ABSTRACT

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Fluorescent YG-microspheres (Polysciences Inc.) were evaluated to simulate *Cryptosporidium* inactivation in a flow-through system that utilizes multiple disinfectants. Experiments were performed in a disinfection process consisting of an ozone primary stage and a secondary free chlorine treatment stage. Impacts of the chemical disinfectant exposure were calculated by tracking the changes in fluorescence distribution with a flow cytometer. Microspheres were initially pretreated to reduce their fluorescence intensity to a level that would allow them to closely mimic *Cryptosporidium.* Microsphere survival ratios (N/No) were calculated by replicating the inactivation of *Cryptosporidium* observed by Driedger *et al.* [6] and selecting an appropriate fluorescence intensity threshold in a histogram analysis. The results from these flow-through experiments suggest that the fluorescence decay of YG-fluorescent microspheres does display synergistic effects when free chlorine is used sequentially with ozone. This study also included the use of numerical models to simulate sequential disinfection processes. Femlab 3.0a and the Segregated Flow Reactor method were used to evaluate sequential disinfection processes. These numerical models were set up to mimic the experimental conditions observed in this research. The sequential disinfection inactivation predicted in these models was similar to the experimental fluorescence decay of the microspheres. However, the models were not effective at predicting fluorescent intensity changes at different intermediate points within the disinfection process stream.

EVALUATING SEQUENTIAL DISINFECTION IN A WATER TREATMENT SYSTEM USING A NON-BIOLOGICAL SURROGATE

by
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BIOGRAPHY

Brannon Heath Richards was born on October 21, 1979 in Hickory, NC. He is the son of Sam and Pat Richards. He graduated from Alexander Central High School in 1998 and decided to pursue a degree in Civil Engineering at North Carolina State University. He graduated in December 2002 with a B.S. in Civil Engineering from North Carolina State University. After graduating, Brannon's interest in environmental engineering led him to pursue a Masters of Science degree in Civil, Construction, and Environmental Engineering (January 2003) and work on environmental issues as a research assistant at North Carolina State University.

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1. Introduction

The primary goal of disinfection in drinking water treatment is to inactivate microorganisms capable of causing waterborne disease outbreaks. This task has become more challenging with the existence of resistant organisms such as *Cryptosporidium*. The ingestion of this protozoan parasite can result in the disease cryptosporidiosis, a potentially life-threatening diarrheal illness in persons with underdeveloped or suppressed immune systems. Due to the dangers associated with *Cryptosporidium*, the EPA has developed rules to limit human exposure through drinking water. The Interim Enhanced Surface Water Treatment Rule (IESWTR) and the Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR) require a 2-log removal for public water systems [29-30]. Additional rules, such as the LT2ESWTR, enhance the monitoring of disinfection by-products, *Cryptosporidium* inactivation, and disinfectant concentration through the entire distribution system [30]. These stricter regulations have forced researchers to focus on the removal of *Cryptosporidium*.

Studies have shown that the use of free or combined chlorine, the most widely used disinfectants, have little or no effect alone on *Cryptosporidium* due to its resistant outer shell [6, 10]. The ability of *Cryptosporidium* to form this resistant outer shell has led researchers to investigate the use of other chemical disinfectants, specifically ozone and chlorine dioxide. The superior efficiencies of these disinfectants have made them the leading chemical disinfectants for inactivating *Cryptosporidium* in drinking water [5, 19].

New approaches have been proposed to reduce ozone and chlorine dioxide dosages, since high concentrations of these disinfectants can lead to the formation of carcinogenic disinfection by products [1, 6, 20, 21, 26-27]. These approaches call for

two disinfectants to be used sequentially in order to inactivate *Cryptosporidium* [4-6, 19-21, 25-27]. Therefore, drinking water facilities can apply ozone or chlorine dioxide as a primary disinfectant and free or combined chlorine as a secondary disinfectant. Li *et al*. [19] have shown that sequential inactivation using ozone followed by free or combined chlorine increased the inactivation of *Cryptosporidium* compared to when these disinfectants were used individually. Driedger *et al.* [6] showed that ozone and free chlorine used sequentially led to a substantially higher *Cryptosporidium* inactivation rate constant for free chlorine than when chlorine was used alone. This synergistic mechanism has been explained by improved chlorine permeation through *Cryptosporidium's* cell wall due to damage caused by the initial ozone exposure [6]. Thus, slower kinetics will be observed if *Cryptosporidium* has a thicker or denser cell wall, since the organism will be more resistant to access for the secondary disinfectant [6].

To date, most *Cryptosporidium* inactivation studies have been done at the bench scale. The problem with designing full-scale reactors is that the CT's (disinfectant concentration x exposure time, based on time that 10% of influent water reaches the effluent) from batch reactors do not account for the hydraulic mixing and short-circuiting of the system. If the system was over-designed, then high disinfectant concentrations would result in excessive treatment cost and greater formation of disinfection by-products. However, reactor under-design could lead to *Cryptosporidium* breakthrough into the drinking water distribution system [23].

To better evaluate *Cryptosporidium* inactivation, direct quantification of disinfection performance is required. Alternatives include development of more accurate CT values by continuously monitoring disinfectant concentration distribution and

hydraulics mixing throughout the reactor. This can be accomplished by performing chemical tracer tests for hydraulic characterization and using inactivation models to simulate organism disinfection behavior [13, 28].

Other alternatives include the use of biological [15] and non-biological [2, 22-23] surrogate indicators. Non-biological indicators are of particular interest, since no special biological facilities are needed. Direct quantification can be made with the surrogate, since it already takes into account system hydraulics. The non-biological surrogate indicators most widely used are fluorescent dye polystyrene microspheres. These non-biological microspheres have been used by Chiou *et al*. [2] to mimic *Giardia* inactivation with ozone disinfection, by Marinas *et al*. [23] to mimic *Cryptosporidium* inactivation with ozone disinfection in batch and full-scale water treatment plants and recently by Baeza and Ducoste [1] to mimic *Cryptosporidium* sequential disinfection in batch reactors. All of these studies have shown promising results in using microspheres to mimic microbial inactivation. However, no study has used microspheres with sequential disinfection in a flow-through system to mimic *Cryptosporidium* inactivation.

The purpose of this study is to evaluate fluorescent Yellow Green (YG)-microspheres to simulate *Cryptosporidium* inactivation in a flow-through system that uses sequential disinfection treatment. To evaluate the efficiency of sequential disinfection systems, calibration was performed in batch reactors in a preceding study by tracking the changes in fluorescence distribution using a flow cytometer. The fluorescence distribution change was matched to a reference *Cryptosporidium* data set – fitted to a Chick Watson and Delayed Chick Watson Model [1]. The Chick Watson and Delayed Chick-Watson models (Equation 1.1) are the most commonly used for describing *Cryptosporidium* decay [11].

$$\frac{N}{No} = \begin{cases} \left(\frac{N}{No}\right)_C \text{ if } Ct \le Ct_{lag} = \frac{1}{k}\ln\left\{\left(\frac{N1}{No}\right)\left(\frac{No}{N}\right)_C\right\} \\ \frac{N1}{No}\exp(-k\,Ct) = \exp\left(-k\left\{Ct - Ct_{lag}\right\}\right) \text{ if } Ct > Ct_{lag} = \frac{1}{k}\ln\left\{\left(\frac{N1}{No}\right)\left(\frac{No}{N}\right)_C\right\} \end{cases} \quad (1.1)$$

$(N/No)_C$ = viability *Cryptosporidium* control (empirical), k= post-shoulder inactivation rate constant, N1/No = intercept with the Y-axis resulting from extrapolation of the pseudo-first order data range, C= disinfectant concentration, t= contact time in min, Ctlag= lag phase CT. For Chick Watson model there is no lag phase, therefore $(N/No)_C$ = N1/No =1.0.

Microsphere application may be limited for a full-scale reactor. Thus, this study also includes the use of numerical models to simulate sequential disinfection processes involving turbulence models for flow field prediction and microbial inactivation kinetic models to represent the microorganisms decay throughout the reactor. The results from the experimental data and model will be used to analyze sequential disinfection of *Cryptosporidium* using ozone followed by chlorine in a flow-through system.

2. Experimental Materials and Methods

2.1. Microspheres

The non-biological surrogates used were YG Fluoresbrite™ fluorescent microspheres with a diameter of 0.94 μm (2% coefficient of variation) and a density of 1.045 g/cm^3 (Figure 1). The commercial microspheres from Polysciences Inc. come stored in an aqueous solution with 2.6% solids content. Preliminary tests showed that the original microspheres contained more fluorescent dye than was required to simulate the inactivation of *Cryptosporidium*. Thus, the microspheres were pretreated with ozone to lower their fluorescence [1, 22]. The pretreatment step will be explained in Section 4.1. The dye had an unknown chemical formula created by Polysciences Inc. However, a previous study showed that the fluorescence of the YG fluoresbrite matches the fluorescence characteristics of fluorescein isothiocyanate [22].

