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Electro-disinfection of Ballast Water

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Electro-disinfection of Ballast Water

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NOMENCLATURE

Acronyms

AOAC – Association of Official Agricultural Chemists

AC – alternating current

AFM – atomic force microscopy

ATP – adenosine triphosphate

BWE– ballast water exchange

BWT–ballast water treatment

BWMA – Ballast Water Management Act

CD – current density

CFR – Code of Federal Regulations

CFU – Colony Forming Unit

DC – direct current

DBPS – disinfectant by products

EEZ – exclusive economic zone

FDA – Food Drug Administration

GAO – United States Government Accountability Office

GSH – glutathione

IEC- International Electrotechnical Committee

IMO – International Maritime Organization

ISO – International Scientific Organization

NISA – National Invasive Species Act

NOBOB – no ballast on board

NPDES – National Pollutant Discharge Elimination System

ORP– oxidative redox potential

PCD – programmed cell death

ROS – reactive oxygen species

SEM – scanning electron microscope

SERC – Smithsonian Environmental Research Center

TEM – transmission electron microscopy

TFM – Lampricide 3-trifluoromethyl-4-nitrophenol

THM – trihalomethanes

TNC – Too numerous to count

USEPA – United States Environmental Protection Agency

UV – ultraviolet

VGP – Vessel General Permit

WET– whole effluent toxicity

Elements

Al – aluminum

Cl_2 – chlorine

H_2O_2 – hydrogen peroxide

$MgSO_4$ – magnesium sulfate

$NaHCO_3$ – sodium bicarbonate

Na_2CO_3 – sodium carbonate

$^{1}O_2$ – singlet oxygen

O_2^{-}• – superoxide radical

O_3 – ozone

OH• – hydroxyl radical

$ONOO^{-}$ – peroxynitrite

Units

A – amperes

A/cm^2 – amps per square centimeter

°C – degrees Celsius

cm – centimeter

ft – feet

g – grams

in – inch

kw – kilowatt

kWh – kilowatt hour

L – liter

L/min – liter per minute

m – meter

m^3 – cubic meters

ma – milliampere

mg/L – milligram per liter

mL – milliliter

mm – millimeter

ppt – parts per thousand

W – watt

μL – microliter

μm – micrometer

Bacteria

E. coli – Escherichia Coli

V. cholerae – Vibrio Cholerae

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ABSTRACT

This research validates electro-disinfection as a potential secondary ballast water treatment technology. Electricity applied to bacteria laden water produced bactericidal effects, reactive oxygen species and chlorine generation which annihilated bacteria. Evaluation of electro-disinfection experiments showed titanium electrodes had the maximum kill efficacy while disinfection with aluminum and stainless steel electrodes had lesser kill efficacy.

A continuous flow electro-disinfection reactor was evaluated utilizing artificial brackish and fresh ballast water. Brackish water had a 100% bacteria kill efficiency utilizing titanium electrodes at a current density of 10 mA/cm^2. Fresh water was augmented with the addition of salt to increase its electrical conductivity from 232 µS/cm to 873 µS/cm to ascertain 100% bacteria kill efficiency with titanium electrodes and a current density of 9.8 mA/cm^2.

Search Words: electro-disinfection, electrolysis, bacteria kill efficacy, ballast water, electrical conductivity, and destruction of pathogenic bacteria

CHAPTER 1

INTRODUCTION

Problem Statement

Shipping industries face the dilemma of reducing ecological risk from ballast water invasive species while maintaining marginal additional operational costs (Endresen et al., 2004). Shipping accounts for two-thirds of all world trade transportation and has been utilizing ballast water since the early 1900s (Endresen et al., 2004). The study of ballast water affecting ecosystems did not occur until the late 1980s. The precursor to these studies was the economic and environmental impacts from comb-jelly fish in the Black Sea and zebra mussels in North America (Drake et al., 2007; IMO, 2008). The United States Government Accountability Office (GAO) estimates the economic cost between 1989 and 2001 of the zebra mussel invasion alone was between seven hundred and fifty million to one billion dollars (GAO, 2005). The pathogenic viable bacteria, *Vibrio cholerae (V. cholerae),* has been documented as being transported by ballast water in ships to the United States in 1991 from South America where there was a pandemic invasion of *V. cholerae* (Bright, 1998).

Ballast water is water that is taken in the substructure of ships in order to control buoyancy, maintain proper stability, and increase maneuverability of the ship in the absence of cargo. At this current time, vessels perform ballast water exchange mid-ocean; however, ballast water in an empty ship is released as cargo is being loaded in ports to adjust the ship's freeboard. Ships that report no-ballast-on-board (NOBOB) can have as much as 200 metric tons of negligible residual water and sediments (Sano et al., 2004). International Maritime Organization

(IMO) states more than ten billion tonnes of ballast water are transferred daily in United States' waterways (IMO, 2009). Sediment communities inside ballast water act as a habitat for dinoflagellate cysts, crabs, shrimp, and bottom dwelling fish as ballast tanks are not cleaned for months to years (Carlton and Holohan, 1998). Ballast water taken from one body of water and discharged into a different body of water is credited for creating biotic homogenous ecosystems and is the predominant cause of species extinction (Rahel, 2002; Sala et al., 2000).

Magnitude of Problem

The United States Government Accountability Office (GAO) declared fifty-three invasive species have been introduced into the Great Lakes within the last thirty years by ballast water and other sources (GAO, 2005). In 2003, IMO noted the rate of successful invasions in the Great Lakes was 66% higher than they were 100 years ago (Matheickal and Raaymakers, 2003). A study was performed on ballast water from sixty-four bulk carriers with the result of the study noting an estimate of 10^{20} bacteria and viruses are annually delivered to and survive in the lower Chesapeake Bay (Drake et al., 2007). Zebra mussels have spread from the Great Lakes region in 1989 into twenty-four states within the United States. This invasive species of mussels attach themselves inside and onto pipes clogging pipes to water municipalities, along with hydroelectric and nuclear power plants (Benson and Raikow, 2009).

The collapse of anchovy fisheries in the areas around the Black Sea and the Sea of Azov have been credited to the introduction of comb-jelly fish, another invasive species (Mitropoulos, 2008).

Legal Perspective of Ballast Water

In 1996, the United States acknowledged ballast water was a problem and established Public Law 104-332 also known as the National Invasive Species Act (NISA). Beginning in 1998, NISA set mandatory ballast water management guidelines consisting of mid-ocean ballast water exchange (BWE) for all ships entering into the Great Lakes waterways with implementation of the law. In 2004, the recommended voluntary guidelines for ballast water management for every maritime ship to exchange ballast became mandatory in the United States for all traffic arriving from outside of the economic exchange zone (EEZ)- defined as two hundred nautical miles from shore- as written in Code of Federal Regulations (CFR) Title 33, Part 151, Subparts C and D (United States, 2001).

Currently, there are only two approved methods of ballast water exchange (BWE) in the United States, flow through or empty and refill. The flow through method of BWE requires three times the ballast tank's volume capacity to be continuously pumped before the final ballast water is held inside the tanks (United States, 2001). The empty and refill method of BWE is for one hundred percent of the ballast water to be emptied and replaced with new ballast water. The empty and refill method has greater kill efficacy in microbial populations of BWE than the flow through method (Carlton and Holohan, 1998). The killing mechanism, "salinity stresses", caused by empty and refill and flow methods should be the causative agent of annihilation of all organisms and bacteria; however, this is not always the case for euryhaline organisms, *Enterobacteria,* and *V. cholerae.*

The United States Environmental Protection Agency (USEPA) requires non-recreational ships larger than seventy-five feet, excluding commercial fishing boats, to possess a National Pollutant Discharge Elimination System (NPDES) permit in order to release on-board ballast

water into the ocean. The Ballast Water Management Act (BWMA) proposed as a law in 2005, if passed would regulate the amount of viable species allowed to be released into aqueous environments during ballast water exchange.

The International Maritime Organization (IMO) recommends BWE to control invasive alien species (IMO, 2003). IMO established G8 guidelines in 2004 that will become international law two years after ratification by thirty countries and currently only sixteen countries have signed this legal agreement (IMO, 2008).

The BWMA is more strenuous than the proposed laws of the IMO G8 guidelines regulating international ballast water, Table 2.1. The criteria presented in Table 2.1 remains the recommended killing efficacy recommendations for ballast water treatment technologies to ascertain and are not enforceable as law at this particular time.

Salinity Tolerant Organisms and Bacteria

Euryhaline organisms are capable of withstanding a wide range of salinities, because their life cycles involve migration from fresh water to marine environments. A renowned euryhaline organism, the sea lamprey, has decimated the fishing industry in the Great Lakes region. Lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), has been developed and utilized since the 1950s to kill sea lamprey larvae in the Great Lakes Region (Jeffrey et al., 1986).

Enterobacteria, also known as coliform bacteria and *V.cholerae* are well adapted to increased salinities (Harvell et al., 2004; Refugio et al., 2005). Enterobacteria are one of the largest bacteria families. Some of the most harmful members of the Enterobacteria family include: *Escherichia coli* (*E. coli*), *Salmonella*, *Shigella*, *Proteus,* and *Serratia* which can cause food poisoning, dysentery, and other bacterial infections (Prescott et al., 2005). The bacteria *V.*

cholerae renown for causing diarrheal illnesses are indigenous to brackish, estuarine, and fresh waters (Refugio et al., 2005).

Treatment Technologies

Many different methods of eradicating animals, bacteria, plants, and viruses in ballast water are being researched and developed to prevent transfer of invasive aquatic species and microorganisms that are harmful to United States' waters. Some current methods of ballast water treatment include filtration, addition of biocides, deoxygenation, heating, ultraviolet (UV) irradiation, and electro-disinfection.

Filtration of ballast water is necessary as the primary treatment method to remove organisms greater than 50 μm. Secondary treatment methods include the use of biocides, deoxygenation, heat, UV irradiation, and electro-disinfection. The addition of chlorine (Cl_2) as a biocide has a proven history in treatment of drinking water in the United States. Cl_2 efficiently kills bacteria and small zooplankton, but sizable concentrations would be necessary to eradicate larger organisms (Daly et al., 2005). Excessive Cl_2 causes disinfectant by-products (DBPS), such as trihalomethanes (THM), that are documented as being carcinogenic. Deoxygenation of ballast Cl_2 water destroys all aerobic organisms; however, deoxygenation creates conditions anaerobic bacteria thrive in while creating more corrosion inside ballast tanks than oxygenated water (Lee et al., 2004). Anaerobic and aerobic bacteria spores are resistant up to temperatures of 100°C and heating causes thermal stresses on ships as well as thermal pollution to the recipient water (Quilez-Badia et al., 2008; Cohen, 1998).

Electro-disinfection Research

The Space and Naval Warfare Systems Command (SPAWAR) funded the ballast water electro-disinfection research program, and the primary contractor, eVenture Technologies, LLC, was subcontracted to the University of New Orleans through Task Order 0099. The objective of this research is to prove electro-disinfection is a cost effective secondary ballast water treatment option. The bench-scale study of electro-disinfection utilizing continuous flow at one liter per minute (L/min) has proven that *Escherichia Coli* (*E.Coli*) and other coliform bacteria alongside microorganisms can be destroyed in both fresh and salt water environments.

Objective and Scope

Electro-disinfection utilizes three processes to destroy bacteria and protozoa: electricity, minimum Cl_2 generation from chlorides, and generated reactive oxygen species (ROS). Electro-disinfection causes minimum degradation to receiving water as the residuals, reactive oxygen species and Cl_2, quickly dissipate. The objective of the electro-disinfection research is to determine the best parameters required to increase the annihilation of detrimental bacteria while minimizing cost by varying voltage, electricity, and electrode metals.

CHAPTER 2

LITERATURE REVIEW

Biodiversity in Ballast Water

Biological invasions are changing earth's ecosystems on a global scale, escalating habitat loss, increasing disease outbreaks, and causing declines in endangered species (Ruiz and Carlton, 2003). Dobbs and Robinson's study of lakes and oceans verified every milliliter (mL) of water contained 10^2 eukaryotic organisms, 10^6 bacteria, 10^7-10^9 viruses, while the same study estimates ballast water contains 10^8-10^9 organisms per liter (Dobbs and Rogerson, 2005). One cubic meter of ballast water is estimated to have a population density of 110 million plankton species (Bai X, et al., 2005). Ruiz and Carlton state studies of ballast water have shown the success of invasions is "density-dependent". The more times an organism is released in the same area, the more likely the species has the ability to successfully colonize (Ruiz and Carlton, 2003).

In 1991, an epidemic from the bacteria *V. cholerae*, serotype O139, occurred in Peru resulting in several million people being infected while ten thousand people died. The proposed original source of the microbe *V.cholerae* was ships' ballast water. Once the infected ballast water was released, it reached the central water drinking system in Peru (Bright, 1998). Discharged ships' ballast water into receiving waters provides ample opportunities for species extinction and pathogenic bacteria introduction, especially amongst repeated trading routes from one region in the world to the next (Ruiz and Carlton, 2003). McCarthy and Khambaty reported in five of nineteen ships examined in Mobile, Alabama; Gulfport and Pascagoula, Mississippi the presence of *V.cholerae* was noted. *V.cholerae* was recovered from the ballast water of all five

ships whose last port of call was in South America. McCarthy and Khambaty's work established fecal coliforms are not a reliable indicator for the presence of *V.cholerae,* Appendix B, Table B.1, B.2 (McCarthy and Khambaty, 1994). No reports of illness from this particular strain of *V.cholerae* that was recovered by McCarthy and Khambaty was noted in North America. This incident illustrates the ability of ships to carry viable disease causing bacteria. It also points out tests analyzing only the presence of fecal coliforms as an indicator of other pathogenic bacteria are inadequate (Drake et al., 2007).

Viruses also can be introduced into receiving waters via ballast water discharge. Viruses, unlike bacteria, are not asexual and require vectors as host cells for propagation of their genetic material (Dobbs and Rogerson, 2005). Mosquitoes are renowned vectors of dengue fever, malaria, and yellow fever viruses (CDC, 2007). Dengue was the most important viral disease transported by mosquitoes affecting humans in the year 1995 (Gubler and Clark, 1995). Larvae of the Asian tiger mosquito were imported to the United States by means of tires being shipped in the 1980s (Vitousek et al, 1996). The Asian tiger mosquito is a carrier of both dengue and yellow fever having the ability to breed in non-traditional habitats (Bright, 1998). By 1992, less than twelve years from its initial introduction into the United States, the Asian tiger mosquito had spread throughout twenty-five states (Vitousek et al., 1996). The documented cases of the Asian tiger mosquitoes being transported through tires does not eliminate the fact mosquito larvae is commonly transported via ballast water.

Euryahline Organisms

Ballast water is credited for the introduction of the sea lamprey, an infamous euryhaline organism. The introduction of the sea lamprey into the Great Lakes region, which decimated the

commercial fishing industry, occurred before the 1950s. From 1991 to 1996, as an alternative to the traditional chemical lampricide, TFM, male sea lampreys sterilized with bisazir were released into Lake Superior to help reduce the number of sea lamprey (Bergstedt et al., 2003).

Two other euryhaline organisms have been introduced into the United States since implementation of mandated ballast water regulations in the Great Lakes in 1993. The invasion of the Ponto-Caspian species amphipod *Echinogammarus ischnus* and the waterflea, *Cercopagis pengoi,* prove current ballast water exchange may be insufficient in preventing invasive aquatic species (Ricciardi and Maclsaac, 2000).

