# GREAT SHIPS INITIATIVE
# BENCH-SCALE TEST FINDINGS
# Technical Report - Public

## Bacoustics Ballast Water Treatment System

## June 10, 2013

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# ABSTRACT

The Great Ships Initiative (GSI) conducts bench-scale (i.e., laboratory scale) status testing services to aid developers of innovative technologies which could have application as ballast water management systems (BWMSs). This report describes findings from GSI bench-scale evaluations of a BWMS proposed by Bacoustics LLC., of Minnetonka, Minnesota. The system utilizes chlorine in conjunction with ultrasound (i.e., sonic energy) and is intended for use as a routine ballast treatment. GSI tested the Bacoustics BWMS to assist with range-finding for effective doses, and determination of chemical fate and chemical degradation.

Tests were conducted on a small, bench-scale working model of the Bacoustics BWMS involving four stages: separation of the incoming test water into two streams—a main stream and a side stream; injection of 100 $\mu$g/L reagent grade sodium hypochlorite (i.e., chlorine) into the side stream; ultrasonic dispersal of the chlorine in the side stream via an ultrasound chamber at a constant level; and finally, recombination of the treated side stream with the main stream flow. Tests were undertaken using two different water types—laboratory water (LW) and high organic content LW (HOC-LW)—and up to three different flow ratios of the side stream versus main stream (25, 90 and 100 %). Varying the side stream flow ratio but not the level of chlorine injected (100 $\mu$g/L) leads to variable doses of chlorine in the main flow after recombination of the two streams.

GSI bench-scale dose effectiveness tests measured the effects of various levels of chlorine and sonic energy on the freshwater algae *Selenastrum capricornutum* and on two species of bacteria—*Escherichia coli* and *Enterococcus faecalis*. Chemical fate tests were undertaken to determine the effect of organic matter and three different diversion ratios of the treated side stream on the concentration of chlorine after recombination of the side stream and main flow. Additional tests were undertaken to measure chlorine degradation over time.

The various treatment combinations of the Bacoustics BWMS tested had little effect on the mortality of the green algae *Selenastrum capricornutum*. The sonic energy treatment alone also did not have any effect on the mortality of *S. capricornutum* nor did it reduce either type of bacteria. Immediate exposure to 100 $\mu$g/L of chlorine in LW significantly reduced both *E. coli* and *E. faecalis*, but only reduced *E. faecalis* significantly in HOC-LW after 48 hours of exposure. Concentrations of total residual chlorine (TRC) in HOC-LW were below detection across all samples, regardless of side stream diversion ratios. In comparison, concentrations of TRC in LW ranged 96 - 356 $\mu$g/L. Chemical degradation of chlorine was influenced by the diversion ratios tested.

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# INTRODUCTION

This technical report presents quantitative findings from GSI bench-scale evaluations of a ballast water management system (BWMS) proposed by Bacoustics LLC., of Minnetonka, Minnesota, with possible application to the Great Lakes. The BWMS utilizes chlorine in conjunction with ultrasound (i.e., sonic energy) to treat ballast water and is intended for use as a routine ballast treatment. GSI undertook these bench-scale tests during 2009 at the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior (UWS) in Superior, Wisconsin. Tests included range-finding evaluations of dose effectiveness and determination of chemical fate and chemical degradation.

# BACKGROUND

## Great Ships Initiative (GSI)

GSI is a regional effort managed by the Northeast-Midwest Institute (NEMWI) devoted to ending the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System and globally. Since its establishment in 2006, GSI has provided independent performance/verification testing services to developers of BWMSs at the bench, land-based and shipboard scales. GSI performs informal "status testing" for systems that are in the research and development stage, and formal certification/verification tests appropriate to market-ready BWMSs.

## Organization

GSI is a project of NEMWI—a Washington, D.C-based private, non-profit, and non-partisan research organization dedicated to the economic vitality, environmental quality, and regional equity of Northeast and Midwest states. The project is carried out collaboratively with contracting entities including the University of Wisconsin-Superior (UW-S), AMI Consulting Engineers, Broadreach Services, and the University of Minnesota-Duluth (UM-D).

Ms. Allegra Cangelosi of NEMWI is GSI's Principal Investigator and Director (GSI PI). A GSI Advisory Committee comprising top-level officials of key stakeholder groups provides direct input to Ms. Cangelosi, advising on GSI award decisions, program direction, finances and fund-raising. The American Great Lakes Ports Association advises the project, assuring that the GSI is meeting the needs of the maritime industry; and coordinating maritime industry and supply chain outreach. Researchers from UW-S's Lake Superior Research Institute (LSRI) and the UM-D's Natural Resources Research Institute (NRRI), among others, provide critical scientific and technical expertise and implementation services to the GSI PI. Dr. Mary Balcer of LSRI is the project's lead zooplankton ecologist. She is also the team leader for LSRI staff engaged in GSI research activities. Dr. Euan Reavie of the NRRI leads all phytoplankton analysis and NRRI staff.

Mr. Matthew TenEyck of LSRI leads all bench-testing and Whole Effluent Toxicity (WET) tests. Ms. Deanna Regan of LSRI is responsible for GSI chemical analysis. Ms. Heidi Saillard of LSRI is responsible for GSI microbial analysis. Ms. Nicole Mays of NEMWI is GSI's Senior Quality Systems Officer and Ms. Kelsey Prihoda of LSRI is GSI's Senior QAQC Officer.

## GSI BENCH-SCALE TESTS

GSI bench-scale tests involve "status testing" to provide BWMS developers insight into the performance of BWMS processes and configurations at early stages of development relative to specific challenge conditions and scenarios. Findings are strictly the performance outcomes of the tests. That is, to maintain its independence as a testing facility, GSI does not engage in discussions with the BWMS developer on, or produce recommendations for, ways to improve the BWMS process subject to testing. Developers apply directly to GSI for these services, and GSI awards "Status Testing" services at no cost except for shipping the subject BWMS to and from the GSI testing facility, and costs involved in the installation and removal of the system from the GSI testing facility.

GSI bench-scale status tests take place year-round at the LSRI. The LSRI is amply equipped with staff expertise and resources to conduct the tests, and has a long history of successfully undertaking similar tests. The overarching goal of GSI bench-scale status testing is to explore dose effectiveness, chemical degradation, residual toxicity, and/or sensitivity to challenge conditions of a proposed BWMS or component thereof.

### GSI Active Substance Degradation Tests

GSI bench-scale active substance degradation tests determine the effect that various water quality or environmental parameters may have on the rate of active substance degradation and/or the rate of formation of disinfection by-products of a BWMS involving active substances. No organisms are used in association with these analytical assays; instead test solutions are analyzed for their concentration of active substance (or active component of the substance). Test results are typically expressed as the percent change in active ingredient concentration.

