Australian Water Recycling
Centre of Excellence

Industry Academic Exchange Program Report

Particles, Pathogens and Micropollutants

A report of a study funded by the
Australian Water Recycling Centre of Excellence

Prof. Karl Linden, August 2014

Particles, Pathogens and Micropollutants

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About the Australian Water Recycling Centre of Excellence

The mission of the Australian Water Recycling Centre of Excellence is to enhance management and use of water recycling through industry partnerships, build capacity and capability within the recycled water industry, and promote water recycling as a socially, environmentally and economically sustainable option for future water security.

The Australian Government has provided $20 million to the Centre through its National Urban Water and Desalination Plan to support applied research and development projects which meet water recycling challenges for Australia's irrigation, urban development, food processing, heavy industry and water utility sectors. This funding has levered an additional $40 million investment from more than 80 private and public organisations, in Australia and overseas.

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School of Civil, Environmental and Chemical Engineering

Particles, Pathogens and Micropollutants

Australian Water Recycling Centre of Excellence Industry Academic Exchange Fellowship 2013

Final Report

11 August 2014

Particles, Pathogens and Micropollutants

Australian Water Recycling Centre of Excellence Industry Academic Exchange Fellowship 2013

RECIPIENT: RMIT University

COLLABORATOR: Melbourne Water

AWRCoE FELLOW: Karl G. Linden, Ph.D., Helen and Huber Croft Professor of Environmental Engineering, Department of Civil, Environmental and Architectural Engineering, University of Colorado Boulder, Colorado, USA.

INTRODUCTION

The goal of the AWRCOE Fellowship Program is to foster industry and academic partnerships by allowing researchers to spend time embedded within the water industry. The Centre has a specific interest in proposals that enhance research linkages between organisations to form strong and lasting industry-academic partnerships, and assist in broadening the current network of research and development relevant to Australia's interests in water recycling.

This particular Fellowship, which involved RMIT University and Melbourne Water, enabled Prof. Linden to spend time in both academia and the water industry. Prof. Linden worked with Prof. Felicity Roddick and her research team at RMIT to support Melbourne Water and enhance their implementation of water recycling projects, specifically in the area of advanced treatment technologies.

Prof. Linden undertook a total of four visits to Australia, three to Melbourne:

- August 25 to September 13, 2013.
- February 1 to February 22, 2014.
- July 14 to July 27, 2014.

During these visits his time was split between Melbourne Water (MW) and RMIT University (RMIT).

He also visited Brisbane 26 April to 6 May 2014, where he participated in the Ozwater'14 conference and undertook several visits, including to The University of Queensland where he gave a public lecture.

The Fellowship was designed to enable Prof. Linden and MW and RMIT University personnel to work together on three specific projects, as well as to have Prof. Linden interact with water industry academics and professionals, postgraduate and undergraduate students.

SPECIFIC PROJECTS

There were three major topic areas on which Prof. Linden worked with personnel from Melbourne Water and RMIT University with associated deliverables. These are summarised below.

1. Problems with the Particle Association of Pathogens

Melbourne Water is currently seeking to revalidate the Western Treatment Plant (WTP) for the production of Class A recycled water as required by the recently released (March 2013) Victorian Department of Health *Guidelines for validating treatment processes for pathogen reduction: Supporting Class A recycled water schemes in Victoria*. The secondary treatment plant at WTP consists of an anaerobic digestion pond, followed by an activated sludge plant and then a series of polishing lagoons. The water is then treated by UV and free chlorination to produce Class A recycled water. While the WTP system was successfully validated in 2005, the recently released

guidelines are more stringent, requiring revalidation of the WTP secondary and tertiary treatment plants. These new guidelines will affect all Victorian plants producing recycled water, hence revalidation work carried on the WTP lagoons will find application elsewhere, e.g. at Barwon Water. As the WTP tertiary system does not include filtration, particles are present in the water and it was required that MW prove that disinfection by both UV and free chlorine can take place in the presence of particles.

Deliverable: Advice, a comprehensive review and editorial assistance was provided by Prof. Linden on the confidential report on *Revalidation of UV treatment at Western Treatment Plant* by Sam Costello of MW. This report has been submitted to the Victorian Department of Health and provides data that demonstrate that there is no viable *Cryptosporidium* in the Class A recycled water from the WTP, i.e. *Cryptosporidium* is not protected from UV disinfection by particles present in the water. Prof. Linden also provided advice to MW regarding Department of Health comments on the report. While final signoff from the Department of Health has not yet been received, MW is confident that this can be expected soon.

2. Publication of Melbourne Water Ozonation Validation Data

The Tertiary Treatment Plant at the MW Eastern Treatment Plant has a novel treatment process which comprises pre-filtration, ozonation, biological media filtration, post-filtration ozonation, UV and free chlorination. Because the enteric virus concentration in the secondary treated water is low, it cannot be used to demonstrate the virus log reductions achieved by ozone. Prof. Linden has worked with MW to identify a suitable surrogate which can be used to demonstrate enteric virus disinfection by ozone. The work involved in-depth analysis of published literature on the subject, as virus appeared to be differentially sensitive to ozone. Prof. Linden was able to demonstrate that an abundant and commonly present micro-organism can be used as the surrogate for virus disinfection by ozone. The work leading to this outcome has been submitted to the journal *Water Research* and Prof. Linden is currently working on the editor's comments. The peer review gained by publication in peer-reviewed journals increases confidence in the work, while simultaneously making it available for use in NatVal 2.2, particularly the project on validation of ozone.

Deliverable: Prof. Linden, in conjunction with MW, to produce two papers for journal publication. Consideration of the data led to the joint decision between MW and Prof. Linden that a single paper which included both the development of the virus surrogate method and the results was the best way of presenting the outcomes of this work. The paper "Establishing surrogate–virus relationships for ozone disinfection of wastewater" was submitted to *Water Research* on February 24, 2014, and is currently being amended in response to the reviewers' comments. A copy of the current working version is provided in Appendix 1.

3. Investigation of ETP Tertiary Treatment Plant Capacity for Micropollutant Removal

Micropollutant studies were carried out for the pilot tertiary plant, and monitoring of the removal capacity of the fully commissioned Tertiary Plant using micropollutants already present in the secondary treated effluent has been conducted. The data from this work is currently being analysed by Prof. Linden and a paper for publication is being prepared. However, the question arises as to the capacity of the Tertiary Plant to remove unexpected spikes in concentration. A number of potential hazards, identified through analysis and a quantitative risk assessment already carried out by MW, were selected and the removal capacity of the Tertiary Plant is being explored at laboratory scale using RMIT facilities and research staff. This work is directed by Prof. Felicity Roddick at RMIT, with input from Prof. Linden and some research funding provided by MW. The outcomes of this work will provide evidence of the ability of the Tertiary Treatment Plant capacity for micropollutant removal and will be of interest to South East Water which supplies the recycled water to users. The ETP treatment process is novel and generally achieves good micropollutant (>85%) removal; this work will provide further evidence of this efficacy and for some analytes which have not been investigated to date. Publication of this work will draw attention to the benefits of

ozonation for wastewater leading to greater choice in treatment units considered for production of recycled water. This work is currently underway.

Deliverable: A paper on the removal of micropollutants already in the secondary effluent by the tertiary treatment process (partially complete), and a report and possibly a paper on the capacity of the treatment process to remove spikes of a representative selection of potentially hazardous chemicals. The target micropollutants have been selected, the methods for their detection in pure water and secondary effluent validated, and preliminary experiments have been undertaken to determine their kinetics of breakdown at different pH in pure water and secondary effluent using an internal reference compound.

INTERACTION WITH THE WATER COMMUNITY

Prof. Linden undertook a wide range of speaking and meeting commitments, fulfilling the agreed tasks of a public lecture at RMIT University, presentations to undergraduate and postgraduate students, and at Ozwater'14, plus several more. He also made major contributions to the NatVal project through discussions with AWRCOE personnel and participation in several NatVal Protocol Development Group meetings during his four visits. A detailed listing is provided in Appendix 2. The major events can be summarised as follows:

Prof. Linden presented a public lecture "Rethinking disinfection in drinking water systems" to about 75 people from metropolitan and regional water utilities, universities and consulting companies at RMIT on February 19, 2014. The lecture was videotaped and is available through the Australian Water Recycling Centre of Excellence webpage and also via a link from the RMIT Water: Effective Technologies and Tools Research Centre webpage. The slides are available from the Victorian branch of the AWA.

The NatVal project benefited from several contributions made by Prof. Linden. These included participation in the NatVal Protocol Development Group (PDG) for the National Validation Framework for Water Treatment Technologies on Sept 12, 2013, and a presentation on "Lessons from the USEPA UV Validation Process". He prepared and delivered a presentation on the "Makings of a Validation Center" for the NatVal PDG on February 12, 2014, which gave an overview of the details of the two major validation centres in the USA for UV and other technology verifications. He also actively participated in the NatVal PDG meeting on 25 July, 2014.

Prof. Linden's contribution to Ozwater'14 was via the planning and presentation of the workshop "Toward National Validation Guidelines for Water Recycling in Australia" at Ozwater'14 with Sue Keay and Mark O'Donohue.

The keynote address "UV Disinfection: New Developments for Small Systems" at the Water Research Australia (Water RA) workshop "Science talks to Industry" was given by Prof. Linden in Melbourne on July 16, 2014. The workshop was attended by approximately 100 members and other water-related industry professionals. The presentation is available through the Water RA website.

Approximately 55 academic staff and postgraduate students from the School of Civil, Environmental and Chemical Engineering at RMIT attended Prof. Linden's presentation "Water Sustainability in Oil and Gas Exploration: Treating Frack Water for Reuse" on Friday 25 July, 2014.

BENEFITS FROM THE FELLOWSHIP

Academic researchers are always keen to see the results of their work translated into practice and to make a positive impact on society. This Fellowship enabled Prof. Linden to build on his connections with MW and RMIT to continue his outreach and knowledge transfer activities in Australia, and to supplement his US- and developing community-based work in disinfection, public health, oxidation processes, and water reuse. As MW is one of the leaders in utilising research innovations to solve important water quality challenges, the Fellowship allowed Prof. Linden to gain an insight into water utility research needs and constraints, and how a water utility applies research. It also provided him the opportunity to build on his understanding of international issues in water quality, stay on the leading edge of practice in water treatment and reuse, and further solidify relationships with academics at RMIT to develop joint research publications and proposals that will benefit further collaborations.

The Fellowship has resulted in one publication, with two others in progress. These encompassed bringing unpublished data on the validation of ozonation processes into the public domain, i.e., demonstration of the use of an abundant and commonly present micro-organism as a surrogate for enteric virus disinfection by ozone, and demonstration of the capability of ozonation to remove continually present and also spikes of potentially hazardous micropollutants. Knowledge dissemination was also achieved via delivery of the keynote presentation at the Water Research Australia (WaterRA) Workshop, contributions to the NatVal PDG meetings and Ozwater'14 workshop, the public lecture and School seminar at RMIT, a lecture to RMIT undergraduate students and mentoring of postgraduate students and postdoctoral researchers at RMIT and MW personnel.

While the benefits to MW, RMIT and Prof. Linden are obvious, the benefits to others will come from publication of the papers and reports that will form the basis for some of the NatVal 2.2 work for the Centre, thus benefiting the water industry, local councils and those carrying out Integrated Water Management. Disinfection of lagoon water in the presence of particles will become more fully understood thus improving risk management of recycled water from lagoon plants. As many regional plants use lagoon processes, many of the regional water authorities will be the beneficiaries from Melbourne Water's validation study.

Discussion regarding future collaboration between Prof. Linden and RMIT has covered two possible areas: extension of the determination and modelling of micropollutant removal in lagoons, particularly at Western Treatment Plant, and, investigation of the impact of disinfection by-products resulting from the chlorination of treated effluent on the marine environment. Prof. Roddick will visit Prof. Linden at University of Colorado Boulder in late November 2014.