United States Ballast Water Laws

The "salinity stress" induced to fresh water organisms during BWE is an effective barrier to possible colonization of bacteria (Carlton and Holohan, 1998). Smithsonian Environmental Research Center (SERC) performed experiments on more than twenty-four different full-size ships from four different vessel types: commercial oil tankers, container ships, bulk carriers, and Navy re-fuelers. SERC's research established BWE produced 80-95% reduction of planktonic organisms from ballast tanks and removed on average 88-99% water in ballast tanks when performed according to regulations (Ruiz and Reid, 2007).

The NISA of 1996 established voluntary, as well as mandatory BWE guidelines for all ships entering into United States' waters (United States, 1996). This act required ships entering into the Great Lakes and the Hudson River from outside of the economic exchange zone (EEZ) to perform mid-ocean exchange of ballast waters prior to entering the EEZ vicinity. The NISA became mandatory in 2004 and is now enforced by the United States Coast Guard out of the EEZ

(United States, 2001). Currently, all ships entering into United States' ports and internal waters are required by law to keep ballast logs of performed BWE.

Numerous petitions have been filed to overturn the Clean Water Act Code of Federal Regulations (CFR) Chapter 40 Section 122.3 which previously excluded "the exception from discharges incidental to the normal operation of a vessel" (EPA Final VGP, 2008). This exclusion existed for thirty-five years allowing boats to release gray water from sinks or laundry, ballast water, and deck runoff from cleaning or rain without National Pollutant Discharge Elimination Permits, until a district court overturned the ruling in September 2006:
"The blanket exemption for discharges incidental to the normal operation of a vessel, contained in 40 CFR 122.3(a), shall be vacated as of September 30, 2008" (EPA NPDES, 2008).
The United States Court of Appeals for the Ninth Circuit upheld this decision (Federal Register, 2008). In 2008, the USEPA established a VGP applicable to vessels that are seventy-nine feet or greater excluding commercial fishing boats. The VGP required all ships that fell into this category to acquire NPDES permits.

The VGP applies to discharges incidental to the normal operation of a vessel and includes twenty-six vessel discharge streams (EPA Final VGP, 2008). The VGP permit is relevant to ballast waters within three miles of United States territorial sea waters and is enforceable as of July 9, 2010 (EPA Federal Register, 2008). The VGP permit does not set effluent numerical limitations on whole effluent toxicity for the experimental ballast water treatment systems. Whole effluent toxicity (WET) testing is implemented for ballast water treatment systems to determine the most environmentally efficient treatment system. The State of Washington utilizes WET testing on treated ballast water as mandated in the Clean Water Act as specified in 40 Code of Federal Regulations (CFR) Part 136 (EPA Federal Register, 2008).

International Maritime Organization Ballast Water Regulations

The United Nations established the International Maritime Organization (IMO) as an agency in 1948 to regulate environmental concerns, legal matters, maritime security, and shipping efficiency (IMO, 2009). In 1988, IMO recognized for the first time ballast water containing aquatic invasive species was capable of altering ecosystems at a port where the ballast water was discharged (IMO, 2004). In 2004, IMO established the International Convention for the Control and Management of Ships' Ballast Water and Sediments. The Convention immediately developed performance standards for discharge of ballast water formally named IMO's Guidelines for Approval of Ballast Water Management Systems; commonly referred to as D-2 standards or G8 guidelines, Table 2.1.

Table 2.1 IMO G8 Guidelines and US BWMA Criteria

Organisms Size Class	IMO G8 Guidelines	US BWMA	California Regulations
Plankton, > 50 µm	< 10 viable organisms / m^3	No detectectable living organisms / m^3	No detectectable living organisms
Plankton, 10-50 µm	< 10 viable organisms / mL	No detectectable living organisms / mL	< 0.01 living organisms / mL
Living organsisms, < 10 µm	No existing standard	No existing standard	< 10^3 bacteria / 100 mL, < 10^4 viruses / 100 mL
Toxicogenic *V. cholerae* (O1 and O139)	< 1 CFU / 100 mL	< 1 CFU / 100 mL	< 1 CFU / 100 mL
Escherichia Coli	< 250 CFU / 100 mL	< 126 CFU / 100 mL	< 126 CFU / 100 mL
Intestinal Enterococci	< 100 CFU / 100 mL	< 33 CFU / 100 mL	< 33 CFU / 100 mL

CFU - Colony Forming Unit

The Convention requires all ships built after 2009 to adhere with the implementation of G8 guidelines based on the ships' ballast water capacity, Table 2.2 (Lloyds Register, 2008). G8 guidelines are highly recommended by the IMO, but are not considered international law. The G8 guidelines will become international law after they are ratified by thirty countries representing 35% of the world's commercial shipping tonnage (IMO, 2005). Currently, only sixteen countries have signed the convention representing 14.24% of the world's shipping

tonnage (IMO, 2008). Implementation of the convention will have a twelve months intermittent period after the convention is signed by thirty countries.

Table 2.2 Implementation Schedule of IMO G8 Guidelines

Ballast Capacity	Before 2009	2009-2011	After 2012
< 1500 m^3	BWE or BWT until 2016; BWT only from 2016	BWT only	BWT only
1500-5000 m^3	BWE or BWT until 2014; BWT only from 2014	BWT only	BWT only
>5000 m^3	BWE or BWT until 2016; BWT only from 2016	BWE or BWT until 2016; BWT only after 2016	BWT only

BWE - Ballast Water Exchange
BWT - Ballast Water Treatment described underneath Approval Processes

Approval Processes

The IMO has a list of approved Flag Administrations known for evaluating and approving ballast water treatments systems in compliance with G8 regulations (Dobroski et al., 2009). The typical time for a flag ship administration to grant an approval certificate is one year to one and one-half years, which includes six months of land based and six months of shipboard trials (Lloyds Register, 2008).

Ballast water treatment technologies may require additional time for an approval certificate if they release active substances that are harmful to aquatic life in receiving waters. Ballast water treatment technologies utilizing active substances, must receive basic approval that fulfills G9 IMO Guidelines, before performing shipboard testing to ensure active substances cause no harm to the environment (Lloyds Register, 2008).

Filtration

Filtration is a physical process of removing sediments, fish, and bacterial pathogens attached to larger organisms and sediments. At the second IMO symposium on ballast water, the following conclusion was made: Primary filtration alone would decrease the rate of successive invasive aquatic species, but it needs to be combined with a secondary treatment to meet proposed G8 guidelines, Table 2.1 (Matheickal and Raaymakers, 2003). Currently, all ships exist with a simple intake filtration mechanism, usually consisting of grates or strainer plates with openings of 1.27 cm (.05 in) to 2.54 cm (1 in) or greater if corrosion persists where water is initially taken into the ballast tanks (Cohen, 1998). Some ships have additional filtration of water through smaller metal screens which removes organic matter and zooplankton as a primary treatment used to enhance secondary treatment options such as biocides, ultra violet (UV) light, heating, and electro-disinfection (Carlton and Holohan, 1998). Filtration of influent water removes ichthyoplakton, invertebrate zooplankton, and the largest phytoplankton and heterotrophic protists; however, filtration of influent water has been unsuccessful in removing most microorganisms in ballast water (Dobbs and Rogerson, 2005). Disk or screens are preferred over traditional granular media for filtration (Dobrosk et al., 2009).

Hydrocyclones

Hydrocyclones are used in ballast water as an alternative to filtration to trap particles in the 50 to 100 μm size range (Dobroski et al., 2009). Hydrocyclones utilize the principle of centrifugation which relies on density differences to separate organisms from sediment. Ballast water is injected at high velocities to impart a vortex that causes heavier particles to move to the outer edges of the cyclone where they become trapped before entering ballast tanks (Dobroski et

al., 2009). The effectiveness of hydrocyclones relies on density of particle, density of ballast water, particle size, speed of rotation, and residence time (Lloyd's Register, 2009). The hindrance of employing hydrocyclone separation is microscopic aquatic organisms have a density less than ballast water, and are very difficult to remove from ballast tank influent water (Dobroski et al., 2009).

Biocides

There are two types of biocides, oxidizing and non-oxidizing. The oxidizing types of biocides are bromine, Cl_2, dioxide, iodine, Peraclean®, and peroxyaceticx acid (Chase, Reilly, and Pederson, 2009; Faimali et al., 2006). Oxidizing types of biocides are added to ballast water through discharge and ballast fill-lines with slight equipment modifications (Daly et al., 2005). Oxidizing biocides are used predominantly in freshwater systems since their biocidal efficacy depletes with increase in organic matter (Chelossi and Faimali, 2006). Non-oxidizing biocides operate similarly to pesticides interfering with the metabolism, reproduction, and physiological processes of organisms (Chase et al., 2009; Chelossi andFaimali et al., 2006). Biocides cause microorganisms' cell death by rupturing the cellular membrane. The treatment efficacy of biocides depends on concentration, exposure time, pH, temperature, and most importantly the type of organism to be eradicated (Perrins et al., 2006; Lloyds Register, 2008). Ballast water containing sediments with higher organic carbon requires increased amounts of biocides to achieve toxicity of organisms (Sano et al., 2004).

Oxidizing Biocides

Chlorine

Venczel et al. demonstrated *Clostridium perfringens* spores exposed to Cl_2 concentrations of 5 mg/L had slower inactivation rates than exposed to mixed oxidant residuals (1997). Excessive amounts of Cl_2 can generate disinfectant by-products (DBPS) such as trihalomethane (THM) which is known for its carcinogenic activity (Pereira, 2000). Cl_2 has been proven to control ballast water organisms within G8 guidelines, but the dosages required to remove organisms in ballast water do not degrade into concentrations low enough to be environmentally benign (Gregg and Hallegraeff, 2007).

Hydrogen Peroxide

Hydrogen peroxide, H_2O_2, generated on site is more cost effective than the generation of ozone (Kuzirian, Terry, Betchel and James, 2001). Hydrogen Peroxide has been recognized for its anti-microbial activity by health professionals for many generations. The foremost advantage of utilizing hydrogen peroxide as a disinfectant is hydrogen peroxide can be generated on site by applying electricity to a brine mixture of water thereby releasing hydrogen peroxide.

Ozone

Industrial applications utilize ozone (O_3) to control large microbial populations, but not to eliminate them (Viitasalo et al., 2005). Large (O_3) generators generate diffused bubbles containing ozone gas into a ballast tank (Chase et al., 2009; Daly et al., 2005). Ozone has a short lifetime of six seconds in seawater because ozone reacts quickly with bromides (Perrins et al., 2006). Ozone in bubbles transforms bromides in sea water to bromines; HOBr and OBr^- operate as disinfectants (Herwig et al., 2006). O_3 reverts back to oxygen (O_2) within hours and the

process is not efficient; 15% of energy is utilized in the conversion O_2 to O_3, while 85% of energy is lost as heat (Hendricks, 2006).

Peraclean Ocean®

Peraclean Ocean® is a biocide utilized in ballast water treatment. *Peraclean Ocean*® is a blend of peracetic acid and hydrogen peroxide, which is relatively stable and produces few harmful byproducts (Lloyd's Register, 2008).

Non-Oxidizing Biocide

SeaKleen®

SeaKleen® is a non-oxidizing biocide developed to be utilized in catfish farming and is currently being marketed for ballast water treatment (Lloyds's Register, 2008). The primary disinfectant agent in *SeaKleen*® is Vitamin K3.

Gregg and Hallegraeff assessed the efficacies of *SeaKleen*® and *Peraclean Ocean*® to establish biocidal activity of these chemicals declined with decrease in temperature (2007). Also noted in their studies, was the biocides biodegradability; inconsistencies between the manufacturer's information and data ascertained in studies proving slower degradation of the biocides (Gregg and Hallegraeff, 2007). *Peraclean Ocean*® was the more biodegradable biocide within two to six weeks after applications of 200 ppm (Gregg and Hallegraeff, 2007).
The primary disadvantage of biocides is necessary storage and handling of dangerous chemicals. The combination of sea water with biocides can cause toxic chemical effects to discharged treated ballast water that affect native organisms (Chase et al., 2009). Biocides have low-capital cost and power consumption; therefore, the chemical costs and storage area causes this particular treatment to be applicable to ships with small ballast capacities (Lloyd's Register, 2008).

Deoxygenation

Oxygen deprivation is being investigated as a ballast water treatment to control corrosion of ballast tanks while killing invasive aquatic species (Lee et al., 2006). Deoxygenation of ballast water as a treatment method has included purging ballast water with nitrogen gas, vacuum chambers, Venturi Oxygen Stripping™, and addition of glucose, sulphide, or nutrient solutions to ballast water (McCollin et al., 2007). The previous listed methodologies of treating ballast water create anaerobic conditions which annihilate aerobic organisms by asphyxiation. However, some bacteria and protists possess metabolic systems that allow them to routinely switch between aerobic and anaerobic environments (Dobbs and Rogerson, 2005).

The amount of oxygen concentration in ballast water should be between 0.2 and 1.0 mg/L to exterminate aquatic organisms while reducing corrosion of ballast tanks (Tamburri and Ruiz, 2005). Lee et al. observed 1020 steel coupons, small rectangular samples of steel, in natural seawater for a year and documented persistence of corrosion was more aggressive under stagnant strict anaerobic conditions, than stagnant aerobic conditions measured by weight loss and instantaneous corrosion rates (2004).

Heat Treatment

The efficacy of heat treatment depends on temperature of ballast water, exposure time, and treatment temperature. Ocean water utilized as a coolant to the main engine on a vessel is recycled and flushed through the ballast tanks to retard the growth of organisms and bacteria. Heated water kills most marine organisms except thermophilic bacteria and bacteria spores (Bai et al., 2005). Bacteria spores generally require more than 100ºC for several minutes to be destroyed (Quilez-Badia et al., 2008). The pathogenic bacterium *Escherichia Coli*, *Salmonella*,

and *Campylobacter* require temperatures of 60-70ºC to inactivate these biological agents from causing disease (Rigby et al., 2004). A compilation of data of various heat treatments by Rigby et al. established that most marine organisms experience mortality at temperatures between 40-45ºC, Appendix B, Table B.3 (2004). The exceptions to these marine organisms were marine bacteria, brine shrimp and rotifer eggs, which required 45-55ºC and longer treatment times. Quilez-Badia et al. (2008) analyzed peer reviewed journal articles of established researchers utilizing heat, and concluded, to successfully treat ballast water, 35ºC must be used for a minimum of twenty hours.

Heat treatment is a promising treatment for ballast water treatment for certain types of ships on long voyages that generate significant amounts of waste heat. However, there exists the problem of ascertaining uniform heating rates of ballast water during cold weather (Boldor et al., 2008). Heat treatment is a viable option for some cruise ships and tankers that generate a large amount of waste heat, while heat treatment is not applicable to bulk carriers that transport large volumes of ballast water and generate a small amount of waste heat (Matheickal and Raaymakers, 2003). The majority of European ships would not be able to utilize heat treatment, because European ships spend 60%-65% of their sea time involved in inner costal travel and adequate waste heat would not be generated due to the brevity of their trips (Endresen et al., 2004).

Another form of heat treatment is the use of microwaves to effectively treat ballast water. The oscillating electric field energy has been proven to alter chemical, biochemical, and physical structures of organisms (Boldor et. al., 2008).

Ultraviolet Radiation

Ultraviolet (UV) light has been utilized for decades to disinfect large volumes of water under high flow rates (Dobbs and Rogerson, 2005). The effectiveness of UV disinfection potential depends on the UV light dose and the amount of turbidity in the water (Perrins et al., 2006). Laroussi et al. (2002) performed laboratory scale experiments on fresh and salt water utilizing UV light; the results were three to four log reductions on cultured and environmental bacteria within thirty to sixty seconds of exposure time to UV light. UV light is absorbed by proteins of a microorganism and a photochemical reaction occurs that alters the DNA. Adsorption of UV at larger dosages ruptures a cell leading to its death (Hendricks, 2006).

