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Effectiveness And Kinetics Of Ferrate As A Disinfectant For Ballast Water

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**EFFECTIVENESS AND KINETICS OF FERRATE AS A
DISINFECTANT FOR BALLAST WATER**

by

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B.S. Bates College, 1985

A thesis submitted in partial fulfillment of the requirements
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in the College of Engineering and Computer Science
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ABSTRACT

The introduction of aquatic nuisance species (ANS) and bacterial pathogens from discharge of ballast water by sea-faring vessels is an ongoing problem that threatens ecosystems and human health. This study investigates the disinfecting capability of ferrate in a marine environment on several organisms listed in international standards for ballast water management. Organisms were grown in a saline solution and were treated with dosages of ferrate ranging from 0.25-5.0 mg/l. A ferrate dose of 5 mg/l resulted in almost complete kill for all organisms tested. Smaller dosages have also been very effective, particularly if all organic material from the nutrient broth used to cultivate them is removed by washing the cells with saline solution. Ferrate appears to act very quickly, with tailing occurring after about 5-15 minutes. Analyses of the data with the CT approach, the Chick-Watson and Hom's models, and an oxidant demand equation derived from equations recommended by the Water Environment Federation (WEF) are in good agreement that ferrate concentration is more important than contact time. The Hom's model appeared to most accurately represent the action of ferrate on these organisms. Salinity and pH did not adversely affect results, and regrowth was not a problem. Two measures to reduce clumping did not eliminate the observed tailing effect, suggesting a different mechanism for this phenomena. These preliminary tests indicate that ferrate could be a very effective disinfectant in the treatment of ballast water, and the short half-life of ferrate is an advantage, since the ballast water is subsequently released into the environment.

This thesis is dedicated to Jerry Lunde for his endless support,
and to all my “best friends”: Cypress, Sherman, Medley, Murphy,
Kaboom!, Flannel, Turbo, and Chaos

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LIST OF ACRONYMS

ANOVA	Analysis of Variance
ANS	Aquatic nuisance species
AOC	Assimilable organic content
ATP	Adenosine triphosphate
awcg	Area with confluent growth
AWWA	American Water Works Association
β	The number of disinfectant-binding sites on an organism
b	WEF experimental regression coefficient
BRP	Bacterial regrowth potential
C_0	Initial concentration of disinfectant
CFU	Colony-forming unit
C_t	Concentration of disinfectant at time t
CT	Contact-time product
d	WEF experimental regression coefficient
DBP	Disinfection byproduct
DOC	Dissolved organic content
EPA	Environmental Protection Agency
FAU	Formazin attenuation unit
HNLC	High-nutrient, low-chlorophyll
IMO	International Maritime Organization
IO	Instant Ocean
k, k_i , and k_2	Experimental rate constants
k'	Chick-Watson die-off constant
k^*	Rate constant
K_d	Michaelis constant
m	Coefficient of importance for time
MEPC	Maritime Environment Protection Committee
MPN	Most probable number
η	An efficiency factor
n	Chick-Watson coefficient of dilution
nd	Not done
NB	Nutrient broth
N_0	Initial bacterial concentration
N_t	Bacterial concentration at time t
NTU	Nephelometric turbidity unit
T	An experimental constant
THM	Trihalomethanes
TOC	Total organic content
UV	Ultraviolet
WEF	Water Environment Federation
WS	Working solution
x	An empirical constant

CHAPTER 1: GENERAL INTRODUCTION

Aquatic invasive species (AIS) are non-native organisms that have been introduced into a body of water. If the organism has a detrimental impact on that water body, then it is also considered an aquatic nuisance species (ANS). Detrimental effects can include loss of biodiversity, reduction in numbers of native species, and disruption of the ecological balance, as well as impacts on commercial, agricultural, recreational, and municipal uses of the water body. Human health can also be impacted, particularly in the case of bacterial, fungal, and viral pathogens.

Ships have long been recognized as a source of ANS. In fact, anti-fouling paint was introduced years ago, in part to combat this problem, making ballast water the primary vector for ANS, including bacterial pathogens (US Environmental Protection Agency, USEPA, 2001). *Vibrio* epidemics in S. America, for example, have been traced to ballast water, and Latin American strains of *V. cholerae*, which lack hemolysin, have been identified in ballast water of cargo ships docked in the American Gulf Coast. The current method of disinfection of ballast water is mid-ocean ballast exchange, which is relatively ineffective (USEPA, 2001). International agencies, in particular the International Maritime Organization (IMO), are encouraging the development of new processes for ballast water treatment, and have established standards for testing of these processes. New techniques and equipment for the preparation of ferrate make it a promising, cost-effective, environmentally-friendly alternative to mid-ocean exchange and other disinfection processes. Ferrate is iron with a valence of +6, making it an extremely powerful oxidant. As a result it inactivates and kills organisms. A company located in Orlando, Florida, Ferrate Treatment Technologies, LLC (“FTT”) has developed

instrumentation to produce ferrate onboard vessels for immediate use as a ballast water disinfectant. Therefore, one objective of this study was to determine whether ferrate could meet the international standards for successful ballast water treatment, including final concentrations of less than 1 CFU/ml of Enterococci, less than 2.5 CFUs/ml of *Escherichia coli*, less than 1 CFU/100 mls of *Vibrio cholerae*, and an unspecified but measured endpoint for coliforms. To this end, pure cultures of *Escherichia coli*, *Klebsiella pneumoniae*, and *V. cholerae*, and a mixed culture of *Enterococcus faecium* and *E. faecilis*, were grown in saline solution to simulate ballast water, and were treated with dosages of ferrate ranging from 0.25-5.0 mg/l.

Different models are used to describe disinfection kinetics. The CT approach, used commonly in drinking water regulations (AWWA, 1999), assumes that equal products of concentrations (C) and contact times (T) will result in equivalent log reductions. The Chick-Watson law relates the survival rate of the target organism to the concentration of disinfectant and a die-off constant (Metcalf & Eddy, 2003). Because of deviations from this law, such as tailing or shoulders caused by media interactions, disinfectant decay, and other factors, this law or equation has been modified by Hom to also consider the effect of time on the rate of disinfection (Hom, 1972). Another approach based on equations recommended by the Water Environment Federation (WEF, 1996) assumes a linear relationship between dosage-response and concentration after initial oxidant demand, for a given time. These approaches to disinfection modeling were applied to a series of dosage-response curves of ferrate acting upon the test organisms in a saline environment, to establish which model best predicted the action of ferrate on these organism. The literature review in Chapter 2 of this thesis delves further into the topic of disinfection modeling, as well as the subjects of ballast water, regrowth, and microbial iron metabolism.

Many variables could affect disinfection on-board, such as pH, salinity, initial bacterial concentration, temperature, and organic content. The first three of these variables were tested for their effect on ferrate disinfection, and the possibility of regrowth was also explored. Results of this testing and of the disinfection and disinfection modeling are presented in Chapter 3, as a paper to be submitted to Water Environment Research. A dose of 5 mg/l resulted in complete disinfection of all organisms tested, and smaller dosages were also very effective. Tailing was consistently observed, and the Hom's model had the highest correlation coefficients.

This leads to the conclusion, discussed further in Chapter 4, that disinfection with ferrate can meet international standards, that the Hom's model can be used to estimate log removals under conditions similar to experimental conditions, and that further testing is warranted. Practical considerations are also discussed, and lead to some of the recommendations for future study outlined in Chapter 5.

CHAPTER 2: LITERATURE REVIEW

2.1 Ballast Water

Ballast water provides stability to ocean-going vessels, and its uptake and discharge allows ships to compensate for cargo loads. Because ballast water is a sample of one ecosystem being transplanted into another, it represents a great potential for biological invasion. American waters receive more than 79 million tonnes of foreign ballast water per year (Carlton et al, 1995), and the number of species carried in these waters is vast. All major marine trophic groups were found in ballast water from Japanese cargo ships in an Oregonian port, such that ballast water acts as a phylogenetically and ecologically nonselective transport vector (Carlton and Geller, 1993). Many recent biological invasions have been attributed to ballast water release, the most famous of which is *Dreissena polymorpha*, the zebra mussel, which was most likely introduced via release of larvae contained in ship ballast water into the Great Lakes in the late 1980s (Hebert et al., 1989). The zebra mussel continues to expand its range, and its tendency to form dense mats has led to pipe fouling at power plants and other facilities (Kovalak et al, 1993).

Bacteria are also among the aquatic nuisance species (ANS) introduced by ballast water discharge. One study found *Vibrio cholerae* 01 in ballast water from five of 19 cargo ships sampled in Alabama and Mississippi (McCarthy and Khambaty, 1994), causing the Food and Drug Administration and US Coast Guard to recommend that ship crews exchange their ballast water in mid-ocean, prior to entry into US ports. Viable counts of the *V. cholerae* were on the order of 10^6 /ml, in salinities ranging from 12 to 32 ppt, again

emphasizing the potential environmental impacts of ballast water. However, open-ocean exchange proved inadequate, and even fresh-water flushing leaves many viable organisms (Hulsmann and Galil, 2001). Because of the growing urgency of this problem, voluntary international guidelines for ballast water management have been established by the Maritime Environment Protection Committee (MEPC) of the International Maritime Organization (IMO) (Raaymakers, 2001), and performance specifications have been set for the successful testing and approval of ballast water management systems.

2.2 Ferrate as a Disinfectant

Ferrate (FeO_4^-) is iron with a valence of +6, which confers upon it a strong tendency to accept electrons, to attain its preferred valence of +2 or +3. Therefore, it acts as an oxidant, like many disinfectants, with a reduction potential of +2.20 V in acidic solution and +0.72 V in alkaline solution (Wood, 1957). This reduction potential makes it a more powerful oxidant in acid than hypochlorite, ozone, hydrogen peroxide, or permanganate (Lee et al, 2004). By oxidizing molecules, it inactivates enzymes, resulting in cell death (Metcalf & Eddy, 2003). Because of its steric resemblance to the phosphate anion, ferrate binds to enzyme phosphoryl groups and therefore site-specifically inactivates phosphatases, dehydrogenases, phosphorylase b, phosphoglucomutase, and other proteins with this functional group. It also specifically oxidizes amino acid residues on muscle phosphorylase, pancreatic ribonuclease, triose phosphate isomerase, and E. coli DNA polymerase-I (Basu et al, 1987). Because the byproduct of ferrate is Fe^{+3} , it can also be

used as a coagulant (Jiang et al, 2002), and for other uses, but its cost of production has hindered its usage.

Disinfection commonly has two goals: the immediate destruction or inactivation of pathogens and persistent inactivation of pathogens through residual action (Xie, 2004). An ideal disinfectant should be toxic to microorganisms, yet non-toxic to higher forms of life. Treatment of ballast water, and other applications to prevent the spread of invasive species, has slightly different goals, since the water is typically treated and then released into the local environment. Because the local species are quite similar to the alien species, the disinfectant should not persist in the environment, to avoid harming the local biota. Also, some higher organisms, such as crustaceans, are considered invasive, so an ideal disinfectant for ballast water would have a broader spectrum of action. Many oxidants have the disadvantage of producing disinfection byproducts (DBPs). For example, trihalomethanes (THMs) are a common DBP of chlorination, and their concentration in drinking water is regulated because of health concerns (Metcalf & Eddy, 2003), although the validity of those concerns is debatable (White, 1999). Ferrate has the advantage that its breakdown product is some form of iron, which is non-toxic to humans except in very large doses. However, other environmental impacts must be considered. For example, to avoid bacterial, algal, and phytoplankton blooms, release of ferrate-treated water should be avoided in the few oceanic areas in which iron appears to be the limiting nutrient for bacterial growth: equatorial Pacific and other high-nutrient, low-chlorophyll (HNLC) areas, upwelling regions, the Southern Ocean, and the sub-Arctic Pacific (Kirchman, 2000). Moreover, even if the breakdown product of the oxidant is not in itself toxic, they can react with constituents in the water to produce environmentally harmful products. THMs, for

example, are formed by the reaction of natural organic matter with chlorine or other oxidants (Metcalf & Eddy, 2003). The presence of natural organic matter also reduces the effectiveness of oxidants. In the case of chlorination, this produces a stepwise phenomena referred to as breakpoint chlorination chemistry, in which the chlorine first reacts with readily oxidizable organic and inorganic material, then with ammonia to form chloramines, and then with less readily oxidizable molecules, including, presumably, the target species, producing a free chlorine residual when oxidizable matter is consumed (Matheickal et al, 2004). Thus, one would expect the action of ferrate to vary with respect to organic content, in terms of both effectiveness and possible DBP formation.

Three general methods of synthesizing ferrate have been used: wet oxidation, dry oxidation, and electrolysis (Lee et al, 2004). Electrolysis involves the anodization of a pure iron electrode in concentrated alkaline solution. The reaction at the cathode is:

In dry oxidation, iron oxides are treated with oxidants at high temperature and pressure to generate ferrate salts, as in the following reaction:

Dry oxidation has the advantage of a one-step, high-yield process (Perfiliev and Sharma, 2004), and shows potential as a green technology to recycle iron oxide wastes from steel manufacturing processes. Wet oxidation shows good potential for small-scale ferrate production, but the requirement for relatively pure chemicals leads to relatively high costs (Lee et al, 2004). Typically, ferric ion is oxidized to ferrate by concentrated

hypochlorite in strong basic solution, after which it is precipitated as potassium ferrate by the addition of excess potassium hydroxide, according to the following reactions:

Ferrate Treatment Technologies has developed a novel, patented, wet oxidation method of ferrate synthesis, using relatively inexpensive chemicals, that can be conducted with equipment onboard a vessel, for on-site ballast treatment, using the following reaction:

Ferrate salts decompose to non-toxic forms of iron in aqueous solution, such as ferric hydroxide, which precipitates according to the following equation (Jiang and Lloyd, 2002):

Ferrate can also decompose to ferric oxyhydroxides, according to the following equation (Schink and Waite, 1980):

The rate of decomposition depends upon many factors, including pH, temperature, and initial ferrate concentration. According to a Pourbaix diagram, ferrate is most stable in an alkaline, highly aerobic environment. It will convert to aqueous ferric and ferrous iron in acidic, aerobic environments, and to non-aqueous $\text{Fe}(\text{OH})_3$ and $\text{Fe}(\text{OH})_2$ precipitates in

alkaline, aerobic-to-somewhat anoxic conditions, and to solid Fe in highly anoxic (i.e. reducing or low oxidation-reduction potential) conditions, across the entire pH range. More dilute solutions result in a decreased driving force towards precipitation (Western Oregon Univ., 2006). Seawater is typically slightly alkaline, so depending on the oxygen levels, decomposing ferrate would be expected to precipitate as a hydroxide or as solid Fe, as long as the pH does not become acidic. Precipitation of ferrate might be a useful method to remove it from treated ballast water, prior to discharge, in order to meet effluent iron standards. These vary widely by state, country, and water classification. Within Florida waters, the maximum iron standard ranges from 0.3-1.0 mg/l (Florida Department of Environmental Protection, 2006).

2.3 Disinfection Modeling

The effectiveness of ferrate must be compared with that of free chlorine and chloramines, as well as other disinfectants, using similar techniques and models. The CT approach, used commonly in drinking water regulations (American Water Works Assn., 1999), looks at combinations of concentrations and contact times. It assumes a linear relationship between contact time and disinfection. This is rarely observed, however. Often, there are tailing and shoulder or lag effects, resulting from disinfectant, environmental, and bacterial characteristics, and numerous modelers have sought to explain these relationships, and to predict disinfection rates from them. The Chick-Watson law relates the survival rate of the target organism to the concentration of disinfectant and a die-off constant (Metcalf and Eddy, 2003). It is expressed as:

$$dN_t/dt = -k' C^n N_t \quad (8)$$

where N_t is the number of organisms at time t , k' is the die-off constant, C is the concentration of disinfectant, and n is a coefficient of dilution which determines the relative importance of disinfectant concentration. The integrated form of this equation is:

$$\ln(N_t/N_0) = -k' C^n t \quad (9)$$

where N_0 is the starting bacterial concentration. When n is greater than 1, concentration is more important than time, and vice versa. Deviations from this law, such as tailing or shoulders caused by media interactions, disinfectant decay, and other factors, result in a non-linear graph of time versus survival (N_t/N_0). A log-log plot can still result in a linear graph for these deviations, provided $C^n t$ is a constant (American Water Works Assn., 1999). In order to better describe deviations from Chick-Watson behavior, Equation 7 was modified by Hom to also consider the non-linear effect of time (Hom, 1972). The integrated form of Hom's equation is:

$$\ln(N_t/N_0) = -k' C^n t^m \quad (10)$$

where m is a coefficient of importance for time. The rate of inactivation decreases with time when m is less than 1. Adding the m coefficient permits further characterization of the effect of time on disinfection efficiency, but might also lead to over-parameterization.

The Hom's equation has been further modified to consider disinfectant decay/demand (Haas and Joffe, 1994). If the decay/demand is first order, then:

$$C_t = C_0 e^{(-k^*t)} \quad (11)$$

where C_0 is initial disinfectant concentration, C_t is the concentration at time t , and k^* is a rate constant. First-order decay appears to be a close approximation for chlorine and ozone decomposition. Applying this to the Hom's equation results in the following:

$$\ln(N_t/N_0) = -k^* C_0^n t^m \eta \quad (12)$$

where η is an efficiency factor to correct for disinfectant loss. The solution requires the incomplete γ function and is therefore difficult to solve by standard computer packages. A simplifying assumption that disinfectant demand is minor results in the following approximation:

$$\eta = [\{1 - e^{(-\psi/m)}\} / (\psi/m)]^m \quad (13)$$

where $\psi = n^*k^*t$. This can then be substituted into Eqn. 12.

A general differential rate law of disinfection states that the rate of inactivation, dN/dt , relates to its components as:

$$dN/dt = -kmN^x C^n t^{m-1} \quad (14)$$

where k is an experimental reaction rate constant, x is an empirical constant, and other variables are as previously defined (Gyurek and Finch, 1998). This equation reduces to the Chick-Watson equation when $m = 1$ and $x = 1$, and to the Hom's equation when $x = 1$.

Furthermore, if first-order rate kinetics are assumed, then:

$$dN/dt = -kmN^xC_0^n e^{-k't} t^{m-1} \quad (15)$$

In this equation, k' is the rate of first-order disinfectant decay. Integrating this, with $m = 1$, results in the Rational model, calculated by Haas et al (1994) as:

$$\log (N/N_0) = - \log [1 + (x-1)*(k/(k'n))*(C_0^n - C_f^n)* N_0^{x-1}] / (x - 1) \quad (16)$$

Yet another variation on the Chick-Watson model includes a lag phase, in which limited inactivation occurs, followed by a first order (i.e. $n = 1$) Chick-Watson equation (Rennecker et al, 2001). The lag phase accounts for observed shoulder effects in inactivation of protozoan cysts. This is similar to the Collins-Selleck model, which also has a biphasic model:

$$\text{For } C_t \leq T, N/N_0 = 1 \quad (17)$$

$$\text{For } C_t \geq T, N/N_0 = (T/(C_t))^n \quad (18)$$

T and n are experimental constants that can be calculated from a linear adjustment of the logarithmic form of Equation 17 (Qualls and Johnson, 1985). This model adjusts for an initial reduction of disinfectant upon contact with the water or medium. Hassen et al (2000) found it necessary to add another experimental constant, m , such that Equation 17 becomes:

$$\text{For } C_t \geq T, N/N_0 = (T/(C_t^m))^n \quad (19)$$

in order for this model to adequately describe inactivation of indicator bacteria (fecal coliforms and fecal streptococci) by chlorine.

The original Selleck model derived from an empirical model to explain the rate of inactivation of coliform bacteria by chlorine. Its linear form is:

$$\log (N/N_0) = -n \log [1 + (C_0/kk')*(1-e^{-k't})] \quad (20)$$

Again, n and k are determined experimentally (Selleck et al, 1978), and it is used primarily to describe initial shoulder effects, followed by a reduction in disinfection (Gyurek and Finch, 1998).

Another model for microbial inactivation by chemical disinfection is analogous to the model for microbial growth, and is therefore referred to as the Monod model (Haas, 1981). It states that:

$$\ln(N_t/N_0) = [(-k_2\beta C)/(C + K_d)]*[t + \{(e^{-k_1 t(C + K_d)} - 1)/(k(C + K_d))\}] \quad (21)$$

In this equation, β is the number of disinfectant-binding sites on the organism, k , k_1 , and k_2 are constants, and C is the liquid concentration of disinfectant, which is distinguished from the concentration of disinfectant at the microbial surface. Also, since it is analogous to Michaelis-Menten enzyme kinetics, K_d is a Michaelis constant, i.e. $K_d = k_{-1}/k_1$. A concentration-dependent lag phase is implicit in this model, and the rate of disinfection is defined as the slope of the linear portion of a plot of $\ln(N_t/N_0)$ vs. t .

An equation recommended by the Water Environment Federation Wastewater Disinfection Manual of Practice (WEF, 1996) has been adapted to describe inactivation of a dinoflagellate alga in ballast water by ozone (Oemcke and van Leeuwen, 2005), in the form:

$$-\log(N/N_0) = b(\log C) + d \quad (22)$$

where C is the oxidant concentration after initial demand has been met, b and d are experimental regression coefficients, and other terms are as described. A steeper slope (i.e. higher b) indicates a higher kill achieved with relatively low doses, and a higher x-intercept indicates a relatively high initial oxidant demand. This is not a rate equation, but a statement of survival as a function of dose, so the coefficients must be determined for each experimental contact time. This equation is referred to as the “oxidant demand” model.

Both the Chick-Watson and Hom’s equations assume that the concentration of disinfectant is constant for the test period or for a given contact time. This is not necessarily valid for some disinfectants, such as free chlorine (Haas, 1979). Assuming that disinfectant concentration follows first-order decay requires the incomplete γ function for an exact solution of the Hom’s model (Haas and Joffe, 1994). However, many studies have found good correlation coefficients using Chick-Watson or Hom’s models for disinfection of various bacteria by free chlorine, implying that the assumptions of these models are valid over the contact times studied (Haas and Karra, 1984), probably because coefficients for time adjust for the loss of disinfectant.

2.4 Mechanisms for Disinfection Kinetics Deviations

As mentioned, a perfectly linear relationship between contact time and disinfection levels is rarely observed, and advances in disinfection modeling have addressed tailing and shoulder effects. A shoulder effect refers to a lag phase in the survival vs. time graph, and

often occurs in organisms that form clumps, such as *Naegleria gruberii* (AWWA, 1999). This is because a colony or plaque-forming unit is not inactivated until all of its member cells are inactivated, causing an initial lag phase. Clumping can also cause tailing, as can several other mechanisms, including:

- Characteristics of the bacterial population, including resistant subpopulations, clumping, or hardening.
- Characteristics of the media, such as shielding by large particles.
- Nature of the disinfectant, such as decay.

Obviously, different classes and species of microorganisms have differing susceptibilities to various disinfectants and disinfection processes. The United States EPA has identified *Mycobacterium avium* as the most disinfectant-resistant waterborne bacteria on its Contaminate Candidate List (Gerba et al, 2003). However, other bacteria are capable of developing resistant subpopulations. For example, a chlorine-resistant subpopulation of fecal coliforms has been identified in the oxidation towers of a Virginia wastewater treatment plant that was experiencing erratic disinfection rates (Scully et al, 1999). The natural variability of a bacterial population favors the development of a resistant subpopulation under adverse conditions.

Hardening is conversion to resistant forms during disinfection. It was initially observed in poliovirus in response to formaldehyde inactivation during preparation of the Salk vaccine (Nathanson and Langmuir, 1963). Bacteria and other microorganisms possess many mechanisms for responding to adverse environmental, including, among some

bacterial species, the formation of spores, a dehydrated life-stage that is resistant to chemical and UV disinfection, as well as pH, temperature, and water moisture conditions.

Shielding can be a major problem in wastewater, which of course has a great deal of particulate matter, and one study found that a critical particle size was necessary to shield coliform bacteria from UV disinfection in secondary wastewater (Emerick et al, 2000).

Shielding is a potential product in any water that contains particles, which includes almost all salt and fresh waters.

Obviously, the problem of disinfectant decay in drinking water has been addressed by the inclusion of a chlorine, or other disinfectant, residual. However, chemicals tend to interact with other chemicals, with organisms, and with the environment, so decay or displacement has been addressed by numerous models. Analysis of a drinking water system using a network water quality model that included disinfectant kinetics and decay lead to a prediction of unacceptable risks from pathogenic bacteria intrusion, even with a detectable chloramine residual (Propato and Uber, 2004). Sohn et al (2004) compared and improved various models of disinfectant decay, and found good predictions for chlorine decay by both empirical power function models and empirical kinetic models. These models are generally very complex. Although the Hom's model assumes constant disinfectant concentration, it can also be used with a geometric mean residual (C_{ave}), if the initial and final disinfectant concentrations (C_0 and C_f) are known, resulting in the equation (Finch et al, 1993):

$$\log(N_t/N_0) = -k(C_{ave})^n t^m \quad (23)$$

Likewise, different models have been used to address different mechanisms of deviation from ideal disinfection behavior. Equation 12, for example, has been used to address the general problem of tailing, but it is difficult to use and highly empirical (AWWA, 1999). If disinfectant concentration is constant, it reduces to Equation 8, which is easier to use. Shoulder effects have been described by a number of models, including a series event, a multitarget, or a diffusional model. Models also try to account for other phenomena that affect disinfection rates by influencing natural die-off rates, such as temperature, salinity, and sunlight. These generally affect the rate constant, k or k' . The Arrhenius equation, for example, predicts a change in the die-off rate constant according to the relationship:

$$k'_T = k'_{20} B^{(T-20)} \quad (24)$$

where T is the temperature of interest in Celsius, k'_{20} is the rate constant at 20° C, and B is an empirical constant related to activation energy and the universal gas content (WEF, 1996), represented as θ in other texts. Equation 23 is only valid for temperatures that are not lethal to bacteria.

2.5 Regrowth

Regrowth measures the ability of disinfectant-damaged microbes to recover through repair mechanisms and reproduce, which is a major issue for drinking water, since even small numbers of pathogens can cause significant harm to the public. It is most often a problem following UV irradiation (WEF, 1996). It is distinguished from aftergrowth,

which is the growth of native microbes within the water distribution system (Escobar et al, 2001). Regrowth is dependent upon the amount of nutrients and organic matter in the medium, which is referred to as bacterial regrowth potential (BRP). Therefore, it correlates with dissolved organic content (DOC) and total dissolved nitrogen (Bolster et al, 2005), total organic content (TOC) (Rizzo, et al, 2004), and assimilable organic content (AOC) (Escobar et al, 2001), a readily usable form of biodegradable organic carbon. Furthermore, the source of the organic matter affects BRP. Freshly derived terrestrial dissolved organic matter (DOM), for example, correlated with a higher BRP than did reservoir water DOM (Page et al, 2002). The authors postulated that this is due to microbial pre-degradation of the reservoir-derived DOM, such that it is a less desirable substrate.

Corrosion products from pipes and other materials can indirectly increase regrowth rates by creating an oxidant demand and therefore reducing the level of disinfection. Likewise, nutrients such as humic acid can also exert an oxidant demand, and therefore increase re-growth rates by serving as food for bacteria and by lowering disinfectant efficiency. Thus, disinfectant levels, corrosion products, and nutrient levels appear to exert a complex interaction upon the growth of *Mycobacterium avium* (Norton et al, 2004). A model to predict the regrowth and aftergrowth potential of drinking water, based on its nutrient potential, has been developed by Jegatheesan et al (2004). It incorporates Monod-type bacterial growth, bacterial death and lysis, and measurements of BDOC fractions to predict the growth potential of indigenous bacteria based on nutritional status of the water. Regrowth can affect the kinetics of disinfection, but generally has a minor impact (WEF, 1996).

2.6 Microbial Utilization of Iron

Iron metabolism in bacteria will affect their susceptibility to ferrate, as well as their use of its reduced forms. Iron is required in a myriad of metabolic processes, by both eukaryotes and prokaryotes. It is, for example, a cofactor for many enzymes, and a major role of iron in mammals and other eukaryotes is as a component of heme, used to transport oxygen. In an aerobic, pH-neutral environment, ferric iron (Fe^{+3}) has a concentration of less than 10^{-18}M , because iron hydroxide, $\text{Fe}(\text{OH})_3$, is insoluble under these conditions (Raymond and Dertz, 2004). Hence, organisms have evolved mechanisms for recognizing, transporting, and storing iron, which results in an even lower concentration of free iron within their circulatory systems. Pathogenic bacteria compete for iron binding with their host by two main strategies. One is by essentially stealing iron from host proteins by binding heme to outer membrane receptors and transporting it into the cell by ABC (ATP-binding cassette) permeases. This strategy can involve hemophores, which bind heme initially and then bind to specific outer membrane receptors (Debarbieux and Wandersman, 2004). The second strategy is the production of siderophores, which are chelating agents that specifically and strongly bind iron and deliver it to bacterial cell surface receptors, which transport it by permeases into the cell. Bacteria exposed to blood produce siderophores, emphasizing the importance of competition with host proteins for iron, and the production of siderophores by bacteria is one aspect of virulence, because the loss of iron to the pathogen inhibits or hinders the host's defenses. There are three broad groups of siderophores, classified by the chelating group: catecholates, hydroxamates, and hydroxycarboxylates. Catecholates, for example, contain catechol, which has two

hydroxyls attached to an aromatic ring; the oxygen atoms bind iron. In gram-positive bacteria, iron-siderophore complexes are recognized by binding proteins anchored to the inner membrane, and transported by ABC permeases into the cell (Raymond and Dertz, 2004). Gram-negative bacteria, on the other hand, possess high-affinity outer membrane receptors that capture iron-siderophore complex and translocate them into the periplasmic space through the Ton complex, which is coupled to the proton-motive force in the cytoplasmic membrane, for eventual transfer into the cell by periplasmic-binding proteins and ATP-dependent membrane transporters (Cohen, 2004). Many bacteria produce more than one type of siderophore. *Escherichia coli*, for example, produces enterobactin, a catecholate siderophore with three catechol groups, giving it six coordinating atoms per molecule, and aerobactin, a carboxylate-hydroxymate mixed siderophore. For a siderophore to be effective, the iron-siderophore complex must be more stable than the iron hydroxide, and it must have comparable or greater affinity for iron than the host transport/storage proteins. For a siderophore to contribute to virulence, its affinity must be greater than that of the host proteins (Raymond and Dertz, 2004).

