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Ozone disinfection of waterborne pathogens and their surrogates: A critical review

Christina M. Morrison [a,*], Samantha Hogard [b,c], Robert Pearce [b,c], Daniel Gerrity [a], Urs von Gunten [d,e], Eric C. Wert [a]

[a] Southern Nevada Water Authority (SNWA), P.O. Box 99954, Las Vegas, NV 89193-9954, USA
[b] Civil and Environmental Engineering Department, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
[c] Hampton Roads Sanitation District, P.O. Box 5911, Virginia Beach, VA 23471-0911
[d] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Ueberlandstrasse 133, CH-8600 Dubendorf, Switzerland
[e] School of Architecture, Civil and Environmental Engineering (ENAC), Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

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ABSTRACT

Viruses, *Giardia* cysts, and *Cryptosporidium parvum* oocysts are all major causes of waterborne diseases that can be uniquely challenging in terms of inactivation/removal during water and wastewater treatment and water reuse. Ozone is a strong disinfectant that has been both studied and utilized in water treatment for more than a century. Despite the wealth of data examining ozone disinfection, direct comparison of results from different studies is challenging due to the complexity of aqueous ozone chemistry and the variety of the applied approaches. In this systematic review, an analysis of the available ozone disinfection data for viruses, *Giardia* cysts, and *C. parvum* oocysts, along with their corresponding surrogates, was performed. It was based on studies implementing procedures which produce reliable and comparable datasets. Datasets were compiled and compared with the current USEPA Ct models for ozone. Additionally, the use of non-pathogenic surrogate organisms for prediction of pathogen inactivation during ozone disinfection was evaluated. Based on second-order inactivation rate constants, it was determined that the inactivation efficiency of ozone decreases in the following order: Viruses >> *Giardia* cysts > *C. parvum* oocysts. The USEPA Ct models were found to be accurate to conservative in predicting inactivation of *C. parvum oocysts and viruses, respectively,* however they overestimate inactivation of *Giardia* cysts at ozone Ct values greater than ~1 mg min L^{-1}. Common surrogates of these pathogens, such as MS2 bacteriophage and *Bacillus subtilis* spores, were found to exhibit different inactivation kinetics to mammalian viruses and *C. parvum* oocysts, respectively. The compilation of data highlights the need for further studies on disinfection kinetics and inactivation mechanisms by ozone to better fit inactivation models as well as for proper selection of surrogate organisms.

1. Introduction

Ozone has been widely applied as a disinfectant for water treatment for more than a century (Rakness, 2015; von Gunten, 2003a; von Sonntag and von Gunten, 2012) and more recently in wastewater and water reuse applications (Burns et al., 2007; Gerrity et al., 2012; von Gunten, 2018). In the United States, a shift to alternative disinfectants (i.e., non-chlorine), including ozonation, was driven by regulations implemented by the United States Environmental Protection Agency (USEPA). These required drinking water utilities to optimize inactivation of protozoan pathogens and viruses relative to the potential formation of disinfection byproducts (DBPs) primarily related to chlorine disinfection (USEPA, 2006, 1999, 1991). Moreover, ozone treatment provides auxiliary benefits including transformation of bulk organic matter and oxidation of trace organics and taste and odor compounds (Camel and Bermond, 1998; Lee et al., 2013; Lee and von Gunten, 2016; von Gunten, 2003b; von Sonntag and von Gunten, 2012).

Viruses, *Giardia* cysts, and *Cryptosporidium parvum* oocysts are all major causes of waterborne diseases that can be uniquely challenging in terms of inactivation/removal during water and wastewater treatment and water reuse (Betancourt, 2019; Efstratiou et al., 2017; Leclerc et al., 2002; Soller et al., 2017). Ozone is acknowledged as an effective

* Corresponding author.
E-mail address: christina.morrison@snwa.com (C.M. Morrison).

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disinfectant for bacteria, encysted protozoans, as well as viruses (von Sonntag and von Gunten, 2012). However, direct comparison of results from ozone disinfection studies is challenging due to the complexity of aqueous ozone chemistry in addition to the variety of different approaches utilized to conduct such studies. The goal of this review is to systematically analyze available ozone disinfection literature by reviewing studies that implemented procedures producing reliable and comparable datasets. Specifically, the review aims to evaluate: (a) bench-scale inactivation kinetics of the three regulated pathogen groups (viruses, *Giardia*, and *Cryptosporidium*) and their potential surrogates in buffered ultrapure water or surface water, (b) mechanisms of inactivation, and (c) current research needs as indicated by data gaps in the ultimate findings of this review.

2. Background

In the United States, the shift to alternative drinking water disinfectants was driven by USEPA treatment regulations. Over time, these regulations specified the required abatement of viruses, *Giardia* cysts, and *C. parvum* oocysts and defined maximum contaminant levels (MCLs) for DBPs. This rigorous approach is unique to the USA and only partially applied in other countries, where ozone is commonly applied as a disinfectant for drinking water treatment (von Sonntag and von Gunten, 2012). Amendments to the Surface Water Treatment Rule (SWTR) required drinking water treatment plants to achieve removal and/or inactivation of at least 4 $\log_{10}$ for virus and 3 $\log_{10}$ for *Giardia* cysts. The SWTR also introduced the "Ct" concept to establish predicted pathogen inactivation levels based on the concentration of the applied disinfectant (C) and the contact time (t), which in the case of full-scale systems is the retention time at which 10% of the volume has passed through the reactor basin, which must be determined via tracer studies (See SI S.1). In response to major outbreaks of cryptosporidiosis traced back to contaminated water resources (Hrudey et al., 2003; Mac Kenzie et al., 1994), the Interim Enhanced Surface Water Treatment Rule, and eventually the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) were introduced, extending removal requirements and Ct models to *C. parvum* oocysts and requiring at least 2 $\log_{10}$ abatement (USEPA, 2006, 1998).

Despite its properties as a strong oxidant and disinfectant, there are several ozone characteristics that make the study of its disinfection efficiency difficult. Ozone is unstable in water and decomposes rapidly, thereby making it difficult to accurately measure and characterize its exposure (von Sonntag and von Gunten, 2012). Furthermore, ozone decomposition leads to the formation of hydroxyl radicals, another powerful oxidant that can contribute to disinfection and/or oxidation (Hoigné and Bader, 1975; Staehelin and Hoigne, 1985; von Gunten, 2003b). Water quality characteristics such as pH, temperature, dissolved organic matter (DOM) concentration/composition, among others, are major drivers of ozone decay and hydroxyl radical formation during ozonation (Elovitz et al., 2000; Staehelin and Hoigne, 1985). Hydroxyl radicals provide a significant benefit during ozonation by enhancing the oxidation of organic constituents (Huber et al., 2003; von Gunten, 2003b), however the impact of hydroxyl radicals on disinfection is not well established. In general, it is assumed that hydroxyl radical exposure can be neglected for disinfection of drinking water because of the typically high efficiency of direct ozone reactions and its diffusion limitation (von Gunten, 2003a).

There are several kinetic models that can be utilized to model the inactivation of a particular organism and the proper model heavily depends on the disinfectant utilized, the target organism, and the method of application (Gyürek and Finch, 1998). The USEPA assumes a second-order (or pseudo first-order in certain cases) rate law (Equation 1) to model the inactivation of viruses, *Giardia* cysts, and *C. parvum* oocysts in water:

$$\frac{dN}{dt} = -kNC \tag{1}$$

where N is the number of viable organisms at time t, C is the concentration of disinfectant at time t, and k is the second-order inactivation rate constant. Integrating the rate equation with the assumption that the disinfectant concentration remains constant over time provides a model in which the disinfectant exposure, or Ct (concentration x contact time, discussed further below), can be used to predict the log-reduction of pathogens:

$$\int_{N_0}^{N} \frac{dN}{N} = -kC \int_{0}^{t} dt \tag{2a}$$

$$\ln\left(\frac{N}{N_0}\right) = -kCt \tag{2b}$$

or

$$log_{10}\left(\frac{N}{N_0}\right) = -\frac{k}{\ln(10)}Ct \tag{2c}$$

There are more complex models that account for non-linear disinfection kinetics (i.e., shouldering and tailing behaviors, see Gyürek and Finch, 1998), however, the necessity to fit anywhere from 1 - 3 empirical constants, with a lack of description as to what these constants physically represent makes such models difficult to apply to full-scale disinfection processes. Therefore, the second-order disinfection model has been regularly utilized to estimate disinfection efficacy despite sometimes obvious and often unexplainable deviations from a linear relationship, particularly when evaluating ozone disinfection. Some of these problems are related to the instability of ozone in water, which complicates the application of the disinfection kinetic models used to describe and predict inactivation of different microorganisms. One case in point is the formation of hydroxyl radicals, which need to be scavenged (e.g., by the addition of tertiary butanol (*t*-BuOH) or DMSO) when trying to isolate the contribution from ozone, specifically (Wolf et al., 2018). Kinetic studies carried out in absence of such a hydroxyl radical scavenger are fundamentally problematic to determine second-order inactivation rate constants. Furthermore, pH and temperature are two factors which need to be carefully monitored, because of their effect on ozone stability and inactivation kinetics (von Sonntag and von Gunten, 2012).

The rapid decay of ozone makes the determination of the Ct value (i.e., ozone exposure) in real reactors more complicated than simply multiplying the final residual concentration and contact time (i.e., the "effluent" method), which underestimates the true ozone exposure due to ozone instability in solution. Ozone decay can be taken into consideration for Ct determination through the following:

$$C(t) = C_0 e^{-k't} \tag{3}$$

where C(t) is the concentration of ozone at time (t), C_0 is the initial ozone concentration, and k' is the first-order ozone decay rate constant. Accounting for time dependence of C in (1) by substituting (3) into (2a) and integrating results in:

$$ln\frac{N}{N_0} = -k\frac{C_0}{k'}\left(1 - e^{-k't}\right) \tag{4}$$

which is equivalent to the area under the ozone decay curve (if ozone exhibits first order decay). The "integral method" requires the collection of a time series of ozone residual measurements throughout the reaction time for accurate calculation of the decay rate constant. Other methods for estimating the area under the ozone decay curve can be utilized to estimate ozone exposure, particularly when ozone does not display first-order decay (von Gunten and Hoigne, 1994).

Alternative methods of Ct calculation encountered in bench-scale ozone disinfection studies include calculating the geometric mean of the initial and final ozone residual and multiplying by the elapsed time, which is a simple and relatively accurate method if ozone displays first-order decay as described above and can also be used at full scale with respect to different reaction chambers (Rakness et al., 2005). Other methods include estimating C as the arithmetic mean of the initial and final ozone residual, as well as simply substituting the final residual. The variety in Ct calculations among studies adds an additional layer of complexity for comparison of data, more so if the Ct calculation method is not specified.

The inactivation rate constants (*k*) utilized to predict microorganism inactivation are typically derived from bench-scale studies evaluating log inactivation vs. Ct. There are several methods for evaluating ozone disinfection at bench-scale, with specific implications associated with each method. Bench-scale studies include (but are not limited to) true batch, semi-batch, and quench-flow reactor systems. True batch tests are conducted by applying a pre-determined dose of aqueous ozone to a closed batch reactor with constant agitation. The aqueous ozone stock solution is produced by bubbling gaseous ozone/oxygen gas mixture through ultrapure water in a chilled reactor to obtain an aqueous ozone concentration in equilibrium to the gas phase concentration according to Henry's law (Hoigne and Bader, 1994; Rakness, 2015; von Sonntag and von Gunten, 2012). Due to the instability of ozone in aqueous solutions, proper determination of ozone exposure during a batch test should include a time series of ozone residual measurements (Hoigne and Bader, 1994; von Sonntag and von Gunten, 2012). Additionally, the added stock solution produces a diluting effect on the test solution which must be accounted for.

