connect the toxic responses with corresponding chemical analyses.[10, 20-22] However, these attempts are limited in the types of actual toxins in wastewater and therefore cannot represent or be extrapolated to reveal the overall toxicity of the wastewater that most often contains multiple toxins.

The objective of this study was to compare the overall mammalian cell cytotoxicity of two sources of wastewaters that have gone through different levels of treatment, before and after two disinfection methods, chlorination and ozonation. Two types of DBPs regulated by the U.S. EPA [23] in drinking water, haloacetic acids (HAAs) and trihalomethanes (THMs), were measured to explore the correlation between the overall cytotoxicity and the regulated DBPs. Haloacetonitrles were also measured for exploration. Two types of analytical mammalian cell cytotoxicity experiments were conducted: (1) rraw wastewater was used as the toxicity treatment agent; (2) concentrated organic matter extracted by XAD-2 and XAD-8 resins was used as the toxicity treatment agent. Experiments using raw wastewaters revealed the overall cytotoxicity of the full strength disinfected waters. Experiments using the organics extracted by XAD-2/XAD-8 resins were carried out only if the full strength wastewater could not induce a significant toxic response in the mammalian cells. The extract obtained using XAD-2/XAD-8 resins enhances the

analytical comparison among samples across a wider range of cytotoxicity. While it is recognized that the compositions of wastewaters are unique at each plant, the current study provides realistic insights into the overall toxic effects of disinfected wastewaters on mammalian cells with and without disinfection. These results may help to identify desirable disinfection technologies to treat wastewaters for agricultural reuse, with regard to reducing adverse toxic biological responses. Additionally, the results may reveal the relationship between cytotoxicity and regulated DBPs.

4.3 Materials and Methods

Source Water Sampling, Characterization, and Processing

Two sources of wastewater were collected: secondary effluent of the Northeast Wastewater Treatment Plant (NEP) in Urbana, Illinois, and effluent from a stabilization pond on a concentrated animal feeding operation (CAFO) swine farm. At the NEP, the raw sewage flows through a preliminary treatment to intercept larger solid debris, a primary treatment to remove solids, a secondary treatment comprised of a trickling filter and an activated sludge unit in series to remove organic matter, a nitrification tower to remove ammonia, and a final disinfection unit to inactivate pathogens. The effluent of the secondary treatment (after the secondary clarifier) was collected for this study. The effluent from the stabilization pond on the swine farm is usually composed of feeding leftovers, runoff from farm cleaning, and animal wastes. The total organic carbon (TOC) was measured by a Shimadzu total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, MD). The absorbance at 254 nm was measured by a Beckman UV−vis spectrophotometer (Beckman Coulter Life Sciences, Indianapolis, IN). The metal species were measured by a PerkinElmer Sciex Elan DRCe ICP-MS (PerkinElmer Life and Analytical Sciences, Norwalk, CT). Ammonia and nitrate nitrogen were measured using Hach kits

(Loveland, CO). Total bromine and iodine were analyzed with ICP-OES (PerkinElmer Life and Analytical Sciences, Norwalk, CT). To accelerate sedimentation of the wastewater in order to eliminate interference of suspended solids, both the secondary effluent and the raw swine farm wastewater samples were further filtered through 0.22 μm glassfiber filters. All samples were stored in dark conditions at −4°C and were used for experiments within a week of collection. Table 4.1 summarizes the water quality parameters for both the secondary effluent and the swine farm wastewater before disinfection.

Disinfection Experiments

Two types of disinfectants were used in different operating modes for both wastewaters. For chlorination, chlorination with and without subsequent dechlorination were conducted on both wastewaters. For experiments with only chlorination, 48 h was passed before sample collection to avoid the addition of a quencher. For chlorination followed by dechlorination, a 15 min of chlorine contact time was allowed before dechlorination. Batch reactors wrapped in aluminum foil were used for the disinfection experiments. These reactors were agitated with magnetic stir bars at 125 rpm. To chlorinate, spectrophotometrically standardized (wavelength = 292 nm, molar absorptivity = 360 M^{-1} cm^{-1}) [24] sodium hypochlorite solution (Ricca chemical, Arlington, Texas) was added to the 0.22 μm glassfiber-filtered wastewaters to below the breakpoint, yielding an applied chlorine dose (mg/L as Cl_2) to TOC (mg C/L) ratio of 1:4, which corresponds to 2 mg/L as Cl_2 for the secondary effluent and 250 mg/L as Cl_2 for the swine farm wastewater. To dechlorinate, reagent grade sodium bisulfite was added to the chlorinated solution at a ratio of 1 mg/L Cl_2 : 1.63 mg/L $NaHSO_3$ to guarantee the absence of residual chlorine. [25]

For ozonation, a transferred ozone dose of 2 mg/L was delivered through a stone diffuser into a semi-batch reactor. Ozone gas was produced by a microchannel plasma ozone generator (EP Purification Inc., IL).[26] Oxygen produced by an oxygen concentrator (Airsep, NY) served as the feedstock gas for the ozone generator and was dried by a silica gel desiccator prior to entering the ozone generator. The exhaust gas was passed to a potassium iodide trap and further vented to a fume hood. A schematic of the setup is provided in the SI, Figure S4.1. A preliminary mass transfer experiment was conducted to determine the mass transfer efficiency of the process, in order to obtain the duration required to deliver 2 mg/L of transferred ozone dose. Details of this experiment can be found in the SI. Briefly, ozone was purged into ozone demand free phosphate buffer at pH 7 at 22°C, and the increase in dissolved ozone concentration was recorded as a function of time. Then coupled with the ozone self-decay rate constant, the dissolved ozone concentration profile was fitted to a mass balance equation to obtain the mass transfer efficiency of the system. Cytotoxicity experiments for ozonation were always conducted one day after the disinfection experiments, to ensure that no residual ozone remained.

Sample Concentration

Experiments using a serial dilution of each wastewater sample were first conducted to determine the cytotoxicity. Full strength wastewaters after disinfection were used to prepare the F12 cell culture medium, which was subsequently filter sterilized (through 0.22 µm filters) and immediately used in cytotoxicity experiments. Further experiments using concentrated samples were carried out only if the full strength wastewater did not induce a significant toxic response in the mammalian cells. These samples were concentrated by adsorption onto the XAD resins. Details about the cleaning of the resins can be found in the SI. After cleaning, 55 mL each of the Soxhlet cleaned XAD 2 (Amberlite XAD 2, Sigma Aldrich, MO) and XAD 8 (Supelite DAX 8,

Sigma Aldrich, MO) resins were packed above a plug of glass wool in a chromatography column. The maximum ratio of water to resins was 770:1 to minimize breakthrough and maximize the adsorption of organics. [27, 28] Samples were acidified to pH < 2 by sulfuric acid prior to being manually poured into the column for extraction, to make sure that carboxylic organics were protonated. The column was eluted with 200 ml of optima chromatography grade ethyl acetate (Fisher Scientific, PA), which was shown to be effective to elute the organics from the XAD resins. [29] The residual water of the XAD ethyl acetate extract was removed using a separatory funnel, followed by passing the hydrophobic fraction through a column of anhydrous sodium sulfate to further reduce the water content. The ethyl acetate eluents were reduced to 1-1.5 mL by a rotary evaporator at 50-60°C and further blown down to dryness by a gentle stream of nitrogen gas. 100 μL of ACS reagent grade dimethyl sulfoxide (DMSO) was used to dissolve the extract, resulting in a 10^5-fold concentration of organics of the original wastewater samples. The sample in DMSO was diluted in F12 +FBS cell culture medium, and the corresponding concentrations were calculated and expressed as a concentration factor of the organics derived from the original volume of the wastewater samples.

