Great Ships Initiative

Procedure No: GSI/SOP/BS/RA/C/3
Issue Date: May 28, 2009
Number of Pages: 09

STANDARD OPERATING PROCEDURE
Procedures for Measuring Organic Carbon in Aqueous Samples

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RECORD OF AMENDMENTS:

No.	Date	Type	No.	Date	Type
1	06/14/2010	Edited definitions to provide consistency among GSI Chemistry SOPs. Added definition for "particulate organic carbon (POC)". Updated "Equipment List" to reflect new "suspended solids modification" to total organic carbon analyzer and increased pore size of filters. Added information regarding storage and archiving of electronic data to "QA/QC" and "Data Storage and Archiving" sections. Updated references.	7		
2	02/16/2011	Divided "Equipment List" into equipment and supplies. Changed "deionized water" to "Milli-Q water" throughout procedure. Added manufacturer for Micro-90® Cleaning Solution. Added sample preparation ¶4.	8		
3	05/20/2011	Added forceps to list of supplies. Added "Sample Handling Requirements" section. Filters are ashed at 400-450°C for at least one hour before use. Edited ¶2.b., ¶4.a., ¶4.e., ¶5.g., and "QA/QC" ¶3. Added reference for TOC Analyzer.	9		
4	07/11/2011	Added text to ¶2.b., ¶3, and ¶4 of the "Procedure". Added ¶4.a. and ¶5.m. to "Procedure".	10		
5			11		
6			12		

STANDARD OPERATING PROCEDURE
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BACKGROUND

The Great Ships Initiative (GSI) is a regional effort devoted to ending the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System and globally. In support of that goal, the GSI has established superlative freshwater ballast treatment evaluation capabilities at three scales—bench, land-based, and on board ship. Each scale is dedicated to addressing specific evaluation objectives. These include:

GSI Bench-Scale Tests

- Range finding for effective treatment dose against diverse freshwater taxa and water quality conditions;
- Generation of freshwater relevant chemical degradation curves; and
- Estimation of residual toxicity given diverse freshwater taxa and water quality conditions.

GSI Land-Based Tests

- Pre-certification testing, i.e., operational and biological performance (including residual toxicity) status-testing given scale-up and a range of challenge conditions; and
- Certification/verification testing, i.e., formal assessment of performance against international and other discharge standards.

GSI Shipboard Tests

- Confirmation of biological and operational treatment performance as expected in the ship environment;
- U.S. Coast Guard Shipboard Technology Evaluation Program (STEP) testing;
- Shipboard type approval testing;
- Ship discharge monitoring; and
- Methods development.

GSI awards its independent status-testing services to candidate systems only if technical and programmatic criteria are met. Decisions are based on third party technical assessments as well as GSI Advisory Committee programmatic input. Testing services are currently offered at no cost to the developer with the exception of transportation and system installation/removal costs. Instead, tests are supported by general project funds which derive from federal and state agency grants, Great Lakes port contributions, and in-kind contributions by local governments and universities.

GSI has no involvement, intellectual or financial, in the mechanics, design or market success of the actual treatment systems it tests. To ensure GSI remains completely independent and is uncompromised by any real or perceived individual or project bias, GSI subjects itself to rigorous quality management policies and procedures. In addition, GSI test activities are subject to rigorous QAQC procedures and documentation. This attention to quality management and QAQC assures the high quality and credible evaluation of both GSI and its findings.

INTRODUCTION

This GSI Standard Operating Procedure (SOP) describes the method used to measure the organic carbon concentration in aqueous samples collected during testing of a prospective ballast treatment system (BTS). This method applies to bench-scale research and development testing, as well as, land-based testing for performance evaluation/certification testing of BTSs. A variety of organic compounds in various oxidation states make up the organic carbon in water and wastewater. To measure the quantity of organically bound carbon, the organic molecules must be separated into single carbon units and converted to a single molecular form that can be measured quantitatively. The Total Organic Carbon (TOC) Analyzer converts organic carbon to carbon dioxide by combustion. The carbon dioxide is then detected by the non-dispersive infrared gas analyzer (NDIR). The NDIR generates a detection signal which is converted to a peak, whose area is calculated by a data processor.

Inorganic carbon (IC) interference is eliminated by acidifying samples to a pH < 2 which converts IC species to carbon dioxide. Purging the sample with purified air prior to analysis removes the carbon dioxide (generated from IC) by volatilization. The difference between total carbon (TC) and IC is TOC.

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).

Dissolved Organic Carbon (DOC): The fraction of total organic carbon (TOC) present in water that passes through a 0.45 μm pore diameter filter (Eaton *et al*., 2005).

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

Inorganic Carbon (IC): The carbonate (CO_3), bicarbonate (HCO_3), and dissolved carbon dioxide (CO_2) present in water (Eaton *et al*., 2005).

