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Kinetic analysis of *Legionella* inactivation using ozone in wastewater

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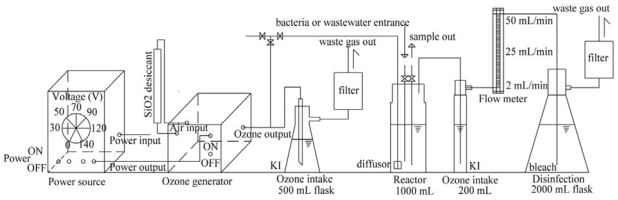
HIGHLIGHTS

- Variations in germ levels can be predicted by the initial O_3 and COD.
- COD is more easily oxidized by ozone than *Legionella*.
- O_3 reduction cannot be compensated proportionally by increasing the O_3 contact time.

GRAPHICAL ABSTRACT



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ABSTRACT

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

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

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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 and De Laat, 2014), N-nitrosodimethylamine (Fabian et al., 2013), brominated trihalomethanes (THMs) (Cimetiere and De Laat, 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 and 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 and von Gunten, 2008; Wim et al., 2010; Gerrity et al., 2011; Zimmermann et al., 2011; Leonardo and 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 (Craik 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 O_3 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 Fig. 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-μm 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) (STA-COL1010, 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 SiO_2 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.

2.2. Chemicals

Yeast extract (BD 212750), 5,5-indigo trisulfonic acid sodium salt (indigo carmine), sodium thiosulfate ($Na_2S_2O_3$), 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}, $Na_2S_2O_3$ 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 $N_aH_2PO_4 \cdot H_2O$ and 35 g H_3PO_4 (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% CO_2 for 2 days to determine the number of living and dead *Legionella*. CO_2 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% CO_2.

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 ($Na_2S_2O_3$ 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