Biological and Chemical Reagents, Chinese Hamster Ovary Cells

Chinese Hamster Ovary (CHO) K1 cell line AS52, clone 11−4−8 was used for all of the cytotoxicity experiments.[30] The CHO cells were maintained in Hams F12 medium containing 5% Fetal Bovine Serum (FBS), 1% antibiotics (100 units/mL sodium penicillin G, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B in 0.85% saline), and 1% L-glutamine at 37°C in a humidified atmosphere containing 5% CO_2.

CHO Cell Chronic Cytotoxicity Assay

The CHO cell chronic cytotoxicity assay measures the reduction in cell density as a function of the concentration of the test samples over a duration of 72 h. [14, 31] A 96-well cell culture microplate (Corning, NY) was used, which allowed for a series of concentrations of the test samples, with each well representing an independent clone of cells and for an independent measurement. Eight wells on the microplate always served as the negative controls comprising the F12 plus FBS medium and 3×10^3 CHO cells, and eight other wells on the same plate always served as the blank control comprising only the F12 plus FBS medium. The remaining wells contained a known concentration of a test sample, F12 plus FBS medium, and 3×10^3 CHO cells for a total volume of 200 μL. The microplate was covered with a sheet of sterile AlumnaSeal film to prevent cross contamination and evaporation and was gently shaken to evenly distribute cells on the plate before being incubated for 72 h in a humidified environment of 37°C and 5% CO_2. After the 72 h period, the medium was aspirated, and the CHO cells were fixed for 10 min using methanol and stained for 10 min using a 1% crystal violet in 50% methanol solution. The microplate was washed, tapped dry, and 50 μL of 75% (v/v) DMSO and 25% (v/v) methanol solution was added to each well. The plate was incubated in the dark at room temperature for 10 min before being analyzed at 595 nm with a SpectraMax microplate reader (Molecular Devices, CA). The absorbance value of each well was recorded, and the averaged absorbance value of the eight blank wells was subtracted from the absorbance value from each well on the microplate. The blank-subtracted absorbance values of the negative controls were defined as 100%. Then the absorbance value of each treatment well was converted into a percentage of the negative control. This process not only normalized the data but also allowed the combination and comparison of data from multiple microplates. For each wastewater concentration, 4−8 replicate clones were analyzed. A concentration-response curve was generated from the obtained data, and a regression

analysis was carried out on the corresponding curve to calculate a LC_{50} value (the sample concentration that induced a cell density that was 50%, as compared to the negative controls) using Origin Pro 9.1 (Northampton, MA). The LC_{50} values were used to quantify the relative cytotoxicity of the different wastewater samples. We employed the OECD Guidelines for the Testing of Chemicals using mammalian cells. [32]

Detection of DBPs

All glassware was thoroughly washed and baked (500°C, 2 h). ACS grade Na_2SO_4 and H_2SO_4, HPLC Plus grade methanol, and *tert*-Butyl methyl ether (MTBE) were purchased from Sigma-Aldrich (St. Louis, MO). THMs, HANs, and HAAs mixed standard was also purchased from Sigma-Aldrich (Table S4.3).

We followed the EPA Method 551.2 with slight modifications. [33, 34] For THM and HAN analysis, 2 mL of MTBE and 8 g Na_2SO_4 were added to a 25 mL sample solution in a 40 mL glass vial capped with PTFE-lined silica septum. The vial was sealed and shaken manually for 3 min and left undisturbed for 5 min. For HAA analysis, 1.5 mL of concentrated H_2SO_4 and 12 g Na_2SO_4 were added to a 25 mL sample solution in a 40 ml glass vial capped with PTFE-lined silica septum before extraction with 3 mL of MTBE (3 min manual shaking). A 2 mL extract was then methylated with 1 mL of 10% H_2SO_4 methanolic solution at 50°C for 2 h. After adding 5 mL of 150 g/L Na_2SO_4 solution and 1 mL of saturated $NaHCO_3$ solution, the extract was subsequently transferred to gas chromatography (GC) vials and kept at 4°C until GC/MS analysis. An Agilent 6850 GC coupled with a 5975 C mass selective detector (MSD) and 6850 series automatic liquid sampler (Agilent, CA) was used for analyzing DBPs. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The column was a fused silica capillary DB-5MS (30 m×0.25 mm×0.25 μm). The analytical conditions to detect THMs and HANs followed the

procedures by Nikolaou et al, [35] and those to detect HAAs followed the study by Xie. [36] A 2 μL sample was introduced into the GC by split injection for THMs and HANs, while for HAAs the injection volume was 3.0 μL under the splitless mode. The oven temperature to detect THMs and HANs was held at 33°C for 5 min, ramped to 130°C at a rate of 10°C /min, held for 1 min, and further increased to 240°C at a rate of 25°C /min for 2 min. The oven temperature for HAAs detection was held at 35°C for 10 min, ramped to 70°C at a rate of 5°C /min, held for 15 min, further increased to 150°C at a rate of 10°C /min, and eventually ramped to 220°C at a rate of 20°C /min for 2 min. The injector temperature was kept at 200°C. The MSD was operated in the SIM mode and the m/z for THMs, HANs, and HAAs followed previous publications. [35, 36] The ion source temperature used was 230°C.

Statistical Analysis

We conducted a bootstrap statistical analysis to generate multiple LC_{50} values by generating multiple concentration-response curves and regression analyses for each curve.[37, 38] Each bootstrap LC_{50} value was converted into a cytotoxicity index value $(LC50^{-1})(10^3)$ in order to conduct an ANOVA test to determine if there were significant differences among the cytotoxicity index values for the wastewaters. This approach allowed us to rank the wastewaters associated with different disinfection methods, from the most cytotoxic to the least cytotoxic. A one-way analysis of variance (ANOVA) test was conducted to determine if each concentration induced a statistically significant level of cell death as compared to the negative control. If a significant F value ($P \leq 0.05$) was obtained, a Tukey multiple means comparison test versus the controls was conducted to identify the lowest concentration factor that was cytotoxic. The power of the test statistic ($1-\beta$) was maintained as ≥ 0.8 at $\alpha = 0.05$. Pairwise comparison of the CTI values was conducted to determine if statistically significant differences existed among

treatments. Pearson product-moment correlation analysis was used to establish possible correlations between the parameters and the CTI values.