If a BWMS does not utilize an active substance, this stage of testing does not apply. Instead, preliminary water chemistry testing may be conducted as needed and include measurement of basic water quality parameters, such as, temperature, pH, conductivity, and dissolved oxygen.

### GSI Dose Effectiveness Tests

GSI bench-scale dose effectiveness tests help determine the range of concentrations of an active substance that is toxic to a variety of robust freshwater zooplankton, algae and

bacteria known to be relatively resilient to stressors. Dose effectiveness test results for zooplankton and algae are typically expressed as percent survival and/or percent mortality, and in the case of resting eggs results are expressed as percent hatch. Where applicable, results may also be expressed in terms of a series of absolute quantifications: $LC_{99}$, i.e., the experimentally derived concentration of an active substance estimated to kill 99 % of the test population following 24 or 48 hours of continuous exposure; No Observed Effect Concentration (NOEC), i.e., the highest concentration of an active substance shown to have no significantly adverse effect on the test population compared to controls; and/or Lowest Observed Effect Concentration (LOEC), i.e., the lowest concentration of an active substance known to have a significantly adverse effect on the test population compared to controls.

**GSI Residual Toxicity Tests**

GSI bench-scale residual toxicity tests help estimate the effect that treated water (following neutralization of the active substance, a degradation period, and/or a dilution step) may have on non-target organisms in the receiving system. These test results are expressed in a manner similar to those for dose effectiveness assays. The principal difference between these tests and dose effectiveness tests is that the concentration of the active substance is adjusted to be consistent with potential discharge levels. The tests are also performed on sensitive organisms rather than robust species.

# METHODS

## Ballast Water Management System (BWMS)

*Treatment and Test Apparatus*

The Bacoustics BWMS utilizes chlorine in conjunction with sonic energy to treat ballast water. The system operates by directing a portion of the incoming ballast water into a side stream. Here, small amounts of chlorine are introduced and the chlorine is dispersed with sonic energy. The chlorinated/sonicated mixture is then reintroduced into the main intake stream (untreated ballast water) prior to the water entering the ballast tank.

GSI bench-scale tests of the Bacoustics BWMS were undertaken on a small scale working model which consisted of four stages (Figure 1). Stage I separated the incoming test water into two streams—a main stream and a side stream. Stage II injected chlorine at a rate of 100 $\mu$g/L into the side stream. Stage III ultrasonically dispersed the chlorine in the side stream via an ultrasound chamber. Stage IV recombined the treated side stream with the main stream flow (Figure 1). The BWMS was equipped with four sample collection ports labelled SP1 through SP4 (Figure 1). SP1 was located in the main stream prior to ballast separation; SP2 was located in the side stream after chlorine injection; SP3 was located in the side stream after the ultrasonic chamber; and SP4 was located in the main stream after recombination of the side stream (Figure 1). The total flow rate

used in the tests was approximately 5.0 gpm. The relative volume of the intake flow diverted to the side stream was variable, leading to variable doses of chlorine in the main flow after recombination.

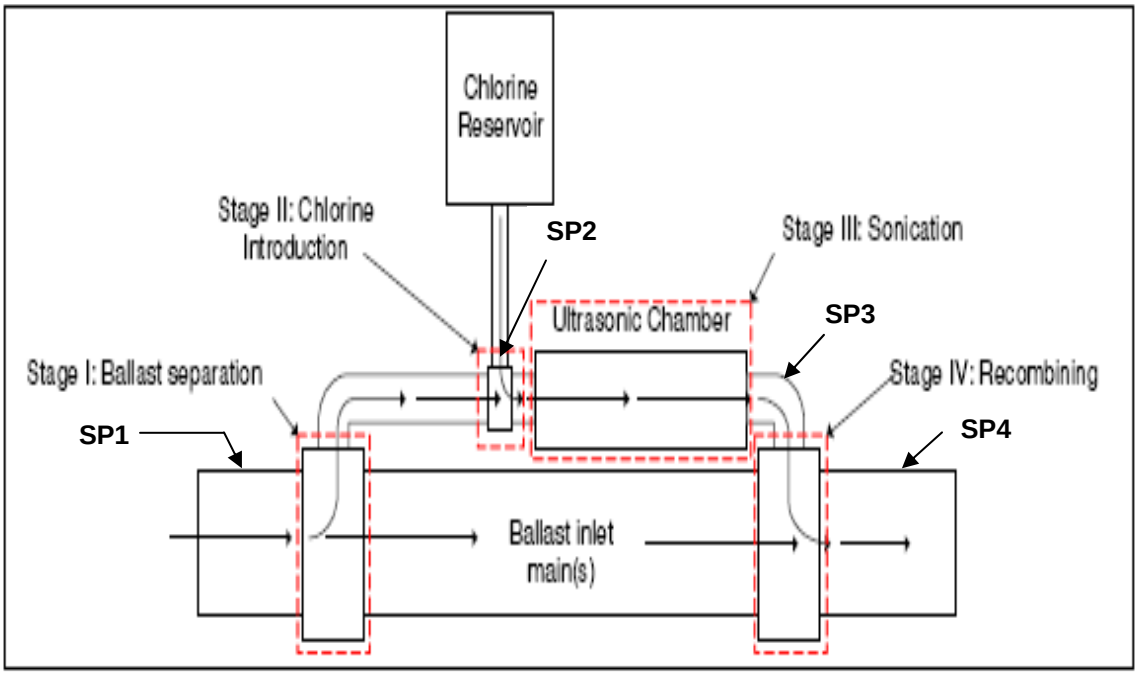


**Figure 1. Schematic of the Small-Scale, Working Model Bacoustics Ballast Water Management System (BWMS), including Sample Collection Ports (SP).**

Experiments took place in an LSRI laboratory equipped with adequate ventilation, electrical connections, and climate control. Test apparatus generally consisted of several 300 mL borosilicate high-form beakers housed within environmental chambers set to the appropriate temperature and light regime.

*Treatment Application*

The treatment apparatus was set up to inject chlorine (i.e., 100 $\mu$g/L reagent grade sodium hypochlorite) and sonic energy at a constant level (i.e., 100 %) into the treated water flow, according to BWMS developer specifications. Tests were undertaken with two different water types (laboratory water, LW, and high organic content laboratory water, HOC-LW) using up to three different flow ratios of the side stream versus main stream (25, 90 and 100 %). Samples were collected at SP1, SP2, SP3, and/or SP4. Test water with no applied chlorine or sonic energy served as the control.

## General Methods

*Sample Water Preparation*

The two experimental water qualities were prepared in the laboratory as follows:

- Laboratory water (LW): Treated Lake Superior water from the City of Superior that was passed through an activated carbon column. Alkalinity ranged from 45 - 50 mg/L as $CaCO_3$. Nonpurgeable organic carbon (NPOC) ranged from 0.5 - 2.0 mg/L. The water is transparent.
- High organic content laboratory water (HOC-LW): LW as described above with addition of tannic (15 mg/L) and humic acids (5 mg/L) to adjust the NPOC to approximately 8 mg/L. The water is stained and has a lower light transmittance when compared to LW. The enriched NPOC level is also reflective of Duluth-Superior Harbor water.