Some pathogenic bacteria can be found in the marine environment. For example, *E. coli*, a well-characterized member of the enteric bacteria and a common cause of gastroenteritis (Brock et al, 1994), is almost ubiquitous. Enteric bacteria are a group of gram-negative, oxidase-negative, nonsporulating, facultatively aerobic, sugar-fermenting bacilli. The *Vibrio* genus is comprised of salt-tolerant species, growing naturally in sea water, that produce a variety of infections, including gastroenteritis, wound and ear infections, and sepsis. *V. cholerae*, the causative agent of cholera, is a small, gram-negative sucrose-fermenting. Serotype O1 is associated with epidemic disease. Species of

Vibrio have two chromosomes, unlike most other bacteria, and genes for multiple iron transport systems are located on both chromosomes. Plasmids can also supply siderophore-coding genes to *Vibrio* species, which can also contribute to virulence. *V. cholerae* uses a catechol siderophore—vibriobactin—as well as a heme receptor and other iron transport systems. Vibriobactin production is highest in environmental isolates, suggesting that this siderophore is more important than the other iron transport systems in the environment. Since heme is a common constituent of vertebrate blood, but not of marine environments, one would expect heme receptors to gain importance in the vertebrate host. *V. vulnificus* is an opportunistic marine pathogen that causes hemorrhagic septicemia in humans and marine animals, with a high associated mortality rate. It uses a catecholate siderophore very similar to vibriobactin—vulnibactin—and a hydroxamate siderophore. *Vibrio* are not particular about where they get their iron; they possess outer membrane receptors that recognize siderophores from other bacteria, including enterobactin from Enterobacteriaceae (DiLorenzo et al, 2004).

In marine environments, iron enters the water by solubilization of the earth's crust, which contains abundant iron (Kirchman, 2000), and by atmospheric deposition of Aeolian dust into the sea (Duce and Tindale, 1991). However, the insolubility of iron oxides limits its availability, and its use by phytoplankton reduces it still more in the marine surface layer. Heterotrophic bacteria account for perhaps 40% of total iron use in the ocean, and iron appears to be the limiting nutrient for bacterial growth in a few oceanic areas: equatorial Pacific and other low-chlorophyll, high-nutrient (HNLC) areas, upwelling regions, the Southern Ocean (Kirchman, 2000), and the sub-Arctic Pacific (Falkowski, 1995). The enzymes involved in nitrate assimilation and N₂ fixation use iron as a

coenzyme, and iron shortage seems to constrain the rates of these processes (Duce and Tindale, 1991). Cyanobacteria—a group of bacteria capable of photosynthesis and also called the blue-green algae (Lehninger, 1982)—have been the best-studied group for response to iron limitation. Changes include an increase in glycogen reserves, reduction in the numbers of some proteins, such as thylakoids, which support photosynthetic processes, and production of novel proteins, including siderophores (Mann, 1995). Ferredoxin, an iron-containing enzyme which carries electrons from chlorophyll to various electron donors (Falkowski, 1995), is replaced by the flavin mononucleotide (FMN)-containing flavodoxin (Lehninger, 1982). Thus, bacteria have strategies to respond to iron limitations, but it does appear to limit microbial growth in some marine environments.

After transport into the microbial cell, ferric iron is reduced to facilitate its removal from the chelating claws of the siderophore, and siderophore-transported iron has been found in the intracellular Fe^{+2} pool within 30 minutes of transport. This is apparently accomplished by siderophore-iron reductases. Intracellular storage of iron is complicated by its chemical properties, which favor the formation of toxic free radicals and insoluble ferric-hydroxide polymers. Mammalian cells therefore store iron as haemosiderin and ferritin, protein shells surrounding an inorganic ferric oxyhydroxide core. Ferritin consists of an oligomeric protein of 24 similar or identical subunits that form a hollow protein shell. The core stores up to 4500 iron atoms as ferric hydroxyphosphate micelles, which are non-toxic and bioavailable. Likewise, bacteria store iron in bacterioferritins, which are very similar to human ferritin, or in ferritin-like proteins. Ferritin is highly conserved across species (Crichton, 2001).

CHAPTER 3 – RESULTS AND DISCUSSION

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3.1 Introduction

The introduction of aquatic nuisance species (ANS) and bacterial pathogens from discharge of ballast water by sea-faring vessels is an ongoing problem that threatens ecosystems and human health. Ballast water provides stability to ocean-going vessels, and its uptake and discharge allows ships to compensate for cargo loads. American waters receive more than 79 million tonnes of foreign ballast water per year (Carlton et al, 1995), and the number of species carried in these waters is vast. All major marine trophic groups were found in ballast water from Japanese cargo ships in an Oregonian port, such that ballast water acts “as a phylogenetically and ecologically nonselective transport vector” (Carlton and Geller, 1993). Many recent biological invasions have been attributed to ballast water release, the most famous of which is *Dreissena polymorpha*, the zebra mussel, which was most likely introduced via release of larvae contained in ship ballast water into the Great Lakes in the late 1980s (Hebert et al., 1989). Zebra mussels continue to expand their range, and their tendency to form dense mats has led to pipe fouling at power plants and other facilities (Kovalak et al, 1993).

Bacteria are also among the aquatic nuisance species (ANS) introduced by ballast water discharge. One study found *Vibrio cholerae* 01 in ballast water from 5 of 19 cargo ships sampled in Alabama and Mississippi (McCarthy and Khambaty, 1994), causing the Food and Drug Administration and US Coast Guard to recommend that ship crews

exchange their ballast water in mid-ocean, prior to entry into US ports. Viable counts of *V. cholerae* were on the order of 10^6 /ml, in salinities ranging from 12 to 32 ppt, again emphasizing the potential environmental impacts of ballast water. However, open-ocean exchange proved inadequate, and even fresh-water flushing leaves many viable organisms in tanks (Hulsmann and Galil, 2001).

Because of the growing urgency of this problem, voluntary international guidelines for ballast water management have been established by the Maritime Environment Protection Committee (MEPC) of the International Maritime Organization (IMO) (Raaymakers, 2001), and performance specifications have been set for the successful testing and approval of ballast water management systems, including:

- The final concentration of Enterococci should be less than 1 CFU/ml,
- The final concentration of *Escherichia coli* should be less than 2.5 CFUs/ml, and
- The final concentration of *Vibrio cholerae* should be less than 1 CFU/100 mls

An exact standard of reduction for coliforms has not been set, but the IMO specifications state that they should be measured in any proposed treatment method.

Disinfection of potable water commonly has two goals: the immediate destruction or inactivation of pathogens, and persistent inactivation of pathogens through residual action (Xie, 2004). An ideal disinfectant should be toxic to microorganisms, yet non-toxic to higher forms of life. Treatment of ballast water, and other applications to prevent the spread of invasive species, has slightly different goals than that of drinking water, since the water is typically treated, and then released into the local environment. Because the local species are quite similar to the aliens, the disinfectant should not persist in the environment, so that the local biota is not harmed. Also, some higher organisms, such as crustaceans, are considered invasive, so an ideal disinfectant for ballast water would have a broader

spectrum of action. One agent that holds promise for ballast water disinfection is ferrate. Ferrate is iron with a valence of +6, which confers upon it a tendency to accept electrons, to attain its preferred valence of +2 or +3. Therefore, it acts as an oxidant, like many disinfectants, with a reduction potential of +2.20 V in acidic solution and +0.72 V in alkaline solution (Wood, 1957). This reduction potential makes it a more powerful oxidant in acid than hypochlorite, ozone, hydrogen peroxide, or permanganate (Lee et al, 2004). By oxidizing other molecules, it inactivates enzymes, resulting in cell death (Metcalf & Eddy, 2003). Because of its steric resemblance to phosphate anion, ferrate binds to enzyme phosphoryl groups and therefore site-specifically inactivates phosphatases, dehydrogenases, phosphorylase b, phosphoglucomutase, and other proteins with this functional group. It also specifically oxidizes amino acid residues on muscle phosphorylase, pancreatic ribonuclease, triose phosphate isomerase, and *E. coli* DNA polymerase-I (Basu et al, 1987). Ferrate can also be used as a coagulant (Jiang et al, 2002), and for other uses, but its cost of production has hindered its commercial usage.

Three general methods of synthesizing ferrate have been used: wet oxidation, dry oxidation, and electrolysis (Lee et al, 2004). Electrolysis involves the anodization of a pure iron electrode in concentrated alkaline solution. The reaction at the cathode is:

In dry oxidation, iron oxides are treated with oxidants at high temperature and pressure to generate ferrate salts, as in the following reaction:

Dry oxidation has the advantage of a one-step process (Perfiliev and Sharma, 2004), and shows potential as a green technology to recycle iron oxide wastes from steel manufacturing processes. Typically, ferric ion is oxidized to ferrate by concentrated hypochlorite in strong basic solution, after which it is precipitated as potassium ferrate by the addition of excess potassium hydroxide. The resultant ferrate sludge is then washed and rinsed many times in alcohols and solvents to obtain a dry, powdered ferrate product according to the following reactions:

Wet oxidation shows good potential for ferrate production, but the requirement for relatively pure chemicals has lead to high costs (Lee et al, 2004). A company located in Orlando, Florida, Ferrate Treatment Technologies, LLC (“FTT”) has solved the high cost problem of prior wet oxidation ferrate synthesis methods, as well as possible issues of instability, packaging, handling, and transport. FTT developed and patented a novel, on site synthesis method, using relatively inexpensive chemicals, and has manufactured ferrate onboard a 67,000 ton container vessel for ballast treatment using both of the following reactions:

Ferrate decomposes to non-toxic forms of iron in aqueous solution according to the following equation:

The rate of decomposition depends upon many factors, including pH, temperature, and initial ferrate concentration (Schink and Waite, 1980). According to a Pourbaix diagram, ferrate is most stable in an alkaline, highly aerobic environment. It will convert to aqueous ferric and ferrous iron in acidic, aerobic environments, and to non-aqueous $\text{Fe}(\text{OH})_3$ and $\text{Fe}(\text{OH})_2$ precipitates in alkaline, aerobic-to-somewhat anoxic conditions, and to solid Fe in highly anoxic (i.e. reducing or low oxidation-reduction potential) conditions, across the entire pH range. More dilute solutions result in a decreased driving force towards precipitation (Western Oregon Univ., 2006). Seawater is typically slightly alkaline, so depending on the oxygen levels, ferrate would be expected to precipitate out as a hydroxide or as solid Fe, as long as the pH does not become acidic. Precipitation of ferrate might be a useful method to remove it from treated ballast water, prior to discharge, in order to meet effluent iron standards. These vary widely by state, country, and water classification. Within Florida waters, the maximum iron standard ranges from 0.3-1.0 mg/l (Florida Department of Environmental Protection, 2006).

Many oxidants have the disadvantage of producing disinfection byproducts (DBPs). For example, trihalomethanes (THMs) are a common DBP of chlorination, and their concentration in drinking water is regulated because of health concerns (Metcalf & Eddy, 2003), although the validity of those concerns is debatable (White, 1999). Ferrate has the advantage that its breakdown product is some form of iron, which is non-toxic to humans

except in very large doses. Even if the breakdown products of the oxidant are not in themselves toxic, they can react with constituents in the water to produce environmentally harmful products. THMs, for example, are formed by the reaction of natural organic matter with chlorine or other oxidants (Metcalf & Eddy, 2003). The presence of natural organic matter also reduces the effectiveness of oxidants. In the case of chlorination, this produces a stepwise phenomena referred to as “breakpoint chlorination chemistry”, in which the chlorine first reacts with readily oxidizable organic and inorganic material, then with ammonia to form chloramines, and then with less readily oxidizable molecules, including, presumably, the target species, producing a chlorine residual after all oxidizable matter is consumed (Matheickal et al, 2004). Likewise, one would expect the action of ferrate to vary with respect to organic content, which creates an additional oxidant demand.

The effectiveness of ferrate must be compared with that of free chlorine and chloramines, as well as other disinfectants, using similar techniques and models. The CT approach, used commonly in drinking water regulations (American Water Works Assn., 1999), looks at combinations of concentrations and contact times. It assumes a linear relationship between contact time and degree of disinfection. This is rarely observed, however. Often, there are “tailing” and “shoulder” or “lag” effects, resulting from disinfectant, environmental, and bacterial characteristics, and numerous modelers have sought to explain these phenomena, and to predict disinfection rates from them. The Chick-Watson law relates the survival rate of the target organism to the concentration of disinfectant and a die-off constant (Metcalf and Eddy, 2003). It is expressed as:

$$dN_t/dt = -k' C^n N_t \quad (8)$$

where N_t is the number of organisms at time t , k' is the die-off constant, C is the concentration of disinfectant, and n is a “coefficient of dilution” which determines the relative importance of disinfectant concentration. The integrated form of this equation is:

$$\ln(N_t/N_0) = -k' C^n t \quad (9)$$

where N_0 is the starting bacterial concentration. When n is greater than 1, concentration is more important than time. Deviations from this law, such as “tailing” or “shoulders” caused by media interactions, disinfectant decay, and other factors, result in a non-linear graph of time versus survival (N_t/N_0). A log-log plot can still result in a linear graph for these deviations, provided $C^n t$ is a constant (American Water Works Assn., 1999). In order to better describe deviations from Chick-Watson behavior, Equation 2 was modified by Hom to also consider the effect of time (Hom, 1972). The integrated form of Hom’s equation is:

$$\ln(N_t/N_0) = -k' C^n t^m \quad (10)$$

where m is a coefficient of importance for time. The rate of inactivation decreases with time when m is less than 1. Adding the m coefficient permits further characterization of the effect of time on disinfection efficiency, but might also lead to over-parameterization.

A general differential rate law of disinfection states that the rate of inactivation, dN/dt , relates to its components as:

$$dN/dt = -k m N^x C^n t^{m-1} \quad (11)$$

where k is an experimental reaction rate constant and other variables are as defined (Gyurek and Finch, 1998). This equation reduces to the Chick-Watson equation when $m = 1$ and $x = 1$, and to the Hom's equation when $x = 1$. Both the Chick-Watson and Hom's equations assume that the concentration of disinfectant is constant for the test period or for a given contact time. This is not necessarily valid for some disinfectants, such as chlorine residual (Haas, 1979). Assuming that disinfectant concentration follows first-order decay requires the incomplete γ function for an exact solution of the Hom's model (Haas and Joffe, 1994). However, many studies have found good correlation coefficients using Chick-Watson or Hom's models for disinfection of various bacteria by free chlorine, implying that the assumptions of these models are valid over the contact times studied (Haas and Karra, 1984).

An equation similar to one recommended by the Water Environment Federation Wastewater Disinfection Manual of Practice (WEF, 1996) has been used to describe inactivation of a dinoflagellate algae in ballast water (Oemcke and van Leeuwen, 2005), in the form:

$$-\log(N/N_0) = b(\log C) + d \quad (12)$$

where C is the oxidant concentration after initial demand has been met, b and d are experimental regression coefficients, and other terms are as described. A steeper slope (i.e. higher b) indicates a higher kill achieved with relatively low doses, and a higher x -intercept indicates a relatively high initial oxidant demand. This is similar to the Chick-Watson model but does not consider time as a variable, so the coefficients must be determined for

each experimental contact time. This equation is referred to as the “oxidant demand” model.

3.2 Methods

Heterotrophic and coliform bacteria were represented by *Escherichia coli* and *Klebsiella pneumoniae* (ATCC #s 11303 and 13833, respectively). The Enterococcus group was represented by a mixed culture of *Enterococcus faecium* and *E. faecilis* (ATCC #s 19434 and 19433, respectively). *Vibrio cholerae* was grown from ATCC #51352 stock. Instant Ocean (IO, Aquarium Systems, Mentor, OH) was added to de-ionized water to produce salt water at 36 ppt per the manufacturer’s instructions. All bacteria were cultured under sterile conditions at room temperature (22°C), with shaking at 25 rpm, in a 5:1 mixture of IO:nutrient broth (8 g/l), for 3-4 days. At time of testing, the bacterial cell suspension was centrifuged for 10 minutes and resuspended in 36 ppt IO (unless otherwise indicated), under sterile conditions. Bacteria were washed twice more in this manner, resuspended in 36 ppt IO, and quantified. *E. faecium*, *E. faecilis*, *Escherichia coli*, and *K. pneumoniae* were quantified by the IDEXX Quanti-Tray (IDEXX Laboratories, Inc., Westbrook, ME) enumeration procedure. *V. cholerae* was quantified by plate culture using nutrient agar (Difco brand, Becton Dickinson & Co., Franklin Lakes, NJ). The suspension of bacteria was subdivided into a number of flasks or 50-ml centrifuge tubes, ferrate was added at a range of dosages with stirring or shaking, and bacterial enumeration was performed again at a number of time intervals for each dosage. At sampling time, an appropriate amount of liquid from each vial was transferred to a 100-ml Coliform Test Vial, with the sodium thiosulfate pellet

already in solution, to stop the oxidation reaction immediately, in a final volume of 100 mls. Colilert-18 substrate for quantification of *K. pneumoniae* and *E. coli* was added prior to transfer to Quanti-trays, which were heat sealed with an IDEXX Quanti-Tray Sealer, and incubated at 35°C for 18 hours. *K. pneumoniae* was quantified by color change, *E. coli* by fluorescence, and *Pseudomonas aeruginosa* was run as a negative control. Combined cultures of *Enterococcus faecilis* and *E. faecium* were quantified by the IDEXX Enterolert Quanti-Tray enumeration procedure. Blue fluorescence was detected with a UV light, after incubation at 41° C for 24 hours. For both Quanti-Tray enumeration procedures, MPN/100 ml was calculated from the most probable number (MPN) tables provided by the manufacturer, and converted to MPN/ml, using the appropriate dilution. When no wells were positive, a value of one was used for calculations (expressed as < value accounting for dilution). Because testing was performed on salt water specimens, the procedure for marine water samples was followed and specimens were diluted at least tenfold with sterile distilled water, i.e. 10 mls of sample in the final volume of 100 mls was the lowest dilution. Untreated cultures of these organisms were run as positive controls, and tap water was run as a negative control. For *V. cholerae*, aliquots for all time points were added to disposable glass tubes containing sodium thiosulfate at a final concentration (after adding treated specimen) of 0.81 mM. Serial dilutions were performed in dilution buffer containing 2.00 mM magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) and 0.312 mM potassium phosphate (KH_2PO_4), and 100-1000 μl was transferred to nutrient agar (Remel brand, Lenexa, KS) plates and spread using a glass spreader. The plates were incubated for 7 days at 35 °C, at which time colonies were counted, and expressed as CFUs/ml. For kinetics, four experiments were run for each organism, and the Chick-Watson kinetic model coefficients were obtained for each

experiment by the method of Metcalf and Eddy (2003), using Microsoft Excel spreadsheets. The Hom's model coefficients were calculated by the method of the American Water Works Association (1999), using multiple linear regression and SPSS statistical software. The oxidant demand coefficients were determined by combining the experimental data for each organism and calculating the coefficients in equation 12 by linear regression for contact times of 1, 5, 15, and 30 mins., using Microsoft Excel. The x-intercept (initial oxidant demand) is solved from the equation for the linear regression, with appropriate conversions from the log values. Slopes were compared by hypothesis testing in regression by the method of Helsel and Hirsch (2002), using a standard Student's t table.

The effect of salinity was investigated by dividing a washed *E. coli* suspension, and bringing it up to equal volumes of either 36 ppt or 10 ppt IO. Various dosages of ferrate ranging from 0.25-5 mg/l were added and sampled at various times. Untreated controls were sampled at the same times for the two salinities, to control for differences in mortality due to salinity without the addition of ferrate, and sodium thiosulfate was also added to untreated controls. The delta log removals were calculated by subtracting untreated controls from the treated controls for each salinity over time. Similarly, the effect of pH was tested by preparing a washed *E. coli* suspension in IO as described above, and measuring the initial pH. One portion of the suspension was then adjusted to a pH 1.5 units higher than the initial pH with 1 N sodium hydroxide, and one portion was adjusted to a pH 1.5 units lower than the initial pH with 1 N hydrochloric acid. Two ferrate dosages, 0.75 and 1.5 mg/L, were tested at various times for the three pHs, and untreated controls were also sampled for each of the conditions (initial pH, acidic pH, and alkaline pH) to adjust for any disinfection effects resulting from the changes in starting pH alone. Delta log removals due to the effect of

ferrate were calculated for each of the pH conditions and dosages by subtracting the change in MPN for the untreated control from the corresponding treated condition. Salinity and pH experiments were done in duplicate with separate ferrate batches. The statistical significance of pH and salinity effects was determined using a fixed effect ANOVA of a multi-factorial design in S-PLUS 2000, using time, dosage, and pH or salinity as independent variables. Anti-clumping effects were investigated by washing *E. coli* three times with IO, as described above, and then either including a low-speed centrifugation (170g for 5 min) and resuspension of the supernatant in an appropriate volume of IO, or intense vortexing after addition of ferrate and just before each sampling event. These were done as two separate experiments and compared simultaneously with normal preparation and treatment, i.e. washing the bacterial culture three times with IO and resuspension in IO, and shaking the specimens prior to sampling. The statistical significance of anti-clumping measures was determined by again using a fixed effect ANOVA of a multi-factorial design in S-PLUS 2000, using time, dosage, and anti-clumping measure (low-speed centrifugation or vortexing vs. normal preparation) as independent variables. Regrowth was measured by growing and resuspending *E. coli* in IO, as above, dosing with 0, 5, or 10 mg/l ferrate for one minute, then quenching with sodium thiosulfate, and quantifying at 15 min. Glucose was added to aliquots from each of these conditions, to a final concentration of 0.2 mg/l, and bacteria were re-quantified at 48 hours.

3.3 Results and Discussion

Log Reductions and Kinetics. The average log reductions achieved at 5 minutes for a 5 mg/l dosage for the test organisms are summarized in Table 1. The number of observation (n) used to obtain these values are given in parentheses for this and subsequent tables. The MPN/ml at various times and dosages for a typical experiment are shown in Figure 1. The corresponding log-log graph of contact time versus survival is shown in Figure 2, to obtain the contact times necessary to obtain a 4-log reduction for each dosage, following Metcalf and Eddy's (2003) procedure for determining the Chick-Watson coefficients, as described in the Methods section. The resultant coefficients for the Chick-Watson and Hom's models for the four organisms are summarized in Table 2, as well as the correlation coefficients. The fact that m does not equal one for any of the organisms indicates deviations from Chick's

Table 1: Log reductions for test organisms for 5 mg/l ferrate dose for 5 min contact time.

Organism	Mean Log Reduction	Standard Deviation
<i>E. coli</i> (n = 4)	6.8	0.3
<i>K. pneumoniae</i> (n = 4)	7.3	0.8
Enterococci (n = 4)	5.5	0.5
<i>V. cholerae</i> (n = 4)	4.4	0.2

Figure 1: Effect of Ferrate Dosage on *E. coli* MPN over Time: Results of One Experiment.

Figure 2: Chick-Watson Analysis of *E. coli* Disinfection Experiment: Results of One Experiment.

Table 2: Coefficients of the Two Kinetic Models for Ferrate on the Study Organisms.

Organism	Chick-Watson Coefficients ¹			Hom Coefficients ¹			
	n	k'	R	n	m	K	R
<i>E. coli</i>	2.1	0.9 +/-	0.78	0.5	0.2	5.4	0.96
	+/- 0.3	0.4	+/- 0.04	+/- 0.2	+/- 0.04	+/- 1.5	+/- 0.02
	(n = 80)	(n = 80)	(n = 110)	(n = 110)	(n = 110)	(n = 110)	(n = 110)
<i>K. pneumoniae</i>	5.4	0.013 +/-	0.67	0.8	0.2	2.9	0.97
	+/- 1.1	0.009	+/- 0.08	+/- 0.2	+/- 0.03	+/- 0.8	+/- 0.01
	(n = 88)	(n = 88)	(n = 112)	(n = 112)	(n = 112)	(n = 112)	(n = 112)
Enterococci	2.4	0.3 +/-	0.78	0.7	0.3	3.0	0.96
	+/- 0.2	0.3	+/- 0.06	+/- 0.1	+/- 0.08	+/- 0.9	+/- 0.02
	(n = 84)	(n = 84)	(n = 108)	(n = 108)	(n = 108)	(n = 108)	(n = 108)
<i>V. cholerae</i>	3.1	0.034 +/-	0.72	0.4	0.15	4.0	0.95
	+/- 0.3	0.02	+/- 0.05	+/- 0.02	+/- .01	+/- 0.3	+/- 0.02
	(n = 63)	(n = 63)	(n = 108)	(n = 108)	(n = 108)	(n = 108)	(n = 108)

¹Means and SDs of 4 experiments/organism.

Law. The shape of the dosage-response curves and the observation that all of the Hom's m coefficients are less than one indicates that tailing is occurring. Furthermore, a Chick-Watson n coefficient of greater than 1 indicates that dosage is more important than time, which is what is observed for all organisms.

The coefficients and correlations obtained from the oxidant demand equation are summarized in Table 3. The fact that the initial oxidant demand (x-intercept) values are highest for 1-min contact times indicates that the initial oxidant demand had not been met until the later contact times. Our slope or b values (2.1 – 6.5) are considerably higher than those of Oemcke and van Leeuwen (2005) (0.2 – 0.5), which theoretically indicates greater inactivation at lower dosages, but it is not valid to compare rates of inactivation of bacteria with a dinoflagellate algae, and the uncommon use of this equation limits other comparisons. The b coefficient is fairly consistent for each organism at the various contact times. Comparing slopes for each organism at the different contact times, at the p = 0.05 level, the 1-min. contact time is different from at least one other time for each organism, perhaps again suggesting that the initial oxidant demand had not been satisfied yet for this contact time.

Table 3: Coefficients for the Oxidant Demand Equation for Ferrate on the Study Organisms.

Organism	Time (min)	b	d	R	Initial Demand
<i>E. coli</i>	1 (n = 20)	3.06	2.43	0.96	0.16
	5 (n = 20)	4.23	3.60	0.97	0.14
	15 (n = 18)	4.54	4.02	0.97	0.13
	30 (n = 12)	4.98	4.44	0.98	0.13
<i>K. pneumoniae</i>	1 (n = 20)	5.27	1.08	0.97	0.62
	5 (n = 20)	6.06	2.23	0.93	0.43
	15 (n = 20)	6.51	2.52	0.95	0.41
	30 (n = 12)	5.55	2.83	0.93	0.31
Enterococci	1 (n = 20)	2.79	1.76	0.88	0.23
	5 (n = 20)	3.53	2.75	0.95	0.17
	15 (n = 16)	3.42	3.04	0.95	0.13
	30 (n = 12)	3.71	3.32	0.94	0.13
<i>V. cholerae</i>	1 (n = 20)	2.10	1.78	0.94	0.14
	5 (n = 20)	2.56	2.54	0.98	0.10
	15 (n = 16)	2.44	2.76	0.95	0.07
	30 (n = 12)	2.66	2.94	0.92	0.08

The only other slopes that differ significantly are the 5 and 30-min contact times for *E. coli*, suggesting again the importance of dosage. This equation is not a rate equation, as is Chick-Watson and Hom's, and in fact only relates survival as a function of dosage for a given contact time. Also, because it is necessary to calculate coefficients for each contact time, this approach seemed more cumbersome than the Hom's model. Furthermore, the consistently higher correlation coefficients obtained with the Hom's equations, summarized in Table 4,

Table 4: Correlation Coefficients for the Disinfection Equations

Organism	R values for the Disinfection Modeling Equations		
	Hom's	Chick-Watson	Oxidant Demand
<i>E. coli</i>	0.96	0.78	0.96-0.98
<i>K. pneumoniae</i>	0.97	0.67	0.93-0.97
Enterococci	0.96	0.79	0.88-0.95
<i>V. cholerae</i>	0.95	0.72	0.92-0.98

indicate that the 3-parameter model is necessary to adequately model the data. Therefore, the following equations best predict the effect of ferrate on the test organisms:

$$E. coli \quad \ln S = -5.4C^{0.5}t^{0.2} \quad (13)$$

$$K. pneumoniae \quad \ln S = -2.9C^{0.8}t^{0.2} \quad (14)$$

$$\text{Enterococci} \quad \ln S = -3.0C^{0.7}t^{0.3} \quad (15)$$

$$V. cholerae \quad \ln S = -4.0C^{0.4}t^{0.2} \quad (16)$$

where t is time in minutes, C is initial ferrate concentration in mg/l, and S is survival of the organism (N_t/N_0).

