Kinetic analysis of *Legionella* inactivation using ozone in wastewater

Jun Li^{1,*}, Kunquan Li², Yan Zhou³, Xuebin Li⁴, Tao Tao⁵

23

24 Abstract

25 *Legionella* inactivation using ozone was studied in wastewater using kinetic
26 analysis and modeling. The experimental results indicate that the relationship between
27 the ozone concentration, germ concentration, and chemical oxygen demand (COD)
28 can be used to predict variations in germ and COD concentrations. The ozone reaction
29 with COD and inactivation of *Legionella* occurred simultaneously, but the reaction
30 with COD likely occurred at a higher rate than the inactivation, as COD is more easily
31 oxidized by ozone than *Legionella*. Higher initial COD concentrations resulted in a
32 lower inactivation rate and higher $\ln N/N_0$. Higher temperature led to a higher
33 inactivation efficiency. The relationship of the initial O_3 concentration and *Legionella*
34 inactivation rate was not linear, and thus, the Ct value required for a 99.99% reduction
35 was not constant. The initial O_3 concentration was more important than the contact
36 time, and a reduction of the initial O_3 concentration could not be compensated by
37 increasing the contact time. The Ct values were compared over a narrow range of
38 initial concentrations; the Ct values could only be contrasted when the initial O_3
39 concentrations were very similar. A higher initial O_3 concentration led to a higher
40 inflection point value for the $\ln N/N_0$ vs C_0t curve. Energy consumption using a plasma
41 corona was lower than when using boron-doped diamond electrodes.

42

43 Keywords:

44

45 Indigo, activation energy, tail phenomena, energy consumption

46

47 Highlights:

48

49 1. Variations in germ levels can be predicted by the initial O₃ and COD.

50 2. COD is more easily oxidized by ozone than *Legionella*.

51 3. O₃ reduction cannot be compensated proportionally by increasing the O₃ contact

52 time.

53

54

1. Introduction

On August 3, 2015, a *Legionnaires'* disease outbreak in New York City led to 81 cases and seven deaths. In the US, approximately 18,000 people are hospitalized each year due to *Legionnaires'* disease. Adults over 65 years old or with chronic lung disease are especially susceptible to *Legionnaires'* disease, and 17% of the infected population dies. Research to mitigate the public health threat of *Legionnaires'* disease is thus of high priority (NBC News, 2015).

Some effective methods for inactivating *Legionella* include chlorination, ultraviolet (UV) irradiation, membrane separation, and ozone disinfection. Chlorination unfortunately leads to the formation of disinfection by-products (DBPs) (Schmalz et al., 2009; Bergmann et al., 2009, 2011), and in some reports, UV irradiation of DBPs showed enhanced formation of cyanogen chloride, haloacetic acids (Wei et al., 2012), halobenzoquinones (Yichao et al., 2013), chloropicrin (Amisha et al., 2011; Cimetiere & De Laat, 2014), N-nitrosodimethylamine (Fabian et al., 2013), brominated trihalomethanes (THMs) (Cimetiere et al., 2014; Aikaterini et al., 2015) and iodinated disinfection by-products (I-DBPs) (Fu-Xiang et al., 2014). Commercial polymeric membranes for water treatment suffer from a trade-off between productivity and selectivity (Jun & Baolin, 2015). Membrane fouling can deteriorate membrane lifespan and thus limits the application of membrane separation technology (Langming et al., 2015). Because ozone is the strongest disinfectant and oxidizer available commercially, ozone systems are being used worldwide to disinfect

drinking and pool water, treat chemical wastewater and cooling-tower water, disinfect agricultural water (hydroponics, spraying of vegetables and fruit), and extend food lifetimes; ozone systems are also used by commercial and institutional laundries. Ozone is increasingly replacing chlorine-based products for disinfection applications due to its extraordinary efficacy in destroying pathogens and the adverse impact of chlorine on the environment and water resources. Ozone has a powerful oxidizing capability and rarely forms DBPs in most wastewater (Jeong et al., 2006; Deborde & von Gunten, 2008; Wim et al., 2010; Gerrity et al., 2011; Zimmermann et al., 2011; Leonardo & Thanh, 2012; Pisarenko et al., 2012; Lee et al., 2013; Sahid et al., 2013; Yongze et al., 2015). Thus, ozone has become a more prominent inactivation technology over the last decade. Ozone is reported to effectively inactivate *Cryptosporidium parvum* oocysts (Craig et al., 2002), but few studies have used ozone to inactivate *Legionella*.

The objectives of this study were to perform a kinetic analysis of *Legionella* inactivation in wastewater using ozone and investigate the effects of various COD and initial O₃ concentrations on *Legionella* inactivation in wastewater as well as the effects of various temperatures on inactivation in deionized water. The energy consumption of *Legionella* inactivation using ozone was also studied.

2. Materials and methods

2.1 Experimental setup

Studies were conducted in semi-batch reactors under laboratory conditions. The reactor system consisted of an adjustable voltage power supply, a flow meter, an ozone generator, a 1-L glass bottle reactor, two waste gas filters, and three waste gas collection bottles (200, 500, 2000 mL), as shown in Figure 1. The *Legionella* suspension was stirred continuously with a magnetic stirrer at 700 rpm (Fisher Isotemp, USA). The output voltage of the power supply could be adjusted from 0 to 140 V. The reactor was immersed in a temperature-controlled water bath. All materials were previously made ozone-demand-free (ODF). Inactivation of bacteria is difficult to measure due to quick inactivation times (Botzenhart et al., 1993). Hence, we first decreased the ozone concentration to a minimum and extended the reaction time to measure variations in ozone and *Legionella* concentrations.