Experimental water temperature was adjusted to the desired level of 25 ± 3 °C by placing the beaker containing the appropriate amount of water in an incubator set to the desired test temperature.

*Chemical Analysis*

Samples for chlorine analysis were collected from the BWMS at the various SPs and stored in 1.0 L Teflon beakers. 20 mL of sample water was then transferred into a smaller 30 mL beaker with 200 $\mu$L of potassium iodide reagent and 200 $\mu$L of acetate buffer reagent added. Samples were analyzed for total residual chlorine (TRC) concentration as soon as possible after collection. Analysis was conducted with a Thermo Orion Mode 97-70 Residual Chlorine Electrode connected to an Orion Model 290A pH/mV/ISE meter.

A 100 mg/L chlorine stock solution was prepared daily by diluting deionized water with 0.175 mL of 6.0 % sodium hypochlorite solution (i.e., Clorox® Bleach) to make 100 mL. Analytical standards, ranging in concentration from 5 - 300 $\mu$g/L, were prepared in deoxygenated deionized water by making dilutions of the 100 mg/L chlorine stock. Potassium iodide reagent and acetate buffer were added to the chlorine containing analytical standards. Chlorine present in the standards/samples oxidizes iodide to iodine in an acidic solution. The iodine concentration after the reaction is equal to the chlorine concentration present before the reaction. A calibration curve plotting log of the chlorine concentration (X-axis) versus the mV response from the Residual Chlorine Electrode (y-axis) is then used to determine TRC concentrations in the samples.

NPOC analysis was conducted on a Shimadzu Model TOC-5050A Total Organic Carbon Analyzer. Samples were filtered in sequence through a Whatman GF/B filter followed by a Millipore 0.45 $\mu$m HA membrane filter. Before analysis, the samples were acidified with concentrated hydrochloric acid to 0.2 %. Samples were then purged with high purity air to remove the inorganic carbon and purgeable organic carbon, and injected into the

analyzer. An organic carbon stock solution was prepared by dissolving 0.2125 g of oven dried anhydrous potassium hydrogen phthalate (KHP) in deionized water, adding 125 $\mu$L of concentrated hydrochloric acid, and diluting to 1000 mL with deionized water. This organic carbon stock solution had a concentration of 1000 mg/L carbon. A series of working standards (i.e., 1.0, 5.0, 10.0 and 100 mg/L carbon) were prepared from the organic carbon stock. Each standard was made to contain 0.2 % hydrochloric acid. The standards were used to generate a calibration curve which was then used to determine the concentration of organic carbon in the samples.

**Experimental Methods**

*Dose Effectiveness Experiments*

GSI dose effectiveness tests measured the effects of chlorine and sonic energy on the freshwater algae *Selenastrum capricornutum* which is known to be resilient to stressors. Dose effectiveness was examined using various combinations of chlorine and sonic energy output in the two test water qualities of LW and HOC-LW. Only two chlorine concentrations, set by the side stream diversion ratio (25 and 100 %), were tested. A control, consisting of 0 $\mu$g/L of chlorine and 0 % sonic energy output, was used to measure background mortality. Table 1 summarizes the water qualities and treatment scenarios analyzed.

In these tests, 2.0 L of *Selenastrum* at a concentration of $1.0x10^8$ cells/mL was added to 1000 L of test water and the treatment apparatus was turned on. The solution flowed through the BWTS at a design rate of 5.0 gpm. Approximately 75.0 mL of solution was then collected at SP4 after 10 minutes.

After collection, samples were immediately placed in complete darkness at a nominal temperature 25.0 ºC ± 1.0 ºC and the test water was not renewed. Observations on algae mortality were used to assess acute toxicity following 0, 24 and 48 hours exposure. Portions of water from each sample were additionally analyzed for TRC at these three time points and for NPOC at 48 hours. The remaining sample was used for other water chemistry analyses such as temperature, pH and dissolved oxygen. Conductivity, hardness and alkalinity analyses were also conducted on selected samples at test initiation and test conclusion. Appropriate analysis methods for each of these parameters were followed.

Statistical analysis was performed with CETIS (v. 1.7.0 revision T, Tidepool Scientific Software, McKinleyville, California) and SigmaStat (v. 3.5, Systat Software Inc., Chicago, IL) statistical software, where applicable. Prior to Analysis of Variance (ANOVA), the data was tested for normality and homogeneity of variance. If data were normally distributed and homogenous, survival was analyzed by ANOVA followed by Dunnett's means comparison test to determine the difference from control data. Statistics were not used to report significances in cases where the results were either 0 % of 100 % survival with no error.

**Table 1. Test Scenarios for GSI Dose Effectiveness Tests of the Bacoustic BWTS involving *Selenastrum capricornutum***

| Water Type | Diversion Ratio of Treated: Untreated Test Water (%) | Injected Chlorine Concentration (μg/L) | Sonic Energy Output (%) |
|---|---|---|---|
| Laboratory Water (LW) | 25 (control) | 0 | 0 |
| | 25 | 0 | 100 |
| | | 100 | 0 |
| | | 100 | 100 |
| | 100 (control) | 0 | 0 |
| | 100 | 0 | 100 |
| | | 100 | 0 |
| | | 100 | 100 |
| High Organic Content Laboratory Water (HOC-LW) | 100 (control) | 0 | 0 |
| | 100 | 0 | 100 |
| | | 100 | 0 |
| | | 100 | 100 |

A second set of GSI dose effectiveness tests measured the effects of chlorine and sonic energy on two species of bacteria—*Escherichia coli* and *Enterococcus faecalis*. Dose effectiveness was examined using various combinations of chlorine and sonic energy output in LW and HOC-LW spiked with test organisms. Only one chlorine concentration, set by the side stream diversion ratio of 100 %, was tested along with two sonic energy outputs of either 0 or 100 %. A control was used to measure background effects due to the test apparatus. Table 2 summarizes the water qualities and treatment scenarios analyzed in these tests.

Prior to testing, freshly prepared streak plates were made using frozen stock cultures of test organisms purchased from ATCC and subsequently stored at - 70 ºC. *E. coli* (ATCC#700609) were grown on the recommend nutrient agar (+ 0.5 % NaCl + 0.01 % nalidixic acid) in an incubator, overnight at 36 ºC. *E. faecalis* (ATCC# 14433) were grown on tryptic soy agar and also incubated overnight at 36 ºC.