4.4 Results and Discussion

Secondary effluent was virtually non-toxic at concentrations close to the original sample (up to 90% concentration of the original samples, Table 4.1, Figure 4.1). Disinfected secondary effluent did not show significant difference in cytotoxicity between the two disinfectants, as compared with the non-disinfected controls, regardless of the mode of disinfection (e.g., chlorination alone vs. chlorination with dechlorination). The independence of cytotoxicity of the whole secondary effluent to disinfectants and disinfection modes could be attributed to the low organics in the secondary effluent (8 mg C/L) that can form chlorination DBPs (C-DBPs) and N-DBPs through reactions with disinfectants. [14, 39] Despite the lack of response to the changing secondary effluent concentration, a trend of slightly increased cytotoxicity was observed for all disinfectants/operating modes except for ozonation (Figure 4.1.). For these secondary effluents we concentrated the organics with or without disinfection using XAD-2/XAD-8 resins. This increased the concentration of the extracted organics which were then analyzed for cytotoxicity using CHO cells.

Ozonation produced treated secondary effluent having the least cytotoxicity compared to chlorination (Figure 4.2. and Table 4.2.). At a transferred ozone dose of 2 mg/L and a residence time of 48 h, the cytotoxicity index value for ozonated secondary effluent was more than 2.4-fold lower than that of the chlorinated and dechlorinated secondary effluent, which was the second least toxic secondary effluent produced after disinfection (Figure 4.3.). This observation agrees with the data trend of the two categories of regulated DBPs (THMs and HAAs), where after ozonation the concentration of total THMs (TTHMs) and total HAAs (THAAs) decreased from

1.14 to 0.24 μg/L and 2.16 to 1.53 μg/L, respectively (Table 4.3.). These experiments uncovered differences between the two chlorination operation modes. Chlorination without dechlorination produced the most toxic effluent, which was significantly different from the non-disinfected secondary effluent. This was likely the result of prolonged disinfection contact time (48 h), which produced the highest amount of THAAs at 12.3 μg/L and probably increased the levels of emerging DBPs (Table 4.3.). Dechlorination after 15 min of chlorine contact time yielded less toxicity than the non-disinfected control. The lowered cytotoxicity may be due to the partial oxidation of organic fractions of the secondary effluent by chlorine, and also that the contact time was not long enough for the formation of excess amount of DBPs. For ozonation, at 2 mg/L of transferred ozone dose, the TTHMs and THAAs reduced from 2.16 to 1.53 μg/L and 1.14 to 0.24 μg/L, respectively, which is consistent with the reduction in cytotoxicity. A further effort to correlate the cytotoxicity with the regulated DBPs for the secondary effluent experiments failed to resolve a significant correlation (r = -0.03, P = 0.97 for TTHMs with CTI; r = 0.73, P = 0.27 for THAAs with CTI). This indicates that other more cytotoxic DBPs beyond the regulated ones, or non-DBP substances, were the forcing agents of the cytotoxicity. To explore this issue, we measured the haloacetonitriles (HANs) to provide insights on the behavior of nitrogenous DBPs that were reported to have high relative contributions to the DBPs-associated toxicity. [14, 40-42] A significant correlation was observed between HANs and the CTI values (r = 0.96, P = 0.04). Therefore, part of the cytotoxicity trend among samples could be related to the concentration of HANs as forcing agents, which is consistent with previous findings. [14, 17] Ozonation reduced the concentration of HANs by two-fold as compared to the non-disinfected samples, the highest of all disinfection treatments, possibly due to the efficient removal of ammonia, one of the precursors to N-DBPs production, from 3.6 to 0.5 mg N/L (Table S4.2.). A summary of the

comparison of cytotoxicity as expressed in CTI values is provided in Figure 4.3, and detailed THMs, HAAs, and HANs concentrations before and after disinfection can be found in the SI.

Several dilutions of the swine farm wastewater were more toxic than any form of the secondary effluent, with or without disinfection (Table 4.2.). This could be reflected in the much higher organics concentration of the swine farm wastewater (1000 mg C/L), which contained runoff of animal forage and feces, both of which were rich in carbon and nitrogen and could have contributed to DBPs precursors. However, it is important to note that there was no significant correlation between TOC and CTI values for neither the secondary effluent (r = 0.93, P = 0.07) nor the swine wastewater (r = 0.58, P = 0.42). Among various disinfectants and modes of disinfection of swine farm wastewater, at 2 mg/L transferred ozone dose and a residence time of 48 h, ozonation produced the lowest cytotoxicity by providing a 17-fold decrease in CTI value compared to the non-disinfected swine farm wastewater (Figure 4.4. and Figure 4.5.). Lowered TTHMs and THAAs from 0.43 to 0.35 μg/L and 1087 to 961 μg/L, respectively after ozonation agreed with this observation. However, HANs almost doubled in concentration after ozonation, which might suggest its less significant role in the swine wastewater system.

Although being the least toxic treated wastewater, ozonated swine farm wastewater still possessed a CTI value 170 times higher than even the most toxic XAD-2/XAD-8 extract of the disinfected secondary effluent, which corresponded to the extended chlorinated sample without dechlorination. Despite the potential to form a number of DBPs due to rich organics that could function as precursors to a variety of C-DBPs and N-DBPs through reactions with disinfectants, [14, 39] different modes of chlorination demonstrated similar cytotoxicity, which was in fact lower than that of the non-disinfected swine farm wastewater. The lowered toxicity is consistent with the results of the chlorinated and dechlorinated secondary effluent and suggests that the

additional chlorination disinfection contact time compared to the dechlorinated samples did not contribute significantly to excessive accumulation of DBPs that could increase the cytotoxicity of the whole swine farm wastewater. This was confirmed by comparing the chlorination with 15 min of contact time and the chlorination with 48 h of contact time in the swine farm wastewater. The extended contact time actually resulted in TTHMs, THAAs, and HANs not higher than the experiment with much shorter contact time of 15 min (Table 4.3.). Therefore, at the applied chlorine and ozone doses, regardless of the disinfectants and disinfection operating mode, any form of disinfection could reduce the cytotoxicity of the swine farm wastewater. This is an added benefit of the disinfection process before the recycled wastewater can be used for any agricultural purposes. Nevertheless, overall for the swine wastewater, and consistent with the secondary effluent experiments, we could not establish a significant correlation between the regulated DBPs and CTI values (r = -0.06, P = 0.94 for TTHMs and CTI; r = 0.52, P = 0.65 for THAAs and CTI). Inconsistent with the observation for the secondary effluent experiment, no significant correlation could be established between the HANs and the CTI values (r = -0.59, P = 0.41), again suggesting the presence of other forcing agents in this highly complex system.