Laboratory Water (LW): City of Superior, Wisconsin municipal water that has been dechlorinated by passage through an activated carbon filter. Sodium sulfite may be added to remove remaining traces of chlorine. Note: Based on data from previous testing, background levels of chlorine from below the limit of detection ($\leq$ 3 μg/L) to 10 μg/L are expected in dechlorinated LW.

Nonpurgeable Organic Carbon (NPOC): The fraction of total organic carbon (TOC) not removed by gas stripping (Eaton *et al*., 2005).

Particulate Organic Carbon (POC): Nondissolved organic carbon, that fraction that would be retained on a 0.45 μm pore diameter filter (Eaton *et al*., 2005). In this method it is defined as the

difference between the NPOC and DOC.

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).

Total Carbon (TC): The combination of inorganic carbon (IC), total organic carbon (TOC), dissolved organic carbon (DOC), and nonpurgeable organic carbon (NPOC) present in water (Eaton *et al*., 2005).

Total Organic Carbon (TOC): All carbon atoms present in water that are covalently bonded in organic molecules (Eaton *et al*., 2005).

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

EQUIPMENT LIST

- Shimadzu Total Organic Carbon Analyzer (Model TOC-5050A) using the suspended solids modification. This modification uses tubing with an inside diameter of 0.8 mm to deliver the sample to the reaction tube.
- Muffle Furnace
- Drying Oven

SUPPLIES

- Aluminum foil
- Anhydrous Potassium Hydrogen Phthalate or 1000 mg/L TOC Standard
- Carrier and purge gas (high purity air, hydrocarbon free)
- Concentrated hydrochloric acid
- Desiccator
- Disposable glass pipettes
- Filtering apparatus (either glass of stainless steel)
- Forceps for handling filters
- Glass sample bottles
- Magnetic stirrer/TFE-coated stirring bars
- Micro-90® Concentrated Cleaning Solution (International Products Corporation; Burlington, NJ)
- Micropipetter and tips
- Milli-Q water (from Millipore Direct 8 system)
- Personal protective equipment (i.e., protective eyewear, laboratory coat, anti-heat gloves, etc.)
- Sample vials/tubes

- Volumetric flasks (100 and 200 mL)
- Volumetric pipets (1, 2, 5 and 10 mL) and pipet bulb
- Whatman GF/F glass fiber filters (effective pore size is 0.7 μm). Filters are ashed at 400-450 °C for at least one hour before use.

SAMPLE HANDLING REQUIREMENTS

1. Collect samples in glass or Teflon containers that have been soaked in Micro cleaning solution, rinsed thoroughly with tap water to remove the cleaning solution and finally rinsed with Milli-Q water.

2. Acidify non-purgable organic carbon (NPOC) samples to a pH of < 2 with concentrated hydrochloric acid (0.2 % HCl). Dissolved organic carbon samples (DOC) are first filtered through a Whatman GF/F filter and then acidified with HCl.

3. Acidified samples may be held in a refrigerator for a maximum of 28 days before analysis. The recommended hold time is ≤ 7 days.

PROCEDURE

Note: All glassware to be used should be soaked for a minimum of 15 minutes in prepared Micro cleaning solution (i.e., 40 mL Micro-90® Concentrated Cleaning Solution: 2 L Milli-Q water), rinsed with hot tap water until no cleaning solution residue remains and rinsed thoroughly with Milli-Q water before use. The GF/F filters used for filtering the sample for the DOC analysis are ashed at 400-450 °C for at least one hour before use. The filters are placed into an aluminum foil envelope and then placed into the muffle furnace and heated to remove organic contaminants. The envelope is closed after removing the envelope from the muffle furnace.

As described in the "Introduction", NPOC values are determined on non-filtered samples. If a DOC concentration is desired, the sample should first be filtered through a Whatman GF/F filter. A filter blank should be analyzed to indicate whether the filtering process is adding organic carbon to the sample. The filter blank is a Milli-Q water sample filtered through the same filter type as used for the sample. The difference between the NPOC and DOC values is reported as POC. Samples must be acidified to 0.2 % with hydrochloric acid before being analyzed.

1. Conduct procedure in a vented work area, taking appropriate health and safety measures.

2. Prepare TOC Stock Solution and Standards:
    a. An organic carbon stock solution can be purchased or prepared in the laboratory. The concentration of the stock solution should be 1000 mg/L TOC. When not in use the stock solution should be refrigerated. Be sure to check the expiration date before using the stock solution.
    b. A total organic carbon stock solution can be prepared by dissolving 0.2125 g of oven-dried (110 °C for 1-2 hours) anhydrous potassium hydrogen phthalate (KHP) in Milli-Q water. The chemical formula for KHP is $C_8H_5KO_4$. Add 200 μL

concentrated hydrochloric acid to a 100 mL volumetric flask to preserve the stock and dilute to volume with Milli-Q water. Be sure the KHP is dissolved completely before moving on to the next step. This organic carbon stock is 1000 mg/L TOC. A new 1000 mg/L stock should be prepared every 6 months. Refrigerate when not in use. Record the preparation of the total organic carbon stock solution in a laboratory notebook or on a datasheet.