One limitation to batch systems is the inability to accurately characterize the initial phase of ozone decay, low-exposure scenarios, and examining fast reacting compounds or microorganisms (Buffle et al., 2006; Hoigne and Bader, 1994). These problems have been addressed using continuous quench-flow systems or reaction systems in which the ozone exposure was controlled by addition of an ozone-reactive compound (e.g., cinnamic acid) (Buffle et al., 2006; Criquet et al., 2015; Czekalski et al., 2016; Torii et al., 2020; Wolf et al., 2018). In quench-flow systems, a mixed sample/ozone solution is pumped with a fixed velocity/flow regime through a thin reaction tube, the length of which can be altered to allow for different contact times. This system is ideal for evaluating fast reacting compounds, however, proper implementation can be challenging, and special consideration should be given to mixing and flow characteristics through the reaction tube. Careful calibration with known ozone reactions is required (Buffle et al., 2006; Criquet et al., 2015).

Semi-batch tests are conducted by applying ozone gas directly to the test solution, allowing the system to reach steady state (i.e., the dissolved ozone in solution remains constant with time), and directly applying organism to the constant gas/ozone mixture (Rakness, 2015). Ozone exposure can be estimated based on the steady state aqueous ozone residual and contact time. A benefit to this approach is the reduction in effort necessary to account for ozone decay within the system. Additionally, there is no need to account for a dilution effect that occurs when dosing with an aqueous stock solution. However, such systems require greater expertise for proper implementation; improper design and implementation can produce misleading results. For instance, if the solution is not fully saturated, the determined kinetics are a mixture of both ozone mass transfer and inactivation. Additionally, for many inactivation processes, the steady-state concentrations in the reactor are very high and require extremely low residence times to achieve realistic ozone exposures, which is often not feasible. Therefore, the preferred method for evaluating inactivation kinetics of microorganisms by ozone is through the use of true-batch systems (for slower inactivating/more resistant microbes such as *C. parvum* oocysts), and modified batch systems (i.e., addition of cinnamic acid) or quench-flow systems (for faster inactivating/less resistant microbes, such as viruses).

There are many parameters that can influence the efficacy of ozone treatment, including pH, temperature, alkalinity, and other intrinsic properties of the water matrix. These parameters generally affect ozone demand/decay or oxidant scavenging kinetics. When pH decreases, ozone becomes more stable (Staehelin and Hoigne, 1985). The role of pH with respect to inactivation of microorganisms is unclear. pH can influence the surface properties of microorganisms (Harden and Harris, 1952; Hsu and Huang, 2002; Michen and Graule, 2010; Righetti and Caravaggio, 1976), however, the extent to which this influences reactivity with ozone is not well understood. Temperature also plays an important role in the stability of ozone in aqueous solutions. Increasing temperature increases the first-order ozone decay rate which therefore reduces the stability of ozone in solution. However, increased temperature also increases the rate of ozone inactivation for microorganisms (Finch and Li, 1999; Li et al., 2001; Roy et al., 1982). Therefore, temperature changes will affect inactivation kinetics in a complex interplay between ozone stability and susceptibility of microorganisms.

Other water quality parameters, such as dissolved organic matter (DOM), are more complex with respect to ozone stability and hence disinfection efficacy (von Gunten, 2003b; von Sonntag and von Gunten, 2012). Therefore, disinfection studies in DOM-containing matrices can be difficult to conduct and interpret. In addition, interaction of microorganisms with organic matter (e.g., coating of membranes) may alter the susceptibility of microorganisms to ozone. In DOM-heavy matrices such as wastewater, it may be difficult to establish an ozone Ct with low ozone doses due to rapid ozone demand/decay. However, these low Ct or 'sub-residual' dosing scenarios can still achieve inactivation of some microorganisms (Gerrity et al., 2012), potentially necessitating alternative crediting frameworks. This is also the case when ozone is supplemented with hydrogen peroxide to preferentially drive the formation of hydroxyl radicals (i.e., advanced oxidation process), drastically reducing (or eliminating) ozone Ct (Acero and von Gunten, 2001). At the bench scale, many of these issues can be avoided by using quench-flow reactors to understand disinfection kinetics in the early reaction phase.

An empirical approach avoiding complications with ozone chemistry relies on non-Ct based frameworks for obtaining disinfection credit in such cases. For example, Gamage et al. (2013) and Gerrity et al. (2012) evaluated the correlation of non-Ct based parameters such as O_3:DOC ratio, ΔUV_{254} absorbance, and ΔTF (total fluorescence) with inactivation of *B. subtilis* spores, *Escherichia coli*, and MS2 during wastewater ozonation. Another study evaluated ΔUV_{254} absorbance and oxidation of carbamazepine as surrogate parameters to evaluate disinfection of viruses in surface water and wastewater (Wolf et al., 2019). Despite the potential value of these emerging non-Ct frameworks, this review focuses on characterizing ozone disinfection efficacy based on the more traditional Ct framework.

3. Methods

3.1. Literature review

Literature was compiled with the goal of analyzing ozone disinfection efficacy for three pathogen groups regulated in water treatment (i.e., viruses, *Giardia* cysts, and *C. parvum* oocysts), and their potential surrogates. Web of Science core collection was searched during September 2020 using search criteria containing a combination of the following key words: Ozon* AND water AND (inactivation OR kinetics OR disinfection) AND (virus OR protozoa OR pathogen OR bacteria OR microb* OR adenovirus OR enterovirus OR calicivirus OR MS2 OR B. subtilis OR giardia OR cryptosporidium). Articles which were peer-reviewed, written in English, and published after the year 1980 were considered for inclusion within the review. Search results were subjected to an initial screening of the titles and abstracts. Articles were included for full-text review if the title and/or abstract indicated (a) ozone was among the disinfectants being analyzed, (b) disinfection in water was evaluated (i.e., excluding ozone as a surface disinfectant or in

the gas phase), and (c) microbial inactivation was analyzed (i.e., excluding proxies for microbial disinfection). The "Materials & Methods" sections of selected articles were evaluated via random assignment between three reviewers. The evaluated articles were organized by (a) whether disinfection kinetics or mechanisms were evaluated, (b) scale of research (i.e., bench-, pilot-, or full-scale), (c) reactor set-up (i.e., batch vs. semi-batch vs. quench-flow vs. miscellaneous), and water matrix (i.e., buffered ultra-purified water vs. surface water vs. wastewater; pH ranges of 5-8; use of hydroxyl radical scavengers). The publications which evaluated disinfection kinetics in buffered ultra-purified water or surface water in batch or quench-flow reactors at bench-scale were selected for further analyses. Due to the aforementioned complexities of ozone in DOM heavy matrices, studies evaluating wastewater were excluded. Articles which evaluated inactivation mechanisms of ozone for the different pathogen groups were also selected for further review. Articles that did not fall into either of these categories were omitted from the review. The reviewing process and the applied selection criteria are summarized in Figure S1. Additionally, while the core literature search was performed in September 2020, there were no additional relevant publications identified in a subsequent search spanning September 2020 through January 2022.

3.2. Data analysis

To explore the state-of-the-art of bench-scale inactivation of viruses, *Giardia* cysts, and *C. parvum* oocysts by ozone, analyses of $\log_{10}$ inactivation as a function of ozone Ct/exposure, as well as reported inactivation rate constants, were conducted. Articles which measured the disinfection kinetics in bench-scale settings (i.e., not inactivation mechanisms) were subject to additional screening which examined (a) the ability of ozone Ct/exposure data to be extracted or calculated directly from the article, (b) the method of ozone Ct/exposure calculation utilized/allowed for with the provided data (i.e., if Ct was not calculated within the manuscript, Ct was calculated to the best of our ability using the provided residual data), (c) proper implementation of the batch reactor or quench-flow reactor set-up, (d) utilization of methods which either directly examine or infer microbe viability (i.e., exclusion of molecular methods), and (e) basic water quality parameters such as temperature and pH.

The analyses consisted of the determination and comparison of ozone Ct/exposure values and corresponding survival ratios (provided as $\log_{10}(N/N_0)$) and second-order inactivation rate constants (k). If applicable, ozone Ct data and second-order inactivation rate constants were extracted directly from the article. If ozone Ct data were provided/calculated using the integral Ct method, this value was prioritized, otherwise priority was given in the following order: geometric mean Ct > arithmetic mean Ct (select studies which only provided Ct information in such form) > final residual ("effluent") Ct. If not provided in a study, second-order inactivation rate constants were calculated in R using least squares linear regression. Datasets with at least five data points were selected for determination of second-order inactivation rate constants. If the resultant inactivation slope (i.e., k) could not be determined statistically different ($p < 0.05$) from 0, the dataset was excluded. If the regression model had a non-significant ($p > 0.05$) y-intercept, the model was fitted through the origin. Otherwise, the y-intercept model was accepted under the assumption that a positive y-intercept provides a more conservative estimate of inactivation based on the resulting reduced slope, and a negative y-intercept indicates the presence of a lag-phase. Data which contained a significant lag-phase (i.e., a phase in which no inactivation occurs despite of ozone Ct/exposure) were fitted to the delayed Chick Watson model (Rennecker et al., 1999), with the Ct_{lag} value provided:

$$ln\frac{N}{N_0} = \begin{cases} 0; if\ Ct \le Ct_{lag} \\ -k(Ct - Ct_{lag}); if\ Ct > Ct_{lag} \end{cases} \quad (5)$$

The USEPA Ct models for ozone disinfection of viruses, *Giardia* cysts, and *C. parvum* oocysts, described in the SWTR and LT2ESWTR were utilized for comparison of the extracted inactivation data:

$$LRV_{virus} = 2.1744 \times 1.0726^{Temp} \times CT \quad (6)$$

$$LRV_{Giardia} = 1.0380 \times 1.0741^{Temp} \times CT \quad (7)$$

$$LRV_{Cryptosporidium} = 0.0397 \times 1.09757^{Temp} \times CT \quad (8)$$

Extracted data were grouped based on organism and temperature, regardless of pH.

4. Results and discussion

4.1. Literature review and data analysis

The aforementioned search criteria yielded 1,399 articles, which were subsequently reviewed through several iterations (Figure S1). This ultimately resulted in 28 articles from which inactivation vs. ozone Ct/exposure data and/or second-order inactivation rate constants were extracted, as well as 10 articles that evaluated ozone inactivation mechanisms for specific pathogen groups. Datasets from each organism were separated into three temperature ranges: 1-9 °C, 10-19 °C, and 20-25 °C. The pH examined in the different datasets ranged from 5 – 8. Due to the overall lack of data, datasets with and without hydroxyl radical scavengers were ultimately grouped together, which could introduce an additional source of variation within datasets, even though the contribution of hydroxyl radical to inactivation is likely minimal.

4.2. Inactivation of viruses

While viruses encompass a variety of morphologies that may influence their resistance to disinfectants (Sigstam et al., 2013), to remain consistent with USEPA categorization, all mammalian virus data were compiled together regardless of viral species. For analyses of mammalian virus surrogates, bacteriophage data were analyzed separately. Six studies met inclusion criteria for the development of $\log_{10}$ inactivation plots and/or inactivation rate constant compilation (Helmer and Finch, 1993; Lim et al., 2010; Sigmon et al., 2015; Torii et al., 2020; Wolf et al., 2018; Young et al., 2020). Six mammalian viruses were evaluated, including coxsackieviruses B3 and B5 (laboratory and environmental strains), poliovirus 1, echovirus 11, human adenovirus 2, and murine norovirus. Bacteriophages MS2, fr, Qβ, GA, φX174, T4, T1, and PRD1 were evaluated. All inactivation data were collected using cell culture most probable number (MPN) or plaque assays respective to each virus type. Experiments were conducted in either batch or quench-flow reactors.

Fig. 1a-d provide an overview of the compiled $\log_{10}$ inactivation of viruses as a function of ozone Ct (i.e., exposure) for three temperature ranges. Overall, viruses are highly reactive with ozone, indicating that efficient inactivation occurs even at very low ozone Ct/exposure. The USEPA models shown as dashed line in Fig. 1a-d provide a conservative estimate of virus inactivation, underestimating inactivation in most cases. The USEPA model was developed using data from Roy et al. (1982), who evaluated poliovirus inactivation using a CSTR. Because of the reactor setup, this study did not pass our article selection process and was therefore not analyzed. Torii et al. (2020) discussed the shortcomings in how the data from this study was utilized for USEPA models, including the use of the average hydraulic residence time as a substitution for the contact time, which would lead to an overestimation of ozone Ct/exposure. Hydraulic considerations, combined with incomplete mixing, and the additional safety factor of 3 applied by the USEPA, may account for the overly conservative nature of the model.

