

STANDARD OPERATING PROCEDURE Procedure for Determining Total Residual Oxidants (TRO) in Water

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RECORD OF AMENDMENTS:

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2			8		
3			9		
4			10		
5			11		
6			12		

STANDARD OPERATING PROCEDURE

Procedure for Determining Total Residual Oxidants (TRO) in Water

BACKGROUND

The [Great Ships Initiative](#) (GSI) is a collaborative effort to end the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System through independent research and demonstration of environmental technology, financial incentives and consistent basin-wide harbor monitoring. To that end, GSI has established research capabilities at three scales—bench, land-based, and shipboard. Each scale is dedicated to addressing specific evaluation objectives, with protocols as consistent with IMO and federal requirements as practicable. Developers of ballast treatment systems apply for GSI research services [online](#), and awards are offered based on an objective review process. GSI incubation/testing will allow meritorious ballast treatment systems to progress as rapidly as possible to an approval-ready and market-ready condition.

GSI bench-scale tests take place year-round at the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI) in Superior, Wisconsin. 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 goals of GSI bench-scale testing are to explore dose-effectiveness, chemical degradation, residual toxicity, and sensitivity to challenge conditions of a proposed ballast treatment method about which little is known. To that end, the tests are “range-finding” missions, to determine the optimal treatment dose/intensity that would maximize effectiveness and minimize residual toxicity. Findings help treatment developers better design an effective system and/or to move to the next stage of treatment evaluation. The tests are also a form of trouble-shooting to encounter possible problems with the proposed treatment in advance of more extensive and larger scale tests.

INTRODUCTION

This GSI Standard Operating Procedure (SOP) describes the procedure used to determine total residual oxidants (TRO) in water—an important analysis technique for prospective ballast treatments involving ozone. In this procedure, a Hach *N,N*-diethyl-*p*-phenylenediamine (DPD) Total Chlorine Reagent powder pillow (Hach Company, Loveland, CO, USA) is added to the water sample. If TROs are present, the sample will develop a red color which is proportional to the TRO concentration. A calibration curve developed using chlorine standards reacted with the Hach DPD reagent can then be used to determine the concentration of TRO (as chlorine) in the sample.

DEFINITIONS

Brackish Water (BW): Synthetic water created from laboratory water (LW) with the addition of commercially prepared salts, such as Instant Ocean, to obtain a salinity of 16 parts per

thousand (as measured by a refractometer).

High Organic Content Laboratory Water (HOC-LW): Synthetic water created from laboratory water (LW) and used as a surrogate in place of Duluth-Superior Harbor water.

Laboratory Water (LW): City of Superior, Wisconsin municipal water that has been dechlorinated by passage through an activated carbon filter. Note: Based on data from previous testing, background levels of chlorine from below the limit of detection ($\leq 3 \mu\text{g/L}$) to $10 \mu\text{g/L}$ are expected in dechlorinated laboratory water, depending on the source of the water.

Prospective Ballast Treatment System (BTS): A system containing an active substance and/or component that mechanically, physically, chemically, or biologically serves to remove, render harmless, or avoid the uptake or discharge of potentially invasive organisms within ballast water (IMO, 2005).

Salt Water (SW): Synthetic water created from laboratory water (LW) with the addition of commercially prepared salts, such as Instant Ocean, to obtain a salinity of 32 parts per thousand (as measured by a refractometer).

EQUIPMENT LIST

- Spectrophotometer capable of analysis at 515 nm.
- Cuvettes, 1 cm.
- Kimwipes.
- Wash bottle with deionized water.
- Volumetric flasks (10 mL, 100 mL).
- 100-1000 μL pipettor with disposable tips.
- 1000-5000 μL pipettor with disposable tips.
- Beakers for sample collection.
- Hach DPD Total Chlorine Reagent Powder Pillows (Cat. No. 21056-69).
- Ultra Bleach (6.0 % sodium hypochlorite).

PROCEDURE

Sample Collection

1. Collect sample water in beakers or sample bottles from the appropriate location(s) depending on the type of test being conducted.
2. Minimize sample agitation to avoid loss of oxidants due to off-gassing from the sample.
3. Collect a minimum of 10 % of samples in duplicate.

Note: Samples containing more than 300 mg/L alkalinity or 150 mg/L acidity as CaCO_3 may not develop the full amount of color, or it may instantly fade. These interferences can be eliminated

by neutralizing the sample to a pH of 6 to 7 with either 1 N sulfuric acid or 1 N sodium hydroxide.

Sample Analysis

1. React samples with the DPD Total Chlorine Reagent Powder Pillow immediately after collection by placing the contents of the powder pillow into the sample beaker before adding the sample). This is especially true of samples that have substances (i.e. organic compounds and reduced forms of iron and manganese) present with which the oxidants readily react.
2. Prepare the spectrophotometer for analysis of the TRO standards and samples. Turn the spectrophotometer on and allow it to warm up. Set the analysis wavelength to 515 nm. Zero the spectrophotometer with deionized water in the cuvette(s).
3. Prepare a 100 mg/L chlorine standard by diluting 0.175 mL of commercially available "Ultra Bleach" (6.0 % sodium hypochlorite) to volume with deionized water in a 100 mL volumetric flask.
4. Use the 100 mg/L chlorine standard to prepare a series of chlorine working standards as indicated in the table 1. All dilutions are made with deionized water.