These equations result in the times required to obtain a 4-log reduction over a range of ferrate dosages, presented in Table 5. *K. pneumoniae* demonstrates the greatest dependence on dosage, as evidenced by the relatively large ratio of times required to achieve 4-log reductions at the low and high dosages in Table 5, summarized in Table 6.

Table 5: Time (in minutes) to Obtain a 4-Log Reduction of the Organisms (extrapolated from experimental data using Hom's model coefficients).

Ferrate Dosage (mg/l)	Organism			
	<i>E. coli</i>	<i>K. pneumoniae</i>	Enterococci	<i>V. cholerae</i>
0.1	4565	3231383	9061	6473
0.2	807	201961	1798	1618
0.4	143	12623	357	405
0.8	25.2	789	70.8	101
1.0	14.4	323	42.1	64.7
2.0	2.6	20.2	8.4	16.2
3.0	0.9	4.0	3.2	7.2
5.0	0.3	0.5	1.0	2.6

Table 6: Ratio of Time Required to Obtain a 4-Log Reduction at 0.1 and 5.0 mg/ Ferrate.

Organism			
<i>E. coli</i>	<i>K. pneumoniae</i>	Enterococci	<i>V. cholerae</i>
15216	6462766	9061	2489

As mentioned, the observation that all of the Hom's m coefficients are less than one and that all of the Chick-Watson n coefficients are greater than one indicates that dosage is more important than time. Haas and Karra (1984) reviewed a number of disinfection studies conducted under demand-free conditions, determined by chemical analyses of the residual, and found that free chlorine generally produced Chick-Watson n values greater than 1 (i.e. tailing), whereas n values of less than 1 (i.e. shoulders) were observed with combined chlorine. However, most of the studies they examined were at lower (2-3) log reductions than this study, which also was not done under demand-free conditions. They observe that the Hom's model did not significantly increase predictive power for most of the experiments, but also note that a less satisfactory agreement with Chick-Watson is likely for higher log reductions, since tailing is a more common occurrence with greater inactivation values. In addition, the k coefficients are experimental rate constants: the higher the k value, the lower the survival. The Chick-Watson k' values cited by Haas and Karra for experiments with *E. coli* range from 0.3 – 30.6 for free chlorine for pH values ranging from 7.0 - 10.7 l/mg-min, and from 2.5×10^{-5} – 1.0 l/mg-min for pH values from 7.0 – 10.5. The Chick-Watson n values range from 0.8 – 1.5 for free chlorine and from 0 – 13.3 for combined chlorine. Thus, this study's n value of 2.1 and the k value of 0.9 for *E. coli* is comparable to the values of either of the other disinfectants, given the wide ranges, but a direct comparison is difficult, given the differences in experimental design, particularly the high salinity of this study.

pH and Salinity. The transferability of these equations to ballast water applications will depend upon several factors, including pH and salinity. The pH of seawater is close to that of the bacterial suspensions in IO (approximately 8), but the pH of ballast water might differ significantly, due to products of corrosion or metabolism of bacteria and other organisms present. The results for a 0.75 mg/l ferrate dosage, shown in Figure 3, indicate that a slightly acidic pH (6.5) enhanced disinfection. Results for 1.5 mg/l ferrate (not shown) were similar ($p < 0.001$ for acidic pH for both dosages), whereas an alkaline pH had a much smaller effect ($p > 0.05$ for both dosages). However, at the 0.75 mg/l dosage, the p value of alkaline vs. starting pH was 0.07, and log reductions appeared to be increasing with time relative to the neutral pH. The effect of the pH change itself was minor for the acidic conditions (a 0.09 log change over the experimental time of 30 minutes) but slightly higher (a 0.25 log reduction) for the alkaline condition. This effect has been subtracted from the effect of ferrate for each time point, as described in the Methods section, to determine the log removals shown in Figure 3. These results are consistent with the higher reduction potential of ferrate in acidic solution, but the effects of the increased stability of ferrate in alkaline solution are less clear, and perhaps are being confused by the higher mortality from alkaline pH and the small sample size (two experiments). Salinity did not have a clear effect on log reductions ($p > 0.05$), as shown in Figure 4.

Figure 3: Effect of pH on Log Reduction of *E. coli* by 0.75 mg/l Ferrate (averages of 2 experiments).

Figure 4: Effect of Salinity on *E. coli* Mortality at Various Ferrate Dosages (averages of 2 experiments).

Initial Bacterial Concentration (N_0). Because the starting bacterial concentration represents an oxidant demand, this also has a potential impact on disinfectant dosage, but the literature on this subject is somewhat contradictory. Rincon and Pulgarin (2004), for example, found that as N_0 increased, the time for inactivation of *E. coli* with illuminated TiO_2 (photocatalysis) increased, or in other words, efficiency of disinfection decreased. Likewise, Greets and Fomichev (1985) found that the disinfection efficiency of ozone on *E. coli* and *P. aeruginosa* (and their phages) improved with a decrease in their initial concentration, and that disinfection seemed to be a function of the concentration of ozone per bacterial cell. On the other hand, Haas and Kaymak (2003) found that as N_0 decreased, the disinfection efficiency of ozone against *Giardia muris* decreased. Figure 5 indicates that a reduction in starting concentration of *K. pneumoniae* corresponded with an increase in log kill by a 1 mg/l ferrate dose. The initial bacterial concentration (N_0) was 5.3×10^7 organisms/ml, and there was a particularly significant increase in log kills with the first log dilution at 5 minutes. After this the log kills seemed to stabilize, although the results at the final time of measurement (10 min. of additional kill, for a final time of 15 min) increased in a linear fashion with dilution until the 1:1000 dilution. This is probably because the final dilution of 1:1000 N_0 resulted in no bacteria detected at the end time, and this was counted as 1 organism according to the procedure outlined in the Methods section, and multiplied by 1000 for the dilution, resulting in the log reduction shown. Thus, the decrease in log reduction for the final dilution appeared to be attributable to the decrease in bacteria available to kill, i.e. the log kill began to decrease simply because there were fewer bacteria to kill. Similar results with respect to the effect

Figure 5: Effect of Initial *K. pneumoniae* Concentration on Effectiveness of a 1 mg/l Ferrate Dosage.

of initial bacterial concentration were obtained for *E. coli* (data not shown). The data indicates that the log kill achieved with a given contact time and dosage decreased as initial bacterial concentration increased. The initial values used in this study were conservative in that they were quite high (approximately 10^7 MPN/ml for *E. coli* and *K. pneumoniae*, and 10^5 MPN/ml for Enterococci). Initial studies by this research group found an MPN for *E. coli* of less than 1 per ml in two locations in waters of the Port of Cape Canaveral, approximately seven orders of magnitude lower than our initial concentrations. Therefore, our estimated dose would be higher than the actual requirement to inactivate the organisms to the required standard.

However, in other countries, researchers are finding higher concentrations of organisms in ballast water, with a great deal of variability. For example, specific oligonucleotide probe analysis of six ballast waters in Singapore Harbour found concentrations of eubacteria, Enterobacteria, *Vibrio* spp, and *E. coli* ranging from 10^4 to 10^6 cells/ml for each of these organism classes (Joachimsthal et al, 2004). It is possible

that holding water promotes breeding of bacteria, but the same team of researchers found slightly lower concentrations in ballast water relative to seawater, and hypothesized that ballast water creates an environment that favors facultative anaerobic bacteria, which might also favor pathogens (Joachimsthal et al, 2003). Therefore, our starting bacterial concentrations might be comparable to, or perhaps slightly higher than, typical ballast water concentrations. Furthermore, the ferrate demand in ballast water due to oxidant-demanding compounds, including inorganics such as corrosion products, and organics needs to be assessed to translate results into the field, along with field testing in a variety of locations.

Anti-clumping. The observation of tailing indicates that clumping of cells might have occurred in the experiments. To investigate this possibility, two anti-clumping procedures—a low-speed centrifugation to remove clumps and intense vortexing—were compared to the standard procedure. The results are presented in Figures 6 and 7. Neither procedure eliminated the observation of tailing, but both procedures appeared to improve the log reductions ($p < 0.05$) over the “normal” preparation. There was a procedural bias in that the anti-clumping procedure was performed before the “normal” preparation procedure, using

Figure 6: Effect of Low Speed Centrifugation to Remove Clumps.

Figure 7: Effect of Vigorous Vortexing to Reduce Potential Clumping.

the same batch of ferrate to eliminate any variability between batches. This meant that if ferrate dissipated during the intervening time the actual dose received by the “normal” preparation would be lower. However, a preliminary study by this lab found ferrate to be stable for hours at 20°C, suggesting that this effect was probably minimal. However, because of the additional step of the low-speed centrifugation, it was impossible to control for N_0 in this comparison, since part of the biomass was removed in centrifugation relative to the “normal” preparation. Because of the anticipated removal of some of the bacteria in this step, this procedure was conducted with three times the volume relative to the “normal” preparation, which resulted in a higher N_0 for the low-speed centrifugation procedure than the “normal” preparation (4.9×10^6 vs. 1.5×10^6 MPN/ml, respectively.) Although the log reductions are expressed relative to the starting concentration for that condition, N_0 is a variable that could have affected the results, as discussed above. However, since centrifugation had a higher initial concentration this difference could not explain the increased log removals observed. This is supported by the fact that vortexing increased removals even though this procedure did not significantly affect the initial bacterial concentration (Figure 7).

Regrowth from a viable but not culturable state. Lastly, possible regrowth of *E. coli* was investigated. Figure 8 summarizes the average results of two experiments. The short contact time of 1 min with a high ferrate dosage of 10 mg/l resulted in irreversible kill, with no regrowth, even with the addition of glucose. The same contact time with a ferrate dose of 5 mg/l did not produce a complete kill, but the number of bacteria continued to decline following the exposure to ferrate, even with the addition of glucose, indicating irreparable damage to the organisms. However, the population with glucose

declined at a slower rate than did the population without glucose, indicating at least some metabolic activity. The data imply that at the higher doses the bacteria were probably killed rather than temporarily impaired and not metabolically active in the enumeration method, but at 5 mg/l there were still some potentially viable organisms. Neither of the disinfected samples showed a rebound in *E. coli* numbers, whether substrate was added or not.

Figure 8: Regrowth of *E. coli* after Ferrate Exposure (average of 2 experiments).

Comparison with Alternative Ballast Water Treatment Processes. The

attractiveness of ferrate as a disinfectant for ballast water requires a comparison with other disinfectants, and the problem of ANS has spurred recent studies of disinfection of seawater. Azanza et al (2001) measured the decimal reduction times (D-values), which is the time to achieve a one-log reduction, of *E. coli* in seawater in response to treatment with ultraviolet (UV) radiation and with chlorine. At 35 ppt salinity and 25°C, they obtained a D-value of

2.0-2.7 min for a 0.5 ppm dosage of chlorine, and a D-value of 0.23-0.37 min for UV radiation at a dosage of $16\text{mWs}^{-1}\text{cm}^{-2}$. Substituting this dosage of ferrate and this level of survival into our Hom's equation for *E. coli* (Equation 13), we obtain a D-value of 0.1 min, which is superior to their values. Oemcke et al (2004) obtained 2- to 3-log reductions at somewhat higher UV doses. The hydroxyl radical, generated by ionization of seawater using a strong electric field, produced at least 2-log reductions of microorganisms (Bai et al, 2003), and heating of ballast water also shows early potential as a ballast water disinfectant (Rigby et al, 2002). Ferrate shows potential as an effective oxidant for ballast water, and merits additional study, as well as cost-effectiveness comparisons with the alternatives after required dosages for all candidates are established.

3.4 Conclusions and Recommendations

A dosage of 5 mg/l ferrate was sufficient to achieve the international standards of less than 1 CFU/100 mls for *V. cholerae*, less than 2.5 CFUs/ml for *E. coli*, and less than 1 CFU/ml for Enterococci, with our experimental initial concentrations of at least 10^4 bacteria/ml and very short contact times, and 4 log removals could be achieved for all organisms with a ferrate dosage of 2 mg/l and a contact time of at least 20 minutes. Both the Chick-Watson and Hom's models indicate that dosage is more important than time, and the Hom's model was superior in predicting the action of ferrate. The tailing effect that was consistently observed in the dosage-response curves suggests that ferrate decomposes rapidly, a desirable trait for subsequent release into the receiving water body, and the natural alkalinity of seawater favors precipitation of the remaining iron for later removal with the

ballast tank sludge, if needed to comply with iron discharge standards,. The disinfection properties of ferrate need to be validated under a variety of field conditions, including a range of initial bacterial concentrations and organic content, but they appear to be significant with various pH values and salinities. Ferrate compares favorably with other candidates for ballast water treatment, and shows promise as an environmentally-friendly, effective disinfectant.

CHAPTER 4 – CONCLUSIONS

All three organisms listed in the international standards for ballast water management—*E. coli*, *Vibrio cholerae*, and Enterococci—were tested. A dosage of 5 mg/l ferrate was sufficient to achieve the international standards of less than 1 CFU/100 mls for *V. cholerae*, less than 2.5 CFUs/ml for *E. coli*, and less than 1 CFU/ml for Enterococci, with our experimental initial concentrations of at least 10^4 bacteria/ml and very short contact times, and four log removals could be achieved for all organisms with a ferrate dosage of 2 mg/l and a contact time of at least 20 minutes. This combination of dosage and contact time would also result in a 4-log reduction of *K. pneumoniae*, which represented coliforms in this study. *K. pneumoniae* showed the greatest dependence on dosage rather than time, but the international standards do not specify an endpoint for this organism, or for coliforms.

Kinetic modeling with three different equations or models, as well as the CT approach, indicated that the dosage of ferrate was more important than the contact time for all four organisms. A tailing effect was consistently observed, and because the Hom's equation is designed to model tailing and shoulder effects, it yielded the highest correlation coefficients. It was also relatively easy to perform using statistical software, and therefore was superior to the other models. Preliminary testing suggested that the tailing effect was not attributable to clumping. It might be the result of precipitation or decomposition of the ferrate, or to some other mechanism, such as resistant subpopulations. Onboard testing should reveal whether this will be a significant problem. If it is the result of rapid decomposition of ferrate, then this is an advantage for subsequent release into the environment.

The 2 mg/l suggested dosage would exceed Florida iron discharge standards, but the natural alkalinity of seawater favors precipitation of the remaining iron within the tank, requiring later removal with ballast tank sludge. It is also possible that lower bacterial concentrations in ballast water will reduce the dosage required, based on the effect of starting bacterial concentration on log reductions noted in Chapter 3.

The disinfection properties of ferrate need to be validated under a variety of field conditions, including a range of initial bacterial concentrations and organic content, but they appear to be significant with various pH values and salinities. Ferrate compares favorably with other candidates for ballast water treatment, and shows promise as an environmentally-friendly, effective disinfectant.

CHAPTER 5 – RECOMMENDATIONS FOR FUTURE STUDY

Obviously, an important next step is onboard testing of ferrate for ballast water disinfection. Ideally, results should be correlated with organic content, bacterial types and concentrations, pH values, temperatures, and other potentially relevant parameters. This would be important not only from a scientific standpoint, but also from a marketing standpoint, in order to extrapolate results to a variety of ballast water conditions encountered worldwide. It is reasonable to expect that field conditions, particularly organic content, might lower observed log reductions, since removal of nutrient broth was required for optimization of experimental procedure (Appendix A). After obtaining acceptable kill rates of a dinoflagellate alga by ozone in ballast water, Oemcke and van Leeuwen (2005) concluded that corrosion products and organic matter in ballast water storage tanks would probably lower efficiency to an unacceptable level, and that the disinfectant would have to be applied during ballast intake. Likewise, ferrate efficiency will depend not only upon field conditions, but also on practical aspects of application.

Precipitation of iron for disposal in the post-treatment sludge should also be explored. This might be necessary to meet simultaneous requirements for disinfection and iron discharge. As mentioned, the maximum iron standard ranges from 0.3-1.0 mg/l within Florida waters, which might make it problematic to achieve sufficient log reductions, depending on bacterial concentrations, organic content, and other factors and constraints. Also as mentioned, iron release might encourage bacterial, algal, and phytoplanktonic blooms in areas where iron is the limiting nutrient. Although the vast volume of ocean water will undoubtedly dilute this effect, the potential for harmful

effects on the environment, and certainly the legal discharge standards, must be addressed. An initial study by another graduate student (Adham Mukkatash) indicated approximately an 85% reduction of ferrate in solution after 30 min at ambient temperature, and the redox equilibria of ferrate favor precipitation, but the iron concentration in solution and in precipitate needs to be established, particularly onboard. The configuration of the onboard ballast water treatment and disposal will probably dictate disinfection optimization. For example, if it is possible to withdraw sediment from the ballast water storage tanks, this might be an easy solution. It might also be easier to treat the ballast intake, as suggested by Oemcke and van Leeuwen (2005), but this might necessitate 100% kill if conditions in the storage tanks favor bacterial growth.

Another practical aspect of ferrate treatment is the determination of the bacterial community that one would expect to encounter in ballast water. Obviously, this will vary greatly, as researchers are discovering, but can probably be narrowed somewhat for a geographical region. Although all of the test organisms seemed to be susceptible to ferrate disinfection, possibly because of common strategies for iron uptake, as mentioned in Chapter 2, these were relatively homogeneous laboratory cultures. Environmental isolates of *Vibrio* have different types and amounts of iron-binding proteins (Chapter 2), but iron uptake by blood-borne strains is better studied than environmental strains, and the uptake of ferrate might differ from that of iron. Ferrate-resistant strains might be encountered in the oceanic environment. Detailed descriptions of the bacterial assemblages, and the relative effectiveness of ferrate on each, would provide interesting scientific information not only on ferrate disinfection, but also on iron uptake by environmental pathogens, and for ballast water researchers worldwide.

All of the practical considerations mentioned are applicable to almost all disinfection processes, so of course another recommendation is for cost-effectiveness comparisons with other ballast water disinfectant candidates. This will depend on the availability of other cost estimates for other disinfectants, but certainly a first step is estimating costs for ferrate disinfection after establishing onboard requirements. Also, estimates of stability and production variability onboard would assist in the evaluation of ferrate as a ballast water disinfectant, as well as other potential applications.

The tailing effect that was consistently observed remains an interesting and unexplained phenomenon. Experiments to elucidate its cause could prove enlightening. For example, to test for resistant subpopulations, as opposed to the precipitation or reduction of ferrate, the bacteria remaining from one disinfection could be disinfected a second time. Resistant subpopulations should result in much lower log reductions with the second disinfection, whereas higher secondary log reductions would suggest that the original ferrate precipitated, decomposed, or otherwise lost its effectiveness.

APPENDIX A: EXPERIMENTAL OUTLINE AND OPTIMIZATION

A.1 Ferrate Experiment Outline

A.1.1 Preparation of Solutions, Reagents, Materials, and Plates.

A. Preparation of R2A agar plates:

Dissolve 14.56 g of R2A agar in 800 mls of distilled water in a 1 l flask. Heat with stirring to dissolve the agar. Cover loosely and autoclave for 15 minutes at 121° C. Allow to cool until it is just cool enough to handle comfortably (45-50° C). Pour about 15 mls of agar into sterile Petri dishes, covering bottom uniformly. Close and allow to solidify for about one hour. Invert and dry overnight, then store in sealed bags at 4° C.

B. Dilution Buffer

1.25 ml stock phosphate solution + 5 ml MgCl-hydrate solution in 1 l H₂O;
autoclave

C. Stock Phosphate Solution

9.89 g potassium phosphate (KH₂PO₄) per l

D. MgCl-Hydrate solution

8.11 g MgCl₂-6H₂O per 100 ml

E. Sodium Thiosulfate 0.081 M

10 g NaThiosulfate in 500 mls water; autoclave

F. Instant Ocean (IO): 1/2 cup/gallon, autoclave

G. Sterile water (autoclaved).

H. Nutrient broth (NB)

8 g in 1 l. Autoclave.

- I. *E. Coli*, or other organism, pellet started in NB per vendor instructions.
Transfer to IO/NB after appropriate incubation time. Incubate 3-5 days, with gentle rocking. Record pH and FAUs/NTUs prior to expt.
- J. Autoclave flasks, beakers, centrifuge vials, etc.

A.1.2 Ferrate Preparation

- A. Ensure water bath is between water level bars.
- B. Dissolve CaOCl_2
 - 1. Measure weight of water cylinder, add 9.8-9.9 g H_2O , re-weigh cylinder.
 - 2. Measure weight of NaOH cylinder, add 32.5-32.6 g NaOH, re-weigh cylinder.
 - 3. Measure 2.698 g of CaOCl .
 - 4. Record all results. Exact measures will be used in spreadsheet (ss).
 - 5. Add water, NaOH, and CaOCl together in reactor. Stir for 90 mins at 7-8 on dial. Parafilm.
- C. Turn on spectrophotometer (switch turns on in 2 steps). Warm up ≥ 15 mins.
- D. Prepare Boric Solution
0.7098 g di-sodium hydrogen phosphate (anhydrous) + 0.3814 g sodium tetraborate decahydrate in 1 l vol. flask.
- E. Get computer/spectrophotometer ready. Go to 001 chem and 510 nm. Mode of operation = scope. Integration period = 19, ave = 1, boxcar = 0. Wash cuvette 3 times w/borate buffer, fill w/borate and insert, and w/spec set to

“scope” click on “reference” and check that peak is within the frame, and “store”. Cover hole, click on “dark”, make sure it’s a straight line, and “store”. Go to mode of operation, “absorbance”, and “scan”. This should be close to zero.

- F. Bring up Excel and ss.
- G. Turn on water bath at approx. 80 min, so that it is at 33° C when ferrate is added.
- H. Slowly add 5 g FeCl_3 to reactor. Set $t=0$.
- I. Every 10 min, add 3 drops ferrate to 50 mls boric acid. Stir. Weigh before and after and enter difference into ss to determine conversion yield.

Maximum is achieved at >80% or over 26 g/l of ferrate.
- J. Use ss to calculate necessary volumes for experiment.
- K. Clean all glassware with 50% HCl and reactor w/10% HCl. Rinse thoroughly w/ diH_2O

A.1.3 Experimental Procedure for HPC

- A. Use working solution (WS) of *E.Coli*, or other organism, incubated in 4:1 IO:NB for 3-5 days at room temp w/gentle shaking. Record pH and FAU/NTU of WS on day of expt.
- B. Prepare appropriate plate controls: open/hood, open/ctr, closed/hood, DB, IO.
- C. Determine appropriate dilutions of WS for TC and HPC.
- D. Apply ferrate per experimental. design, typically 5 mg/l final concentration in 200 mls. Ensure that all mixtures are being adequately stirred.

- E. At appropriate times and concentrations, stop reaction by reducing ferrate with sodium thiosulfate.
- F. Make serial dilutions w/dilution buffer. Plate onto R2A agar plates at appropriate dilutions.
- G. Allow plates to dry for 10-15 minutes. Invert and incubate at 26° C for 3-7 days.

A.1.4 Quantification of HPC

- A. Count all colonies.
- B. Optimum colony counts are 30-300. Less than 30 is statistically unreliable, and greater than 300 results in crowding, which inhibits growth.
- C. Multiply by dilution factor.
- D. Note that final numbers are expressed as cfu/ml. Since 0.1 ml of sample is typically plated, this counts as a dilution and must be included in the final calculation.

A.1.5 Experimental Procedure and Quantification for Quanti-Trays/TC (total coliform)/EC (*E. coli*)

- A. Salt water specimens must be diluted at least 1:10. To ensure that the sample is quenched immediately, have the sodium thiosulfate pellet already dissolved in an amount of sterile water appropriate for the dilution.

- B. 100 mls of diluted sample are mixed with one pack of substrate reagent in a sterile vessel, which is then capped and shaken till substrate dissolves.
Colilert-18 substrate is used for *K. pneumoniae* and *E. coli*; Enterolert is used for Enterococci.
- C. This mixture is poured into a Quant-Tray and sealed in the sealer.
- D. The trays are incubated at 35° C for 18 hours (Colilert-18) or 41° C for 24 hours (Enterolert).
- E. Results are read as follows for Colilert-18:
Colorless or very lightly yellow = negative
Clearly yellow = positive
E. coli will also fluoresce under uv light.
- F. Results are read as follows for Enterolert under uv light:
Colorless = negative
Clearly fluorescent = positive
- G. The number of positive small and large wells is compared to the chart to determine most probable number (MPN).
- H. This gives the MPN in the 100 ml sample. Divide by 100 to obtain MPN/ml, and multiply by any appropriate dilutions.

A.1.6 Sterile Technique—A Few Notes

The idea is to prevent any contamination of samples and procedures. Therefore:

- Use sterile gloves.
- Wipe down surfaces with disinfectant.

- Perform testing in biological safety cabinet (BSC).
- Flame vessel (e.g. bottle) openings before pouring.
- Get all reagents and equipment ready in BSC before beginning procedure.
- Do not touch agar or liquids.

A.2 Optimization of Experimental Procedure

A.2.1 Effect of Stirring or Vortexing

A.2.1.1 Effect of Stirring

Summary: The effect of stirring on disinfection effectiveness was investigated by treating aliquots of a suspension of *K. pneumoniae* in IO, in glass flasks on a stir plate, with 2 mg/l ferrate for three conditions: no stirring (NS), moderate stirring (MS, low speed on stir plate), or vigorous stirring (VS, high speed on stir plate, such that a vortex was apparent). Reaction aliquots were quenched at 5 and 15 mins, and *K. pneumoniae* was quantified by HPC. Figure A-1 summarizes the results, which indicate that stirring markedly increases the log reductions. Vigorous stirring is recommended for faster, greater log reductions.

Figure A-1: Effect of Stirring on Ferrate Effectiveness on *K. pneumoniae* Mortality.

A.2.1.2 Effect of Vortexing

Summary: The effect of vortexing on disinfection effectiveness was investigated by treating aliquots of a suspension of *E. coli* in IO in 50-ml disposable centrifuge tubes with 0.75-4 mg/l ferrate for two conditions: normal preparation (inverting tube after ferrate treatment and immediately prior to sampling) and vigorous vortexing (vortexing on high setting after ferrate treatment and immediately prior to each sampling event). Reaction aliquots were quenched at various times, and *E. coli* was quantified by the Quanti-Tray method. Figure A-2 summarizes the results, which indicate that vortexing markedly increased the log reductions. Vortexing is recommended for faster, greater log reductions.

Figure A-2: Effect of Vortexing on Ferrate Effectiveness on *E. coli* Mortality.

A.2.2 Effect of Washing of Bacterial Suspension

Summary: The effect of washing to remove NB on disinfection effectiveness was investigated by spinning a suspension of *E. coli* in 5:1 IO:NB once, resuspending an aliquot in IO (“1X Wash”, and re-spinning an aliquot and resuspending an aliquot in IO (“2X Wash”). This was repeated one more time (“3X Wash”). These aliquots were then treated with 0.5 mg/l ferrate, and reaction aliquots were quenched at 5 min. *E. coli* was quantified by the Quanti-Tray method, and compared with the starting bacterial concentration (N_0) for each of the three conditions. Figure A-3 summarizes the results, which indicate that washing repeatedly increased the log reductions. It should be noted that N_0 was not the same for these three conditions, but had a fairly small range of $3.2 \times 10^5 - 2.3 \times 10^6$ organisms/ml, and there is about a 2 log increase. Washing at least two times is recommended for greater log reductions.

Figure A-3: Effect of Washing on *E. coli* Mortality by 0.5 mg/l Ferrate.

A.2.3 Effect of Delay in Processing

Summary: Because large experiments with many stopped reactions at various times and dosages required a delay in processing, this experiment investigated whether this delay would impact results. The effect of delay was investigated by performing a small dosage-response experiment with two dosages and several contact times done in duplicate. The reactions were stopped at the appropriate times and dilutions by adding aliquots to thiosulfate in solution in 100-ml Coliform Test Vial, as described in the Methods section of Chapter 3. Samples were then either processed as quickly as possible (“Immed.”) or after a 3-hr delay (“Wait”). Results are graphed in Figure A-4 and indicate that a delay in process would not adversely affect results.

Figure A-4: Effect of Delay in Processing on *E. coli* Log Reductions.

**APPENDIX B: SUMMARY GRAPHS OF DOSE RESPONSE
CURVES FOR TEST ORGANISMS TO FERRATE DISINFECTION**

Each graph represents means and SDs of four experiments.

Figure B-1: Summary of Dose Response Curves for *E. coli*.

Figure B-2: Summary of Dose Response Curves for *K. pneumoniae*.

Figure B-3: Summary of Dose Response Curve for Enterococci.

Figure B-4: Summary of Dose Response Curves for *V. cholerae*.

**APPENDIX C: SAMPLE CALCULATIONS OF KINETIC MODEL
CONSTANTS AND PH AND SALINITY EFFECTS**

C.1 Calculations for Chick-Watson and Hom's Coefficients

The following are the series of calculations conducted to obtain the Chick-Watson and Hom's coefficients for one experiment, in this case for *E. coli*, experiment # 79. These calculations were conducted for each of four dosage-response experiments for each of the four organisms. Please see Appendix E for raw data, or spreadsheets for all experiments are available upon request.

1. Quanti-Tray wells were read and tabulated per the procedure in Appendix A for each dosage and time. For example, in this experiment, for 0.25 mg/l ferrate at one min., two dilutions were done, 10^3 and 10^6 . For the lower dilution, all 49 large and 48 small wells were positive, which according to the MPN table results in a value of >2419.6. For the 10^6 dilution, 45 large and 15 small wells were positive, which corresponds to a value of 157.6 organisms per 100 mls of sample. This value is used, since the 10^3 dilution value is outside the linearity of the test procedure. It is then multiplied by the dilution and divided by 100 to obtain organisms (MPN) per ml, such that $(157.6 \times 10^6)/100 = 157.6 \times 10^4$ organisms or MPN/ml. Please note that *V. cholerae* was done by plate method, so colonies on plates were instead counted, in duplicate for each dilution, to produce CFU/ml values. All other calculations were the same. Figure C-1 shows the graph of results from these readings and calculations, for experiment #79.