Electro-chlorination

The production of Cl_2 products to disinfect water is a popular technology employed by water treatment plants to treat drinking water (Parker et al., 2007). The water passes through an electrolytic generator which transforms the chlorides into Cl_2 to disinfect the water. Diao et al. (2004) determined generation of Cl_2 by products had a stronger killing efficiency of *E. coli* than conventional chlorination by the addition of biocides. Further advantages of electro-chlorination it eliminates transport and storage of Cl_2 as all Cl_2 is produced on site.

Electro-disinfection

Electro-disinfection has been utilized by municipalities to treat drinking water, and in food processing industries for its bactericidal properties to eradicate microorganisms. Microorganisms encompass a large class of eukaryotic and prokaryotic cells that cannot be seen without a microscope. Eukaryotic cells have inside organelles, contain a nucleus, and have a membrane compartment that contains genetic material (Prescott et al., 2005). Eukaryotic

microorganisms include algae, fungi, protozoa, plant and animal cells. Prokaryotes include all classes of bacteria with the typical size of one to five micrometers long (Brock et al, 1994). Because prokaryotic cells are smaller, non-spherical, and have a thicker cell membrane they are able to resist higher levels of electricity (Zimmermann and Neil, 1996).

The cell membrane for both prokaryotic and eukaryotic cells acts as a selective barrier to absorb food and nutrients. Death occurs from transmembrane pores allowing loss of important cell components and destruction of chemical gradients (Drees, Abbaszadegan, and Maier, 2003). Electroporlation describes the process when electricity causes excessive opening of transmembrane pores until death of the microorganism occurs.

Voltage

Eukaryotic cells are more susceptible to lower voltages of 1 kV/cm than prokaryotic cells which require 15 to 20 kV/cm of direct current at 25ºC in order for them to lyses (Sale and Hamilton, 1968). Zimmermann and Neil state prokaryotic cells have larger osmotic pressures inside their cell walls and the applied electrical voltages causes them to lyses at voltages smaller than 15-20 kV/cm (Zimmermann and Neil, 1996). Temperature is another important parameter in the lyses of cells. Zimmerman et al. observed eukaryotic cells at a temperature of 37ºC lyses at 0.5 V (Zimmermann and Neil, 1996; Zimmermann and Coster, 1975). Irreversible permeabilization of the bacterium *Legionella* has been observed utilizing twenty repetitive direct current (DC) pulses at ten milli-second intervals at 550 V/cm (Teissié et al., 2002). Electric field effects unaccompanied by other parameters are insufficient in killing high populations of bacteria and viruses to fulfill the drinking water standards in the United States (Drees et al., 2003).

Current

If water contains chlorides, electrolysis will convert chloride ions to Cl_2. DC has a continuous unidirectional current flow while alternating current (AC) has an electrical current that periodically changes direction. AC produces less electrolytic processes within the water than DC; therefore, less chlorides are transformed into Cl_2 using AC (Park et. al., 2004). Jeong et al. (2007) performed a study and validated the destruction of *E. coli* utilizing AC strictly from the production of hydroxyl radicals as no Cl_2 was generated at the end of their experiment.

Oxidative Radicals effects on Eukaryotic Cells

Chlorine's existence as a bactericide has been utilized repeatedly as a final disinfection step in the production of safe drinking water. The presence of chlorides in the water after electrolysis can cause electro-generation of bactericidal oxides such as O•-, OH•, O_3, Cl_2, ClO•-, HO_2-, HOCl, Cl_2O, and oxidized carbonate and sulphate species (Hallegraff et al., 1997; Patermarakis and Fountoukidis, 1990). The generated Cl_2 acts as residual disinfectant, unlike oxygen and hydrogen peroxide radicals which are created near the electrode surface and have an existence of nanoseconds (Patermarakis and Fountoukidis, 1990; Kim et al., 2006). Dress et al. (2003) proved mixed oxidants were responsible for the amelioration of bacteria in electrochemical disinfection by seeding the laboratory created water consisting of bacteria with the antioxidant reduced glutathione (GSH). Both eukaryotic and prokaryotic cells produce reduced GSH to protect themselves intracellulary and extracellurary from alkylating agents, free radicals, and oxidative stress.

Eukaryotes having higher levels of cellular organization contain mitochondria whereas prokaryotes lack this organelle. The mitochondria are correlated to the "powerhouses" of the

cell because it generates adenosine triphosphate (ATP), which is utilized as chemical energy. The mitochondrion consists of inner and outer membranes. The inner membrane is impermeable to ions allowing it to have its own transmembrane potential which projects inward into folds called cristae (McKee and McKee, 2003; Martinez-Huitle and Brillas, 2008). The outer membrane of the mitochondria is smooth and contains an anion voltage dependent channel that is responsible for transport of ions and metabolites across the outer membrane (Martinez-Huitle and Brillas, 2008). Oxygen easily diffuses across cells because of its solubility in non-polar lipid core membranes (McKee and McKee, 2003). Eukaryotic cells can contain up to more than a thousand mitochondria to drive the energy consuming processes. However, some yeasts, unicellular algae, and trypanosome protozoa have a single giant tubular mitochondrion (Prescott et al., 2005).

The mitochondrion utilizes gaseous oxygen in a process known as oxidative phosphorylation to extract chemical energy from the breakdown of nutrients (McKee and McKee, 2003). Another function of the mitochondria is the initiation and execution of apoptotic and necrotic cell death by means of ROS overwhelming cells antioxidant capacity (Ferris et al., 2005). ROS has been proven as initial precursor to activate programmed cell death (PCD) in nine species of protozoa, mammalian cells, and unicellular eukaryotes (Martinez-Huitle and Brillas, 2009). ROS eventually disintegrate after annihilating bacteria, protozoa, and other pollutants into its final residual state of CO_2, H_2O, and inorganic salts (Bai et al., 2005).

Oxidative phosphorylation involves five multiprotein complexes and at complexes I and III of the respiratory chain, reactive oxygen species are generated (Ott, et al., 2007). ROS are necessary as carrier molecules to mediate essential biochemical reactions for adenosine triphosphate (ATP) production in aerobic organisms. The most common ROS are the superoxide

radical (O_2^-•), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radical (OH•) (Prescott et al., 2005). A radical is an atom or group of atoms that contain one or more pairs of unpaired ions (McKee and McKee, 2003). These ROS when produced in excess are toxic to cellular components; causative agents of cell ageing, cell death, enzyme inactivation, mitochondrial DNA mutations, and polysaccharide depolymerization (Ott et al., 2007; McKee and McKee, 2003). One to five percent of the molecular oxygen consumed by the mitochondria is converted into superoxide radicals (Ott et al., 2007; Yoneda, Katsumata, Hayakawa, Tanka, and Ozawa, 1995).

The first ROS formed during reduction of oxygen inside the inner membrane of the mitochondria is the superoxide radical (O_2^-•) at complex I. The presence of antimycin in the mitochondria suppresses cellular respiration and makes complex III an important generator of the superoxide radical (O_2^-•) (Ott et al., 2007). The superoxide radical may react unintentionally with nitric oxide to form highly reactive peroxynitrite (Ott et al., 2007; McKee and McKee, 2003).

$$O_2^- \bullet + NO \bullet \rightarrow ONOO^-$$

Peroxynitrite generates irreversible mitochondrial respiration and damage to mitochondria components complexes I, II, IV, and V (Fleury et al., 2002). The superoxide radical (O_2^-•) is very soluble and mildly reactive in an aqueous environment where it reacts with itself to produce hydrogen peroxide (H_2O_2) to continue cellular respiration activities (Fleury et al., 2002; McKee and McKee, 2003).

$$2H^+ + 2O_2^- \bullet \rightarrow O_2 + H_2O_2$$

Hydrogen peroxide (H_2O_2) has limited reactivity which allows it to easily become dispersed as it moves within the cell. The biochemical reaction of hydrogen peroxide (H_2O_2) with any transition metals, the most common being Iron II, produces a hydroxyl radical (OH•). The hydroxyl radical (OH•) is the strongest oxidant amongst all of the ROS (Zorov, et al., 1997). The contact time required for microorganisms to be destroyed with application of OH• was 10^5 times lower with greater kill efficiency than that for the same dosage of Cl_2 (Jeong, Kim, and Yoon, 2006; McKee and McKee, 2003).

$$Fe_2 + H_2O_2 \rightarrow Fe^{3+} + OH\bullet + OH^-$$

The highly excited singlet oxygen ($^1O_2$) can be formed from either the superoxide radical (O_2^-•) or from the organic peroxides (ROOH) as shown below (McKee and McKee, 2003):

$$2O_2^- \bullet + 2H^+ \rightarrow H_2O_2 + {}^1O_2$$

$$2ROOH \rightarrow 2ROH + {}^1O_2$$

Singlet oxygen in aqueous media is short lived and can cause cellular devastation (Morris, 1976). Eukaryotic cells have an immunological response to invading bacteria and fungi known as respiratory burst. During respiratory burst the cell accelerates its production of hydrogen peroxide (H_2O_2) and superoxide radical (O_2^-•) to disintegrate perceived or actual invading bacteria (McKee and McKee, 2003).

Reactive Oxygen Species on Prokaryotic Cells

Morphological features resembling PCD has been demonstrated to occur in organisms without mitochondria (Martinez-Huitle and Brillas, 2009). PCD occurs in the development processes of bacteria, on occurrence when antibiotics are used to destroy cells, and when cells

are exposed to other harmful conditions (Lewis, 2000). During respiratory burst plant, animal, and some microbes excrete ROS to eradicate invading bacteria (Imlay, 2003).

Various families of bacteria submersed in the same concentrations of O_2^-• and H_2O_2 experience different effects of oxidative stress on its cells (Imlay, 2003). Most bacteria encounter a significant amount of mutagenic DNA damage if not eradication from a ten minute exposure to millimolar levels of H_2O_2 (Imlay, 2003). ROS causes *Escherichia coli* (*E. coli*) to aggregate together as a self protection mechanism (Lewis, 2000).

Electro-disinfection's biocidal capability overshadowed the disinfection potential of Cl_2 and ozone on strains of *E. coli* as shown by the scanning electron microscopy (SEM) in Appendix B Figure B.1 (Kim et al., 2006). As shown in the SEM image electrodisinfection at 16 mA/cm^2 initial aggregation of the bacteria occurred, and at 25 mA/cm^2 the bacteria amalgamated together into a coalesced arrangement (Kim et al., 2006).

The annihilation of *E. coli* using electro-disinfection on a laboratory scale has been demonstrated to increase with the amount of current density applied Appendix B Figure B.2 (Jeong et al., 2006). Morphological changes to *E. coli* at optimum current density acquired by transmission electron microscopy (TEM) and atomic force microscopy (AFM) are in Appendix B Figure B.3 (Jeong, et al., 2006). Electric-pulse techniques have been shown to work on laboratory scale, but there exists no data on electricity on larger scale ballast water operations (Dobbs and Rogerson, 2005).

Microorganism Influenced Corrosion

Microorganisms can create a biofilm that leads to 'interior hull fouling' inside ballast tanks (Drake et al., 2005). The conventional approach to treating this microbiologically

influenced corrosion (MIC) has been to use oxidizing and non-oxidizing biocides (Little et al., 2006). Costerton et al. (1994) reported biofilm bacteria are resistant to antibiotics and biocides at levels 500 to 5,000 times higher than those needed to kill planktonic cells of the same species. Costerton et al. (1994) experiments established biofilm bacteria are killed by low doses of antibiotics when exposed to a DC electric field of 15 $\mu A/cm^2$ to 2.1 mA/cm^2.

CHAPTER 3

ECONOMICS

A 1991 study performed by Smith et al. (1996) of biological invasions affecting the shipping industry, established large commercial vessels transported and released 2.4 million gallons per hour of foreign water into United States' waters. Numerous vessels require the capability to treat ballast water in quantities of 2000 to 20,000 m^3/hr (Rigby, Hallegraeff, and Taylor, 2004).

The majority of the technologies currently on the market were designed to treat ballast water with flow rates of 250 m^3/hr, which are the flow rates of the first phase of ballast water of ships under stringent ballast water regulations (Lloyd's Register, 2008). In 2008, only three ballast water treatment technologies held approval certificates granted by Flagships under oversight of the IMO: Alfa Laval Tumba AB, Hamann AG, and NEI Treatment Systems LLC, Appendix B Table B4 (Lloyd's Register, 2008). Other perspective ballast water treatment technologies expected to receive type approval certificates within the next twelve to eighteen months along with estimates of capital and operational cost, Table 3.1 (Lloyd's Register, 2008).

Alfa Laval Tumba AB treatment technologies incorporates filtration followed by ultraviolet radiation augmented with a titanium oxide photocatalyst. Alfa Laval Tumba did not provide the IMO with any initial estimates of capital and operational cost, Appendix B Table B.4 (a). Alfa Laval Tumba received a type approval certificate in 2008 (Lloyd's Register, 2008).

Hamann AG utilizes two steps in their filtration process followed by application of the biocide Peraclean®Ocean to ballast water. Hamann AG provided IMO with the operational cost

of $200 for 1000 m^3/hr, Table B.4 (b). Hamann AG also obtained a type approval certificate in 2008 and did not provide any additional fiscal information (Lloyd's Register, 2008).

NEI Treatment Systems LLC treatment technology consists of deoxygenation and cavitation. NEI Treatment Systems LLC has an operational cost $150 for 1000 m^3/hr, Appendix B Table B.4 (c). NEI Treatment Systems LLC has capital costs for 200 m^3/hr and 2000 m^3/hr at $360 and $690, respectively. NEI Treatment Systems LLC acquired their type approval certificate in 2007 (Lloyd's Register, 2008).

An economic analysis could not be performed for ballast water treatment technologies on the market, because the companies that submitted estimated costs did not provide additional details on the assumptions that allowed them to arrive at their estimates, Table 3.1. Also, the costs will decrease as the ballast water laws become enforced and the demand for ships to treat ballast increases.

Hi Tech Marine Pty. Ltd. is the only technology listed by Lloyd's Register using heat treatment, Table 3.1. Hi Tech Marine Pty. Ltd. has high capital costs while asserting low operational costs, because of the broad assumption of utilizing ship's waste heat. A second type of ballast water treatment technology utilizes electrolysis and electrocoagulation. Severn Trent De Nora or Techross would be the most economical depending on whether electrolysis is being performed on freshwater or saltwater. Severn Trent De Nora employs residual Cl_2 neutralization after disinfecting the ballast water. The third predominant ballast water treatment technology is utilizing UV irradiation to disinfect ballast water. Marenco is the most economical company utilizing UV treatment of ballast water. Only two ballast water treatment companies utilize deoxygenation. They are MH Systems Inc. and Nutech O3. Nutech 03 is the most economical

ozone ballast water treatment technology; however, Lloyds Register placed a footnote stating, "manufacturer states $.007 per treatment" (Lloyds Register, 2008).