The data points which cluster directly at or below the USEPA models for temperatures ranging from 20 – 25 °C (Fig. 1c) belong to a single study examining murine norovirus inactivation. These data imply that

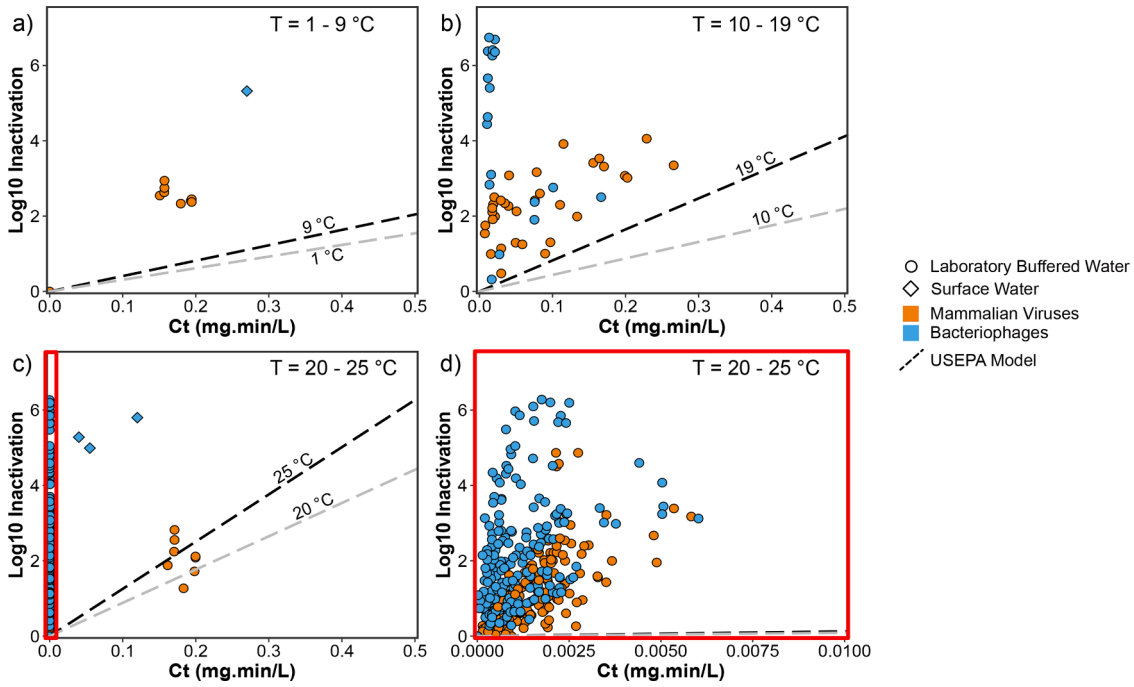

Fig. 1. Virus inactivation by ozone at pH 5-8: $\log_{10}$ inactivation as a function of ozone Ct for viruses at (a) 1-9 °C, (b) 10-19 °C, (c) 20-25 °C (d) 20-25 °C (magnified to highlight low Ct range).

murine noroviruses may be relatively less susceptible to ozone inactivation than other mammalian viruses. However, the ozone Ct/exposure values determined from this study may be inaccurate. Specifically, the study suggests that with ozone decay constants upwards of 5 min^{-1} and an applied ozone dose of 1 mg L^{-1}, there was still measurable ozone residual at the end of the experimental contact times (greater than two minutes). With these parameters a residual ozone concentration of 4.5×10^{-5} mg/L can be calculated, which is far below the detection for ozone in aqueous solution (Eq. 3) (Bader and Hoigné, 1981). This may indicate potential interferences in the ozone residual measurements, or other issues with the experimental set up, which would lead to less reliable ozone decay data. Nevertheless, as the only representative of the virus family *Caliciviridae,* which contains notable human pathogen human norovirus, it was necessary to include the data in this review.

Interestingly, mammalian viruses appear slightly more resistant to ozone than most bacteriophages at all temperature ranges. Figures S2a,b (SI) indicate that bacteriophage MS2 and closely related ssRNA phages (fr, GA, Qβ) (blue symbols in Figure S2) are the least resistant to ozone, while the DNA phages T4, PRD1, and φX174 are more resistant (red symbols in Figure S2). Bacteriophages, particularly MS2, are often utilized as surrogates for enteric, mammalian virus inactivation during water/wastewater treatment due to the ease of culturing and enumeration as compared to mammalian viruses (Amarasiri et al., 2017). The results from our assessment indicate that MS2 may provide a slight overestimation of viral pathogen inactivation by ozone. Bacteriophage T4 or φX174 may be more suitable for conservatively predicting virus inactivation by ozone, however further research is necessary.

The apparent differences in resistance towards ozone between bacteriophages and mammalian viruses are reflected within the compiled second-order inactivation rate constants (*k*), particularly at lower temperatures (Table 1). Inactivation rate constants for bacteriophages are 1-2 orders of magnitude higher than for mammalian viruses at temperatures from 1-19 °C, though this is based on a low number of data points (n=4 for mammalian viruses, n=5 for bacteriophages). This pattern of the range persists at 20-25 °C, however, the median values between the two datasets are comparable (2.3-4.7 x 10^3 mg^{-1} min^{-1} L).

When broken down by individual datasets (Table 2), the majority of the second-order rate constants for both bacteriophages and mammalian viruses fall in the range of 10^3 – 10^4 mg^{-1} min^{-1} L for 20-25 °C. Second-order rate constants outside of this range correspond to bacteriophages MS2 and Qβ on the higher end (1.2 – 3.1 x 10^4 mg^{-1} min^{-1} L) and the mammalian coxsackievirus B5 and murine norovirus at the lower end (0.33 – 6.1 x 10^2 mg^{-1} min^{-1} L). As previously described, the second-order rate constants associated with murine norovirus should be treated with caution. Disregarding murine norovirus, the higher and lower range of second-order inactivation rate constants are all associated with replicate datasets. MS2, Qβ, and coxsackievirus B5 all have additional measured second-order inactivation rate constants which fall within 10^3 – 10^4 mg^{-1} min^{-1} L. Many of the second-order inactivation rate constants were determined in studies utilizing either traditional batch systems, or highly sophisticated systems which allow for the evaluation of very low Ct ranges (<0.01 mg min L^{-1}) (Torii et al., 2020; Wolf et al., 2018; Young et al., 2020). The differences between these reactor systems may influence the variability experienced within the datasets. However, it should be noted that second-order rate constants for the reactions between ozone and specific chemical species have been

Table 1
Range of second-order inactivation rate constants, *k,* (base e, $mg^{-1}min^{-1}L$, $M^{-1}s^{-1}$ provided in parenthesis) of mammalian viruses and bacteriophages by ozone in buffered ultra-purified water.

	Mammalian Viruses				Bacteriophages			
T (°C)	n	Median	Min	Max	n	Median	Min	Max
1 - 9	2	-	2.1 x 10^1 (1.7 x 10^4)	2.9 x 10^1 (2.3 x 10^4)	2	-	1.7 x 10^3 (1.3 x 10^6)	2.4 x 10^3 (1.9 x 10^6)
10 - 19	2	-	1.5 x 10^1 (1.2 x 10^4)	3.4 x 10^1 (2.7 x 10^4)	4	1.4 x 10^3 (1.1 x 10^6)	8.5 x 10^2 (6.8 x 10^5)	3.7 x 10^3 (3.0 x 10^6)
20 - 25	18	2.1 x 10^3 (1.7 x 10^6)	3.3 x 10^1 (2.6 x 10^4)	4.4 x 10^3 (3.6 x 10^6)	11	4.7 x 10^3 (3.8 x 10^6)	1.5 x 10^3 (1.2 x 10^6)	3.1 x 10^4 (2.5 x 10^7)

Table 2
Second-order inactivation rate constants (k, base e) by ozone for specific viral group datasets in ultra-purified buffered waters. CA = *trans*-cinnamic acid, *t*-BuOH = *tert*-butanol.

Organism	Genome	Viral Family	Temp., °C	pH	k, mg^{-1} min^{-1} L	k, $M^{-1}s^{-1}$	Notes	Reference
MS2	ssRNA	*Fiersviridae*	2	6.5	1.7×10^3	1.3×10^6	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
MS2	ssRNA	*Fiersviridae*	2	8.5	2.4×10^3	1.9×10^6	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
Murine Norovirus	ssRNA	*Caliciviridae*	5	7	2.1×10^1	1.7×10^4	Batch, see discussion in main text	Lim et al. (2010)
Murine Norovirus	ssRNA	*Caliciviridae*	5	5.6	2.9×10^1	2.3×10^4	Batch, see discussion in main text	Lim et al. (2010)
MS2	ssRNA	*Fiersviridae*	12	6.5	2.0×10^3	1.6×10^6	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
MS2	ssRNA	*Fiersviridae*	12	8.5	3.7×10^3	3.0×10^6	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
MS2	ssRNA	*Fiersviridae*	16	7.5	8.5×10^2	6.8×10^5	Batch	Sigmon et al. (2015)
PRD1	dsDNA	*Tectoviridae*	16	7.5	6.3×10^1	5.1×10^4	Batch	Sigmon et al. (2015)
Coxsackievirus B5	ssRNA	*Picornaviridae*	16	7.5	1.5×10^1	1.2×10^4	Batch	Sigmon et al. (2015)
Human Adenovirus	dsDNA	*Adenoviridae*	16	7.5	3.4×10^1	2.7×10^4	Batch	Sigmon et al. (2015)
GA	ssRNA	*Fiersviridae*	22	7	9.4×10^3	7.5×10^6	Quench Flow	Torii et al. (2020)
MS2	ssRNA	*Fiersviridae*	22	7	1.6×10^4	1.2×10^7	Quench Flow	Torii et al. (2020)
MS2	ssRNA	*Fiersviridae*	22	6.5	1.3×10^4	1.0×10^7	*t*-BuOH, Quench Flow	Wolf et al. (2018)
MS2	ssRNA	*Fiersviridae*	22	8.5	4.7×10^3	3.8×10^6	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
MS2	ssRNA	*Fiersviridae*	22	6.5	2.4×10^3	1.9×10^6	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
fr	ssRNA	*Fiersviridae*	22	7	1.2×10^4	9.7×10^6	Quench Flow	Torii et al. (2020)
phiX174	ssDNA	*Microviridae*	22	7	3.9×10^3	3.1×10^6	Quench Flow	Torii et al. (2020)
phiX174	ssDNA	*Microviridae*	22	6.5	1.5×10^3	1.2×10^6	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
Qβ	ssRNA	*Fiersviridae*	22	7	3.1×10^4	2.5×10^7	Quench Flow	Torii et al. (2020)
Qβ	ssRNA	*Fiersviridae*	22	6.5	4.1×10^3	3.3×10^6	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
T4	dsDNA	*Myoviridae*	22	6.5	1.6×10^3	1.3×10^6	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
Coxsackievirus B3	ssRNA	*Picornaviridae*	22	7	3.5×10^3	2.8×10^6	Quench Flow	Torii et al. (2020)
Coxsackievirus B3	ssRNA	*Picornaviridae*	22	7	2.3×10^3	1.8×10^6	Environmental culture, Quench flow	Torii et al. (2020)
Coxsackievirus B3	ssRNA	*Picornaviridae*	22	7	1.4×10^3	1.1×10^6	Environmental culture, Quench flow	Torii et al. (2020)
Coxsackievirus B3	ssRNA	*Picornaviridae*	22	7	1.2×10^3	9.6×10^5	Environmental culture, Quench flow	Torii et al. (2020)
Coxsackievirus B5	ssRNA	*Picornaviridae*	22	7	4.4×10^3	3.6×10^6	Quench Flow	Torii et al. (2020)
Coxsackievirus B5	ssRNA	*Picornaviridae*	22	6.5	3.1×10^3	2.5×10^6	Environmental culture, *t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
Coxsackievirus B5	ssRNA	*Picornaviridae*	22	7	2.4×10^3	1.9×10^6	Environmental culture, Quench flow	Torii et al. (2020)
Coxsackievirus B5	ssRNA	*Picornaviridae*	22	6.5	2.3×10^3	1.8×10^6	*t*-BuOH, batch (w/ CA)	Young et al. (2020)
Coxsackievirus B5	ssRNA	*Picornaviridae*	22	7	2.3×10^3	1.8×10^6	Environmental culture, Quench flow	Torii et al. (2020)
Coxsackievirus B5	ssRNA	*Picornaviridae*	22	7	1.8×10^3	1.4×10^6	Environmental culture, Quench flow	Torii et al. (2020)
Coxsackievirus B5	ssRNA	*Picornaviridae*	22	6.5	6.1×10^2	4.9×10^5	Environmental culture, *t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
Coxsackievirus B5	ssRNA	*Picornaviridae*	22	6.5	5.5×10^2	4.4×10^5	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
Echovirus 11	ssRNA	*Picornaviridae*	22	6.5	2.4×10^3	1.9×10^6	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
Echovirus 11	ssRNA	*Picornaviridae*	22	6.5	2.0×10^3	1.6×10^6	*t*-BuOH, batch (w/ CA)	Young et al. (2020)
Human Adenovirus	dsDNA	*Adenoviridae*	22	6.5	1.1×10^3	9.0×10^5	*t*-BuOH, batch (w/ CA)	Wolf et al. (2018)
Murine Norovirus	ssRNA	*Caliciviridae*	20	5.6	4.0×10^1	3.2×10^4	Batch, see discussion in main text	Lim et al. (2010)
Murine Norovirus	ssRNA	*Caliciviridae*	20	7	3.3×10^1	2.6×10^4	Batch, see discussion in main text	Lim et al. (2010)
Poliovirus 1	ssRNA	*Picornaviridae*	22	7	3.7×10^3	2.9×10^6	Quench Flow	Torii et al. (2020)

shown to vary up to a factor of 5 for different studies (von Sonntag and von Gunten, 2012). Therefore, the variability determined within the virus dataset (up to 8-fold within the same viral species) is not too outstanding.