Wastewater was obtained from the effluent of the Urbana & Champaign Sanitary District Plant in Urbana, Illinois, USA. Wastewater was filtered through a 0.20- μ m membrane filter to remove bacteria before experiments and stored in a 4 °C refrigerator until use. Deionized water was obtained from an Environmental Engineering and Science laboratory at the University of Illinois at Urbana-Champaign (205 N Mathews Ave, Urbana, Illinois, USA). The ozone concentration C and contact time t necessary for a 99.99% reduction were used to establish a Ct value. Excess ozone gas was discharged into a gas absorption bottle with a 2% KI solution to quench excess ozone gas immediately and avoid excess ozone discharge into the environment.

Ozone gas was produced using a plasma corona from an ozone generator (EP Purification, Inc., Champaign, Illinois USA) (STACOL1010, IL, USA). Using

microcavity plasma technology developed at the University of Illinois, this ozone generator is efficient, robust, and over ten-fold smaller in footprint mass and volume than conventional ozone technology. The instrument produces highly uniform gas streams, is extremely compact and offers great flexibility in ozone production capacity. Inlet air was filtered with a dry SiO₂ desiccant, as wet air decreases the production efficiency of the generator. A power supply offered voltages ranging from 0 to 140 V; a higher voltage produces a higher outlet ozone concentration.

Figure 1. Experimental setup of the reactor.

2.2 Chemicals

Yeast extract (BD 212750), 5,5-indigo trisulfonic acid sodium salt (indigo carmine), sodium thiosulfate (Na₂S₂O₃), potassium iodide (KI), potassium hydroxide (KOH), and phosphoric acid were purchased from the Sigma-Aldrich Company (USA).

A stock solution of Indigo Reagent was prepared by dissolving 0.6 g L⁻¹ (1 mM) potassium indigo trisulfonate in 20 mM phosphoric acid. This solution was replaced after long-term storage when absorbance at 600 nm dropped below 80% of the initial value. The KI concentration was 0.12 mol L⁻¹, Na₂S₂O₃ concentration was 0.0063 mol L⁻¹, KOH concentration was 1.79 mol L⁻¹ and phosphoric acid concentration was 8.67 mol L⁻¹.

2.3 Preparation of bacteria and phosphate buffer solutions

Legionella pneumophila subsp. (ATCC[®] 33152[™]) was purchased from the American Type Culture Collection (ATCC[®]), Manassas, Virginia, USA. *Legionella* was cultured overnight using #1099 CYE broth at 37 °C for 12 h with continuous shaking at 200 rpm. *Legionella* growth was controlled at exponential phase to minimize *Legionella* death because dead *Legionella* also consume ozone. It is difficult to calculate what percentage of *Legionella* are dead and what fraction of ozone is consumed by dead *Legionella*. The *Legionella* culture solution was washed three times with phosphate buffer (PBS) to minimize the background Chemical Oxygen Demand (COD) from *Legionella* culture components, such as CYE, which interfere with measurements of ozone activity. The *Legionella* culture solution was centrifuged at 5000 rpm for 10 min, and the resulting cell pellets were collected and resuspended in 25 mL of PBS in a 50-mL tube. The *Legionella* suspensions were diluted with PBS (pH 2: 28 g NaH₂PO₄·H₂O and 35 g H₃PO₄ (85%) dissolved in 1 L distilled water). These highly concentrated 25-mL suspensions of *Legionella* were used in experiments. After ozone disinfection, all *Legionella* samples were incubated in solid media plates at 37 °C and 5% CO₂ for 2 days to determine the number of living and dead *Legionella*. CO₂ is helpful for *Legionella* growth. To research the effect of different initial ozone concentrations and contact times on *Legionella* inactivation, *Legionella* in solution must be determined after every experiment. A 200-μL sample of *Legionella* was plated on solid media and incubated at 37 °C and 5% CO₂.

The pH was adjusted with KOH, and 2-mL samples were removed with a sterile syringe and added to a test tube containing 2 mL of sodium thiosulfate solution (Na₂S₂O₃ of 1.5 g L⁻¹) to quench the residual ozone.

2.4 Analytical methods

Legionella cell populations were assayed following Standard Methods for the Examination of Water and Wastewater (APHA, 1989). Dissolved ozone concentrations were determined following the indigo method of Bader (Bader & Hoigné, 1981) at 600nm and UV absorbance of ozone at 258 nm based on a molar absorptivity of 2900 M⁻¹ cm⁻¹ (Hoigné & Bader, 1976). The pH of the solution was determined with a pH meter (AB15, Fisher Scientific, USA).

Any uncertainty in the concentration of the indigo reagent did not affect the accuracy of the analytical method because it was based on standard curves for changes in absorbance per added mole of ozone (Bader & Hoigné, 1981).

Legionella was counted by plating on solid media.

2.5 Kinetic analysis equations

The kinetic analysis of *Legionella* in wastewater was analyzed according to the following process:

The ozone germicidal rate is expressed using Equation (1).

$$r_{gem} = \frac{dN_{gem}}{dt} = -k_{gem}N_{gem}C_{O_3,b} \quad (1)$$

where r_{gem} is the ozone germicidal rate in CFU mL⁻¹ s⁻¹; k_{gem} is the ozone germicidal rate coefficient in L mg⁻¹ s⁻¹; N_{gem} is the germ concentration in CFU mL⁻¹; and $C_{O_3,b}$ is the bulk O₃ concentration in mg L⁻¹.