CONCLUSION

The goal of the Fellowship was to foster industry and academic partnerships. This was achieved through primarily Prof. Linden working with personnel at Melbourne Water and academics at RMIT University on three specific projects involving the particle association of pathogens, publication of Melbourne Water ozonation data, and the capacity of the ETP tertiary treatment process for micropollutant removal. This work has contributed to understanding of the efficacy of the wastewater treatment processes, particularly on the impact of particles on UV disinfection and the application of ozone for disinfection and micropollutant removal, and has resulted in the submission of one peer reviewed journal paper and the preparation of two other papers is in progress. These outcomes will form the basis for some of the NatVal 2.2 work for the AWRCOE, and so benefit the water industry, local councils and those conducting integrated water management.

Other benefits from the Fellowship were that Prof. Linden was able to interact with other members of the water industry and academia through public presentations, one-on-one discussions and visits to other universities. Several of these connections are scheduled for subsequent collaboration.

APPENDIX 1: PAPER

"Establishing surrogate – virus relationships for ozone disinfection of wastewater"

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Manuscript Draft

Manuscript Number:

Title: Establishing Surrogate - Virus Relationships for Ozone Disinfection of Wastewater

Article Type: Research Paper

Keywords: recycled water; reuse; phage; pathogens; human virus

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Abstract: Pressure on fresh water resources is leading many municipalities to plan for more extensive reuse of wastewater to replace or off-set freshwater needs. One of the major concerns for water reuse is the potential transmission of human pathogenic agents such as viruses, bacteria, and protozoa. Therefore, safe reuse of wastewater requires high levels of pathogen inactivation. Ozone is a very effective disinfectant for viruses and has distinct benefits over other forms of disinfectants in that it increases the clarity of water and can oxidize some chemical contaminants in water. However, because of the combination of the high ozone demand of most wastewaters and rapid reaction kinetics with viruses, the determination of ozone dose in wastewater and recycled water treatment is not well defined. Various surrogates are used as indicator organisms for human pathogenic viruses in water reuse practice. However, there is little information on the relationship between surrogates and human pathogenic viruses for ozone disinfection in wastewater. In this study, we compared the ozone inactivation kinetics of several surrogates (E. coli, coliphage T1, T4, PRD-1, φ174, and MS2) and human pathogenic viruses (poliovirus 1, ecovirus 11, coxsackievirus B5, and adenovirus 2) in carefully controlled experiments over a range of pH and temperature levels typical of secondary effluent wastewater. A reduction equivalent dose method was used to compare the inactivation of the microorganisms studied across waters with varying ozone demand. The inactivation of all viruses and surrogates studied was greater than 4 log at ozone Ct levels of less than 1 (mg/L)-min. Among the surrogates tested, E. coli and PRD-1 were identified as suitable surrogates for human pathogenic viruses in ozone disinfection of wastewater.

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Cover Letter, For Editor only

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March 28, 2014

Editor-in-Chief, Water Research

Dear Editor,
Please find attached via online submission the manuscript "Establishing surrogate – virus relationships for ozone disinfection of wastewater" for your consideration to publish in the journal *Water Research*.

This research was funded by Melbourne Water who has under development, major water recycling schemes in the State of Victoria in Australia. Their Eastern Treatment plant has ozone as a treatment process for wastewater disinfection and they are obtaining some credit for Cryptosporidium inactivation. They were interested in the use of ozone in wastewater for obtaining credit for inactivation of viruses. They have been working with the State of Victoria Health Department to validate ozonation as a disinfection process for a few years. However the fact that it is very difficult to measure a persistent ozone residual in wastewater led them to fund a study that would develop a series of surrogates to represent virus inactivation and carry out carefully controlled disinfection experiments to determine the rapid inactivation kinetics for viruses during ozonation.

While various surrogates are used as indicator organisms for human pathogenic viruses in water reuse practice, there is little information on the relationship between surrogates and human pathogenic viruses for ozone disinfection in wastewater. In this study, we devised experiments to compare the ozone inactivation kinetics of commonly used surrogates and a series of human pathogenic viruses. We also developed a reduction equivalent dose method to compare the inactivation of the microorganisms studied across waters with varying ozone demand, common in wastewater. This method has the potential to become a standard for evaluating ozonation of wastewater.

This research work is original from our laboratory and has not been published before. We believe this manuscript is of interest to the readership of *Water Research* especially for those interested in disinfection, wastewater treatment, and ozonation applications.

We look forward to your review of this manuscript. Please contact me [phone (303-492-4798) or email (karl.linden@colorado.edu)] for all future correspondence.

Sincerely,

Karl Linden

Karl Linden, Ph.D.; Helen and Huber Croft Professor of Environmental Engineering

*Highlights (for review)

Establishing surrogate – virus relationships for ozone disinfection of wastewater

HIGHLIGHTS

- We investigate the comparative ozone disinfection of common surrogates and viruses
- High ozone demand of wastewater required new data analysis approaches
- Inactivation of all viruses was > 4 log at ozone Ct levels of < 1 (mg/L)-min
- *E. coli* and PRD-1 were the best viral surrogates in wastewater ozone disinfection

ESTABLISHING SURROGATE – VIRUS RELATIONSHIPS FOR OZONE DISINFECTION OF WASTEWATER

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ABSTRACT

Pressure on fresh water resources is leading many municipalities to plan for more extensive reuse of wastewater to replace or off-set freshwater needs. One of the major concerns for water reuse is the potential transmission of human pathogenic agents such as viruses, bacteria, and protozoa. Therefore, safe reuse of wastewater requires high levels of pathogen inactivation. Ozone is a very effective disinfectant for viruses and has distinct benefits over other forms of disinfectants in that it increases the clarity of water and can oxidize some chemical contaminants in water. However, because of the combination of the high ozone demand of most wastewaters and rapid reaction kinetics with viruses, the determination of ozone dose in wastewater and recycled water treatment is not well defined. Various surrogates are used as indicator organisms for human pathogenic viruses in water reuse practice. However, there is little information on the relationship between surrogates and human pathogenic viruses for ozone disinfection in wastewater. In this study, we compared the ozone inactivation kinetics of several surrogates (*E. coli*, coliphage T1, T4, PRD-1, ϕ174, and MS2) and human pathogenic viruses (poliovirus 1, ecovirus 11, coxsackievirus B5, and adenovirus 2) in carefully controlled experiments over a range of pH and temperature levels typical of secondary effluent wastewater. A reduction equivalent dose method was used to compare the inactivation of the microorganisms studied across waters with varying ozone demand. The inactivation of all viruses and surrogates studied was greater than 4 log at ozone Ct levels of less than 1 (mg/L)-min. Among the surrogates tested, *E. coli* and PRD-1 were identified as suitable surrogates for human pathogenic viruses in ozone disinfection of wastewater.

42 Keywords: inactivation, wastewater, recycled water, reuse, phage, pathogens, adenovirus,
43 echovirus, coxsackievirus, poliovirus

Introduction

Ozonation of water and wastewater for disinfection is a beneficial method to improve microbiological quality and protect public health. Hundreds of US water utilities and thousands of European utilities depend on ozonation to meet increasingly stringent regulations. However, while ozonation of drinking water is widely used, the use of ozone for the disinfection of wastewater is much more narrow. Wastewater ozone facilities make up less than 2.5% of the number of drinking water utilities using ozone disinfection in the United States (Oneby et al., 2010). As beneficial reuse of wastewater in high exposure non-drinking applications such as irrigation of open space, residential third pipe and edible agriculture increases, the microbiological quality of that water must be protective of public health. The transmission of viruses through wastewater irrigation is an area of concern for regulators. Due to the varying nature of the presence of viruses in wastewater and the difficulty in monitoring for infective viruses in real time, there is a need to understand the comparative inactivation of viral surrogates along side pathogenic viruses. Surrogates that are conservative in their inactivation compared to pathogenic viruses could be used to indicate effectiveness for disinfection using ozone. The objectives of this study were to investigate the relationship between ozone disinfection of a suite of pathogenic viruses and a number of proposed surrogates to determine if the inactivation of a viral surrogate could indicate effective inactivation of pathogenic viruses. The research also focused on defining the ozone dose-response kinetics of the microorganisms studied, while addressing the complexities of defining the ozone dose under varying ozone demand water matrices.

Background

Viruses

The US EPA first reported ozone dose (concentration x time, Ct) values for disinfection of viruses in 1991 (USEPA 1991) and subsequently re-reported these values in 2002 and 2003 manuals (USEPA 2002, 2003). These values were originally published by Roy (1982) and Vaughn (1987) and indicate that at higher temperatures, the required Ct decreases, while within the range of most natural waters, pH does not affect ozone performance. Under the worse case conditions of cold temperature (<1°C), 4 log inactivation of viruses requires a Ct of 1.8 (mg/L)-min, whereas for a temperature of 25°C the Ct is 0.3. While these reports cite Ct values generalized for all viruses, the literature for inactivation of specific viruses indicates more variability in Ct. Ideally the Ct value is an integration of concentration (ozone residual) over time, although this is not always reported. In the literature, the Ct values are typically noted as the initial concentration multiplied by exposure time (providing a very conservative Ct) or as average concentration (if reported such as in a flow through system) multiplied by time.

In lab or drinking water quality matrices, virus inactivation was typically rapid. Poliovirus is very sensitive to ozone disinfection, with the literature indicating greater than 4 log inactivation at ozone Ct values of 0.13 (mg/L)-min or lower (Shin and Sobsey 2003; Emerson et al., 1982). Cell associated polioviruses were inactivated to greater than 4 log at a Ct of 0.8 (mg/L)-min (Emerson et al., 1982). Vaughn et al. (1987) reported human and simian rotavirus inactivation of 5 log under 4°C conditions and pH values of 6 to 8 for ozone doses of 0.04 (mg/L)-min. Vaughn et al., (1990) also reported that Hepatitis A virus was very susceptible to ozone with more than 5 log inactivation achieved at a Ct of less than 1.0 (mg/L)-min. Adenovirus was very susceptible

to ozone with more than 4 log inactivation achieved at a Ct of 0.07-0.6 (mg/L)-min (Thurston, et al., 2005). Feline calicivirus was reached more than 4 log inactivation at a Ct of 0.01-0.03 (mg/L)-min (Thurston, et al., 2005). Roy (1982) studied ozonation of 2 types of Echoviruses and found a Ct of 0.3 (mg/L)-min resulted in a log inactivation of 2.5 to 3.5. They also reported coxsackievirus required a Ct of 0.3 for log inactivation of 3 to 4. Cell associated coxsackievirus required a higher Ct of 1.3 (mg/L)-min to achieve 4 log reduction (Emerson et al., 1982). Chang and Snyder (1974) reported echovirus 12 to be the most resistant virus to ozone, with poliovirus 2 as the second and coxsackie B5 and coxsackie B3 as the third and fourth most resistant in ozone demand free water and in treated river water. Echovirus 29 was the most susceptible, followed by adenovirus 79 and poliovirus 1 and 3.

Surrogates

Surrogates for viruses in ozone studies, such as added phage, bacteria or native organisms have been studied with mixed results. *E. coli* were found to be very sensitive to ozone but the reported specific sensitivity varied between researchers. Hunt and Mariñas (1999) found 6 log inactivation at less than 0.01 (mg/L)-min Ct, whereas Tanner et al. (2004) reported 4 log inactivation at 0.25 Ct. Native coliform studies also indicated sensitivity to ozone with less than 2 coliforms per 100 mL detected after a Ct of 1 in wastewater (Ishida et al., 2008) and 0.2 mg/L in natural water (Keller *et al.*, 1974). In cases where viruses and coliform were monitored simultaneously, < 2 coliform per 100 mL always corresponded with complete virus inactivation.