The slight differences in susceptibility between viral groups may be explained through inactivation mechanisms. Due to the differences in capsid morphology, genome composition, and replication mechanisms for different virus groups (e.g., enteroviruses, caliciviruses, coliphages, etc.), it is impossible to assign a singular ozone inactivation mechanism for viruses as a generalized group at this stage of the knowledge. Studies evaluating ozone inactivation mechanisms for the viral genus *Enterovirus* (family *Pircornaviridae*) are the most abundant. Roy et al. (1981) found RNA damage to account for the majority of inactivation for poliovirus 1. Torrey et al. (2019) came to a similar conclusion for echovirus 11, which was additionally confirmed by Young et al. (2020) for coxsackievirus B5. Results from this study also indicate that multiple genome hits are likely required for inactivation of echovirus 11 and coxsackievirus B5. Despite this, capsid damage cannot be fully ignored as a potential contributor to ozone inactivation, as current methodologies limit the ability to fully differentiate between the two. It is possible that differences in capsid composition/morphology may be an important factor for the differences in inactivation kinetics between genetically similar isolates of the same viruses determined in (Torii et al., 2021). Ozone inactivation mechanisms for viruses relevant to public health outside of *Picornaviridae*, such as human adenoviruses and *Caliciviruses*, have yet to be determined.

In contrast, Kim et al. (1980) was able to confirm capsid damage during ozone treatment of bacteriophage f2, though due to the analytical limitations of the time, it cannot be concluded that this is the primary inactivation mechanisms of ozone over genome damage. Bacteriophage f2, along with bacteriophage fr, are no longer considered distinct virus species, and have since been merged into the viral species MS2 (van Regenmortel et al., 2000). Therefore, it is appropriate to interpret these results as descriptive of MS2. MS2 belongs to the viral family *Fiersviridae* (formerly *Leviviridae*), which includes other closely related ssRNA bacteriophages in this review such as Qβ and GA (Callanan et al., 2020; Stockdale et al., 2020). Further research utilizing updated methods for MS2 and closely related bacteriophages could potentially explain their increased susceptibility to ozone disinfection compared to *Enteroviruses*. DNA bacteriophages φX174, T4, and PRD1, which behaved more similar to the mammalian viruses, are all taxonomically distinct from MS2 and other members of *Fiersviridae* up to the order of virus realm (ICTV, 2020). Therefore, it is inappropriate to assume that ozone inactivation mechanisms for these particular bacteriophages would be similar without further investigations. In general, more research is needed to explore the inactivation mechanisms and kinetics of DNA phages as well as mammalian DNA viruses (such as human adenoviruses). Based on similar inactivation mechanisms and kinetics a better surrogate than MS2 could be selected to assess viral inactivation during ozonation.

4.3. Inactivation of Giardia spp.

Seven studies investigating inactivation of *Giardia spp.* met the specific analysis criteria for extracting $\log_{10}$ inactivation vs. ozone Ct/

exposure data (Finch et al., 1993b; Haas and Kaymak, 2003; Labatiuk et al., 1994, 1992a, 1992b, 1991; Li et al., 2004). Of these seven studies, only two second-order inactivation rate constants are available for review (Haas and Kaymak, 2003; Labatiuk et al., 1992a). The majority of studies evaluated *Giardia muris*, a surrogate for the human pathogen *Giardia lamblia. G. muris* is advantageous in the laboratory setting as it is non-pathogenic to humans and maintains reproducible patterns of infection in mouse models (Belosevic and Faubert, 1983). There is conflicting evidence of the relative resistance of *G. muris* compared to *G. lamblia*, with some studies indicating higher resistance to ozone (Wickramanayake et al., 1985), while others finding no statistical differences (Finch et al., 1993b). Three different methods (*in vivo* mouse and gerbil models and *in vitro* excystation) were utilized to quantify *Giardia* survival ratios in the selected studies, which could lead to variability in study conclusions (Labatiuk et al., 1991). However, due to the overall lack of available data, studies using either of these methods were included for evaluation.

Compiled $\log_{10}$ inactivation vs. ozone Ct/exposure data at different temperature ranges are provided in Fig. 2a-c. All three temperature ranges indicate significant inactivation of *G. muris* cysts in the lower Ct ranges (< 1 mg min L^{-1}) with a tailing effect for higher ozone exposures. This effect is apparent regardless of the experimental matrix. Because of the tailing effect, the USEPA models (dashed lines in Fig. 2) overestimate inactivation of *Giardia* cysts for an ozone Ct $>$ ~1-2 mg min L^{-1} for most temperatures.

The USEPA model was developed from a single study that did not pass through the systematic review protocol due to its use of a semi-batch reactor set up (Wickramanayake et al., 1985; USEPA, 1991). Additionally, this study was performed at a single temperature (5 °C), which was used for extrapolation of inactivation models to higher temperatures. Based on the limited dataset used to develop the model, it is expected that it does not accurately predict *Giardia* cyst inactivation for the data analyzed in this review. This tailing effect highlights one of the weaknesses of utilizing a linear kinetic model for predicting inactivation.

The two available second-order inactivation rate constants for *G. muris* cysts are provided in Table 3. The values differ by over a factor of 10, and seemingly indicate higher efficiency at lower temperatures.

Table 3
Second-order inactivation rate constants, *k* (base e, $mg^{-1}min^{-1}L$, $M^{-1}s^{-1}$ provided in parenthesis) of *Giardia muris* cysts by ozone in buffered ultra-purified water.

	Giardia muris cysts	
T (°C)	n	Median
1 - 9	0	-
10 - 19	1	3.7×10^1 (3.0×10^4)
20 - 25	1	1.0×10^0 (8.3×10^2)

However, it should be noted that the second-order inactivation rate constant for T=10-19 °C (Haas and Kaymak, 2003) was determined at very low Ct values (all < 1 mg min L^{-1}), and therefore is reflective of the initial rapid inactivation phase evident prior to tailing. The data used to calculate second- order rate constants for T=20-25 °C (from Labatiuk et al., 1992a), examined higher Ct values, and therefore exhibited considerable tailing, which resulted in a poor model fit ($R^2 = 0.374$). Omitting data points influenced by tailing increased the second-order rate constant to 3.73 mg^{-1} min^{-1} L but remains almost exactly one order of magnitude lower than the second-order rate constants determined by Haas and Kaymak (2003). The compiled data indicate that a linear kinetic model may be inappropriate for estimating inactivation of *Giardia* cysts by ozone at higher Ct/exposure.

Studies examining the mechanism of *Giardia* cyst inactivation have reported that ozone produces severe degradation of the inner membranous layer of the cyst wall, irregular vacuole formation, and detachment of the trophozoite within the cyst (Li et al., 2004). These changes in cyst morphology were consistent with cyst inactivation. However, it remains unclear if the observed tailing effect is related to the inactivation mechanism by ozone. Labatiuk et al. (1992b) determined that *Giardia* cysts are rapidly inactivated within the first two minutes of ozonation, with little inactivation occurring thereafter, even in the presence of considerable ozone residual. Additionally, it was observed that ozone decreased in efficiency with lower concentrations of *Giardia* cysts, which would be the scenario encountered after the rapid initial inactivation (Haas and Kaymak, 2003). Regardless of the potential cause of the tailing effect, it is evident that at least 1 $\log_{10}$ inactivation of *Giardia* cysts can be obtained by an ozone exposure of ~1 mg min L^{-1}.

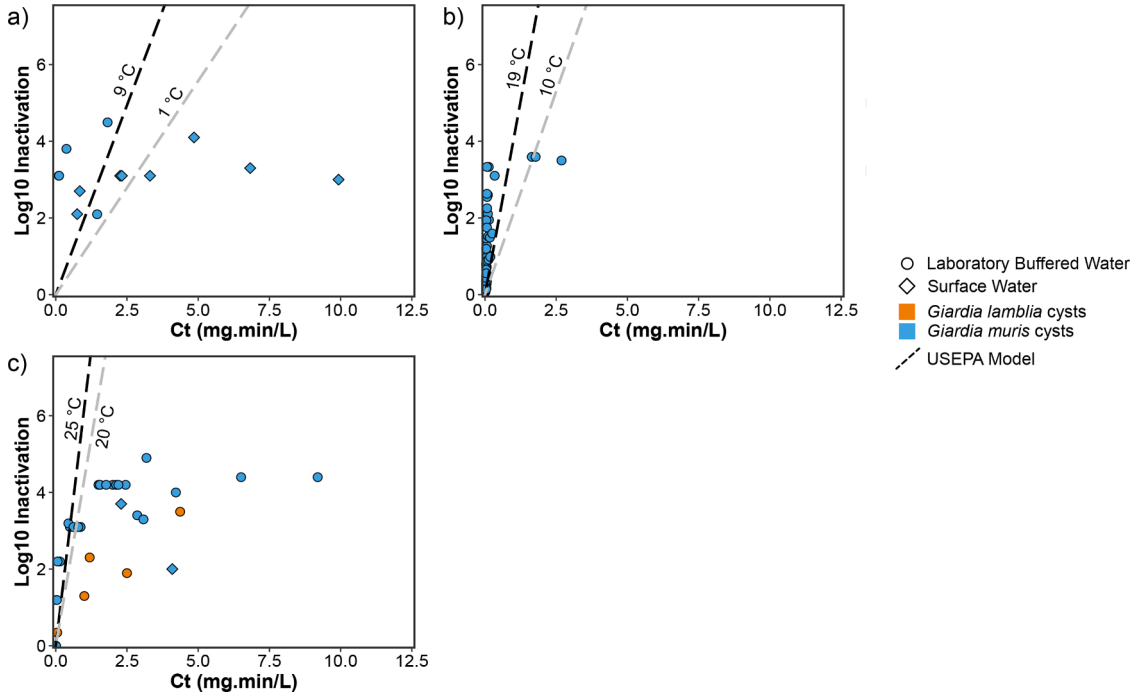

Fig. 2. *Giardia spp.* cyst inactivation by ozone at pH 5-8: $\log_{10}$ inactivation as a function of ozone Ct for *Giardia spp.* cysts at (a) 1-9 °C, (b) 10-19 C, (c) 20-25 °C.