Two 100 mL cultures per species were then prepared by swabbing bacteria from the appropriate streaked plate and adding the swabs to the appropriate broth in a sterile flask. Flasks were placed in a 37 ºC shaking water bath overnight. The two flasks of each species were combined and a portion was collected to measure absorbance. Following, 110 mL of each of the cultures were then centrifuged at 200 RCF for 10 minutes in sterile centrifuge tubes. Supernatant was poured off and discarded and bacteria were resuspended in sterile deionized water before adding to the test water tank containing approximately 1,100 L of LW, thereby creating LW spiked with test organisms.

To begin testing, the BWMS was turned on and the LW flowed through the system at a design rate of 5.0 gpm. Four tests were conducted according to the test scenarios detailed in Table 2. Three replicate samples were collected in 1 L sterile polypropylene bottles or flasks from both the influent (SP1) and effluent ports (SP4). Each replicate was divided equally among three 250 mL sterile polypropylene bottles; one for each exposure period of 0, 24, and 48 hours. The 0 hour samples were analyzed immediately as described below while the remaining bottles were placed in 25 ºC shaking water baths (Orbit Shaker), with caps loosened, until their exposure period was reached. Three replicates of LW, filtered through a Millipore Stericup (0.22 $\mu$m pore size, sterile vacuum filtration unit) were used as a negative control or procedural blank (LST) which also ran the duration of the test coincident with the samples

Once the four LW tests were complete, HOC-LW test water was prepared by adding tannic acid and humic acid to the remaining previously spiked 500 L of 25 ºC LW as described above. The same test scenarios were used for the four HOC-LW water tests (table 2). At each exposure period of 24 and 48 hours, in the same order as in test initiation; portions were collected first for microbial analysis and then for chemical analysis.

For analysis, an aliquot of the sample water was diluted using peptone saline diluent (PSD), and 1 mL portions of the dilutions were then added to two pre-sterilized 100 mL vessels containing sodium thiosulfate to neutralize any remaining chlorine. Untreated samples were further diluted to 100 mL with sterile deionized water. The appropriate media capsule (i.e., Colilert® or Enterolert™) was then added to each 100 mL sample and mixed. The sample was poured into a Quanti-Tray/2000, and sealed using the Quanti-Tray sealer. All trays were incubated well-side down for 24 hours—Colilert® trays at 35 ºC and Enterolert™ trays at 41 ºC. A blank was also run for each analysis time point. In addition, 10 % of the samples were also analyzed in duplicate.

After incubation, Quanti-Trays were examined under a handheld UV light (Spectroline E-series, 6 watt, 110 volt), and the number of small and large fluorescing (positive) wells was recorded. The Quanti-Tray/2000 Most Probable Number (MPN) table was referenced in order to determine the MPN per 100 mL. MPN is a common method of obtaining quantitative data on concentrations of discrete items from positive/negative (incidence) data and in this case correlates well with colony forming units (cfus) per mL.

SigmaStat (v. 3.5, Systat Software Inc., Chicago, IL) was used to compare treatment groups in these tests, with the MPN of each treatment group replicate at each time point. A value of half the lower limit of quantification (LLOQ) was used for replicates having an MPN below the LLOQ. At each time point (i.e., 0, 2, 24, and 48 hours) treatment group means were compared using a one-way repeated measures analysis of variance (RM ANOVA). If the data passed the normality and equal variance tests, the Dunnet method for multiple comparisons was used to determine if significant ($p<0.05$) differences existed between the treatment group means. If the data did not pass the normality test, the data were compared using the Friedman RM ANOVA on Ranks and

Tukey's Test for pairwise multiple comparisons. Treatment group means were also compared using one-way RM ANOVA.

**Table 2. Test Scenarios for GSI Dose Effectiveness Tests of the Bacoustic BWMS involving *Escherichia coli and Enterococcus faecalis* Spiked Test Water.**

| Water Type | Diversion Ratio of Treated:Untreated Test Water (%) | Injected Chlorine Concentration ($\mu$g/L) | Sonic Energy Output (%) |
|---|---|---|---|
| Laboratory Water (LW) | 100 (control) | 0 | 0 |
| | 100 | 0 | 100 |
| | | 100 | 0 |
| | | 100 | 100 |
| High Organic Content Laboratory Water (HOC-LW) | 100 (control) | 0 | 0 |
| | 100 | 0 | 100 |
| | | 100 | 0 |
| | | 100 | 100 |

*Chemical Fate and Degradation Experiments*

GSI bench-scale chemical fate tests were undertaken to determine the effect of organic matter and three different diversion ratios of the treated side stream on the concentration of chlorine after recombination with the mainstream. Tests were performed using LW and HOC-LW and with and without sonic energy. Table 3 describes the water qualities and treatment scenarios analyzed in these tests.

**Table 3. Test Scenarios for GSI Chemical Fate Experiments of the Bacoustic BWMS.**

| Water Type | Diversion Ratio of Treated:Untreated Test Water (%) | Injected Chlorine Concentration ($\mu$g/L) | Sonic Energy Output (%) |
|---|---|---|---|
| Laboratory Water (LW) | 25 (control) | 0 | 0 |
| | 25 | 100 | 100 |
| | 90 | 100 | 100 |
| | 100 (control) | 0 | 0 |
| | 100 | 100 | 100 |
| High Organic Content Laboratory Water (HOC-LW) | 25 | 100 | 100 |
| | 90 | 100 | 100 |
| | 100 (control) | 0 | 0 |
| | 100 | 100 | 100 |

Additional tests were undertaken to measure chlorine degradation over time. In these tests, samples were analyzed for their concentration of TRC and NPOC daily for up to five days. Table 4 describes the water qualities and treatment scenarios analyzed.

**Table 4. Test Scenarios for GSI Chemical Degradation Experiments of the Bacoustic BWMS.**

| Water Type | Diversion Ratio of Treated:Untreated Test Water (%) | Injected Chlorine Concentration ($\mu$g/L) | Sonic Energy Output (%) |
|---|---|---|---|
| Laboratory Water (LW) | 25 | 100 | 100 |
| | 100 | 100 | 100 |

# FINDINGS

## Dose Effectiveness Experiments

The proposed BWMS process involving chlorine and sonic energy had little effect on the freshwater algae *S. capricornutum* in these tests, and effects that were detected resulted from chlorine exposure alone (Table 5). In LW, mortality ranged from 0 – 12.4 % and was highest in the treatment involving 100 $\mu$g/L chlorine in combination with 0 % sonic energy for the 25 % diversion ratio. In HOC-LW, mortality was lower overall, ranging from 0 – 0.4 % across all treatment combinations.