Figure C-1: Dosage-Response Curve for Sample Calculation.

2. The starting bacterial concentration (N_0 , which was calculated by the same procedure) was then divided by the value for each dosage and time (N_t) and the log of this is taken to obtain the log reduction for each dosage and time, as shown in Figure C-2.
3. To calculate the Chick-Watson constants, the negative natural log of N_t/N_0 vs. contact time was graphed log-log, as shown in Figure C-3, per the method of Metcalf & Eddy (2003). Trend lines were included for ease of reading.

Figure C-2: Log Reduction Graph for Sample Calculation.

Figure C-3: Chick-Watson Analysis for Sample Calculation: Step 1

4. For a 4-log removal, this corresponds with $N_t/N_0 = 0.0001$, so $-\ln(N_t/N_0) = 9.2$.

Thus, for each dosage, the contact time needed to achieve this log reduction was determined from Figure 19, as shown in Table C-1.

**Table C-1: Contact Times Needed
for Each Dosage (Part 2 of
Chick-Watson Analysis)**

Time to 99.99% Kill (min)	Ferrate Dosage (mg/l)
28	0.75
11	1.5
3	3
1	5

5. This was then plotted log-log, as shown in Figure C-4. A trend line was again included for ease of interpretation.
6. From this, the Chick-Watson n coefficient is equal to the negative of the inverse of the slope. The y -intercept was estimated from the graph (in this case as 5), and k is then calculated from the equation:

$$k = -\ln(N_t/N_0)/\exp^{(n \ln(y-int))}$$

Since the slope in this case is -0.57, then $n = 1.76$. Then, since $N_t/N_0 = 0.0001$ for a 4-log reduction, $k = 0.55$. This was done for each experiment for each organism, generating average n and k values.

Figure C-4: Part 2 of Chick-Watson Analysis for Sample Calculation (4-log reduction).

7. To generate Hom's coefficients, according to the method of the American Water Works Association (1999), three columns of values were generated for each time (t) and dosage (concentration, C) of ferrate: $\ln(t)$, $\ln(C)$, and $\ln(-\ln(N_t/N_0))$. These were then entered into an SPSS input file, and a linear regression was run with $\ln(t)$ and $\ln(C)$ as independent variables and $\ln(-\ln(N_t/N_0))$ as the dependent variable. From this, the coefficient for $\ln(t) = m$, the coefficient for $\ln(C) = n$, and $\exp^{(\text{coeff. for constant})} = k$. For experiment #79, Hom's $m = 0.236$, $n = 0.619$, and $k = 3.963$. Means and SDs were calculated for the four experiments.
8. Since the Chick-Watson law states that $\ln(N_t/N_0) = -k'C^nN_t$, actual vs. predicted values were compared by graphing $\ln(N_t/N_0)$ (actual, or experimental) against $-k'C^nN_t$ (predicted, from the calculated coefficients), as shown in Figure C-5. The correlation coefficient, R, was calculated by Excel.

Figure C-5: Chick-Watson Model vs. Actual Data for Sample Calculation.

9. Likewise, the Hom's model states that $\ln(N_t/N_0)$ (actual) = $k'C^n t^m$ (predicted), so, these were graphed, as shown in Figure C-6, and R was calculated in Excel.
10. Means and SDs for R values for both models were generated for each organism.

Figure C-6: Hom's Model vs. Actual Data for Sample Calculation.

C.2 Calculations for Oxidant Demand Coefficients

The oxidant demand equations were applied to the same dosage-response experiments used to generate the Chick-Watson and Hom's coefficients. However, since coefficients must be determined for separate time points, the four experiments for each organism were combined in order to generate enough data points.

1. N_t/N_0 was calculated, as in section C.1, for each concentration, and this was done for each time. Because of the closeness of time points, the rest of the analysis was done for 1, 5, 15, and 30 mins only.
2. $\log(C)$ and $-\log(N_t/N_0)$ were calculated and graphed. Linear regression was performed to obtain slope, intercept, and variance (R^2). Figure C-7 shows the resultant graph and equation for *K. pneumoniae* at $t = 1$ min.

Figure C-7: Graph for *K. pneumoniae* to Obtain Oxidant Demand Coefficients at 1 min.

3. The correlation coefficient R was calculated as the square root of the variance.
4. The slope is equal to the b coefficient, and the y-intercept is the d coefficient.

The x-intercept (initial oxidant demand) is solved from the equation for the linear regression, with appropriate conversions from the log values. I.e. $N_t/N_0 = 1$ (to correspond with point where survival begins to decline from unity), so -
 $\log(N_t/N_0) = 0$, then $x = (y-d)/b$, and initial oxidant demand = 10^x .

5. To determine whether the slopes (b coefficient) differed significantly for the different contact times, the equation:

$$t = (b_1 - b_1^*) / (s / \text{sq.rt.}(SS_x)) \quad (1)$$

was used to test the null hypothesis that $b_1 - b_1^* = 0$. In this equation, SS_x is the sum of squares for x (where x is log of ferrate concentration, C, and $SS_x = \sum (x - x_{\text{avg}})^2$), b_1^* is a slope other than b_1 , in this case, a slope for a different contact time, and s is the standard error of the regression. This can be calculated as the square root of the mean square error (MSE), which is defined as:

$$MSE = s^2 = (SS_y - b_1 S_{xy}) / (n - 2) \quad (2)$$

where :

$$SS_y = \sum (y - y_{\text{avg}})^2$$

$$S_{xy} = \sum (x - x_{\text{avg}})(y - y_{\text{avg}})$$

and n = number of observations

This is equivalent to the Excel function for the standard error of the regression of y and x (“STEYX”). Thus, for the example given in Figure 22 for *K. pneumoniae* at 1 min, these calculations result in the values given in Table C-2.

6. This was done for all contact times, and the slope of each one was compared with all the others (which becomes b_1^* in equation 1). The degrees of freedom for

each comparison was calculated as $n + n_1^* - 2$ where n_1^* is the number of observations for b_1^* . This was used to obtain the null hypothesis t values (t_{crit})

Table C-2: Sample Calculations for Oxidant Demand Equation Parameters and Statistics.

C (mg/l)	N/N ₀	log C	log(N/N ₀)	(x-x _{avg}) ²	Parameter	Value
0.75	0.4433	-0.12	0.35	0.241	b	5.27
1.5	0.0213	0.18	1.67	0.036	d	1.08
3	0.0011	0.48	2.98	0.012	n	20
4	0.0001	0.60	4.12	0.056	R ²	0.94
5	0.0000	0.70	5.65	0.111	R	0.97
0.75	0.4261	-0.12	0.37	0.241	x _{avg}	0.37
1.5	0.0255	0.18	1.59	0.036	SS _x	1.824
3	0.0010	0.48	3.02	0.012	s	0.413
4	0.0002	0.60	3.69	0.056		
5	0.0000	0.70	4.92	0.111		
0.75	0.2768	-0.12	0.56	0.241		
1.5	0.0063	0.18	2.20	0.036		
3	0.0002	0.48	3.68	0.012		
4	0.0001	0.60	3.98	0.056		
5	0.0000	0.70	5.19	0.111		
0.75	0.0897	-0.12	1.05	0.241		
1.5	0.0063	0.18	2.20	0.036		
3	0.0003	0.48	3.56	0.012		
4	0.0001	0.60	4.16	0.056		
5	0.0000	0.70	5.16	0.111		

from a standard Student's t test table. Plugging these values into equation 1 results in the values given in Table C-3.

Table C-3: Sample Calculations for Hypothesis Testing of Oxidant Demand Slope Comparisons.

b*(5min)	b*(15min)	b*(30min)
6.06	6.51	5.55
df(5min)	df(15min)	df(30min)
38	38	30
tcrit	tcrit	tcrit
2.025	2.025	2.042
tcalc(vs 5')	tcalc(vs 15')	tcalc(vs 30')
-1.816	-3.062	-0.636
Significant at p = 0.05 (alpha =0.025) level?		
no	yes	no

7. This was done for all organisms for the 1, 5, 15, and 30 min contact times.

C.3 Calculations for pH and Salinity Effects

1. MPNs at various times and dosages were determined for ferrate-treated and -untreated specimens, and the log reduction was calculated relative to the starting bacterial concentration, N_0 . For example, for the -1.5 pH condition (please see Section 3.1.2 for procedure outline), two different dilutions— 10^{-1} and 10^{-3} —were done at $t = 30$ min. The MPN/100 ml for these was 920.8 and 7.5, respectively, which calculated to 92 and 75 MPN/ml (N_t), respectively, after considering the dilution. If, as in this example, more than one dilution resulted in a value within linearity of the test, then, in general, an average of these, or the value that resulted in the smoothest curve, or the more conservative value was used. The log reduction was then calculated relative to N_0 ($\log (N_0/N_t)$). The same was done for the untreated specimen at this time point. Thus, for the example, the log reduction for the treated specimen was 5.72 and 0.09 for the untreated specimen, resulting in a delta log reduction of 5.63.
2. The delta log reductions were then tabulated for each condition. For pH, the delta log reductions for each pH condition and time point were tabulated for each dosage. For salinity, the delta log reductions for each salinity condition, time point, and dosage were tabulated.
3. These were then entered into S-PLUS 2000 and a fixed effect ANOVA of a multi-factorial design was run, using time, dosage, and pH or salinity as independent variables, and delta log reduction as the dependent variable.
4. Results are summarized in Appendix D.

APPENDIX D: SUMMARY OF STATISTICS

D.1 Summary of Statistics: Chick-Watson and Hom's Coefficients

Table D-1: Means and SDs for Chick-Watson and Hom's Coefficients.

Organism	Expt. #	CW means and SDs			Hom's means and SDS				Model vs Actual	
		n	k'	R	n	M	k	R	Rhom	Rcw
<i>E. coli</i>	79	1.756	0.545	0.793	0.619	0.236	3.963	0.962	0.970	0.793
	80	1.917	0.616	0.766	0.667	0.194	4.306	0.940	0.950	0.765
	92	2.545	1.394	0.822	0.291	0.150	6.896	0.973	0.965	0.822
	93	2.113	0.904	0.722	0.393	0.163	6.443	0.971	0.966	0.736
	mean	2.083	0.865	0.776	0.493	0.186	5.402	0.962	0.963	0.779
	sd	0.341	0.385	0.043	0.180	0.038	1.482	0.015	0.009	0.037
<i>K. pneumoniae</i>	86	6.939	0.004	0.566	1.025	0.229	2.257	0.973	0.970	0.606
	87	5.246	0.008	0.636	0.933	0.248	2.223	0.974	0.979	0.632
	88	4.841	0.013	0.723	0.718	0.225	3.459	0.960	0.969	0.708
	89	4.529	0.025	0.746	0.696	0.185	3.796	0.980	0.980	0.721
	mean	5.389	0.013	0.668	0.843	0.222	2.934	0.972	0.975	0.667
	sd	1.074	0.009	0.083	0.162	0.026	0.813	0.008	0.006	0.056
Enterococci	74	2.284	0.108	0.841	0.895	0.399	1.815	0.972	0.967	0.793
	75	2.737	0.057	0.805	0.627	0.247	2.795	0.984	0.948	0.765
	81	2.323	0.441	0.737	0.687	0.224	3.808	0.960	0.958	0.823
	82	2.230	0.688	0.709	0.738	0.249	3.572	0.936	0.964	0.736
	mean	2.394	0.324	0.773	0.737	0.280	2.998	0.963	0.958	0.794
	sd	0.232	0.297	0.061	0.115	0.080	0.899	0.020	0.010	0.029
<i>V. cholerae</i>	94	2.786	0.041	0.651	0.413	0.152	4.336	0.934	0.934	0.651
	95	3.037	0.023	0.733	0.427	0.139	4.092	0.963	0.960	0.733
	96	3.404	0.012	0.713	0.413	0.168	3.626	0.931	0.931	0.713
	97	2.801	0.061	0.778	0.376	0.146	4.092	0.963	0.966	0.778
	mean	3.007	0.034	0.719	0.407	0.151	4.037	0.948	0.948	0.719
	sd	0.289	0.021	0.053	0.022	0.012	0.297	0.018	0.018	0.053

D.2 Summary of Statistics: Oxidant Demand Equation Coefficients and Hypothesis Testing

D.2.1 Oxidant Demand Coefficients and Hypothesis Testing for *E. coli*

time (min)	n	s	SS _x	b	d	R ²	x _{avg}	b* (1min)	b* (5min)	b* (15min)	b* (30min)	df (1min)	df (5min)	df (15min)	df (30min)
1	20	0.388	3.296	3.06	2.43	0.92	0.25		4.231	4.539	4.976		38	36	30
5	20	0.465	3.296	4.23	3.60	0.94	0.25	3.057		4.539	4.976	38		36	30
15	18	0.514	3.099	4.54	4.02	0.95	0.20	3.057	4.231		4.976	36	36		28
30	12	0.335	1.367	4.98	4.44	0.96	-0.004	3.057	4.231	4.539		30	30	28	

t _{calc} (vs 1')	t _{calc} (vs 5')	t _{calc} (vs 15')	t _{calc} (vs 30')	t _{crit}	t _{crit}	t _{crit}	t _{crit}	Significant at p = 0.05 (alpha =0.025) level?			
	-4.997	-5.872	-8.106		2.025	2.03	2.042		yes	yes	yes
4.997		-1.125	-2.847	2.025		2.03	2.042	yes		no	yes
5.872	1.125		-1.539	2.03	2.03		2.048	yes	no		no
8.106	2.847	1.539		2.042	2.042	2.048		yes	yes	no	

D.2.2 Oxidant Demand Coefficients and Hypothesis Testing for *K. pneumoniae*

time (min)	n	s	SS _x	b	d	R ²	x _{avg}	b* (1min)	b* (5min)	b* (15min)	b* (30min)	df (1min)	df (5min)	df (15min)	df (30min)
1	20	0.413	1.824	5.27	1.08	0.94	0.58		6.058	6.511	5.553		38	38	30
5	20	0.754	1.824	6.06	2.23	0.87	0.37	5.273		6.511	5.553	38		38	30
15	20	0.679	1.824	6.51	2.52	0.90	0.37	5.273	6.058		5.553	38	38		30
30	12	0.580	0.725	5.55	2.83	0.87	0.18	5.273	6.058	6.511		30	30	30	

t _{calc} (vs 1')	t _{calc} (vs 5')	t _{calc} (vs 15')	t _{calc} (vs 30')	t _{crit}	t _{crit}	t _{crit}	t _{crit}	Significant at p = 0.05 (alpha =0.025) level?			
	-1.816	-3.062	-0.636		2.025	2.025	2.042		no	yes	no
1.816		-0.854	0.854	2.025		2.025	2.042	no		no	no
3.062	0.854		1.717	2.025	2.025		2.042	yes	no		no
0.636	-0.854	-1.717		2.042	2.042	2.042		no	no	no	

D.2.3 Oxidant Demand Coefficients and Hypothesis Testing for Enterococci

time (min)	n	s	SS _x	b	d	R ²	$\bar{x}_{avg}$	b* (1min)	b* (5min)	b* (15min)	b* (30min)	df (1min)	df (5min)	df (15min)	df (30min)
1	20	0.743	4.189	2.79	1.76	0.77	0.13		3.529	3.423	3.706		38	34	30
5	20	0.550	4.189	3.53	2.75	0.91	0.13	2.788		3.423	3.706	38		34	30
15	16	0.462	2.995	3.42	3.04	0.91	-0.02	2.788	3.529		3.706	34	34		26
30	12	0.462	1.232	3.71	3.32	0.89	-0.18	2.788	3.529	3.423		30	30	26	

t _{calc} (vs 1')	t _{calc} (vs 5')	t _{calc} (vs 15')	t _{calc} (vs 30')	t _{crit}	t _{crit}	t _{crit}	t _{crit}	Significant at p = 0.05 (alpha =0.025) level?			
	-2.346	-1.997	-2.509		2.025	2.033	2.042		yes	no	yes
2.346		0.397	-0.576	2.025		2.033	2.042	yes		no	no
1.997	-0.397		-0.891	2.033	2.033		2.056	no	no		no
2.509	0.576	0.891		2.042	2.042	2.056		yes	no	no	

D.2.4 Oxidant Demand Coefficients and Hypothesis Testing for *Vibrio cholerae*

time (min)	n	s	SS _x	b	d	R ₂	x _{avg}	b* (1min)	b* (5min)	b* (15min)	b* (30min)	df (1min)	df (5min)	df (15min)	df (30min)
1	20	0.304	2.837	2.10	1.78	0.88	0.28		2.555	2.438	2.662		38	34	30
5	20	0.212	2.837	2.55	2.54	0.96	0.28	2.098		2.438	2.662	38		34	30
15	16	0.300	2.237	2.44	2.76	0.90	0.17	2.098	2.555		2.662	34	34		26
30	12	0.347	0.983	2.66	2.94	0.85	0.032	2.098	2.555	2.438		30	30	26	

t _{calc} (vs 1')	t _{calc} (vs 5')	t _{calc} (vs 15')	t _{calc} (vs 30')	t _{crit}	t _{crit}	t _{crit}	t _{crit}	Significant at p = 0.05 (alpha =0.025) level?			
	-2.975	-1.788	-2.392		2.025	2.033	2.042		yes	no	yes
2.975		0.726	-0.530	2.025		2.033	2.042	yes		no	no
1.788	-0.726		-0.879	2.033	2.033		2.056	no	no		no
2.392	0.530	0.879		2.042	2.042	2.056		yes	no	no	

D.3 Summary Statistics for pH and Salinity Effects and Anti-Clumping Measures

Table D-2: Summary of pH Statistics for 0.75 mg/l Ferrate Dosage.

Comparison: All three pH condtions.					
Variable	DF	Sum of Squares	Mean Square	F Value	Pr (F)
pH	2	5.86	2.93	54.5	2.7×10^{-8}
Time	3	10.45	3.48	63.6	8.9×10^{-10}
Residuals	18	0.98	0.054		
Comparison: Acid vs. neutral pH.					
Variable	DF	Sum of Squares	Mean Square	F Value	Pr (F)
pH	1	3.80	3.80	56.8	1.2×10^{-5}
Time	3	7.61	2.54	37.9	4.3×10^{-6}
Residuals	11	0.74	0.067		
Comparison: Alkaline vs. neutral pH.					
Variable	DF	Sum of Squares	Mean Square	F Value	Pr (F)
pH	1	0.071	0.071	4.01	0.07
Time	3	4.84	1.61	90.5	5.0×10^{-8}
Residuals	11	0.19	0.017		

Table D-3: Summary of pH Statistics for 1.5 mg/l Ferrate Dosage.

Comparison: All three pH condtions.					
Variable	DF	Sum of Squares	Mean Square	F Value	Pr (F)
pH	2	5.93	2.96	129.3	2.1×10^{-11}
Time	3	10.1	3.36	146.6	7.8×10^{-13}
Residuals	18	0.41	0.023		
Comparison: Acid vs. neutral pH.					
Variable	DF	Sum of Squares	Mean Square	F Value	Pr (F)
pH	1	4.51	4.51	244.8	7.3×10^{-9}
Time	3	7.10	2.37	128.6	7.6×10^{-9}
Residuals	11	0.20	0.018		
Comparison: Alkaline vs. neutral pH.					
Variable	DF	Sum of Squares	Mean Square	F Value	Pr (F)
pH	1	0.00092	0.00092	0.041	0.84
Time	3	5.91	1.97	86.8	1.0×10^{-7}
Residuals	11	0.25	0.023		

Table D.3-3: Summary of Statistics for Salinity Effect

Variable	DF	Sum of Squares	Mean Square	F Value	Pr (F)
Salinity	1	0.36	0.36	3.58	0.063
Time	3	24.7	8.23	81.4	<10 ⁻⁸
Dosage	4	369	92.3	913.0	<10 ⁻⁸
Residuals	71	7.18	0.10		

Table D-4: Summary of Statistics for Anti-Clumping (AC) Measures.

Comparison: Low-Speed Centrifugation vs. Normal Preparation.					
Variable	DF	Sum of Squares	Mean Square	F Value	Pr (F)
AC Measure	1	4.36	4.36	16.7	2.1×10^{-4}
Dosage	3	37.7	12.6	47.9	$<10^{-10}$
Time	1	4.31	4.31	16.5	2.2×10^{-4}
Residuals	40	10.5	0.26		
Comparison: Vortexing vs. Normal Preparation.					
Variable	DF	Sum of Squares	Mean Square	F Value	Pr (F)
AC Measure	1	5.94	5.94	8.20	0.0067
Dosage	3	32.7	10.9	15.1	1.2×10^{-6}
Time	1	21.4	21.4	29.5	3.2×10^{-6}
Residuals	39	28.2	0.72		

APPENDIX E: RAW EXPERIMENTAL DATA

Experiment numbers correspond to numbers in the lab notebooks, which are numbered chronologically. Raw data can also be supplied as Excel files upon request. Gaps in the numbering sequences in this appendix are attributable to experiments to establish protocol or to experiments of questionable success. Experiments are arranged here by purpose. Nd means “not done”, awcg means “area with confluent growth”.

E.1 Dosage Response Experiments with E. coli

Expt. #	23				
Date of Expt.:	4/12/2005				
Date HPC read:	4/15/2005				
Purpose:	Investigate effect of time on disinfection w/2 mg/l ferrate				
WS:	E. Coli in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O				
Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10^-n)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		0			0
QC open, counter		0			0
dilution buffer		0			0
Instant Ocean		nd			nd
All specs. w/ferrate stopped w/TS; all concs. are 2 mg/ l					
WS 0'	6	18	22	20	2.00E+07
WS 0'	5	206	199	202.5	2.03E+07
2 mg/l, 10'	3	129	153	141	1.41E+05
2 mg/l, 25'	3	58	69	63.5	6.35E+04
2 mg/l, 25'	4	7	5	6	6.00E+04
2 mg/l, 50'	2	158	177	167.5	1.68E+04
3 mg/l, 50'	3	18	35	26.5	2.65E+04

2 mg/l, 75'	2	72	76	74	7.40E+0 3
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Expt. #:	30
Date of Expt.:	5/3/2005
Date HPC read:	5/6/2005
Purpose:	Investigate effect of time on effectiveness of disinfection w/5 mg/l ferrate
WS:	E Coli in IO:NB, 600 mls as a 5:1 dilution, grown for 2 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		0			0
QC open, counter		3			3
dilution buffer		0			0
Instant Ocean		nd			nd
All specs. w/ferrate stopped w/TS; all concs. are 5 mg/ l					
WS 0'	6	63	66	64.5	6.45E+07
WS 0'	7	6	9	7.5	7.50E+07
5 mg/l, 15'	1	2	1	1.5	1.50E+01
5 mg/l, 15'	1,1ml	35	cg	35	3.50E+01
5 mg/l, 60'	1	0	0	0	0.00E+00
5 mg/l, 60'	1, 1 ml	0	0	0	0.00E+00

Expt. #:	32
Date of Expt.:	5/3/2005
Date HPC read:	5/17/2005
Purpose:	Investigate effect of time on effectiveness of disinfection w/5 mg/l ferrate
WS:	E Coli in IO:NB, 600 mls as a 5:1 dilution, grown for 2 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		0			0
QC open, counter		1			1
dilution buffer		nd			nd
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 5 mg/ l					
WS 0'	5	125	119	122	1.22E+07
WS 0'	6	23	14	18.5	1.85E+07
5 mg/l, 15'	1	18	16	17	1.70E+07
5 mg/l, 15'	1, 1 ml	38	cg	38	3.80E+01
5 mg/l, 60'	1	0	0	0	0.00E+00
5 mg/l, 60'	1, 1 ml	0	0	0	0.00E+00

Expt. #:	34
Date of Expt.:	5/3/2005
Date HPC read:	5/6/2005
Purpose:	Investigate effect of time on effectiveness of disinfection w/5 mg/l ferrate
WS:	<i>E. Coli</i> in IO:NB, 600 mls as a 5:1 dilution, grown for 2 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		nd			nd
QC open, counter		nd			nd
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 5 mg/ l					
WS 0'	5	111	130	120.5	1.21E+07
WS 0'	6	15	19	17	1.70E+07
5 mg/l, 15'	1	0	0	0	0.00E+00
5 mg/l, 15'	1, 1 ml	3	0	1.5	1.50E+00
5 mg/l, 60'	1	0	0	0	0.00E+00
5 mg/l, 60'	1, 1 ml	0	0	0	0.00E+00

Expt. #:	45
Date of Expt.:	5/25/2005
Date HPC read:	5/30/2005
Purpose:	Investigate short contact times for disinfection w/2 mg/l ferrate
WS:	E Coli in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 100 mls 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		nd			nd
QC open, counter		2			2
dilution buffer		nd			nd
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 2 mg/ l					
WS 0'	6	57	52	54.5	5.45E+07
WS 0'	7	18	4	11	1.10E+08
2'	1	29	30	29.5	2.95E+02
5'	1	1	6	3.5	3.50E+01
10'	1,1 ml	0	3	1.5	1.50E+00
30'	1, 1 ml	1	0	0.5	5.00E-01

Expt. #:	51
Date of Expt.:	6/7/2005
Date HPC read:	6/13/2005
Purpose:	Investigate short contact times for disinfection w/2 mg/l ferrate
WS:	E Coli in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 200 mls 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		nd			nd
QC open, counter		3			3
dilution buffer		nd			nd
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 2 mg/ l					
WS 0'	5	53	51	52	5.20E+06
WS 0'	6	5	6	5.5	5.50E+06
2'	1	9	13	11	1.10E+02
5'	1	2	2	2	2.00E+01
10'	1,1 ml	awcg	2	2	2.00E+00
30'	1, 1 ml	1	0	0	0.00E+00

Expt. #:	53
Date of Expt.:	6/15/2005
Date HPC read:	6/21/2005
Purpose:	Investigate short contact times for disinfection w/1.5 mg/l ferrate
WS:	E Coli in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 100 mls 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		nd			nd
QC open, counter		7			7
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 1.5 mg/ l					
WS 0'	5	34	30	32	3.20E+06
WS 0'	6	1	5	3	3.00E+06
2'	2	16	19	17.5	1.75E+03
5'	1	102	93	97.5	9.75E+02
8'	2	2	5	3.5	3.50E+02
10'	1	24	27	25.5	2.55E+02
15'	1	8	20	14	1.40E+02

Expt. #:	59
Date of Expt.:	6/27/2005
Date HPC read:	7/1/2005
Purpose:	Investigate short contact times of 0.5 mg/l ferrate on <i>E. coli</i>
WS:	<i>E. coli</i> in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O

Heterotrophic Plate Count					
Specimen/QC	Dilution (10 ⁻ⁿ)	Number of Colonies		Ave. Count	Colony Count
		Plate 1	Plate 2		
QC open, hood		1			1
QC closed, hood		nd			nd
QC open, counter		1			1
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 0.5 mg/ l					
WS 0'	5	38	35	36.5	3.65E+06
WS 0'	6	1	3	2	2.00E+06
1'	4	46	41	43.5	4.35E+05
2'	4	15	23	19	1.90E+05
3'	3	105	99	102	1.02E+05
4'	3	42	42	42	4.20E+04
5'	3	21	33	27	2.70E+04
7'	3	25	28	26.5	2.65E+04
10'	3	30	26	28	2.80E+04

Date of Expt:	7/1/2005									
Expt #:	63									
Date read:	7/2/2005									
Purpose:	Dosage Response Curve for E. coli									
WS:	E.coli grown in 5:1 IO:NB for 5 days, spun, washed X3 and resuspended in 5:1 IO/diH2O									
Conc.(mg/l)	time (min)		lg wls pos	sm wls pos	MPN/ 100ml		calc MPN/ml		log redctn.	
		dilution				MPN/ml		C*T		
0	0	5	49	36	866.4	866400	866400	0	N/A	
0	0	7	7	0	7.5	750000	750000	0	N/A	
0	0	9	1	0	1	10000000	10000000	0	N/A	
0.25	1	1	49	48	>2419.6	>24.2	>24.2	0.25	N/A	
0.25	1	3	49	47	2419.5	24195	24195	0.25	1.52	
0.25	1	5	21	6	34.5	34500	34500	0.25	1.37	
0.25	2	3	49	46	1986.3	19863	19863	0.5	1.61	
0.25	2	5	15	4	22.3	22300	22300	0.5	1.56	
0.25	3	1	49	48	>2419.6	>24.2	>24.2	0.75	N/A	
0.25	3	3	49	43	1413.6	14136	14136	0.75	1.76	
0.25	3	5	16	2	21.3	21300	21300	0.75	1.58	
0.25	5	3	49	44	1553.1	15531	15531	1.25	1.72	
0.25	5	5	14	0	16.1	16100	16100	1.25	1.70	
0.75	1	1	49	48	>2419.6	>24.2	>24.2	0.75	N/A	
0.75	1	4	38	3	72.7	7270	7270	0.75	2.05	
0.75	2	1	49	48	>2419.6	>24.2	>24.2	1.5	N/A	
0.75	2	4	27	2	40.4	4040	4040	1.5	2.30	
0.75	3	1	49	48	>2419.6	>24.2	>24.2	2.25	N/A	
0.75	3	4	16	2	21.3	2130	2130	2.25	2.58	
0.75	5	1	49	48	>2419.6	>24.2	>24.2	3.75	N/A	
0.75	5	4	10	1	12.1	1210	1210	3.75	2.82	
1.5	1	1	49	48	>2419.6	>24.2	>24.2	1.5	N/A	
1.5	1	4	15	1	18.7	1870	1870	1.5	2.64	
1.5	2	1	49	45	1732.9	173.29	173.29	3	3.67	
1.5	2	3	12	1	14.6	146	146	3	3.74	
1.5	3	1	49	44	1553.1	155.31	155.31	4.5	3.72	
1.5	3	3	9	0	9.8	98	98	4.5	3.92	
1.5	5	1	49	37	920.8	92.08	92.08	7.5	3.94	
1.5	5	3	6	0	6.3	63	63	7.5	4.11	