4.4. Inactivation of Cryptosporidium parvum oocysts and surrogates

Eight studies met the criteria for inclusion for log_{10} inactivation vs. ozone Ct/exposure analysis of *C. parvum* oocysts (Cho and Yoon, 2007; Craik et al., 2003; Finch et al., 1993a; Finch and Li, 1999; Gyurek et al., 1999; Lewin et al., 2001; Li et al., 2001; Wohlsen et al., 2007), with six of the studies reporting second-order inactivation rate constants (Cho and Yoon, 2007; Craik et al., 2003; Finch et al., 1993a; Gyurek et al., 1999; Lewin et al., 2001; Li et al., 2001). The evaluated studies utilized either *in vivo* mouse models or *in vitro* excystation to quantify inactivation of *C. parvum* oocysts. In studies where both *in vivo* and *in vitro* methods were utilized, *in vivo* data were preferentially selected for analysis due to evidence of *in vitro* methods underestimating inactivation (Bukhari et al., 2000; Finch et al., 1993a). While the studies specifically utilized *C. parvum*, the stocks were largely obtained from different sources or batches. In addition to *C. parvum* oocysts, data evaluating disinfection of *Bacillus subtilis* spores from nine studies were also evaluated to assess its potential as a surrogate for *C. parvum* oocysts (Cho et al., 2006, 2003a, 2003b, 2002; Choi et al., 2007; Craik et al., 2002; Dow et al., 2006; Driedger et al., 2001).

C. parvum oocyst inactivation data for three temperature ranges are provided in Fig. 3a-c. *C. parvum* oocysts exhibit greater resistance to ozone than both viruses and *Giardia*. The data do not provide evidence of a lag-phase or tailing at low or high ozone Cts, respectively. The USEPA models (dashed lines) are closely aligned with the inactivation data from the compiled studies, providing the most accurate prediction of inactivation of the three selected pathogen group models. The model over-predicts *C. parvum* oocyst inactivation for 8% of the 135 total data points. Even though this is a good agreement, it is unclear if these deviations from the model are due to natural variability of the organism (thus indicating the model is not conservative to oocyst inactivation) or due to variability in experimental methods.

The USEPA model for *C. parvum* oocysts was developed from the data of four studies (Li et al., 2001; Oppenheimer et al., 2000; Owens et al., 2000; Rennecker et al., 1999). Only one of the four studies was included in our analysis (Li et al., 2001), as the remainder of the studies either utilized semi-batch systems, evaluated inactivation at pilot-scale, or were not published in a peer-reviewed article (i.e., only project report); all of which were selective constraints of the literature review process in this article. The *C. parvum* oocyst inactivation models developed by USEPA utilized a less stringent safety factor due to concerns related to bromate formation. Therefore, it is expected that the model would provide a less conservative prediction of inactivation in comparison to the virus model.

The inactivation efficiency of *B. subtilis* during ozonation was also examined (Fig. 3d-f, blue symbols). In contrast to *C. parvum* oocysts, there is an apparent shouldering, or inactivation lag-phase, at each temperature. This lag-phase appears to change with temperature, but with no clear pattern (i.e., the lag-phase in the 10 -19 °C range appears larger than both 5 – 9 °C and 20 – 25 °C), despite a clear temperature relationship determined in Driedger et al. (2001). This could be due to the grouping of data regardless of pH, which has been previously demonstrated to influence the lag-phase (Cho et al., 2003b, 2003a, 2002; Dow et al., 2006). It is also possible that additional water quality parameters may be influencing the lag. Dow et al. (2006) evaluated parameters influencing the lag-phase of *B. subtilis* inactivation and did

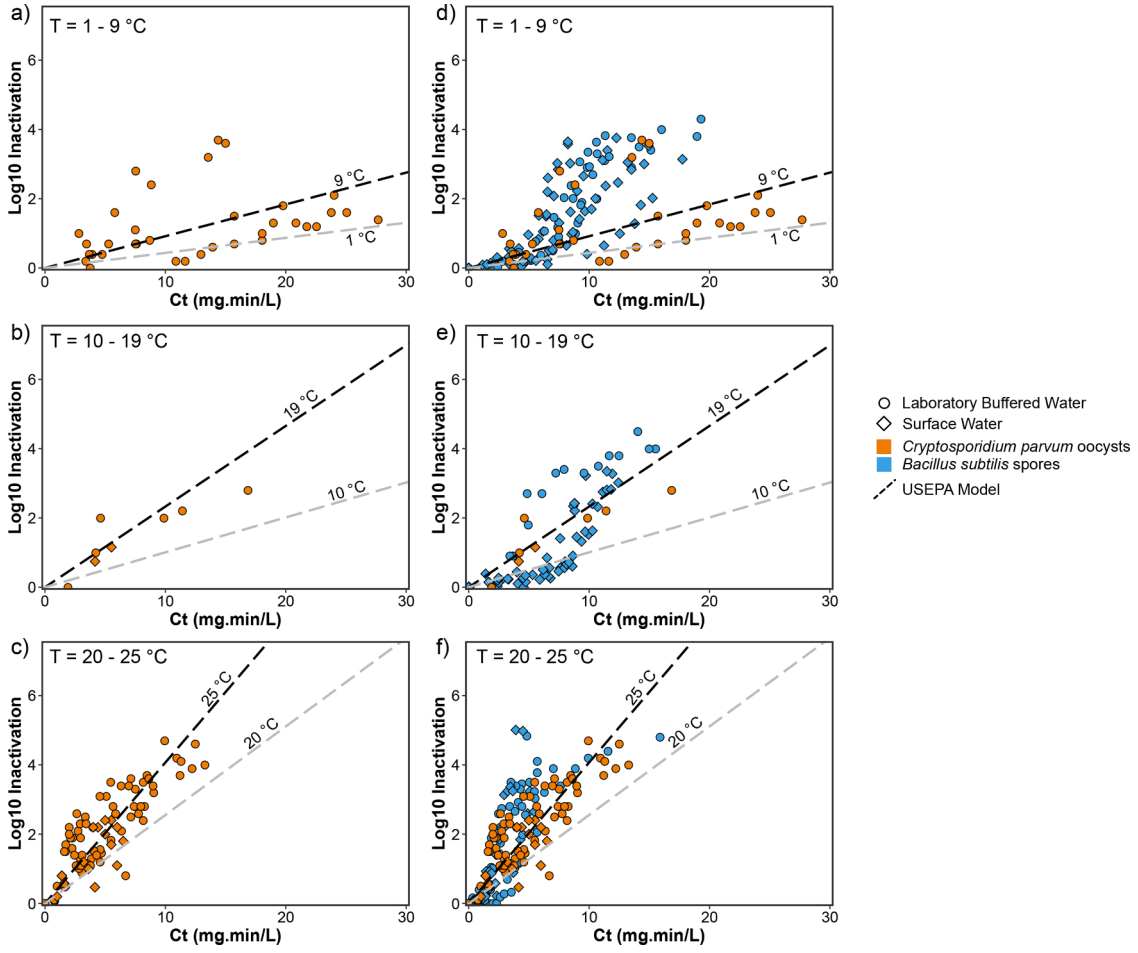

Fig. 3. *C. parvum* oocyst inactivation by ozone at pH 5-8: log_{10} inactivation as a function of ozone Ct for *C. parvum* oocysts at (a) 1-9 °C, (b) 10-19 °C, (c) 20-25 °C, *C. parvum* oocysts and *B. subtillis* spores at (d) 1-9 C, (e) 10-19 °C, and (f) 20-25 °C.

not find any significant influence of bulk DOC concentration or turbidity, however, differences in DOM composition were not considered. It is currently unclear which water quality parameter, if any, may be influencing the extent of the lag-phase.

Table 4 provides second-order inactivation rate constants for both *C. parvum* oocysts and *B. subtilis* spores, respectively, using either the second-order linear model or delayed Chick-Watson model. Overall, second-order inactivation rate constants for *C. parvum* oocysts are much lower than for viruses or *Giardia* cysts, indicating that it is much more resistant to ozone. In addition to second-order rate constants in buffered solutions, values in various synthetic or natural surface waters are also provided for each organism. Based on limited data for both *C. parvum* oocysts and *B. subtilis* spores, it is not clear that natural or synthetic surface water significantly influences the inactivation rate. The median second-order inactivation rate constants for both organisms in natural/synthetic surface water fall within the ranges determined in buffered solutions, albeit this is based on very limited data in the case of *C. parvum* oocysts.

To evaluate *B. subtilis* spores as a surrogate for *C. parvum* oocyst inactivation, the ozone Ct/exposure for 2 $\log_{10}$ inactivation were calculated using the respective inactivation models for direct comparison. For the temperature range of 20 – 25 °C, a Ct/exposure of 4.8 – 8.8 mg min L^{-1} would be necessary for 2 $\log_{10}$ inactivation of *C. parvum* oocysts. However, for *B. subtilis*, a Ct ranging from 0.4 to 12.6 mg min L^{-1} is required. The wide variety of factors influencing *B. subtilis* spore inactivation exclude it as a surrogate for *C. parvum oocyst* inactivation, in agreement with Driedger et al. (2001).

The consistent lag-phase for *B. subtilis* spores, but not *C. parvum* oocysts, may provide potential evidence of different ozone inactivation mechanisms. Shouldering can be indicative of a multiple-hit inactivation mechanism, in which multiple areas of an organism must be damaged by the disinfectant prior to its inactivation (Kimball, 1953). The shouldering phase dissipates once the necessary number of targets are destroyed within the organism, usually leading to an exponential phase of inactivation. This contrasts with a single-hit mechanism, in which the destruction of one target results in the inactivation of the organism (i.e., exponential disinfection begins immediately upon application). It has been found that the inner-membrane of the *B. subtilis* spore is likely the site of lethal damage, with ozone damage to the spore coat likely necessary prior to destruction of the inner-membrane (Young and Setlow, 2004). However, it cannot be ruled out that the damage to the inner membrane is limited by ozone diffusion through the spore coat. Regardless, the ultimate effect remains the same in which the spore coat provides an initial resistance to disinfection, resulting in a perceived lag-phase and thus supports the use of a delayed Chick-Watson model.

The mechanism of ozone inactivation for *C. parvum* oocysts has not been evaluated previously, to the best of our knowledge. The oocyst wall is likely composed of an outer glycocalyx layer followed by a lipid hydrocarbon layer, a layer of cysteine-rich proteins, and ultimately a structural polysaccharide layer, underneath which exists the sporozoite (Jenkins et al., 2010). The *C. parvum* sporozoite cannot survive without its oocyst, and it is unable to complete its life cycle until it reaches the gut of a host, excysts, and attaches to intestinal lining (Smith et al., 2005). Encysting is a lifecycle requirement for *C. parvum*. This is in contrast with *B. subtilis* which can freely exist as a vegetative bacterial cell without its spore; spore formation is a survival mechanism for low-nutrient environmental conditions (Sella et al., 2014). These differences in life cycles and the ensuing differences in cell structures likely influence the discrepancy in ozone inactivation kinetics between the two distinct organisms. Interestingly, studies have determined that *in-vitro* excystation predicts less inactivation of *C. parvum oocysts* when compared to *in-vivo* mouse models for similar ozone exposures (Bukhari et al., 2000; Finch et al., 1993a), i.e., some sporozoites successfully excyst when provided gut-mimicking conditions, however, they may lack the capability of completing their life cycle once excysted. This could indicate damage to the sporozoite by ozone, however, further research would be necessary to verify this. In contrast to previous studies, the results from this review did not indicate a large discrepancy between *in-vivo* and *in-vitro* methods (Figure S3, SI).

4.5. Limitations of previous studies and research needs

In this review, there were several limitations associated with the comparison of inactivation data from different sources. First and foremost, a large portion of disinfection studies were excluded during the review process due to the use of non-batch or non-quench-flow reactor designs. In particular, many of the excluded bench-scale studies utilized semi-batch reactors. This drastically reduced the number of studies for data extraction (69 of 210 articles omitted for this reason). Semi-batch reactors were excluded from examination due to the inherent difficulty in proper application of such systems (Finch et al., 2001). Moreover, as a reviewer, it can be difficult to assess proper implementation of semi-batch systems due to the limited information provided within manuscripts. A more unified approach to studying ozone disinfection kinetics would help to increase the availability of comparable data produced in future studies.