Phage f2 appeared to be very sensitive to ozone. More than 7 log inactivation was achieved at an ozone Ct of 0.08 (mg/L)-min (Kim *et al.*, 1980) while MS2 coliphage was also sensitive with a

6 log inactivation at a Ct of 0.4 or less (Tanner et al., 2004; Shin and Sobsey, 2003; Finch and Fairbairn, 1991; Ishida et al., 2008).

The spores of the anaerobe *Clostridium perfringens* were also shown to be extremely resistant to ozone. On a Ct basis, Tyrell (1995) reported only 0.2 log inactivation at a Ct of 0.3 (mg/L)-min in wastewater. An applied ozone dose of almost 30 mg/L resulted in a 1.7 log inactivation after 10 minutes in a wastewater matrix (Xu et al., 2002).

Ozone Disinfection in Wastewater

The USEPA Wastewater Disinfection Manual (1986) recommends an absorbed (transferred) ozone dose of 15-20 mg/L to achieve 2.2 total coliforms/100 mL in a filtered nitrified effluent and a dose of 3-5 mg/L to achieve 200 cfu/100 mL fecal coliforms. Of the 5 ozone disinfection wastewater plants built since 1985, the designed target transferred doses range between 4 and 8 mg/L (Oneby, 2010). Gehr and Nicell (1996) presented data showing applied ozone doses of 17 to 20 mg/L reduced fecal coliform by 98%. The calculated Ct required to achieve 4 log reduction was 2.9 (mg/L)-min and, interestingly, 3 log reduction was achieved in the absence of any ozone residual (calculated Ct of zero). Xu *et al.* (2002) illustrated a few important points when ozonating wastewater. They suggest that the transferred ozone dose is the critical parameter in ozonation of wastewater, not the Ct as proposed for natural waters. After single step filtration, they reported that ozonation can meet stringent California Title 22 standards for reuse, including total inactivation of viruses. Burns et al. (2007) reported on ozone doses required for virus inactivation in wastewater where an applied ozone dose of 3, 5, and 8 mg/L resulted in an ozone concentration after 30 seconds of <0.1, 0.6, and 2.5 mg/L respectively.

133 Based on ozone concentration over time, Ct values were calculated and disinfection credits for
134 Giardia and viruses were determined from the EPA tables used for drinking water disinfection -
135 5 mg/L applied ozone (equivalent to a Ct = 0.3 (mg/L)-min) was calculated to be capable of
136 achieving a 6 log virus inactivation credit.
137
138 Ishida et al. (2008) based their data on a transferred ozone dose and correlated a 5 log poliovirus
139 inactivation to 6.5 log MS2 inactivation in wastewater, to meet California Title 22 standards. A
140 transferred ozone dose of 3-5 mg/L was sufficient to achieve the 6.5 log MS2 inactivation for
141 contact times above 10 seconds in microfiltered effluent. In media filtered effluent, a transferred
142 ozone dose of greater than 7, after 10 seconds, was required to achieve the same level of
143 inactivation.
144
145 Overall, in clean water, both viruses and bacteria or phage-type surrogates are inactivated very
146 quickly and at low Ct levels, generally well below 0.5 (mg/L)-min and in all cases, including
147 cell-associated viruses, below 1.5 (mg/L)-min. In the few studies performed in wastewater, the
148 inactivation was somewhat slower. However, the wastewater studies did not typically have
149 clearly defined Ct calculations, so a precise comparative evaluation is not possible. The use of
150 MS2 phage as a surrogate for viruses in wastewater was cautioned against by Helmer and Finch
151 (1993) who noted that MS2 was overly sensitive to ozone. Conversely, the use of a spore such
152 as *Clostridium* is not relevant to virus inactivation due to its extreme resistance to ozonation, and
153 Xu et al. (2002) recommend against this. There is thus a need for a comparative study of virus
154 and surrogate inactivation by ozone to determine if a suitable relationship exists that would allow
155 for confidence in approving virus disinfection credits based on inactivation of spiked or native

surrogates.

MATERIALS AND METHODS

Selection of Viruses

Based on the literature review, and practicalities of virus propagation, the following viruses were chosen:

- Poliovirus 1 (PV1) - is the most extensively investigated of the enteric viruses in the literature and its inclusion allows comparison with previous data.
- Coxsackievirus B5 (CVB5) – Due to aggregation at pH values typical of secondary effluent this virus may have added resistance to ozone disinfection, and it is known to be more resistant to free chlorine than HAV or PV1.
- Adenovirus 2 (Ad2) – represents double stranded DNA viruses, which may exhibit variation in their resistance to disinfection compared to single stranded RNA or DNA viruses.
- Echovirus 11 (EV-11) – allows comparison to the other two enteroviruses (PV1 and CVB5) and to previous studies.

The following microorganisms were chosen to evaluate as surrogates:

- *E. coli* – based on previous literature and its importance as a wastewater indicator organism
- MS2 coliphage – based on its extensive use as a viral surrogate in disinfection studies
- T1 and T4 phage – double stranded DNA phage, providing diversity of structure among phage examined

- ΦX174 phage – a single stranded DNA phage
- PRD-1 phage – a double stranded DNA phage that has shown high resistance to UV disinfection

Methods for Generating and Measuring Ozone Stock Solutions

Ozone was produced by an ozone generator; either a Wedeco GSO30, an Orec 03V5-0, or an Ozone Solutions TG-40. In all cases, ozone was bubbled into a continuously mixed batch of laboratory grade ultrapure (DI) water, which was cooled to 4°C. Ozone was bubbled into the reactor for at least 30 minutes before the concentration of the solution was determined. Ozone concentration was determined by measuring absorbance at 258 nm in a 1-cm quartz cuvette. A laboratory grade ultrapure water was used as a zero control. A 4:1 dilution of the ozone stock was required to keep the solution within the range of the UV absorbance instrument (Hach DR5000). The solution was prepared by pipetting 3 mL of deionized water into the quartz cuvette and placing it into the spectrophotometer. Ozone stock solution (1 mL) was then pipetted into the cuvette with a consistent and quick motion, making sure that the tip was inserted into the water to avoid volatilization and to assist in mixing. The absorbance reading was immediately taken. When the absorbance of a 4x dilution was at or above 1.000, an 8x dilution was used, adding 0.50 mL ozone stock solution to 3.5 mL of DI water.

Ozone concentration was then calculated using the Beer-Lambert Law:

$$\frac{mg\ O_3}{L} = \frac{\Delta A}{b * \varepsilon} * f$$

Where:

201 ΔA = *difference in* UV_{258} *absorbance between sample and blank*

202 b = *path length of cell, cm*

203 ε = *molar absorption coefficient of ozone at 258 nm (3100* $M^{-1}cm^{-1}$*)*

204 $f = 48{,}000\ mg\ O_3\ mol^{-1}$

205

206 ## Solutions and Glassware Preparation

207 Ozone demand free (ODF) water was used to make phosphate buffered saline (PBS) solution and
208 sodium thiosulfate. To prepare ODF water, ozone gas was bubbled directly into 2,000 mL of
209 ultrapure laboratory grade water for 30 minutes while stirring, with chemicals added after water
210 preparation. Solution was allowed to sit for 3 days, or a full day after the absence of ozone was
211 confirmed. ODF glassware was made by filling containers with > 15 ppm ozone solution and
212 letting them sit for over 3 hours (often overnight), at which point containers were emptied and
213 dried in a 100°C furnace.

214 ## Wastewater Shipping

215 Secondary effluent filtered by ultrafiltration membranes to minimize biological activity was
216 shipped frozen from Melbourne Water's Eastern Treatment Plant (ETP) in Melbourne Australia.
217 Before use, water was thawed at 4°C and filtered using sterile glassware and Whatman GF/F
218 0.7 micron glass fiber filters previously baked at 450°C for 3h to remove any particles formed
219 during storage. Filtered water was stored in baked glassware before use.

220

221 The water quality characteristics for ETP Wastewater were measured and reported in Table 1.
222 Water quality was not altered for experiments and all wastewater disinfection experiments were

223 conducted at the natural pH of 7.96 and at 16°C. All methods conformed to those in Standard
224 Methods (APHA *et al.*, 2012).
225
226 **Table 1 goes here**
227
228 ## Experimental Procedure
229 The target microbe stock was spiked into a completely mixed vessel containing enough volume
230 (typically 100 to 300 mL) of PBS or wastewater for the creation of ozone decay curves and for
231 performing disinfection experiments. Batch reactors (5-20 mL) held in a constant temperature
232 water bath were filled from this stock solution using a 10 mL pipette just prior to ozone dosing.
233 ## Ozone Dosing
234 The standard batch solution ozone test (SOT) method was used to apply the ozone dose to the
235 batch reactor (Hoigné and Bader, 1994). Ozone from the concentrated stock was pipetted into
236 the batch reactor, using the same procedure used to measure the ozone stock solution
237 concentration. Sample dilution was minimized by using a high concentration (70-80 ppm) ozone
238 stock solution. Dilution factors for wastewater and clean water disinfection studies are illustrated
239 in Figures S1 and S2 in the Supplementary Information. Dilution factors associated with clean
240 water disinfection were about an order of magnitude lower than for wastewater, even though a
241 lower ozone stock concentration was used for clean water ozone doses.
242 ## Sampling
243 Sampling for ozone residual and microbiological assays was performed in the same manner;
244 sample was extracted at regular intervals from the batch reactor beginning 4s before the desired

245 time point and inserted into the ozone quenching solution at the desired time point. Two ozone
246 quenching solutions were used: Indigo dye (in three variations described below) was used for
247 measuring ozone residual and 0.03% sodium thiosulfate (STS) was used in an equal volume to
248 the ozonated sample as a quenching agent for microbiological samples (concentration achieved
249 rapid complete quenching while minimizing any toxicity to the virus cell culture assays, data not
250 shown). When sampling for microbiological assays using STS as a quenching agent, samples
251 were vortexed immediately following the sampling to ensure that the solution was completely
252 mixed and that the ozone had reacted completely with the STS. The first time point and
253 subsequent sampling intervals were 10s from the start of ozone dosing.
254

255 ## Measuring Ozone Residual

256 Ozone residual concentrations were measured using the Indigo Colorimetric Method (Bader and
257 Hoigné, 1981; APHA et al., 2012). Indigo solutions I, II and modified II was used for ozone
258 concentrations in the range of 0.01 to 0.1 ppm, 0.05 to 0.5 ppm, and higher than 0.3 ppm,
259 respectively. In wastewater at high ozone doses, a combination of the variations in indigo
260 methods was used to create a curve covering their complete respective range. The stock indigo
261 solution was stable for four months whereas the Indigo I and Indigo II solutions were freshly
262 made each week (APHA *et al.*, 2012). The method described in Standard Methods was scaled
263 down from 100 mL to work in 1 mL polystyrene cuvettes where 0.9 mL of sample was added to
264 0.1 mL of Indigo I solution to determine residuals between 0.01 and 0.1 ppm O_3.
265

266 ## Microbiological Methods

267

Phage Propagation and Enumeration

The propagation and enumeration of phages used in this study followed similar procedures other than utilizing different hosts for different phages. The phage / host pairs were MS2/*E. coli* C3000, T1 and T4/*E. coli* B, ΦX174/*E. coli* CN13, and PRD-1/ *Salmonella typhimurium* LT2. First, phages were grown and enumerated in their appropriate hosts by the double agar layer technique (Adams, 1959). The top agar layer exhibiting confluent lysis of the host cells was harvested by scraping into a small amount of PBS, and phages were extracted by homogenizing in an equal volume of chloroform. The supernatant was recovered following low speed (4,000 X g) centrifugation for 30 minutes at 4°C.