2.2. Experimental apparatus

The flow-through sequential disinfection system consisted of four bench scale reactors in series: two initial ozone contactors followed by a chlorine-mixing chamber and finally, a chlorine baffled contactor. Influent water was filtered and deionized by a Bantam Deionizer, to remove free and combined chlorine as well as other organic compounds that react with ozone and chlorine. The influent water was pumped to the 1st ozone contactor. Tests were performed with the source water to make sure that the free and combined chlorine concentrations were zero. However, some ammonium may have been released into the pilot-scale system. Thus, additional measurements were performed within the pilot system following free chlorine addition and will be discussed later. The

pretreated microspheres were injected with an influent peristaltic pump flowing to the initial two ozone bubble column contactors (cocurrent-countercurrent sequence). The experiments were performed with two influent flows, 11.0 and 15.5 ml/s. The water-microsphere mixture, already exposed to ozone, flowed by gravity and entered the chlorine mixing chamber, where it was thoroughly mixed with a free chlorine solution. The water was then pumped by a peristaltic pump from the mixer into the chlorine contactor, where the water was eventually removed by gravity. The flow of the water/microsphere mixture is shown in Figure 2.

2.2.1. Ozone experimental apparatus: The ozone contactors were 60 cm tall with a diameter of 7.5 cm. At the bottom of the contactors was a fritter diffuser through which the ozone gas was injected. The water/microsphere mixture flowed cocurrent with the ozone in the first contactor and countercurrent to the ozone in the second contactor. A Model G-11 Ozone-Pacific corona discharge generator produced ozone with dried industrial air. The ozone gas was fed at a flow rate of 10 SCFH (standard cubic feet per hour). The ozone concentration was controlled with a variable output knob that operates from 0 to 100%. All experiments were performed at room temperature (20 ± 2 °C).

2.2.2. Chlorine experimental apparatus: A 12 cm diameter mixing chamber was used to blend the chlorine with the water/microsphere mixture after ozone treatment. Chlorine was injected with a peristaltic pump into the mixer at a rate of 0.255 ml/s, where a stirrer was used to create a complete mix condition. The chlorine mixer contained a fixed volume of 910 cm^3. The effluent water was pumped by a second peristaltic pump into the chlorine contactor. The chlorine contactor had an overall width of 61 cm and an

overall length of 37.5 cm. The water height within the contactor was 13.5 cm. The contactor had three baffles running from east to west and three baffles running north to south inside the contactor. The three baffles running east to west split the contactor into four equal sections of 61 cm by 9 cm. The three baffles running north to south promoted additional top to bottom mixing. These vertical baffles ran from the top of the contactor to a height 2 cm above the bottom of the contactor. Figure 2 displays the baffles located within the chlorine contactor.

2.3. Ozone disinfection:

In this research, ozone was applied to the microspheres to perform two tasks. Ozone was first used to pretreat the microspheres so that the fluorescent decay would closely mimic the inactivation kinetics of *Cryptosporidium.* Ozone was later used to act as the primary disinfectant in the flow-through disinfection process.

2.3.1. Microsphere pretreatment and Threshold determination:

One liter of 0.01 M phosphate buffer at pH 7 was placed in a semi-batch reactor. Ozone was bubbled through the reactor for at least 20 minutes to ensure a steady state concentration of 0.30 ± 0.03 mg/L. The ozone concentration was measured (50 mL of sample) by an Indigo Pocket Colorimetric Method, Hach Co. (Loveland, Co). After a steady state ozone concentration was reached, approximately 2.0×10^{10} microspheres suspended in 50 mL of pH 7 buffer were injected into the system. The reaction was carried out for a period of 90 minutes; then the knob was turned to 0%, and air was bubbled for 20 minutes to remove the residual ozone in the microspheres batch. The pretreated microspheres were stored at 4°C.

Following microsphere pretreatment, a batch test was performed to determine the threshold value for the pretreated microspheres. The batch test was performed much the same as the microsphere pretreatment. Again, ozone gas was bubbled into a semi-batch reactor containing one liter of 0.01 M phosphate buffer at pH 7, until a steady state concentration of 0.30 ± 0.03 mg/L was achieved. After a steady state concentration was achieved, 50 mL of pretreated microspheres were injected into the system. The samples were immediately quenched with 1 mL of 0.1 N sodium thiosulfate to stop all reactions with ozone. Samples were taken at time intervals of 30 sec, 60 sec, 2 min, 3 min, 4 min, and 6 min. These sample times were selected such that the microspheres fluorescent intensity decay could be monitored for a CT of 1.4 mg*min/L (approximately 1-log inactivation). The fluorescent intensity decay over this six minute time period was used to select a threshold value which allowed the fluorescent microspheres to mimic the *Cryptosporidium* reference data of Driedger *et al.* [6].

2.3.2. Primary ozone disinfection:

Ozone was bubbled into the first and second ozone contactors as described in Section 2.2.1. Experiments were performed at ozone concentrations of 0.35 +/- 0.03 mg/L in the first reactor and 0.75 +/- 0.03 mg/L in the second reactor, to achieve 1-log of microspheres' fluorescence decay for the steady-state flow equal to 15.3 ml/sec. Ozone concentrations within the second ozone contactor were higher because an equal amount of ozone gas was bubbled between the two contactors, and the second contactor received much of the effluent ozone from the first reactor. Low flow experiments were performed with ozone concentrations of 0.20 +/- 0.03 mg/L in the first reactor and 0.38 +/- 0.03 mg/L in the second reactor to achieve 1-log of fluorescence decay for the microspheres.

Experimental measurements of the ozone concentration at different points along the column height were performed to ensure that the target concentration was reached. After steady state was achieved in the reactor, the water/microsphere mixture was pumped into the 1st ozone contactor with a microsphere concentration of 70 ug/L. Samples of 50 mL were taken from the effluent of the 2nd reactor to monitor the extent of fluorescence decay prior to injection of chlorine. The ozone in the samples was quenched by adding 4 mL of 0.1N sodium thiosulfate (Fisher Scientific). Due to the low microsphere concentrations, the 50 mL samples were centrifuged at 3500 rpm for 10 minutes. The concentrated samples were then stored at 4 ºC and analyzed within 24 hours.

2.4. Sequential disinfection:

Sequential disinfection consisted of an ozone primary stage and a free chlorine secondary stage. The methodology for the ozone primary disinfection was explained in Section 2.3.2. After contact with ozone, the microspheres flowed by gravity into the chlorine mixing chamber. Ozone concentrations within the chlorine mixing chamber were approximately 0.06 mg/L.

The free chlorine secondary disinfection stage was performed at room temperature (20 ± 2 °C). A 1 g/L free chlorine stock solution was prepared with 5% sodium hypochlorite (ACROS Organics). The stock solution was added to the mixing chamber to produce free chlorine concentrations of approximately 16.5 +/- 1.0 mg/L as Cl_2 during high flow conditions and 12.4 +/- 1.0 mg/L as Cl_2 during low flow conditions. Combined chlorine concentrations were also measured within the reactor system to determine the affect of the released ammonium. Combined chlorine concentrations of approximately 1.0 +/- 0.1 mg/L were measured during both low and high flow

conditions. Previous experiments have shown that combined chlorine has little effect on the fluorescence decay of the microspheres [1].

The microspheres, already mixed with the free chlorine, were then pumped to the chlorine contactor. Ozone concentrations were measured at several locations within the baffled chlorine contactor and no ozone was measured within the contactor. Free chlorine concentrations, within the contactor, were measured by the DPD Pocket Colorimetric Method, Hach Co. (Loveland, Co). Samples of 40 mL were taken from several locations. The free chlorine reaction was stopped with 4 mL of 0.1 N sodium thiosulfate. CT values of 510 mg*min/L were targeted for the chlorine disinfection process. This amount of chlorine exposure was needed to achieve 1-log of inactivation during chlorine sequential disinfection.

Microsphere samples were taken from multiple locations in the flow-through system. The sampling locations are shown in Figure 2. The sampling methodology of the dye-microspheres was similar to tracer tests. However the microsphere surrogate method was more straightforward, since it only required the collection of one sample instead of many samples during tracer tests [2]. The samples were quenched with 0.1 N sodium thiosulfate in order to remove any remaining ozone or free chlorine within the samples. Due to the sample microsphere low concentrations, the samples were centrifuged at 3500 rpm for 10 minutes. The concentrated samples were stored at 4ºC and analyzed within 24 hours.