**Table 5. Mean Percent Mortality (Standard Deviation) of the Green Algae *Selenastrum capricornutum* Exposed to Various Combinations of Chlorine and Sonic Energy in Two Water Types (Laboratory Water and Challenge Water) Over a 48 Hour Experimental Period.**

| Water Type | Diversion Ratio (%) | Chlorine Concentration ($\mu$g/L) | Sonic Energy Output (%) | Mortality (%) 0 Hours | 24 Hours | 48 Hours |
|---|---|---|---|---|---|---|
| Laboratory Water (LW) | 25 (control) | 0 | 0 | 0 | 0.3 (0.5) | 0.3 (0.4) |
| | 25 | 0 | 100 | 0 (0) | 0 (0) | 0.6 (1.0) |
| | | 100 | 0 | 0 (0) | 8.6 (8.4) | 12.4 (11.4) |
| | | 100 | 100 | 0.5 (0.4) | 7.6 (5.4) | 3.1 (1.2) |
| | 100 (control) | 0 | 0 | 0 (0) | 0 (0) | 0.4 (0.8) |
| | 100 | 0 | 100 | 0.3 (0.4) | 0.3 (0.5) | 0.5 (0.5) |
| | | 100 | 0 | 0.3 (0.5) | 3.3 (1.9) | 2.1 (2.3) |
| | | 100 | 100 | 0 (0) | 1.8 (1.7) | 1.9 (1.8) |
| High Organic Content Laboratory Water (HOC-LW) | 100 (control) | 0 | 0 | 0.4 (0.8) | 0 (0) | 0 (0) |
| | 100 | 0 | 100 | 0 (0) | 0.4 (0.6) | 0 (0) |
| | | 100 | 0 | 0 (0) | 0 (0) | 0.4 (0.7) |
| | | 100 | 100 | 0 (0) | 0 (0) | 0 (0) |

In addition to mortality, sample water was analyzed for its concentration of TRC at 0, 24 and 48 hours. For tests involving no treatment with chlorine, the initial concentration of TRC ranged 8.5 - 11.9 $\mu$g/L in LW, but was below detection, i.e., < 2.3 $\mu$g/L, in HOC-LW (Table 6). By 24 hours, TRC in LW had also decreased to below detection (Table 6).

For tests involving treatment with 100 $\mu$g/L of chlorine, TRC initially ranged 81.6 – 107 $\mu$g/L in LW, though was below detection in HOC-LW (Table 6). TRC in LW continued to decline over the 48 hour test period, yet was still above detection, i.e., 9.9 - 11.3 $\mu$g/L, at 48 hours (Table 6).

NPOC levels at 48 hours in LW, ranged from 2.8 to 3.3 mg/L across all treatments (Table 7). The concentration of NPOC in HOC-LW was higher, ranging 10.2 – 10.7 mg/L (Table 7).

**Table 6. Mean (Standard Deviation) Concentration of Total Residual Chlorine (TRC) in Sample Water Used for GSI Dose Effectiveness Tests Involving the Green Algae *Selenastrum* and Collected at 0, 24 and 48 Hours.**

| Water Type | Diversion Ratio (%) | Chlorine Concentration (µg/L) | Sonic Energy Output (%) | Total Residual Chlorine (µg/L) | | |
|---|---|---|---|---|---|---|
| | | | | 0 Hours | 24 Hours | 48 Hours |
| Laboratory Water (LW) | 25 (control) | 0 | 0 | 11.9 (0.9) | < 2.3 | < 2.3 |
| | 25 | 0 | 100 | 8.5 (0.2) | < 2.3 | < 2.3 |
| | | 100 | 0 | 107 (1.4) | 21.8 (2.3) | 9.9 (0.4) |
| | | 100 | 100 | 87.2 (2.8) | 18.3 (0.7) | 11.0 (1.2) |
| | 100 (control) | 0 | 0 | 8.6 (0.2) | < 2.3 | < 2.3 |
| | 100 | 0 | 100 | 10.4 (0.7) | < 2.3 | < 2.3 |
| | | 100 | 0 | 96.8 (2.9) | 16.0 (1.4) | 11.3 (1.6) |
| | | 100 | 100 | 81.3 (4.9) | 16.2 (1.7) | 11.3 (2.4) |
| High Organic Content Laboratory Water (HOC-LW) | 100 (control) | 0 | 0 | < 2.3 | < 2.3 | < 2.3 |
| | 100 | 0 | 100 | < 2.3 | < 2.3 | < 2.3 |
| | | 100 | 0 | < 2.3 | < 2.3 | < 2.3 |
| | | 100 | 100 | < 2.3 | < 2.3 | < 2.3 |

**Table 7. Mean (Standard Deviation) Concentration of Nonpurgeable Organic Carbon (NPOC) in Sample Water Used for GSI Dose Effectiveness Tests Involving the Green Algae *Selenastrum capricornutum* and Collected at 48 Hours.**

| Water Type | Diversion Ratio (%) | Chlorine Concentration (µg/L) | Sonic Energy Output (%) | Nonpurgeable Organic Carbon (NPOC) (mg/L) |
|---|---|---|---|---|
| Laboratory Water (LW) | 25 (control) | 0 | 0 | 3.0 |
| | 25 | 0 | 100 | 2.8 |
| | | 100 | 0 | 3.3 |
| | | 100 | 100 | 3.1 |
| | 100 (control) | 0 | 0 | 3.0 |
| | 100 | 0 | 100 | 3.0 |
| | | 100 | 0 | 3.0 |
| | | 100 | 100 | 3.0 |
| High Organic Content Laboratory Water (HOC-LW) | 100 (control) | 0 | 0 | 10.2 |
| | 100 | 0 | 100 | 10.6 |
| | | 100 | 0 | 10.7 |
| | | 100 | 100 | 10.6 |

Results of GSI bench-scale dose effectiveness tests involving chlorine and sonic energy on the two types of bacteria, *E. coli* and *E. fecalis* are presented in Tables 8 and 9, respectively. Again, sonic energy alone had no detectable effect. Control tests conducted in LW yielded MPNs of approximately 9.5E+05 per 100 mL of *E. coli* and 1.1E+06 per 100 mL of *E. faecalis* (Tables 8 and 9). Similarly, control tests conducted in HOC-LW yielded MPNs of approximately 1.1E+06 per 100 mL of *E. coli* and 5.7E+06 per 100 mL of *E. faecalis* (Tables 8 and 9).

Both *E. coli* and *E. faecalis* were significantly ($p$<0.05) reduced when compared to controls following 48 hours exposure to 100 $\mu$g/L of chlorine in LW (Tables 8 and 9). *E. coli* was also significantly ($p$<0.05) reduced when compared to controls following 0 and 24 hours exposure to 100 $\mu$g/L of chlorine in HOC-LW, but not following 48 hours exposure (Table 8).

In contrast, *E. faecalis* was only significantly ($p$<0.05) reduced when compared to controls following 48 hours exposure to 100$\mu$g/L of chlorine in HOC-LW (Table 9). The sonic energy treatment alone did not reduce either type of bacteria (Tables 8 and 9).