The ozone decay rate is expressed using Equation (2).

$$r_{O_3,d} = \sum_{i=1}^n r_i \quad (2)$$

where $r_{O_3,d}$ is the ozone decay rate in mg s^{-1} and r_i is the ozone decay rate under the influence of the amount of matter oxidized by ozone in mg s^{-1} .

The ozone mass transfer rate is subject to the film diffusion principle, shown in Equation (3).

$$r_{O_3,f} = k_g (C_{O_3,g} - C_{O_3,b}) \quad (3)$$

where $r_{O_3,f}$ is the ozone mass transfer rate in mg s^{-1} ; k_g is the ozone film diffusion coefficient in L s^{-1} ; $C_{O_3,g}$ is the ozone gas concentration in gas at the gas-liquid in mg L^{-1} ; and $C_{O_3,b}$ is the bulk O_3 concentration in liquid in mg L^{-1} .

Equation (1) is integrated to give Equation (4).

$$\ln \frac{N_{gem}}{N_{gem,0}} = -k_{gem} C_{O_3,b} t \quad (4)$$

where N_{gem} is the germ concentration in CFU mL^{-1} ; $N_{gem,0}$ is the initial germ concentration in CFU mL^{-1} ; k_{gem} is the ozone germicidal rate coefficient in $\text{L mg}^{-1} \text{s}^{-1}$; $C_{O_3,b}$ is the bulk O_3 concentration in liquid in mg L^{-1} ; and t is the ozone contact time in seconds.

When $C_{O_3,b}$ is not constant, Equation (5) can be used to express two parameters on the right side of Equation (13).

$$Ct = \int_0^T \frac{C_{O_3,b}}{T} t dt \quad (5)$$

where C is the ozone concentration in mg L^{-1} ; T is the ozone contact time in seconds; t is the ozone contact time in seconds, ranging from 0 to T ; and $C_{O_3,b}$ is the bulk O_3 concentration in liquid in mg L^{-1} . Note that in equation (5), T is different from t on the

integral.

In equation (5), $C_{O_3,b}$ can be approximated by the ozone application rate in $\text{mg O}_3 \text{ L}^{-1} \text{ min}$ (assuming continuous application of ozone), which is obtained by the total mass of ozone applied divided by the volume of water and contact time, or, $\text{mO}_3 / (\text{VT})$. However, a more correct procedure is to establish ozone depletion kinetics and replace $C_{O_3,b}$ with an equation that relates the ozone concentration to time.

Time was included in the integral of the equation to consider a continuous range of ozone contact times. For example: the ozone concentration applied (total mass of ozone divided by the volume of water in the reactor): 7.8 mg L^{-1} . Contact Time: 10 min. Ozone application rate: $0.78 \text{ mg O}_3 \text{ L}^{-1} \text{ min}^{-1}$.

$$Ct = \int_0^T \frac{C_{O_3,b}}{T} t dt = \int_0^{10} \frac{7.8}{10} t dt = 0.78 \times \frac{1}{2} \times t^2 = 0.78 \times \frac{1}{2} \times 100 = 39 \text{ mg L}^{-1} \text{ min}$$

Ozone mass balance is expressed using Equation (6).

$$Q_g (G_{O_3,in} - G_{O_3,out}) = r_{O_3,f} = -r_{O_3,d} \quad (6)$$

where Q_g is the ozone gas flow rate in L s^{-1} ; $G_{O_3,in}$ is the influent gas-phase ozone concentration in mg L^{-1} ; $G_{O_3,out}$ is ozone concentration in the off-gas in mg L^{-1} ; $r_{O_3,f}$ is the ozone mass transfer rate in mg s^{-1} ; and $r_{O_3,d}$ is the ozone decay rate in mg s^{-1} .

Equation (7) can be obtained after Equation (3) is substituted into Equation (6).

$$Q_g (G_{O_3,in} - G_{O_3,out}) = k_g (C_{O_3,g} - C_{O_3,b}) \quad (7)$$

The bulk O_3 concentration is solved in Equation (8). $C_{O_3,g}$ is related to $G_{O_3,out}$ by the Henry's law.

$$C_{O_3,b} = C_{O_3,g} - \frac{1}{k_g} Q_g (G_{O_3,in} - G_{O_3,out}) \quad (8)$$

Because the ozone decay rate is related to the ozone, germ, and COD concentrations, Equation (9) is inferred by reasonable analysis.

$$r_{O_3,d} = -k_{O_3,COD} C_{O_3,b} COD - k_{O_3,gem} C_{O_3,b} N_{gem} \quad (9)$$

where $r_{O_3,d}$ is the ozone decay rate in mg s^{-1} ; $k_{O_3,COD}$ is the ozone decay coefficient related to chemical oxygen demand (COD) in $\text{L}^2 \text{mg}^{-1} \text{s}^{-1}$; $C_{O_3,b}$ is the bulk O_3 concentration in liquid in mg L^{-1} ; COD is the initial chemical oxidization demand of wastewater in mg L^{-1} ; $k_{O_3,gem}$ is the ozone decay coefficient relating to the germ in $\text{mL L CFU}^{-1} \text{s}^{-1}$; and N_{gem} is the germ concentration in CFU mL^{-1} .