Table 1. Chlorine Working Standards.

Standard Concentration (mg/L TRO as Cl ₂)	Volume of 100 mg/L Standard (mL)	Final Volume (mL)
0.0	0	10
0.5	0.050	10
1.0	0.100	10
2.0	0.200	10
3.0	0.300	10

5. Transfer 10 mL of each standard into a 30 mL beaker. Add the contents of one Hach DPD Total Chlorine Reagent Powder Pillow to each solution. Swirl the beaker to help dissolve the reagent. Allow the color to develop for 3 minutes. The absorbance of the solution should be read within 6 minutes of when the DPD powder pillow was added to the liquid.
6. Rinse the sample cuvette with several small portions of the standard to be analyzed. Fill the cuvette at least two-thirds full with the solution to be analyzed. Wipe the outside of the cuvette with a Kimwipe to remove any moisture or finger prints. (Note: cuvettes should only be handled on the upper portion of the cuvette.) Check to see that the cuvette walls are clean and dry before inserting it into the spectrophotometer.

7. Place the cuvette into the spectrophotometer. Read and record the absorbance of the solution. Repeat the procedure for each standard.
8. Collect the samples to be analyzed in pre-cleaned beakers. Immediately transfer 10 mL of the sample, or a smaller aliquot for high concentration samples, into a 30 mL beaker to which the contents of a Hach DPD Total Chlorine Reagent Powder Pillow has been added. If an aliquot of sample less than 10 mL is used, dilute the sample volume to 10 mL with deionized water. Allow the sample to react with the reagent for at least 3 minutes. Read the absorbance of the solution within 6 minutes of adding the sample to the reagent.
9. Repeat steps 6 and 7 for each sample being analyzed.
10. Prepare a calibration curve using the concentrations of the standards as the “x” values and the associated corrected absorbance values as the “y” values. Calculate the corrected absorbance values by subtracting the absorbance of the 0.0 mg/L standard (blank) from each of the other standards and samples. Note: If the water samples are colored or turbid, an aliquot of the water sample that has not been treated with the oxidizing agent should be used to prepare a water specific blank. In this situation, 10 mL of the untreated sample is added to a DPD Total Chlorine Reagent Powder Pillow and mixed. After three minutes the absorbance of the solution is measured. Calculate corrected absorbance values for the colored or turbid water samples by subtracting the absorbance of this water specific blank from the sample(s). The calibration curve can be prepared in Excel or on a calculator equipped with that capability.
11. Use the slope of the line and the y-intercept value determined from the calibration curve to determine the TRO concentrations of the samples. The equation for a straight line is rearranged to allow solving for the TRO concentrations of the samples.

$$y = mx + b$$

where: y = corrected absorbance

m = slope of the line

x = TRO concentration as Cl_2

b = y-intercept of the line

The equation is rearranged to solve for concentration as follows:

$$x = \frac{y - b}{m}$$

If it is desired to report the TRO values as bromine, the TRO value as chlorine is multiplied by a factor of 2.25. This may be more appropriate for saltwater samples.

QUALITY ASSURANCE/QUALITY CONTROL

1. Conduct all quality assurance/quality control procedures according to the GSI/QAPP/1 - Quality Assurance Project Plan (QAPP) for Great Ships Initiative Bench-Scale and Land-

Based Biological Tests (2009). Analyze data to ensure that all applicable data quality criteria are met.

2. Collect and analyze in duplicate at least 10 % of the samples to document sampling and analytical variability. Whenever possible, spike at least 10 % of the samples with a spiking solution containing chlorine. Reference standards are not available for TRO.
3. Follow all procedures outlined in this SOP. Any deviations known ahead of time must be approved by the GSI Lead Investigator for Bench-Scale Studies. Any deviations made during the experiment must be recorded and also approved by the GSI Lead Investigator for Bench-Scale Studies as soon as practicable.
4. Record data on data collection forms or in specific laboratory notebooks. All instrument data output) and data forms must be stored in a project-specific three-ring binder. Ensure hard copies of instrument data output and data collection forms are scanned and stored electronically.

DATA STORAGE AND ARCHIVING

1. Store and archive data according to GSI/QAPP/1 - Quality Assurance Project Plan (QAPP) for Great Ships Initiative Bench-Scale and Land-Based Biological Tests (2009).
2. Archive all hard- and electronic-copies of data and records generated for a period of five years.

REFERENCES AND RELATED DOCUMENTS

Cangelosi AA (2006). RDTE Facility for the Great Ships Initiative (GSI) (OAR-SG-2006-20000364). Project Proposal to the National Oceanic and Atmospheric Administration/U.S. Fish and Wildlife Service. Northeast-Midwest Institute, Washington D.C.

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Eaton AD, Clesceri LS, Rice EW & Greenberg AE (2005). DPD Colorimetric Method. Standard Methods for the Examination of Water and Wastewater: Method 4500-Cl G, 4-67 to 4-68.

Great Ships Initiative Standard Operating Protocols: <http://www.nemw.org/GSI/protocols.htm>.

Great Ships Initiative website: www.greatshipsinitiative.org.

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International Maritime Organization (IMO) (2005). Guidelines for Approval of Ballast Water Management Systems (G8) Adopted by Resolution MEPC.125 (53). London, England.