2.5. Flow cytometer analysis

Cytometry is a method to differentiate and count cells and microparticles. The flow cytometer model used for measuring the microspheres fluorescence was a Becton Dickinson FACSCalibur[+]. Microspheres showed the highest signal on the FL1 (FITC fluorescence) detector. The flow cytometer is restricted by two FITC resolutions: 256 or 1024 channels. Settings were adjusted to see the microspheres at a resolution of 256 channels. Gating was performed in a dot/density plot using the characteristic forward (FSC) versus orthogonal light scatter (SSC) for eliminating any extraneous element. The light scattering properties, FSC and SSC were used to assess differences in the particles' morphology. FSC was related to the particle size, while the SSC was related to the internal granularity and complexity [1]. At least 10,000 events-microspheres were acquired per sample. Flow cytometer settings used in this research are displayed in Table 1.

2.5.1. Histogram analysis:

The fluorescence intensity results were obtained as histograms. Histograms were analyzed by a compatible shareware software called WinMDI Version 2.8 - (Windows Multiple Document Interface for Flow Cytometry) developed by Joe Trotter, Scripps Institute [14].

The microspheres survival ratio (N/No) was calculated [23] by selecting a threshold or marker where the N/No ratio was equal to the number of events with fluorescence intensity greater than the threshold, divided by the total number of events in

[+] Flow cytometer: B.D. Model FACSCalibur, Microbiology and Immunology. College of Veterinary Medicine. NCSU Raleigh NC 27606.

the entire histogram. The threshold value corresponded to a boundary between the beads representing the viable and non-viable *Cryptosporidium* cysts. Survival ratios were normalized by the survival ratio at time zero of each experiment. Figure 3 displays the histograms obtained with the software. Using Figure 3A symbols, the survival ratio was described by the following equation:

$$\frac{N}{No} = \left(\frac{M0 - M1}{M0}\right) \Big/ \left(\frac{N}{No}\right)_{Ct=0\ min\text{-}mg/L} \tag{2.1}$$

(Mo-M1) represents the number of microspheres-events with fluorescence intensity greater than the threshold. Mo represents the number of microspheres-events in the entire histogram. Figure 3B displays an example of fluorescence intensity histograms after an ozone treatment of 0.28 mg/L. The movement to the left of the histograms produced the decay of the survival ratio (N/No) that was used to develop the inactivation curves (i.e., N/No versus CT (mg-min/L) disinfection values).

3. Numerical Methods

In this study, numerical methods were used to simulate the sequential disinfection process, including turbulence models for flow field predictions and microbial inactivation kinetic models to represent the microorganism's response to disinfectants.

3.1. Flow field simulation:

Turbulent flow involves random velocity and pressure fluctuations where fluid motion is more complex and unsteady. Virtually all flows of engineering interest are turbulent, including the flows used in this study. Various experiments were performed to

determine the water hydraulics and retention times within the different reactors. The tracer test pulse method was used to determine the mixing and water hydraulics within the reactor. To perform the pulse tracer tests, a known concentration and volume of potassium permanganate dye was injected into the system. Samples of effluent water were taken at an interval of time small enough to capture the shape of the residence time density function [7]. The samples were collected, and the absorbance of each sample was determined to find the concentration of dye at different time intervals. Quality assurance was performed on the collected effluent dye concentration that included an assessment of the mass recovered. Only tracer tests that achieved at least a 95% mass recovery were retained. Using the concentrations and corresponding time intervals, the retention time of each component of the disinfection system as well as the residence time density function were found using the following equations:

$$\bar{\tau}_{RTD} = \sum_{i=0}^{i\max-1} \left[\frac{f(t_i)*t_i + f(t_{i+1})*t_{i+1}}{2} \right] (t_{i+1} - t_i) \tag{3.1}$$

$$f(t_i) = \frac{c(t_i)}{area} \tag{3.2}$$

$$area = \sum_{i=0}^{i\max-1} \left[\frac{c(t_{i+1}) + c(t_i)}{2} \right] (t_{i+1} - t_i) \tag{3.3}$$

The residence times computed from Equation 3.1 are shown in Table 2.

The turbulent flow within the reactor was predicted using Femlab 3.0a. Femlab is a commercially available numerical model for solving engineering problems based on partial differential equations (PDEs). When solving the PDEs, Femlab uses the finite element method.

The two-equation k-ε model was used to determine the turbulent flow characteristics within the reactor. Launder and Spalding [17] version of the two-equation k-ε model used in this study is shown below:

Continuity:

$$\frac{\partial \overline{U}_i}{\partial x_i} = 0 \tag{3.4}$$

Kinematic eddy viscosity:

$$\nu_t = C_\mu k^2 / \varepsilon \tag{3.5}$$

Turbulence kinetic energy:

$$\frac{\partial k}{\partial t} + \overline{U_j}\frac{\partial k}{\partial x_j} = \nu_t\left(\frac{\partial \overline{U_i}}{\partial x_j} + \frac{\partial \overline{U_j}}{\partial x_i}\right)\frac{\partial \overline{U_i}}{\partial x_j} + \frac{\partial}{\partial x_j}\left[(\nu + \frac{\nu_t}{\sigma_k})\frac{\partial k}{\partial x_j}\right] - \varepsilon \tag{3.6}$$

Dissipation rate:

$$\frac{\partial \varepsilon}{\partial t} + \overline{U_j}\frac{\partial \varepsilon}{\partial x_j} = C_{\varepsilon 1}\frac{\varepsilon}{k}\nu_t\left(\frac{\partial \overline{U_i}}{\partial x_j} + \frac{\partial \overline{U_j}}{\partial x_i}\right)\frac{\partial \overline{U_i}}{\partial x_j} - C_{\varepsilon 2}\frac{\varepsilon^2}{k} + \frac{\partial}{\partial x_j}\left[(\nu + \nu_t/\sigma_\varepsilon)\frac{\partial \varepsilon}{\partial x_j}\right] \tag{3.7}$$

Reynolds stresses:

$$-\overline{u_i u_j} = \nu_t\left(\frac{\partial \overline{U_i}}{\partial x_j} + \frac{\partial \overline{U_j}}{\partial x_i}\right) - \frac{2}{3}k\delta_{ij} \tag{3.8}$$

The empirical constants used in this model are shown below and were not optimized in this study:

$C_{\varepsilon 1} = 1.44,\ C_{\varepsilon 2} = 1.92,\ C_\mu = 0.09,\ \sigma_k = 1.0,\ \sigma_\varepsilon = 1.3$

The flow field predicted by this model is shown in Figure 4. The conditions used in the flow field simulation are shown in Table 3.

3.2. Microbial inactivation:

Modeling of the decay and inactivation of the microorganism was performed following the simulation of the turbulent flow field. Figure 5 displays the common

microbial survival curves that are used for different microorganisms. These inactivation kinetic models include the Chick-Watson, Rational, Selleck, and Series-Event models. In this study, two models were used to capture the inactivation of *Cryptosporidium,* the Chick-Watson and the Series-Event model. The Chick-Watson model is used if the microbial survival curve follows the traditional exponential log kill profile. The Series-Event model is used to describe survival curves with an initial shoulder followed by an exponential kill. The Chick-Watson model was used to describe the inactivation kinetics during chlorine treatment and the Series-Event model was used to describe the inactivation kinetics during primary ozone treatment. The disinfection rate and disinfection survival rate for these two models is shown below. During sequential disinfection the Chick-Watson model was extended to capture the two sloped curve observed by Driedger *et al.* [6]. The disinfection survival equation for this model is also shown below.

Disinfection rate equations:

Chick-Watson $r = - kCN$ (3.9)

Series-Event $r_k = kCN_{i-1} - kCN_i$ (3.10)

Disinfection survival equations:

Chick-Watson $N/N_0 = \exp(-kCt)$ (3.11)

Series-Event $$N/N_0 = \exp(-kCt)\left[\sum_{\kappa=0}^{n-1}\frac{(kCt)^{\kappa}}{\kappa!}\right]$$ (3.12)

Chick-Watson (sequential) $N/N_0 = (1-p)*\exp(-kCt) + p*\exp(-kCt)$ (3.13)

In the above equations, k represents the inactivation rate constant, C is the disinfectant concentration, N is the number of live microorganisms, t is the contact time, and p is the fraction of microorganisms following a specific inactivation rate constant. The inactivation rate constants used in this research are shown in Table 4. The rate constants for both ozone and chlorine inactivation were taken from Driedger *et al.* [6].