Date of Expt:	7/6/2005									
Expt #:	64									
Date read:	7/7/2005									
Purpose:	Dosage Response Curve for E. coli									
WS:	E.coli grown in 5:1 IO:NB for 5 days, then spun, washed X3 and resuspended in 5:1 IO/diH2O									
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	C*T	log redctn.	
0	0	3	49	48	>2419.6	>24.2	>24.2	0	N/A	
0	0	5	25	3	37.9	37900	37900	0	N/A	
0	0	7	0	0	0	0	100000	0	N/A	
0.25	1	1	49	48	>2419.6	>24.2	>24.2	0.25	N/A	
0.25	1	3	45	30	250.4	2504	2504	0.25	1.18	
0.25	1	5	6	0	6.3	6300	6300	0.25	0.78	
0.25	2	3	44	26	199.3	1993	1993	0.5	1.28	
0.25	2	5	4	1	5.2	5200	5200	0.5	0.86	
0.25	3	1	49	47	2419.6	241.96	241.96	0.75	2.19	
0.25	3	3	44	23	182.9	1829	1829	0.75	1.32	
0.25	3	5	3	0	3.1	3100	3100	0.75	1.09	
0.25	5	3	41	19	132	1320	1320	1.25	1.46	
0.25	5	5	0	0	0	0	1000	1.25	1.58	
0.75	1	1	49	47	2419.6	241.96	241.96	0.75	2.19	
0.75	1	4	3	0	3.1	310	310	0.75	2.09	
0.75	2	1	49	42	1299.65	129.97	129.97	1.5	2.46	
0.75	2	4	3	0	3.1	310	310	1.5	2.09	
0.75	3	1	49	36	866.4	86.64	86.64	2.25	2.64	
0.75	3	4	1	0	1	100	100	2.25	2.58	
0.75	5	1	48	21	285.1	28.51	28.51	3.75	3.12	
0.75	5	4	1	0	1	100	100	3.75	2.58	
1.5	1	1	40	9	95.9	9.59	9.59	1.5	3.60	
1.5	1	3	3	0	3.1	31	31	1.5	3.09	
1.5	2	1	26	1	36.9	3.69	3.69	3	4.01	
1.5	2	3	3	0	3.1	31	31	3	3.09	
1.5	3	1	14	1	17.3	1.73	1.73	4.5	4.34	
1.5	3	3	1	0	1	10	10	4.5	3.58	
1.5	5	1	5	0	5.1	0.51	0.51	7.5	4.87	
1.5	5	3	0	0	0	0	10	7.5	3.58	

Date of Expt:	7/6/2005									
Expt #:	65									
Date read:	7/7/2005									
Purpose:	Dosage Response Curve for E. coli									
WS:	E.coli grown in 5:1 IO:NB for 5 days, then spun, washed X3 and resuspended in 5:1 IO/diH2O									
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	C*T	log redctn.	
0	0	3	49	48	>2419.6	>24.2	>24.2	0	N/A	
0	0	5	25	3	37.9	37900	37900	0	N/A	
0	0	7	1	0	1	100000	100000	0	N/A	
0.25	1	1	49	48	>2419.6	>24.2	>24.2	0.25	N/A	
0.25	1	3	48	36	574.8	5748	5748	0.25	3.24	
0.25	1	5	11	0	12.2	12200	12200	0.25	2.91	
0.25	2	3	48	30	436	4360	4360	0.5	3.36	
0.25	2	5	10	0	11	11000	11000	0.5	2.96	
0.25	3	1	49	48	>2419.6	>24.2	>24.2	0.75	N/A	
0.25	3	3	46	25	241.5	2415	2415	0.75	3.62	
0.25	3	5	4	1	5.2	5200	5200	0.75	3.28	
0.25	5	3	45	16	162.4	1624	1624	1.25	3.79	
0.25	5	5	2	0	2	2000	2000	1.25	3.70	
0.75	1	1	49	48	>2419.6	>24.2	>24.2	0.75	N/A	
0.75	1	4	4	0	4.1	410	410	0.75	4.39	
0.75	2	1	49	35	816.4	81.64	81.64	1.5	5.09	
0.75	2	4	2	0	2	200	200	1.5	4.70	
0.75	3	1	49	29	579.4	57.94	57.94	2.25	5.24	
0.75	3	4	2	0	2	200	200	2.25	4.70	
0.75	5	1	48	24	328.2	32.82	32.82	3.75	5.48	
0.75	5	4	2	0	2	200	200	3.75	4.70	
1.5	1	1	36	4	67.7	6.77	6.77	1.5	6.17	
1.5	1	3	3	0	3.1	31	31	1.5	5.51	
1.5	2	1	22	3	32.3	3.23	3.23	3	6.49	
1.5	2	3	1	0	1	10	10	3	6.00	
1.5	3	1	7	2	9.6	0.96	0.96	4.5	7.02	
1.5	3	3	0	0	0	0	1000	4.5	4.00	
1.5	5	1	2	0	2	0.2	0.2	7.5	7.70	
1.5	5	3	0	0	0	0	1000	7.5	4.00	

Date of Expt:	8/17/2005								
Expt #:	79								
Date read:	8/18/2005								
Purpose:	Dosage Response Curve for E. coli								
WS:	E.coli grown in 5:1 IO:NB for 3 days, spun, washed X3 and resuspended in 5:1 IO/diH2O								
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	C*T	log redctn.
0	0	nd	nd	nd	nd	nd	nd	0	N/A
0	0	7	45	15	157.6	15760000	15760000	0	N/A
0	0	5	49	48	>2419.6	>24.2	>24.2	0	N/A
0.25	1	3	49	48	>2419.6	>24.2	>24.2	0.25	N/A
0.25	1	6	49	28	547.5	5475000	5475000	0.25	0.46
0.25	2	3	49	48	>2419.6	>24.2	>24.2	0.5	N/A
0.25	2	6	48	19	260.3	2603000	2603000	0.5	0.78
0.25	3	3	49	48	>2419.6	>24.2	>24.2	0.75	N/A
0.25	3	6	48	15	218.7	2187000	2187000	0.75	0.86
0.25	5	3	49	48	>2419.6	>24.2	>24.2	1.25	N/A
0.25	5	6	48	10	178.9	1789000	1789000	1.25	0.94
0.25	15	3	49	48	>2419.6	>24.2	>24.2	3.75	N/A
0.25	15	6	47	9	155.3	1553000	1553000	3.75	1.01
0.25	30	3	49	48	>2419.6	>24.2	>24.2	7.5	N/A
0.25	30	5	49	22	387.3	387300	387300	7.5	1.61
0.75	1	2	49	48	>2419.6	>24.2	>24.2	0.75	N/A
0.75	1	5	49	16	275.5	275500	275500	0.75	1.76
0.75	2	2	49	48	>2419.6	>24.2	>24.2	1.5	N/A
0.75	2	5	46	1	109.8	109800	109800	1.5	2.16
0.75	3	2	49	48	>2419.6	>24.2	>24.2	2.25	N/A
0.75	3	5	31	4	52.9	52900	52900	2.25	2.47
0.75	5	2	49	48	>2419.6	>24.2	>24.2	3.75	N/A
0.75	5	5	20	0	24.9	24900	24900	3.75	2.80
0.75	15	2	49	48	>2419.6	>24.2	>24.2	11.25	N/A
0.75	15	4	25	3	37.9	3790	3790	11.25	3.62
0.75	30	2	49	48	>2419.6	>24.2	>24.2	22.5	N/A
0.75	30	4	15	0	17.5	1750	1750	22.5	3.95
1.5	1	4	49	19	325.5	32550	32550	1.5	2.69
1.5	2	4	45	17	167.4	16740	16740	3	2.97
1.5	3	2	49	48	>2419.6	>24.2	>24.2	4.5	N/A
1.5	3	4	40	3	80.9	8090	8090	4.5	3.29
1.5	5	4	23	1	31.3	3130	3130	7.5	3.70
1.5	15	3	47	4	130.9	1309	1309	22.5	4.08
1.5	30	3	10	1	12.1	121	121	45	5.11
3	1	3	49	31	648.8	6488	6488	3	3.39
3	2	3	49	13	235.9	2359	2359	6	3.82
3	3	3	42	9	107.6	1076	1076	9	4.17
3	5	3	39	2	74.4	744	744	15	4.33
3	15	3	10	1	12.1	121	121	75	5.11
5	1	3	43	9	114.5	1145	1145	5	4.14
5	2	3	14	1	17.3	173	173	10	4.96
5	3	3	5	0	5.2	52	52	15	5.48
5	5	1	18	7	30.7	3.07	3.07	0	6.71

Date of Expt:	8/17/2005								
Expt #:	80								
Date read:	8/18/2005								
Purpose:	Dosage Response Curve for E. coli								
WS:	E.coli grown in 5:1 IO:NB for 3 days, spun, washed X3 and resuspended in 5:1 IO/diH2O								
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	C*T	log redctn.
0	0	nd	nd	nd	nd	nd	nd	0	N/A
0	0	7	45	15	157.6	15760000	15760000	0	N/A
0	0	5	49	48	>2419.6	>24.2	>24.2	0	N/A
0.25	1	3	49	48	>2419.6	>24.2	>24.2	0.25	N/A
0.25	1	6	49	29	579.4	5794000	5794000	0.25	0.43
0.25	2	3	49	48	>2419.6	>24.2	>24.2	0.5	N/A
0.25	2	6	48	28	396.8	3968000	3968000	0.5	0.60
0.25	3	3	49	48	>2419.6	>24.2	>24.2	0.75	N/A
0.25	3	6	49	15	261.3	2613000	2613000	0.75	0.78
0.25	5	3	49	48	>2419.6	>24.2	>24.2	1.25	N/A
0.25	5	6	48	12	193.5	1935000	1935000	1.25	0.91
0.25	15	3	49	48	>2419.6	>24.2	>24.2	3.75	N/A
0.25	15	6	47	7	145	1450000	1450000	3.75	1.04
0.25	30	3	49	48	>2419.6	>24.2	>24.2	7.5	N/A
0.25	30	5	49	44	1553.1	1553100	1553100	7.5	1.01
0.75	1	2	49	48	>2419.6	>24.2	>24.2	0.75	N/A
0.75	1	5	41	15	119.1	119100	119100	0.75	2.12
0.75	2	2	49	48	>2419.6	>24.2	>24.2	1.5	N/A
0.75	2	5	18	0	21.8	21800	21800	1.5	2.86
0.75	3	2	49	48	>2419.6	>24.2	>24.2	2.25	N/A
0.75	3	5	14	1	17.3	17300	17300	2.25	2.96
0.75	5	2	49	48	>2419.6	>24.2	>24.2	3.75	N/A
0.75	5	5	10	1	12.1	12100	12100	3.75	3.11
0.75	15	2	49	48	>2419.6	>24.2	>24.2	11.25	N/A
0.75	15	4	26	3	39.9	3990	3990	11.25	3.60
0.75	30	2	49	48	>2419.6	>24.2	>24.2	22.5	N/A
0.75	30	4	22	2	30.9	3090	3090	22.5	3.71
1.5	1	4	44	17	154.1	15410	15410	1.5	3.01
1.5	2	4	27	2	40.4	4040	4040	3	3.59
1.5	2	2	49	46	1986.3	1986.3	1986.3	3	3.90
1.5	3	4	16	0	18.9	1890	1890	4.5	3.92
1.5	5	3	46	8	137.6	1376	1376	7.5	4.06
1.5	15	3	33	4	58.3	583	583	22.5	4.43
1.5	30	3	28	1	41	410	410	45	4.58
3	1	3	49	27	517.2	5172	5172	3	3.48
3	2	3	36	11	82.3	823	823	6	4.28
3	3	3	29	2	44.8	448	448	9	4.55
3	5	1	49	32	686.7	68.67	68.67	15	5.36
3	15	1	46	18	190.4	19.04	19.04	75	5.92
5	1	3	43	9	114.5	1145	1145	5	4.14
5	2	3	18	1	23.1	231	231	10	4.83
5	3	3	7	0	7.5	75	75	15	5.32
5	5	1	22	0	28.2	2.82	2.82	0	6.75

Date of Expt:	9/13/2005							
Expt #:	92							
Date read:	9/14/2005							
Purpose:	Dosage Response Curve for E. coli							
WS:	E.coli grown in 5:1 IO:NB for 5 days, spun, washed X3, & resuspended in 5:1 IO/diH2O							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	log redctn.
0	0	7	44	14	141.4	14140000	14140000	N/A
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A
0.75	1	3	nd	nd	nd	nd	nd	nd
0.75	1	4	48	13	201.4	20140	20140	2.85
0.75	2	3	nd	nd	nd	nd	nd	nd
0.75	2	4	38	4	74.9	7490	7490	3.28
0.75	3	3	nd	nd	nd	nd	nd	nd
0.75	3	4	33	8	65.7	6570	6570	3.33
0.75	5	3	nd	nd	>2419.6	>24.2	>24.2	N/A
0.75	5	4	20	3	28.8	2880	2880	3.69
0.75	15	1	nd	nd	nd	nd	nd	nd
0.75	15	3	48	20	272.3	2723	2723	3.72
0.75	30	1	nd	nd	nd	nd	nd	nd
0.75	30	3	44	8	118.7	1187	1187	4.08
1.5	1	2	nd	nd	nd	nd	nd	nd
1.5	1	4	46	9	142.1	14210	14210	3.00
1.5	2	4	18	1	23.1	2310	2310	3.79
1.5	3	3	29	8	54.5	545	545	4.41
1.5	5	3	17	1	21.6	216	216	4.82
1.5	15	3	7	2	9.6	96	96	5.17
1.5	30	3	4	0	4.1	41	41	5.54
3	1	3	48	16	275.5	2755	2755	3.71
3	1	5	nd	nd	nd	nd	nd	nd
3	2	3	35	7	70.3	703	703	4.30
3	2	5	nd	nd	nd	nd	nd	nd
3	3	2	46	11	151.5	151.5	151.5	4.97
3	5	2	24	0	31.7	31.7	31.7	5.65
3	15	2	5	1	6.3	6.3	6.3	6.35
3	30	2	3	0	3.1	3.1	3.1	6.66
4	1	3	42	9	107.6	1076	1076	4.12
4	1	2	nd	nd	nd	nd	nd	nd
4	2	2	46	14	167	167	167	4.93
4	2	2	nd	nd	nd	nd	nd	nd
4	3	2	33	10	69.5	69.5	69.5	5.31
4	5	2	14	2	18.5	18.5	18.5	5.88
4	15	2	4	0	4.1	4.1	4.1	6.54
4	30	2	nd	nd	nd	nd	nd	nd
5	1	2	48	15	218.7	218.7	218.7	4.81
5	2	2	38	9	86.2	86.2	86.2	5.21
5	3	2	19	5	29.8	29.8	29.8	5.68
5	5	2	2	2	4.1	4.1	4.1	6.54
5	15	1	5	0	5.2	0.52	0.52	7.43

Date of									
Expt: 9/13/2005									
Expt #: 93									
Date read: 9/14/2005									
Purpose: Dosage Response Curve for E. coli									
E.coli grown in 5:1 IO:NB for 5 days, spun, washed X3 and resuspended in 5:1									
WS: IO/diH2O									
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	log redctn.	
0	0	nd	nd	nd	nd	nd	nd	N/A	
0	0	7	44	14	141.4	14140000	14140000	N/A	
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A	
0.75	1	3	49	48	>2419.6	>24.2	>24.2	N/A	
0.75	1	5	46	17	184.2	184200	184200	1.89	
0.75	2	3	nd	nd	nd	nd	nd	nd	
0.75	2	4	49	25	461.1	46110	46110	2.49	
0.75	3	3	nd	nd	nd	nd	nd	nd	
0.75	3	4	43	12	124.6	12460	12460	3.05	
0.75	5	3	nd	nd	>2419.6	>24.2	>24.2	N/A	
0.75	5	4	30	6	53.7	5370	5370	3.42	
0.75	15	1	nd	nd	nd	nd	nd	nd	
0.75	15	3	43	8	111.2	1112	1112	4.10	
0.75	30	1	nd	nd	nd	nd	nd	nd	
0.75	30	3	42	7	101.7	1017	1017	4.14	
1.5	1	2	nd	nd	nd	nd	nd	nd	
1.5	1	4	31	7	58.1	5810	5810	3.39	
1.5	2	4	6	0	6.3	630	630	4.35	
1.5	3	3	28	3	44.1	441	441	4.51	
1.5	5	3	15	1	18.7	187	187	4.88	
1.5	15	3	5	0	5.2	52	52	5.43	
1.5	30	3	2	0	2	20	20	5.85	
3	1	3	30	2	47.1	471	471	4.48	
3	1	5	nd	nd	nd	nd	nd	nd	
3	2	3	20	0	24.9	249	249	4.75	
3	2	5	nd	nd	nd	nd	nd	nd	
3	3	2	37	6	75.1	75.1	75.1	5.27	
3	5	2	19	3	27.2	27.2	27.2	5.72	
3	15	2	5	1	6.3	6.3	6.3	6.35	
3	30	2	1	1	2	2	2	6.85	
4	1	3	21	2	29.2	292	292	4.69	
4	1	2	nd	nd	nd	nd	nd	nd	
4	2	3	5	0	5.2	52	52	5.43	
4	2	2	nd	nd	nd	nd	nd	nd	
4	3	2	16	1	20.1	20.1	20.1	5.85	
4	5	2	4	2	6.2	6.2	6.2	6.36	
4	15	2	2	0	2	2	2	6.85	
4	30	2	nd	nd	nd	nd	nd	nd	
5	1	2	44	9	122.3	122.3	122.3	5.06	
5	2	2	15	2	19.9	19.9	19.9	5.85	
5	3	2	5	0	5.2	5.2	5.2	6.43	
5	5	2	1	0	1	1	1	7.15	
5	15	1	4	0	4.1	0.41	0.41	7.54	

E.2 Dosage Response Experiments for *K. pneumoniae*

Expt. #:	31
Date of Expt.:	5/3/2005
Date HPC read:	5/6/2005
Purpose:	Dosage response of <i>K. pneumoniae</i> to 5 mg/l ferrate
WS:	<i>K. pneumoniae</i> in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		0			0
QC open, counter		3			3
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 5 mg/ l					
WS 0'	6	34	38	36	3.60E+07
WS 0'	7	1	4	2.5	2.50E+07
5 mg/l, 15'	1	58	57	57.5	5.75E+02
5 mg/l, 15'	2	13	8	10.5	1.05E+01
5 mg/l, 60'	1	cont	0	cont	N/A
5 mg/l, 60'	1, 1 ml	0	0	0	0.00E+00

Expt. #:	33
Date of Expt.:	5/3/2005
Date HPC read:	5/6/2005
Purpose:	Dosage response of <i>K. pneumoniae</i> to 5 mg/l ferrate
WS:	<i>K. pneumoniae</i> in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		0			0
QC open, counter		1			1
dilution buffer		nd			nd
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 5 mg/ l					
WS 0'	6	29	33	31	3.10E+07
WS 0'	7	3	1	2	2.00E+07
5 mg/l, 15'	1	4	6	5	5.00E+01
5 mg/l, 15'	1, 1ml	48	awc g	48	4.80E+01
5 mg/l, 60'	1	0	0	0	0.00E+00
5 mg/l, 60'	1, 1 ml	0	0	0	0.00E+00

Expt. #:	35
Date of Expt.:	5/3/2005
Date HPC read:	5/6/2005
Purpose:	Dosage response of <i>K. pneumoniae</i> to 2 mg/l ferrate
WS:	<i>K. pneumoniae</i> in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		nd			nd
QC open, counter		nd			nd
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 5 mg/ l					
WS 0'	6	35	30	32.5	3.25E+07
WS 0'	7	2	5	3.5	3.50E+07
5 mg/l, 15'	1	0	0	0	0.00E+00
5 mg/l, 15'	1, 1ml	2	5	3.5	3.50E+00
5 mg/l, 60'	1	0	0	0	0.00E+00
5 mg/l, 60'	1, 1 ml	0	0	0	0.00E+00

Expt. #:	60
Date of Expt.:	6/27/2005
Date HPC read:	7/1/2005
Purpose:	Short contact times of 0.5 mg/l ferrate on <i>K. pneumoniae</i>
WS:	<i>K. pneumoniae</i> in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O

Heterotrophic Plate Count					
Specimen/QC	Dilution (10 ⁻ⁿ)	Number of Colonies		Ave. Count	Colony Count
		Plate 1	Plate 2		
QC open, hood		1			1
QC closed, hood		nd			nd
QC open, counter		1			1
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 0.5 mg/ l					
WS 0'	4	87	80	83.5	8.35E+05
WS 0'	5	12	10	11	1.10E+06
1'	4	38	41	39.5	3.95E+05
2'	4	18	27	22.5	2.25E+05
3'	3	140	129	134.5	1.35E+05
4'	3	77	79	78	7.80E+04
5'	3	58	33	45.5	4.55E+04
7'	3	24	30	27	2.70E+04
10'	2	208	246	227	2.27E+04

Date of Expt:	9/1/2005							
Expt #:	86							
Date read:	9/2/2005							
Purpose:	Dosage Response Curve for <i>K. pneumoniae</i>							
WS:	<i>K. pneumoniae</i> grown in 5:1 IO:NB for 3 days, spun, washed X3, and resuspended in 5:1 IO/diH2O							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/100ml	MPN/ml	calc MPN/ml	log redctn.
0	0	3	49	48	nd	nd	nd	N/A
0	0	7	32	7	60.9	6090000	6090000	N/A
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A
0.75	1	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	1	6	47	24	270	2700000	2700000	0.35
0.75	2	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	2	6	43	16	139.1	1391000	1391000	0.64
0.75	3	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	3	6	38	7	81.6	816000	816000	0.87
0.75	5	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	5	6	17	2	22.8	228000	228000	1.43
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A
0.75	15	5	45	16	162.4	162400	162400	1.57
0.75	30	2	49	48	>2419.6	>24.2	>24.2	N/A
0.75	30	5	42	17	133.6	133600	133600	1.66
1.5	1	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	1	4	49	42	1299.7	129970	129970	1.67
1.5	2	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	2	5	22	2	30.9	30900	30900	2.29
1.5	3	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	3	4	48	17	238.2	23820	23820	2.41
1.5	5	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	5	5	9	0	9.8	9800	9800	2.79
1.5	15	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	15	4	34	11	74.8	7480	7480	2.91
1.5	30	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	30	4	23	5	36.8	3680	3680	3.22
3	1	4	35	4	64.4	6440	6440	2.98
3	2	2	49	29	579.4	579.4	579.4	4.02
3	3	2	49	17	290.9	290.9	290.9	4.32
3	3	4	3	0	3.1	310	310	4.29
3	5	2	44	19	163.1	163.1	163.1	4.57
3	15	1	46	33	319.9	31.99	31.99	5.28
3	30	1	39	16	109	10.9	10.9	5.75
4	1	3	28	4	45.7	457	457	4.12
4	2	1	49	37	920.8	92.08	92.08	4.82
4	3	1	46	28	268.2	26.82	26.82	5.36
4	5	1	43	15	135.4	13.54	13.54	5.65
4	15	1	21	2	29.2	2.92	2.92	6.32
5	1	1	43	15	135.4	13.54	13.54	5.65
5	2	1	27	3	42	4.2	4.2	6.16
5	3	1	18	1	23.1	2.31	2.31	6.42
5	5	1	9	0	9.8	0.98	0.98	6.79
5	15	1	3	1	4.1	0.41	0.41	7.17

Date of Expt:	9/1/2005							
Expt #:	87							
Date read:	9/2/2005							
Purpose:	Dosage Response Curve for <i>K. pneumoniae</i>							
WS:	<i>K. pneumoniae</i> grown in 5:1 IO:NB for 3 days, spun, washed X3, and resuspended in 5:1 IO/diH2O							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/100ml	MPN/ml	calc MPN/ml	log redctn.
0	0	3	49	48	nd	nd	nd	N/A
0	0	7	32	7	60.9	6090000	6090000	N/A
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A
0.75	1	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	1	6	47	23	259.5	2595000	2595000	0.37
0.75	2	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	2	6	41	16	122.2	1222000	1222000	0.70
0.75	3	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	3	6	32	7	60.9	609000	609000	1.00
0.75	5	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	5	5	47	15	191.8	191800	191800	1.50
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A
0.75	15	5	43	7	108.1	108100	108100	1.75
0.75	30	2	49	48	>2419.6	>24.2	>24.2	N/A
0.75	30	4	49	33	727	72700	72700	1.92
1.5	1	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	1	4	49	44	1553.1	155310	155310	1.59
1.5	2	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	2	4	49	37	920.8	92080	92080	1.82
1.5	3	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	3	4	49	25	461.1	46110	46110	2.12
1.5	5	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	5	4	49	18	307.6	30760	30760	2.30
1.5	15	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	15	4	30	5	52	5200	5200	3.07
1.5	30	2	49	48	>2419.6	>24.2	>24.2	N/A
1.5	30	4	29	6	51.2	5120	5120	3.08
3	1	4	33	4	58.3	5830	5830	3.02
3	2	4	23	0	29.9	2990	2990	3.31
3	3	2	49	48	>2419.6	>24.2	>24.2	N/A
3	3	4	14	1	17.3	1730	1730	3.55
3	5	4	8	1	9.7	970	970	3.80
3	15	3	9	1	10.9	109	109	4.75
3	30	3	1	1	2	20	20	5.48
4	1	3	42	14	123.4	1234	1234	3.69
4	2	3	27	4	43.5	435	435	4.15
4	3	3	6	1	7.4	74	74	4.92
4	5	1	44	31	230	23	23	5.42
4	15	1	41	13	113	11.3	11.3	5.73
5	1	1	49	33	727	72.7	72.7	4.92
5	2	1	46	22	217.8	21.78	21.78	5.45
5	3	1	40	14	109.5	10.95	10.95	5.75
5	5	1	15	6	24.7	2.47	2.47	6.39
5	15	1	12	0	13.5	1.35	1.35	6.65

Date of Expt:	9/6/2005							
Expt #:	88							
Date read:	9/7/2005							
Purpose:	Dosage Response Curve for <i>K. pneumoniae</i>							
WS:	<i>K. pneumoniae</i> grown in 5:1 IO:NB for 3 days, spun, washed X3, and resuspended in 5:1 IO/diH2O							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/100ml	MPN/ml	calc MPN/ml	log redctn.
0	0	3	49	48	nd	nd	nd	N/A
0	0	7	48	23	313	31300000	31300000	N/A
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A
0.75	1	3	nd	nd	nd	nd	nd	nd
0.75	1	6	49	36	866.4	8664000	8664000	0.56
0.75	2	3	nd	nd	nd	nd	nd	nd
0.75	2	6	48	15	218.7	2187000	2187000	1.16
0.75	3	3	nd	nd	nd	nd	nd	nd
0.75	3	6	25	3	37.9	379000	379000	1.92
0.75	5	3	nd	nd	nd	nd	nd	nd
0.75	5	6	6	1	7.4	74000	74000	2.63
0.75	15	2	nd	nd	nd	nd	nd	nd
0.75	15	6	4	0	4.1	41000	41000	2.88
0.75	30	6	leakd	leakd	leaked	nd	nd	nd
0.75	30	3	49	46	1986.3	19863	19863	3.20
1.5	1	2	nd	nd	nd	nd	nd	nd
1.5	1	4	49	46	1986.3	198630	198630	2.20
1.5	2	2	nd	nd	nd	nd	nd	nd
1.5	2	4	49	36	866.4	86640	86640	2.56
1.5	3	2	nd	nd	nd	nd	nd	nd
1.5	3	4	48	29	416	41600	41600	2.88
1.5	5	2	nd	nd	nd	nd	nd	nd
1.5	5	4	46	22	217.8	21780	21780	3.16
1.5	15	2	nd	nd	nd	nd	nd	nd
1.5	15	4	43	17	143	14300	14300	3.34
1.5	30	2	nd	nd	nd	nd	nd	nd
1.5	30	3	49	40	1119.9	11199	11199	3.45
3	1	3	49	31	648.8	6488	6488	3.68
3	2	3	48	25	344.1	3441	3441	3.96
3	3	4	nd	nd	nd	nd	nd	nd
3	3	2	49	43	1413.6	1413.6	1413.6	4.35
3	5	2	48	30	436	436	436	4.86
3	15	2	47	13	178.5	178.5	178.5	5.24
3	30	2	35	12	80.5	80.5	80.5	5.59
4	1	3	48	24	328.2	3282	3282	3.98
4	2	2	49	34	770.1	770.1	770.1	4.61
4	3	2	45	19	178	178	178	5.25
4	5	2	33	8	65.7	65.7	65.7	5.68
4	15	1	32	2	52.1	5.21	5.21	6.78
5	1	2	48	13	201.4	201.4	201.4	5.19
5	2	1	49	20	344.8	34.48	34.48	5.96
5	3	1	32	13	71.9	7.19	7.19	6.64
5	5	1	4	2	6.2	0.62	0.62	7.70
5	15	1	2	0	2	0.2	0.2	8.19