At any reaction time t , Equation (10) is obtained by the combination of the Equations (6), (7) and (9).

$$k_g (C_{O_3,g} - C_{O_3,b}) = -k_{O_3,COD} C_{O_3,b} COD - k_{O_3,gem} C_{O_3,b} N_{gem} \quad (10)$$

Equation (11) is inferred by Equation (10).

$$C_{O_3,g} = \left(1 - \frac{k_{O_3,COD} COD + k_{O_3,gem} N_{gem}}{k_g} \right) C_{O_3,b} \quad (11)$$

Equation (12) is inferred by Equation (11).

$$C_{O_3,b} = \frac{C_{O_3,g}}{1 - \frac{k_{O_3,COD} COD + k_{O_3,gem} N_{gem}}{k_g}} \quad (12)$$

Equation (13) is obtained by the combination of Equations (5), (8), (11) and (12).

$$Ct = \int_0^T \frac{C_{O_3,b}}{T} dt = \int_0^T \left[C_{O_3,g} - \frac{1}{k_g} Q_g (G_{O_3,in} - G_{O_3,out}) \right] \times \frac{1}{T} \times t dt = \int_0^T \frac{C_{O_3,g}}{1 - \frac{k_{O_3,COD} COD + k_{O_3,gem} N_{gem}}{k_g}} \times \frac{1}{T} \times t dt \quad (13)$$

Equation (14) is obtained by combining Equations (4) and (13).

$$\ln \frac{N_{gem}}{N_{gem,o}} = -k_{gem} \int_0^T \frac{C_{O_3,g}}{1 - \frac{k_{O_3,COD} COD + k_{O_3,gem} N_{gem}}{k_g}} \times \frac{1}{T} \times t dt \quad (14)$$

The COD of all of the equations above is the initial COD concentration.

After the coefficients in Equation (14) (k_{gem} , $C_{O3,g}$, $k_{O3,COD}$, $k_{O3,gem}$, and k_g) are obtained from experiment, the equation can be used to predict germ and COD concentration variations. These equations were obtained through careful mathematical analyses.

2.6 Energy consumption equation

Energy consumption depends on the voltage, desired inactivation level, and disinfection time. Energy consumption was calculated by Equation 15:

$$E \left(\frac{\text{kWh}}{\text{m}^3} \right) = \frac{U \times I \times t}{V} \quad (15)$$

where E is the energy consumption in kW h m⁻³; U is the voltage in volts; I is the current in amperes; t is the treatment time in h; and V is the volume of the treated water in m³.

3 Results and discussion

3.1 Kinetic analysis of *Legionella* inactivation in wastewater

To verify the accuracy of Equation (14), the error range between real values and simulated values using Equation (14) was analyzed. Because the tail phase was long and the germ reduction quantity was small, the tail phase is not important in germ inactivation, especially for engineering applications. Therefore, we focused on data from before the tail phase.

The limit of quantification for the UV absorbance of ozone at 258 nm is 16.5 µg L⁻¹ (UV-2450, Shimadzu, Japan). Thus, C₀=33 µg L⁻¹ was used below. N₀=3.91×10⁷

CFU mL⁻¹ was the maximum *Legionella* concentration after incubation. Thus,
 $N_0=3.91 \times 10^7$ CFU mL⁻¹ was used below. Of the tested temperatures 7, 11, 16, and
 22 °C, the ozone disinfection efficiency was lowest at 7 °C, as shown in section 3.3.
 Thus 7 °C was used below.

Figure 2 (a) shows the effect of various initial COD concentrations (COD₀=0.75,
 1.5, 2.25 and 3 mg L⁻¹) on the inactivation rate of *Legionella* in wastewater at 7 °C,
 $N_0=3.91 \times 10^7$ CFU mL⁻¹, and C₀=33 µg L⁻¹. Only data before the tail phase was used
 to compare real and simulated values using Equation (14). Through many experiments,
 the following coefficient values were obtained: $k_{gem}=0.423$ L mg⁻¹ s⁻¹, $C_{O3,g}=1.3$ mg
 L⁻¹, $k_{O3,COD}=-9454013k_{O3,gem}$, and $k_g=-50668876k_{O3,gem}$. These coefficient values
 were substituted into Equation (14) to give Equation (16).

$$\ln \frac{N_{gem}}{N_{gem,o}} = -0.423 \int_0^T \frac{1.3}{1 - 0.1866 \times COD + \frac{1}{50668876} \times N_{gem}} \times \frac{1}{T} dt \quad (16)$$

Equation (16) relates the germ concentration and COD concentration for COD₀=
 3 mg L⁻¹, 7 °C, $N_0=3.91 \times 10^7$ CFU mL⁻¹, and C₀=33 µg L⁻¹. Equation (16) was used to
 calculate the simulated value. The real value was obtained from Figure 2 (a). The
 simulated values at t=2, 3, 4, and 5 min were calculated using Equation (16) to give
 simulated values ($\ln \frac{N_{gem}}{N_{gem,o}}$) of -1.017, -1.526, -1.994, and -2.421, respectively. The
 real values at t=2, 3, 4, and 5 min were -1.027, -1.541, -2.054, and -2.568,
 respectively, as obtained from Figure 2 (a). The error ranges of the simulated values
 were thus calculated as 1%, 1%, 2.9%, and 5.7% of real values at t=2, 3, 4, and 5 min,
 respectively. Equations (14) and (16) are thus quite accurate, and Equation (14) can

be used to predict variations in germ and COD concentrations.