These data indicate that secondary effluent is preferred for agricultural reuse over swine farm runoff regardless of the disinfectants. If the swine farm wastewater is to be considered for agricultural reuse, such as during drought seasons in certain regions, further studies should be conducted to validate its safety. Chlorination is capable of reducing the cytotoxicity of both tested wastewaters, which not only depends on the operation modes but also the constituents of the wastewaters. Ozonation consistently lowered the cytotoxicity of wastewaters, regardless of the source. As a result, if technology and economy permit, ozonation may hold the most promise in reducing the overall cytotoxicity of wastewater. While in general more regulated DPBs in

drinking water were produced after disinfection except for ozonation, the reduced overall cytotoxicity to mammalian cells suggests that for wastewater disinfection emphasis is needed on substances beyond the regulated DBPs. For instance, TTHMs are commonly used in epidemiology studies to monitor potential adverse health impacts.[9, 43-46] However, as seen in this study and a previous one,[9] a reduced cytotoxicity accompanying an elevated level of TTHMs revealed that monitoring TTHMs alone may not have enough resolving power to identify forcing agents responsible for toxicity and adverse health effects. [47] Moreover, correlations that were significant for the secondary effluent could not be applied to the swine farm wastewater, suggesting the importance of variability in water quality. Therefore, the current findings should be further validated with more wastewaters from diverse sources. Although the specific causative agent(s) in the wastewaters of the observed cytotoxicity were not clear, quantitative, comparative cytotoxicity analyses provides a good estimate of the overall adverse biological impacts.

4.5 List of Figures and Tables

Table 4.1. Water quality summary of secondary effluent and swine farm wastewater.

Water quality parameter	Units	Secondary effluent	Swine farm wastewater
pH	-	7	8.5
Ammonia	mg N/L	3.6	3320
Nitrate	mg N/L	8.96	23.03
TOC	mg C/L	8	1010
SUVA	L/mg C/m	1.8	3.3
UV_{254}	1/m	14.4	3300
COD	mg O_2/L	20	998
Ag	µg/L	0	0
As	µg/L	1.9	2
Cd	µg/L	0	0
Cr	µg/L	2.1	23
Hg	µg/L	1.8	0
Total bromine	mg/L	1	2
Total iodine	mg/L	828	1431

Table 4.2. Induction of Chronic Cytotoxicity in CHO Cells by the secondary effluent and the swine farm wastewater.

Wastewater sample	Lowest cytotoxic concn. factor [a]	r^2 [b]	LC_{50} (concn. factor) [c]	ANOVA test statistics[d]
XAD secondary ww w/o disinfect.	30×	0.94	39.6×	$F_{10,33} = 50.5$; $P \leq 0.001$
XAD secondary ww Cl+deCl	20×	0.98	50.1×	$F_{10, 32} = 169.7$; $P \leq 0.001$
XAD secondary ww Cl 48 h	20×	0.98	32.4×	$F_{10,33} = 200.9$; $P \leq 0.001$
XAD secondary ww ozonation	110×	0.96	124.9×	$F_{10,33} = 78.7$; $P \leq 0.001$
Whole swine farm ww w/o disinfect.	0.01×	0.87	0.02×	$F_{10,43} = 27.8$; $P \leq 0.001$
Whole swine farm ww Cl+deCl	0.02×	0.97	0.02×	$F_{10,77} = 272.4$; $P \leq 0.001$
Whole swine farm ww Cl 48 h	0.02×	0.98	0.02×	$F_{10,77} = 333.1$; $P \leq 0.001$
Whole swine farm ww ozonation	0.1×	0.91	0.2×	$F_{10,77} = 78.9$; $P \leq 0.001$

a. The lowest cytotoxic concentration factor was the lowest concentration factor of the corresponding wastewater samples that produced a statistically significant cytotoxic response in the dose-response curve, as compared to the negative controls.

b. r^2 is the coefficient of determination for the regression analysis that derived LC_{50} values.

c. The LC_{50} value is the sample concentration factor that induced a cell density that was 50% of the negative controls.

d. The degrees of freedom for the between-groups and residual associated with the calculated *F*-test results and the resulting probability value.

Table 4.3. Characterization of DBPs from swine wastewater and secondary effluent.

wastewater source	compound	conc. w/o disinfection (μg/L)	conc. w/ chlorination 48 hrs (μg/L)	conc. w/ chlorination 15 min (μg/L)	conc. w/ ozonation (μg/L)
swine wastewater	total haloacetic acids	1087.54 ± 58.25	1950.27 ± 76.58	166301.2 ± 129.13	960.75 ± 39.67
	total trihalomethanes	0.43 ± 0.02	2.65 ± 0.69	110.13 ± 2.46	0.35 ± 0.03
	total haloacetonitriles	12.52 ± 3.25	20.40 ± 3.68	32.59 ± 2.98	23.26 ± 1.86
secondary effluent	total haloacetic acids	2.16 ± 0.12	12.30 ± 0.99	4.91 ± 0.24	1.53 ± 0.21
	total trihalomethanes	1.14 ± 0.06	0 ± 0	2.26 ± 0.18	0.24 ± 0.02
	total haloacetonitriles	21.25 ± 2.79	48.36 ± 3.46	18.62 ± 3.01	10.29 ± 2.32

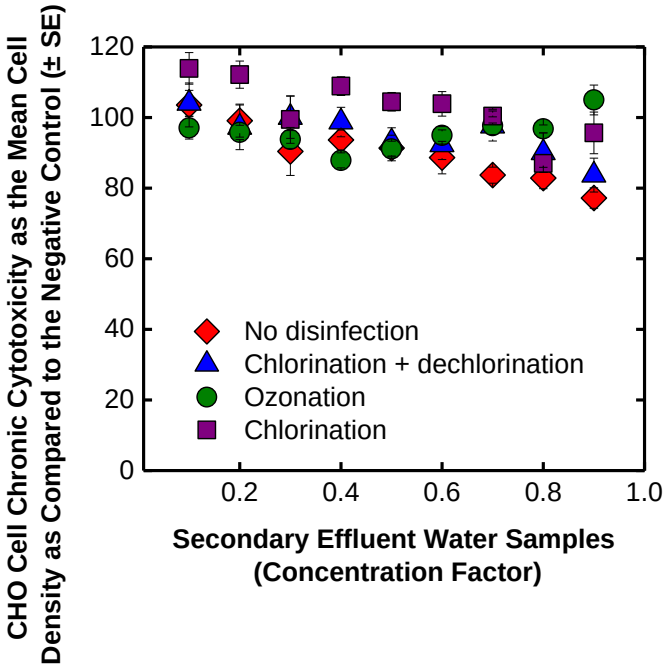

Figure 4.1. The cytotoxicity dose-response data from all of the whole secondary effluent samples with or without treatment.