E. coli Propagation and Enumeration

The procedures for the propagation and enumeration of *E. coli* CN13 and F amp were identical. The streak plate technique was used for colony isolation for each disinfection experiment; a loop was mixed into 50 mL of tryptic soy broth (TSB) with shaking incubation at 37°C until the optical density was around 0.800 absorbance units, which corresponds to late log growth phase and approximately 10^9 CFU/mL. The desired volume was centrifuged for 15 minutes and then washed with PBS (filled with PBS, re-dispersed, re-centrifuged), and then washed/centrifuged three more times to isolate clean bacteria from TSB before spiking into stock solution. Spread plating was used to enumerate *E. coli*, using 100 mm nutrient agar plates and 0.10 mL of sample with triplicate plates for each sample dilution. Plates were incubated at 37°C for 24 hours. Three PBS blanks were run with every experiment.

Adenovirus Propagation and Assay

Adenovirus 2 (Ad2, ATCC VR-846) was obtained from the American Type Culture Collection (Manassas, VA) and maintained on the A549 cell line. The propagation of adenovirus was

291 similar to enteroviruses described below except a different cell line (A549) was used.
292 Adenovirus was assayed by 50% Tissue Culture Infectious Dose ($TCID_{50}$) method on confluent
293 layers of A549 cells grown in 24-well tissue culture plates. Briefly, serial dilutions of sample
294 were performed in PBS and 100 µL of each dilution was added to each well for a total of 4 wells
295 per dilution. During infection, sample inocula were incubated with the cell monolayers for
296 1 hour at 37°C and 5% CO_2, carefully moving the plates horizontally every 15 minutes to ensure
297 even distribution of the inoculum. After 1 hour of infection, maintenance media was added to
298 the cell monolayers. Maintenance media consisted of complete F12k minimal media with 2%
299 heat inactivated fetal bovine serum. The infectivity of Ad2 was determined by observing CPE
300 on the confluent A549 cells over 14 days following inoculation of disinfected samples and
301 controls.

302 **Coxsackievirus, Poliovirus, and Echovirus Propagation and Assay**

303 Echovirus 11 (EV-11; ATCC VR-31), and coxsackievirus B5 (CVB5; Faulkner, ATCC VR-185)
304 were obtained from the American Type Culture Collection (Manassas, VA). Poliovirus 1 (PV1;
305 strain LSc-2ab) was obtained from Mark Sobsey at the University of North Carolina Chapel Hill.
306 Viruses were maintained on BGM (Buffalo Green Monkey Kidney) cell line monolayers with
307 Minimum Essential Medium (MEM) containing 5% calf serum (CS; HyClone Laboratories,
308 Logan, UT) at an incubation temperature of 37°C with 5% CO_2. These viruses were propagated
309 by inoculating stock viruses into cell monolayers that were ~90% confluent. Following the
310 observation of $\geq$ 90% destruction of the monolayer, the cell culture flasks were frozen (at -80°C)
311 and thawed (at 37°C) three successive times to release the viruses from the host cells. Cell lysate
312 was mixed with equal volume of chloroform, vortexed vigorously for 1 min, and then

313 centrifuged at 2,500 g for 15 minutes at 4°C. The top aqueous layer containing the virus was
314 carefully removed using a pipette and the purified viruses were stored at -80°C until use.
315
316 Viral titrations for PV1, EV-11, and CVB5 were performed using 10-fold serial dilution plaque-
317 forming assays described by Bidawid *et al.* (2003). Briefly, host cell monolayers in 6-well tissue
318 culture plates were inoculated with 0.1 ml volumes of 10-fold serial dilutions (in duplicate) of
319 the virus stock and incubated at 37°C for 1 hour to allow for virus adsorption to the cells.
320 Following this incubation period, 3 ml of a molten solution of MEM containing 1.5% Bacto agar,
321 2% FBS, 1 M HEPES buffer, 7.5% sodium bicarbonate, 10 mg/ml kanamycin, 100x antimycotic
322 (HyClone Laboratories, Logan, UT), and 200 mM glutamine (Glutamax; HyClone Laboratories,
323 Logan, UT) was added as an overlay to each well and allowed to solidify. The plates were then
324 incubated at 37°C with 5% CO2 for 2 days. Following this incubation, the agar overlays were
325 removed and the cell monolayers were stained with 0.5% crystal violet (Sigma-Aldrich, St.
326 Louis, MO) dissolved in ultrapure water and mixed 1:1 with 95% ethanol. The plaques (clearings
327 in the cell monolayer) were counted to enumerate infectious viruses.

328 ## Calculating Ozone Dose

329 Two different approaches were used for calculating ozone dose using the ozone residuals values
330 generated by the Indigo method: discrete summation Ct (Hunt and Mariñas, 1999) and extended
331 T_{10} Ct (EPA, 2003). These methods are described in detail in the supplementary materials.

332 ## Ct Approaches used in this Study

333 While both the discrete summation and Extended T_{10} Ct calculation methods were used in this
334 study, the two methods can yield quite different results due to the difference in how they address
335 applied ozone dose and initial ozone demand. The choice of Ct calculation influences

interpretation of the results with respect to the relative resistance of the viruses and surrogates. This issue, including a recommendation for the most appropriate method, is discussed in the following sections.

RESULTS

Water quality characteristics for ETP Wastewater are found in ***Table 1***. Water quality was not altered in any way for experiments. All wastewater disinfection experiments were conducted at the natural pH of 7.96 and at 16 C.

Ozone Inactivation of Virus Surrogates in Clean Water: pH and Temperature

Several bacteriophage were chosen as potential virus surrogates, including MS2, T1, T4, PRD-1, and ΦX174 along with two strains of *E. coli*: CN13 and F amp. Surrogates were tested in a matrix of six different conditions over two temperatures and three pH values. Figure 1 illustrates the results of the surrogate disinfection experiments performed in clean buffered water.

Figure 1 goes here

The majority of the data are bunched together in the top left indicating high inactivation at low ozone doses. *E. coli* and most phages showed similar resistance while PRD-1 and ΦX174 were slightly more resistant than the other surrogates at very low Ct values; however, increased ozone demand associated with impurities in phage stock may have contributed to this. Regardless of any ozone demand or calculation method biases, the required ozone Ct for high levels of surrogate inactivation was well below 1 (mg/L)-min. Neither temperature in the 16 to 23°C range nor pH in the range 6 to 8 significantly affected ozone disinfection (data not shown) at

these very low Ct values, a finding supported by US EPA (1991) who indicated only very minor inactivation differences over these ranges.

Ozone Inactivation of Candidate Viruses: Defining the Dose-response in Clean Water

Virus data presented in Figure 2 indicate that Ad2 appears to be the most resistant virus to ozone. However, due to varying levels of impurity in virus stocks, the ozone demand may bias the discrete summation Ct calculation. When ozone dose is plotted using the Extended T_{10} calculation method, which reduces the impact of the ozone demand of the solution on Ct, Ad2 was still the most resistant microorganism tested. Regardless of any ozone demand or calculation method biases, the required ozone Ct for high levels (3-4 log and greater) of virus inactivation was well below 1 (mg/L)-min.

Figure 2 goes here

Ozone Inactivation of Candidate Viruses and Surrogates: Defining the Dose-Response in Eastern Treatment Plant wastewater

The dose-responses of surrogates and viruses in ETP wastewater was evaluated using relatively resistant surrogates (PRD-1, ΦX174, F amp *E. coli*, and CN13 *E. coli*) and viruses (PV1, Ad2, and CVB5). The ozone-dose-response of the surrogates and viruses using both the Discrete Summation Ct calculation method and the Extended T_{10} Ct method are illustrated in Figures 3 and 4, respectively.

Figure 3 shows that phage appear to be more resistant to ozone than *E. coli* in wastewater, although phage stock imparts ozone demand while *E. coli* stock does not, which would result in an apparent increase in resistance in the case of phage.

Figure 3 goes here

Figure 4 goes here

Similar to clean water disinfection experiments, adenovirus appeared to be the most resistant one among the viruses tested. Due to the high ozone demand of the adenovirus solution compared to the other viruses tested, however, a higher purity adenovirus stock was obtained (courtesy of Clancy Environmental Consultants). This stock, labeled "LD" (low demand), was also tested in the ETP wastewater to investigate the issue of virus stock demand in biasing of the results. When using the Discrete Summation Ct method, the differences between the low and high demand stock impact the apparent inactivation kinetics of the adenovirus (see Figure 4 top). Interestingly, these results do not differ as significantly when using the Extended T_{10} Ct calculation method. As noted in the Supplementary Information, the Extended T_{10} method reduces the impact of the ozone demand of the water because it uses a calculated C_1 dose (*i.e.* theoretical ozone concentration at time T_0 based on first-order ozone decay kinetics) as opposed to the applied ozone dose, which is used for the Discrete Summation Ct method. This has the effect of minimizing the impact of the initial ozone demand on the Ct calculation and therefore compressing the dose response. Data in *Figure 4 bottom* are bunched up below 0.04 (mg/L)-min. In Figure 3, the Extended T_{10} method results in phages that have higher ozone demand appearing to be less resistant to ozone than *E. coli*, which has almost no ozone demand.

In ETP wastewater, when either the Extended T_{10} or Discrete Summation method is used, adenovirus appears to be the most resistant of the three viruses. However, looking at these Extended T_{10} Ct data, it is clear that the viruses in general are susceptible to ozone at very low Ct values (less than 0.1 (mg/L)-min) and there was not an appreciable difference in sensitivity between them.

The Ozone Demand Issue and Its Implications

The issue of varying ozone demand for stock solutions of spiked microorganisms and its impact on apparent ozone dose-response kinetics has largely been ignored in the literature. The first indication that ozone demand is an important consideration is the difference in ozone disinfection efficiency between clean water versus wastewater. In wastewater, surrogates appear to exhibit increased resistance compared to clean water results. In theory, an organism should have the same response to a disinfectant regardless of the matrix it is in, unless there is an unexpected effect of that matrix (particle shielding for instance), or the measurement of the disinfectant dose is affected by the matrix, leading to data that cannot be compared. Note that the ETP wastewater was pre-filtered and therefore particle shielding is not considered relevant for these results.

This effect is clear as illustrated in *Figure 5*. In ETP wastewater, a Discrete Summation Ct of around 1.0 (mg/L)-min was required for 4 log inactivation of *E. coli* compared to less than 0.040 (mg/L)-min in clean buffered water. This discrepancy in the dose-response data for the exact strains and stocks of *E. coli* is caused by the ozone demand exerted by wastewater organic

425 matter and other constituents rapidly competing for (scavenging) ozone molecules, thus affecting
426 the calculation of Ct.
427
428 **Figure 5 goes here**
429
430 *E. coli* appears to exhibit increased resistance in wastewater compared to clean buffered water
431 when the Discrete Summation Ct is used. This fact provides an obstacle in any ozone
432 experiment that has to be overcome. The effects of varying ozone demand on data interpretation
433 can be neutralized in a number of ways. One way is to prepare surrogate and virus stocks with
434 the least demand possible. However, for many microorganisms such as phage, stocks produced
435 with a titer sufficiently high to demonstrate greater than 4 log inactivation inevitably retain some
436 of the organic matter associated with propagation and thus introduce some level of ozone
437 demand to the test water matrix.
438
439 Results of wastewater disinfection of *E. coli* and these two high-demand and "apparently-
440 resistant" phages presented in Figure 3 indicate a Discrete Summation Ct of approximately
441 0.8 mg/L-min was required for 4 log inactivation of *E. coli*, whereas the two phages tested
442 required a Ct of greater than 1.5 (mg/L)-min for 4 log inactivation. However, the quantification
443 of Ct is clearly affected by the water oxidant demand. Practically, in the SOT experiments, it is
444 nearly impossible to collect multiple samples over the first 10 seconds of the ozone contact time
445 to measure residual ozone, such that in the case of comparing a high and low ozone demand
446 water the calculated Ct may differ greatly. An illustration of this effect is provided in Figure S3

447 and S4. If we could measure the concentration after a time frame of 1 second or shorter and use
448 this as the C_1 value, these differences would be much less significant.
449
450 One method to normalize samples with varying ozone demand is to use an "internal standard"
451 microorganism. This was achieved by combining two different microbial surrogates, or a
452 surrogate and a virus, in the same background water to directly compare their ozone dose-
453 response under identical demand conditions in a wastewater sample.