Date of Expt:	9/6/2005							
Expt #:	89							
Date read:	9/7/2005							
Purpose:	Dosage Response Curve for <i>K. pneumoniae</i>							
WS:	<i>K. pneumoniae</i> grown in 5:1 IO:NB for 3 days, spun, washed X3, and resuspended in 5:1 IO/diH2O							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/100ml	MPN/ml	calc MPN/ml	log redctn.
0	0	3	49	48	nd	nd	nd	N/A
0	0	7	48	23	313	31300000	31300000	N/A
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A
0.75	1	3	nd	nd	nd	nd	nd	nd
0.75	1	6	47	25	280.9	2809000	2809000	1.05
0.75	2	3	nd	nd	nd	nd	nd	nd
0.75	2	6	43	16	139.1	1391000	1391000	1.35
0.75	3	3	nd	nd	nd	nd	nd	nd
0.75	3	6	28	4	45.7	457000	457000	1.84
0.75	5	3	nd	nd	nd	nd	nd	nd
0.75	5	6	17	0	20.3	203000	203000	2.19
0.75	15	2	nd	nd	nd	nd	nd	nd
0.75	15	6	10	1	12.1	121000	121000	2.41
0.75	30	6	8	0	8.6	86000	86000	2.56
0.75	30	3	49	46	1986.3	19863	19863	3.20
1.5	1	2	nd	nd	nd	nd	nd	nd
1.5	1	4	49	46	1986.3	198630	198630	2.20
1.5	2	2	nd	nd	nd	nd	nd	nd
1.5	2	4	49	34	770.1	77010	77010	2.61
1.5	3	2	nd	nd	nd	nd	nd	nd
1.5	3	4	49	25	461.1	46110	46110	2.83
1.5	5	2	nd	nd	nd	nd	nd	nd
1.5	5	4	46	22	217.8	21780	21780	3.16
1.5	15	2	nd	nd	nd	nd	nd	nd
1.5	15	4	47	14	185	18500	18500	3.23
1.5	30	2	nd	nd	nd	nd	nd	nd
1.5	30	3	49	37	920.8	9208	9208	3.53
3	1	3	49	36	866.4	8664	8664	3.56
3	2	3	46	24	233.3	2333	2333	4.13
3	3	4	nd	nd	nd	nd	nd	nd
3	3	2	49	33	727	727	727	4.63
3	5	2	47	25	280.9	280.9	280.9	5.05
3	15	2	40	4	83.3	83.3	83.3	5.57
3	30	2	19	1	24.6	24.6	24.6	6.10
4	1	3	47	18	214.2	2142	2142	4.16
4	2	3	36	3	65.7	657	657	4.68
4	3	2	49	16	275.5	275.5	275.5	5.06
4	5	2	35	6	68.3	68.3	68.3	5.66
4	15	1	37	8	79.8	7.98	7.98	6.59
5	1	2	49	11	214.3	214.3	214.3	5.16
5	2	1	49	34	770.1	77.01	77.01	5.61
5	3	1	45	9	131.4	13.14	13.14	6.38
5	5	1	2	0	2	0.2	0.2	8.19
5	15	1	0	0	0	0	0.1	8.50

E.3 Dosage Response Experiments for Enterococci

WS (27-29):	<i>Enterococcus faecilis</i> and <i>faecium</i> grown in 5:1 IO:NB for 5 days, spun, washed, and resuspended in 5:1 IO/diH2O							
Date of Expts:	5/3/2005							
Date read:	5/5/2005							
				lg wells pos	sm wells pos			log redctn
Condition	conc (mg/l)	time (min)	dilution			MPN	MPN/ml	
Expt. #:	27							
WS	0	0	6	44	10	125.9	1259000	0
15 min	5	15	1	12	1	14.6	1.46	5.9
60 min	5	60	1	0	0	0	0	6.1
Expt. #:	28							
WS		0	7	10	1	12.1	1210000	0
15 min	5	15	1	9	2	11	1.1	6.0
60 min	5	60	1	0	0	0	0	6.1
Expt. #:	29							
WS	0	6	41	12	110	1100000	1100000	0
15 min	15	1	3	0	3.1	0.31	0.31	6.6
60 min	60	1	0	0	0	0	1	6.0

Date of Expt:	8/4/2005							
Expt #:	74							
Date read:	8/5/2005							
Purpose:	Dosage Response Curve for Enterococci							
WS:	Enterococci grown in 5:1 IO:NB for 6 days, spun, washed X3, and resuspended in 5:1 IO/diH2O							
Conc.(mg/l)	time (min)		lg wls pos	sm wls pos	MPN/ 100ml		calc MPN/ml	log redctn.
		dilution				MPN/ml		
0	0	3	49	48	>2419.6	>2419.6	>2419.6	N/A
0	0	5	46	24	233.3	233300	233300	N/A
0	0	7	5	0	5	500000	500000	N/A
0.25	1	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.25	1	4	49	46	1986.3	198630	198630	0.07
0.25	2	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.25	2	4	49	43	1413.6	141360	141360	0.22
0.25	3	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.25	3	4	49	40	1119.9	111990	111990	0.32
0.25	5	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.25	5	4	49	34	770.1	77010	77010	0.48
0.25	15	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.25	15	4	49	21	365.4	36540	36540	0.81
0.25	30	3	49	47	2419.6	24196	24196	0.98
0.75	1	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.75	1	4	47	27	304.4	30440	30440	0.88
0.75	2	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.75	2	4	45	18	172.6	17260	17260	1.13
0.75	3	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.75	3	4	42	8	104.6	10460	10460	1.35
0.75	5	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.75	5	4	30	3	48.7	4870	4870	1.68
0.75	15	1	49	48	>2419.6	>2419.6	>2419.6	N/A
0.75	15	4	14	0	16.1	1610	1610	2.16
0.75	30	2	49	41	1203.3	1203.3	1203.3	2.29
1.5	1	3	49	35	816.4	8164	8164	1.46
1.5	2	3	48	22	298.7	2987	2987	1.89
1.5	3	1	49	48	>2419.6	>2419.6	>2419.6	N/A
1.5	3	3	43	12	124.6	1246	1246	2.27
1.5	5	2	49	33	727	727	727	2.51
1.5	15	1	49	40	1119.9	111.99	111.99	3.32
1.5	30	1	49	33	727	72.7	72.7	3.51
3	1	2	49	44	1553.1	1553.1	1553.1	2.18
3	2	1	49	46	1986.3	198.63	198.63	3.07
3	3	1	49	38	980.4	98.04	98.04	3.38
3	5	1	48	29	416	41.6	41.6	3.75
3	15	1	39	11	95.9	9.59	9.59	4.39
5	1	1	49	47	2419.6	241.96	241.96	2.98
5	2	1	48	20	272.3	27.23	27.23	3.93
5	3	1	29	4	48	4.8	4.8	4.69
5	5	1	9	1	10.9	1.09	1.09	5.33
5	3	1	45	9	131.4	13.14	13.14	6.38
5	5	1	2	0	2	0.2	0.2	8.19
5	15	1	0	0	0	0	0.1	8.50

Date of Expt:	8/4/2005								
Expt #:	75								
Date read:	8/5/2005								
Purpose:	Dosage Response Curve for Enterococci								
WS:	Enterococci grown in 5:1 IO:NB for 6 days, spun, washed X3, and resuspended in 5:1 IO/diH2O								
Conc.(mg/l)	time (min)		lg wls pos	sm wls pos	MPN/ 100ml		calc MPN/ml	log redctn.	
		dilution				MPN/ml			
0	0	3	49	48	>2419.6	>2419.6	>2419.6	N/A	
0	0	5	46	24	233.3	233300	233300	N/A	
0	0	7	5	0	5	500000	500000	N/A	
0.25	1	2	49	48	>2419.6	>2419.6	>2419.6	N/A	
0.25	1	4	49	35	816.4	81640	81640	0.46	
0.25	2	2	49	48	>2419.6	>2419.6	>2419.6	N/A	
0.25	2	4	49	29	579.4	57940	57940	0.60	
0.25	3	2	49	48	>2419.6	>2419.6	>2419.6	N/A	
0.25	3	4	48	28	396.8	39680	39680	0.77	
0.25	5	2	49	48	>2419.6	>2419.6	>2419.6	N/A	
0.25	5	4	49	22	387.3	38730	38730	0.78	
0.25	15	2	49	48	>2419.6	>2419.6	>2419.6	N/A	
0.25	15	4	48	21	285.1	28510	28510	0.91	
0.25	30	3	49	46	1986.3	19863	19863	1.07	
0.75	1	2	49	48	>2419.6	>2419.6	>2419.6	N/A	
0.75	1	4	47	18	206.4	20640	20640	1.05	
0.75	2	2	49	48	>2419.6	>2419.6	>2419.6	N/A	
0.75	2	4	45	15	157.6	15760	15760	1.17	
0.75	3	2	49	48	>2419.6	>2419.6	>2419.6	N/A	
0.75	3	4	42	4	93.2	9320	9320	1.40	
0.75	5	2	49	48	>2419.6	>2419.6	>2419.6	N/A	
0.75	5	4	36	3	65.7	6570	6570	1.55	
0.75	15	1	49	48	>2419.6	>2419.6	>2419.6	N/A	
0.75	15	4	22	0	28.2	2820	2820	1.92	
0.75	30	2	49	44	1553.1	1553.1	1553.1	2.18	
1.5	1	3	49	34	770.1	7701	7701	1.48	
1.5	2	3	48	20	272.3	2723	2723	1.93	
1.5	3	1	49	48	>2419.6	>2419.6	>2419.6	N/A	
1.5	3	3	40	12	106.7	1067	1067	2.34	
1.5	5	2	46	34	331.4	331.4	331.4	2.85	
1.5	15	1	49	40	1119.9	111.99	111.99	3.32	
1.5	30	1	49	27	517.2	51.72	51.72	3.65	
3	1	2	49	44	1553.1	1553.1	1553.1	2.18	
3	2	1	49	47	2419.6	241.96	241.96	2.98	
3	3	1	49	43	1413.1	141.31	141.31	3.22	
3	5	1	49	26	488.4	48.84	48.84	3.68	
3	15	1	49	15	261.3	26.13	26.13	3.95	
5	1	1	49	46	1986.3	198.63	198.63	3.07	
5	2	1	49	33	727	72.7	72.7	3.51	
5	3	1	38	10	88.6	8.86	8.86	4.42	
5	5	1	18	7	30.7	3.07	3.07	4.88	
5	3	1	45	9	131.4	13.14	13.14	6.38	
5	5	1	2	0	2	0.2	0.2	8.19	
5	15	1	0	0	0	0	0.1	8.50	

Date of Expt:	8/23/2005							
Expt #:	81							
Date read:	8/24/2005							
Purpose:	Dosage Response Curve for Enterococci							
WS:	Enterococci grown in 5:1 IO:NB for 6 days, spun, washed X3, and resuspended in 5:1 IO/diH2O							
Conc.(mg/l)	time (min)		lg wls pos	sm wls pos	MPN/ 100ml		calc MPN/ml	log redctn.
		dilution				MPN/ml		
0	0	3	49	47	2419.6	24196	24196	N/A
0	0	5	40	5	85.7	85700	85700	N/A
0	0	7	1	0	1	100000	100000	N/A
0.25	1	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.25	1	5	28	0	39.5	39500	39500	0.34
0.25	2	2	49	46	1986.3	1986.3	1986.3	1.63
0.25	2	5	17	0	20.3	20300	20300	0.63
0.25	3	2	49	47	2419.6	2419.6	2419.6	1.55
0.25	3	5	12	0	13.5	13500	13500	0.80
0.25	5	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.25	5	5	8	0	8.6	8600	8600	1.00
0.25	15	2	47	34	403.4	403.4	403.4	2.33
0.25	15	5	7	0	7.5	7500	7500	1.06
0.25	30	2	49	43	1413.6	1413.6	1413.6	1.78
0.25	30	5	5	0	5.2	5200	5200	1.22
0.75	1	2	49	46	1986.3	1986.3	1986.3	1.63
0.75	1	5	5	0	5.2	5200	5200	1.22
0.75	2	2	49	44	1553.1	1553.1	1553.1	1.74
0.75	2	5	3	0	3.1	3100	3100	1.44
0.75	3	2	49	32	686.7	686.7	686.7	2.10
0.75	3	5	0	0	0	0	1000	1.93
0.75	5	2	48	15	218.7	218.7	218.7	2.59
0.75	5	5	0	1	1	1000	1000	1.93
0.75	15	2	30	6	53.7	53.7	53.7	3.20
0.75	15	4	0	0	0	0	100	2.93
0.75	30	2	26	3	39.9	39.9	39.9	3.33
0.75	30	4	0	0	0	0	100	2.93
1.5	1	2	43	4	99	99	99	2.94
1.5	2	2	25	0	33.6	33.6	33.6	3.41
1.5	2	4	0	0	0	0	100	2.93
1.5	3	2	14	1	17.3	17.3	17.3	3.69
1.5	5	1	45	9	131.4	13.14	13.14	3.81
1.5	15	1	42	6	98.8	9.88	9.88	3.94
1.5	30	1	22	3	32.3	3.23	3.23	4.42
3	1	1	48	17	238.2	23.82	23.82	3.56
3	2	1	21	8	37.3	3.73	3.73	4.36
3	3	1	17	1	21.6	2.16	2.16	4.60
3	5	1	14	2	18.5	1.85	1.85	4.67
3	15	1	5	1	6.3	0.63	0.63	5.13
5	1	1	17	5	26.6	2.66	2.66	4.51
5	2	1	4	2	6.2	0.62	0.62	5.14
5	3	1	2	0	2	0.2	0.2	5.63
5	5	1	1	0	1	0.1	0.1	5.93
5	15	1	0	0	0	0	0.1	8.50

Date of Expt:	8/23/2005							
Expt #:	82							
Date read:	8/24/2005							
Purpose:	Dosage Response Curve for Enterococci							
WS:	Enterococci grown in 5:1 IO:NB for 6 days, spun, washed X3, and resuspended in 5:1 IO/diH2O							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	log redctn.
0	0	3	49	47	2419.6	24196	24196	N/A
0	0	5	40	5	85.7	85700	85700	N/A
0	0	7	1	0	1	100000	100000	N/A
0.25	1	2	49	48	>2419.6	>2419.6	>2419.6	N/A
0.25	1	5	31	6	56.3	56300	56300	0.18
0.25	2	2	49	46	1986.3	1986.3	1986.3	1.63
0.25	2	5	22	0	28.2	28200	28200	0.48
0.25	3	2	48	47	960.6	960.6	960.6	1.95
0.25	3	5	16	1	20.1	20100	20100	0.63
0.25	5	2	48	40	689.3	689.3	689.3	2.09
0.25	5	5	7	1	8.5	8500	8500	1.00
0.25	15	2	49	44	1553.1	1553.1	1553.1	1.74
0.25	15	5	5	1	6.3	6300	6300	1.13
0.25	30	2	49	43	1413.6	1413.6	1413.6	1.78
0.25	30	5	6	0	6.3	6300	6300	1.13
0.75	1	2	49	47	2419.6	2419.6	2419.6	1.55
0.75	1	5	6	1	7.4	7400	7400	1.06
0.75	2	2	47	38	472.1	472.1	472.1	2.26
0.75	2	5	6	0	6.3	6300	6300	1.13
0.75	3	2	45	30	250.4	250.4	250.4	2.53
0.75	3	5	3	0	3.1	3100	3100	1.44
0.75	5	2	43	26	182.3	182.3	182.3	2.67
0.75	5	5	0	0	0	0	1	4.93
0.75	15	2	32	3	53.8	53.8	53.8	3.20
0.75	15	4	0	0	0	0	1	4.93
0.75	30	2	18	5	28.1	28.1	28.1	3.48
0.75	30	4	0	0	0	0	1	4.93
1.5	1	2	24	0	31.7	31.7	31.7	3.43
1.5	2	2	10	2	13.2	13.2	13.2	3.81
1.5	2	4	0	0	0	0	1	4.93
1.5	3	2	9	0	9.8	9.8	9.8	3.94
1.5	5	1	40	7	90.8	9.08	9.08	3.97
1.5	15	1	39	6	83.6	8.36	8.36	4.01
1.5	30	1	25	1	35	3.5	3.5	4.39
3	1	1	18	7	30.7	3.07	3.07	4.45
3	2	1	18	2	24.3	2.43	2.43	4.55
3	3	1	15	4	22.3	2.23	2.23	4.58
3	5	1	12	1	14.6	1.46	1.46	4.77
3	15	1	4	1	5.2	0.52	0.52	5.22
5	1	1	15	3	21.1	2.11	2.11	4.61
5	2	1	9	2	12	1.2	1.2	4.85
5	3	1	3	0	3.1	0.31	0.31	5.44
5	5	1	0	1	1	0.1	0.1	5.93

E.4 Dosage Response Experiments for *Vibrio cholerae*

Date of Expt:	12/6/05						
Expt #:	94						
Date read:	12/13/05						
WS:	Vibrio grown in 5:1 IO:NB for 5 days, then washed X3 w/IO, then 1x low speed centrifugation, and resuspended in 5:1 IO:NB						
		Number of Colonies			Colony Count		
Specimen/QC	Dilution	Plate 1	Plate 2	ave count			
QC open, hood		0			0		
QC closed, hood		0			0		
QC open, counter		nd			nd		
dilution buffer		0			0		
Instant Ocean		nd			nd		
all specs. w/ferrate stopped w/TS							
			Number of Colonies				
Conc (mg/l)	Time (min)	Dilution	Plate 1	Plate 2	Ave. Count	Calc. Count	Log redctn.
0	0	0	6	0	0	100000	N/A
0	0	0	4	0	1	5000	N/A
0	0	0	3	23	19	21000	N/A
0.5	1	3	4	5	4.5	4500	0.67
0.5	1	1	141	awcg	141	1410	1.17
0.5	2	3	0	0	0	1000	1.32
0.5	2	1	84	88	86	860	1.39
0.5	3	3	0	4	2	2000	1.02
0.5	3	1	49	cont	49	490	1.63
0.5	5	3	0	0	0	1000	1.32
0.5	5	1	28	22	25	250	1.92
0.5	15	3	0	0	0	1000	1.32
0.5	15	1	23	21	22	220	1.98
0.5	30	2	1	4	2.5	250	1.92
0.5	30	0	87	nd	87	87	2.38
1	1	3	0	0	0	1000	1.32
1	1	1	8	12	10	100	2.32
1	2	3	0	0	0	1000	1.32
1	2	1	2	7	4.5	45	2.67
1	3	3	0	0	0	1000	1.32
1	3	1	0	9	4.5	45	2.67
1	5	3	0	0	0	1000	1.32
1	5	1	2	6	4	40	2.72
1	15	3	0	0	0	1000	1.32
1	15	1	1	0	0.5	5	3.62
1	30	2	0	0	0	100	2.32
1	30	0	2	7	4.5	4.5	3.67

Conc (mg/l)	Time (min)	Dilution	Number of Colonies		Ave. Count	Calc. Count	Log redctn.
			Plate 1	Plate 2			
2.5	1	3	0	0	0	1000	1.32
2.5	1	1	3	2	2.5	25	2.92
2.5	2	3	0	0	0	1000	1.32
2.5	2	1	3	0	1.5	15	3.15
2.5	3	2	0	0	0	100	2.32
2.5	3	0	2	5	3.5	3.5	3.78
2.5	5	2	0	0	0	100	2.32
2.5	5	0	6	awcg	6	6	3.54
2.5	15	2	0	0	0	100	2.32
2.5	15	0	2	3	2.5	2.5	3.92
2.5	30	1	0	0	0	10	3.32
2.5	30	0	2	nd	2	2	4.02
4	1	2	0	0	0	100	2.32
4	1	0	9	11	10	10	3.32
4	2	2	0	0	0	100	2.32
4	2	0	0	7	3.5	3.5	3.78
4	3	1	0	0	0	10	3.32
4	3	0	4	awcg	4	4	3.72
4	5	0	0	0	0	1	4.32
4	15	0	0	0	0	1	4.32
5	1	2	0	0	0	100	2.32
5	1	0	5	6	5.5	5.5	3.58
5	2	2	0	0	0	100	2.32
5	2	0	2	8	5	5	3.62
5	3	0	2	0	1	1	4.32
5	5	0	0	0	0	1	4.32

Date of Expt:	12/6/05						
Expt #:	95						
Date read:	12/13/05						
WS:	V. cholerae grown in 5:1 IO:NB for 5 days, then washed X3 w/IO, then 1x low speed centrifugation, and resuspended in 5:1 IO:NB						
		Number of Colonies					
Specimen/QC	Dilution	Plate 1	Plate 2	ave count	Colony Count		
QC open, hood		0			0		
QC closed, hood		0			0		
QC open, counter		nd			nd		
dilution buffer		0			0		
Instant Ocean		nd			nd		
all specs. w/ferrate stopped w/TS							
			Number of Colonies				
Conc (mg/l)	Time (min)	Dilution	Plate 1	Plate 2	Ave. Count	Calc. Count	Log redctn.
0	0	6	0	0	0	100000	N/A
0	0	4	0	1	0.5	5000	N/A
0	0	3	23	19	21	21000	N/A
0.5	1	3	4	5	4.5	4500	0.92
0.5	1	1	141	awcg	141	1410	1.32
0.5	2	3	0	0	0	1000	1.15
0.5	2	1	84	88	86	860	1.35
0.5	3	3	0	4	2	2000	1.15
0.5	3	1	49	cont	49	490	1.38
0.5	5	3	0	0	0	1000	1.32
0.5	5	1	28	22	25	250	1.48
0.5	15	3	0	0	0	1000	1.32
0.5	15	1	23	21	22	220	1.66
0.5	30	2	1	4	2.5	250	2.32
0.5	30	0	87	nd	87	87	2.44
1	1	3	0	0	0	1000	1.32
1	1	1	8	12	10	100	2.19
1	2	3	0	0	0	1000	1.32
1	2	1	2	7	4.5	45	2.18
1	3	3	0	0	0	1000	1.32
1	3	1	0	9	4.5	45	2.42
1	5	3	0	0	0	1000	1.32
1	5	1	2	6	4	40	2.72
1	15	3	0	0	0	1000	1.32
1	15	1	1	0	0.5	5	2.92
1	30	2	0	0	0	100	2.32
1	30	0	2	7	4.5	4.5	2.83

Conc (mg/l)	Time (min)	Dilution	Number of Colonies		Ave. Count	Calc. Count	Log redctn.
			Plate 1	Plate 2			
2.5	1	3	0	0	0	1000	1.32
2.5	1	1	3	2	2.5	25	2.67
2.5	2	3	0	0	0	1000	1.32
2.5	2	1	3	0	1.5	15	2.78
2.5	3	2	0	0	0	100	2.32
2.5	3	0	2	5	3.5	3.5	3.48
2.5	5	2	0	0	0	100	2.32
2.5	5	0	6	awcg	6	6	3.54
2.5	15	2	0	0	0	100	2.32
2.5	15	0	2	3	2.5	2.5	3.62
2.5	30	1	0	0	0	10	3.32
2.5	30	0	2	nd	2	2	3.85
4	1	2	0	0	0	100	2.32
4	1	0	9	11	10	10	3.19
4	2	2	0	0	0	100	2.32
4	2	0	0	7	3.5	3.5	3.48
4	3	1	0	0	0	10	3.32
4	3	0	4	awcg	4	4	3.42
4	5	0	0	0	0	1	4.32
4	15	0	0	0	0	1	4.32
5	1	2	0	0	0	100	3.32
5	1	0	5	6	5.5	5.5	3.28
5	2	2	0	0	0	100	2.32
5	2	0	2	8	5	5	3.72
5	3	0	2	0	1	1	3.85
5	5	0	0	0	0	1	4.62

Date of Expt:	12/8/05						
Expt #:	96						
Date read:	12/15/05						
WS:	V. cholerae grown in 5:1 IO:NB for 7 days, then washed X3 w/IO, then 1x low speed centrifugation, and resuspended in 5:1 IO:NB						
		Number of Colonies					
Specimen/QC	Dilution	Plate 1	Plate 2	ave count	Colony Count		
QC open, hood		0			0		
QC closed, hood		0			0		
QC open, counter		nd			nd		
dilution buffer		0			0		
Instant Ocean		0			0		
all specs. w/ferrate stopped w/TS							
			Number of Colonies				
Conc (mg/l)	Time (min)	Dilution	Plate 1	Plate 2	Ave. Count	Calc. Count	Log redctn.
0	0	6	0	0	0	100000	N/A
0	0	4	1	3	2	20000	N/A
0	0	3	16	13	14.5	14500	N/A
0.5	1	3	2	3	2.5	2500	0.76
0.5	1	1	139	144	141.5	1415	1.01
0.5	2	3	0	0	0	1000	1.16
0.5	2	1	76	62	69	690	1.32
0.5	3	3	1	2	1.5	1500	0.99
0.5	3	1	55	61	58	580	1.40
0.5	5	3	0	0	0	1000	1.16
0.5	5	1	28	30	29	290	1.70
0.5	15	3	0	0	0	1000	1.16
0.5	15	1	29	28	28.5	285	1.71
0.5	30	2	5	1	3	300	1.68
0.5	30	0	awcg	52	52	52	2.45
1	1	3	2	0	1	1000	1.16
1	1	1	13	19	16	160	1.96
1	2	3	0	0	0	1000	1.16
1	2	1	16	12	14	140	2.02
1	3	3	0	0	0	1000	1.16
1	3	1	5	11	8	80	2.26
1	5	3	0	0	0	1000	1.16
1	5	1	5	4	4.5	45	2.51
1	15	3	0	0	0	1000	1.16
1	15	1	0	4	2	20	2.86
1	30	2	0	2	1	100	2.16
1	30	0	9	nd	9	9	3.21

Conc (mg/l)	Time (min)	Dilution	Number of Colonies		Ave. Count	Calc. Count	Log redctn.
			Plate 1	Plate 2			
2.5	1	3	1	0	0.5	500	1.46
2.5	1	1	12	10	11	110	2.12
2.5	2	3	0	0	0	1000	1.16
2.5	2	1	7	8	7.5	75	2.29
2.5	3	2	0	0	0	100	2.16
2.5	3	0	7	5	6	6	3.38
2.5	5	2	0	0	0	100	2.16
2.5	5	0	5	awcg	5	5	3.46
2.5	15	2	0	0	0	100	2.16
2.5	15	0	8	2	5	5	3.46
2.5	30	1	0	0	0	10	3.16
2.5	30	0	4	nd	4	4	3.56
4	1	2	0	0	0	100	2.16
4	1	0	20	16	18	18	2.91
4	2	2	1	0	0.5	50	2.46
4	2	0	awcg	7	7	7	3.32
4	3	1	0	0	0	10	3.16
4	3	0	13	14	13.5	13.5	3.03
4	5	0	9	0	4.5	4.5	3.51
4	15	0	0	3	1.5	1.5	3.99
5	1	1	1	1	1	10	3.16
5	1	0	12	14	13	13	3.05
5	2	2	0	0	0	100	2.16
5	2	0	6	0	3	3	3.68
5	3	0	2	0	1	1	4.16
5	5	0	2	0	1	1	4.16

Date of Expt:	12/8/05						
Expt #:	97						
Date read:	12/15/05						
WS:	V. cholerae grown in 5:1 IO:NB for 7 days, then washed X3 w/IO, then 1x low speed centrifugation, and resuspended in 5:1 IO:NB						
		Number of Colonies			Colony Count		
Specimen/QC	Dilution	Plate 1	Plate 2	ave count			
QC open, hood		0			0		
QC closed, hood		0			0		
QC open, counter		nd			nd		
dilution buffer		0			0		
Instant Ocean		0			0		
all specs. w/ferrate stopped w/TS							
		Number of Colonies					
Conc (mg/l)	Time (min)	Dilution	Plate 1	Plate 2	Ave. Count	Calc. Count	Log redctn.
0	0	6	0	0	0	100000	N/A
0	0	4	1	3	2	20000	N/A
0	0	3	16	13	14.5	14500	N/A
0.5	1	3	0	1	0.5	500	0.30
0.5	1	1	78	75	76.5	765	0.12
0.5	2	3	0	0	0	1000	0.00
0.5	2	1	49	52	50.5	505	0.30
0.5	3	3	0	0	0	1000	0.00
0.5	3	1	34	41	37.5	375	0.43
0.5	5	3	0	0	0	1000	0.00
0.5	5	1	26	15	20.5	205	0.69
0.5	15	3	0	0	0	1000	0.00
0.5	15	1	13	10	11.5	115	0.94
0.5	30	2	3	1	2	200	0.70
0.5	30	0	awcg	nd	nd	nd	nd
1	1	3	0	0	0	1000	0.00
1	1	1	22	17	19.5	195	0.71
1	2	3	0	0	0	1000	0.00
1	2	1	13	17	15	150	0.82
1	3	3	0	0	0	1000	0.00
1	3	1	0	9	4.5	45	1.35
1	5	3	0	0	0	1000	0.00
1	5	1	4	4	4	40	1.40
1	15	3	0	0	0	1000	0.00
1	15	1	1	5	3	30	1.52
1	30	2	0	0	0	100	1.00
1	30	0	16	nd	16	16	1.80