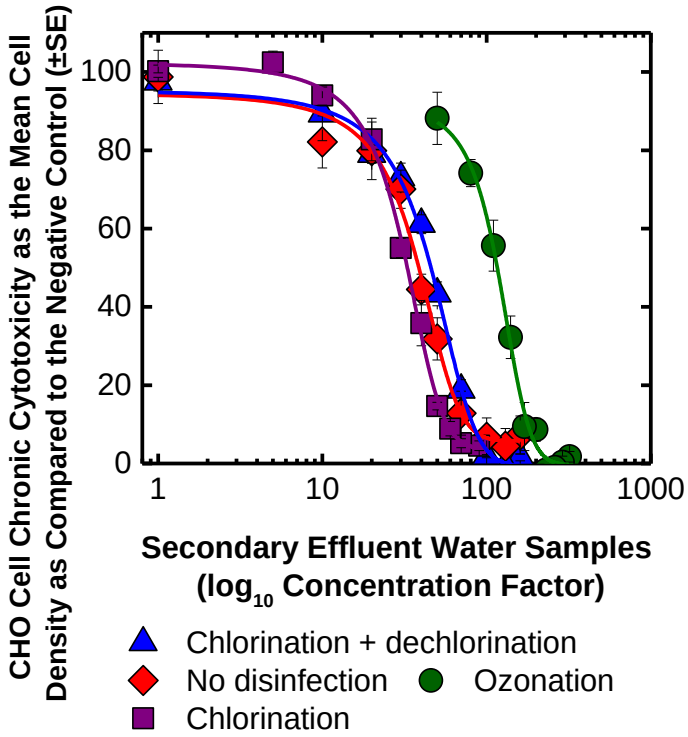

Figure 4.2. The cytotoxicity dose-response data from all of the XAD extract of the secondary effluent samples with or without treatment.

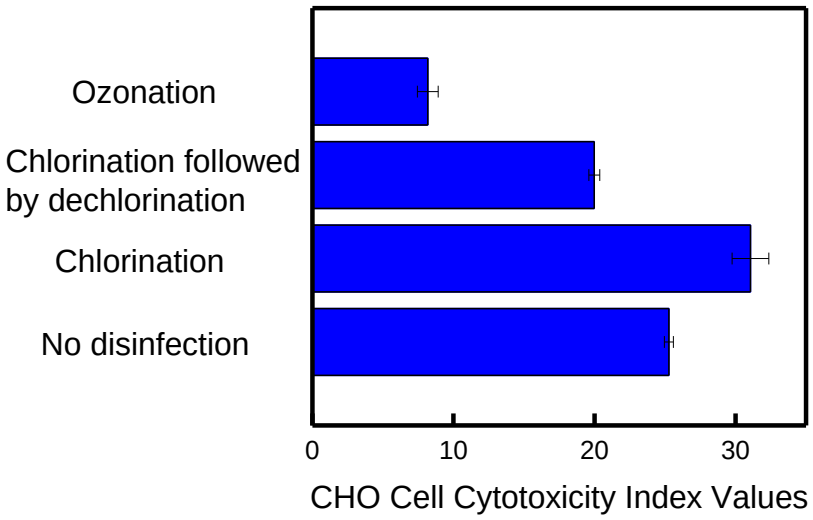

Figure 4.3. Comparison of the mean CHO cell cytotoxicity index values for the secondary effluent samples. Ozonated samples were the least cytotoxic amongst all samples. Cytotoxicity ranking: Samples chlorinated for 48 h > Non-disinfected samples > Chlorinated and dechlorinated samples > Ozonated samples. Index values are expressed in arbitrary units. Error bars correspond to the standard error.

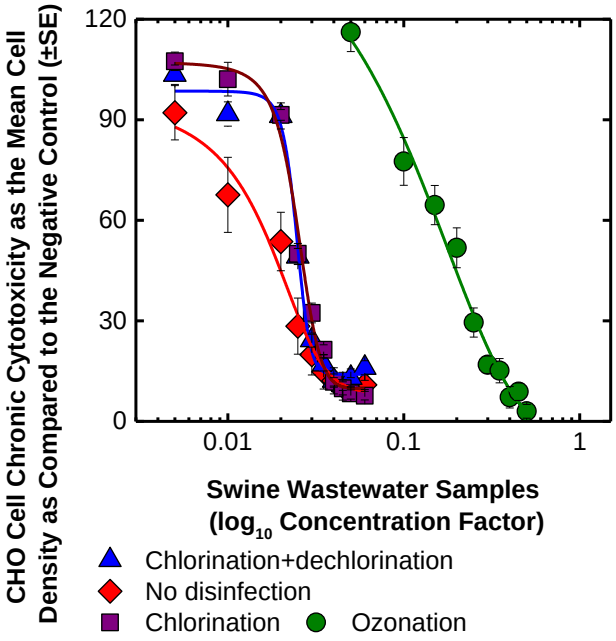

Figure 4.4. The cytotoxicity dose-response data from all of the swine farm wastewater samples with or without treatment.

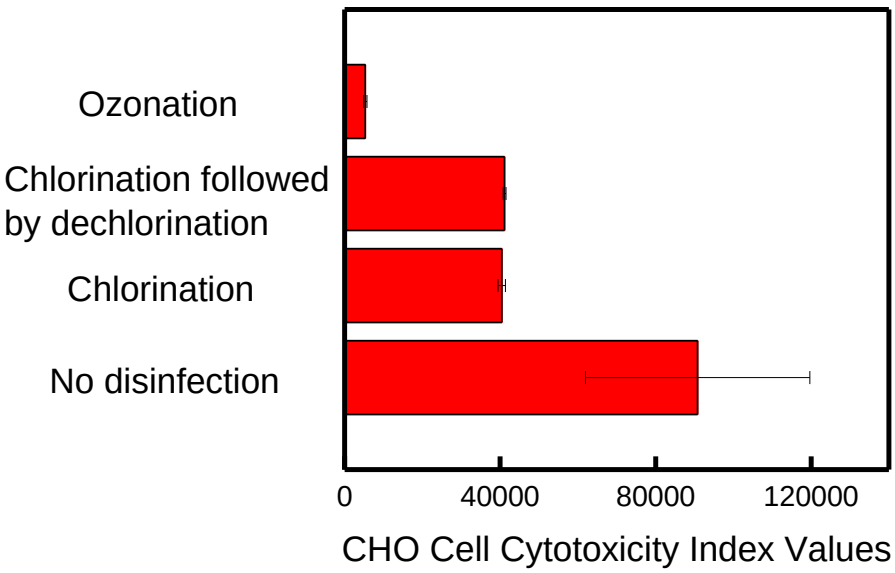

Figure 4.5. Comparison of the mean CHO cell cytotoxicity index values for the swine farm wastewater samples. Cytotoxicity ranking: Non-disinfected samples > Chlorinated and dechlorinated samples = Samples chlorinated for 48 h > Ozonated samples. Index values are expressed in arbitrary units. Error bars correspond to the standard error.