Table 3.1 Costs of Commercial Ballast Water Treatment Systems (Lloyd's Register, 2008)

Manufacturer	Treatment Processes	Active Substance Basic / Final	System Approval SB / LB	Capital Cost, ($k) 200 / 2000 ($m^3$/hr)	Oper. Cost, ($1000) m^3/hr	Power Requirment $\frac{kW}{1000\frac{m^3}{hr}}$
Alfa Laval Tumba AB	Filt, and UV/ TiO_2	07-2007 / 07-2007	04-2008 / 04-2008	NA	NA	NA
ATG Willand	Filt and UV	–	–	NA	NA	NA
Ecochlor Inc	Cl (as ClO_2)	10-2008 / 07-2009*	Ongoing / 06-2008	500 / 800	80	NA
Electrichlor Inc	Filt and EL/EC	–	–	350 / NA	19	>10
Environmental Technologies Inc	Filt ,O_3, and US	–	–	NA / 500	5	70
Gauss	Filt and UV	–	–	NA	NA	NA
Greenship	HC and EL/EC	10-2008 / 07-2009*	06-2008 / 10-2007	300 / 2000	NA	30
Hamann AG	HC, Filt, and PAA	03-2006 / 04-2008	06-2007 / 06-2007	NA	200	NA
Hitachi	Coag and Filt	04-2008 / –	07-2008 / 06-2008	NA / 400	NA	NA
Hi Tech Marine Pty Ltd	Heat	–	–	780 / 1600	0 **	5.8
Hyde Marine Inc. *Hyde Guardian*	Filt and UV	–	11-2008 / 11-2008	NA	10	75
Hyde Marine Inc. *SeaKleen™*	*SeaKleen™*	–	–	NA	NA	NA
JFE Engineering Corporation	Filt, Cl as (Cl_2), and RES	10-2008 / 07-2009	10-2009 / 10-2009	NA	40	7.7
Marenco Technology Group Inc.	Filt and UV	–	2007 / 2007	145 / 175	0.6-1.0	60
Mahle NFV GmbH	Filt and UV	–	2009 / 2009	NA	NA	60
MH Systems Inc.	Deoxy	–	07-2009 / 12-2008	650 / 950	60	10-18
Mitsui Engineering & Shipbuilding	O_3 and Cav	10-2006 / 07-2009*	03-2009 / 02-2008	NA	NA	NA
NEI Treatment Systems LLC	Deoxy and Cav	***	***	360 / 690	150	25
Nutech 03	O_3	07-2007 / 10-2008*	2008 / 2008	250 / 450	0.007	10
Oceansaver AS	Filt, Deoxy, and Cav	04-2008 / 10-2008*	08-2008 / 10-2007	288 / 1600	NA	80-100
Optimarin AS	Filt and UV	–	01-2009 / 05-2008	430 / 1800	NA	220
Panasia	Filt, UV/ OH	04-2008 / –	03-2009 / 10-2008	NA	NA	194
Qwater	Filt and US	–	–	NA	NA	NA
Resource Ballast Technology	Filt, O_3, EL/EC, and Cav	04-2008 / 2009*	2008 / 2008	200 / 500	NA	13-20
RWO Marine	Filt, EL/EC/OH	10-2006 / 07-2009*	03-2009 / 09-2007	NA	NA	80 Sw, 120 Fw
Severn Trent De Nora	EL/EC and RES	2009* / 2009*	2009 / 01-2007*	350 / 500	13	113
Siemens	Filt and EL/EC	–	–	400 / 600	20-30	60-130

Cav- Cavitation, Cl- Chlorination, ClO_2- Cl2 dioxide, Coag- Coagulent(with metallic particles), Deox- Deoxygenation, EL/EC- Electrolysis/Electrochlorination, Filt- Filtration, Fw-Freshwater, HC- Hydrocyclon, O_3-Ozonation, PAA- Peracetic acid, RES- Residual Cl2 neutralization, Sw- Saltwater, US- Ultrasound, UV- Ultraviolet irradiation

* dates projected by manufacturer
** assumes waste heat utilized
*** tests are comparable to IMO 'G8' ballast water protocol stating to have been completed prior to introduction of G8 protocol

CHAPTER 4

METHODOLOGY

Experimental Setup

The Space and Naval Warfare Systems Command (SPAWAR) funded the ballast water research program, and the primary contractor, eVenture Technologies, LLC, was subcontracted to the University of New Orleans through Task Order 0099. This funding allowed the University of New Orleans to purchase electro-disinfection equipment to perform experiments on ballast water treatment.

The purchased equipment when assembled became referred to as the electro-disinfection system as it was proven to annihilate laboratory seeded challenge water that contained *Escherichia Coli* (*E. coli*) and heterotrophic bacteria. The electro-disinfection system included a motorized fluid pump, manual hydraulic fluid pump, DC power rectifier, and an electrochemical disinfector. Water was forced at 1 (L/min) continuously by the Baldor Reliance Vector Drive Motor Master Micropump, model number IDMN 3538, through electrodes within the electro-disinfection system with applied voltage to disinfect the water, Figures 4.1 and 4.2. Ecolotron Inc. manufactured the electrochemical disinfector made of steel with internal dimensions of 30.48 cm (12 in) height, 17.78 cm (7 in depth), and 52.07 cm (20.5 in) length. Nine electrode plates, each with a dimension 17.5 cm x 17.5 cm, were placed parallel inside the electro-disinfection system. The electrodes had an opening of 1.94 cm (.470 in) and a length of 10.16 cm (4 in) permitting flow of water through the parallel plates of the electro-disinfection system, Figure 4.1 (Andrade, 2009). The electrodes of the electro-disinfection system were arranged

alternatively so there were five anodes and four cathodes. Plastic plates of 10.16 cm (.470 in) width were used as separators between the electrodes with an outer width of 17.5 cm X 17.5 cm and a diameter of 10.5 cm, Figure 4.2 (Andrade, 2009).

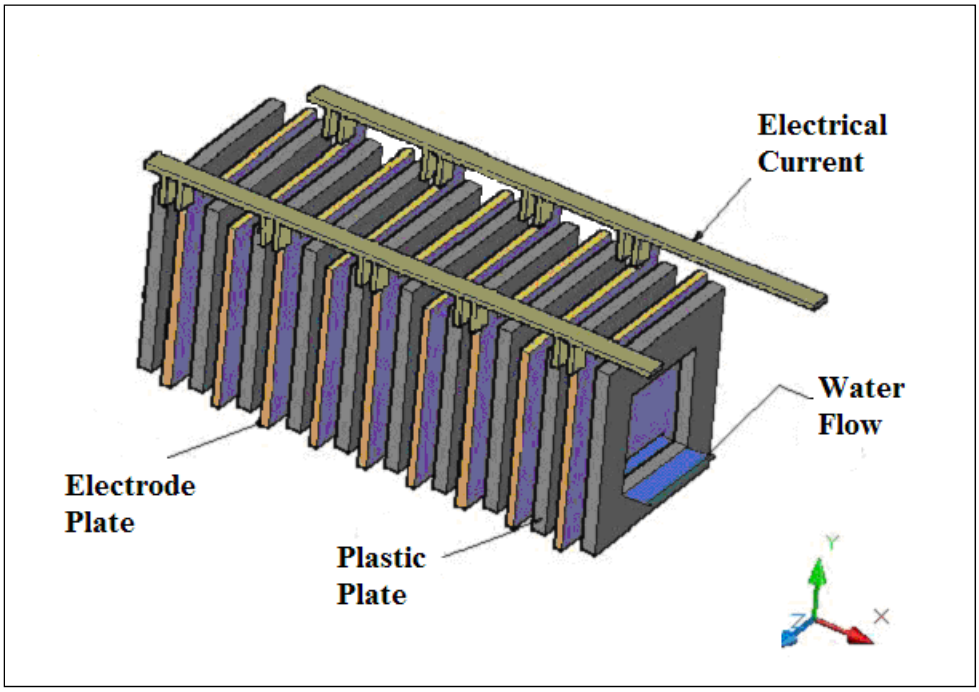


Figure 4.1 Oblique View of Electro-disinfection Unit

The separators between the electrodes within the electro-disinfection system permitted the production of hydrogen and oxygen gases to safely escape while minimizing electrical resistance (Trasatti and Wendt, 1990). The manual hydraulic fluid pump ENERPAC model P39 allowed zero-gap configuration between the anodes, cathodes, and plastic plate separators. The electrical current split water molecules, and the plastic separators allowed hydrogen gases to be produced at the anode while oxygen gases were produced at the cathode.

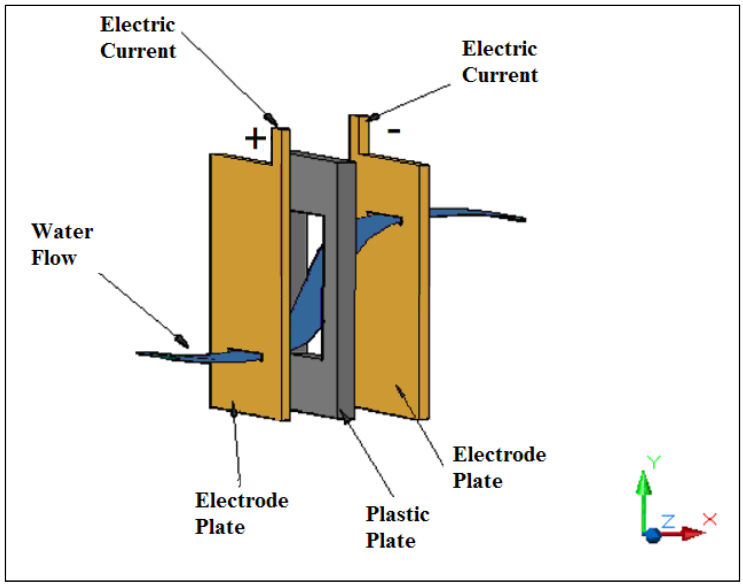
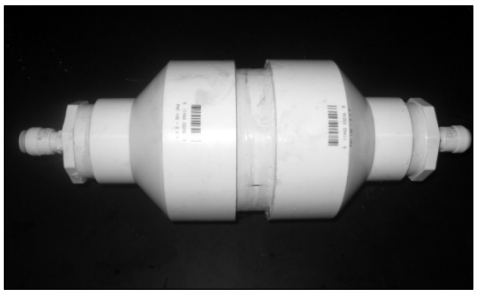
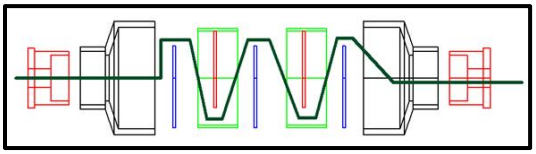


Figure 4.2 Close Up of Electrodes and Separator in Electro-disinfection Unit

A control reactor was constructed out of pvc pipe to operate in a comparable manner as the electro-disinfection unit, Figure 4.3 (a). The main distinction between the two reactors was absence of electricity applied to the water inside the control reactor. The inner diagram of the control reactor illustrates the replication of the water's flow pattern to the electro-disinfection unit, Figure 4.3 (b) (La Motta et al., 2009).



(a)



(b)

Figure 4.3 (a) Caption Control Reactor (b) Control Reactor Inner Flow Diagram

The disinfection equipment had a homogenous parallel electric field except at the fringes of the electrodes. The DC rectifier, BK Precision model 1791, allowed the voltage and amperage

to be manipulated to determine the best parameters to produce the highest kill efficiency of bacteria and zooplankton. The BK Precision model was calibrated by the company in November of 2009 and requires only once a year calibration of amperage and voltage. The electrical wire connecting the rectifier to the experimental setup was 0.3175 cm (0.125 in) diameter and 3.05 m (10 ft) length, which allows the presumption of negligible losses of electricity.

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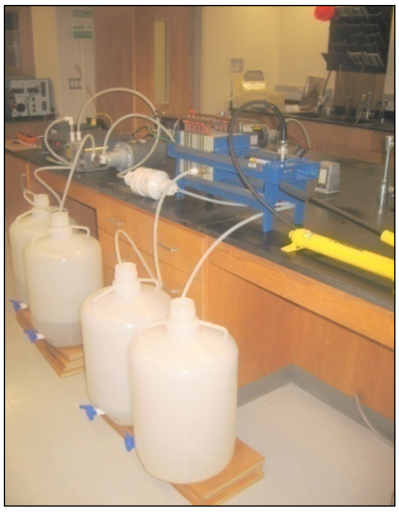
(a)



(b)

Figure 4.4 (a) Caption of Electro-disinfection Unit (b) Caption of Electro-disinfection Test

All analytical analyses were performed in triplicate within a two hour window prior to and following each electro-disinfection test, Figure 4.4 (a) and (b). All test water was stirred on a Lab-Line Instruments Inc. King Size Magnestir before, during, and after all electro-disinfection tests to ensure the protozoa were evenly distributed. Water was collected for all laboratory analyses described below in three autoclaved one liter beakers. The same beakers were utilized for after test analyses after autoclaving them at 125°C for thirty minutes to ensure sterilization.

Conductivity, dissolved oxygen, oxidative reduction potential, pH, and temperature were measured utilizing Thermo Scientific Orion 5 Star Plus Benchtop model number 1119000. The Thermo Scientific Orion 5 Star Plus Benchtop meter was calibrated by Thermo Scientific in house test equipment to abide by International Scientific Organization (ISO) and International Electrotechnical Commission (IEC), ISO 9001:2000 and ISO/IEC 17025:2005, along with all U.S. Pharamcopeia standards. The meter was calibrated in December 2009 and the calibration accreditation was plausible up to one year. The dissolved conductivity, dissolved oxygen, ORP, and pH meter was re-calibrated the day of every test to ensure compliance within the calibration standards range due to temperature variations.

The chlorides were measured by titration method utilizing Hach Drop Kit 8-P catalog number 1440-01 for the low range method (0-100 mg/L) of chlorides. The Hach DR 2800 portable spectrophotometer measured total Cl_2, free and combined Cl_2, utilizing methods equivalent to USEPA Method 330.5 and Standard Method 4500-Cl G for drinking water and wastewater analyses. Cl_2 in brackish water experiments was measured utilizing Hach Method 10070 for a range from (0.1 to 10 mg/L). In cases where the Cl_2 was above 10 mg/L range the sample was diluted fifty percent with deionized water and then the measurement was taken. Fresh water tests produced less Cl_2, and Hach Method 8167 was employed for a range from (0.2 to 2.00 mg/L).

The killing efficiency of bacteria was determined by using 3M™ Petrifilm™ Ecoli/Coliform Count Plates. These dry rehydratable petrifilm plates are recognized by the Association of Official Agricultural Chemists (AOAC), Food Drug Administration (FDA), and International Standards Organization (ISO) for determining bacteria in vegetables, meat, diary, and processed foods. The petrifilm plate has a square foam plastic bottom 10.16 cm X 10.16 cm

(4 inches X 4 inches) inside with a circular area of 20 cm^2 (7.874 in^2). The circular area contains a gel substance that upon application of liquid activates nutrients essential to the growth of coliform and *E. coli* bacteria. A thin plastic film with gridlines that had the same dimensions as the plastic bottom plate acted as a cover to the 3M™ Petrifilm™ plate. This thin film protected the growth area prior to and after inoculation period with gridlines which aided in enumeration of bacteria. All of the plates were stored in air tight containers prior to inoculation with bacteria. One milliliter of water was applied on them as specified by 3M™ Petrifilm™ and the plates were incubated at 35°C. The nutrient agar had Violet Red Bile dye that was activated after addition of water, which differentiated the coliform from *E. coli* bacteria. Coliform and *E. coli* bacteria enumeration were taken 24 hours and 48 hours from the time of initial incubation, respectively. The coliform bacteria were observed as red dots while the *E. coli* were blue dots surrounded by translucent air pockets, Figure 4.5.