The inactivation of *Cryptosporidium* in the flow-through disinfection system was evaluated in Femlab and using the Segregated Flow Reactor (SFR) method. Femlab 3.0a computed the transport and inactivation of *Cryptosporidium* using the turbulent convective-diffusion-reaction equation. This method views the microorganisms as a continuous reacting tracer with reaction kinetics based on Equations 3.9 and 3.10. Microbial inactivation based on the SFR approach was calculated using the residence time distribution function in the following equation:

$$\mathrm{N/N_{0\ Effluent}} = \int_0^{T\max} [(\mathrm{N/N_0})(t)]_{bench} * E(t)dt \qquad (3.14)$$

$(\mathrm{N/N_0})(t)$ is the bench-scale survival rate at time t and $E(t)$ is the normalized residence time density function (RTD).

The SFR model assumes that each parcel behaves as a completely mixed batch reactor that remains completely segregated, therefore not mixing with other fluid parcels. In addition, SFR assumes that the kinetics are first order for each reactor. This last assumption was relaxed for the purposes of this research. The SFR was used here to determine if a simple modeling approach could characterize the sequential disinfection process. Equation 3.14 will be used to compute the effluent log inactivation at four locations in the disinfection system; following the 1st ozone contactor, 2nd ozone contactor, mixing chamber and the baffled chlorine contactor.

4. Experimental Results.

4.1. Microspheres pretreatment and Threshold determination:

The microspheres used in this research had an initial fluorescence intensity which was greater than the fluorescence intensity needed to mimic the *Cryptosporidium* inactivation reference data. Therefore, the microspheres were pretreated so that the initial fluorescence intensity could be reduced. The amount of pretreatment needed for the microspheres to accurately mimic the *Cryptosporidium* reference data was found in an earlier study by Baeza and Ducoste [1].

All pretreatment experiments in this study were performed with ozone. The fluorescence average intensity decay with ozone concentrations of 0.29 and 0.66 mg/L for ozone pretreated microspheres at pH 7 and 20 ± 2ºC is illustrated in Figure 6. Previous experiments have shown that the pretreatment stage is easier to control at lower ozone concentrations. Thus, concentrations in the range of 0.30 +/- 0.03 mg/L were used in all pretreatment experiments [1]. The duration of ozone pretreatment was approximately 90 minutes. Earlier experiments have shown that this exposure time works best for representing *Cryptosporidium* inactivation.

Following the pretreatment of the microspheres, a batch test was performed in the same manner as the pretreatment test. A threshold value was determined from the pretreatment batch step and was later used for the sequential disinfection inactivation. This threshold value was found by simulating the Chick-Watson kinetic model for *Cryptosporidium* inactivation observed by Driedger *et al.* [6]. The threshold value that produced results, which closely mimicked the inactivation observed by Driedger [6], was found by using the least squares method and comparing the different threshold results to

the reference data. This threshold value was used for the remainder of the experiments performed with a particular batch of pretreated microspheres. Figure 7 displays an example of a batch test following ozone pretreatment. This figure shows how only one threshold value can most accurately mimic the *Cryptosporidium* reference data.

4.2. Primary disinfection with ozone:

Ozone disinfection experiments were carried out at pH 6 and at a temperature of 20 ± 2ºC. Experiments were performed with two separate velocities, a high velocity of 1.233 m/s (15.5 ml/s) and a low velocity of 0.875 m/s (11.0 ml/s). Results for the microsphere fluorescence decay with ozone are shown in Table 5. The conditions for each experiment are also shown in Table 5. Primary ozone disinfection experiments were performed after ozone pretreatment. Primary ozone disinfection was used to achieve approximately 1-log of microsphere fluorescence decay. Histogram analysis (Section 2.5.1) was used to develop these tables by selecting the appropriate threshold that matched the reference inactivation rate. Several factors could affect the threshold value for a particular experiment. Inequality in thresholds could be due to varying ozone concentrations or varying pretreatment times during the pretreatment stage. In addition, varying microsphere size and varying dye concentrations within the microspheres could affect threshold values.

In Table 5, the results show that a microsphere fluorescence decay of approximately one-log was achieved during primary ozone treatment. The flow rate and ozone concentration in the two column ozonators was designed to achieve approximately one-log reduction to mimic the primary ozone treatment of *Cryptosporidium* from Driedger et al. [6]. The data in Table 5 from the ozone primary treatment stage in a flow-

through system suggests that the microspheres were able to achieve the target *Cryptosporidium* inactivation level.

4.3. Secondary chlorine disinfection:

Table 6 displays the results as well as the conditions of secondary free chlorine disinfection experiments. Sampling locations are shown in Figure 2. N/No was determined using the histogram analysis technique (Section 2.5.1). The threshold values used in the free chlorine stage were the same as the threshold values used in the ozone primary disinfection stage. The results were normalized by N/No ratio at time zero of the chlorine stage. The normalization was done due to the variability of the microspheres' initial survival ratio following primary treatment, which ranged from 0.131 to 0.194.

In Table 6, the microspheres exposed to chlorine without any ozone primary treatment show a somewhat fast initial fluorescence decay. This slightly high fluorescence decay may be due to an area of recirculation located at the beginning of the baffled chlorine contactor, which may have caused additional exposure time. However, when chlorine was applied after ozone primary treatment, the microspheres, like the *Cryptosporidium* reference data [6], showed an improved inactivation or fluorescence decay. Table 6 demonstrated that the sequential inactivation tests have a much faster initial fluorescence decay and a greater total fluorescence decay than the non-sequential disinfection tests. This was most likely due to chlorine's ability to quickly degrade exposed dye on the microspheres.

The initial ozone exposure broke down outer layers of the microsphere, exposing dye on the inner layers. The chlorine reacted quickly with the exposed dye and then slowly penetrated the remaining layers and reacted with the dye in the inner layers. The

results of a previous study showed that ozone is able to erode the polymer matrix, allowing the free chlorine to readily react with the dye available on the layers now exposed after ozone treatment [1]. When compared to the chlorine-only disinfection tests, the flow-through results with ozone followed by chlorine demonstrated the fluorescence microspheres ability to mimic synergistic effects.

5. Numerical Results.

5.1. Flow simulation:

The experimental flow field was simulated in a 2-D hydrodynamic model using Femlab 3.0a. Two flow velocities were used in this model to simulate *Cryptosporidium* inactivation: a low flow velocity of 0.006875 m/s (11.0 mL/s) and a high flow velocity of 0.0096875 m/s (15.5 mL/s). The flow field obtained using a velocity of 0.0096875 m/s is shown in Figure 4. A simulated tracer test was performed in Femlab 3.0a to determine the residence times within our 2-D model. The simulated tracer was based on solving the turbulent convection diffusion equation for an inert chemical species and simulating a step input tracer test. The chemical species concentration was monitored at the effluent of the individual reactors. The simulated tracer test was performed with both velocities used in this research and was performed for each individual reactor. The residence times from the model are also shown in Table 2. The residence times from the model were similar to the residence times generated experimentally. Differences in the residence times between the experimental and numerical results are likely due to model simplifications (i.e. 2-D flow simulation). Although not shown here, differences were

also found in the shape of the residence time density function, further suggesting that the 2-D flow simulation did not completely capture the 3-D macroscale mixing characteristics of the different reactors.

5.2. Microbial inactivation:

Cryptosporidium inactivation was modeled using both the Chick-Watson and the Series-event model. The Series-event model was used for describing microbial inactivation when ozone was used as the disinfectant. Previous research has shown that *Cryptosporidium* inactivation with chlorine follows the Chick-Watson model [1]. Table 3 shows model conditions for both low and high flow. Model conditions, including ozone and chlorine concentrations and decay, closely resembled the conditions created experimentally. The results given by the two models for the overall disinfection flow-through process are shown in Table 7.

In Table 7, the Femlab results display a final *Cryptosporidium* inactivation, following ozone disinfection that under-predicts the experimental fluorescence intensity for the high flow rate, while over-predicting the experimental fluorescence intensity for the low flow rate. The Femlab model further over-predicts the experimental results particularly at all three locations in the baffled contactor. The fast initial inactivation of the microspheres observed experimentally with chlorine was not fully captured in the model. This is most likely due to the inability of the 2-D model to capture the complex 3-D mixing conditions, in particular at the beginning of the reactor where a perforated baffle is located. Thus, the microspheres display a quicker initial fluorescence decay experimentally. However, the final simulated fluorescence intensity is similar to the fluorescence decay of the microspheres observed experimentally.

In addition to the inability of the 2-D model to capture the 3-D mixing conditions, the model is also based on only one inactivation rate kinetic, which was obtained from Driedger et al [6] and is based on one ozone CT (CT of 1.4 mg*min/L). However, in this flow-through system, each microorganism will spend a different amount of time within the ozone contactors, thus experiencing a range of CT values. Different CT values will lead to different inactivation rate constants for the secondary chlorine disinfectant. Therefore, if a microorganism spends more time in the ozone contactors it will be subject to more ozone exposure and will most likely degrade more quickly once exposed to chlorine. The opposite is true if the microorganism is initially exposed to less primary ozone. Thus, inactivation kinetics for the secondary disinfectant used in this model may not completely capture the sequential disinfection experienced in a flow-through system.