**Table 8. Average (n=3, ± Standard Error) Most Probable Number (MPN) of *E. coli* per 100mL in Dose Effectiveness Experiments Involving Chlorine and Sonic Energy.**

| Water Type | Diversion Ratio (%) | Chlorine Concentration ($\mu$g/L) | Sonic Energy Output (%) | Most Probable Number (MPN) | | |
|---|---|---|---|---|---|---|
| | | | | 0 Hours | 24 Hours | 48 Hours |
| Laboratory Water (LW) | 100 (control) | 0 | 0 | 9.5E+05 (8.6E+04) | 4.1E+05(1.1E+05) | 1.5E+04 (1.1E+05) |
| | 100 | 0 | 100 | NM | 4.9E+06(7.1E+06) | 2.4E+06 (5.0E+05) |
| | | 100 | 0 | NM | < 2.0 * | < 2.0 * |
| | | 100 | 100 | 1.3 (0.3) * | < 2.0 * | < 2.0 * |
| High Organic Content Laboratory Water (HOC-LW) | 100 (control) | 0 | 0 | 1.1E+06 (3.3E+04) | 1.0E+07 (8.2E+06) | 8.9E+06 (1.7E+06) |
| | 100 | 0 | 100 | NM | 1.4E+07 (6.7E+05) | 1.1E+07 (1.4E+06) |
| | | 100 | 0 | NM | 6.0E+06 (2.6E+05) * | 3.4E+06 (8.3E+05) |
| | | 100 | 100 | 6.9E+05 (8.6E+04) * | 6.1E+06(6.4E+05) * | 5.7E+06 (2.1E+06) |

NM = Not measured.
*The differences in the mean values among the treatment groups are greater than would be expected by chance; by Dunnett's Method there is a statistically significant difference ($p$<0.05) when compared to the control.

**Table 9. Average (n=3, ± Standard Error) Most Probable Number (MPN) of *E. faecalis* per 100mL in Dose Effectiveness Experiments Involving Chlorine and Sonic Energy.**

| Water Type | Diversion Ratio (%) | Chlorine Concentration ($\mu$g/L) | Sonic Energy Output (%) | Most Probable Number (MPN) | | |
|---|---|---|---|---|---|---|
| | | | | 0 Hours | 24 Hours | 48 Hours |
| Laboratory Water (LW) | 100 (control) | 0 | 0 | 1.1E+06 (1.6E+05) | DE | 8.1E+04 (8.0E+04) |
| | 100 | 0 | 100 | NM | DE | 4.4E+04 (2.9E+04) |
| | | 100 | 0 | NM | DE | < 2.0 * |
| | | 100 | 100 | 14.8 (11.6) * | DE | < 2.0 * |
| High Organic Content Laboratory Water (HOC-LW) | 100 (control) | 0 | 0 | 5.7E+06 (7.1E+04) | 3.3E+06 (1.2E+06) | 3.6E+06 (1.2E+06) |
| | 100 | 0 | 100 | NM | 4.1E+06 (6.5E+05) | 2.8E+06 (2.8E+05) |
| | | 100 | 0 | NM | 2.0E+06 (1.6E+06) | 8.1E+05 (4.7E+05) * |
| | | 100 | 100 | 2.4E+06 (1.9E+04) | 9.8E+05 (1.2E+05) | 8.2E+04 (4.7E+04) * |

NM = Not measured.
DE = Dilution error.
*The differences in the mean values among the treatment groups are greater than would be expected by chance; by Dunnett's Method there is a statistically significant difference ($p$<0.05) when compared to the control.

For tests involving no treatment with chlorine (i.e. control and sonic energy only), the initial concentration of TRC ranged 6.1–7.7 $\mu$g/L in LW, but was below detection, i.e., < 2.3 $\mu$g/L, in HOC-LW (Table 10). By 48 hours TRC in LW had also decreased though was slightly above detection, ranging 2.4 – 3.8 $\mu$g/L (Table 10). For tests involving treatment with 100 $\mu$g/L of chlorine, TRC initially ranged 82.0 – 90.3 $\mu$g/L in LW, though was below detection in HOC-LW (Table 10). TRC in LW continued to decline over the 48 hour test period, yet was still above detection, i.e., 5.3 – 21.0 $\mu$g/L, at 48 hours (Table 10).

In addition to TRC, sample water was analyzed for its concentration of NPOC at 48 hours. In LW, the concentration of NPOC ranged 0.6 – 0.9 mg/L across all treatments (Table 11). In comparison, the concentration of NPOC in HOC-LW was higher, ranging 7.7 – 8.2 mg/L (Table 11).

**Table 10. Mean (Standard Deviation) Concentration of Total Residual Chlorine (TRC) in Sample Water Used for GSI Dose Effectiveness Tests Involving *E. coli* and *E. faecalis* and Collected at 0, 24 and 48 Hours.**

| Water Type | Diversion Ratio (%) | Chlorine Concentration (µg/L) | Sonic Energy Output (%) | Total Residual Chlorine (µg/L) | | |
|---|---|---|---|---|---|---|
| | | | | 0 Hours | 24 Hours | 48 Hours |
| Laboratory Water (LW) | 100 (control) | 0 | 0 | 6.1 (0.9) | 5.2 (1.1) | 2.4 |
| | 100 | 0 | 100 | 7.7 (1.5) | 4.0 (0.4) | 3.8 |
| | | 100 | 0 | 90.3 (4.3) | 40.4 (3.4) | 21.0 (7.6) |
| | | 100 | 100 | 82.0 (2.7) | 30.4 (3.3) | 5.3 (2.3) |
| High Organic Content Laboratory Water (HOC-LW) | 100 (control) | 0 | 0 | < 2.3 | < 2.3 | < 2.3 |
| | 100 | 0 | 100 | < 2.3 | < 2.3 | < 2.3 |
| | | 100 | 0 | < 2.3 | < 2.3 | < 2.3 |
| | | 100 | 100 | < 2.3 | < 2.3 | < 2.3 |

**Table 11. Mean (Standard Deviation) Concentration of Nonpurgeable Organic Carbon (NPOC) in Sample Water Used for GSI Dose Effectiveness Tests Involving *E. coli* and *E. faecalis* and Collected at 48 Hours.**

| Water Type | Diversion Ratio (%) | Chlorine Concentration (µg/L) | Sonic Energy Output (%) | Nonpurgeable Organic Carbon (NPOC) (mg/L) |
|---|---|---|---|---|
| Laboratory Water (LW) | 100 (control) | 0 | 0 | 0.9 |
| | 100 | 0 | 100 | 0.7 |
| | | 100 | 0 | 0.6 |
| | | 100 | 100 | 0.7 |
| High Organic Content Laboratory Water (HOC-LW) | 100 (control) | 0 | 0 | 7.7 |
| | 100 | 0 | 100 | 8.0 |
| | | 100 | 0 | 8.0 |
| | | 100 | 100 | 8.2 |

## Chemical Fate and Degradation Experiments

Results of GSI chemical fate tests on the BWMS system and its components are presented in Tables 12 and 13 which detail NPOC and TRC concentrations for the range of treatment scenarios analyzed. For tests conducted in LW, concentrations of NPOC were low, ranging 0.2 – 0.6 mg/L in the influent and 0.2 – 0.9 mg/L in the effluent, regardless of treatment (Table 12). As expected, NPOC concentrations in HOC-LW were higher ranging 7.1 – 7.7 mg/L in the influent and 6.8 – 7.6 mg/L in the effluent, regardless of treatment (Table 12).