3.2 Effect of the COD concentration on the inactivation rate of *Legionella* in wastewater

The effect of various initial COD concentrations ($COD_0=0.75, 1.5, 2.25$ and 3 mg L^{-1}) on the inactivation rate of *Legionella* in wastewater was investigated at 7°C , $N_0=3.91\times 10^7 \text{ CFU mL}^{-1}$, and $C_0=33 \text{ }\mu\text{g L}^{-1}$, as shown in Figure 2 (a). At $t=5 \text{ min}$ and $C_0t=165 \text{ }\mu\text{g L}^{-1} \text{ min}$, the *Legionella* inactivating efficiency was 99.9998%, 99.5912%, 96.7395%, and 92.3273% at initial COD concentrations of 0.75, 1.5, 2.25 and 3 mg L^{-1} , respectively. The fastest *Legionella* inactivation rate was 1.3×10^5 , 1.29×10^5 , 1.26×10^5 , and $1.2\times 10^5 \text{ CFU s}^{-1}$ at initial COD concentrations of 0.75, 1.5, 2.25 and 3 mg L^{-1} , respectively. After 5 min, the *Legionella* inactivation rate became very slow with tail phenomena. The relationship between the initial COD concentration and *Legionella* inactivation rate is shown in Figure 2 (b), suggesting that higher initial COD concentrations result in lower inactivation rates. The relationship between the initial COD concentrations and $\ln N/N_0$ is shown in Figure 2 (c), suggesting that higher initial COD concentrations result in higher $\ln N/N_0$ values. N is the germ concentration in CFU mL^{-1} , which is the same as N_{gem} in Equation 1, 4, 10, 12, 13, and 14. N_0 is the initial germ concentration in CFU mL^{-1} , which is the same as $N_{\text{gem},0}$ at Equation 4 and 14.

Figure 2 (a) shows that the *Legionella* inactivation efficiency was 99.5912% and 99.8769% at $t=5 \text{ min}$ and 25 min , respectively, at 7°C with an initial ozone concentration $C_0=33 \text{ }\mu\text{g L}^{-1}$ in wastewater with $COD_0=1.5 \text{ mg L}^{-1}$. Based on the

experiments above and Figure 2 (a), the results show that *Legionella* inactivation reached 99.999% at an initial O_3 concentration of $33 \mu g L^{-1}$ ($C_0=33 \mu g L^{-1}$), initial COD concentration of $0.75 mg L^{-1}$ ($COD_0=0.75 mg L^{-1}$), and O_3 contact time of 25 min ($t=25 min$).

Comparing Figure 2 (a) to Figure 3, the *Legionella* inactivation rate was more than 30-fold higher in deionized water than in wastewater ($COD_0=1.5 mg L^{-1}$): *Legionella* inactivation occurred in 10 seconds in deionized water, but took 5 minutes in wastewater, indicating that the ozone reaction with COD and inactivation of *Legionella* occurred simultaneously. The reaction with COD likely occurs at a higher rate than inactivation. The lower activation energy and ORP of COD does not necessarily result in an increased reaction rate, which depends on the reaction kinetics, and hence, COD is more easily oxidized by ozone than *Legionella*.

Figure 2 (a) Effect of the initial COD concentrations on $\ln N/N_0$ at 7 °C, $N_0=3.91 \times 10^7$ CFU mL⁻¹, and $C_0=33$ µg L⁻¹; (b) *Legionella* inactivation rate vs. initial COD concentration; (c) $\ln N/N_0$ vs initial COD concentration.

3.3 Effect of temperature on the inactivation rate of *Legionella* with ozone in deionized water

Temperature is an important factor affecting the ozone concentration. To study the effect of temperature on the inactivation rate of *Legionella*, experiments were performed in deionized water.

Figure 3 shows the experimental results under the conditions of $N_0=1.70 \times 10^6$ CFU mL⁻¹, $C_0=16.5$ µg L⁻¹, and pH 7 at 7, 11, 16, and 22 °C. The results showed that the *Legionella* inactivation efficiency was 98.32%, 99.03%, 99.52%, and 99.79% at 7, 11, 16 and 22 °C, respectively, at $C_0t=65$ µg L⁻¹ s in deionized water.

This result showed that higher temperature led to a higher inactivation efficiency and rate, and lower temperature led to a lower ozone decay rate and lower inactivation efficiency, even if the ozone solubility increased when the temperature decreased. This result was completely different from the prediction that the inactivation efficiency would increase when the temperature decreased because of an increase in ozone solubility. We expected that a higher ozone concentration should lead to a higher inactivation efficiency, but the opposite was observed, possibly because low temperature decreases ozone activity and collisions between ozone and bacterial surfaces.

Figure 3 also shows that the *Legionella* inactivation efficiency was 98.32% and

99.05% at $t=10$ s and 50 s at 7 °C with an initial ozone concentration $C_0=16.5 \mu\text{g L}^{-1}$ in deionized water, respectively, indicating a tailing phenomenon. Even if the ozone contact time was increased several fold, the inactivation efficiency would increase very little. Thus, the initial ozone concentration C_0 is more important than the ozone contact time t for bacterial inactivation.

Figure 3. Inactivation of *Legionella* at various temperatures in deionized water at pH 7.

3.4. Effect of various initial O_3 concentrations on *Legionella* inactivation in wastewater

The *Legionella* inactivation efficiency was lowest at 7 °C among 7, 11, 16 and 22 °C based on the last experiment, and thus, 7 °C was used in this experiment.

From section 3.2, the *Legionella* inactivating efficiency was lowest at an initial COD concentration of 3 mg L^{-1} among 0.75, 1.5, 2.25 and 3 mg L^{-1} , and thus, an initial COD concentration of 3 mg L^{-1} was used in this experiment. The initial bacteria concentration $N_0=3.91 \times 10^7 \text{ CFU mL}^{-1}$ was the highest concentration observed in the initial experiments, and thus, $N_0=3.91 \times 10^7 \text{ CFU mL}^{-1}$ was used in this experiment. In other words, the most challenging conditions of those tested preliminarily were used to test ozone disinfection efficiency.