For the SFR model, the results in Table 7 show that the model, following ozone disinfection, under-predicts the experimental fluorescent intensity for the high flow condition and over-predicts the experimental fluorescent intensity for the low flow conditions. Both the high and low flow rate effluent chlorine disinfection levels were under predicted with the SFR approach. As discussed earlier, the SFR model assumes that each parcel of fluid is a completely mixed batch reactor and is completely independent of other fluid parcels that all undergo a first order reaction. This is not the case since the ozone reaction kinetics has a lag phase prior to following 1^{st} order kinetics (i.e. series event kinetics). In addition, the kinetics for the secondary chlorine disinfectant significantly departed from 1^{st} order kinetics as displayed by Equation 3.13. Though this assumption was relaxed so that this model could be used, the fact that not all parcels follow first order reaction kinetics does affects this models ability to accurately predict the microbial inactivation levels. Finally, the chlorine concentration near the

injection point in the chlorine mixer may not be the same as the concentration at other locations downstream from that point, abandoning one of the assumptions of the SFR approach.

Table 8 displays a direct comparison of the model performance under non-sequential and sequential conditions for chlorine secondary disinfection. Both the Femlab and SFR approaches were able to show the higher inactivation level when applying two sequential disinfectants compared to chlorine alone. However, for the non-sequential conditions, both models predict a higher effluent fluorescence intensity value than the experimental results regardless of the flow rate. Again this deviation is likely due to the modeling assumptions described earlier. The differences in inactivation observed during secondary free chlorine were more noticeable at the start of the chlorine exposure. Femlab did not predict a fast initial inactivation once the microorganisms were exposed to chlorine, as was seen experimentally. Both models did predict final inactivation levels that were similar to the final inactivation achieved experimentally for the low flow rate. The SFR model seemed to show a faster initial decay for the sequential condition, which can be seen by looking at the inactivation following the mixing chamber within this model. However, the SFR greatly overestimated the final inactivation for the high flow rate sequential condition.

As mentioned earlier the disinfection kinetic constants for chlorine were based on an initial treatment with ozone equivalent to a CT of 1.4 mg*min/L at pH 7 and 20ºC. Microspheres in the flow-through reactor system may experience varying amounts of ozone exposure. Therefore, the microspheres entering the chlorine mixing chamber may have a range of CT values. This most likely resulted in different inactivation rates once the microspheres were exposed to chlorine. Those microspheres exposed to less ozone

(i.e. CT = 1.0 mg*min/L) may not have been inactivated as quickly by chlorine; whereas the microspheres exposed to more ozone (i.e. CT = 2.0 mg*min/L) may have been inactivated quicker by chlorine and follow different non-linear kinetics.

A range of disinfection kinetic curves based on the different CT values achieved in the system would be needed in order to quantify microorganism inactivation. Therefore, the inactivation in the numerical models may not have accurately reflected the experimental research, since the microorganisms that entered the baffled chlorine contactor were all assumed to have been only exposed to a CT of 1.4 mg*min/L, and thus assumed to be inactivated by the same disinfection kinetics (i.e. Equation 3.13).

6. Summary.

Experimental research was conducted to demonstrate the use of YG Fluoresbrite^{TM} microspheres as a non-biological surrogate for *Cryptosporidium* disinfection under sequential disinfection conditions in a flow-through water treatment system. Microsphere inactivation survival ratios (N/No) were determined using the histogram analysis method in order to relate microsphere fluorescence intensity decay to *Cryptosporidium* inactivation. Tests were performed at two flow rates in a system that consisted of ozone as a primary disinfectant followed by chlorine as a secondary disinfectant. Experimental analysis of the microspheres fluorescence decay using two disinfectants demonstrated that the YG microsphere could imitate enhanced secondary disinfectant performance and mimic the behavior of *Cryptosporidium* sequential disinfection. However, a histogram analysis must be performed following each

microsphere pretreatment step to determine the threshold value needed for the microspheres to closely resemble the *Cryptosporidium* inactivation kinetics. This optimal threshold value must be used for all of the experiments conducted with the specific microsphere batch.

Numerical research was also conducted to simulate *Cryptosporidium* sequential disinfection in a flow-through water treatment system. A commercially available numerical program called Femlab 3.0a and the Segregated Flow Reactor (SFR) method were used to model the microbial inactivation process. Overall ozone and chlorine inactivation achieved in the model were similar to the inactivation achieved experimentally. However, the models did not capture the fast initial inactivation observed by the microspheres when first exposed to chlorine. The models only showed a slightly higher initial followed by slower linear kinetics; whereas the experiments showed a fast initial inactivation followed by a tailing region.

The numerical results did demonstrate that inactivation with chlorine is enhanced when chlorine is used sequentially with primary ozone treatment and that the fluorescence decay of the YG-microsphere can be used to mimic *Cryptosporidium* inactivation under sequential disinfection conditions. This marks the first time sequential inactivation has been modeled using numerical representation of a flow-through system. However, more work is needed to understand how to incorporate varying amounts of primary ozone exposure in a flow-through system into the model so that the changing secondary disinfectant inactivation kinetics can be adapted numerically.

The primary disadvantage to using non-biological microspheres to mimic *Cryptosporidium* inactivation in a full-scale drinking water treatment facility is the high cost of microspheres. However, with additional research, the numerical models from this

research could be used to simulate sequential disinfection in a full-scale water treatment facility.

7. Future Work.

In this study, fluorescent microspheres were used to evaluate the efficiency of sequential disinfection using ozone as a primary disinfectant and free chlorine as a secondary disinfectant, in a flow-through system. The results from this research were compared to numerical models, generated using reference *Cryptosporidium* data, in order to characterize and optimize sequential disinfection processes in a full-scale drinking water treatment facility. However, in order to better understand disinfection processes in a flow-through system, additional work will need to be performed. Batch studies need to be conducted to characterize *Cryptosporidium* sequential disinfection with varying CT values for the primary disinfectant. Disinfection kinetic curves need to be determined for a variety of primary disinfectant concentrations and exposure times. The results from this work could be mimicked using fluorescent microspheres (see Baeza/Ducoste [1]). The range of CT values and disinfection kinetic curves could then be used to improve model performance. In addition to improving the disinfection kinetic response of the secondary disinfectant, a 3-D model should be created to properly represent the 3-D turbulent mixing that occurs within this flow-through system. The data obtained from these studies could be used to characterize and optimize sequential disinfection processes for a full-scale water treatment facility.

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- Microbiology and Immunology. College of Veterinary Medicine where the flow cytometer analyses were done.

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Table 1: Flow cytometer settings.

Detector/Amps				
Parameter	**Detector**	**Voltage**	**Amp.Gain**	**Mode**
P1	FSC	EO2	4.16	Log
P2	SSC	564	1.00	Linear
P3	FL1	999	9.99	Linear
Primary parameter: FSC - Value: 192, Secondary parameter: SSC - Value: 279				

Detector notation: FSC (forward light scatter, related to cell size), SSC (90° light scatter, related to the internal granularity or complexity of the particle), FL1 (Green-FITC fluorescence)

Table 2: Residence times for experiments and Femlab 3.0a model.

Lab Reactor Residence Times				
Flow (ml/sec)	Ozone Contactor 1 RT (sec)	Ozone Contactor 2 RT (sec)	Mixing Chamber RT (sec)	Chlorine Contactor RT (sec)
15.5	142	142	64	1880
11	204	204	98	2670
Femlab Residence Times				
Flow (ml/sec)	Ozone Contactor 1 RT (sec)	Ozone Contactor 2 RT (sec)	Mixing Chamber RT (sec)	Chlorine Contactor RT (sec)
15.5	122	122	59	1884
11	175	175	91	2702

Table 3: Femlab 3.0a flow field simulation and microbial inactivation conditions.