4.6 Supplementary Information

Ozonation setup

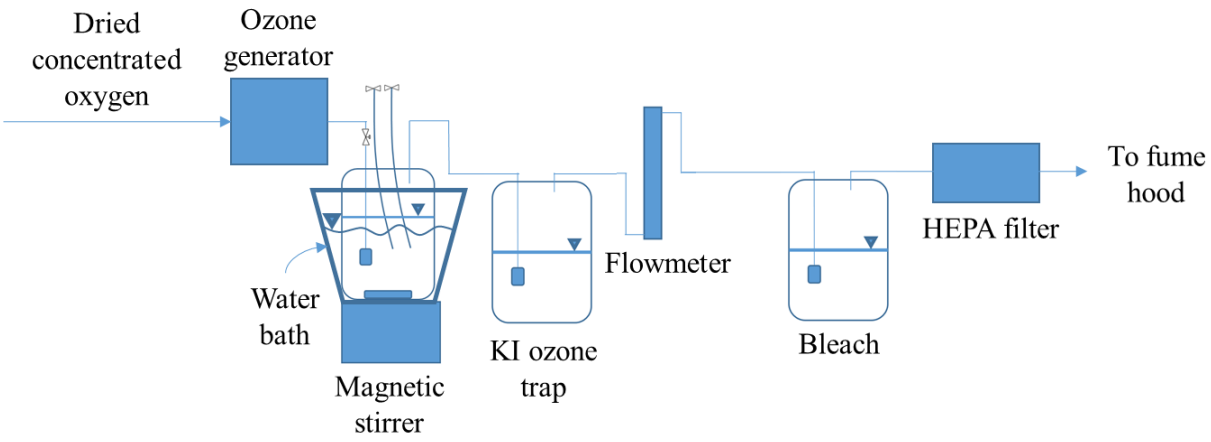

Figure S4.1. Schematic of the ozonation system.

Cleaning of the XAD Resins

The XAD 2 and XAD 8 resins were independently cleaned using Soxhlet extractions with a sequence of 400 ml methanol, 400 ml ethyl acetate, and 400 ml of methanol. The extraction duration for each solvent was 24 hours. After cleaning, the XAD 2 and XAD 8 resins were stored separately in methanol at 4 ^{o}C until use. All solvents used for the Soxhlet extractions were of optima chromatography grade.

Delivering the Determined Transferred Ozone Dose

Applied ozone dose is defined as:

$$\int_{t_0}^{t} \frac{Q_{gin} C_{gin}}{V_L} dt \qquad S(4.1)$$

where t is the time elapsed, C_{gin} is the ozone concentration in the outlet of the ozone generator, Q_{gin} is the ozone gas flow rate, V_L is the volume of solution in the semi-batch reactor, and m is the Henry's law constant at 23 ^{o}C.

Transferred ozone dose is defined as:

$$\int_{t_0}^{t} \frac{Q_{gin}}{V_L} (C_{gin} - C_{gout}) dt \qquad S(4.2)$$

where C_{gout} is the ozone concentration at the exhaust outlet of the semi-batch reactor.

Alternatively S(4.2) can be expressed using the concept of Mass Transfer Efficiency (MTE) as:

$$\int_{t_0}^{t} \frac{Q_{gin}}{V_L} \left(C_{gin} - C_{gout}\right) dt = \text{MTE} \times \text{applied ozone dose} \qquad S(4.3)$$

$$= \text{MTE} \times \int_{t_0}^{t} \frac{Q_{gin} C_{gin}}{V_L} dt$$

Since transferred ozone dose can also be expressed as:

$$\int_{t_0}^{t} K_L a \left(\frac{C_{gi}}{m} - C_L\right) dt \qquad S(4.4)$$

we can therefore have the following relationship:

$$\text{MTE} \times \int_{t_0}^{t} \frac{Q_{gin} C_{gin}}{V_L} dt = \int_{t_0}^{t} K_L a \left(\frac{C_{gi}}{m} - C_L\right) dt \qquad S(4.5)$$

To determine the time required to deliver a preferred transferred ozone dose (2 mg/L) at a given ozone mass production rate (4 g/h), the term $K_L a$ in S(4.5), i.e. the volumetric liquid-phase mass transfer coefficient must be known. To do so, the first order ozone self-decay rate constant k was first calculated from the reported half-life of ozone in water at 23 ^{o}C and pH 7,[48] which

was approximately 15 minutes. The calculated k was 0.046 min^{-1}. Ozone was added continuously to the buffered organic-free solution, and the dissolved ozone concentration C_L was measured at different times and subsequently fitted to the solution to the mass balance equation for batch reactors S(4.6), as given in S(4.7).[49]

$$\frac{dC_L}{dt} = K_L a\left(\frac{C_{gi}}{m} - C_L\right) - kC_L \qquad \text{S(4.6)}$$

$$C_L = \frac{\exp\left(t(K_L a+k)\right)K_L a\frac{C_{gi}}{m} - K_L a\frac{C_{gi}}{m}}{\exp(t(K_L a+k))(K_L a+k)} \qquad \text{S(4.7)}$$

where C_{gi} is the ozone concentration at the interphase, and m is the Henry's law constant. The fitted value of $K_L a$ was 0.194 min^{-1} ($R^2 > 0.98$). An example of the fitting curve is shown in Figure S4.1.

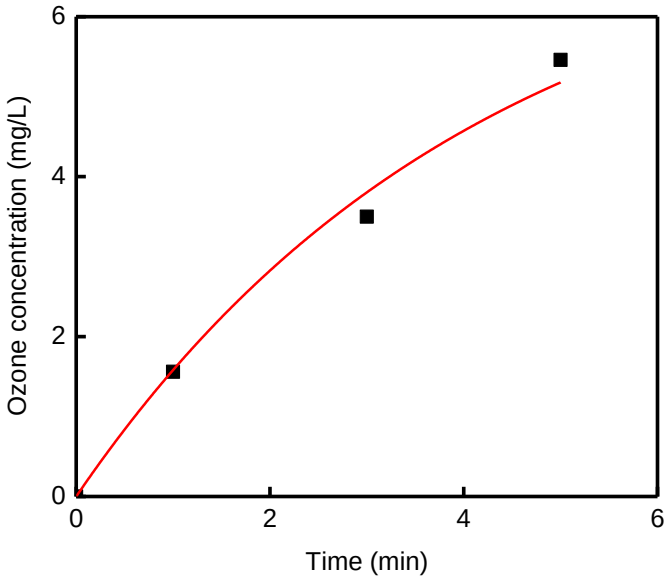

Figure S4.2. Examples of profile of ozone concentration during constant flow rate purging into buffer solution at pH 7 at 23 °C.