454 ## Normalizing Variable Ozone Demand Samples

455 Because ozone demand introduced by microorganism stock solutions confounds the calculation
456 of an accurate Ct, an approach was developed to normalize for varying ozone demand by
457 combining two or more microorganisms (e.g., *E. coli* and PRD-1 phage) including one with little
458 to no ozone demand, and demonstrating comparative inactivation in the same water matrix.
459
460 *Figure 6* shows the results of the combined *E. coli* - PRD-1 batch experiment. Compared to
461 *E. coli* alone, the Ct required for *E. coli* inactivation in ETP wastewater increased when
462 combined with the PRD-1 phage. This illustrates the bias that is associated with the impurities in
463 surrogate and viral stock solutions, despite the fact that only small amounts (e.g. 0.15 mL of
464 PRD-1 stock in 200 mL) were applied. Surprisingly, these demands were significant even when
465 spiked into wastewater, which generally had a high ozone demand of its own.
466
467 **Figure 6 goes here**

From these data it is clear that (i) the apparent higher resistance of the PRD-1 to ozone, compared to *E. coli*, is due to the ozone demand of the PRD-1 stock solution, and (ii) it is difficult to measure an accurate Ct in high ozone demand water.

Transferred ozone dose has been suggested as a good way to measure ozone dose in wastewater (Xu et al., 2002, Ishida *et al.*, 2008), however, it may give misleading interpretations when using Hoigné and Bader's SOT method. While for some engineering decisions the transferred ozone dose for a given level of inactivation of indigenous (non-spiked) microbes in a specific water may be useful, variations in the ozone demand of the water due to fluctuations in water quality would need to be accounted for. Comparing inactivation data on the basis of Ct would appear to be a better method than transferred ozone dose. The Ct bias from varying stock solution ozone demand typically occurs within the first 10 seconds of the experiment. Because 10 seconds is the first time point used by the Discrete Summation method together with the applied dose, the Discrete Summation Ct value does not reflect the true character of the decay curve within the first 10 seconds. This method and the Extended T_{10} Ct method are compared below as methods to represent ozone dose.

Ozone Reduction Equivalent Dose Concept

To ultimately compare dose-responses across a suite of microbes with varying ozone demand and arrive at a true ozone dose-response relationship for a microorganism, each organism under investigation was tested in a combined water matrix with an *E. coli* reference microbe. *E. coli* is used as a normalizing factor to indicate the actual dose to which organisms inside the complex water matrix were being subjected, and thus could be normalized against. This is analogous to

491 the use of reduction equivalent doses in UV disinfection tests. *E. coli* F amp was chosen as the
492 indicator species since the ozone demand associated with *E. coli* alone is negligible compared to
493 that of virus and surrogate stocks, its addition does not have an impact on somatic phage or virus
494 assays, and it can be easily enumerated in the laboratory. *Figure 7* shows *E. coli* combination
495 experiments for PRD-1 phage and for CB5 and PV1 viruses.
496
497 **Figure 7 goes here**
498
499 As expected, the observed inactivation of *E. coli* as a function of Discrete Summation Ct
500 decreased with increasing ozone demand in various mixtures as was exemplified in comparing
501 the clean water and wastewater experiments in *Figure 5*. The magnitude and variability of
502 ozone demand in wastewater during these combined experiments can be seen in *Figure 8*, which
503 depicts ozone residuals at 10 seconds and 30 seconds after various applied ozone doses.
504 Although Richard, 1994 and others suggest the use of 30 seconds as the initial ozone residual
505 measurement for measuring SOT ozone demand, Figure 8 shows that ozone residual at
506 10 seconds may me more informative since ozone residual at 30 seconds is more frequently
507 below detection limit. Ozone demand in wastewater is significant on its own, and in addition
508 varied according to the amount of ozone demanding constituents in the virus or surrogate stock
509 solutions.
510
511 **Figure 7 goes here**
512

513 In each combined batch, there was no significant difference between the resistance to ozone of
514 *E. coli* and that of PV-1, Ad2 and CVB5 (*E. coli* combinations with: PV-1 $p < 0.05$, Ad2 $p <$
515 0.001 and CVB5 $p < 0.00005$).
516
517 **Figure 8 goes here**
518
519 *E. coli* was used to normalize the ozone exposure (Ct) across the suite of varying ozone
520 demanding waters tested, as shown in ***Figure 8***. This was done using the following method
521 based on testing *E. coli* and poliovirus as an example:
522 1. A normalizing factor was determined based on making the combined batch *E. coli* +
523 poliovirus logarithmic inactivation curve line up with that of *E. coli* alone.
524 2. The same normalizing factor was then applied to the poliovirus-only logarithmic
525 inactivation curve to generate an *E. coli* reduction equivalent inactivation curve for
526 poliovirus.
527 The normalized data based on discrete summation Ct, shown in the top portion of ***Figure 9***,
528 gives a better idea of the ozone resistance of surrogates and viruses relative to one another.
529 These data suggest that both PRD-1 and *E. coli* are appropriate surrogates for inactivation of
530 viruses in wastewater. More importantly, it illustrates that these data contain no outliers
531 regarding ozone sensitivity. It indicates that inactivation of the surrogates would also represent
532 similar inactivation of viral pathogens.
533
534 **Comparing Ozone Dose Response Using Discrete Summation and Extended T_{10} Ct**
535 **Methods**
536 The bottom portion of ***Figure 9*** uses the Extended T_{10} Ct calculation method with the data from
537 the combined virus-*E. coli* experiments. While the Extended T_{10} method can minimize the

538 impact of varying ozone demand of test waters, the bottom portion of *Figure 9* shows that the
539 Extended T_{10} calculation is also sensitive to ozone demand but in a very different way. The
540 microbes in the higher ozone demand stocks are interpreted as being very susceptible to ozone
541 and those in the lower ozone demand stocks as more resistant. While these data lend further
542 evidence to the fact that viruses are very susceptible to ozone at low exposures and that exposure
543 above 0.1 (mg/L)-min Ct effectively inactivate all viruses tested, the use of Extended T_{10} Ct
544 calculation may also lead to misinterpretation of the data. The *E. coli* alone, which has an
545 insignificant contribution to ozone demand, is an outlier to the right, suggesting that its
546 inactivation requires a higher ozone exposure. Higher-demand viruses are bunched at the left,
547 suggesting that their inactivation would require a lower ozone exposure. If there is significant
548 virus inactivation occurring during the consumption of ozone demand over the first initial few
549 seconds, this portion of the Ct calculation would not be accounted for in the Ct.
550
551 Data from combined experiments also suggest that use of the Extended T_{10} Ct calculations
552 causes the shape of apparent ozone dose response curves to differ based on different oxidant
553 demand conditions. While Extended T_{10} calculations themselves may reduce ozone demand
554 bias, this Ct calculation method prevents the data from being as easily normalized as the discrete
555 summation data were normalized in the upper portion of *Figure 9*.
556
557 While it is difficult to ascertain exactly what dose is required for virus inactivation, it is clear that
558 the viruses are no more resistant to ozone than the surrogates tested. Furthermore, significant (>
559 3 to 4 log) inactivation occurs at a Ct of approximately 1 (mg/L)-min using the discrete
560 summation method (which is clearly conservative) and also occurs at a Ct of less than

561 0.1 (mg/L)-min using the Extended T_{10} method (which may be an underestimate). In the context
562 of creating regulations, the weight of all the data in the literature and the findings of this research
563 indicate that in order to achieve a virus inactivation of >4 log, the ozone Ct exposure lies
564 somewhere between 0.1 and 1.0 (mg/L)-min.
565
566 **Figure 9 goes here**
567
568 Proposed ozone Ct values for varying levels of inactivation of the viruses and surrogates
569 examined in this study are presented in *Table 2*. While these data are presented for a specific
570 wastewater, because the spiked-stock for each microorganism's ozone demand impact was
571 accounted for, the relative sensitivities of the viruses and surrogates can be used to select an
572 appropriate surrogate to demonstrate varying levels of virus inactivation for use in any water.

573 Conclusions

574 Ozone is highly effective for disinfection of virus in filtered secondary effluent. Neither pH nor
575 temperature, examined within the typical ranges of natural waters (pH 6 to 8 and temperature of
576 16-23 °C), affected ozone disinfection. Discrete Summation Ct, Extended T_{10} Ct, and transferred
577 ozone dose could all be appropriate methods for monitoring disinfection performance provided
578 that any ozone demand bias is eliminated. When a variable ozone demand is present, the most
579 appropriate method for neutralizing the false impact on Ct comparisons among different
580 microorganisms is to utilize the Extended T_{10} Ct calculation method, as supported by the US
581 EPA (2010). A method employing the reduction equivalent dose concept was presented to
582 normalize ozone Ct data between samples with varying ozone demand.

583

584 *E. coli* was identified as a good candidate for use as a surrogate for virus disinfection by ozone.
585 Similarly, PRD-1 phage was found to be a good candidate as a surrogate for pathogenic viruses
586 in ozone disinfection, however, care should be taken to reduce the PRD-1 stock demand if it is to
587 be used in field scale testing.

588 ## References

589 Adams, M.H. (1959) *Bacteriophage*. Interscience Publishers, New York, pp. 450 – 456.

590 American Public Health Association. (2012). *Standard Methods for the Examination of Water*
591 *and Wastewater*; 22nd Edition.

592 Bader H., Hoigné, J. (1981) Determination of ozone in water by the indigo method. *Water*
593 *Research;* Vol. 15, pp. 449 – 456.

594 Bidawid, S., Malik, N., Adegunrin, O., Sattar, S.S., Farber, J.M. (2003). A feline kidney cell
595 line-based plaque assay for feline calicivirus, a surrogate for Norwalk virus. *Journal of*
596 *Virological Methods*; Vol. 107, pp. 163 – 167.

597 Burns, N., Hunter, G., Jackman, A, Hulsey, R, Coughenour, J., Walz, T. (2007) The Return of
598 Ozone and the Hydroxyl Radical to Wastewater Disinfection. *Ozone: Science and*
599 *Engineering*; Vol. 29, No. 4, pp. 303 – 306.

600 Chang, P.E., Snyder, J.E. (1974) Relative resistance of eight human enteric viruses to ozonation
601 in Saugatucket River water. *Aquatic Applications of Ozone Workshop*. International
602 Ozone Association, Boston, MA (Cited in Keller et al. 1974)

603 Emerson, M., Sproul, O., Buck, C. (1982) Ozone inactivation of cell-associated viruses. *Applied*
604 *and Environmental Microbiology*; Vol. 43, No. 3, pp. 603.

605 Finch, G., Fairbairn, N. (1991) Comparative Inactivation of Poliovirus Type 3 and MS2
606 Coliphage in Demand-Free Phosphate Buffer by Using Ozone. *Applied and*
607 *Environmental Microbiology*; Vol. 57, No. 11, pp. 3121.

608 Gehr, R., Nicell, J. (1996) Pilot Studies and Assessment of Downstream Effects of UV and
609 Ozone Disinfection of a Physicochemical Wastewater. *Water Quality Research;* Vol. 31,
610 No. 2, pp. 263-281.