3. Prepare a series of working standards (see Table 1) by pipetting the appropriate amount of the 1000 mg/L organic carbon stock into 200 mL volumetric flasks, adding 400 μL concentrated hydrochloric acid and diluting to 200 mL with Milli-Q water. Working standards must be remade every 3 months or when evidence of a change in concentration occurs. Refrigerate standards when not in use. Record the preparation of the working standards in a laboratory notebook or on a datasheet.

Table 1. Working Standards.

TOC Conc. (mg/L)	Volume Pipetted (mL)	Volume Conc. Acid (μL)	Final Volume (mL)	Final Conc. (mg/L)
1000	10.0	400	200	50
1000	5.0	400	200	25
1000	2.0	400	200	10
1000	1.0	400	200	5.0
1000	0.5	400	200	2.5

4. Prepare Samples:
   a. Shake the sample bottle containing the organic carbon sample thoroughly and then pour a small volume (10-20 mL) of the sample into a small Micro cleaned bottle or test tube. This is the NPOC sample.
   b. Place a GF/F filter rough side up in the Micro-cleaned filtering apparatus. Position apparatus onto a Micro cleaned filtering flask, attach the vacuum hose and turn on the pump. The vacuum pump should be adjusted to 7-9 inches of mercury vacuum. Rinse with ~50 mL of Milli-Q water. Tap the side of the apparatus a few times to make sure water is not trapped anywhere.
   c. Disconnect the vacuum hose, shut off the pump, remove the filter apparatus, and empty the flask. Rinse the flask with Milli-Q water.
   d. Reposition filter apparatus onto flask and reattach the vacuum hose. Turn pump back on and pour a small amount of sample (30-50 mL) into filter apparatus taking care not to let it pour down the side of the sample bottle. Tap the side of the apparatus a few times to make sure the sample isn't trapped anywhere.
   e. Disconnect the vacuum hose, shut off the pump, remove the filter apparatus, and empty the flask.
   f. Reposition filter apparatus onto flask and reattach the vacuum hose. Turn pump back on and filter the remaining sample, taking care not to let any sample pour down the side of the sample bottle.

g. Disconnect the vacuum hose, shut off the pump, remove the filter apparatus, and pour a small amount out of the flask into the drain to rinse the neck of the flask. This filtered portion is for the DOC analysis.
h. Disassemble the filtering apparatus, remove the used filter, reassemble and rinse three times with Milli-Q water. Rinse the flask three times with Milli-Q water also.
i. Both the NPOC and DOC samples need to be acidified to ~ 0.2 % before analyzing on the TOC Analyzer.
j. Repeat steps a-i above for each sample.

5. Analyze Standards and Samples using the Shimadzu Total Organic Carbon Analyzer:
   a. Open the main valve on the air cylinder. Adjust the valve on the regulator to provide a pressure of 85 psi. The Carrier Gas flow rate should be 150 mL/min. and the Sparge Gas flow should be 100 mL/min. The Carrier Gas flow should start as soon as the instrument is turned on. Note: The Sparge Gas flow can only be checked when a sample is being sparged.
   b. Turn the TOC analyzer on using the switch on the left hand side of the instrument.
   c. Use the **F1** key to get to the **Main Menu**. Press **3 Enter** to get to **General Conditions**, then use the **Down Arrow** key to get to the **Furnace On/Off** position. Press **1 Enter**. Return to the **Main Menu** by pressing **F2**.
   d. Go to **Monitor** by pressing **6 Enter** to monitor the progress of the warm-up. When each of the conditions on the right of the screen reads OK, the instrument is ready to begin analysis. This will take approximately 30 minutes.
   e. Prepare a blank by acidifying a volume of Milli-Q water to 0.2 % with concentrated hydrochloric acid.
   f. Return to the **Main Menu** when the instrument warm-up is complete by pressing the **F2** key and then **Sample Measurement**. Do not alter anything on the screen that follows.
   g. Fill a sample tube about two-thirds full with blank solution. Place the instrument's sampling tube into the blank solution and press **F1** to move on to the next screen. The **Cycle Mode** and **Non-stop Mode** should read **2** (off). Press **Start** to analyze the blank. This procedure cleans the instrument. After the sample has run, repeat the procedure using the same sample. If the area of the peak is greater than 3000, consult the GSI Chemist or Laboratory Manager.
   h. Develop a calibration curve or use one that has been stored in the instrument. If a stored curve is used, a blank and at least one standard should be checked before any samples are analyzed. The check standard (e.g., 10.0 mg/L) result should fall within 10 % of the actual concentration. If not, re-run the standard. If this second analysis also fails, the standard should be remade and reanalyzed. If the newly prepared standard is found to have a reported value within 10 % of the actual concentration, analysis of samples can begin. If not, a new set of standards should be prepared and a new calibration curve generated before proceeding to analysis of samples
   i. Prepare a new calibration curve by returning to the **Main Menu** by pressing the **F2** key and entering **1** for **Calibration**. Enter the exact concentration of the

standards prepared in place of the 0.0, 2.5, 5.0 and 10.0 mg/L. Table 2 provides an example of the data to be entered.