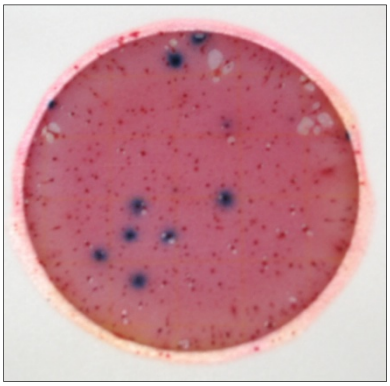


Figure 4.5 Caption 3M Plate for Bacteria Enumeration

Microscopic identification of protozoa viability before and after each electro-disinfection test was performed in triplicate. The following analytical equipment utilized were: Forty Reichert stereomicroscope, Omano Fluorscent Microscope (FL400), and a digital microscope Jentopik ProgRes® C5 camera. Two hundred microliters (µL) were initially dispensed onto a

Structure Probe Inc. Sedgewick-Rafter counting chamber with inner dimensions of 50 mm X 20 mm and a depth capacity of 1 milliliter (mL) for all microscopic analyses. On the occasion the protozoa were innumerable, smaller dilutions were made until an accurate count of protozoa was ascertained.

All laboratory analytical analyses previously described are shown below in Table 4.1 along with their scientific precision:

Table 4.1 Measurements with Scientific Precision

Parameter	Method and Precision
Current	BK Precision: 0.2% * (reading) ± 10 r
Voltage	BK Precision: 0.2% * (reading) ± 10 r
Chlorides	Hach 8P Drop Kit ± 1 drop (0.4 mL)
Total Cl_2 (0-10 mg/L)	Hach 10070 ± 0.1 mg/L
Total Cl_2 (0-2 mg/L)	Hach 8167 ± 0.02 mg/L
Conductivity	Orion 5 Star ± 2-5% * (reading)
Dissolved Oxygen	Orion 5 Star ± 2-5% * (reading)
Oxidative Reduction Potential	Orion 5 Star ± 2-5% * (reading)
pH	Orion 5 Star ± 2-5% * (reading)
Temperature	Orion 5 Star ± 2-5% * (reading)
Coliform	Petrifilm ± 1 colony

Experimental Plan

The experimental plan was to determine the best set of electrodes, voltage, and current to electro-disinfect the water to fulfill the criteria listed in the Ballast Water Management Act (BWMA) 2005. The created challenge water for all tests was seeded with *E. coli* and heterotrophic bacteria to indicate biological contamination. The freshwater tests were

augmented with salinity to negate water's poor ionic conductivity. The salt acted as a conductive electrolyte lessening the resistance of water to electricity allowing higher amperage to be ascertained.

CHAPTER 5

RESULTS AND DISCUSSION

Foreword to Tests

This electro-disinfection research was categorized into brackish and fresh water experiments to better assess the disinfection potential in those aqueous environments. The brackish water was collected from the south shore of Lake Pontchartrain. The fresh water was seeded with bacteria from Marrero Wastewater Treatment Plant's effluent and diluted with demineralized water. All water was collected within the same week tests were executed and aerated in the laboratory. On the occasion either the test or control water had a scarcity of protozoa, the water was seeded from the stock culture of *Daphnia Magna*, salt rotifers, and fresh water rotifers from the laboratory.

Preliminary experiments performed at the beginning of this research demonstrated titanium electrodes had a higher kill efficacy while generating zero precipitate. Stainless steel and aluminum electrodes produced yellow (ferric hydroxide) and white (aluminum hydroxide) precipitate, respectively. In this case, treated water would require further filtration prior to its release into an aqueous environment to remove the precipitate, a hindrance in proving economic feasibility. The data ascertained from the preliminary tests were conclusive, but not run with controls to conserve test materials. Therefore, the initial tests that were conducted for this research were critical in choosing to work solely with titanium electrodes, but are not included in Appendix A.

Brackish Water Tests

This research demonstrated electro-disinfection is a practical option for treatment of secondary ballast water, because of high percentage of bacteria annihilation, Table 5.1. The aluminum electrode utilized during the experiment tabulated below had excellent killing efficacy in this experiment; however, the killing efficacy could have been attributed to Cl_2 generation from this test. Cl_2 was not accurately measured for some of the tests indicated below by the inequality symbols, Table 5.1. The reason for this was the Cl_2 concentration was outside of the spectrophotometer's measuring range and additional reagents had to be purchased.

Table 5.1 Collocation of Bacteria Killing Efficacies for Brackish Water Tests

Electrode	pH	Temp. (°C)	Cond. (µS/cm)	Cond. (ppt)	Current (A)	Voltage (V)	Cl_2 (mg/L)	Bacteria Killing Efficiency (%)	
								Heterotrophic	*E. coli*
Alumnium	6	22	8171	4.8	10.2	24.3	> 3.5	100	100
Alumnium	7	19	9072	5.8	10.1	18.6	> 3.5	100	100
Titanium	6	19	7628	4.6	10.2	25.2	> 3.5	97.9	100
Titanium	8.6	19.7	1148	0	10.1	23.5	13.40	100	100

This research illustrated the possibility the generation of Cl_2 by electricity could be contributing to destruction of the bacteria. Toxicity tests were conducted to verify Cl_2 is the primary disinfectant agent responsible for all of the bacteria annihilation in brackish water tests that were performed, Appendix A Experiments #1-4.

The Cl_2 toxicity tests consisted of five liters of identical untreated ballast water being set aside prior to the electro-disinfection tests. The untreated ballast water was continuously stirred and minute amounts of Cl_2 was added and held at one minute detention intervals before the water was transferred to 3M agar media plates and incubated. Viable bacteria results for the electro-disinfected water and applied toxicity tests, Figures 5.1-5.4 and Appendix A Experiment #4.

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	Cl_2 (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Avg.	**8.62**	**1 549**	**19.7**	**8.62**	**0.01**	**24 833**	**867**
Treated Effluent Avg.	**9.30**	**1 148**	**19.8**	**12.29**	**13.40**	**0**	**0**

Toxicity of bacteria to one minute exposure to Cl_2:

Dosage Cl_2 applied (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/100mL)
5.20	0	0
6.32	0	0
11.84	0	0
13.76	0	0
14.16	0	0
14.24	0	0
14.64	0	0
14.88	0	0
15.20	0	0
15.28	0	0
15.96	0	0
16.80	0	0
17.04	0	0
17.80	0	0

Figure 5.1 Cl_2 Toxicity Test Results on Brackish Water

(a) (b) (c)

Figure 5.2 (a) Before Electro-disinfection (b) After Electro-disinfection (c) After Cl_2 Tests

These Cl_2 test results are very significant because a broad range of Cl_2 (mg/L) were performed at one-half of the amount of Cl_2 that was generated in the electro-disinfection process and no viable bacteria were noted in the results. The results of the Cl_2 tests suggest residual Cl_2 neutralization would have to be implemented to ballast water treated with electricity in order to

be in compliance with EPA standards, thereby, increasing the potential cost of ballast water treatment.

Fresh Water Tests

Fresh water tests that were conducted for this research initially did not have 90% killing efficacy. Upon examination of the brackish water tests and deliberation, it was hypothesized there was not enough salt in the water to allow sufficient conductivity of electricity to annihilate bacteria at 90%. Experiments were performed to determine the best combination of salts to increase the conductivity of water while minimizing the generation of chlorine. The salts evaluated were magnesium sulfate ($MgSO_4$), sodium bicarbonate ($NaHCO_3$), and sodium carbonate (Na_2CO_3). The salts were evaluated individually and in a one to one combination with other salts. The evaluation of $MgSO_4$, $NaHCO_3$, and Na_2CO_3 tests to determine Cl_2 generation are not seen in Appendix A, because the only parameter measured in these electro-disinfection tests was the generation of Cl_2. The tests verified that the type of salt utilized during the tests was insignificant because equivalent amounts of chlorine were produced during the tests. The water's pH was also raised when performing electro-disinfection tests using carbonate salts as noted in footnote, Table 5.2 and Experiment #12. Of the salts utilized during the tests, $MgSO_4$was the most commonly known and most economical; therefore, all future electro-disinfection tests were performed utilizing magnesium sulfate, Table 5.2.

After salt was added to the water, conductivity increased in the water, thereby, decreasing the resistance of water and allowing electricity to flow and electrocute bacteria. The first fresh water test with conductivity of 232 μS/cm was ineffective in destroying *E. coli* as the quantity of bacteria were too numerous to count (TNC), Table 5.2 and Appendix A Experiment #6. As

shown below a series of tests were run utilizing $MgSO_4$ with the results showing the minimum conductivity of water would have to be 867 µS/cm to have 100% killing efficacy, Table 5.2 and Appendix A Experiments #6-15.

Table 5.2 Synopsis Fresh Water Tests using $MgSO_4$ to increase conductivity

pH	Temp. (°C)	Added $MgSO_4$ (grams)	Cond. (µS/cm)	Current (A)	Voltage (V)	Cl_2 (mg/L)	Bacteria Killing Efficiency (%)	
							Heterotrophic	*E. coli*
7.75	19.8	12.82	1406	10.1	23.9	2.53	100	100
8.47	21.1	9.62	1271	10.6	27.1	1.66	83.2	93.2
8.4	24.2	8.60	1036	10	32.3	0.52	99.9	100
8.60	22.7	6.00	1007	10	31.6	1.35	99.7	100
9.94	21.0	6.82*	873	10	36.8	1.56	100	100
8.68	23.5	4.00	875	10	31.2	1.31	100	100
8.41	22.3	2.00	755	10	39.3	0.89	98.7	100
8.41	25.0	0**	623	10	49.7	0.51	91.2	93.3
8.41	25.4	1.07	473	10	61.3	0.06	56.7	0
8.03	18.5	0	232	4.5	27.1	0.47	80.1	TNC

* Test was performed with $MgSO_4$ and Na_2CO_3

** No salt was added because when diluting fresh water with laboratory grade demineralized water was spent contributing to ions in the water

TNC – Too numerous to count

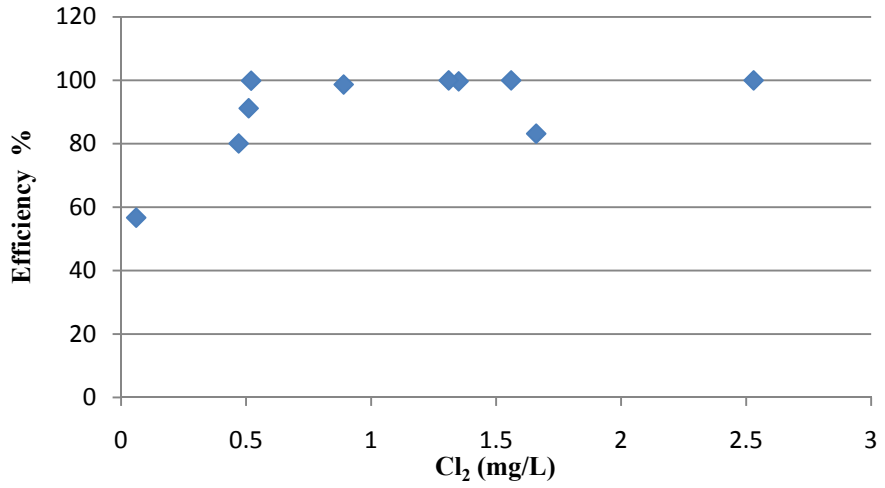


Figure 5.3 Bacteria Killing Efficiency for Fresh Water Tests

The fresh water experiments conducted determined that a minimum presence of 0.5 mg/L Cl_2 is necessary to destroy bacteria, Figure 5.1. Another Cl_2 toxicity test was performed at the same specifications described above to determine the percentage of bacteria killing efficiency attributed to the generation of Cl_2. The fresh water's salinity in both the control and treated influents was increased to 873 µS/cm. The treated water had a 99.7% heterotrophic bacteria killing efficiency while the control Cl_2 test had a 54.4% killing efficiency. The 45.3% difference between the two tests validates other electrochemical processes are occurring within the water and contributing to the annihilation of bacteria.

As the amount of salt increased the quantity of Cl_2 generated increased in a non-linear amount, Figure 5.4. Electrolysis of fresh water consisting of chlorides within the range of 35-52 mg/L had no linear correlation between salinity range and the created Cl_2, Figure 5.5. However, fresh water with a range 80-90 mg/L of chlorides had an exceptionally linear correlation between the salinity of the fresh water and the amount of Cl_2 produced, Figure 5.6.

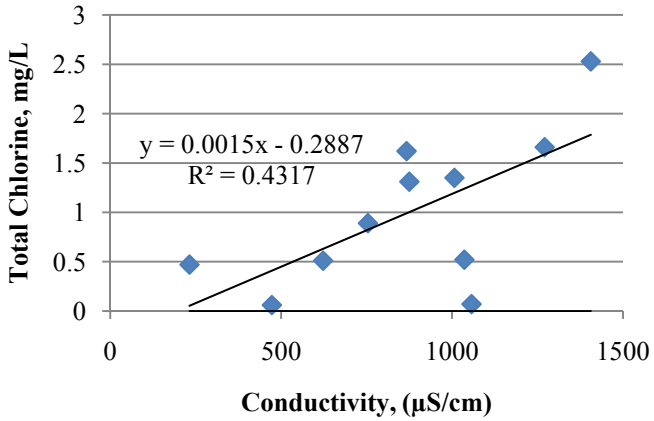


Figure 5.4 Total Cl_2 Generated from Fresh Water Tests

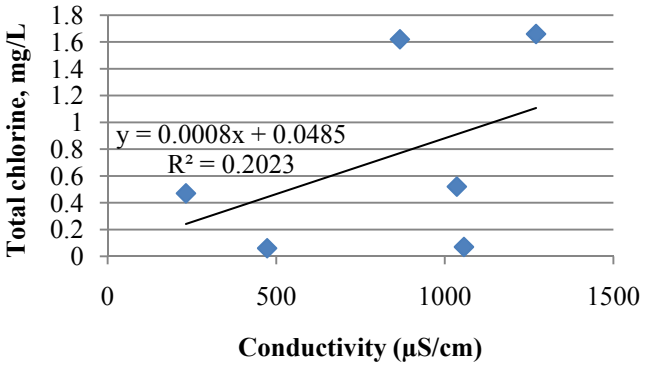


Figure 5.5 Generated Cl_2 from Cl^- range 35-52 mg/L

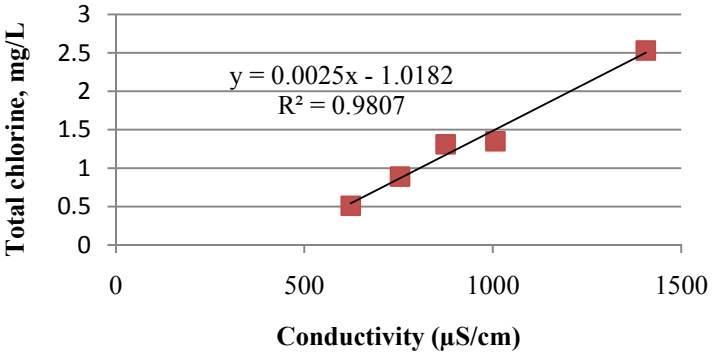


Figure 5.6 Generated Cl_2 from Cl^- range 80-90 mg/L

Another interesting fresh water test was performed with *Daphnia Magna* (*D. Magna*), which are often utilized as an indicator species to test an ecosystem's toxins, Figure 5.7 (b). Both the control and test water contained 12 liters of water in each bucket: with three broods of eggs in each bucket and a population of 108 *D. Magna* per liter of water. After the tests, a viable count of *D. Magna* was performed twice for both the control and treated effluent; first by placing the effluent water in a beaker and looking for any mobility of viable *D. Magna.* The second evaluation for mobility was performed by sieving the effluent water slowly and again observing

for any mobility of viable *D. Magna.* After these observational tests, the control and treated effluents had 417 and 0 viable *D. Magna*, respectively. *D. Magna* eggs were placed in optimum breeding environments and observed for the standard hatching time of twenty nine days. The following results occurred: two broods of *D. Magna* eggs hatched from the control water, and zero *D. Magna* eggs hatched from the treated water, Appendix A Experiment #5.