611 Hoigne, J. (1994) Characterization of water quality criteria for ozone processes. Part I: Minimal
612 set of analytical data. *Ozone Science and Engineering*; Vol. 16, Issue 2, pp. 113-120.
613 Hoigne, J., Bader, H. (1994) Characterization of water quality criteria for ozone processes. Part
614 II: Lifetime of added ozone. *Ozone Science and Engineering*; Vol. 16, Issue 2, pp. 121-
615 134.
616 Hunt, N.K., Mariñas, B. (1999) Inactivation of Escherichia coli with ozone: chemical and
617 inactivation kinetics. *Water Research*; Vol. 33, No. 11, pp 2633 – 2641.
618 Ishida, C., Salveson, A., Robinson, K., Bowman, R., Snyder, S. (2008) Ozone disinfection with
619 the HiPOXTM reactor: streamlining an "old technology" for wastewater reuse. *Water*
620 *Science and Technology*; Vol. 58, No.9, pp 1765.
621 Keller, J.W., Morin, R.A., Schaffernoth, T.J. (1974) Ozone Disinfection Pilot Plants Studies at
622 Laconia, New Hampshire. *Journal of the American Water Works Association; Vol.* 66,
623 pp. 730.
624 Kim, C.H., Gentile, D.M., Sproul, 0.J. (1980) Mechanism of ozone inactivation of bacteriophage
625 f2. *Applied and Environmental Microbiology*; Vol. 39, pp 210 – 218.
626 Oneby, M., Bromley, C., Borchardt, J., Harrison, D. (2010) Ozone Treatment of Secondary
627 Effluent at U.S. Municipal Wastewater Treatment Plants. *Ozone: Science and*
628 *Engineering*; Vol. 32, pp. 43-55.
629 Richard, Y. (1994) Ozone Water Demand Test. *Ozone Science and Engineering*; Vol. 16, Issue
630 4, pp. 355-365.
631 Roy, D., Englebrecht, R.S., Chian, E.S.K. (1982) Comparative inactivation of six enteroviruses
632 by ozone. *Journal of the American Water Works Association*; Vol. 74, pp. 660 – 664.

633 Shin, G-A., Sobsey, M. (2003) Reduction of Norwalk Virus, Poliovirus 1, and Bacteriophage
634 MS2 by Ozone Disinfection of Water. *Applied and Environmental Microbiology*; Vol.
635 67, No. 9, pp. 3975 – 3978.
636 Tanner, B., Kuwahara, S., Gerba, C., Reynolds, K. (2004) Evaluation of electrochemically
637 generated ozone for the disinfection of water and wastewater. *Water Science and*
638 *Technology*; Vol. 50, No. 1, pp. 19 – 25.
639 Thurston, J., Haas, C., Jacangelo, J., Gerba, C. (2005) Inactivation of enteric adenovirus and
640 feline calicivirus by ozone. *Water Research*; Vol. 39, pp. 3650 – 3656.
641 USEPA (1986) Design Manual: Municipal Wastewater Disinfection, Office of Water (4606M),
642 EPA 625-I-86-021.
643 USEPA (1991) Guidance Manual for Compliance with the Filtration and Disinfection
644 Requirements for Public Water Systems using Surface Water Sources, Office of Drinking
645 Water, EPA CN-69-01-6989, March 1992.
646 USEPA (2001) Chapter 16: Procedures for Detecting Coliphages, In *USEPA Manual of Methods*
647 *for Virology*, Office of Research and Development, EPA 600-4-84-013 (N16), June 2001.
648 USEPA (2002) Addendum to USEPA (1999) Alternative Disinfectants and Oxidants Guidance
649 Manual, Office of Water (4607), EPA 815-R-99-014, April 1999.
650 USEPA (2003) LT1ESWTR Disinfection Profiling and Benchmarking Technical Guidance
651 Manual, Office of Water (4606M), EPA 816-R-03-004, May 2003.
652 USEPA (2010) Long Term 2 Enhanced Surface Water Treatment Rule Toolbox Guidance
653 Manual, Office of Water (4606), EPA 815-D-09-001, April 2010.
654 Vaughn, J. M., Chen, Y.-S., Lindberg, K., Morales, D. (1987) Inactivation of human and simian
655 rotaviruses by ozone. *Applied and Environmental Microbiology*; Vol. 53, pp. 2218 –

656 2221.
657 Vaughn, J., Chen, Y-S., Novotny, J., Strout, D. (1990) Effects of ozone treatment on the
658 infectivity of hepatitis A virus. *Canadian Journal of Microbiology*; Vol. 36, pp. 557.
659 Xu, P., Janex, M-L., Savoye, P., Cockx, A., Lazarova, V. (2002) Wastewater disinfection by
660 ozone: main parameters for process design. *Water Research*; Vol. 36, pp 1043 – 1055.

661

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Table and Figure Titles

Table 1: Water quality characteristics of filtered Eastern Treatment Plant wastewater for disinfection experiments.

Table 2. Normalized Ct requirements for specified log inactivation levels of viruses and surrogates in wastewater at pH=7.96 and at 16°C

Figure 1. Ozone apparent dose response curves for phages in clean water based on discrete summation Ct. Note the shadowed data points indicate the maximum log inactivation, which is a function of the starting concentration.

Figure 2. Ozone disinfection dose-response curves for selected viruses in laboratory buffered water at two temperatures based on discrete summation Ct (top plot) and Extended T_{10} Ct (bottom plot).

Figure 3. Ozone dose response curves surrogates in ETP wastewater effluent based on (top) Discrete Summation Ct and (bottom) Extended T_{10} Ct. Wastewater effluent tested at 16°C and a pH of 7.96.

Figure 4. Ozone dose response curves of viruses in ETP wastewater effluent based on (top) discrete summation Ct and (bottom) extended T_{10} Ct method. Wastewater experiments were run at 16°C and a pH of 7.96. HD and LD refer to High Demand and Low Demand stocks. Note X-axis scales differ due to calculation method.

Figure 5. E. coli apparent dose response curves for clean water (CW and no-fill markers) compared to ETP wastewater effluent (WW and solid markers) using Discrete Summation Ct calculation methods. Water quality conditions are noted in the legend.

Figure 6. Ozone apparent dose responses for surrogates in ETP wastewater effluent at 16°C, pH of 7.96, in a combined batch based on Discrete Summation Ct. Dose response for F amp E. coli tested alone is shown in hollow triangles for reference.

Figure 7: Apparent dose response curves for three combination experiments in ETP wastewater effluent at 16°C and pH of 7.96 with viruses and surrogates using E. coli as an indicator species based on Discrete Summation Ct.

Figure 8: Ozone residual at 10 seconds and 30 seconds as a function of initial ozone dose in ETP wastewater effluent at 16°C and an unaltered pH of 7.96.

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716 wastewater at 16°C and an unaltered pH of 7.96 with Discrete Summation Ct values (top) and
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720

Table 1
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Table 1: Water quality characteristics of filtered Eastern Treatment Plant wastewater for disinfection experiments.

Parameter	Raw Value	Units
pH	7.96	pH
Total Organic Carbon (TOC)	17.1	mg-C/L
Total Dissolved Nitrogen (TDN)	9.5	mg-N/L
Nitrate	3.92	mg-N/L
Nitrite	0.189	mg-N/L
Turbidity	0.1	NTU
UV 254	0.130	Abs

Table 2
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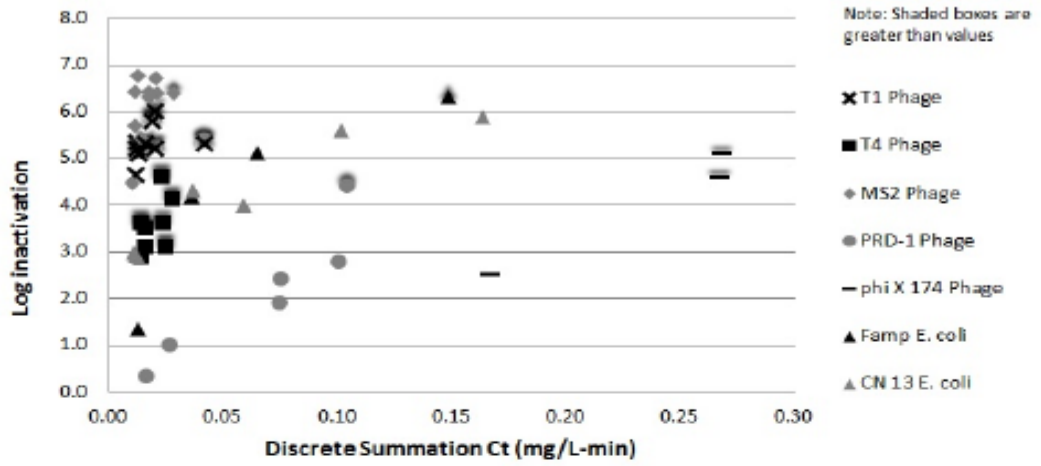
Table 2. Normalized Ct requirements for specified log inactivation levels of viruses and surrogates in wastewater at pH=7.96 and at 16°C

	E. coli-normalized Ct for wastewater (mg/L)-min			
log inactivation	**1**	**2**	**3**	**4**
E. coli	0.48	0.65	0.82	0.98
Coxsackievirus B5 (CVB5)	0.32	0.51	0.71	0.90***
Poliovirus 1 (PV-1)	0.47	0.58	0.68	0.78**
Adenovirus 2 (Ad2)	0.40	0.51	0.70-0.90*	0.77-1.10*[+]
ΦX174	0.33	0.45	0.57	0.69
PRD-1	0.43	0.63	0.83	1.00

* Range of Cts reflect different separate batch experiments
** 0.789 (mg-min)/L gave > 4.16 log inactivation of PV-1
*** 0.844 (mg-min)/L gave a 3.19 log inactivation and 1.115 (mg-min)/L gave > 5.57 log inactivation
[+]1.10 (mg-min)/L gave 4.74 log inactivation of Ad2

Figure 1



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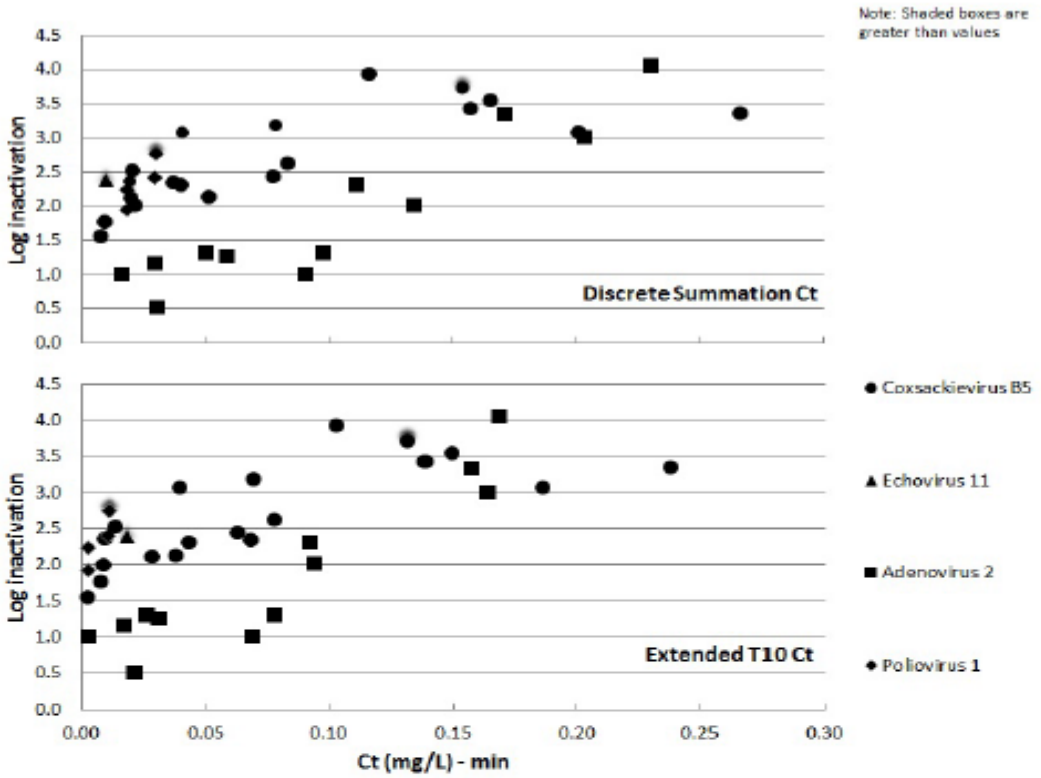