The higher NPOC concentrations in HOC-LW were found to affect chlorine concentrations. In this water type, concentrations of TRC were below detection, i.e., < 2.3 $\mu$g/L, across all samples regardless of treatment (Table 12). In comparison, concentrations of TRC in LW ranged 96 - 357 $\mu$g/L. The levels of TRC measured immediately after chlorine injection were highest in the lowest diversion ratio tested (Table 13). Though treatment TRC levels were somewhat lower in effluent, they were still higher than controls, ranging 66 – 77 $\mu$g/L (Table 13).

**Table 12. Nonpurgeable Organic Carbon (NPOC) Concentrations Measured in Chemical Degradation Experiments of the Bacoustic BWMS.**

| Water Type | Diversion Ratio (%) | Injected Chlorine Concentration ($\mu$g/L) | Sonic Energy Output | Nonpurgeable Organic Carbon (mg/L) | |
|---|---|---|---|---|---|
| | | | | Influent | Effluent |
| Laboratory Water (LW) | 25 (control) | 0 | 0 | 0.6 | 0.9 |
| | 25 | 100 | 100 | 0.5 | 0.5 |
| | 90 | 100 | 100 | 0.2 | 0.2 |
| | 100 (control) | 0 | 0 | 0.2 | 0.3 |
| | 100 | 100 | 100 | 0.2 | 0.3 |
| High Organic Content Laboratory Water (HOC-LW) | 25 | 100 | 100 | 7.7 | 7.5 |
| | 90 | 100 | 100 | 7.6 | 7.6 |
| | 100 (control) | 0 | 0 | 7.1 | 6.8 |
| | 100 | 100 | 100 | 7.4 | 7.3 |

**Table 13. Total Residual Chlorine (TRC) Concentrations Measured in Chemical Degradation Experiments of the Bacoustic BWMS.**

| Water Type | Diversion Ratio (%) | Injected Chlorine Concentration ($\mu$g/L) | Sonic Energy Output | Total Residual Chlorine Concentration ($\mu$g/L) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Influent | Post-Chlorine | Post-Sonic | Effluent |
| Laboratory Water (LW) | 25 (control) | 0 | 0 | 5.0 | 4.8 | 4.2 | 2.7 |
| | 25 | 100 | 100 | 6.9 | 357 | 278 | 70 |
| | 90 | 100 | 100 | 7.3 | 97 | 85 | 79 |
| | 100 (control) | 0 | 0 | 3.5 | < 2.3 | < 2.3 | < 2.3 |
| | 100 | 100 | 100 | 4.0 | 96 | 65 | 66 |
| High Organic Content Laboratory Water (HOC-LW) | 25 | 100 | 100 | < 2.3 | < 2.3 | < 2.3 | < 2.3 |
| | 90 | 100 | 100 | < 2.3 | < 2.3 | < 2.3 | < 2.3 |
| | 100 (control) | 0 | 0 | < 2.3 | < 2.3 | < 2.3 | < 2.3 |
| | 100 | 100 | 100 | < 2.3 | < 2.3 | < 2.3 | < 2.3 |

For samples analyzed for their degradation of chlorine in LW over five days, there was a slight difference in the rate of degradation between the lower and higher diversion ratios (Figure 2). In the 25 % diversion ratio tests, samples exhibited a slower initial rate of degradation compared to the 100 % diversion ratio tests (Figure 2).