The effect of various initial O_3 concentrations on *Legionella* inactivation in wastewater was investigated at 7 °C, initial bacteria concentration $N_0=3.91 \times 10^7 \text{ CFU}$

mL⁻¹, and initial COD concentration of 3 mg L⁻¹, as shown in Figure 4 (a), and the results showed that a higher initial O₃ concentration led to a higher inactivation efficiency. The inactivation rate increases with the increasing initial concentration of ozone, and the ozone contact time required for 99.99% reduction was shorter as initial concentration of ozone increased. The fastest *Legionella* inactivation rates were 1.2×10⁵, 1.95×10⁶, and 3.9×10⁶ CFU s⁻¹ at initial O₃ concentrations of 33, 165, and 330 µg L⁻¹ (7 °C and N₀=3.91×10⁷ CFU mL⁻¹), respectively. When the initial O₃ concentration increased 10-fold from 33 to 330 mg L⁻¹, the *Legionella* inactivation rate increased 32.5-fold, showing that the relationship of the initial O₃ concentration to the *Legionella* inactivation rate is not linear. The Ct value required for a 99.99% reduction was not constant because it decreased consistently with the increasing initial concentration of ozone. This phenomenon was found in all experiments under all conditions and confirms that the initial O₃ concentration is more important than the contact time and that a reduction in the initial O₃ concentration cannot be compensated by increasing the ozone contact time. It also shows that Ct values can only be compared within a narrow range of initial concentrations, considering the influence of temperature and other factors. In addition, Ct values from different experiments can only be contrasted when the initial O₃ concentrations are very similar.

The three curves comparing C₀t and lnN/N₀ in Figure 4 (a) show three different inflection points. The inactivation rate was fastest before each inflection point. After each inflection point, the inactivation rate was lower and tail phenomena were observed. The relationship between C₀ and lnN/N₀ at the inflection points of the curves was analyzed in Figure 4 (b), suggesting a linear relationship between C₀ and lnN/N₀ at the inflection points. This indicates that higher initial O₃ concentrations led

414 to higher inflection point values in the $\ln N/N_0$ vs C_0t curve or, generally speaking, that
415 the initial O_3 concentration affected the inflection point.

Figure 4 (a) The effect of various initial O₃ concentrations on *Legionella* inactivation; (b) relationship of C₀ and lnN/N₀ at the inflection point; (c) energy requirement for complete inactivation of *Legionella* (10⁷ CFU mL⁻¹ cells) in deionized water; (d) energy requirement for complete inactivation *Legionella* (10⁷ CFU mL⁻¹ cells) in wastewater at a COD concentration of 0.75 mg L⁻¹.

3.5 Energy consumption

Energy consumption was monitored during the entire disinfection process. Figure 4 (c) shows the energy consumption under deionized water conditions of 0.072 kW h m⁻³ at voltage of 50 V and flow rate of 25 mL min⁻¹ to achieve a 7-log reduction of *Legionella* in 1 min. In the case of a higher voltage at 110 V and flow rate of 25 mL min⁻¹, the time needed to achieve a 7-log reduction was relatively short (20 seconds) and the energy consumption was 0.041 kW h m⁻³.

Cano et al. (2012) showed that 0.2 kW h m⁻³ energy consumption was required to completely inactivate *E. coli* (N₀=10³ CFU mL⁻¹) using a boron-doped diamond electrode. Kerwick et al. (2005) and Haaken et al. (2012) showed that an energy consumption between 1.0 and 2.6 kW h m⁻³ was required to completely inactivate *E. coli* (N₀=10³–10⁵ CFU mL⁻¹), which is not economical for applications using a boron-doped diamond electrode. Compared to these studies, this research shows that energy consumption using the plasma corona from an ozone generator (STACOL1010, IL, USA) was lower than systems using a boron-doped diamond electrode.

Figure 4 (d) shows the energy consumption in wastewater. The energy requirement was 1.815 kW h m⁻³ at a voltage of 50 V, flow rate of 25 mL min⁻¹ and

COD of 0.75 mg L⁻¹ to achieve a 7-log reduction in *Legionella* in 25 minutes. In the case of a higher voltage of 110 V, flow rate of 25 mL min⁻¹ and COD of 0.75 mg L⁻¹, the time needed to achieve a 7-log reduction was relatively short (1 minute), and therefore, the energy consumption was 0.123 kW h m⁻³. The results show that energy consumption in wastewater was much higher than in deionized water.

4 Conclusions

The *Legionella* inactivation kinetics in wastewater can be expressed mathematically. The equations presented here can be used to predict variations in germ and COD concentrations and are accurate enough for research use.

The ozone reaction with COD and inactivation of *Legionella* occurred simultaneously. COD oxidization by ozone is easier than with *Legionella*. Higher initial COD concentrations resulted in a lower inactivation rate. The lower activation energy and ORP of COD does not necessarily result in an increased reaction rate, which depends on the reaction kinetics. Higher temperature led to higher inactivation efficiency and rate, even if the ozone solubility increased when the temperature decreased.

The relationship of the initial O₃ concentration and *Legionella* inactivation rate was not linear, and the Ct value required for a 99.99% reduction of bacteria was not constant. The initial O₃ concentration is more important than the O₃ contact time, and a reduction in the initial O₃ concentration cannot be compensated by a proportional increase in the O₃ contact time. A comparison of Ct values can be made within a narrow range of initial O₃ concentrations.