VIABILITY OF MICROORGANISMS:

	Live *Daphnia Magna* (count)
Treated Effluent	0
Control Effluent	417
5 day Treated Effluent	0
5 day Control Effluent	4

RESULTS AFTER STANDARD HATCHING TIME:

Live *Daphnia Magna*	(count)
Treated Effluent	0
Control Effluent	2

	pH	Temp (°C)	D.O. (mg/L)
Influent Avg.	7	20	6.41
Treated Effluent Avg.	7	21	9.01
Control Influent Avg.	7	20	6.46
Conotrol Effluent Avg.	7	20	6.35

(a)

(b)

Figure 5.7 (a) Electro-disinfection Test on *D. Magna* (b) *D. Magna* at 20X Magnification

In an effort to replicate a test executed by Park et al. (2004) that used AC, and annihilated 100% *Vibrio parahaemolyticus* and generated zero Cl_2, a new electro-disinfection test was performed outside the scope of the original research. This test utilized alternating current at 1 ampere and 50 hertz to reduce the electrolysis time of the artificial ballast water and to minimize the generation of Cl_2. To reproduce the short treatment time reported by Park et al. (2004), only two titanium electrodes were placed inside of the electro-disinfection reactor instead of the traditional nine electrodes. The shortened retention time increased the water flow rate to 5.8

(L/min) instead of the usual 1 (L/min). The water was augmented with magnesium sulfate from an original conductivity of 353 µS/cm to 1206 µS/cm to lessen the resistance of water and maximize the water current carrying ability. See Figure 5.8 and Appendix A Experiment #16. This electro-disinfection test had 16% and 50% killing efficacy of bacteria and protozoa, respectively, as shown in Figure 5.8. The killing effiency can be contributed to the electrochemical processes that occurred in the electro-disinfection unit, because no Cl_2 was generated during the test.

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Avg.	**8.62**	**1 206**	**16.5**	**9.71**	**0**	**375**	**197 333**	**0**
Treated Effluent Avg.	**9.37**	**1 197**	**20.6**	**9.37**	**0**	**368**	**164 666**	**0**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa	**Treated Effluent Protozoa**
(1) 1 rotifer, 1 amoeba, & 1 filamentous organism	3	
(2) 1 rotifer & 10 ostrocads	11	
(3) 1 rotifer, 1 amoeba, & 1 ostrocad	3	
(4) 1 amoeba & 3 ostrocads	4	
(5) 1 rotifer & 1 stalked cilitate	2	
(6) 2 rotifers		2
(7) 1 rotifer		3
(8) 1 rotifer & 1 amoeba		2
(9) 2 ostrocads		2
(10) 1 amoeba & 1 stalked cilitate		2
Avg.	**2**	**1**

Figure 5.8 Electro-disinfection Test Utilizing Alternating Current

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Stronger enforcements are impending for destroying bacteria laden water via a secondary water treatment from ships' ballast tanks that is jettisoned into United States waterways and coastlines. Presently there are several methods being utilized to filter and treat primary influent ballast water to prevent the spread of invasive aquatic species and to protect ecosystems. Currently, none of today's technologies can operate unaccompanied by another technology to achieve the killing efficiency specified in IMO's ballast regulations and in United States ballast laws.

Electricity breaking oxygen compounds create ROS which can be attributed to a high annihilation rate of bacteria and *D. Magna* in ballast water. Generated Cl_2 from treatment of brackish water by electricity is the predominant factor in destroying bacteria in brackish waters. Fresh water (low salinity) chlorides that have been transformed into Cl_2 are within EPA's scope of tolerance for discharge into aqueous waters. This research demonstrates Cl_2 along with ROS can be utilized for disinfection of bacteria in a cost effective manner.

Electro-disinfection is a marketable technology that needs modifications and additional research to make it a more efficient process to treat ballast water. The recommendation from evaluations of electro-disinfection research is more studies should be conducted on larger applications of electro-disinfection on ballast water that would be jettisoned into fresh and brackish water systems. The main focus of this additional research should be the minimization of Cl_2 generation when disinfecting brackish or sea water, to eliminate the need of dechlorination

using, for instance, alternating current. This last alternative would require an entirely new research project.

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APPENDIX A EXPERIMENTAL DATA

EXPERIMENT #1

Water: Brackish water obtained from London Canal connected to Lake Pontchartrain
Date: Feburary 15, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

OPERATION PARAMETERS:

Sample Volume (L)	40
Current Density (mA/cm^2)	10.0
Volts	25.2
Amps	10.2
kWh/m^3	4.3

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/100mL)
Influent Beginning	6	7 200	19	24 400	0
Influent End	6	7 356	19	19 300	100
Influent Avg.	**6**	**7 278**	**19**	**21 850**	**50**
Treated Effluent Beginning	7	7 800	21	933	0
Treated Effluent End	6	7 456	22	0	0
Treated Effluent Avg.	**7**	**7 628**	**22**	**467**	**0**
Control Influent Beginning	7	8 051	19	10 400	100
Control Influent End	7	8 023	19	8 600	0
Control Influent Avg.	**7**	**8 037**	**19**	**9 500**	**50**
Control Effluent Beginning	7	8 023	19	31 900	200
Control Effluent End	7	7 897	19	31 500	0
Control Effluent Avg.	**7**	**7 960**	**19**	**31 700**	**100**
5 day Treated Effluent	6	7 421	18	30 300	0
5 day Treated Effluent	6	7 312	18	32 400	0
5 day Treated Effluent Avg.	**6**	**7 367**	**18**	**31 350**	**0**
5 day Control Effluent	6	8 010	18	17 400	0
5 day Control Effluent	6	7 997	18	16 700	0

EXPERIMENT #2

Water: Brackish obtained from London Canal connected to Lake Pontchartrain
Date: Feburary 27, 2009
Electrodes: Nine alumnium electrodes
Power Source: Direct Current

OPERATION PARAMETERS:

Contact Time, minutes	1
Sample Volume (L)	40
Current Density (mA/cm^2)	9.9
Volts	18.6
Amps	10.1
kWh/m^3	6.9

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	Turbidity (NTU)	Coliforms (CFU/100 mL)
Influent Beginning	7	9 012	20	8	5 200
Influent Middle	7	8 896	20	8	4 200
Influent End	7	9 023	20	8	5 000
Influent Avg.	**7**	**8 977**	**20**	**8**	**4 800**
Treated Effluent Beginning	6	9 045	19	31	0
Treated Effluent Middle	6	9 089	19	28	0
Treated Effluent End	6	9 081	19	25	0
Treated Effluent Avg.	**6**	**9 072**	**19**	**28**	**0**
5 day Treated Effluent	6	9 078	21	0	0
5 day Treated Effluent	6	9 063	21	1	0
5 day Treated Effluent	6	9 092	21	3	1 500
5 day Treated Effluent Avg.	**6**	**9 078**	**21**	**1.33**	**500**

EXPERIMENT #3

Water: Brackish water obtained from London Canal connected to Lake Pontchartrain
Date: March 4, 2009
Electrodes: Nine alumnium electrodes
Power Source: Direct Current

OPERATION PARAMETERS:

Sample Volume (L)	12
Current Density (mA/cm^2)	10.0
Volts	24.3
Amps	10.2
kWh/m^3	4.1

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/100mL)
Influent Beginning	6	7 308	19	26 100	200
Influent Middle	7	7 411	20	36 200	100
Influent End	6	9 415	19	38 800	0
Influent Avg.	**6**	**8 044**	**19**	**33 700**	**100**
Treated Effluent Beginning	7	7 510	22	0	0
Treated Effluent Middle	7	7 491	21	0	0
Treated Effluent End	6	9 512	24	0	0
Treated Effluent Avg.	**7**	**8 171**	**22**	**0**	**0**
Control Influent Beginning	7	9 488	19	35 300	100
Control Influent Middle	7	9 531	19	35 800	300
Control Influent End	7	9 506	19	35 000	0
Control Influent Avg.	**7**	**9 508**	**19**	**35 367**	**133**
Control Effluent Beginning	7	10 011	19	34 600	100
Control Effluent Middle	7	9 899	19	33 900	100
Control Effluent End	7	9 702	19	34 500	100
Control Effluent Avg.	**7**	**9 871**	**19**	**34 333**	**100**
5 day Treated Effluent	6	10 203	20	0	0
5 day Treated Effluent	6	10 487	20	0	0
5 day Treated Effluent	6	9 261	21	0	0
5 day Treated Effluent Avg.	**6**	**9 983**	**20**	**0**	**0**
5 day Control Effluent	6	9 265	20	28 500	0
5 day Control Effluent	6	9 871	20	8 300	0
5 day Control Effluent	6	9 999	20	7 100	100

	pH	Conductivity (µS/cm)	Temp (°C)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/100mL)
Influent Avg.	6	8 045	19	33 700	100
Treated Effluent Avg.	7	8 171	22	0	0
Control Influent Avg.	7	9 508	19	35 367	133
Conotrol Effluent Avg.	7	9 871	19	34 333	100
5 day Treated Effluent Avg.	6	9 984	20	0	0
5 day Control Effluent Avg.	6	9 712	20	14 633	33

EXPERIMENT #4

Water: Brackish water obtained from London Canal connected to Lake Pontchartrain
Date: May 19, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

OPERATION PARAMETERS:

Sample Volume (L)	18
Current Density (mA/cm^2)	9.9
Volts	23.5
Amps	10.1
kWh/m^3	4.0

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	Cl_2 (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	8.69	1 165	19.6	8.69	0.02	26 400	900
Influent Middle	8.76	1 808	19.8	8.76	0	24 900	800
Influent End	8.42	1 675	19.8	8.42	0	23 200	900
Influent Avg.	**8.62**	**1 549**	**19.7**	**8.62**	**0.01**	**24 833**	**867**
Treated Effluent Beginning	9.17	1 149	19.7	12.23	14.24	0	0
Treated Effluent Middle	9.41	1 147	19.8	12.46	10.08	0	0
Treated Effluent End	9.32	1 149	19.9	12.19	15.88	0	0
Treated Effluent Avg.	**9.30**	**1 148**	**19.8**	**12.29**	**13.40**	**0**	**0**

Toxicity of bacteria to one minute exposure to Cl_2:

Dosage Cl_2 applied (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/100mL)
5.20	0	0
6.32	0	0
11.84	0	0
13.76	0	0
14.16	0	0
14.24	0	0
14.64	0	0
14.88	0	0
15.20	0	0
15.28	0	0
15.96	0	0
16.80	0	0
17.04	0	0
17.80	0	0

EXPERIMENT #5

Water: Fresh water
Date: Feburary 16, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

Implanted: 108 *Daphnia Magna* per Liter in Control and Treated Waters
3 broods of eggs from Control and Treated Waters in optimal breeding environment

OPERATION PARAMETERS:

Sample Volume (L)	12
Current Density (mA/cm^2)	1.3
Volts	55
Amps	1.3
kWh/m^3	1.2

	pH	Temp	D.O.
Influent Avg.	7	20	6.41
Treated Effluent Avg.	7	21	9.01
Control Influent Avg.	7	20	6.46
Conotrol Effluent Avg.	7	20	6.35

LAB ANALYSIS:

	pH	Temp (°C)	D.O. (mg/L)
Influent Beginning	7	20	6.14
Influent Middle	7	20	6.39
Influent End	7	20	6.71
Influent Avg.	**7**	**20**	**6.41**
Treated Effluent Beginning	7	21	9.00
Treated Effluent Middle	7	20	8.89
Treated Effluent End	6	21	9.14
Treated Effluent Avg.	**7**	**21**	**9.01**
Control Influent Beginning	7	20	6.81
Control Influent Middle	7	20	6.09
Control Influent End	7	20	6.49
Control Influent Avg.	**7**	**20**	**6.46**
Control Effluent Beginning	7	20	6.37
Control Effluent Middle	6	20	6.47
Control Effluent End	7	20	6.21
Control Effluent Avg.	**7**	**20**	**6.35**
5 day Treated Effluent	7	19	6.68
5 day Treated Effluent	7	18	7.04
5 day Treated Effluent	7	19	7.23
5 day Treated Effluent Avg.	**7**	**19**	**6.98**
5 day Control Effluent	7	19	6.94
5 day Control Effluent	7	19	6.71

(Cont.) EXPERIMENT #5

VIABILITY OF MICROORGANISMS:

	Live *Daphnia Magna* (count)
Treated Effluent	0
Control Effluent	417
5 day Treated Effluent	0
5 day Control Effluent	4

RESULTS AFTER STANDARD HATCHING TIME:

Live *Daphnia Magna*	(count)
Treated Effluent	0
Control Effluent	2

EXPERIMENT #6

Water: Fresh water
Date: May 22, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

OPERATION PARAMETERS:

Sample Volume (L)	18
Current Density (mA/cm^2)	4.4
Volts	27.1
Amps	4.5
kWh/m^3	2.03

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	ORP (mv)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	8.21	232	18.8	10.79	271.4	0	45	100 300	1 100
Influent Middle	8.14	229	18.7	10.32	257.2	0	35	102 500	900
Influent End	8.05	229	18.8	10.48	269.4	0	35	98 700	1 000
Influent Avg.	**8.13**	**230**	**18.8**	**10.53**	**266.0**	**0**	**38**	**100 500**	**1 000**
Treated Effluent Beginning	8.23	231	18.6	11.94	-117.9	0.44	35	20 000	1 000
Treated Effluent Middle	7.95	231	18.4	11.88	-127.2	0.5	30	22 100	1 000
Treated Effluent End	7.92	232	18.6	11.68	-118.5	0.48	35	17 800	900
Treated Effluent Avg.	**8.03**	**232**	**18.5**	**11.83**	**-121.2**	**0.47**	**33**	**19 967**	**967**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa (count/200 µL)	**Treated Effluent Protozoa (count/10 µL)**
(1) 1 rotifer & 1 blood worm	2	
(2) 1 rotifer & 1 amoeba	2	
(3) 1 rotifer & 2 amoeba	3	
(4) 2 rotifers	2	
(5) 1 amoeba & 1 ostrocad		2
(6) 1 stalked cilitate & 2 amoeba		3
(7) 2 rotifers & 1 amoeba		2
Avg.	**2**	**2**

EXPERIMENT #7

Water: Fresh water
Date: June 26, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

Addition: 12.82 grams of $MgSO_4$ to increase water's original conductivity from 556 µS/cm to 1 416 µS/cm

OPERATION PARAMETERS:

Sample Volume (L)	18
Current Density (mA/cm^2)	9.9
Volts	23.9
Amps	10.1
kWh/m^3	4.0