Boundary Conditions with Flow = 11.0 mL/sec			
Inflow	v: velocity (m/s)	Ko: turbulent kinetic energy (m^2/s^2)	Eo: Dissipation (m^2/s^3)
	0.006875	(0.02*v)^2	(0.1643/0.09)*Ko^(3/2)
Logarithmic wall function	layer thickness (m)		
	wall width/9000		
Outflow	Pressure (Pa)		
	0		
Boundary Conditions with Flow = 15.5 mL/sec			
Inflow	v: velocity (m/s)	Ko: turbulent kinetic energy (m^2/s^2)	Eo: Dissipation (m^2/s^3)
	0.0096875	(0.02*v)^2	(0.1643/0.09)*Ko^(3/2)
Logarithmic wall function	layer thickness (m)		
	wall width/9000		
Outflow	Pressure (Pa)		
	0		

Femlab 3.0a model conditions for high flow

	Average Velocity x 10^-3 (m/s)	Avg. Ozone concentration (mg/L)	Avg. Chlorine concentration (mg/L)
Ozone Contactor 1	5.46	0.35	0
Ozone Contactor 2	5.46	0.75	0
Chlorine Mixing Chamber	4.76	0	16.42
Baffled Contactor Location 1	4.27	0	16.39
Baffled Contactor Location 2	4.25	0	16.35
Baffled Contactor Location 3	4.25	0	16.31

Femlab 3.0a model conditions for low flow

	Average Velocity x 10^-3 (m/s)	Avg. Ozone concentration (mg/L)	Avg. Chlorine concentration (mg/L)
Ozone Contactor 1	3.75	0.20	0
Ozone Contactor 2	3.75	0.38	0
Chlorine Mixing Chamber	3.38	0	12.84
Baffled Contactor Location 1	3.08	0	12.82
Baffled Contactor Location 2	3.01	0	12.70
Baffled Contactor Location 3	3.00	0	12.59

Table 4: Kinetic constants (k) for *Cryptosporidium* single disinfectant and sequential disinfection. Reported by Driedger *et al.* [6].

For single disinfectant			
Using ozone: Shape: characterized by a very small lag phase and follows a Series-Event model.			
k (20ºC) [L/mg-min]	1.502	N1/No	-
Using free chlorine: Shape: characterized by an initial lag phase and follows a Chick-Watson model.			
k (20ºC) [L/mg-min]	0.00134	N1/No	1.49

Sequential disinfections: secondary free chlorine treatment
- Level of ozone pretreatment: a CT of 1.4 mg-min/L
- Shape: double slope, characterized by an initial faster decline and a secondary slower inactivation decay.

	First slope	**Second slope**
Factor	6	2
k (20ºC) [L/mg-min]	0.00804	0.00268

Table 5: Ozone primary disinfection tests and experimental conditions.

Pretreatment: 90 min at 0.29 ± 0.03 mg O3/L, pH 7.0 and 20 ± 2°C				
Experiment	Flow (ml/sec)	Ozone Concentration (mg/L)	Threshold Fitted	Final N/No
HF-1	15.5	0.75	80	0.150
HF-2	15.5	0.75	40	0.160
HF-3	15.5	0.74	40	0.194
LF-1	11.0	0.38	130	0.157
LF-2	11.0	0.39	110	0.145
LF-3	11.0	0.39	105	0.131

Table 6: Chlorine primary and secondary disinfection tests and experimental conditions.

Chlorine non-sequential disinfection						
Pretreatment: 90 min at 0.29 ± 0.03 mg O3/L, pH 7.0 and 20 ± 2°C						
Experiment	HF-1	HF-2	HF-3	LF-1	LF-2	LF-3
Flow (ml/sec)	15.5	15.5	15.5	11.0	11.0	11.0
Chlorine Concentration (mg/L)	16.2	16.8	17.4	12.8	12.2	13.1
Threshold Fitted	110	40	72	116	48	96
Initial N/No	1.000	1.000	1.000	1.000	1.000	1.000
N/No Location 1	0.765	0.738	0.761	0.799	0.795	0.785
N/No Location 2	0.594	0.533	0.600	0.483	0.583	0.515
N/No Location 3	0.335	0.282	0.306	0.211	0.248	0.237
Chlorine sequential disinfection						
Pretreatment: 90 min at 0.29 ± 0.03 mg O3/L, pH 7.0 and 20 ± 2°C						
Experiment	HF-1	HF-2	HF-3	LF-1	LF-2	LF-3
Flow (ml/sec)	15.5	15.5	15.5	11.0	11.0	11.0
Chlorine Concentration (mg/L)	15.8	16.4	17.1	12.8	12.2	13.1
Threshold Fitted	80	40	40	130	110	105
Initial N/No	1.000	1.000	1.000	1.000	1.000	1.000
N/No Location 1	0.463	0.406	0.247	0.316	0.320	0.320
N/No Location 2	0.224	0.241	0.212	0.232	0.227	0.217
N/No Location 3	0.209	0.134	0.164	0.081	0.119	0.072

Table 7: Inactivation observed in the numerical models compared to experimental research results.

	N/No (Total) for Low Flow Conditions			N/No (Total) for High Flow Conditions		
Sequential Disinfection	Femlab 3.0a	Experimental with 95 % CI in bold	SFR	Femlab 3.0a	Experimental with 95 % CI in bold	SFR
	1.000	1.000	1.000	1.000	1.000	1.000
Ozone 1 (SE Model)	0.808	-	0.717	0.703	-	0.639
Ozone 2 (SE Model)	0.176	0.144 **(.129-.159)**	0.178	0.104	0.168 **(.142-.194)**	0.116
Chlorine Mixer (CW)	-	-	0.150	-	-	0.100
Chlorine Location 1 (CW)	0.153	0.046 **(.042-.050)**	-	0.089	0.061 **(.048-.074)**	-
Chlorine Location 2 (CW)	0.064	0.033 **(.028-.037)**	-	0.048	0.038 **(.033-.042)**	-
Chlorine Location 3 (CW)	0.029	0.013 **(.009-.017)**	0.012	0.021	0.028 **(.020-.036)**	0.008

Table 8: Sequential and Non-sequential disinfection comparisons.

	N/No (Total) for Low Flow Conditions			N/No (Total) for High Flow Conditions		
	Femlab 3.0a	Experimental with 95 % CI in bold	SFR	Femlab 3.0a	Experimental with 95 % CI in bold	SFR
Chlorine Inactivation Non-sequential Disinfection (Chick-Watson)						
	1.000	1.000	1.000	1.000	1.000	1.000
Chlorine Mixer	-	-	0.972	-	-	0.976
Chlorine Location 1	0.980	0.793 **(.785-.801)**	-	0.976	0.755 **(.738-.771)**	-
Chlorine Location 2	0.850	0.527 **(.470-.585)**	-	0.849	0.576 **(.534-.618)**	-
Chlorine Location 3	0.730	0.232 **(.211-.254)**	0.491	0.740	0.308 **(.278-.338)**	0.515
Chlorine Inactivation Sequential Disinfection (Chick-Watson Sequential)						
	1.000	1.000	1.000	1.000	1.000	1.000
Chlorine Mixer	-	-	0.845	-	-	0.862
Chlorine Location 1	0.869	0.319 **(.316-.321)**	-	0.856	0.372 **(.245-.499)**	-
Chlorine Location 2	0.364	0.225 **(.217-.234)**	-	0.462	0.226 **(.209-.242)**	-
Chlorine Location 3	0.165	0.091 **(.062-.119)**	0.084	0.202	0.169 **(.126-.212)**	0.072

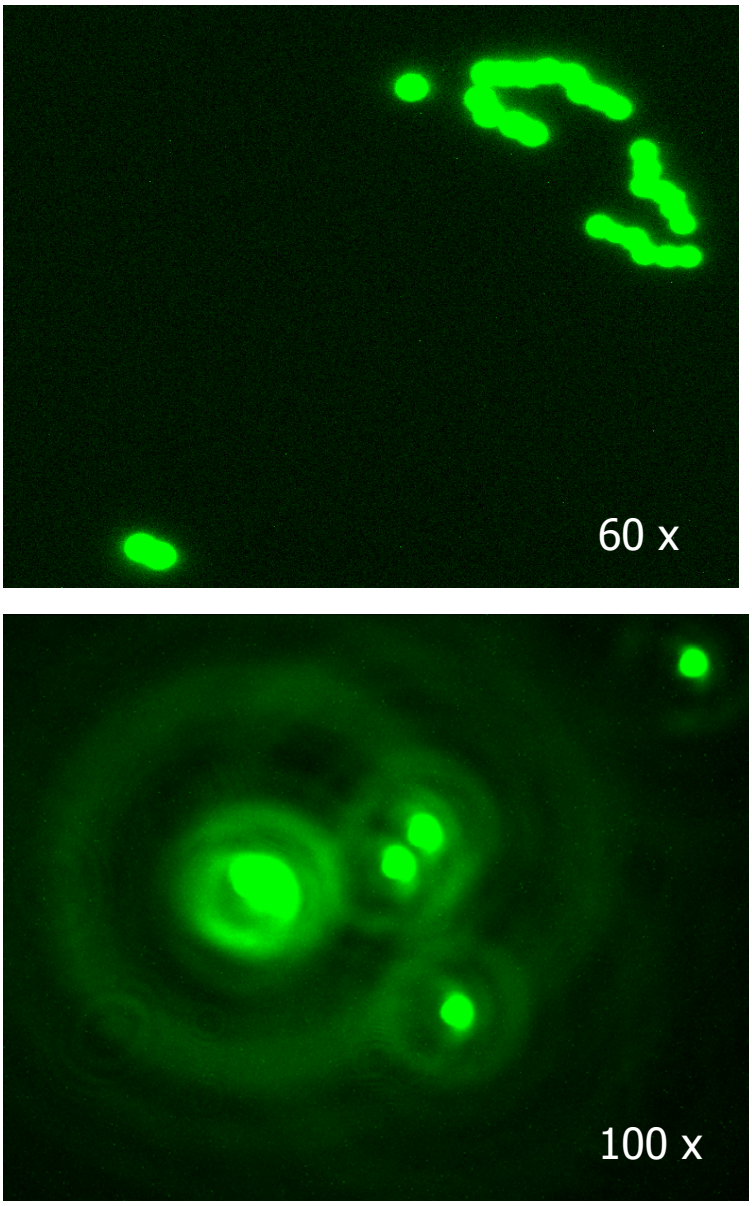


Figure 1: YG Fluoresbrite™ fluorescent microspheres of 0.94 μm diameter seen in an epifluorescence microscope.