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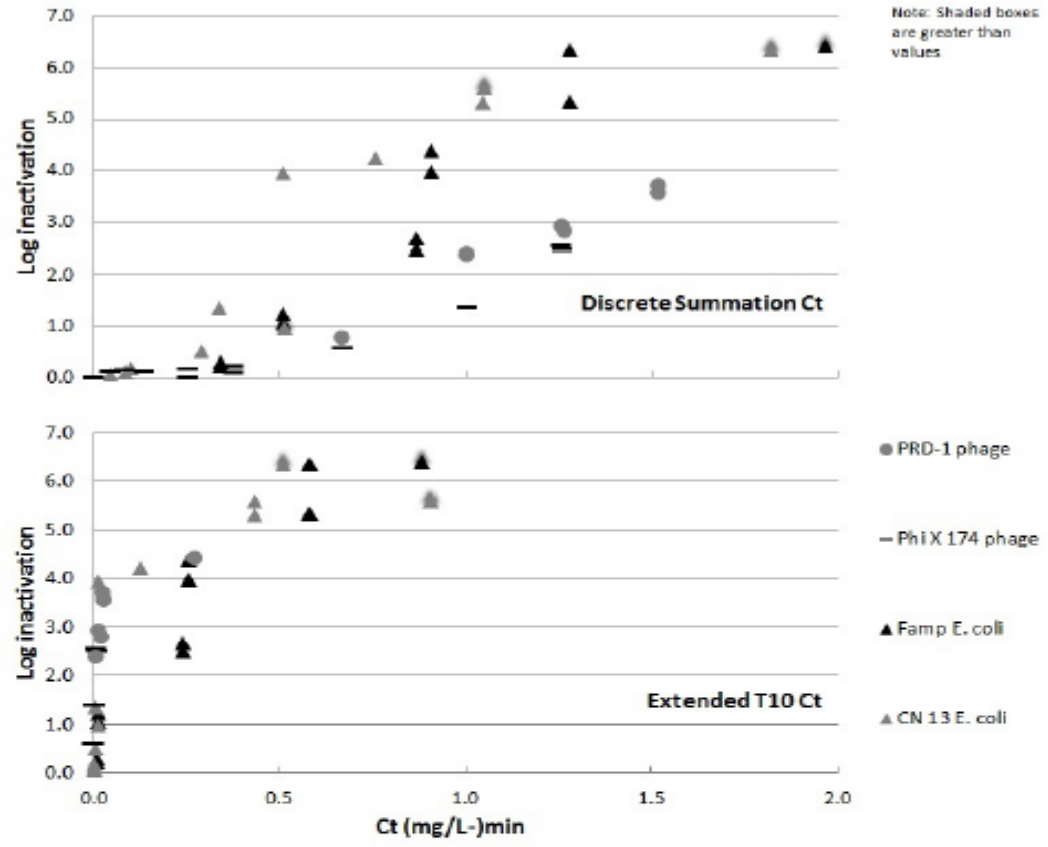

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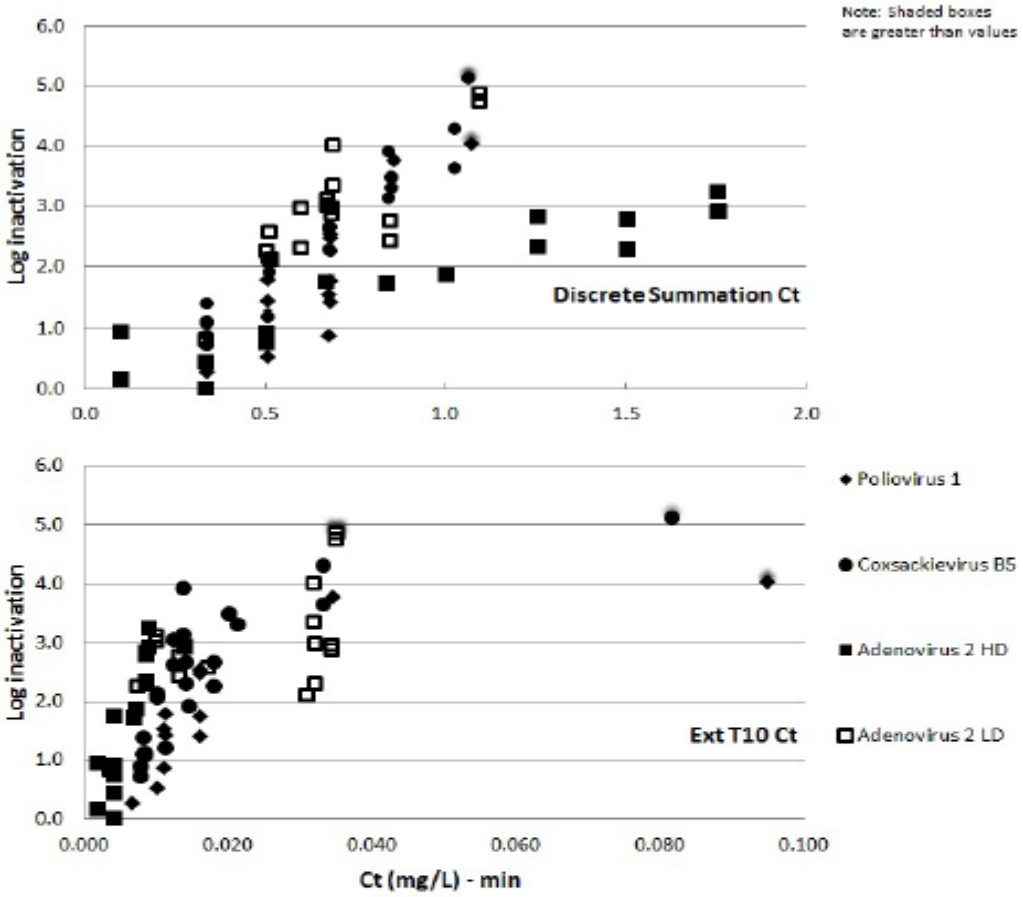

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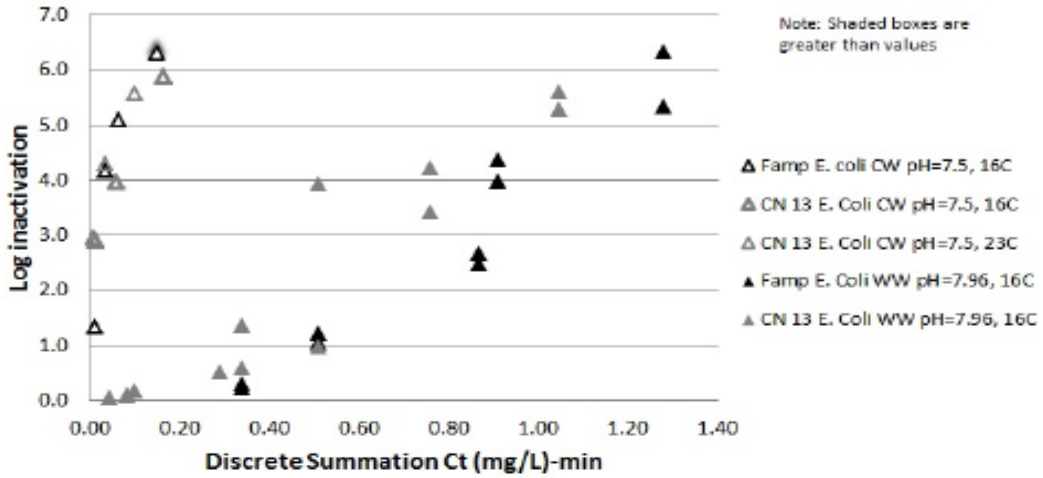

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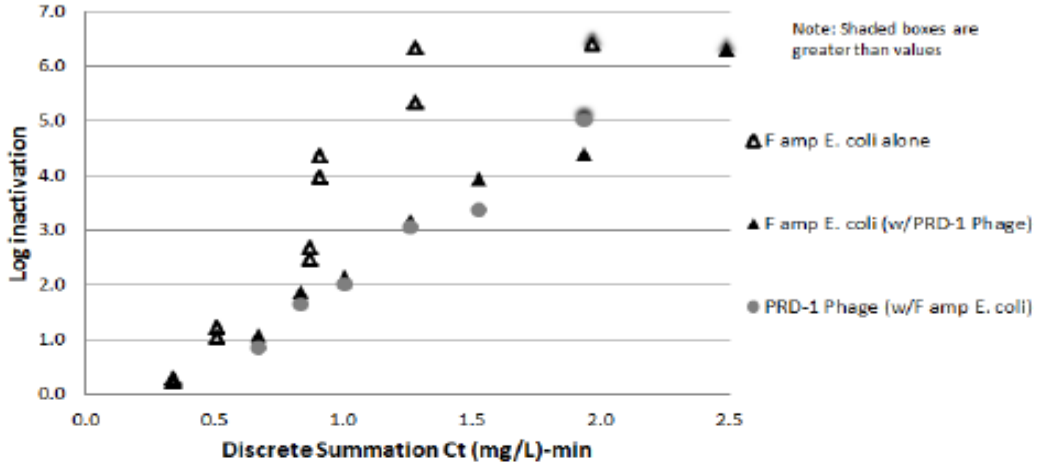

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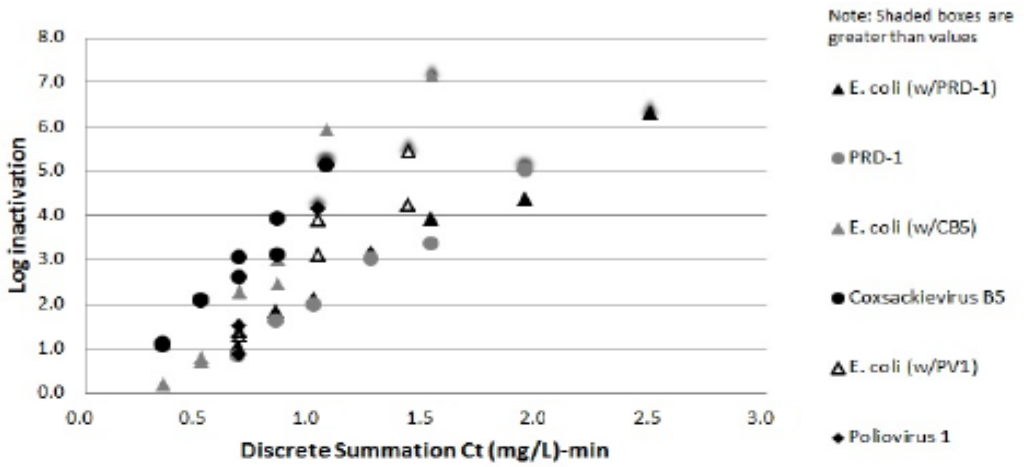