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	7.68	1 411	19.5	6.23	0	85	199 300	4 400
Influent Middle	7.69	1 417	18.9	5.54	0	85	197 000	3 900
Influent End	7.70	1 419	19.3	5.60	0	80	202 600	4 300
Influent Avg.	**7.69**	**1 416**	**19.2**	**5.79**	**0**	**83**	**199 633**	**4 200**
Treated Effluent Beginning	7.74	1 408	19.6	10.33	2.60	85	0	0
Treated Effluent Middle	7.76	1 405	19.3	10.12	2.60	85	0	0
Treated Effluent End	7.75	1 406	20.6	10.56	2.40	85	0	0
Treated Effluent Avg.	**7.75**	**1 406**	**19.8**	**10.34**	**2.53**	**85**	**0**	**0**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa (count/20 µL)	**Treated Effluent Protozoa (count/10 µL)**
(1) 3 rotifers, 2 amoeba, & 1 ostrocad	6	
(2) 3 rotifiers, 1 amoeba, & 1 stalked cilitate	5	
(3) 3 rotifers, 1 amoeba, & 1 flagellate	5	
(4) 2 rotifers, 1 amoeba, & 4 flagellates	7	
(5) 1 rotifer, 2 amoeba, 1 flagellate & 1 stalked cilitate	5	
(6) 1 ostrocad		1
(7) 1 amoeba		1
		0
		0
		0
Avg.	**6**	**0**

EXPERIMENT #8

Water: Fresh water
Date: July 11, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

Addition: 6.00 grams of $MgSO_4$ to increase water's original conductivity of 657 µS/cm to 1 009 µS/cm

OPERATION PARAMETERS:

Sample Volume (L)	18
Current Density (mA/cm^2)	9.8
Volts	31.6
Amps	10.0
kWh/m^3	5.3

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	8.32	1 008	19.4	6.89	0	90	1 066	0
Influent Middle	7.03	1 010	19.4	7.03	0	90	1 086	0
Influent End	6.97	1 010	19.5	6.97	0	90	1 070	0
Influent Avg.	**7.44**	**1 009**	**19.4**	**6.96**	**0**	**90**	**1 074**	**0**
Treated Effluent Beginning	8.63	1 007	22.8	10.38	0	85	3	0
Treated Effluent Middle	8.58	1 006	22.5	10.63	0	80	6	0
Treated Effluent End	8.6	1 006	22.7	10.37	0	85	0	0
Treated Effluent Avg.	**8.60**	**1 007**	**22.7**	**10.46**	**0**	**83**	**3**	**0**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa (count/20 µL)	**Treated Effluent Protozoa (count/10 µL)**
(1) 3 rotifers & 1 ostrocad	4	
(2) 3 rotifiers & 1 amoeba	4	
(3) 2 rotifers & 1 ostrocad	3	
(4) 3 rotifers	3	
		0
		0
		0
Avg.	**4**	**0**

EXPERIMENT #9

Water: Fresh water
Date: July 14, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

Addition: 4.00 grams of $MgSO_4$ to increase water's orginial conductivity from 634 µS/cm to 876 µS/cm

OPERATION PARAMETERS:

Sample Volume (L)	18
Current Density (mA/cm^2)	9.8
Volts	31.2
Amps	10.0
kWh/m^3	5.2

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	8.37	873	19.2	7.35	0	85	119 700	6 700
Influent Middle	8.38	877	19.2	7.47	0	85	118 300	7 700
Influent End	8.38	877	19.3	7.37	0	90	116 300	7 000
Influent Avg.	**8.38**	**876**	**19.2**	**7.40**	**0**	**87**	**118 100**	**7 133**
Treated Effluent Beginning	8.69	876	23.5	9.36	1.31	80	0	0
Treated Effluent Middle	8.68	875	23.5	9.79	1.32	85	0	0
Treated Effluent End	8.67	875	23.4	8.91	1.31	80	0	0
Treated Effluent Avg.	**8.68**	**875**	**23.5**	**9.35**	**1.31**	**82**	**0**	**0**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa (count/200 µL)	**Treated Effluent Protozoa (count/10 µL)**
(1) 2 rotifers, 1 amoeba, & 1 stalked cilitate	4	
(2) 2 rotifers & 3 flagellates	5	
(3) 2 rotifers & 2 ostrocads	4	
(4) 2 rotifers, 1 amoeba, & 1 stalked cilitate	4	
(5) 1 rotifer, 1 ostrocad, & 3 stalked cilitates	5	
(6) 1 stalked cilitate		1
(7) 1 stalked cilitate		1
		0
		0
		0
Avg.	**4**	**0**

EXPERIMENT #10

Water: Fresh water
Date: July 15, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

Addition: 2.00 grams of $MgSO_4$ to increase water's orginial conductivity from 588 µS/cm to 716 µS/cm

OPERATION PARAMETERS:

Sample Volume (L)	18.0
Current Density (mA/cm^2)	9.8
Volts	39.3
Amps	10.0
kWh/m^3	6.6

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	8.38	715	19.5	6.32	0	85	140 300	3 000
Influent Middle	8.39	716	19.5	6.40	0	90	141 700	3 300
Influent End	8.38	716	19.4	6.45	0	85	132 300	3 100
Influent Avg.	**8.38**	**716**	**19.5**	**6.39**	**0**	**87**	**138 100**	**3 133**
Treated Effluent Beginning	8.45	756	24.0	8.82	0.89	85	3 000	0
Treated Effluent Middle	8.39	754	21.8	8.62	0.88	80	1 300	0
Treated Effluent End	8.39	754	21.2	8.58	0.89	85	1 000	0
Treated Effluent Avg.	**8.41**	**755**	**22.3**	**8.67**	**0.89**	**83**	**1 767**	**0**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa (count/200 µL)	**Treated Effluent Protozoa (count/10 µL)**
(1) 1 rotifer & 3 filamentous organisms	4	
(2) 1 rotifier, 1 filamentous organism, & 1 amoeba	5	
(3) 1 rotifer, 1 filamentous organism, & 1 amoeba	3	
(4) 1 rotifer, 1 filamentous organism, & 1 unknown protozoa	3	
(5) 2 rotifers & 1 filamentous organism	3	
(6) 2 filamentous organisms, 1 amoeba, & 1 stalked cilitate	4	3
(7) 2 filamentous organisms		1
(8) 1 filamentous organism		1
(9) 2 stalked cilitates		2
(10) 1 stalked cilitate		1
Avg.	**4**	**7**

EXPERIMENT #11

Water: Fresh water
Date: July 16, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

OPERATION PARAMETERS:

Sample Volume (L)	18.0
Current Density (mA/cm^2)	9.8
Volts	49.7
Amps	10.0
kWh/m^3	8.3

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	8.20	626	19.1	6.06	0	85	117 300	0
Influent Middle	8.21	626	19.2	6.24	0	85	119 700	0
Influent End	8.24	626	19.3	6.00	0	90	118 300	0
Influent Avg.	**8.22**	**626**	**19.2**	**6.10**	**0**	**87**	**118 433**	**0**
Treated Effluent Beginning	8.41	623	25.1	9.04	0.5	80	20 000	1 000
Treated Effluent Middle	8.41	624	24.9	9.49	0.52	85	22 100	1 000
Treated Effluent End	8.43	622	24.9	9.32	0.51	85	17 800	900
Treated Effluent Avg.	**8.41**	**623**	**25.0**	**9.28**	**0.51**	**83**	**19 967**	**967**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa (count/200 µL)	**Treated Effluent Protozoa (count/10 µL)**
(1) 1 rotifer, 1 amoeba, & 3 filmamentous organisms	5	
(2) 1 rotifer, 1 amoeba, 1 filamentous organism, & 1 stalked cilitate	4	
(3) 1 rotifer, 1 amoeba, & 2 stalked cilitates	4	
(4) 1 rotifer, 1 filamentous organism, & 2 stalked cilitates	4	
(5) 1 ostrocad, 2 amoeba, & 1 filamentous organism	4	
(6) 3 filamentous organisms & 1 stalked cilitate		4
(7) 1 rotifer, 1 filamentous organism, & 1 amoeba		1
(8) 1 rotifer, 1 filamentous organism, & 1 stalked cilitate		1
(9) 2 stalked cilitates & 1 amoeba		2
(10) 1 rotifer, 1 filamentous organism, & 1 amoeba		2
Avg.	**4**	**2**

EXPERIMENT #12

Water: Fresh water
Date: July 20, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

Addition: 3.41 grams of $MgSO_4$
3.41 grams of Na_2CO_3 to increase water's original conductivity from 420 µS/cm to 879 µS/cm

OPERATION PARAMETERS:

Sample Volume (L)	18
Current Density (mA/cm^2)	9.8
Volts	36.8
Amps	10.0
kWh/m^3	6.1

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	10.01	878	19.9	6.19	0	40	448 000	47 000
Influent Middle	10.05	879	19.9	6.67	0	35	456 000	38 000
Influent End	10.04	879	19.9	6.70	0	40	437 000	45 000
Influent Avg.	**10.03**	**879**	**19.9**	**6.52**	**0**	**38**	**447 000**	**43 333**
Treated Effluent Beginning	9.94	868	21.0	9.72	1.56	35	1 000	0
Treated Effluent Middle	9.95	875	21.0	9.46	1.57	30	1 000	0
Treated Effluent End	9.94	876	21.0	8.92	1.56	35	2 000	0
Treated Effluent Avg.	**9.37**	**873**	**21.0**	**9.37**	**1.56**	**33**	**1 333**	**0**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa (count/200 µL)	**Treated Effluent Protozoa (count/10 µL)**
(1) 1 rotifer & 1 flagellate	2	
(2) 1 stalked cilitate	1	
(3) 2 stalked cilitates	2	
(4) 1 stalked cilitate	1	
(5) 2 stalked cilitates	0	2
(6) 1 stalked cilitate	0	1
(7) 1 stalked cilitate	0	1
		0
		0
Avg.	**1**	**1**

(Cont.) EXPERIMENT #12

Toxicity of bacteria to one minute Cl_2 detention time:

Dosage Cl_2 applied (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/100 mL)
0.46	4 030	460
0.65	3 400	420
1.10	2 140	385
1.30	1 875	285
1.41	1 760	300
1.38	1 643	263
1.44	1 805	320
1.50	2 100	290
2.39	1 053	210

EXPERIMENT #13

Water: Fresh water
Date: July 27, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

Addition: 1.07 grams of $MgSO_4$ to increase water's orginial conductivity from 313 µS/cm to 415 µS/cm

OPERATION PARAMETERS:

Sample Volume (L)	18
Current Density (mA/cm^2)	8.5
Volts	61.3
Amps	8.7
kWh/m^3	8.9

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	8.38	413	19.4	7.38	0	45	288 000	5 000
Influent Middle	8.36	415	19.0	7.34	0	45	280 000	5 000
Influent End	8.35	417	18.7	7.41	0	45	282 000	7 000
Influent Avg.	**8.36**	**415**	**19.0**	**7.38**	**0**	**45**	**283 333**	**5 666**
Treated Effluent Beginning	8.42	468	24.7	12.79	0.05	45	124 000	7 000
Treated Effluent Middle	8.41	474	25.8	11.03	0.06	45	119 000	6 000
Treated Effluent End	8.40	476	25.7	11.30	0.06	50	125 000	5 000
Treated Effluent Avg.	**8.41**	**473**	**25.4**	**11.71**	**0.06**	**47**	**122 667**	**6 000**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa (count/200 µL)	**Treated Effluent Protozoa (count/10 µL)**
(1) 1 rotifers & 1 stalked cilitate	2	
(2) 1 amoeba & 1 filamentous organism	2	
(3) 1 rotifer	1	
(4) 1 stalked cilitate	1	
(5) 1 rotifer		1
(6) 1 stalked cilitate		1
(7) 1 rotifer & 1 amoeba		2
(8) 1 amoeba & 1 filamentous organism		2
(9) 1 amoeba		1
Avg.	**5**	**1**

EXPERIMENT #14

Water: Fresh water
Date: July 29, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

Addition: 8.60 grams of $MgSO_4$ to increase water's orginial conductivity from 657 µS/cm to 1 011 µS/cm

OPERATION PARAMETERS:

Sample Volume (L)	18
Current Density (mA/cm^2)	9.8
Volts	32.3
Amps	10.0
kWh/m^3	5.38

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	ORP (mv)	D.O. (mg/L)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	8.41	1 009	21.2	336.1	5.85	0	55	49 900	2 900
Influent Middle	8.40	1 011	20.8	343.3	5.16	0	50	48 700	2 900
Influent End	8.40	1 014	21.3	314.0	5.51	0	50	50 300	2 600
Influent Avg.	**8.40**	**1 011**	**21.1**	**331.1**	**5.51**	**0**	**52**	**49 633**	**2 600**
Treated Effluent Beginning	8.38	1 036	24.9	-138.9	8.89	0.55	45	1 000	0
Treated Effluent Middle	8.41	1 036	23.7	-136.1	8.79	0.50	50	1 000	0
Treated Effluent End	8.40	1 036	24.0	-135.7	8.42	0.52	45	0	0
Treated Effluent Avg.	**8.40**	**1 036**	**24.2**	**-136.9**	**8.70**	**0.52**	**47**	**666**	**0**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa (count/200 µL)	**Treated Effluent Protozoa (count/200 µL)**
(1) 5 rotifers & 1 amoeba	6	
(2) 4 rotifiers & 1 amoeba	5	
(3) 2 rotifers, 1 flagellate, & 1 stalked cilitate	4	
(4) 3 rotifers & 1 flagellate	4	
(5) 3 rotifers & 1 amoeba	4	
		0
		0
		0
		0
		0
Avg.	**5**	**0**

EXPERIMENT #15

Water: Fresh water
Date: July 31, 2009
Electrodes: Nine titanium electrodes
Power Source: Direct Current

Addition: 9.62 grams of $MgSO_4$ to increase water's original conductivity from 584 µS/cm to 1 260 µS/cm

OPERATION PARAMETERS:

Sample Volume (L)	18.0
Current Density (mA/cm^2)	10.4
Volts	27.1
Amps	10.6
kWh/m^3	4.8

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	ORP (mv)	D.O. (mg/L)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	8.57	1 256	19.3	280.0	9.70	0	45	168 000	15 000
Influent Middle	8.55	1 261	19.3	270.2	9.85	0	50	174 000	16 000
Influent End	8.55	1 262	19.3	276.7	9.74	0	45	177 000	13 000
Influent Avg.	**8.56**	**1 260**	**19.3**	**275.6**	**9.76**	**0**	**47**	**173 000**	**14 667**
Treated Effluent Beginning	8.48	1 270	20.9	-126.7	12.35	1.68	45	27 000	1 000
Treated Effluent Middle	8.48	1 275	21.0	-131.4	12.05	1.65	45	29 000	2 000
Treated Effluent End	8.46	1 269	21.3	-128.9	10.65	1.64	50	31 000	0
Treated Effluent Avg.	**8.47**	**1 271**	**21.1**	**-129.0**	**11.68**	**1.66**	**47**	**29 000**	**1 000**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa (count/200 µL)	**Treated Effluent Protozoa (count/200 µL)**
(1) 1 filamentous organism & 1 stalked cilitate	2	
(2) 1 rotifiers & 1 amoeba	2	
(3) 1 stalked cilitate	1	
(4) 2 amoeba	2	
(5) 1 filamentous organism & 1 amoeba	2	
		0
		0
		0
		0
		0

EXPERIMENT #18

Water: Fresh water
Date: September 22, 2009
Electrodes: Two titanium electrodes
Power Source: Alternating Current
Flow Rate: 5.8 (L/min)

Addition: 40.58 grams of $MgSO_4$ to increase water's original conductivity from 358 µS/cm to 1 206 µS/cm

OPERATION PARAMETERS:

Sample Volume (L)	40.0
Current Density (mA/cm^2)	70.0
Volts	1.1
Amps	1.0
kWh/m^3	0.003