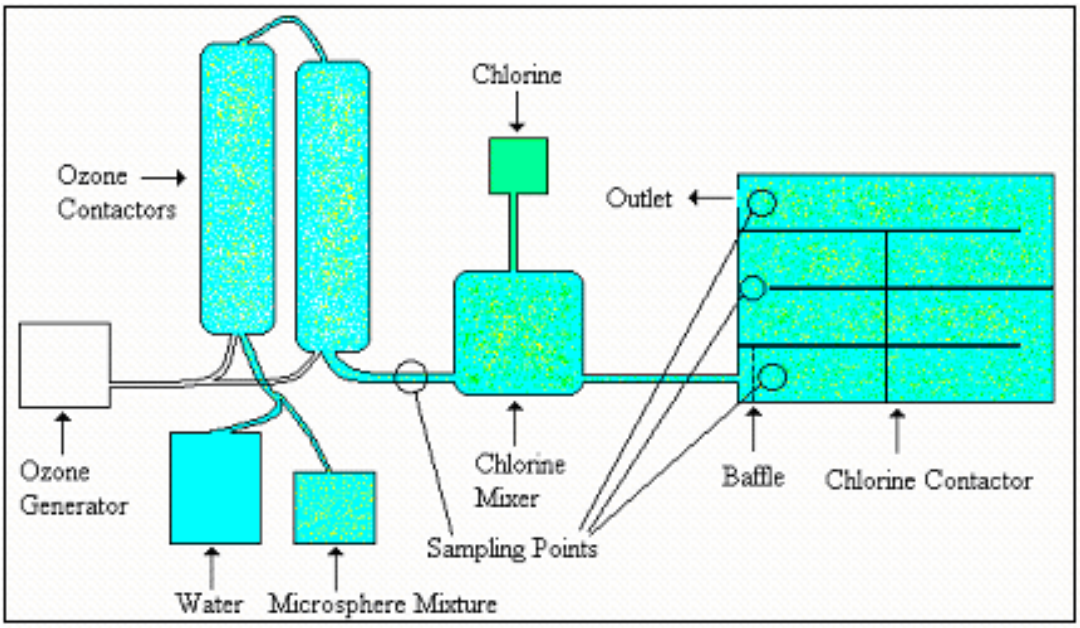
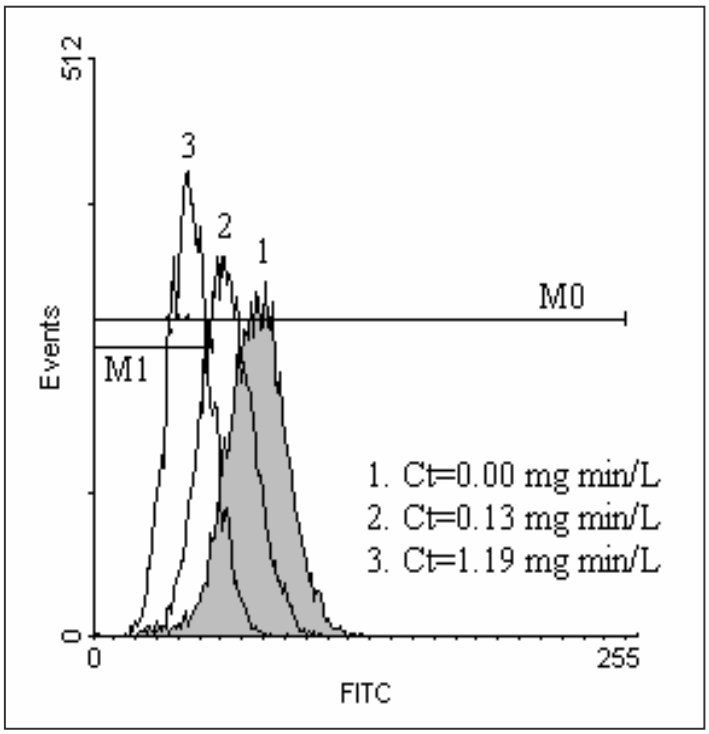


Figure 2: The flow-through system consists of an initial double ozone column contactor followed by a chlorine-mixing chamber and finally, a chlorine baffled contactor.

A)



B)

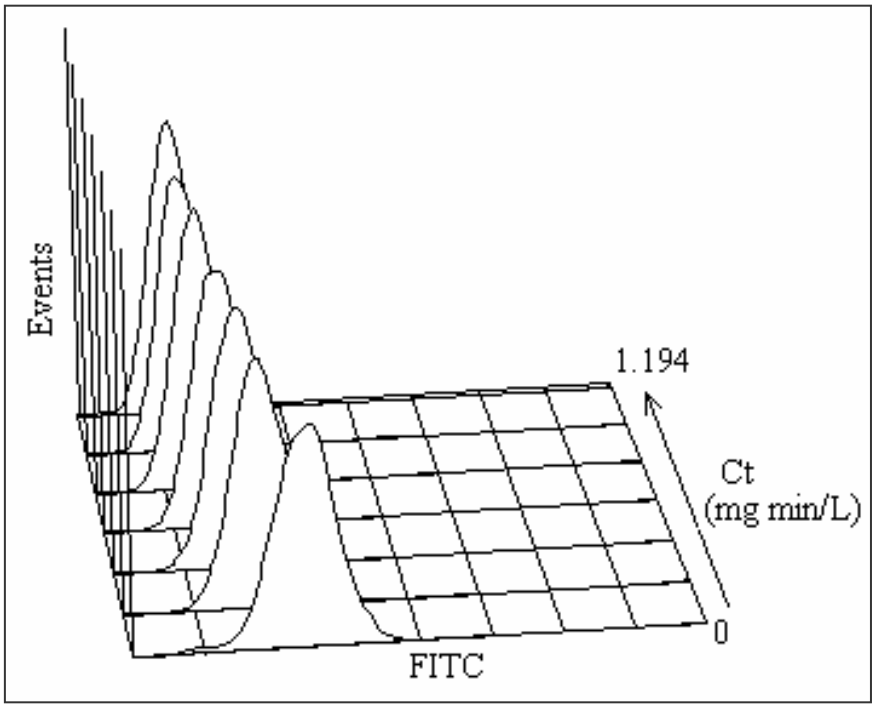


Figure 3: Fluorescence intensity histogram analysis. A) Figure descriptive of the N/No survival ratio calculation. B) Example of microsphere histograms for ozone treatment at pH 7 and 20 ± 2 ºC [1].