Conc (mg/l)	Time (min)	Dilution	Number of Colonies		Ave. Count	Calc. Count	Log redctn.
			Plate 1	Plate 2			
2.5	1	3	0	0	0	1000	0.00
2.5	1	1	7	9	8	80	1.10
2.5	2	3	0	0	0	1000	0.00
2.5	2	1	4	8	6	60	1.22
2.5	3	2	0	0	0	100	1.00
2.5	3	0	18	awcg	18	18	1.74
2.5	5	2	0	0	0	100	1.00
2.5	5	0	5	7	6	6	2.22
2.5	15	2	0	0	0	100	1.00
2.5	15	0	2	6	4	4	2.40
2.5	30	1	0	0	0	10	2.00
2.5	30	0	0	0	0	1	3.00
4	1	2	0	0	0	100	1.00
4	1	0	11	10	10.5	10.5	1.98
4	2	2	0	0	0	100	1.00
4	2	0	6	1	3.5	3.5	2.46
4	3	1	0	0	0	10	2.00
4	3	0	4	2	3	3	2.52
4	5	0	3	0	1.5	1.5	2.82
4	15	0	0	0	0	1	3.00
5	1	2	1	0	0.5	50	1.30
5	1	0	21	awcg	21	21	1.68
5	2	2	0	0	0	100	1.00
5	2	0	11	9	10	10	2.00
5	3	0	2	0	1	1	3.00
5	5	0	0	1	0.5	0.5	3.30

E.5 Experiments on Effect of N_0

Expt. #:	43
Date of Expt.:	5/13/2005
Date HPC read:	5/17/2005
Purpose:	Investigate effect of N_0 on disinfection w/2 mg/l ferrate
WS:	E Coli in IO:NB, 600 mls as a 5:1 dilution, grown for 2 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10^{-n})	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		0			0
QC open, counter		5			5
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 2 mg/ l					
WS 0'	6	28	32	30	3.00E+07
WS 0'	7	3	2	2.5	2.50E+07
N_0 , 15'	1	118	125	121.5	1.22E+03
N_0 , 30'	1	9	10	9.5	9.50E+01
1:10 N_0 , 15'	1,1 ml	0	0	0	0.00E+00
1:10 N_0 , 30'	1, 1 ml	0	0	0	0.00E+00
1:100 N_0 , 15'	1, 1 ml	0	0	0	0.00E+00
1:100 N_0 , 30'	1, 1 ml	0	0	0	0.00E+00
1:1000 N_0 , 15'	1, 1 ml	0	0	0	0.00E+00
1:1000 N_0 , 30'	1, 1 ml	0	0	0	0.00E+00

Expt. #:	48
Date of Expt.:	6/1/2005
Date HPC read:	6/6/2005
Purpose:	Effect of N ₀ on effectiveness of 2 mg/l ferrate on <i>K. pneumoniae</i>
WS:	<i>K. pneumoniae</i> in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH ₂ O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		nd			nd
QC open, counter		2			2
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 2 mg/l					
WS 0'	6	76	84	80	8.00E+07
WS 0'	7	11	17	14	1.40E+08
N ₀ , 5'	5	25	26	25.5	2.55E+06
N ₀ , 20'	4	48	45	46.5	4.65E+05
1:10 N ₀ , 5'	1	112	107	109.5	1.10E+03
1:10 N ₀ , 20'	1	3	11	7	7.00E+01
1:100 N ₀ , 5'	1	9	6	7.5	7.50E+01
1:100 N ₀ , 20'	1, 1 ml	4	7	5.5	5.50E+00
1:1000 N ₀ , 5'	1, 1 ml	5	0	2.5	2.50E+00
1:1000 N ₀ , 20'	1, 1 ml	0	0	0	0.00E+00

Expt. #:	52
Date of Expt.:	6/7/2005
Date HPC read:	6/12/2005
Purpose:	Investigate effect of N_0 on disinfection w/1.5 mg/l ferrate
WS:	<i>E Coli</i> in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O

		Number of Colonie s			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		nd			nd
QC open, counter		3			3
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 1.5 mg/ l					
WS 0'	6	53	51	52	5.20E+07
WS 0'	7	6	5	5.5	5.50E+07
N_0 , 10'	3	160	175	167.5	1.68E+05
N_0 , 20'	3	11	10	10.5	1.05E+04
1:10 N_0 , 10'	1, 1 ml	2	awcg	2	2.00E+00
1:10 N_0 , 20'	1, 1 ml	0	0	0	0.00E+00
1:100 N_0 , 10'	1, 1 ml	1	1	1	1.00E+00
1:100 N_0 , 20'	1, 1 ml	0	1	0.5	5.00E-01
1:1000 N_0 , 10'	1, 1 ml	0	0	0	0.00E+00
1:1000 N_0 , 20'	1, 1 ml	0	0	0	0.00E+00

Expt. #:	54
Date of Expt.:	6/15/2005
Date HPC read:	6/21/2005
Purpose:	Investigate effect of N ₀ on disinfection w/0.5 mg/l ferrate
WS:	E Coli in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH ₂ O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		nd			nd
QC open, counter		7			7
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 0.5 mg/ l					
WS 0'	6	34	30	32	3.20E+07
WS 0'	7	1	5	3	3.00E+07
N ₀ , 5'	2	104	99	101.5	1.02E+04
N ₀ , 15'	2	80	76	78	7.80E+03
1:10 N ₀ , 5'	1	102	93	97.5	9.75E+02
1:10 N ₀ , 15'	1	8	20	14	1.40E+02
1:100 N ₀ , 5'	1	4	3	3.5	3.50E+01
1:100 N ₀ , 15'	1	3	1	2	2.00E+01
1:1000 N ₀ , 5'	1, 1 ml	6	3	4.5	4.50E+00
1:1000 N ₀ , 15'	1, 1 ml	0	0	0	0.00E+00

Expt. #:	57
Date of Expt.:	6/17/2005
Date HPC read:	6/23/2005
Purpose:	Effect of N ₀ on effectiveness of 1 mg/l ferrate on <i>K. pneumoniae</i>
WS:	<i>K. pneumoniae</i> in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, spun, washed, and resuspended in 5:1 IO:dH ₂ O

Heterotrophic Plate Count					
Specimen/QC	Dilution (10 ⁻ⁿ)	Number of Colonies		ave count	Colony Count
		Plate 1	Plate 2		
QC open, hood		0			0
QC closed, hood		nd			nd
QC open, counter		4			4
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 2 mg/ l					
WS 0'	6	53	52	52.5	5.25E+07
WS 0'	7	5	3	4	4.00E+07
N ₀ , 5'	3	508	476	492	4.92E+05
N ₀ , 10'	3	33	34	33.5	3.35E+04
1:10 N ₀ , 5'	1	42	awcg	42	4.20E+02
1:10 N ₀ , 10'	1	15	24	19.5	1.95E+02
1:100 N ₀ , 5'	1	3	4	3.5	3.50E+01
1:100 N ₀ , 10'	1, 1 ml	2	2	2	2.00E+00
1:1000 N ₀ , 5'	1, 1 ml	3	0	1.5	1.50E+00
1:1000 N ₀ , 10'	1, 1 ml	0	0	0	0.00E+00

E.6 Experiments on Procedure Optimization

E.6.1 Effect of Delay in Processing

Date of Expt:	7/15/2005									
Expt #:	66									
Date read:	7/16/2005									
Purpose:	Evaluate any effect on delay in processing									
WS:	E.coli grown in 5:1 IO:NB for 5 days, then spun, washed X3 and resuspended in 5:1 IO/diH2O									
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	Condition	log redctn.	
0	0	3	49	48	>2419.6	>24.2	>24.2	I	N/A	
0	0	7	48	18	248.9	24890000	24890000	I	N/A	
0	0	5	49	48	>2419.6	>24.2	>24.2	I	N/A	
0.75	1	2	49	47	2419.6	2419.6	2419.6	I	4.01	
0.75	1	5	14	2	18.5	18500	18500	I	3.13	
0.75	5	1	49	48	>2419.6	>24.2	>24.2	I	N/A	
0.75	5	3	49	29	579.4	5794	5794	I	3.63	
0.75	15	1	49	48	>2419.6	>24.2	>24.2	I	N/A	
0.75	15	3	48	19	260.3	2603	2603	I	3.98	
1.5	1	1	49	48	>2419.6	>24.2	>24.2	I	N/A	
1.5	1	4	5	0	5.2	520	520	I	4.68	
1.5	5	1	49	48	>2419.6	>24.2	>24.2	I	N/A	
1.5	5	3	12	1	14.6	146	146	I	5.23	
1.5	15	1	49	42	1299.7	129.97	129.97	I	5.28	
0	0	7	48	16	218.7	21870000	21870000	W	0.06	
0	0	5	49	48	>2419.6	>24.2	>24.2	W	N/A	
0.75	1	2	49	48	>2419.6	>24.2	>24.2	W	N/A	
0.75	1	5	20	2	27.5	27500	27500	W	2.96	
0.75	5	1	49	48	>2419.6	>24.2	>24.2	W	N/A	
0.75	5	3	48	24	328.2	3282	3282	W	3.88	
0.75	15	1	49	48	>2419.6	>24.2	>24.2	W	N/A	
0.75	15	3	45	37	312.3	3123	3123	W	3.90	
1.5	1	1	49	48	>2419.6	>24.2	>24.2	W	N/A	
1.5	1	4	2	0	2	200	200	W	5.09	
1.5	5	1	49	48	>2419.6	>24.2	>24.2	W	N/A	
1.5	5	3	10	0	11	110	110	W	5.35	
1.5	15	1	49	42	1299.7	129.97	129.97	W	5.28	

E.6.2 Experiments on Effect of Stirring

Expt. #:	47
Date of Expt.:	5/25/2005
Date HPC read:	5/30/2005
Purpose:	Investigate effect of stirring on disinfection w/2 mg/l ferrate
WS:	E Coli in IO:NB, 600 mls as a 5:1 dilution, grown for 4 days on shaker table at RT, then spun and resuspended in 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		nd			nd
QC open, counter		2			2
dilution buffer		nd			nd
Instant Ocean		0			0
all specs. w/ferrate stopped w/TS; all concs. are 1.5 mg/l					
WS 0'	6	53	54	53.5	5.35E+07
WS 0'	7	8	4	6	6.00E+07
NS 5'	4	29	30	29.5	2.95E+05
NS 15'	1	15	15	15	1.50E+02
MS 5'	1	5	2	3.5	3.50E+01
MS 15'	1,1 ml	1	1	1	1.00E+00
VS 5'	1,1 ml	0	2	1	1.00E+00
VS 15'	1, 1 ml	0	0	0	0.00E+00

Expt. #:	50
Date of Expt.:	6/1/2005
Date HPC read:	6/6/2005
Purpose:	Investigate effect of stirring on disinfection of <i>K. pneumoniae</i> w/2 mg/l ferrate
WS:	<i>K. pneumoniae</i> in IO:NB, 600 mls as a 5:1 dilution, grown for 3 days on shaker table at RT, then spun and resuspended in 100 mls 5:1 IO:dH2O

Heterotrophic Plate Count					
		Number of Colonies			
Specimen/QC	Dilution (10 ⁻ⁿ)	Plate 1	Plate 2	ave count	Colony Count
QC open, hood		0			0
QC closed, hood		nd			nd
QC open, counter		2			2
dilution buffer		0			0
Instant Ocean		nd			nd
all specs. w/ferrate stopped w/TS; all concs. are 2 mg/l ferrate					
WS 0'	5	120	94	107	1.07E+07
WS 0'	6	17	26	21.5	2.15E+07
NS 5'	4	31	30	30.5	3.05E+05
NS 15'	3	13	18	15.5	1.55E+04
MS 5'	2	153	139	146	1.46E+04
MS 15'	1	25	22	23.5	2.35E+02
VS 5'	2	33	36	34.5	3.45E+03
VS 15'	1	25	28	26.5	2.65E+02

E.6.3 Experiment on Effect of Washing

Expt. #:	55					
Date of Expt.:	6/17/2005					
Date HPC read:	6/19/2005					
Purpose:	Effect of washing on effectiveness of 1 mg/l ferrate on <i>K. pneumoniae</i>					
WS:	<i>K. pneumoniae</i> in IO:NB, 600 mls as a 5:1 dilution, grown for 5 days on shaker table at RT, spun, washed, and resuspended in 5:1 IO:dH2O					
Condition	WS (MPN)	dilution	MPN	5' (MPN)	dilution	MPN
1X wash	1732.9	6	1.73E+07	4.1	7	4.10E+05
2X wash	1119.9	6	1.12E+07	30.5	4	3.05E+03
3X wash	629.4	6	6.29E+06	18.9	4	1.89E+03

E.7 Regrowth Experiments

Date of Expt:	7/28/2005							
Expt #:	90							
Date read:	7/29/2005							
Purpose:	Regrowth of E. coli after ferrate disinfection							
WS:	E.coli grown in 5:1 IO:NB for 4 days, spun, washed X3 and resuspended in 5:1 IO/diH2O							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	log redctn.
0	15 min	3	49	48	nd	nd	nd	nd
0	15 min	7	3	0	3.1	310000	310000	N/A
0	15 min	5	48	15	218.7	218700	218700	N/A
5	15 min	1	47	10	160.7	16.07	16.07	4.13
5	15 min	3	3	0	3.1	31	31	3.85
10	15 min	1	0	0	0	0	0.1	6.34
0	48 hr	4	49	39	1046.2	104620	104620	0.32
0	48 hr	7	1	0	1	100000	100000	0.34
0 + glu	48 hr	5	48	28	396.8	396800	396800	-0.26
0 + glu	48 hr	8	0	0	0	0	1000000	-0.66
5	48 hr	1	5	0	5.2	0.52	0.52	5.62
5	48 hr	3	0	0	0	0	10	4.34
5 + glu	48 hr	1	29	3	46.4	4.64	4.64	4.67
5 + glu	48 hr	3	0	0	0	0	10	4.34
10	48 hr	1	0	0	0	0	0.1	6.34
10 + glu	48 hr	1	0	0	0	0	0.1	6.34

Date of Expt:	7/28/2005							
Expt #:	91							
Date read:	7/29/2005							
Purpose:	Regrowth of E. coli after ferrate disinfection							
WS:	E.coli grown in 5:1 IO:NB for 4 days, spun, washed X3 and resuspended in 5:1 IO/diH2O							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	log redctn.
0	15 min	3	49	48	nd	nd	nd	nd
0	15 min	7	3	0	3.1	310000	310000	N/A
0	15 min	5	48	15	218.7	218700	218700	N/A
5	15 min	1	45	7	123.6	12.36	12.36	4.25
5	15 min	3	0	0	0	0	10	4.34
10	15 min	1	0	0	0	0	0.1	6.34
0	48 hr	4	49	39	1046.2	104620	104620	0.32
0	48 hr	7	1	0	1	100000	100000	0.34
0 + glu	48 hr	5	48	28	396.8	396800	396800	-0.26
0 + glu	48 hr	8	0	0	0	0	1000000	-0.66
5	48 hr	1	11	1	13.4	1.34	1.34	5.21
5	48 hr	3	0	0	0	0	10	4.34
5 + glu	48 hr	1	33	4	58.3	5.83	5.83	4.57
5 + glu	48 hr	3	0	1	1	10	10	4.34
10	48 hr	1	0	0	0	0	0.1	6.34
10 + glu	48 hr	1	0	0	0	0	0.1	6.34

E.8 Experiments on Effect of Salinity

Date of Expt:	8/25/2005									
Expt #:	84, Effect of Salinity									
Date read:	8/26/2005									
WS:	E.coli grown in 5:1 IO:NB for 2 days,spun, washed X3, & resuspended in 5:1 IO/diH2O									
Condition:	36 ppt									
	time		lg wls	sm wls	MPN/		calc	log	delta	
Conc.(mg/l)	(min)	dilution	pos	pos	100ml	MPN/ml	MPN/ml	redctn.	log redctn.	
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A		
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	0	7	39	6	83.6	8360000	8360000	N/A		
0	2	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	2	7	40	4	83.3	8330000	8330000	0.00		
0	5	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	5	7	40	5	85.7	8570000	8570000	-0.01		
0	15	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	15	7	39	6	83.6	8360000	8360000	0.00		
0	30	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	30	7	38	5	77.1	7710000	7710000	0.04		
0.25	2	3	49	48	>2419.6	>24.2	>24.2	N/A		
0.25	2	6	44	25	193.6	1936000	1936000	0.64	0.63	
0.25	5	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.25	5	6	32	22	89.5	895000	895000	0.97	0.98	
0.25	15	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.25	15	6	31	14	70.5	705000	705000	1.07	1.07	
0.25	30	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.25	30	5	47	24	270	270000	270000	1.49	1.46	
0.75	2	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	2	4	47	28	316.9	31690	31690	2.42	2.42	
0.75	5	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	5	4	31	5	54.6	5460	5460	3.19	3.20	
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	15	4	19	2	25.9	2590	2590	3.51	3.51	
0.75	30	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	30	4	17	2	22.8	2280	2280	3.56	3.53	
1.5	2	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	2	4	38	16	103.4	10340	10340	2.91	2.91	
1.5	5	2	49	44	1413.6	1413.6	1413.6	3.77	3.78	
1.5	5	4	10	1	12.1	1210	1210	3.84	3.85	
1.5	15	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	15	3	29	8	54.5	545	545	4.19	4.19	
1.5	30	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	30	4	3	0	3.1	310	310	4.43	4.40	
3	2	1	49	44	1553.1	155.31	155.31	4.73	4.73	
3	5	1	49	31	648.8	64.88	64.88	5.11	5.12	
3	15	1	46	25	241.5	24.15	24.15	5.54	5.54	
3	30	1	16	2	21.3	2.13	2.13	6.59	6.56	
5	2	1	22	0	28.2	2.82	2.82	6.47	6.47	
5	5	1	10	0	11	1.1	1.1	6.88	6.89	
5	15	1	0	0	0	0	0.1	7.92	7.92	
5	30	1	0	0	0	0	0.1	7.92	7.89	

Expt #:	84									
Condition:	10 ppt									delta log redctn.
Conc.(mg/l)	time (min)		lg wls pos	sm wls pos	MPN/ 100ml		calc MPN/ml	log redctn.		
		dilution			MPN/ml					
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A		
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	0	7	43	11	121.1	12110000	12110000	N/A		
0	2	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	2	7	44	9	122.3	12230000	12230000	0.00		
0	5	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	5	7	44	11	129.6	12960000	12960000	-0.19		
0	15	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	15	7	42	12	116.9	11690000	11690000	-0.15		
0	30	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	30	7	42	13	120.1	12010000	12010000	-0.16		
0.25	2	3	49	48	>2419.6	>24.2	>24.2	N/A		
0.25	2	6	46	25	241.5	2415000	2415000	0.70	0.70	
0.25	5	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.25	5	6	39	27	141.7	1417000	1417000	0.93	1.12	
0.25	15	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.25	15	6	37	9	82	820000	820000	1.17	1.31	
0.25	30	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.25	30	5	48	33	501.2	501200	501200	1.38	1.54	
0.75	2	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	2	4	48	43	791.5	79150	79150	2.18	2.19	
0.75	5	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	5	4	45	34	284.1	28410	28410	2.63	2.82	
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	15	4	29	2	44.8	4480	4480	3.43	3.58	
0.75	30	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	30	4	21	1	27.9	2790	2790	3.64	3.79	
1.5	2	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	2	4	40	17	62.7	6270	6270	3.29	3.29	
1.5	5	2	49	45	1732.9	1732.9	1732.9	3.84	4.03	
1.5	5	4	19	0	23.3	2330	2330	3.72	3.91	
1.5	15	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	15	3	24	2	34.5	345	345	4.55	4.69	
1.5	30	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	30	3	19	1	24.6	246	246	4.69	4.85	
3	2	1	49	39	1046.2	104.62	104.62	5.06	5.07	
3	5	1	42	14	123.4	12.34	12.34	5.99	6.18	
3	15	1	25	4	39.3	3.93	3.93	6.49	6.63	
3	30	1	8	0	8.6	0.86	0.86	7.15	7.31	
5	2	1	29	5	49.6	4.96	4.96	6.39	6.39	
5	5	1	15	2	19.9	1.99	1.99	6.78	6.97	
5	15	1	0	0	0	0	0.1	8.08	8.23	
5	30	1	0	0	0	0	0.1	8.08	8.24	

Date of Expt:	8/25/2005								
Expt #:	85, Effect of salinity								
Date read:	8/26/2005								
WS:	E.coli grown in 5:1 IO:NB for 5 days,spun, washed X3, & resuspended in 5:1 IO/diH2O								
Condition:	36 ppt								
Conc.(mg/l)	time (min)		lg wls pos	sm wls pos	MPN/ 100ml		calc MPN/ml	log redctn.	delta log redctn.
		dilution				MPN/ml			
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A	
0	0	6	49	34	770.1	7701000	7701000	N/A	
0	2	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	2	7	38	4	74.9	7490000	7490000	0.01	
0	5	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	5	7	40	3	80.9	8090000	8090000	-0.02	
0	15	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	15	7	38	6	79.4	7940000	7940000	-0.01	
0	30	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	30	7	37	5	73.3	7330000	7330000	0.02	
0.25	2	3	49	48	>2419.6	>24.2	>24.2	N/A	
0.25	2	6	43	14	131.7	1317000	1317000	0.77	0.75
0.25	5	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.25	5	6	26	4	41.4	414000	414000	1.27	1.29
0.25	15	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.25	15	6	18	0	21.8	218000	218000	1.55	1.56
0.25	30	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.25	30	5	47	16	198.9	198900	198900	1.59	1.57
0.75	2	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	2	4	46	36	355.5	35550	35550	2.34	2.32
0.75	5	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	5	4	37	9	82	8200	8200	2.97	2.99
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	15	4	20	1	26.2	2620	2620	3.47	3.48
0.75	30	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	30	3	46	18	190.4	1904	1904	3.61	3.59
1.5	2	2	48	46	870.4	870.4	870.4	3.95	3.93
1.5	2	4	9	4	14.2	1420	1420	3.73	3.72
1.5	5	2	49	35	816.4	816.4	816.4	3.97	4.00
1.5	5	4	5	1	6.3	630	630	4.09	4.11
1.5	15	1	47	13	178.5	17.85	17.85	5.63	5.65
1.5	15	3	3	0	3.1	31	31	5.40	5.41
1.5	30	1	44	6	111.9	11.19	11.19	5.84	5.82
1.5	30	3	2	0	2	20	20	5.59	5.56
3	2	1	49	46	1986.3	198.63	198.63	4.59	4.58
3	2	3	13	1	16	160	160	4.68	4.67
3	5	1	46	22	217.8	21.78	21.78	5.55	5.57
3	15	1	35	4	64.4	6.44	6.44	6.08	6.09
3	30	1	12	1	14.6	1.46	1.46	6.72	6.70
5	2	1	4	1	5.2	0.52	0.52	7.17	7.16
5	5	1	2	0	2	0.2	0.2	7.59	7.61
5	15	1	1	0	1	0.1	0.1	7.89	7.90
5	30	1	0	0	0	0	0.1	7.89	7.87

Expt #:	85									
Condition:	10 ppt									delta
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	log redctn.	log redctn.	delta log redctn.
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A		
0	0	5	nd	nd	nd	nd	nd	nd		
0	0	6	49	37	920.8	9208000	9208000	N/A		
0	2	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	2	7	44	9	122.3	12230000	12230000	-0.12		
0	5	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	5	7	44	11	129.6	12960000	12960000	-0.23		
0	15	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	15	7	42	12	116.9	11690000	11690000	-0.18		
0	30	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	30	7	42	13	120.1	12010000	12010000	-0.19		
0.25	2	3	49	48	>2419.6	>24.2	>24.2	N/A		
0.25	2	6	42	12	120.1	1201000	1201000	0.88		1.01
0.25	5	3	49	48	>2419.6	>24.2	>24.2	N/A		N/A
0.25	5	6	26	1	36.9	369000	369000	1.40		1.62
0.25	15	3	49	48	>2419.6	>24.2	>24.2	N/A		N/A
0.25	15	6	22	1	29.5	295000	295000	1.49		1.68
0.25	30	3	49	48	>2419.6	>24.2	>24.2	N/A		N/A
0.25	30	5	44	28	211	211000	211000	1.64		1.83
0.75	2	2	49	48	>2419.6	>24.2	>24.2	N/A		N/A
0.75	2	4	47	43	550.4	55040	55040	2.22		2.35
0.75	5	2	49	48	>2419.6	>24.2	>24.2	N/A		N/A
0.75	5	4	45	20	183.5	18350	18350	2.70		2.93
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A		N/A
0.75	15	4	23	2	32.4	3240	3240	3.45		3.63
0.75	30	2	49	48	>2419.6	>24.2	>24.2	N/A		N/A
0.75	30	4	21	1	27.9	2790	2790	3.52		3.71
1.5	2	2	49	48	>2419.6	>24.2	>24.2	N/A		N/A
1.5	2	4	40	17	62.7	6270	6270	3.17		3.29
1.5	5	2	49	45	1732.9	1732.9	1732.9	3.73		3.95
1.5	5	4	14	0	16.1	1610	1610	3.76		3.98
1.5	15	1	49	48	>2419.6	>24.2	>24.2	N/A		N/A
1.5	15	3	21	1	27.9	279	279	4.52		4.70
1.5	30	1	49	48	>2419.6	>24.2	>24.2	N/A		N/A
1.5	30	3	9	0	9.8	98	98	4.97		5.17
3	2	1	49	43	1413.6	141.36	141.36	4.81		4.94
3	5	1	44	16	149.7	14.97	14.97	5.79		6.02
3	15	1	36	4	67.7	6.77	6.77	6.13		6.31
3	30	1	12	3	16.9	1.69	1.69	6.74		6.93
5	2	1	25	2	36.4	3.64	3.64	6.40		6.53
5	5	1	7	0	7.5	0.75	0.75	7.09		7.32
5	15	1	3	0	3.1	0.31	0.31	7.47		7.65
5	30	1	0	0	0	0	0.1	7.96		8.16

E.9 Experiments on Effect of pH

Date of Expt:	8/9/2005									
Expt #:	77									
Date read:	8/10/2005									
Purpose:	Effect of pH									
WS:	E.coli grown in 5:1 IO:NB for 4 days,spun, washed X3, & resuspended in 5:1 IO/diH2O									
Condition:	N (Normal pH)									
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	log redctn.	delta log redctn.	
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A		
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	0	7	49	22	387.3	38730000	38730000	N/A		
0	2	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	2	7	49	25	461.1	46110000	46110000	-0.08		
0	5	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	5	7	49	21	365.4	36540000	36540000	0.03		
0	15	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	15	7	49	24	435.2	43520000	43520000	-0.05		
0	30	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	30	7	48	24	328.2	32820000	32820000	0.07		
0.75	2	3	49	48	>2419.6	>24.2	>24.2	N/A		
0.75	2	5	49	36	866.4	866400	866400	1.65	1.73	
0.75	5	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	5	5	45	15	157.6	157600	157600	2.39	2.37	
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	15	5	41	6	93.3	93300	93300	2.62	2.67	
0.75	30	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	30	5	24	0	31.7	31700	31700	3.09	3.02	
1.5	2	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	2	4	49	29	579.4	57940	57940	2.83	2.90	
1.5	5	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	5	3	49	34	770.1	7701	7701	3.70	3.68	
1.5	15	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	15	3	47	18	214.2	2142	2142	4.26	4.31	
1.5	30	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	30	3	47	12	172.3	1723	1723	4.35	4.28	

Expt #:	77									
Condition:	-pH (Acidic pH)									delta
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	log redctn.	log redctn.	
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A		
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	0	7	49	26	488.4	48840000	48840000	N/A		
0	2	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	2	7	49	23	410.6	41060000	41060000	0.08		
0	5	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	5	7	49	23	410.6	41060000	41060000	0.08		
0	15	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	15	7	49	25	461.1	46110000	46110000	0.02		
0	30	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	30	7	48	28	396.8	39680000	39680000	0.09		
0.75	2	3	49	47	2419.6	24196	24196	3.31		
0.75	2	5	49	20	344.8	344800	344800	2.15	2.08	
0.75	5	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	5	5	21	2	29.2	29200	29200	3.22	3.15	
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	15	5	3	0	3.1	3100	3100	4.20	4.17	
0.75	30	2	49	45	1732.9	1732.9	1732.9	4.45	4.36	
0.75	30	5	1	0	1	1000	1000	4.69	4.60	
1.5	2	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	2	4	42	7	101.7	10170	10170	3.68	3.61	
1.5	5	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	5	3	40	2	78.5	785	785	4.79	4.72	
1.5	15	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	15	3	24	0	31.4	314	314	5.19	5.17	
1.5	30	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	30	3	10	0	11	110	110	5.65	5.56	

Expt #:	77								
Condition:	+pH (Alkaline pH)								delta
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	log redctn.	log redctn.
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A	
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	0	7	49	30	613.1	61310000	61310000	N/A	
0	2	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	2	7	49	24	435.2	43520000	43520000	0.15	
0	5	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	5	7	49	25	461.1	46110000	46110000	0.12	
0	15	7	49	48	>2419.6	>24.2	>24.2	N/A	
0	15	7	49	22	387.3	38730000	38730000	0.20	
0	30	7	49	48	>2419.6	>24.2	>24.2	N/A	
0	30	7	49	20	344.8	34480000	34480000	0.25	
0.75	2	3	49	48	>2419.6	>24.2	>24.2	N/A	
0.75	2	5	49	42	1299.7	1299700	1299700	1.67	1.52
0.75	5	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	5	5	48	26	360.9	360900	360900	2.23	2.11
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	15	5	47	5	135.4	135400	135400	2.66	2.46
0.75	30	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	30	5	19	1	24.6	24600	24600	3.40	3.15
1.5	2	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
1.5	2	4	49	36	866.4	86640	86640	2.85	2.70
1.5	5	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
1.5	5	3	49	44	1553.1	15531	15531	3.60	3.47
1.5	15	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
1.5	15	3	48	31	360.9	3609	3609	4.23	4.03
1.5	30	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
1.5	30	3	47	11	166.4	1664	1664	4.57	4.32