LAB ANALYSIS:

	pH	Conductivity (µS/cm)	Temp (°C)	D.O. (mg/L)	Cl_2 (mg/L)	Chlorides (mg/L)	Coliforms (CFU/100 mL)	*E. Coli* (CFU/ 100mL)
Influent Beginning	8.61	1 203	16.7	9.71	0	365	763 000	0
Influent Middle	8.63	1 206	16.3	9.75	0	375	770 000	0
Influent End	8.62	1 209	16.4	9.66	0	385	757 000	0
Influent Avg.	**8.62**	**1 206**	**16.5**	**9.71**	**0**	**375**	**197 333**	**0**
Treated Effluent Beginning	8.68	1 194	20.5	9.36	0	370	66 800	0
Treated Effluent Middle	8.63	1 199	20.6	9.41	0	360	691 000	0
Treated Effluent End	8.65	1 197	20.8	9.34	0	375	684 000	0
Treated Effluent Avg.	**9.37**	**1 197**	**20.6**	**9.37**	**0**	**368**	**164 666**	**0**

IDENTIFICATION OF VIABLE MICROORGANISMS:

	Influent Protozoa	**Treated Effluent Protozoa**
(1) 1 rotifer, 1 amoeba, & 1 filamentous organism	3	
(2) 1 rotifer & 10 ostrocads	11	
(3) 1 rotifer, 1 amoeba, & 1 ostrocad	3	
(4) 1 amoeba & 3 ostrocads	4	
(5) 1 rotifer & 1 stalked cilitate	2	
(6) 2 rotifers		2
(7) 1 rotifer		3
(8) 1 rotifer & 1 amoeba		2
(9) 2 ostrocads		2
(10) 1 amoeba & 1 stalked cilitate		2
Avg.	**2**	**1**

APPENDIX B PUBLISHED EXPERIMENTAL DATA

Table B.1 Ships' Water Parameters that Conveyed *V. cholerae* (McCarthy and Khambaty, 1994)

Ship	Water source	pH[a]	Salinity (ppt)[a]	Concn of fecal coliforms (CFU/100 ml)	Presence of *V. cholerae*
1	Bilge			1.2×10^2	−
	Sewage			2.7×10^4	−
2	Bilge			ND[b]	−
	Sewage			0	−
3	Bilge			ND	−
	Sewage			2.8×10^4	−
4	Ballast	7	32	0	−
	Sewage			0	−
5	Ballast	7	17	0	−
	Sewage			0	−
6	Ballast	7	30	1.2×10^1	−
	Bilge			ND	−
	Sewage			3.0×10^6	−
7	Ballast	6	32	3.0	−
	Bilge			ND	−
8	Ballast	7	13	2.0×10^1	+
	Fire main	7	14	5.8×10^2	+
9	Ballast	6	14	0	+
	Bilge			0	+
	Sewage			1.5×10^5	+
10	Ballast	7	32	0	+
	Bilge			0	+
11	Ballast	7	31	0	−
	Sewage			0	−
12	Ballast	7	28	0	−
	Sewage			0	−
13	Ballast	7	26	0	−
	Sewage			0	−
14	Ballast	7	26	0	−
15	Ballast	7	32	0	−
16	Ballast	7	18	9.0×10^1	−
17	Ballast	7	21	5.1×10^2	−
18	Ballast	6	20	0	+
19	Ballast	6	12	0	+

Table B.2 Ship's Record's that transported *V. cholera* (McCarthy and Khambaty, 1994)

Ship	Date[a]	Location	LPC[b]
1	10/30/91	Mobile, Ala.	Japan
2	10/30/91	Mobile, Ala.	South Africa
3	10/31/91	Mobile, Ala.	Mexico
4	11/01/91	Mobile, Ala.	Nova Scotia
5	11/01/91	Mobile, Ala.	Venezuela
6	11/05/91	Mobile, Ala.	Louisiana
7	11/05/91	Mobile, Ala.	Puerto Rico
8	11/06/91	Mobile, Ala.	Brazil
9	11/07/91	Mobile, Ala.	Colombia
10	11/19/91	Pascagoula, Miss.	Chile
11	11/25/91	Gulfport, Miss.	Venezuela
12	11/25/91	Gulfport, Miss.	Venezuela
13	11/26/91	Mobile, Ala.	Venezuela
14	11/26/91	Mobile, Ala.	Venezuela
15	12/02/91	Mobile, Ala.	Venezuela
16	12/03/91	Mobile, Ala.	Venezuela
17	12/03/91	Mobile, Ala.	Venezuela
18	06/11/92	Mobile, Ala.	Puerto Rico
19	06/12/92	Mobile, Ala.	Brazil

a- month/date/year (Date)
b- Last Port of Call (LPC)

Table B.3 Collocation of Data from Heat Treatment (Rigby et al., 2004)

Organism	Acute (secs-mins)	Chronic (hrs-days)	Reference
MARINE BACTERIA *Vibrio cholerae*	≥55°C	45°C, 2-3 hrs in seawater but survived in nutrient broth	McCarthy 1996 Desmarchelier and Wong 1998
MICROALGAE			
Diatoms *Skeletonema costatum , Detonula pumila,Pseudo-nitzschia cuspidata, Thalassiosira rotula*	35 °C, 30-60 min		Marshall and Hallegraeff (original data); Forbes and Hallegraeff 2001
Small diatoms *Amphora, Navicula jeffreyi*	35 °C, 5 hr (*Nitzschia paleaceae* survived)		Forbes and Hallegraeff 2002
Raphidophyte *Heterosigma akashiwo*	35 °C, 5 hr		Marshall and Hallegraeff (original data)
Picoplankton *Nannochloropsis oculata*	42.5 °C, 3 hr		Marshall and Hallegraeff (original data)
Chlorophyte *Dunaliella tertiolecta*	42.5 °C, 24 hr		Marshall and Hallegraeff (original data)
Dinoflagellate *Amphidinium carterae*	35 °C, 30 min		Marshall and Hallegraeff (original data)
Dinoflagellate *Alexandrium*	45 °C, 3 min		Montani et al. 1995
Alexandrium catenella dinocysts	42 °C, 30 min	38 °C, 4.5hr	Hallegraeff et al.1997
Gymnodinium catenatum dinocysts	40-45 °C, 30-60 sec	35-37.5 °C, 1-2 hr	Hallegraeff et al.1997
SEAWEED *Undaria pinnatifida* spores	35-40 °C, 0.9-42 min		Mountfort et al.1999
MOLLUSCS			
Dreissena polymorpha (adult)	36°C, 10 min	32°C , 3hr 33°C,1.5hr	Jenner and Janssen-Mommen 1992
Crassostrea gigas (larvae)	40-48 °C, 6-97 min		Mountfort et al.1999
Crassostrea virginica	48.5 °C		Sellers and Stanley 1989
Mytilus edulis	40 °C, 0,33 hr		Johnson et al. 1983
Corbicula fluminea	44 °C (instantaneous)		Graney et al. 1983
Perna viridis	43 °C, 30 min		
STARFISH *Coscinasterias calamaria* (larvae)	39-44 °C, 1-35 min		Mountfort et al.1999
CRUSTACEAN *Artemia salina* (hydrated eggs)	42.5 °C, 48 h (dry eggs survive)		Marshall and Hallegraeff (original data)
ROTIFER *Brachionus*	42.5 °C, 1h (eggs survive)		Marshall and Hallegraeff (original data)

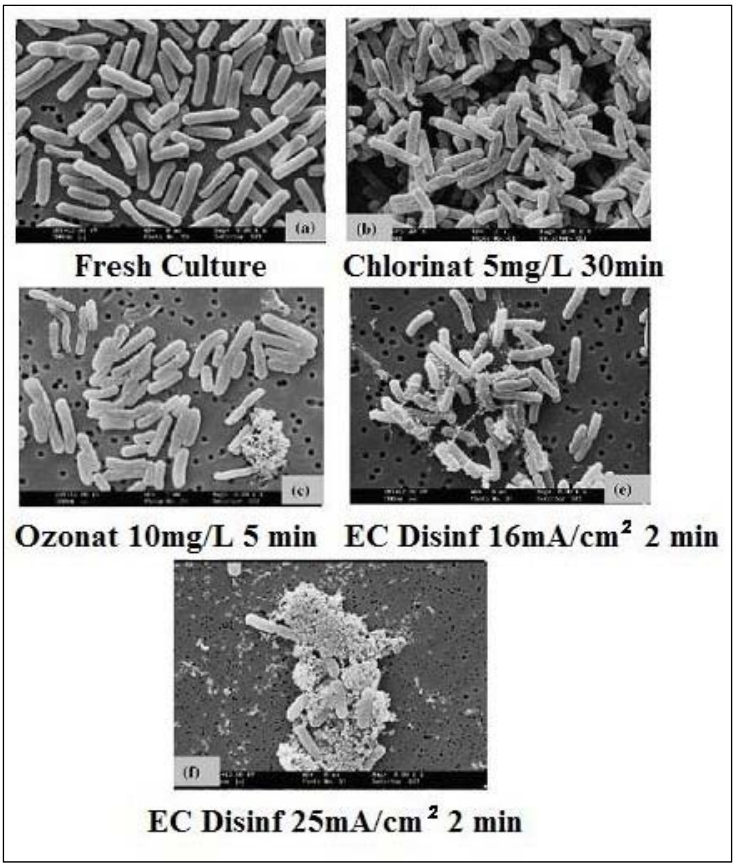

Figure B.1 Scanning electron Microscopy Disinfection Methods (Kim et al., 2006)

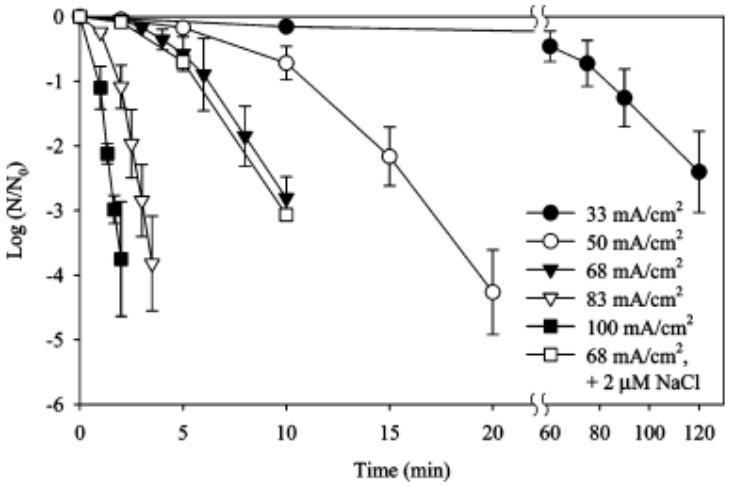

FIGURE 1. Effect of current density on *E. coli* inactivation ($[E. coli]_0 = 10^5$ CFU/mL, $[KH_2PO_4]_0 = 0.2$ M, pH = 7.1, 25 °C).

Figure B.2 Current effect on *E. coli* (Jeong et al., 2004)

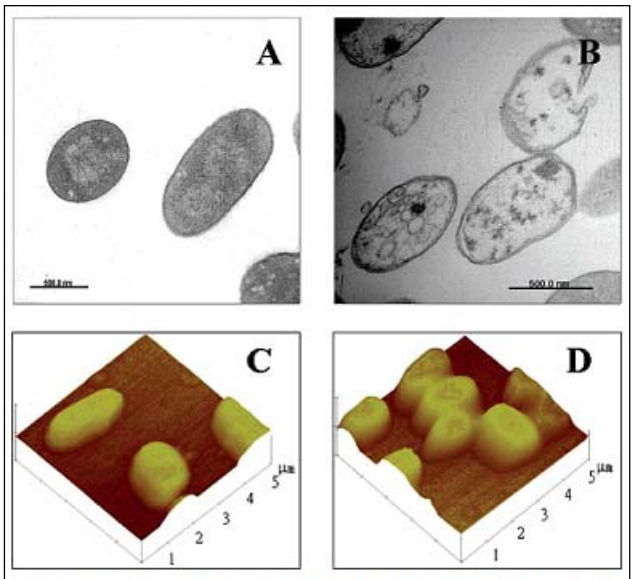

FIGURE 3. Morphological change of *E. coli* cells resulting from the electrolysis at 100 mA/cm² for 5 min ($[E. coli]_0 = 10^8$ CFU/mL, $[KH_2PO_4]_0 = 0.2$ M, pH = 7.1, 25 °C, (A) before electrolysis (TEM), (B) after electrolysis (TEM), (C) before electrolysis (AFM), (D) after electrolysis (AFM)).

Figure B.3 TEM and AFM Microscopy of Electro-disinfection on *E. coli* (Jeong et al., 2006)

Table B.4 Economics of Three Approved Systems by IMO (Lloyd's Register, 2008)

Supplier **Alfa Laval Tumba AB** — **Partner(s)** Wallenius
Process *Pureballast*: Filtration + Ultraviolet/TiO_2 — **Country** Norway
System used Ballasting + discharging — **Web site** www.alfalaval.com

Active substance approval (if applicable) Basic	Final	System approval Shipboard	Landbased	Test site	Type approval certificate	Commercially available	Units installed	Projected production Units/y
07/2007*	07/2007*	04/2008	04/2008	NIVA	27/06/2008	2006	5	**

Capacity 1000 m^3/h	Footprint, m^2 for unit capacity of: 200 m^3/h	2000 m^3/h	Maximum height m	Capex, $k 200 m^3/h	2000 m^3/h	Opex $per 1000 m^3/h	Company formed	No. employees
5	3	12	3	NA	NA	NA	1883	9500

Power requirement kW / 1000 m^3/ h	Additional services	Comments
NA	Air, water (rinsing)	*Basic and final approval granted MEPC 56 **According to an evaluation of potential growth to 2016, manufacturing not seen as a limiting factor

(a)

Supplier **Hamann AG** — **Partner(s)** EVONIC Industries
Process 2 step filtration and peracetic acid (Peraclean®Ocean) — **Country** Germany
System used Ballasting — **Web site** www.hamannag.com

Active substance approval (if applicable) Basic	Final	System approval Shipboard	Landbased	Test site	Type approval certificate	Commercially available	Units installed	Projected production Units/y
03/2006*	04/2008**	06/2007	06/2007	NIOZ	10/06/2008	Since 2006	2	65

Capacity 1000 m^3/h	Footprint, m^2 for unit capacity of: 200 m^3/h	2000 m^3/h	Maximum height m	Capex, $k 200 m^3/h	2000 m^3/h	Opex $per 1000 m^3/h	Company formed	No. employees
0.05-2	4.3	64#	2.2-2.9	NA	NA	200	1970	94

Power requirement kW / 1000 m^3/ h	Additional services	Comments
NA	NA	* Basic approval 24/03/06 (MEPC 54/2/12 annex 5) **Final approval 04/04/2008 (MEPC 57/2/10 annex 7) #Footprint includes pipework

(b)

Supplier **NEI Treatment Systems LLC** — **Partner(s)** Mitsubishi Kakoki Kaishi Ltd (Japan), Samgong Co. (Korea)
Process Deoxygenation + cavitation — **Country** US
System used Ballasting — **Web site** www.nei-marine.com

Active substance approval (if applicable) Basic	Final	System approval Shipboard	Landbased	Test site	Type approval certificate	Commercially available	Units installed	Projected production Units/y
-	-	-	-	NOAA	10/2007	2006	5	200

Capacity 1000 m^3/h	Footprint, m^2 for unit capacity of: 200 m^3/h	2000 m^3/h	Maximum height m	Capex, $k 200 m^3/h	2000 m^3/h	Opex $per 1000 m^3/h	Company formed	No. employees
>10	3	6	2.6	360	690	150	1997	5

Power requirement kW / 1000 m^3/ h	Additional services
25	Air and water

(c)

VITA

Kathleen McCraven was born in Natchez, MS. She completed a Bachelor of Science degree in biological engineering at Mississippi State University in 2007.