Acknowledgments

The authors would like to thank Professor Thanh Helen Nguyen and Shaoying Qi for supervision and assistance, and Hanting Wang for English editing. The authors would also like to thank Reviewer #3, #4 and #5 for their comments and helping to settle many problems. Many thanks to EP Purification, Inc. for supplying an ozone generator. This project has been supported by a National Science Foundation of USA CAREER grant to T.H.N. (0954501); the Key Research Items of Zhejiang Educational Committee, China (Grant No. Z201119987); the General Research Items of the Natural Science Foundation of Zhejiang Province, China (Grant No. Y5110280); the Natural Science Foundation of China (Grant No. 51102136); and the Doctoral fund of Ministry of Education of China (Grant No. 20110097120021).

References

- Aikaterini, Spiliotopoulou, Kamilla, M.S. Hansen, Henrik, R. Andersen, 2015. Secondary formation of disinfection by-products by UV treatment of swimming pool water. *Sci. Total Environ.* 520, 96-105.
- Amisha, D. Shah, Aaron, D. Dotson, Karl, G. Linden, William, A. Mitch, 2011. Impact of UV disinfection combined with chlorination/chloramination on the formation of halonitromethanes and haloacetonitriles in drinking water. *Environ. Sci. Technol.* 45 (8), 3657-3664.
- APHA (American Public Health Association), 1989. Standard Methods for the

489 Examination of Water and Wastewater, 17th edition. Port City Press, Baltimore,
 490 Maryland, USA.

491 Bader, H., Hoigné, J., 1981. Determination of ozone in water by the indigo method.
 492 Water Res. 15, 449-456.

493 Bergmann, M. E. H., Iourtchouk, T., Rollin, J., 2011. The occurrence of bromate and
 494 perbromate on BDD anodes during electrolysis of aqueous systems containing
 495 bromide: first systematic experimental studies. J. Appl. Electrochem. 41,
 496 1109-1123.

497 Bergmann, M. E. H., Rollin, J., Iourtchouk, T., 2009. The occurrence of perchlorate
 498 during drinking water electrolysis using BDD anodes. Electrochim. Acta, 54,
 499 2102-2107.

500 Botzenhart, K., Tarcson, G. M., Ostruschka, M., 1993. Inactivation of bacteria and
 501 coliphages by ozone and chlorine dioxide in a continuous flow reactor. Water Sci.
 502 Tech. 27 (3-4), 363-370.

503 Cano, A., Cañizares, P., Barrera-Díaz, C., Sáez, C., Rodrigo, M. A., 2012. Use of
 504 conductive-diamond electrochemical-oxidation for the disinfection of several
 505 actual treated wastewaters. Chem. Eng. J. 211, 463-469.

506 Craik, S. A., Smith, D. W., Belosevic, M., Chandrakanth, M., 2002. Use of *Bacillus*
 507 *subtilis* spores as model microorganisms for ozonation of *Cryptosporidium*
 508 *parvum* in drinking water treatment. J. Environ. Eng. Sci. 1, 173-186.

509 Deborde, M., von Gunten, U., 2008. Reactions of chlorine with inorganic and organic
 510 compounds during water treatment-kinetics and mechanisms: a critical review.

511 Water Res. 42, 13-51.

512 Fabian, Soltermann, Minju, Lee, Silvio, Canonica, Urs, von Gunten, 2013. Enhanced
 513 N-nitrosamine formation in pool water by UV irradiation of chlorinated
 514 secondary amines in the presence of monochloramine. Water Res. 47 (1), 79-90.

515 Fu-Xiang, Tian, Bin, Xu, Yi-Li, Lin, Chen-Yan, Hu, Tian-Yang, Zhang, Nai-Yun,
 516 Gao, 2014. Photodegradation kinetics of iopamidol by UV irradiation and
 517 enhanced formation of iodinated disinfection by-products in sequential oxidation
 518 processes. Water Res. 58, 198-208.

519 Gerrity, D., Gamage, S., Holady, J. C., Mawhinney, D. B., Quinones, O., Trenholm, R.
 520 A., Snyder, S. A., 2011. Pilot-scale evaluation of ozone and biological activated
 521 carbon for trace organic contaminant mitigation and disinfection. Water Res. 45
 522 (5), 2155-2165.

523 Haaken, D., Dittmar, T., Schmalz, V., Worch, E., 2012. Influence of operating
 524 conditions and wastewater-specific parameters on the electrochemical bulk
 525 disinfection of biologically treated sewage at boron-doped diamond (BDD)
 526 electrodes. Desalinat. Water Treat. 46, 160-167.

527 Hoigné, J., Bader, H. 1976. The role of hydroxyl radical reactions in ozonation
 528 processes in aqueous solutions. Water Res. 10, 377-386.

529 Jeong, J., Kim, J. Y., Yoon, J., 2006. The role of reactive oxygen species in the
 530 electrochemical inactivation of microorganisms. Environ. Sci. Technol. 40,
 531 6117-6122.

532 Jun, Yin, Baolin, Deng, 2015. Polymer-matrix nanocomposite membranes for water

533 treatment. *J. Membr. Sci.* 479, 256-275.

534 Kerwick, M. I., Reddy, S. M., Chamberlain, A., Holt, D. M., 2005. Electrochemical
 535 disinfection, an environmentally acceptable method of drinking water
 536 disinfection. *Electrochim. Acta.* 50, 5270-5277.