Date of Expt:	8/9/2005									
Expt #:	78									
Date read:	8/10/2005									
Purpose:	Effect of pH									
WS:	E.coli grown in 5:1 IO:NB for 4 days,spun, washed X3, & resuspended in 5:1 IO/diH2O									
Condition:	N (Normal pH)									
	time		lg wls	sm wls	MPN/		calc	log	delta	
Conc.(mg/l)	(min)	dilution	pos	pos	100ml	MPN/ml	MPN/ml	redctn.	log redctn.	
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A		
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	0	7	49	22	387.3	38730000	38730000	N/A		
0	2	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	2	7	49	25	461.1	46110000	46110000	-0.08		
0	5	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	5	7	49	21	365.4	36540000	36540000	0.03		
0	15	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	15	7	49	24	435.2	43520000	43520000	-0.05		
0	30	5	49	48	>2419.6	>24.2	>24.2	N/A		
0	30	7	48	24	328.2	32820000	32820000	0.07		
0.75	2	3	49	48	>2419.6	>24.2	>24.2	N/A		
0.75	2	5	49	39	1046.2	1046200	1046200	1.57	1.64	
0.75	5	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	5	5	46	22	217.8	217800	217800	2.25	2.22	
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	15	5	37	3	69.1	69100	69100	2.75	2.80	
0.75	30	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
0.75	30	5	27	1	38.9	38900	38900	3.00	2.93	
1.5	2	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	2	4	49	34	770.1	77010	77010	2.70	2.78	
1.5	5	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	5	3	49	39	1046.2	10462	10462	3.57	3.54	
1.5	15	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	15	3	48	19	260.3	2603	2603	4.17	4.22	
1.5	30	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A	
1.5	30	3	42	15	126.7	1267	1267	4.49	4.41	

Expt #:	78								
Condition:	-pH (Acidic pH)								delta
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	log redctn.	log redctn.
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A	
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	0	7	49	26	488.4	48840000	48840000	N/A	
0	2	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	2	7	49	23	410.6	41060000	41060000	0.08	
0	5	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	5	7	49	23	410.6	41060000	41060000	0.08	
0	15	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	15	7	49	25	461.1	46110000	46110000	0.02	
0	30	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	30	7	48	28	396.8	39680000	39680000	0.09	
0.75	2	3	49	47	2419.6	24196	24196	3.31	
0.75	2	5	49	24	435.2	435200	435200	2.05	1.97
0.75	5	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	5	5	18	0	21.8	21800	21800	3.35	3.27
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	15	5	5	1	6.3	6300	6300	3.89	3.86
0.75	30	2	49	46	1986.3	1986.3	1986.3	4.39	4.30
0.75	30	5	2	0	2	2000	2000	4.39	4.30
1.5	2	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
1.5	2	4	39	3	76.7	7670	7670	3.80	3.73
1.5	5	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
1.5	5	3	34	1	55.7	557	557	4.94	4.87
1.5	15	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
1.5	15	3	20	1	26.2	262	262	5.27	5.25
1.5	30	1	49	37	920.8	92.08	92.08	5.72	5.63
1.5	30	3	7	0	7.5	75	75	5.81	5.72

Expt #:	78								
Condition:	+pH (Alkaline pH)								delta
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/ 100ml	MPN/ml	calc MPN/ml	log redctn.	log redctn.
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A	
0	0	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	0	7	49	30	613.1	61310000	61310000	N/A	
0	2	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	2	7	49	24	435.2	43520000	43520000	0.15	
0	5	5	49	48	>2419.6	>24.2	>24.2	N/A	
0	5	7	49	25	461.1	46110000	46110000	0.12	
0	15	7	49	48	>2419.6	>24.2	>24.2	N/A	
0	15	7	49	22	387.3	38730000	38730000	0.20	
0	30	7	49	48	>2419.6	>24.2	>24.2	N/A	
0	30	7	49	20	344.8	34480000	34480000	0.25	
0.75	2	3	49	48	>2419.6	>24.2	>24.2	N/A	
0.75	2	5	49	45	1732.9	1732900	1732900	1.55	1.40
0.75	5	3	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	5	5	49	28	547.5	547500	547500	2.05	1.93
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	15	5	47	5	135.4	135400	135400	2.66	2.46
0.75	30	2	49	48	>2419.6	>24.2	>24.2	N/A	N/A
0.75	30	5	14	3	19.7	19700	19700	3.49	3.24
1.5	2	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
1.5	2	4	49	26	488.4	48840	48840	3.10	2.95
1.5	5	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
1.5	5	3	49	30	613.1	6131	6131	4.00	3.88
1.5	15	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
1.5	15	3	48	14	209.8	2098	2098	4.47	4.27
1.5	30	1	49	48	>2419.6	>24.2	>24.2	N/A	N/A
1.5	30	3	40	4	83.3	833	833	4.87	4.62

E.10 Anti-Clumping Measures

Date of Expt:	12/13/2005							
Expt #:	98							
Date read:	12/14/2005							
Purpose:	Effect of anti-clumping measures							
WS:	E.coli grown in 5:1 IO:NB for 5 days, spun, washed X3 and resuspended in 5:1 IO/diH2O							
Condition:	N (Normal preparation)							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/100ml	MPN/ml	calc MPN/ml	log redctn.
0	0	nd	nd	nd	nd	nd	nd	N/A
0	0	5	49	42	1299.7	1299700	1299700	N/A
0	0	7	15	0	17.5	1750000	1750000	N/A
0.75	1	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	1	6	7	1	8.5	85000	85000	1.25
0.75	2	3	49	43	1413.6	14136	14136	2.03
0.75	2	6	1	0	1	10000	10000	2.18
0.75	3	3	49	37	920.8	9208	9208	2.22
0.75	3	6	0	0	0	0	10000	2.18
0.75	5	3	49	24	435.2	4352	4352	2.54
0.75	5	6	0	0	0	0	10000	2.18
0.75	15	3	49	23	410.6	4106	4106	2.57
0.75	15	6	0	0	0	0	10000	2.18
0.75	30	3	48	22	298.7	2987	2987	2.71
1.5	1	2	nd	nd	nd	nd	nd	nd
1.5	1	4	30	2	47.1	4710	4710	2.51
1.5	2	3	41	5	90.6	906	906	3.23
1.5	3	3	32	2	52.1	521	521	3.47
1.5	5	3	28	3	44.1	441	441	3.54
1.5	15	3	20	4	30.1	301	301	3.70
1.5	30	3	12	0	13.5	135	135	4.05
3	1	3	48	18	248.9	2489	2489	2.79
3	1	5	nd	nd	nd	nd	nd	nd
3	2	3	24	1	33.1	331	331	3.66
3	2	5	nd	nd	nd	nd	nd	nd
3	3	2	46	9	142.1	142.1	142.1	4.03
3	5	2	37	10	84.2	84.2	84.2	4.26
3	15	2	35	1	58.6	58.6	58.6	4.42
3	30	2	17	2	22.8	22.8	22.8	4.83
4	1	3	36	8	75.9	759	759	3.30
4	1	2	nd	nd	nd	nd	nd	nd
4	2	3	25	2	36.4	364	364	3.62
4	2	2	nd	nd	nd	nd	nd	nd
4	3	2	36	5	69.7	69.7	69.7	4.34
4	5	2	24	6	40.2	40.2	40.2	4.58
4	15	1	46	17	184.2	18.42	18.42	4.92

Expt #:	98							
Condition:	Anti-clumping/Low-speed centrifugation							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/100ml	MPN/ml	calc MPN/ml	log redctn.
0	0	nd	nd	nd	nd	nd	nd	N/A
0	0	6	49	26	488.4	4884000	4884000	N/A
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	1	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	1	6	3	0	3.1	31000	31000	2.20
0.75	2	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	2	6	2	0	2	20000	20000	2.39
0.75	3	3	49	46	1986.3	19863	19863	2.39
0.75	3	6	0	0	0	0	10000	2.69
0.75	5	3	49	43	1413.6	14136	14136	2.54
0.75	5	6	0	0	0	0	10000	2.69
0.75	15	2	49	48	>2419.6	>24.2	>24.2	N/A
0.75	15	5	14	0	16.1	16100	16100	2.48
0.75	30	3	49	32	686.7	6867	6867	2.85
1.5	1	2	nd	nd	nd	nd	nd	nd
1.5	1	3	49	44	1553.1	15531	15531	2.50
1.5	2	2	49	46	1986.3	1986.3	1986.3	3.39
1.5	3	2	49	17	290.9	290.9	290.9	4.23
1.5	5	2	49	17	290.9	290.9	290.9	4.23
1.5	15	2	46	14	167	167	167	4.47
1.5	30	2	44	15	145.5	145.5	145.5	4.53
3	1	2	49	46	1986.3	1986.3	1986.3	3.39
3	1	5	nd	nd	nd	nd	nd	nd
3	2	2	48	18	248.9	248.9	248.9	4.29
3	2	5	nd	nd	nd	nd	nd	nd
3	3	2	23	3	34.1	34.1	34.1	5.16
3	5	2	20	4	30.1	30.1	30.1	5.21
3	15	2	16	1	20.1	20.1	20.1	5.39
3	30	1	44	8	118.7	11.87	11.87	5.61
4	1	2	46	18	190.4	190.4	190.4	4.41
4	1	2	nd	nd	nd	nd	nd	nd
4	2	2	31	5	54.6	54.6	54.6	4.95
4	2	2	nd	nd	nd	nd	nd	nd
4	3	2	22	0	28.2	28.2	28.2	5.24
4	5	1	48	17	238.2	23.82	23.82	5.31
4	15	1	46	6	129.1	12.91	12.91	5.58

Date of Expt:	12/13/2005							
Expt #:	99							
Date read:	12/14/2005							
Purpose:	Effect of anti-clumping measures							
WS:	E.coli grown in 5:1 IO:NB for 5 days, spun, washed X3 and resuspended in 5:1 IO/diH2O							
Condition:	N (Normal preparation)							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/100ml	MPN/ml	calc MPN/ml	log redctn.
0	0	nd	nd	nd	nd	nd	nd	N/A
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A
0	0	5	49	21	365.4	365400	365400	0.00
0.75	1	3	48	45	870.4	8704	8704	1.62
0.75	1	6	5	1	6.3	63000	63000	0.76
0.75	2	3	47	43	571.7	5717	5717	1.81
0.75	2	6	0	0	0	0	10000	1.56
0.75	3	3	47	31	357.8	3578	3578	2.01
0.75	3	6	0	0	0	0	10000	1.56
0.75	5	3	47	22	249.5	2495	2495	2.17
0.75	5	6	0	0	0	0	10000	1.56
0.75	15	3	45	19	177.9	1779	1779	2.31
0.75	15	6	0	0	0	0	10000	1.56
0.75	30	3	44	17	154.1	1541	1541	2.37
1.5	1	2	nd	nd	nd	nd	nd	nd
1.5	1	4	47	30	343.6	34360	34360	1.03
1.5	2	3	42	20	144.5	1445	1445	2.40
1.5	3	3	39	14	103.6	1036	1036	2.55
1.5	5	3	35	9	74.3	743	743	2.69
1.5	15	3	17	0	20.3	203	203	3.26
1.5	30	3	10	0	10	100	100	3.56
3	1	3	47	12	172.3	1723	1723	2.33
3	1	5	nd	nd	nd	nd	nd	nd
3	2	3	nd	nd	nd	nd	nd	nd
3	2	5	nd	nd	nd	nd	nd	nd
3	3	2	46	6	129.1	129.1	129.1	3.45
3	5	2	32	9	64.5	64.5	64.5	3.75
3	15	2	26	0	35.5	35.5	35.5	4.01
3	30	2	11	1	13.4	13.4	13.4	4.44
4	1	3	44	18	158.5	1585	1585	2.36
4	1	2	nd	nd	nd	nd	nd	nd
4	2	3	nd	nd	nd	nd	nd	nd
4	2	2	32	3	53.8	53.8	53.8	3.83
4	3	2	29	2	44.8	44.8	44.8	3.91
4	5	2	20	0	24.9	24.9	24.9	4.17
4	15	1	29	0	41.6	4.16	4.16	4.94

Expt #:	99							
Condition:	Anti-clumping/Vortexing							
Conc.(mg/l)	time (min)	dilution	lg wls pos	sm wls pos	MPN/100ml	MPN/ml	calc MPN/ml	log redctn.
0	0	nd	nd	nd	nd	nd	nd	N/A
0	0	5	49	21	365.4	365400	365400	N/A
0	0	3	49	48	>2419.6	>24.2	>24.2	N/A
0.75	1	3	49	45	1732.87	17328.7	17328.7	1.32
0.75	1	6	47	36	436.6	4366000	4366000	-1.08
0.75	2	3	49	43	1413.6	14136	14136	1.41
0.75	2	6	3	0	0	0	10000	1.56
0.75	3	3	43	33	219.8	2198	2198	2.22
0.75	3	6	0	0	0	0	10000	1.56
0.75	5	3	39	19	117.4	1174	1174	2.49
0.75	5	6	0	0	0	0	10000	1.56
0.75	15	2	47	15	191.8	191.8	191.8	3.28
0.75	15	5	0	0	0	0	1000	2.56
0.75	30	3	21	4	31.8	318	318	3.06
1.5	1	2	nd	nd	nd	nd	nd	nd
1.5	1	4	49	32	686.7	68670	68670	0.73
1.5	2	3	43	11	121.1	1211	1211	2.48
1.5	3	3	31	5	52.9	529	529	2.84
1.5	5	3	11	1	13.4	134	134	3.44
1.5	15	3	0	0	0	0	10	4.56
1.5	30	3	0	0	0	0	10	4.56
3	1	3	48	29	436	4360	4360	1.92
3	1	5	nd	nd	nd	nd	nd	nd
3	2	3	48	17	238.2	2382	2382	2.19
3	2	5	nd	nd	nd	nd	nd	nd
3	3	2	19	2	24.6	24.6	24.6	4.17
3	5	2	11	0	12.2	12.2	12.2	4.48
3	15	2	0	0	0	0	1	5.56
3	30	1	0	0	0	0	0.1	6.56
4	1	3	44	21	172.7	1727	1727	2.33
4	1	2	nd	nd	nd	nd	nd	nd
4	2	2	19	9	34	34	34	4.03
4	2	2	nd	nd	nd	nd	nd	nd
4	3	2	4	3	7.2	7.2	7.2	4.71
4	5	2	1	0	1	1	1	5.56
4	15	1	0	0	0	0	0.1	6.56

LIST OF REFERENCES

- American Water Works Assn., 1999. Water Quality and Treatment: A Handbook of Community Water Supplies, 5th ed.; McGraw-Hill, Inc.: NY, NY.
- Azanza, M.P.V., Azanza, R.V., Gedaria, A.I., Sententa, H.G., Idjao, M.V., 2001. Decimal reduction times of *Pyrodictum bahamense* var *compressum* and *Escherichia coli* in chlorine- and ultraviolet-treated seawater. Letters in Appl. Microbiol. 33, 371-376.
- Bai, M.; Zhang, Z., Bai, X., Zhou, X., Deng, S., Wang, N., 2003. The killing of microorganisms in ship as ballast water using hydroxyl radicals. Oceanologia et Limnologia Sinica 34, 484-489.
- Basu, A., Williams, K.R., Modak, M.J., 1987. Ferrate oxidation of *Escherichia coli* DNA polymerase-I. J. Biol. Chem. 262, 9601-9607.
- Bolster, C.H., Bromley, J.M., Jones, S.H., 2005. Recovery of chlorine-exposed *Escherichia coli* in estuarine microcosms. Environ. Sci. Technol. 39, 3083-3089.
- Brock, T.D., Madigan, M.T., Martinko, J.M., Parker, J., 1994. Biology of Microorganisms, 7th ed. Prentice Hall, Englewood Cliffs, NY.

- Cohen, G.N., 2004. Microbial Biochemistry. Kluwer Acad. Pubs., Boston.
- Crichton, R., 2001. Inorganic Biochemistry of Iron Metabolism: From Molecular Mechanisms to Clinical Consequences, 2nd ed. John Wiley & Sons, NY.
- Carlton, J.T., Reid, D., van Leeuwen, H., 1995. The Role of Shipping in the Introduction of Nonindigenous Aquatic Organisms to the Coastal Waters of the United States (other than the Great Lakes) and an Analysis of Control Options (Tech. Rep. No. CG-D11-95, US Coast Guard, Washington DC) in Ruiz, G.M., Rawlings, T.K., Dobbs, F.C., Drake, L.A., Mullady, T., Huq, A., Colwell, R.R., 2000. Global spread of microorganisms by ships. *Nature* 408, 49-50.
- Carlton, J.T., Geller, J.B., 1993. Ecological roulette: The global transport of nonindigenous marine organisms. *Science* 261, 78-82.
- Debarbieux, L., Wandersman, C., 2004. Hemophore-dependent heme acquisition systems, pp 38-47 in Iron Transport in Bacteria, Crosa, J.H., Mey, A.R., Payne, S.M., eds. ASM Press, Wash., DC.
- Di Lorenzo, M., Stork, M., Alice, A.F., Lopez, C.S., Crosa, J.H., 2004. *Vibrio*, pp 241-255 in Iron Transport in Bacteria, Crosa, J.H., Mey, A.R., Payne, S.M., eds. ASM Press, Wash., DC.

Duce, R.A., Tindale, N.W., 1991. Atmospheric transport of iron and its deposition in the ocean. *Limnol. Oceanogr.* 36, 1715-1726, in *Microbial Ecology of the Oceans*, Kirchman, D.L., ed., 2000, Wiley-Liss, NY.

Emerick, R.W., Loge, F.J., Ginn, T., Darby, J.L., 2000. Modeling the inactivation of particle-associated coliform bacteria. *Water Environ. Res.* 72, 432-438.

Escobar, I.C., Randall, A.A., Taylor, J.S., 2001. Bacterial growth in distribution systems: effect of assimilable organic carbon and biodegradable dissolved organic carbon. *Environ. Sci. Technol.* 35, 3442-3447.

Falkowski, P.G., 1995. Towards understanding the molecular ecology of phytoplankton photosynthesis, pp 17-38 in *Molecular Ecology of Aquatic Microbes*, I Joint, ed. Springer-Verlag, NY.

Florida Department of Environmental Protection, 2006. Surface Water Quality Standards, Chapter 62-302. <http://www.dep.state.fl.us/legal/Rules/shared/62-302.pdf> (accessed March 2006).

Gerba, C.P., Nwachuku, N., Riley, K.R., 2003. Disinfection resistance of waterborne pathogens on the United States Environmental Protection Agency's Contaminant Candidate List (CCL). *Aqua* 52, 81-94.

- Greets, N.V., Fomichev, A.Y., 1985. The dependence of ozone antibacterial and antiphage action on the concentration of cells and phage particles in the reaction medium. *Mikrobiologiya* 54, 410-413.
- Gyurek, L.L., Finch, G.R., 1998. Modeling water treatment chemical disinfection kinetics. *J. Environ. Eng.* 124, 783-793.
- Haas, C.N., 1981. Rational approaches in the analysis of chemical disinfection kinetics. *Chemistry in Water Reuse*, Vol. 1, Ann Arbor Science, Ann Arbor, MI.
- Haas, C.N., 1979. Kinetics of bacterial deactivation with chlorine. *J. Environ. Eng.* 105, 1198-1199.
- Haas, C.N., Heath, M., Jacangelo, J., Anmangandla, U., Joffe, J., Hornberger, J.C., Glicker, J., 1994. Development and validation of rational design methods of disinfection. American Water Works Assn. and AWWA Research Foundation, Denver, CO.
- Haas, C.N., Joffe, J., 1994. Disinfection under dynamic conditions: Modification of Hom's model for decay. *Environ. Sci. Technol.*, 28, 1367-1369.
- Haas, C.N., Karra, S.B., 1984. Kinetics of microbial inactivation by chlorine: Review of results in demand-free systems. *Water Res.*, 18, 1443-1449.

- Haas, C.N., Kaymak, B., 2003. Effect of initial microbial density on inactivation of *Giardia muris* by ozone. *Water. Res.* 37, 2980-2988.
- Hassen, A., Heyouni, A., Shayeb, H., Cherif, M., Boudabous, A., 2000. Inactivation of indicator bacteria in wastewater by chlorine—a kinetics study. *Biores. Tech.* 72, 85-93.
- Hebert, P. D. N., Muncaster, B.W., Mackie, G. L., 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): A new mollusc in the Great Lakes. *Can. J. Fish. Aquat. Sci.*, 46, 1587-1591.
- Hom, L.W., 1972. Kinetics of chlorine disinfection in an ecosystem. *J. Sanit. Eng. Div. Am. Soc. Civ. Engrs.* 98, 183-193.
- Helsel, D.R., Hirsch, R.M., 2002. Statistical Methods in Water Resources, Chapter 3 in the Techniques of Water-Resources Investigations of the United States Geological Survey, Book 4, Hydrologic Analysis and Interpretation.
<http://water.usgs.gov/pubs/twri/twri4a3> (accessed January 2006).
- Hulsmann, N., Galil, B.S., 2001. The effects of freshwater flushing on marine heterotrophic protists—Implications for ballast water management. *Marine Pollution Bulletin* 42, 1082-1086.

Jiang, J.-Q., Lloyd, B., 2002. Review: Progress in the development and use of ferrate salt as an oxidant and coagulant for water and wastewater treatment. *Water Res.* 36, 1397-1408.

Joachimsthal, E.L., Ivanov, V., Tay, J.-H., Tay, S.T.-L., 2004. Bacteriological examination of ballast water in Singapore Harbour by flow cytometry with FISH. *Marine Pollution Bulletin* 49, 334-343.

Joachimsthal, E.L., Ivanov, V., Tay, J.-H., Tay, S.T.-L., 2003. Flow cytometry and conventional enumeration of microorganisms in ships' ballast water and marine samples. *Marine Pollution Bulletin* 46, 308-313.

Kirchman, D.L., 2000. Uptake and Regeneration of Inorganic Nutrients by Marine Heterotrophic Bacteria, pp 261-288 *in* Kirchman, D.L., ed., *Microbial Ecology of the Oceans*. Wiley-Liss: NY, NY.

Kovalak, W.P., Longton, G.D., Smithee, D., 1993. Infestation of Power Plant Water Systems by the Zebra Mussel (*Dreissena polymorpha pallas*) *in* Nalepa, T.F.; Schloesser, D.W. eds., *Zebra Mussels: Biology, Impacts, and Control*. Lewis Pub.: Boca Raton, FL.

- Jegatheesan, V., Kastl, G., Fisher, I., Chandy, J., Angles, M., 2004. Modeling bacterial growth in drinking water: Effect of nutrients. J. Am. Water Works Assoc. 96, 129-141.
- Lee, Y., Cho, M., Kim, J.Y., Yoon, J., 2004. Chemistry of ferrate (Fe(VI)) in aqueous solution and its applications as a green chemical. J. Ind. Eng. Chem. 10, 161-171.
- Lehninger, A.L., 1982. Principles of Biochemistry. Worth Pub., NY.
- Mann, N.H., 1995. How do cells express nutrient limitation at the molecular level? pp 171-190 in Molecular Ecology of Aquatic Microbes, Joint, I., ed. Springer-Verlag, NY.
- Matheickal, J., Selvakumar, P., Weitz, F., Mahmud, H., Daly, L., Reinhart, D.R.; Sharma, V.K., Ching Thin, L.L., 2004. "Singapore's R&D efforts in developing innovative ballast water treatment technology with a special emphasis on Fe-VI (Ferrate) as a potential secondary disinfection chemical," presented at the Ballast Water Treatment Symposium, Singapore, May 2004.
- McCarthy, S.A., Khambaty, F.M., 1994. International Dissemination of Epidemic *Vibrio cholerae* by Cargo Ship Ballast and Other Nonpotable Waters. Appl. & Environ. Microbiol. 60, 2597-2601.

- Metcalf & Eddy, Inc., 2003. Wastewater Engineering: Treatment and Reuse, 4th ed. McGraw-Hill, NY.
- Nathanson, N., Langmuir, A.D., 1963. The Cutter Incident: Poliomyelitis following formaldehyde-inactivated poliovirus vaccination in the United States during the spring of 1955. II. Relationship of poliomyelitis to Cutter vaccine. *American Journal of Epidemiology* 78:29-60.
- Norton, C.D., LeChevallier, M.W., Falkinham, J.O., 2004. Survival of *Mycobacterium avium* in a model distribution system. *Water Res.* 38:1457-1466.
- Oemcke, D., Parker, N., Mountfort, D., 2004. Effect of UV irradiation on viability of micro scale and resistant forms of marine organisms: Implications for the treatment of ships' ballast water. *J. Marine Environ. Eng.* 7, 153-171.
- Oemcke, D., van Leeuwen, J., 2005. Ozonation of the marine dinoflagellate alga *Amphidium* sp—Implications for ballast water disinfection. *Water Res.* 39, 5119-5125.
- Page, D.W., van Leeuwen, J.A., Spark, K.M., Drikas, M., Withers, N., D.E. Mulcahy, 2002. Effect of alum treatment on the trihalomethane formation and bacterial regrowth potential of natural and synthetic waters. *Water Res.* 36, 4884-4892.

- Perfiliev, Y.D., Sharma, V.K., 2004. Ferrate (VI) synthesis: Dry and wet methods. Proceedings of the International Symposium Innovative Ferrate(VI) Technology in Water and Wastewater Treatment; Prague, Czech Republic, May 31; ICT Press, Institut of Chem. Tech., Prague, 32-37.
- Propato, M., Uber, J.G., 2004. Vulnerability of water distribution systems to pathogen intrusion: How effective is a disinfectant residual? Environ. Sci. Technol. 38, 3713-3722.
- Qualls, R.G., Johnson, J.D., 1985. Modelling and efficiency of ultraviolet disinfection systems. Water Res. 19, 1039-1046.
- Raaymakers, S., 2001. 1st International Ballast Water Treatment Standards Workshop Report, March 28-30, 2001, report. <http://globallast.imo.org> (accessed March 2006).
- Raymond, K.N., Dertz, E.A., 2004. Biochemical and Physical Properties of Siderophores, pp 3-17 in Iron Transport in Bacteria, JH Crosa, AR Mey, and SM Payne, eds. ASM Press, Wash., DC.
- Rennecker, J.L., Kim, J.-H., Corona-Vasquez, B., Marinas, B.J., 2001. Role of disinfectant concentration and pH in the inactivation kinetics of *Cryptosporidium*

parvum oocysts with ozone and monochloramine. Environ. Sci. Technol. 35, 2752-2757.

Rigby, G.R., Hallegraeff, G.M., Sutton, C., 2002. Novel ballast water heating technique offers cost-effective treatment to reduce the risk of global transport of harmful marine organisms. Marine Ecol. Progress Series 191, 289-293.

Rincon, A.-G., Pulgarin, C., 2004. Bactericidal action of illuminated TiO₂ on pure *Escherichia coli* and natural bacterial consortia: Post-irradiation events in the dark and assessment of the effective disinfection time. Appl. Catalysis B: Environ. 49, 99-112.

Rizzo, L., Belgiorno, V., Napoli, R.M.A., 2004. Regrowth evaluation of coliform bacteria injured by low chlorine doses using selective and nonselective media. J. Environ. Sci. and Health 39, 2081-2092.

Schink, T., Waite, T.D., 1980. Inactivation of f2 virus with ferrate (VI). Water Res. 14, 1705-1717.

Scully, F.E., Jr., Hogg, P.A., Kennedy, G., Lewicki, C., Rule, A.M., Soffriti, J.G., 1999. Development of **Disinfection**-Resistant Bacteria During Wastewater Treatment. Water Environ. Res., 71, 277-281.

Selleck, R.E., Saunier, B.M., Collins, H.F., 1978. Kinetics of bacterial deactivation with chlorine. J. Environ. Eng. 104, 1197-1212.

Sohn, J., Amy, G., Cho, J., Lee, Y., Yoon, Y., 2004. Disinfectant decay and disinfection by-products formation model development: chlorination and ozonation by-products. Water Res. 38, 2461-2478.

US Environmental Protection Agency, 2001. Aquatic Nuisance Species in Ballast Water Discharge: Issue and Options, Office of Water, Washington, DC
<http://www.socp.org/ballast/papers/EPA> (accessed June 2006).

WEF, 1996. Wastewater Disinfection: Manual of Practice FD-10. Water Environment Federation.

Western Oregon University, 2006. Pourbaix Diagrams.
www.wou.edu/las/physci/ch412/pourbaix.htm (accessed March 2006).

White, C.G. 1999. Handbook of Chlorination and Alternative Disinfectants, 4th ed. John Wiley & Sons, NY.

Wood, R.H., 1957. The heat, free energy and entropy of the ferrate (VI) ion. J. Amer. Chem. Soc. **80**, 9, 2038-2041.

Xie, Y.F., 2004. Disinfection Byproducts in Drinking Water: Formation, Analysis, and Control. Lewis Pub.: NY, NY.