In studies utilizing similar approaches, there were still many systematic differences that made comparison difficult, such as differences in reactor volumes utilized (5 mL to 5 L) and ozone residual measurement methods (direct absorbance at 260 nm vs. indigo trisulfonate at 600 nm), the use of hydroxyl radical scavengers (Table S1). Additionally, the purity level of the microbial stocks can influence results and skew interpretation due to the interactions between the organic matter present in microbial growth media and ozone (Mesquita et al., 2010;

Table 4
Second-order inactivation rate constants, *k* (base e, $mg^{-1}min^{-1}L$, $M^{-1}s^{-1}$ provided in parenthesis) and Ct_{lag} (mg min L^{-1}) values (when applicable) of *C. parvum* oocysts and *B. subtilis* spores by ozone in buffered ultra-purified water (or similarly low DOC matrix) and natural or synthetic surface water.

		***C. parvum* oocysts**				***B. subtilis* spores**						
			***k*, mg^{-1} min^{-1} L ($M^{-1}s^{-1}$)**				***k*, mg^{-1} min^{-1} L ($M^{-1}s^{-1}$)**			**Ct_{lag}, mg min L^{-1}**		
	***T* (°C)**	**n**	**Median**	**Min**	**Max**	**n**	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**
Laboratory Buffer	*1 - 9*	1	1.1×10^{-1} (9.0×10^{1})	-	-	2	-	6.9×10^{-1} (5.5×10^{2})	1.2×10^{0} (9.6×10^{2})	-	3.4	5.1
	10 - 19	0	-	-	-	1	(9.3×10^{-1}) (7.4×10^{2})	-	-	2.6	-	-
	20 - 25	4	7.2×10^{-1} (5.7×10^{2})	5.3×10^{-1} (2.4×10^{2})	9.6×10^{-1} (7.7×10^{2})	21	1.7×10^{0} (1.3×10^{3})	4.3×10^{-1} (3.4×10^{2})	5.2×10^{0} (4.2×10^{3})	1.2	0.3	2.0
Natural/synthetic surface water	*1 - 9*	0	-	-	-	4	1.2×10^{0} (9.8×10^{2})	9.7×10^{-1} (7.8×10^{2})	2.0×10^{0} (1.6×10^{3})	5.2	4.6	10
	10 - 19	0	-	-	-	2	-	1.6×10^{0} (1.3×10^{3})	2.8×10^{0} (2.2×10^{3})		2.7	6.1
	20 - 25	1	9.0×10^{-1} (7.2×10^{2})	-	-	11	2.9×10^{0} (2.3×10^{3})	1.3×10^{0} (1.1×10^{3})	6.3×10^{0} (5.1×10^{3})	1.4	0.6	2.9

Wolf et al., 2018). Furthermore, the method in which ozone Ct is determined can influence data interpretation. Of the 1,038 data points collected for $\log_{10}$ inactivation vs. ozone Ct, 72 data points had sufficient information to estimate ozone Ct using the four methods encountered in this review (integral, geometric mean, final residual, and arithmetic mean). Assuming integration of the ozone residual curve provides the most accurate estimation of ozone exposure, comparison of this value with the alternative approaches determined that the geometric mean Ct method provided the closest estimate of Ct/exposure based on residual sum of squares. Fig. 4 demonstrates the ability of the geometric mean Ct method to accurately estimate the ozone exposure, which is to be expected as long as ozone exhibits first-order decay in a specific matrix and there is a final residual ozone concentration > 0 (Gyürek and Finch, 1998). In contrast, as shown in Fig. 4, the arithmetic mean overestimates and the final residual ("effluent") method underestimates the ozone exposure. While arithmetic mean deviates less from the integral method when compared to the "effluent" method, it requires the same input values as the geometric mean (i.e., C_0, C, and t). Therefore, there is no practical reason to utilize the arithmetic over the geometric mean. Figure S4 (SI) provides a conceptual overview of the different ozone Ct estimation methods.

It should be mentioned that while we have demonstrated only slight deviations in the different Ct estimation methods, these quick estimation methods begin to deviate considerably with increasing ozone decay rates, such as those seen during wastewater treatment. Therefore, it is not recommended to employ these estimation methods under such conditions. If an ozone decay curve cannot be collected for whatever reason, a more accurate estimation consists of estimating the first order ozone decay rate constant with the initial and final ozone residual concentration measurements and time elapsed between the two:

$$k' = -\frac{ln\left(\frac{C}{C_0}\right)}{t} \tag{10}$$

The calculated value for k' can then be substituted into (4). However, this method also requires an assumption of first-order ozone decay. Integration of an ozone residual time series is recommended as best practice.

Studies were also eliminated if they did not provide sufficient information to confidently compare either $\log_{10}$ inactivation and Ct or second-order inactivation rate constants, either through lack of ozone decay data, missing pertinent water quality data (i.e., temperature, pH, etc.), lack of sufficient reactor set-up description, improper microbiological data reporting, among others. It is recommended that ozone disinfection studies should provide the water quality and system characteristics previously described in Hoigné (1994) and Hoigné and Bader (1994) as necessary for general ozone related studies. Such characteristics include water temperature, pH, DOC concentration, alkalinity, among others. Additionally, disinfection studies should provide organism strain/identifier, cultivation and purification techniques, initial spike concentrations, and particularly the method used to handle data which fall below limits of detection (i.e., avoiding substitution with detection limit).

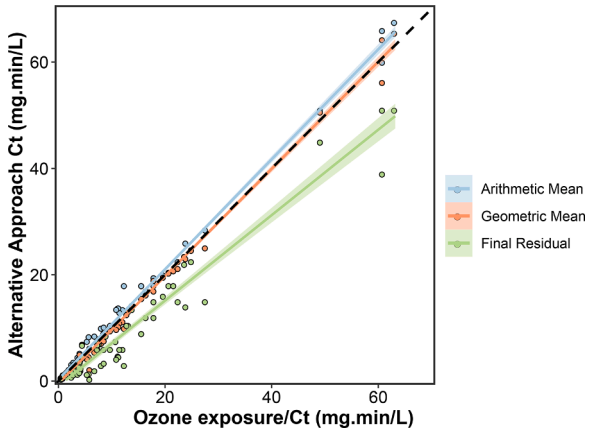

Fig. 4. Comparison of Ct calculation methods (at ozone decay rates < 0.5 min^{-1}) based on 72 data points from the literature search which provided enough information to calculate Ct with the four different approaches (integration of residual curve, i.e., "true" ozone exposure, geometric mean, arithmetic mean, and final residual). Orange symbols indicate the geometric mean Ct calculation. Green symbols indicate the final residual (effluent) Ct calculation. Blue symbols indicate arithmetic mean Ct calculation. Linear regression lines with 95% confidence intervals are also provided. The dashed line represents a scenario in which the alternative Ct approach perfectly predicts the ozone exposure determined via integration of the ozone residual curve.

While the evaluation of virus inactivation via ozone has progressed through time with increasingly sophisticated techniques, studies on *Giardia* cysts and *C. parvum* oocysts have stalled, with the most recent publications meeting our selection criteria published more than 14 years ago. After several outbreaks of cryptosporidiosis initially spurred interest in this topic (Fox and Lytle, 1996), support for such research waned and is still currently lacking. This is likely in large part due to limitations in cultivating and assessing viability of each organism, which require *in vivo* animal models. However, particularly in the case of *Giardia* which deviated the furthest from the USEPA models based on data within this review, new data would be of great benefit, particularly in discerning the cause of the apparent tailing effect.

Similarly, ozone inactivation kinetics evaluated at varying temperature ranges were sparse, with the majority of studies included within this review evaluating inactivation at 20-25 °C. Furthermore, very few studies evaluated inactivation at varying temperatures within the same study. As described previously, systematic differences between studies make direct comparisons of data difficult. Therefore, it is difficult to provide confident assertations regarding the effect of temperature on disinfection efficacy based on these studies alone. Once again, this was strikingly evident for *Giardia* cysts in which *k* values for the 10-19 °C range appear significantly larger than the 20-25 °C, which is contrary to what might be expected.

There is a lack of mechanistic explanations for the different inactivation kinetics exhibited by the different organisms, largely due to the lack of studies evaluating inactivation mechanisms of ozone in general, particularly for encysted protozoans. Ozone inactivation mechanisms are summarized in Fig. 5. However, there have been advances in the study of viral inactivation mechanisms. Torrey et al. (2019) and Young et al. (2020) provide novel methodologies for estimating viral inactivation mechanisms, particularly relating to genome destruction. These methods could be applied to other virus species, and potentially validate the differences in inactivation mechanisms between MS2 and closely related bacteriophages and other ssRNA mammalian viruses, as well as provide justification for potentially better surrogate candidates such as φX174 or T4.

It is important to recognize that while the reported kinetics of surrogate bacteriophages and mammalian viruses appear to deviate from one another, when compared to Ct requirements for *Giardia* cysts and *C. parvum* oocysts, those apparent differences become negligible (Fig. 6). Specifically, drinking water systems designed for 2 $\log_{10}$ inactivation of *C. parvum* oocysts will require a significant ozone Ct/exposure (i.e., much greater than 1 mg min L^{-1}), which would ensure considerable inactivation of mammalian viruses and bacteriophages ($\gg 2$ $\log_{10}$ at 20 °C). However, in situations where little to no ozone exposure is achieved, such as in sub-residual water reuse applications or with ozone AOP (i.e., supplemented with H_2O_2), these differences could become relevant, and thus further exploration into alternate virus surrogates is warranted, particularly with the increasing interest in ozone for water reuse applications and the significant virus inactivation requirements in such systems.

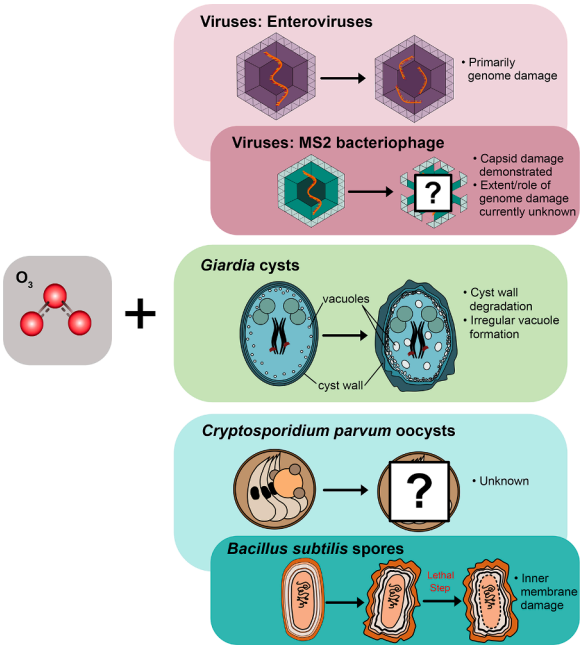

Fig. 5. Summary of ozone inactivation mechanisms for each pathogen group and associated surrogates.

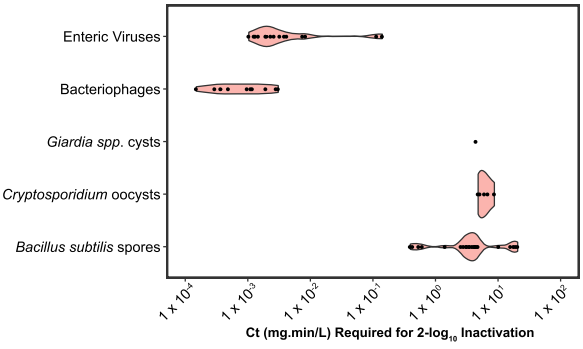

Fig. 6. Ct required for 2 $\log_{10}$ inactivation of different organisms with ozone as determined by compiled inactivation rate constants at 20-25 °C. For data see Tables 1-3. Shaded area around points provides insight into the distribution of Ct values.

5. Conclusions

Based on a critical review of ozone disinfection the following conclusions can be drawn:

- Ozone is an effective disinfectant against chlorine resistant pathogens such as *C. parvum* oocysts, can effectively inactivate other pathogens such as *Giardia* cysts, and performs rapidly against viruses.
- USEPA models which were developed more recently using multiple datasets (*C. parvum* oocysts) were more accurate than earlier models developed using a single dataset (viruses and *Giardia* cysts). Comparison of the data compiled in this review suggests the need for improvements in USEPA models for viruses and *Giardia* cysts.
- Further research on the applicability of non-pathogenic surrogates such as MS2 and *B. subtilis* spores is necessary. The data compiled in this review indicate that these organisms do not accurately reflect the behavior of the pathogens they represent. Studies directly evaluating pathogenic organisms are costly and time consuming, therefore further investigation of appropriate surrogates may help advance the knowledge, and ultimately the prediction, of ozone disinfection.
- Greater emphasis should be placed on minimizing differences in ozone disinfection study approaches and development of standard protocols. This would allow for better comparability between studies and allow for greater consensus on ozone inactivation of pathogens.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118206.