MTE can now be calculated from S(4.8) using a finite difference approach as given in S(4.9), to be 1.32%:

$$\mathrm{MTE} = \frac{V_L K_L a}{Q_{gin} C_{gin}} \int_{t_0}^{t} \left(\frac{C_{gi}}{m} - C_L\right) dt \qquad \text{S(4.8)}$$

$$\mathrm{MTE} = \frac{V_L K_L a}{Q_{gin} C_{gin}} \sum \left(\frac{C_{gi}}{m} - C_{Li}\right) \Delta t \qquad \text{S(4.9)}$$

The wastewater samples to be disinfected were therefore ozonated for exactly 136 seconds to deliver a transferred ozone dose of 2 mg/L.

DMSO Background Cytotoxicity

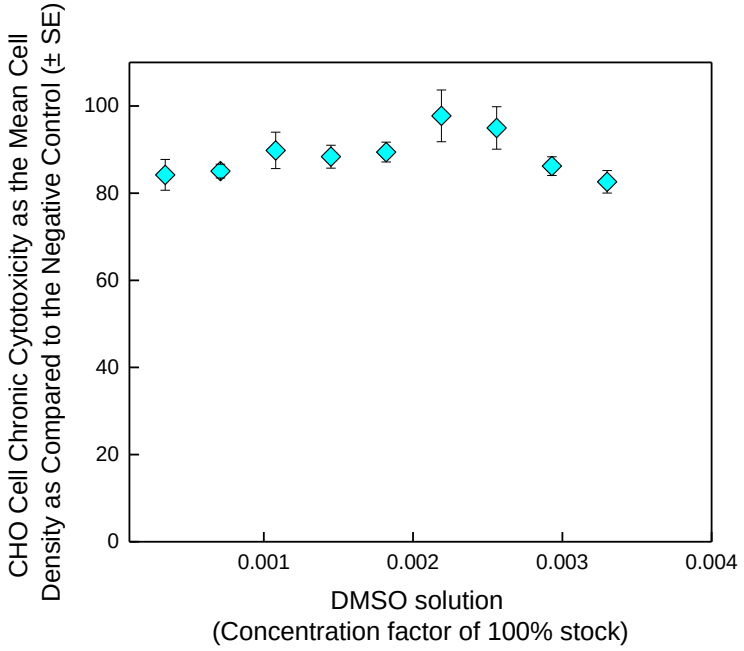

Figure S4.3. Cytotoxicity of DMSO solution within the concentration range used in experiments.

Table S4.1. Characterization of swine wastewater, DBPs and selected parameters.

compound	conc. w/o disinfection (μg/L)*	conc. w/ chlorination 48 hrs (μg/L)*	conc. w/ chlorination 15 min (μg/L)*	conc. w/ ozonation (μg/L)*
haloacetic acids				
monochloroacetic acid	0	0	0	0
dichloroacetic acid	0	76.421	65.773	0
trichloroacetic acid	0	5.7079	6.8419	3.7843
monobromoacetic acid	0	0	0	0
dibromoacetic acid	0	0	0.95	0.95
tribomoacetic acid	0	0.2297	164761.8	0
bromodichloroacetic acid	0	0	0	0
dibromochloroacetic acid	1087.275	1839.086	1454.289	955.092
bromochloroacetic acid	0.2604	28.8276	11.5644	0.9192
trihalomethanes				
chloroform	0	0.715	109.417	0.3454
bromodichloromethane	0.4271	1.9337	0.7151	0
dibromochloromethane	0	0	0	0
bromoform	0	0	0	0
haloacetonitrile				
dibromoacetonitrile	0	3.720625	0	0
bromochloroacetonitrile	12.51687	16.6781	31.24034	23.2568
trichloroacetonitrile	0	0	0	0
dichloroacetonitrile	0	0	1.349404	0
inorganic parameters				
ammonia nitrogen (mg N/L)	3320	950	2720	2560
total organic carbon (mg C/L)	1010.17	2100.50	1564.33	1332.83

* Unit is μg/L except for inorganic parameters

Table S4.2. Characterization of secondary effluent, DBPs and selected parameters.

compound	w/o disinfection (μg/L)*	w/ chlorination 48 hrs (μg/L)*	w/ chlorination 15 min (μg/L)*	w/ ozonation (μg/L)*
haloacetic acids				
monochloroacetic acid	0	0	0	0
dichloroacetic acid	1.069	2.415	2.936	0.859
trichloroacetic acid	0	8	0	0
monobromoacetic acid	0	0	0	0
dibromoacetic acid	0	0	0	0
tribomoacetic acid	0	0.2297	0.2297	0
bromodichloroacetic acid	0	0	0	0
dibromochloroacetic acid	0	0.5415	0.646	0
bromochloroacetic acid	1.086	1.1136	1.0992	0.6684
trihalomethanes				
chloroform	0.8272	0	1.5752	0.242
bromodichloromethane	0.3137	0	0.6809	0
dibromochloromethane	0	0	0	0
bromoform	0	0	0	0
haloacetonitrile				
dibromoacetonitrile	3.004741	1.810741	0	0
bromochloroacetonitrile	18.24709	46.55025	18.12219	10.29395
trichloroacetonitrile	0	0	0	0
dichloroacetonitrile	0	0	0.501856	0
inorganic parameters				
ammonia nitrogen (mg N/L)	3.6	5.6	1.8	0.5
total organic carbon (mg C/L)	8.00	8.14	6.75	5.48

* Unit is μg/L except for inorganic parameters

Table S4.3. The DBPs standards used in experiments.

No.	type of standards	species	concentration
1	THMs	chloroform	2000 μg/mL of each component in MTBE
		bromodichloromethane	
		dibromochloromethane	
		bromoform	
2	HANs	bromochloroacetonitrile	2000 μg/mL of each component in MTBE
		dibromoacetonitrile	
		dichloroacetonitrile	
		trichloroacetonitrile	
3	HAAs	monochloroacetic acid	2000 μg/mL of each component in MTBE
		bromoacetic acid	
		dichloroacetic acid	
		bromochloroacetic acid	
		trichloroacetic acid	
		dibromoacetic acid	
		bromodichloroacetic acid	
		dibromochloroacetic acid	
		tribromoacetic acid	

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CHAPTER 5

CONCLUSIONS

5.1 Conclusion

The findings in this thesis are summarized here.