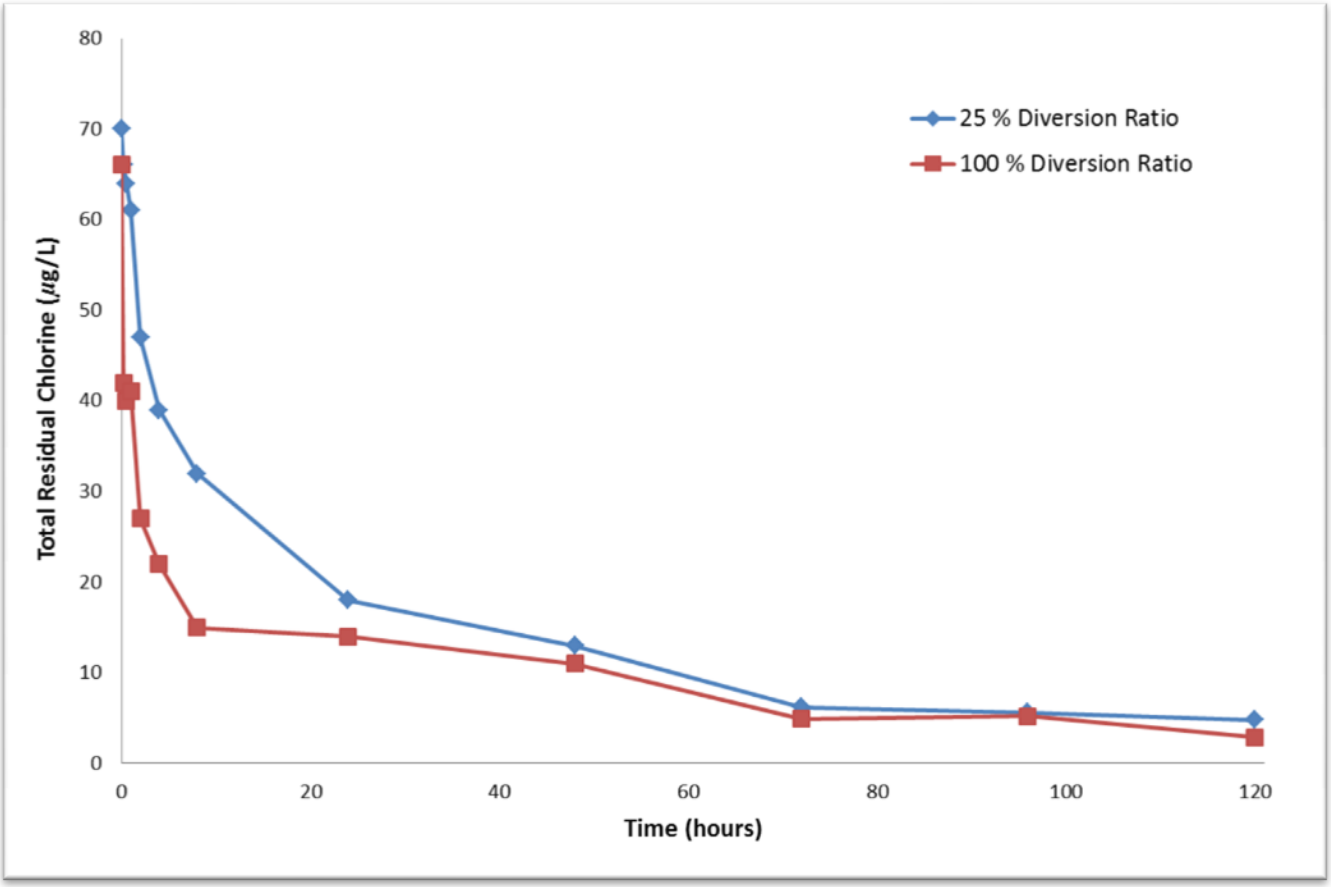


**Figure 2. Degradation of 100 µg/L of Chlorine in 25 % and 100 %Diversion Ratio Tests Conducted in Laboratory Water (LW) Over a 5 Day (264 Hour) Test Period.**

# GSI QUALITY MANAGEMENT

## Standard Operating Procedures (SOPs)

Standard operating procedures (SOPs) were used to implement all test activities. This facilitates consistent conformance to technical and quality system requirements and increases data quality. The SOPs include both programmatic and technical processes and procedures such as organism culturing; sample collection, labeling, analysis and custody; and safety. Table 14 outlines the GSI SOPs utilized for these tests. Any deviations made to SOPs during the experiment were recorded and also approved by the GSI Lead On-Site Investigator for Bench-Scale Studies as soon as practicable, as well as communicated to the GSI Senior QAQC officer.

**.Table 14. GSI Bench-Scale Standard Operating Procedures (SOPs) Utilized for Tests of the Bacoustics Bench-Scale BWMS.**

| SOP Category | Subcategory | SOP Title | SOP Code |
|---|---|---|---|
| General | Administration | Procedure for Record Keeping | GSI/SOP/G/A/RK/1 |
| General | Administration | Procedure for Data Entry, Data Quality Control and Database Management | GSI/SOP/G/RA/DM/1 |
| General | Administration | Procedures for Good Documentation Practices | GSI/SOP/G/A/RK/3 |
| Research Activities | Sample Custody | Procedure for Custody of GSI Samples | GSI/SOP/G/RA/SC/1 |
| Research Activities | Sample Custody | Procedure for Labeling GSI Bench-Scale Samples | GSI/SOP/G/RA/SC/4 |
| Research Activities | Culturing | Procedure for Culturing *Selenastrum capricornutum* | GSI/SOP/BS/RA/CU/4 |
| Research Activities | Chemical Degradation | Bench-Scale Procedure for Examining the Aquatic Degradation of Active Substance(s) in a Ballast Treatment System | GSI/SOP/BS/RA/CD/1 |
| Research Activities | Chemical Degradation | Procedure for Examining the Degradation of Active Substance(s) in a Ballast Treatment System using Large-Volume Influent and Effluent Tanks | GSI/SOP/BS/RA/CD/2 |
| Research Activities | Dose Effectiveness | Procedure for Assessing Dose Effectiveness of a Ballast Treatment System Using Selenastrum capricornutum | GSI/SOP/BS/RA/DE/5 |
| Research Activities | Chemistry | Procedure for Determining Total Residual Oxidants (TRO) in Water | GSI/SOP/BS/RA/C/2 |
| Research Activities | Chemistry | Procedures for Measuring Organic Carbon in Aqueous Samples | GSI/SOP/BS/RA/C/3 |
| Research Activities | Chemistry | Procedure for Analyzing Total Residual Chlorine Concentrations in Water | GSI/SOP/BS/RA/C/6 |
| Research Activities | Microbiology | Procedure for the Detection and Enumeration of Enterococcus using Enterolert™ | GSI/SOP/BS/RA/MA/3 |
| Research Activities | Microbiology | Procedure for the Detection and Enumeration of Total Coliforms and E. coli Using IDEXX's Colilert® | GSI/SOP/BS/RA/MA/4 |

## Quality Assurance/Quality Control (QA/QC)

### *Analytical*

Test conditions were monitored daily for parameters that might affect the outcome of the test (i.e., temperature, and dissolved oxygen). Daily and weekly calibration of test meters ensured optimal performance.

Approximately 10 % of all samples were collected and analyzed in duplicate. The results of the duplicate analysis were consistent with GSI QAQC criteria. The following table (Table 15) provides results of the QC samples for TRC analysis.

**Table 15. Duplicate Agreement and Spike Recovery Agreement for Total Residual Chlorine (TRC) Analysis.**

| Total Residual Chlorine (TRC) Analysis –QC Data | |
|---|---|
| Duplicate Agreement (%) | Spike Recovery (%) |
| Mean = 95.2 ± 4.1% (n=21) | Mean = 101.2 ± 4.7% (n=25) |

*Microbial*

The average initial concentration of bacteria in LW tests yielded MPNs of approximately 9.4E+05 *E. coli* per 100 mL and 1.0E+06 *E. faecalis* per 100 mL of influent water. Similarly, HOC-LW tests yielded MPNs of approximately 7.6E+05 *E. coli* per 100 mL and 5.5E+05 *E. faecalis* per 100 mL in the influent water. All media blanks and sterile water blanks for the Colilert® and Enterolert™ tests were negative.

**Data Audits, Management and Archiving**

Data were recorded on data collection forms or in specific laboratory notebooks. The GSI Senior QAQC Officer performed inspections of datasheet, logbooks, recorded measurements, and instrumentation used during the tests. All hard- and electronic-copies of data and records will be maintained by LSRI and archived for a period of five years.

## CONCLUSION

The dose effectiveness test results show that the sonic energy component of the Bacoustic bench-scale BWMS had no effect on the organisms analyzed in these tests, and did not add to the chlorine-alone effect when the two components were activated in unison. The various treatment combinations of the BWMS tested had little effect on the mortality of the green algae *S. capricornutum*. However, any treatment combination that included chlorine and water with low organic matter (i.e., LW) immediately reduced both bacteria types. In contrast, only forty-eight hours exposure to chlorine in HOC-LW significantly reduced *E. faecalis*.

In the chemical fate experiments, diversion ratio had no influence on the chlorine concentration when recombined downstream. In comparison, concentrations of chlorine in LW were highest immediately after chlorine injection in the lowest diversion ratio tested. As can be expected, the rate of chlorine degradation was influenced by the diversion ratios tested.