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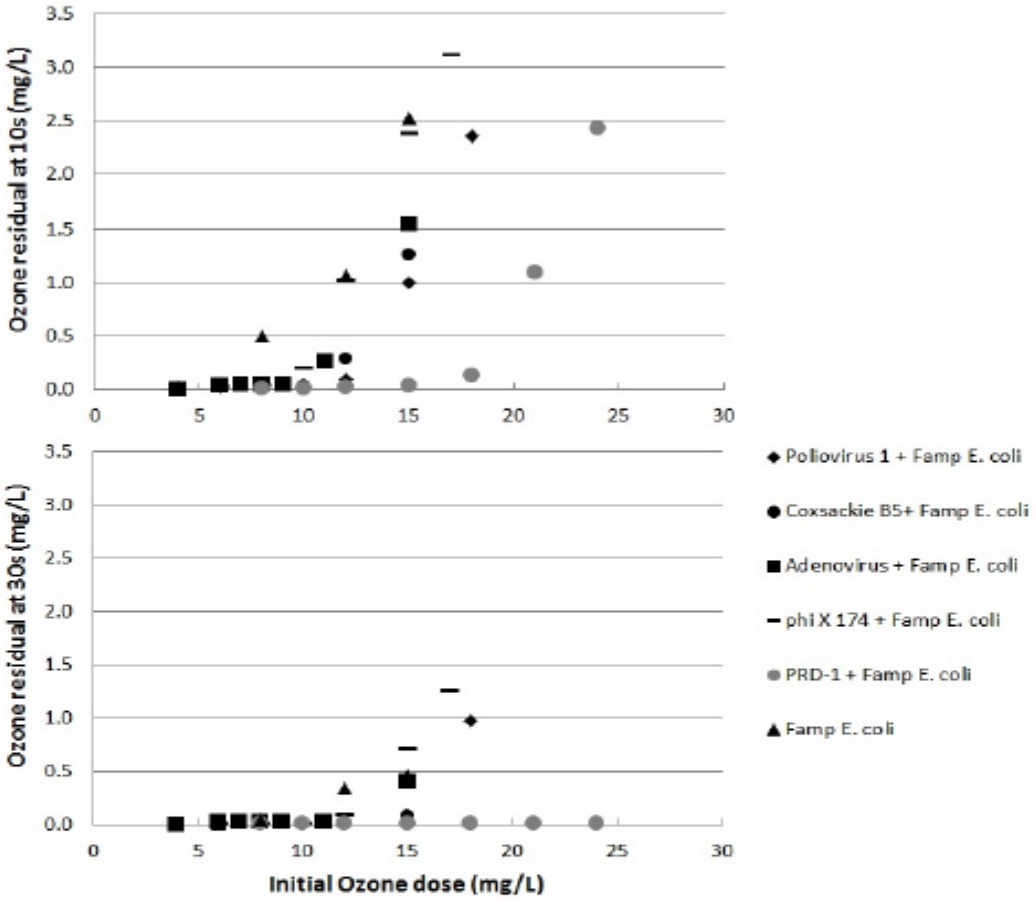

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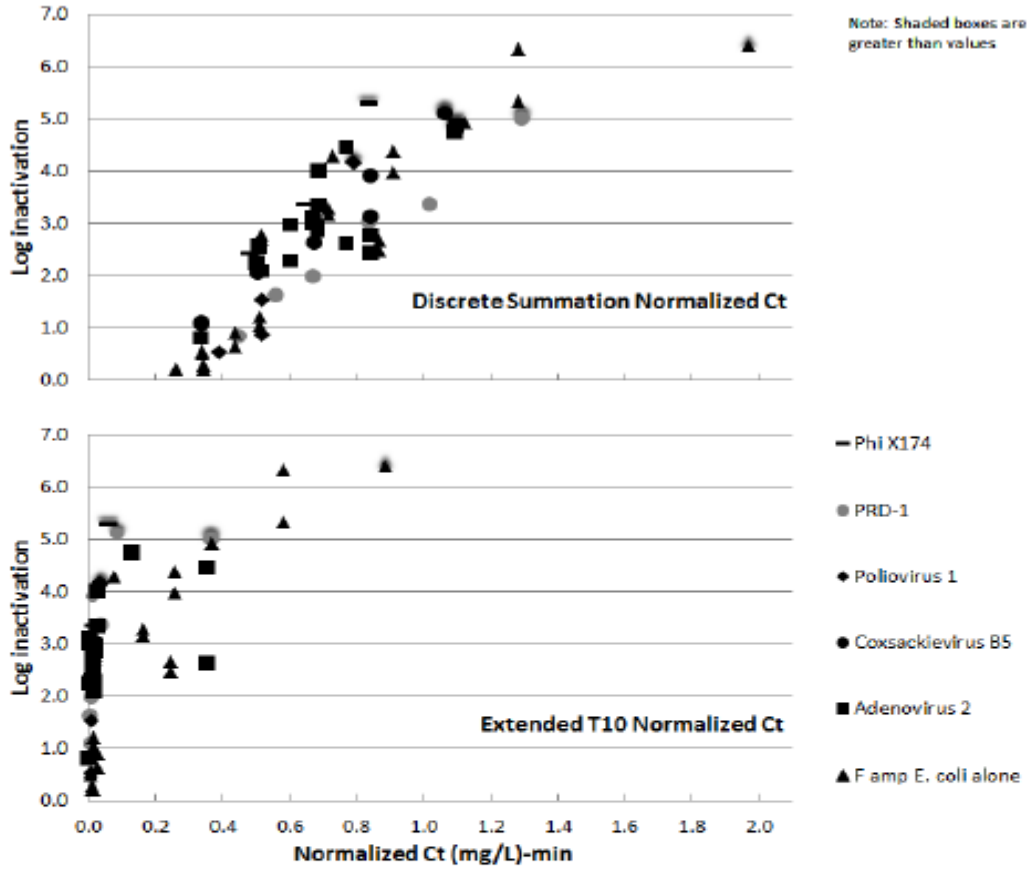

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Electronic Supplementary Material (for online publication only)
Click here to download Electronic Supplementary Material (for online publication only): Supplemental Information-Sigmon3-14.

APPENDIX 2: Summary of interactions with MW and RMIT personnel, and the water community.

1. MELBOURNE WATER ACTIVITIES

Prof Linden visited Eastern Treatment Plant to see the full scale tertiary process and gain a better understanding of how his work for MW had been implemented and current issues which need resolution.

Regular meetings with MW scientists including Prof Judy Blackbeard (Manager, Water Recycling Research, Strategic Planning) Clare McAuliffe (Senior Process Engineer) Sam Costello (Project Planner), John Mieog and Suzie Sarkis (Team Leader, Drinking Water Quality Planning). Discussions included:

- Pathogen association with particles leading to reduced efficacy of disinfection;
- Emerging contaminants in the wastewater treated at Eastern and Western Treatment Plants; and
- Ozonation and UV processes for treatment efficacy.

As part of NatVal 2.2, provided expert scientific and editorial advice to Clare McAuliffe of MW regarding the writing of the validation protocol for ozone.

Presented a seminar on 5 September to the MW research group providing an overview of the research projects he is involved in, focusing on topics relevant to MW.

Reviewed NatVal roadmap reports to prepare for the NatVal meeting in Melbourne on September 12, 2013.

Wrote workshop proposal for Ozwater'14 on validation processes, in collaboration with AWRCoE, which was accepted for presentation on April 30, 2014 titled "Toward National Validation Guidelines for Water Recycling in Australia".

Revalidation of UV treatment at Western Treatment Plant: Reviewed materials and data for the particles and pathogens report prepared by Sam Costello (MW). Provided comprehensive review and edited the report submitted to the Victorian Department of Health defending the data that indicate there is no particle association problem with *Cryptosporidium* at the WTP.

Met with Dr Paul Monis from SA Water, Judy Blackbeard (MW) and Sam Costello (MW) to review the plans for the PhD level study of particle association of pathogens. Contributed to revision of the research plan and provided background information and previous publications to the group.

Met with Assoc. Prof Stuart Khan (UNSW), Felicity Roddick, Judy Blackbeard, Yufei Wang, and Linhua Fan to review the PhD project "Improving modelling and prediction of removal of micropollutants during wastewater treatment " that Yufei Wang will be working on at RMIT with Felicity Roddick, and involving Stuart Khan. Discussed presentation by Stuart of WERF report by Dickenson et al. (2010) and contributed to discussions on photolysis of targeted pollutants in the WTP and modelling the decay of contaminants of interest in biological system.

Visited and toured the Melbourne Water Western Treatment Plant (WTP) with Felicity Roddick, Judy Blackbeard, Yufei Wang, and Stuart Khan.

2. RMIT ACTIVITIES

Presentation to the water-related postgraduate students, held discussions about their projects, including some one-on-one discussions.

Discussion with Prof Felicity Roddick, and Linhua Fan, Thang Nguyen and Prita Puspita about other research projects being undertaken at RMIT and toured RMIT laboratory facilities.

With Felicity Roddick began planning for the upcoming project on the ozonation of spiked Eastern Treatment Plant effluent for micropollutant removal, including initial discussion on choice of micropollutants to study.

Made plans for the next visit during February 1 to 21, 2014 including plans for public lecture at RMIT and running of laboratory experiments for ozonation of spiked ETP effluent.

On 10 September gave a lecture to 4th year Chemical Engineering students undertaking the Advanced Environmental Engineering elective on Advanced Oxidation Processes for Wastewater Treatment.

Met with Prita Puspita and Felicity Roddick to discuss the ozonation study to be carried out at RMIT, to meet some of the needs of Melbourne Water as part of the Fellowship grant. Provided guidance on the methods and literature available for the study to determine ozonation rate constants for specific compounds targeted by Melbourne Water.

Presented lecture to about 75 people from metropolitan and regional water utilities, universities and consulting companies at RMIT on “Rethinking disinfection in drinking water systems” on February 19, 2014. The lecture was videotaped and is available through the Australian Water Recycling Centre of Excellence webpage and also via a link from the RMIT Water: Effective Technologies and Tools Research Centre webpage. The slides are available from the Victorian branch of the AWA.

Met with Muhammad Umar to discuss post-PhD plans and strategies for obtaining a post-doc. Offered to help make connections to faculty in USA

Met with Muhammad Umar and Felicity Roddick to discuss ozonation of micropollutant results on 18 and 24 July.

Met with Judy Blackbeard, Linhua Fan, Felicity Roddick and PhD student Yufei Wang to discuss the modelling of micropollutant removal in WTP lagoons project 23 July.

3. NatVal ACTIVITIES

Participated in NatVal Protocol Development Group (PDG) for the National Validation Framework for Water Treatment Technologies on September 12, 2013. Presented at that meeting on “Lessons from the USEPA UV Validation Process”.

Reviewed materials provided by Sue Keay for NatVal Protocol Development Group meeting that was held on February 12, 2014.

Prepared a presentation on the “Makings of a Validation Centre” and gave at the NatVal PDG meeting on February 12 at MW.

Held a teleconference with Sue Keay and Mark O’Donohue to review plans for the Ozwater’14 workshop on “Toward National Validation Guidelines for Water Recycling in Australia”.

Visited AWRCoE and met with Mark and some representatives from the Queensland Heath Department to discuss the state of regulation regarding recycled water and the work of NatVal, on May 5, 2014.

Participated in NatVal PDG meeting on 25 July, 2014.

4. OTHER OUTREACH ACTIVITIES

Prof Linden presented a lecture to students and academic staff at the University of Queensland, hosted by Prof Jurg Keller and his Advanced Water Management Centre on May 5, 2014. The lecture was on "Rethinking Disinfection in Drinking Water Systems" and was followed by a lively discussion. During his visit, Prof Linden met with doctoral and post-doctoral students of Prof. Keller to advise them on some UV-related research, toured the laboratory facilities, and had lunch with a group from the Advanced Water Management Centre.

Visited Profs Mikel Duke and Stephen Gray at Victoria University on 15 July 2014 to tour the laboratories and discussed research collaborations on membranes and membrane pre-treatment to minimize fouling. Following up with them to support proposal development and potential co-advising of a student in the near future.

Gave keynote speech “UV Disinfection: New Developments for Small Systems” at the WaterRA workshop “Science talks to Industry” on July 16. Attended day 2 of workshop on July 17 and interacted with attendees. The workshop was attended by approximately 100 members and other water-related industry professionals. The presentation is available through the WaterRA website.

Had discussion with Carolyn Madden and Susan Crosher of South East Water regarding the potential environmental impacts on marine ecosystem of chlorinated disinfection by-products from wastewater on July 23.

Discussed UV disinfection issues and regulations with Vanora Mulvenna of Victorian Department of Health on July 24.