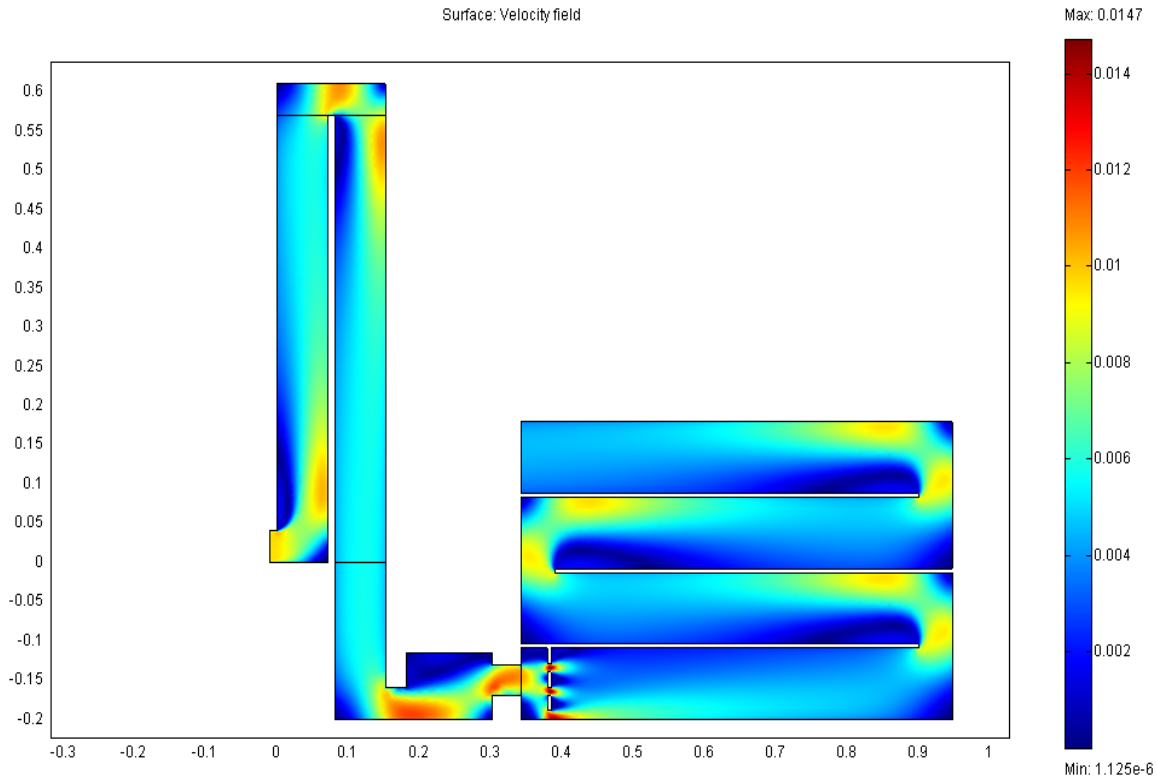


Figure 4: Flow field velocity contour predicted by Femlab 3.0a with an initial velocity of 0.0096875 m/s.

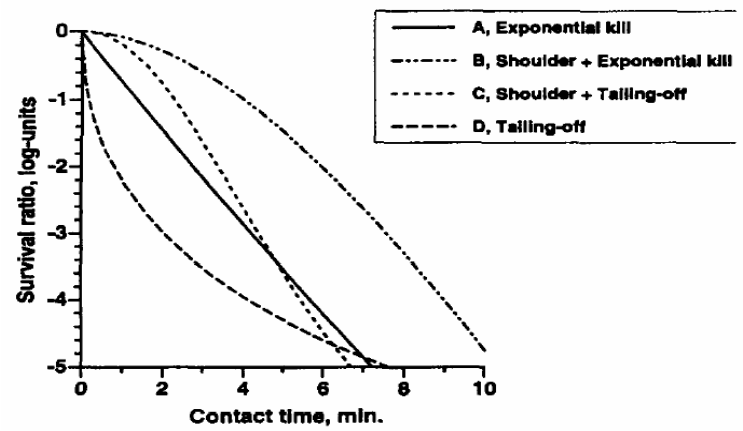


Figure 5: Common microbial survival curves for different microorganisms [11].

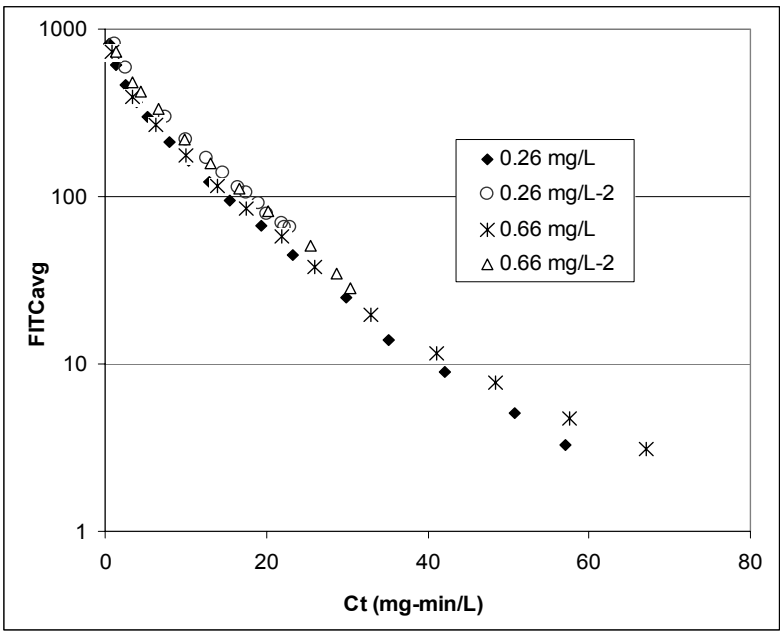


Figure 6: Ozone pretreatment of microspheres at pH 7 and 20 ± 2 Cº [1].

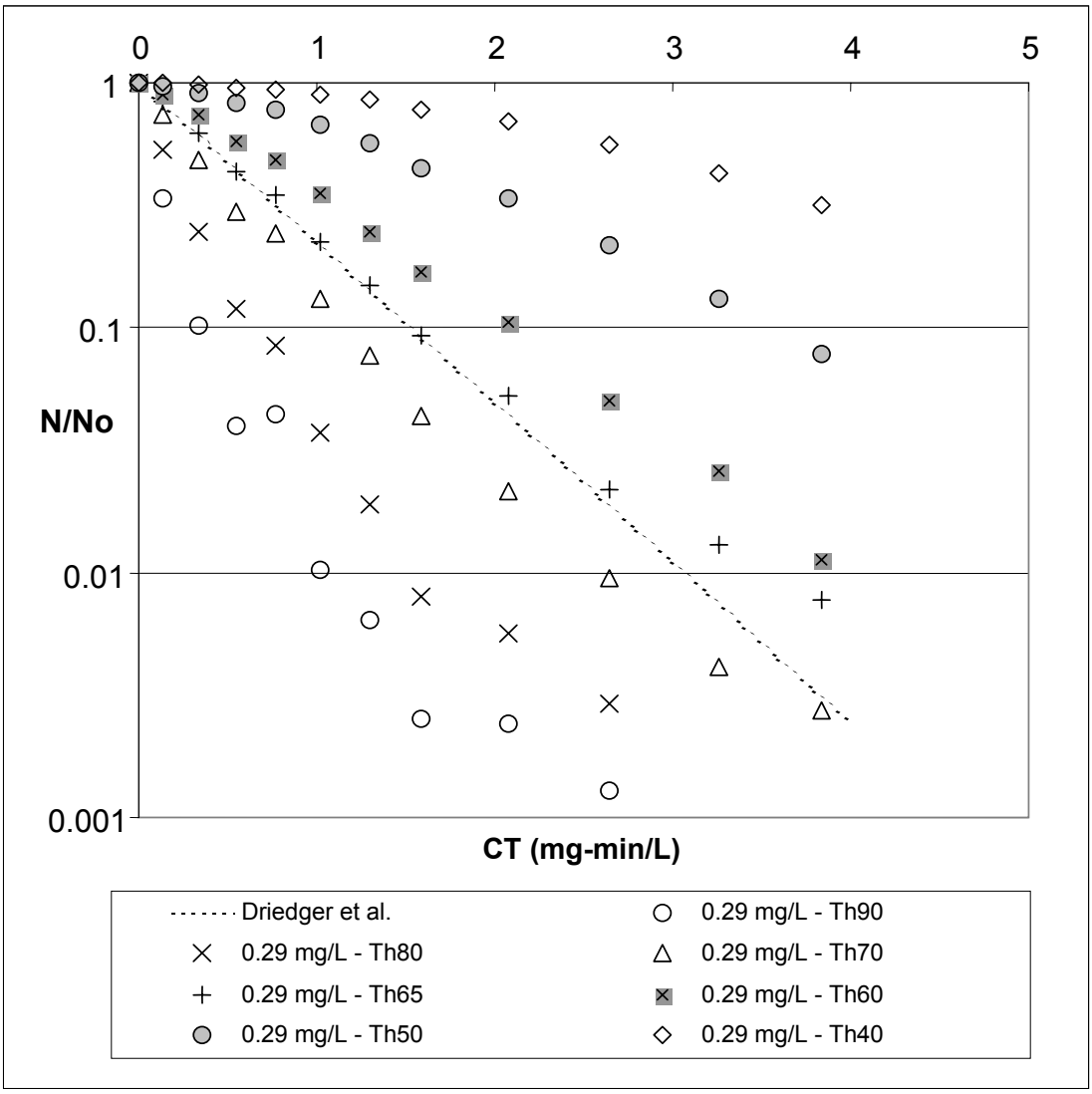


Figure 7: Microspheres inactivation with ozone at pH 7, 20 ± 2 Cº, and for a duration of 90 min. Inactivation ratio curve variability depending on the fluorescence intensity threshold selected. Reference inactivation curve based on Driedger *et al.* [6] (Table 3) [1].