537 Langming, Bai, Heng, Liang, John, Crittenden, Fangshu, Qu, An, Ding, Jun, Ma,
 538 Xing, Du, Shadong, Guo, Guibai, Li, 2015. Surface modification of UF
 539 membranes with functionalized MWCNTs to control membrane fouling by
 540 NOM fractions. *J. Membr. Sci.* 492, 400-411.

541 Lee, Y., Gerrity, D., Lee, M., Encinas Bogeat, A., Salhi, E., Gamage, S., Trenholm, R.
 542 A., Wert, E. C., Snyder, S. A., von Gunten, U., 2013. Prediction of
 543 micropollutant elimination during ozonation of municipal wastewater effluents:
 544 use of kinetic and water specific information. *Environ. Sci. Technol.* 47 (11),
 545 5872-5881.

546 Leonardo, Gutiérrez, Thanh, H. Nguyen, 2012. Interactions between rotavirus and
 547 Suwannee River organic matter: Aggregation, deposition, and adhesion force
 548 measurement. *Environ. sci. technol.* 46 (16), 8705-8713.

549 Cimetiere, N., De Laat, J., 2014. Effects of UV-dechloramination of swimming pool
 550 water on the formation of disinfection by-products: a lab-scale study. *Microchem.*
 551 *J.* 112, 34-41.

552 Pisarenko, A. N., Stanford, B. D., Yan, D., Gerrity, D., Snyder, S. A., 2012. Effects of
 553 ozone and ozone/peroxide on trace organic contaminants and NDMA in drinking
 554 water and water reuse applications. *Water Res.* 46 (2), 316-326.

555 Sahid, L. Rosado-Lausell, Hanting, Wang, Leonardo, Gutiérrez, Ofelia, C
 556 Romero-Maraccini, Xi-Zhi, Niu, Karina, Y. H., Gin, Jean-Philippe, Croué,
 557 Thanh, H. Nguyen, 2013. Roles of singlet oxygen and triplet excited state of
 558 dissolved organic matter formed by different organic matters in bacteriophage
 559 MS2 inactivation. *Water Res.* 47 (14), 4869-4879.

560 Schmalz, V., Dittmar, T., Haaken, D., Worch, E., 2009. Electrochemical disinfection
 561 of biologically treated wastewater from small treatment systems by using
 562 boron-doped diamond (BDD) electrodes – contribution for direct reuse of
 563 domestic wastewater. *Water Res.* 43, 5260-5266.

564 Wei, Liu, Zaili, Zhang, Xin, Yang, Yiyue, Xu, Yongmei, Liang, 2012. Effects of UV
 565 irradiation and UV/chlorine co-exposure on natural organic matter in water. *Sci.*
 566 *Total Environ.* 414, 576-584.

567 Wim, T. M. Audenaert, Manly, Callewaert, Ingmar, Nopens, Jan, Cromphout, Robert,
 568 Vanhoucke, Ann, Dumoulin, Pascal, Dejans, Stijn, W.H. Van Hulle, 2010.
 569 Full-scale modelling of an ozone reactor for drinking water treatment. *Chem.*
 570 *Eng. J.* 157, 551-557.

571 Yichao, Qian, Wei, Wang, Jessica, M. Boyd, Minghuo, Wu, Steve, E. Hrudey,
 572 Xing-Fang, Li, 2013. UV-induced transformation of four halobenzoquinones in
 573 drinking water. *Environ. Sci. Technol.* 47 (9), 4426-4433.

574 Yongze, Liu, Jin, Jiang, Jun, Ma, Yi, Yang, Congwei, Luo, Xiaoliu, Huangfu,
 575 Zhongkai, Guo, 2015. Role of the propagation reactions on the hydroxylradical
 576 formation in ozonation and peroxone (ozone/hydrogen peroxide) processes.

577 Water Res. 68, 750-758.

578 Zimmermann, S. G., Wittenwiler, M., Hollender, J., Krauss, M., Ort, C., Siegrist, H.,
579 von Gunten, U., 2011. Kinetic assessment and modeling of an ozonation step for
580 full-scale municipal wastewater treatment: micropollutant oxidation, by-product
581 formation and disinfection. Water Res. 45 (2), 605-617.

582 NBC News, 2015. New York *Legionnaires'* Outbreak Sickens 81, Kills.
583 <http://www.nbcnews.com/health/health-news/new-york-legionnaires-outbreak-sickens>
584 -71-n403301.

585

1
2

3 Figure 1. Experimental setup of the reactor unit.

4
5

1
2

3 Figure 1. Experimental setup of the reactor unit.

4

5

1
2 (a)

3
4 (b)

5
6 (c)

7 Figure 2 (a) Effect of initial COD concentrations on $\ln N/N_0$ at 7°C , $N_0=3.91 \times 10^7 \text{ CFU}$
8 mL^{-1} , and $C_0=33 \mu\text{g L}^{-1}$; (b) Legionella inactivation rate vs. initial COD concentration;
9 (c) $\ln N/N_0$ vs. initial COD concentration.

1
 2 Figure 3. Inactivation of Legionella at various temperatures in deionized water at pH
 3 7.
 4

(a)

(b)

(c)

(d)

Figure 4 (a) The effect of the various initial O_3 concentrations on Legionella inactivation; (b) Relationship of C_0 and $\ln N/N_0$ at inflection point of curve; (c) Energy requirement of a complete inactivation Legionella of 10^7CFU mL^{-1} cells in deionized water; (d) Energy requirement of a complete inactivation Legionella of 10^7CFU mL^{-1} cells in wastewater with 0.75mg L^{-1} COD concentration.