Up to five $\log_{10}$ of *L. pneumophila* was inactivated in wastewater using the microchannel plasma ozonator. The operation mode of the microchannel plasma ozonator that yielded the lowest energy consumption per $\log_{10}$ *L. pneumophila* inactivation was high initial dissolved ozone concentration and high driving voltage (120V). This condition is required likely due to the significantly faster inactivation of *L. pneumophila* at higher ozone doses. Contrary to a previous finding [1], the CT concept for disinfection design appeared to be valid. A CT equation was therefore developed to predict the *L. pneumophila* inactivation by ozone in wastewater under the tested conditions. The inactivation kinetics of *L. pneumophila* in organic-free solution depended strongly on the temperature and was less effective at lower temperature, which is contrary to previous findings that ozone was either behaving similarly at temperature from 25 to 45 ^{o}C [2], or that ozone was more effective at 5 compared to 15 ^{o}C [3]. However, in the presence of WOM the inactivation kinetics was independent of temperature. High loadings of WOM was found to require higher initial dissolved ozone concentration in order to achieve the same inactivation goal. Contact time may have a positive correlation with *L. pneumophila* inactivation in wastewater, which contradicts a previous finding that it has no impact on bacterial inactivation in wastewater [1]. This was possibly the result of low levels of dissolved ozone below detection that rendered the contact time seemingly irrelevant. These results delivered a package that not only provided guidance on the implementation of the microchannel plasma ozone generator for

secondary effluent disinfection, but also a better understanding of ozonation technology for wastewater disinfection in general.

A combined LCA and QMRA analysis further showed that for five out of six environmental impact categories, the microchannel plasma ozonation disinfection system provided more human health protection as compared to the traditional chlorination with dechlorination system. This is attributed to the former's low energy consumption and the higher susceptibility of the pathogens to ozone than to chlorine. Despite different profiles of consumed electricity fuel sources in Florida, California, and Texas, the microchannel plasma ozonation system consistently offset more disability adjusted life years (equivalent to providing greater human health protection) as compared to the chlorination disinfection system given the same degree of treatment. These results demonstrated that the microchannel plasma ozonation system is better than the chlorination (with dechlorination) system for landscaping reuse disinfection, from the perspective of human health protection.

Chronic cytotoxicity assays were used to evaluate the change in toxicity of wastewaters after alternative disinfection technologies (ozonation vs. chlorination). The swine farm wastewater was found to be approximately 2000 times more cytotoxic than the secondary effluent. Both the microplasma ozonation and chlorination were capable of reducing the overall cytotoxicity. Microplasma ozonation consistently reduced the mammalian cell cytotoxicity of the wastewaters by as much as 10 times. Although chlorination had the potential to lower the cytotoxicity when dechlorination was followed, the toxicity reduction was still more prominent for the microplasma ozonation. While the relationship between reduced cytotoxicity and the modification or reduction of specific compound(s) is unknown, regulated DBPs may not be the dominating forcing agents.

5.2 Contributions

This research developed a framework for the prediction of dissolved ozone concentration in wastewaters, based on input parameters such as the initial ozone concentration, initial TOC loading, Henry's law constant at different temperatures, and gas phase ozone concentrations. Contrary to previous studies, wastewater disinfection using ozone appeared to follow the CT concept, which is a convenient way to predict inactivation as well as to determine the dosage of disinfectants for a desired level of inactivation. Contrary to a previous study, it was observed that inactivation depended on HRT, as long as the residual ozone was available. These observations not only facilitate the implementation of the microplasma ozone generation system, but as well better our understandings of the ozone disinfection of wastewaters at large.

Additionally, the identification of the disinfection technology that provided more overall protection of human health – microplasma ozonation, as compared to chlorination plus dechlorination, suggests a potential future focus on the implementation of this novel technology. The method and concept to generate the ranges of operation for both the microplasma ozonation, as well as chlorination with dechlorination could function as the first step towards the development of a guideline that not only focuses on the mere aspect of fulfilling the inactivation target for a disinfection treatment process, but also the overall picture of human health protection.

This thesis also explored the potential changes in wastewaters toxicity on mammalian cells after disinfection, comparing microplasma ozonation and chlorination (with and without dechlorination). From a mammalian cell cytotoxicity point of view, the identification of the less toxic secondary wastewater suggested that it is a safer option for purposes that involve contact with people, such as agricultural reuse, as compared to the more toxic but widely available

runoff wastewater from swine farms. The observation that ozonation consistently lowered the toxicity of both sources of wastewaters suggested that ozonation should be considered as one of the candidates for wastewater disinfection, particularly wastewaters that may come in contact with people, such as during an irrigational reuse scenario. Chlorination is capable of reducing the cytotoxicity, but careful attention has to be put on operational conditions such as the presence or absence of a dechlorination step. Based on the study of this part of the thesis, it is suggested that regulations should consider incorporating more than the regulated DBPs.

5.3 Future Prospects

This thesis provided experimental and simulation work that aimed to better understand ozone disinfection in wastewater treatment in general, with a focus on the microplasma ozonation technology in particular. Numerous areas stand out from the current study to be extended into future studies.

Since the actual reactions involved between ozone and various components in the wastewater, such as WOM, are likely to be very complex and numerous, models incorporating more details in specific reactions would likely improve the modeling results. The current model assumed a lumped second order reaction between the WOM and ozone based on literature [1, 4], which could be improved by incorporating more precise reaction orders. For instance, Farooq et al. obtained satisfactory results by deriving a 3/2 overall order for the ozonation reaction between residual ozone and organic matter, with 1/2 order with respect to the organic matter and first order with respect to the residual ozone. However, it should be noted that reaction orders are pollutant specific and are subject to change with specific water source [5]. In addition, besides the reaction order, one future prospect for the modelling work could be taking into account the unaccounted for reactions. As mentioned, reactions with ozone in wastewater matrix is

numerous, and they not only consume ozone, such as with ferrous and nitrite ions, but also inhibit the decay of ozone. For instance, carbonate and bicarbonate species could react with hydroxyl radicals, which are important intermediates during the cycle of ozone decomposition, thus quenching the decomposition process [6].

Additionally, due to the difficulty of physically measure the wastewater that contains numerous interference such as WOM and bacteria, the developed model could only be indirectly validated by the goodness of fit to the Chick-Watson model. Therefore future efforts could also focus on developing methods or sensors that can detect low levels of ozone in wastewaters.

Regarding the LCA and QMRA part of the thesis, currently the three cultural approaches, i.e. individualist, hierarchist, and egalitarian approaches (ranking from the most liberal to the most conservative) are only applicable towards the LCA part of the analysis. Therefore it would be very helpful to incorporate similar characterization factors based on cultural conservativeness for the QMRA analysis. Moreover, future work could also incorporate chemicals into the QMRA analysis because apart from the microbial pathogens that could impact human health, various chemicals in the disinfected water, such as DBPs, could also have a significance on the overall human health impact. Although strictly speaking it would not be called QMRA due to the inclusion of chemical risk assessment.

For the cytotoxicity part of the work, certain regulated contaminants, such as N-Nitrosodimethylamine (NDMA), do no demonstrate cytotoxicity in the 72 hrs chronic cytotoxicity assay [7]. Therefore future efforts could detect the concentration of such contaminants in wastewater and compare it with regulated levels in order to generate a sense of potential risks. Moreover, due to the very complicated nature of the swine wastewater, future efforts could focus on quantitatively and qualitatively identifying all potential DBPs beyond the regulated ones, as

well as non-DBPs, and correlate them with the observed overall cytotoxicity in order to identify ones that have the most significant impact.

5.4 References

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