References

Acero, J.L., von Gunten, U., 2001. Characterization of oxidation processes: ozonation and the AOP O3/H2O2. J. Am. Water Work. Assoc. 93, 90–100. https://doi.org/10.1002/j.1551-8833.2001.tb09311.x.

Amarasiri, M., Kitajima, M., Nguyen, T.H., Okabe, S., Sano, D., 2017. Bacteriophage removal efficiency as a validation and operational monitoring tool for virus reduction in wastewater reclamation. Review. Water Res. 121, 258–269. https://doi.org/10.1016/j.watres.2017.05.035.

Bader, H., Hoigné, J., 1981. Determination of ozone in water by the indigo method. Water Res 15, 449–456. https://doi.org/10.1016/0043-1354(81)90054-3.

Belosevic, M., Faubert, G.M., 1983. Giardia muris : correlation between oral dosage , course of infection , and trophozoite distribution in the mouse small intestine. Expermental Parisitology 56, 93–100.

Betancourt, W., 2019. Part three: specfic exreted pathoges: environmental and epidemology aspects - cryptospororidum spp. Glob. Water Pathog. Proj. 7, 1–49.

Buffle, M.O., Schumacher, J., Salhi, E., Jekel, M., von Gunten, U., 2006. Measurement of the initial phase of ozone decomposition in water and wastewater by means of a continuous quench-flow system: Application to disinfection and pharmaceutical oxidation. Water Res 40, 1884–1894. https://doi.org/10.1016/j.watres.2006.02.026.

Bukhari, Z., Marshall, M.M., Korich, D.G., Fricker, C.R., Smith, H.V., Rosen, J., Clancy, J. L., 2000. Comparison of Cryptosporidium parvum viability and infectivity assays following ozone treatment of oocysts. Appl. Environ. Microbiol. 66, 2972–2980. https://doi.org/10.1128/AEM.66.7.2972-2980.2000.

Burns, N., Hunter, G., Jackman, A., Hulsey, B., Coughenour, J., Walz, T., 2007. The return of ozone and the hydroxyl radical to wastewater disinfection. Ozone Sci. Eng. 29, 303–306. https://doi.org/10.1080/01919510701463206.

Callanan, J., Stockdale, S.R., Shkoporov, A., Draper, L.A., Ross, R.P., Hill, C., 2020. Expansion of known ssRNA phage genomes: from tens to over a thousand. Sci. Adv. 6 https://doi.org/10.1126/sciadv.aay5981.

Camel, V., Bermond, A., 1998. The use of ozone and associated oxidation processes in drinking water treatment. Water Res 32, 3208–3222. https://doi.org/10.1016/S0043-1354(98)00130-4.

Cho, M., Chung, H., Yoon, J., 2003a. Quantitative evaluation of the synergistic sequential inactivation of Bacillus subtilis spores with ozone followed by chlorine. Environ. Sci. Technol. 37, 2134–2138. https://doi.org/10.1021/es026135h.

Cho, M., Chung, H., Yoon, J., 2003b. Disinfection of water containing natural organic matter by using ozone-initiated radical reactions. Appl. Environ. Microbiol. 69, 2284–2291. https://doi.org/10.1128/AEM.69.4.2284-2291.2003.

Cho, M., Chung, H., Yoon, J., 2002. Effect of pH and importance of ozone initiated radical reactions in inactivating Bacillus subtilis spore. Ozone Sci. Eng. 24, 145–150. https://doi.org/10.1080/01919510208901605.

Cho, M., Kim, J.H., Yoon, J., 2006. Investigating synergism during sequential inactivation of Bacillus subtilis spores with several disinfectants. Water Res 40, 2911–2920. https://doi.org/10.1016/j.watres.2006.05.042.

Cho, M., Yoon, J., 2007. Quantitative evaluation and application of Cryptosporidium parvum inactivation with ozone treatment. Water Sci. Technol. 55, 241–250. https://doi.org/10.2166/wst.2007.008.

Choi, Y., Cho, M., Lee, Y., Choi, J., Yoon, J., 2007. Inactivation of Bacillus subtilis spores during ozonation in water treatment plant: Influence of pre-treatment and consequences for positioning of the ozonation step. Chemosphere 69, 675–681. https://doi.org/10.1016/j.chemosphere.2007.05.045.

Craik, S.A., Smith, D.W., Belosevic, M., Chandrakanth, M., 2002. Use of Bacillus subtilis spores as model microorganisms for ozonation of Cryptosporidium parvum in drinking water treatment. J. Environ. Eng. Sci. 1, 173–186. https://doi.org/10.1139/S02-012.

Craik, S.A., Smith, D.W., Chandrakanth, M., Belosevic, M., 2003. Effect of turbulent gas-liquid contact in a static mixer on Cryptosporidium parvum oocyst inactivation by ozone. Water Res 37, 3622–3631. https://doi.org/10.1016/S0043-1354(03)00285-9.

Criquet, J., Rodriguez, E.M., Allard, S., Wellauer, S., Salhi, E., Joll, C.A., von Gunten, U., 2015. Reaction of bromine and chlorine with phenolic compounds and natural organic matter extracts - electrophilic aromatic substitution and oxidation. Water Res 85, 476–486. https://doi.org/10.1016/j.watres.2015.08.051.

Czekalski, N., Imminger, S., Salhi, E., Veljkovic, M., Kleffel, K., Drissner, D., Hammes, F., Bürgmann, H., von Gunten, U., 2016. Inactivation of antibiotic resistant bacteria and resistance genes by ozone: from laboratory experiments to full-scale wastewater treatment. Environ. Sci. Technol. 50, 11862–11871. https://doi.org/10.1021/acs.est.6b02640.

Dow, S.M., Barbeau, B., von Gunten, U., Chandrakanth, M., Amy, G., Hernandez, M., 2006. The impact of selected water quality parameters on the inactivation of Bacillus subtilis spores by monochloramine and ozone. Water Res 40, 373–382. https://doi.org/10.1016/j.watres.2005.10.018.

Driedger, A., Staub, E., Pinkernell, U., Mariñas, B., Köster, W., von Gunten, U., 2001. Inactivation of bacillus subtilis spores and formation of bromate during ozonation. Water Res 35, 2950–2960. https://doi.org/10.1016/S0043-1354(00)00577-7.

Efstratiou, A., Ongerth, J.E., Karanis, P., 2017. Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2011–2016. Water Res 114, 14–22. https://doi.org/10.1016/j.watres.2017.01.036.

Elovitz, M.S., von Gunten, U., Kaiser, H.P., 2000. Hydroxyl radical/ozone ratios during ozonation processes. II. The effect of temperature, pH, alkalinity, and DOM properties. Ozone Sci. Eng. 22, 123–150. https://doi.org/10.1080/01919510008547216.

Finch, G.R., Black, E.K., Gyurek, L., Belosevic, M., 1993a. Ozone inactivation of Cryptosporidium parvum in demand-free phosphate buffer determined by in vitro excystation and animal infectivity. Appl. Environ. Microbiol. 59, 4203–4210. https://doi.org/10.1128/aem.59.12.4203-4210.1993.

Finch, G.R., Black, E.K., Labatiuk, C.W., Gyurek, L., Belosevic, M., 1993b. Comparison of Giardia lamblia and Giardia muris cyst inactivation by ozone. Appl. Environ. Microbiol. 59, 3674–3680. https://doi.org/10.1128/aem.59.11.3674-3680.1993.

Finch, G.R., Haas, C.N., Oppenheimer, J.A., Gordon, G., Trussell, R.R., 2001. Design criteria for inactivation of Cryptosporidium by ozone in drinking water. Ozone Sci. Eng. 23, 259–284. https://doi.org/10.1080/01919510108962010.

Finch, G.R., Li, H., 1999. Inactivation of cryptosporidium at 1°C using ozone or chlorine dioxide. Ozone Sci. Eng. 21, 477–486. https://doi.org/10.1080/01919512.1999.10382886.

Fox, K.R., Lytle, D.A., 1996. Milwaukee's crypto outbreak: investigation and recommendations. J. Am. Water Works Assoc. 88, 87–94.

Gamage, S., Gerrity, D., Pisarenko, A.N., Wert, E.C., Snyder, S.A., 2013. Evaluation of process control alternatives for the inactivation of escherichia coli, ms2 bacteriophage, and bacillus subtilis spores during wastewater ozonation. Ozone Sci. Eng. 35, 501–513. https://doi.org/10.1080/01919512.2013.833852.

Gerrity, D., Gamage, S., Jones, D., Korshin, G.V, Lee, Y., Pisarenko, A., Trenholm, R.A., von Gunten, U., Wert, E.C., Snyder, S.A., 2012. Development of surrogate correlation models to predict trace organic contaminant oxidation and microbial inactivation during ozonation. Water Res 46, 6257–6272. https://doi.org/10.1016/j.watres.2012.08.037.

Gyürek, L.L., Finch, G.R., 1998. Modeling Water Treatment Chemical Disinfection Kinetics. J. Environ. Eng. 124, 783–793.

Gyurek, L.L., Li, H., Belosevic, M., Finch, G.R., 1999. Ozone inactivation kinetics of cryptosporidium in phosphate buffer. J. Environ. Eng. 125, 913–924.

Haas, C.N., Kaymak, B., 2003. Effect of initial microbial density on inactivation of Giardia muris by ozone. Water Res 37, 2980–2988. https://doi.org/10.1016/S0043-1354(03)00112-X.

Harden, V.P., Harris, J.O., 1952. The isoelectric point of bacterial cells. J. Bacteriol. 65, 198–202.

Helmer, R.D., Finch, G.R., 1993. Use of MS2 coliphage as a surrogate for enteric viruses in surface waters disinfected with ozone. Ozone Sci. Eng. 15, 279–293. https://doi.org/10.1080/01919519308552490.

Hoigné, J., 1994. Characterization of water quality criteria for ozonation processes. part i:minimal set of analytical data. Ozone Sci. Eng. 16, 113–120. https://doi.org/10.1080/01919519408552416.

Hoigne, J., Bader, H., 1994. Characterization of water quality criteria for ozonation processes. part ii: lifetime of added ozone. Ozone Sci. Eng. 16, 121–134. https://doi.org/10.1080/01919519408552417.

Hoigné, J., Bader, H., 1975. Ozonation of water : role of hydroxyl radicals as oxidizing intermediates. Science 190, 782–784, 80-.

Hrudey, S.E., Payment, P., Huck, P.M., Gillham, R.W., Hrudey, E.J., 2003. A fatal waterborne disease epidemic in Walkerton, Ontario: Comparison with other waterborne outbreaks in the developed world. Water Sci. Technol. 47, 7–14. https://doi.org/10.2166/wst.2003.0146.

Hsu, B.M., Huang, C., 2002. Influence of ionic strength and pH on hydrophobicity and zeta potential of Giardia and Cryptosporidium. Colloids Surfaces A Physicochem. Eng. Asp. 201, 201–206. https://doi.org/10.1016/S0927-7757(01)01009-3.

Huber, M.M., Canonica, S., Park, G.Y., von Gunten, U., 2003. Oxidation of pharmaceuticals during ozonation and advanced oxidation processes. Environ. Sci. Technol. 37, 1016–1024. https://doi.org/10.1021/es025896h.

ICTV, 2020. Virus taxonomy: The ICTV report on virus classification and taxon nomenclature [WWW Document]. Int. Comm. Virus Taxon. URL https://talk.ictvonline.org/ictv-reports/ictv_online_report/.

Jenkins, M.B., Eaglesham, B.S., Anthony, L.C., Kachlany, S.C., Bowman, D.D., Ghiorse, W.C., 2010. Significance of wall structure, macromolecular composition, and surface polymers to the survival and transport of Cryptosporidium parvum oocysts. Appl. Environ. Microbiol. 76, 1926–1934. https://doi.org/10.1128/AEM.02295-09.

Kim, C.K., Gentile, D.M., Sproul, O.J., 1980. Mechanism of ozone inactivation of bacteriophage f2. Appl. Environ. Microbiol. 39, 210–218. https://doi.org/10.1128/aem.39.1.210-218.1980.

Kimball, A.W., 1953. The fitting of multi-hit survival curves. Biometrics 9, 201–211.

Labatiuk, C.W., Belosevic, M., Finch, G.R., 1994. Inactivation of giardia muris using ozone and ozone-hvdrogen peroxide. Ozone Sci. Eng. 16, 67–78. https://doi.org/10.1080/01919519408552381.

Labatiuk, C.W., Belosevic, M., Finch, G.R., 1992a. Evaluation of high level ozone inactivation of giardia muris using an animal infectivity model. Ozone Sci. Eng. 14, 1–12. https://doi.org/10.1080/01919519208552314.

Labatiuk, C.W., Belosevic, M., Finch, G.R., 1992b. Factors influencing the infectivity of Giardia muris cysts following ozone inactivation in laboratory and natural waters. Water Res 26, 733–743. https://doi.org/10.1016/0043-1354(92)90004-N.

Labatiuk, C.W., Schaefer, F.W., Finch, G.R., Belosevic, M., 1991. Comparison of animal infectivity, excystation, and fluorogenic dye as measures of Giardia muris cyst inactivation by ozone. Appl. Environ. Microbiol. 57, 3187–3192. https://doi.org/10.1128/aem.57.11.3187-3192.1991.

Leclerc, H., Schwartzbrod, L., Dei-Cas, E., 2002. Microbial agents associated with waterborne diseases. Crit. Rev. Microbiol. 28, 371–409. https://doi.org/10.1080/1040-840291046768.

Lee, Y., Gerrity, D., Lee, M., Bogeat, A.E., Salhi, E., Gamage, S., Trenholm, R.A., Wert, E.C., Snyder, S.A., von Gunten, U., 2013. Prediction of micropollutant elimination during ozonation of municipal wastewater effluents: use of kinetic and water specific information. https://doi.org/10.1021/es400781r.

Lee, Y., von Gunten, U., 2016. Advances in predicting organic contaminant abatement during ozonation of municipal wastewater effluent: reaction kinetics, transformation products, and changes of biological effects. Environ. Sci. Water Res. Technol. 2, 421–442. https://doi.org/10.1039/c6ew00025h.

Lewin, N., Craik, S., Li, H., Smith, D.W., Belosevic, M., 2001. Sequential inactivation of Cryptosporidium using ozone followed by free chlorine in natural water. Ozone Sci. Eng. 23, 411–420. https://doi.org/10.1080/01919510108962024.

Li, H., Gyurek, L.L., Finch, G.R., Smith, D.W., Belosevic, M., 2001. EFFECT of temperature on ozone inactivation of cryptosporidium parvum in oxidant demand-free phosphate buffer. J. Environ. Eng. 127, 456–467.

Li, Y., Smith, D.W., Belosevic, M., 2004. Morphological changes of Guardia lamblia cysts after treatment with ozone and chlorine. J. Environ. Eng. Sci. 3, 495–506.

Lim, M.Y., Kim, J.M., Lee, J.E., Ko, G., 2010. Characterization of ozone disinfection of murine norovirus. Appl. Environ. Microbiol. 76, 1120–1124. https://doi.org/10.1128/AEM.01955-09.

Mac Kenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S., Blair, K.A., Peterson, D.E., Kazmierczak, J.J., Addiss, D.G., Fox, K.R., Rose, J.B., Davis, J.P., 1994. A massive outbreak in milwaukee of cryptosporidium transmitted through the public water supply. N. Engl. J. Med. 331, 161–167.

Mesquita, M.M.F., Stimson, J., Chae, G.T., Tufenkji, N., Ptacek, C.J., Blowes, D.W., Emelko, M.B., 2010. Optimal preparation and purification of PRD1-like bacteriophages for use in environmental fate and transport studies. Water Res 44, 1114–1125. https://doi.org/10.1016/j.watres.2009.11.017.

Michen, B., Graule, T., 2010. Isoelectric points of viruses. J. Appl. Microbiol. 109, 388–397. https://doi.org/10.1111/j.1365-2672.2010.04663.x.

Oppenheimer, J.A., Aieta, M.E., Trussell, R.R., Jacangelo, J.G., Najm, I.., 2000. Evaluation of cryptosporidium inactivation in natural waters.

Owens, J.H., Miltner, R.J., Rice, E.W., Johnson, C.H., Dahling, D.R., Schaefer, F.W., Shukairy, H.M., 2000. Pilot-scale ozone inactivation of cryptosporidium and other microorganisms in natural water. Ozone Sci. Eng. 22, 501–517. https://doi.org/10.1080/01919510009408793.

Rakness, K.L., 2015. Ozone in drinking water treatment: process design, operation, and optimization. American Water Works Association.

Rakness, K.L., Najm, I., Elovitz, M., Rexing, D., Via, S., 2005. Cryptosporidium log-inactivation with ozone using effluent CT10, geometric mean CT10, extended integrated CT10 and extended CSTR calculations. Ozone Sci. Eng. 27, 335–350. https://doi.org/10.1080/01919510500250267.

Rennecker, J.L., Mariñas, B.J., Owens, J.H., Rice, E.W., 1999. Inactivation of cryptosporidium parvum oocysts with ozone. Water Res 33, 2481–2488. https://doi.org/10.1016/S0043-1354(99)00116-5.

Righetti, P.G., Caravaggio, T., 1976. Isoelectric points and molecular weights of proteins. J. Chromatogr. 127, 1–28.

Roy, D., Chian, E.S.K., Engelbrecht, R.S., 1982. Mathematical model for enterovirus inactivation by ozone. Water Res 16, 667–673. https://doi.org/10.1016/0043-1354(82)90089-6.

Roy, D., Wong, P.K.Y., Engelbrecht, R.S., Chian, E.S.K., 1981. Mechanism of enteroviral inactivation by ozone. Appl. Environ. Microbiol. 41, 718–723.

Sella, S.R.B.R., Vandenberghe, L.P.S., Soccol, C.R., 2014. Life cycle and spore resistance of spore-forming Bacillus atrophaeus. Microbiol. Res. 169, 931–939. https://doi.org/10.1016/j.micres.2014.05.001.

Sigmon, C., Shin, G.A., Mieog, J., Linden, K.G., 2015. Establishing surrogate - virus relationships for ozone disinfection of wastewater. Environ. Eng. Sci. 32, 451–460. https://doi.org/10.1089/ees.2014.0496.

Sigstam, T., Gannon, G., Cascella, M., Pecson, B.M., Wigginton, K.R., Kohn, T., 2013. Subtle differences in virus composition affect disinfection kinetics and mechanisms. Appl. Environ. Microbiol. 79, 3455–3467. https://doi.org/10.1128/AEM.00663-13.

Smith, H.V., Nichols, R.A.B., Grimason, A.M., 2005. Cryptosporidium excystation and invasion: Getting to the guts of the matter. Trends Parasitol 21, 133–142. https://doi.org/10.1016/j.pt.2005.01.007.

Soller, J.A., Eftim, S.E., Warren, I., Nappier, S.P., 2017. Microbial risk analysis evaluation of microbiological risks associated with direct potable reuse 5, 3–14. https://doi.org/10.1016/j.mran.2016.08.003.

Staehelin, J., Hoigne, J., 1985. Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. Environ. Sci. Technol. 19, 1206–1213. https://doi.org/10.1021/es00142a012.

Stockdale, C.J., Adriaenssens, E.M., Kuhn, J.H., Pallen, M., Rumnieks, J., Shkoporov, A., Draper, L.A., Ross, R.P., Hill, C., 2020. Rename one class (Leviviricetes - formerly Allassoviricetes), rename one order (Norzivirales - formerly Levivirales), create one new order (Timlovirales), and expand the class to a total of six families, 420 genera and 883 species. Int. Comm. Taxon. Viruses.

Torii, S., Itamochi, M., Katayama, H., 2020. Inactivation kinetics of waterborne virus by ozone determined by a continuous quench flow system. Water Res 186, 116291. https://doi.org/10.1016/j.watres.2020.116291.

Torii, S., Miura, F., Itamochi, M., Haga, K., Katayama, K., Katayama, H., 2021. Impact of the heterogeneity in free chlorine, uv254, and ozone susceptibilities among coxsackievirus b5 on the prediction of the overall inactivation efficiency. Environ. Sci. Technol. 55, 3156–3164. https://doi.org/10.1021/acs.est.0c07796.

Torrey, J., von Gunten, U., Kohn, T., 2019. Differences in viral disinfection mechanisms as revealed by quantitative transfection of echovirus 11 genomes. Appl. Environ. Microbiol. 85, 1–14. https://doi.org/10.1128/AEM.00961-19.

USEPA, 2006. Long term 2 enhanced surface water treatment rule. United States Environ. Prot. Agency 1–375.

USEPA, 1999. Alternative disinfectants and oxidants guidance manual. United States Environ. Prot. Agency 1–328.

USEPA, 1998. National primary drinking water regulations: interim enhanced surface water treatment. United States Environ. Prot. Agency 69478–69521. https://doi.org/10.1016/0196-335x(80)90058-8.

USEPA, 1991. Guidance manual for compliance with the filtration and disinfection requirements for public water systems using surface water sources. United States Environ. Prot. Agency 1–580.

van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R., Wickner, R.B., 2000. Virus taxonomy. Seventh report of the international committee on taxonomy of viruses, international committee on virus taxonomy.

von Gunten, U., 2018. Oxidation processes in water treatment: are we on track? Environ. Sci. Technol. 52, 5062–5075. https://doi.org/10.1021/acs.est.8b00586.

von Gunten, U., 2003a. Ozonation of drinking water : part II . disinfection and by-product formation in presence of bromide , iodide or chlorine. Water Res 37, 1469–1487.

von Gunten, U., 2003b. Ozonation of drinking water: part i. oxidation kinetics and product formation. Water Res 37, 1443–1467. https://doi.org/10.1016/S0043-1354(02)00457-8.

von Gunten, U., Hoigne, J., 1994. Bromate formation during ozonization of bromide-containing waters: interaction of ozone and hydroxyl radical reactions. Environ. Sci. Technol. 28, 1234–1242. https://doi.org/10.1021/es00056a009.

von Sonntag, C., von Gunten, U., 2012. Chemistry of ozone in water and wastewater treatment: from basic principles to applications, chemistry of ozone in water and wastewater treatment. From Basic Principles Appl. https://doi.org/10.2166/9781780400839.

Wickramanayake, G.B., Rubin, A.J., Sproul, O.J., 1985. Effects of ozone and storage temperature on Giardia cysts. J. /Am. Water Work. Assoc. 77, 74–77. https://doi.org/10.1002/j.1551-8833.1985.tb05591.x.

Wohlsen, T., Stewart, S., Aldridge, P., Bates, J., Gray, B., Katouli, M., 2007. The efficiency of ozonated water from a water treatment plant to inactivate cryptosporidium oocysts during two seasonal temperatures. J. Water Health 5, 433–440. https://doi.org/10.2166/wh.2007.039.

Wolf, C., Pavese, A., von Gunten, U., Kohn, T., 2019. Proxies to monitor the inactivation of viruses by ozone in surface water and wastewater effluent. Water Res 166, 115088. https://doi.org/10.1016/j.watres.2019.115088.

Wolf, C., von Gunten, U., Kohn, T., 2018. Kinetics of inactivation of waterborne enteric viruses by ozone. Environ. Sci. Technol. 52, 2170–2177. https://doi.org/10.1021/acs.est.7b05111.

Young, S., Torrey, J., Bachmann, V., Kohn, T., 2020. Relationship between inactivation and genome damage of human enteroviruses upon treatment by UV254, free chlorine, and ozone. Food Environ. Virol. 12, 20–27. https://doi.org/10.1007/s12560-019-09411-2.

Young, S.B., Setlow, P., 2004. Mechanisms of bacillus subtilis spore resistance to and killing by aqueous ozone. J. Appl. Microbiol. 96, 1133–1142. https://doi.org/10.1111/j.1365-2672.2004.02236.x.