Table 2. Data for Calibration Curve.

Type	1st Cal. Curve	2nd Cal. Curve
1st Std. Conc.	0.0 mg/L	0.0 mg/L
2nd Std. Conc.	2.5 mg/L	10.0 mg/L
3rd Std. Conc.	5.0 mg/L	25.0 mg/L
4th Std. Conc.	10.0 mg/L	50.0 mg/L
Range	X 1	X 5
Inj. Volume	53 μL	26 μL
No. Injects	3	2
Max. No. Injects	3	2
Sparge Time	3 min	3 min

Note: If you make changes to the conditions, you have to press **Enter** for the instrument to accept the changes.

j. Press **F1** for **Next** after entering the calibration curve conditions. The instrument will prompt you to **Set 1st Std Vessel, Insert Sampling Tube**. Put the sampling tube into the blank sample vial and press **Start**. The instrument will analyze that standard and prompt you to analyze the subsequent standards. When all the standards in the 1st curve have been analyzed; develop a 2nd calibration curve (suggested concentrations 0.0, 10.0, 25.0 and 50.0 mg/L). The screen will automatically adjust the range and inject volume for the TOC concentrations. Do not change the range value and the inject volume value if they are different from those shown here. Go through the analysis for the 2nd standard curve.
k. Return to the **Main Menu** and go to **Sample Measurement** by pressing **2 Enter**. Enter the # of the 0-10 mg/L calibration curve to be used as the 1st calibration curve under the **NPOC** column. Press **Enter**. Enter the # of the 0-50 mg/L calibration curve to be used as the 2nd calibration curve under the NPOC column. Press **Enter**. This allows the sample to be analyzed on the 0-10 mg/L curve initially. If the concentration is higher than 10 mg/L, it will be rerun on the higher standard curve. Press **F1** to move on to the next screen.
l. The **Cycle Mode** and **Non-stop Mode** should read **2** (off). Place the sampling tube in the sample container and press **Start**. After the sample has been analyzed, press **F1** (**Next**). The sample data will be printed as a hardcopy. Write the identity of the sample next to the print-out that it corresponds to. Continue the sample analysis until concentrations have been determined for all samples.
m. Analyze a standard after every 10 samples and after analysis of the final sample.
n. To end, go to the **Main Menu**, enter **General Conditions** and turn the furnace off by entering **2** next to the **Furnace On/Off** line. Return to the **Main Menu**. Press **7 Enter** to enter the **Standby** options screen. Enter **1** (**Next**) to **Finish/Running**.

Press **F1** for **Standby**. The screen will inform you of how much time remains before the instrument can be shut off.

o. Turn the power switch for the instrument off when the instrument display indicates it can be shut off. **Do not shut the instrument off prematurely**. This wait is necessary to allow the instrument to cool down.
p. Close the main valve on the air tank. Turn the secondary valve on the air tank counterclockwise until it is loose.

QUALITY ASSURANCE/QUALITY CONTROL

1. Conduct all QAQC procedures according to *GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests (2011) or GSI/QAQC/QAPP/BS/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Bench-Scale Tests (2010).*

2. Analyze data to ensure that all applicable data quality criteria are met.

3. 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 TOC spiking solution. A certified reference standard will be analyzed at least once each day samples are analyzed to document performance of analytical instrumentation.

4. Follow all procedures outlined in this SOP. Any SOP amendments known ahead of time must be approved by the GSI Lead On-Site Investigator (for Land-Based or Bench-Scale Studies) and communicated to the GSI Senior QAQC Officer. Any deviations made during the experiment must be recorded, communicated to the GSI Senior QAQC Officer and also approved by the GSI Lead On-Site Investigator as soon as practicable.

5. Record data on data collection forms or in specific laboratory notebooks. All instrument data output (e.g., chromatograms, absorbance scans, and/or measurements) and data forms must be stored in a project-specific three-ring binder. Ensure hard copies of all raw data (e.g., instrument data output and data collection forms) collected during treatment technology performance evaluation/certification testing are scanned and stored electronically on the LSRI secured Local Area Network (LAN). The requirement for a backup, electronic copy of raw data is only implemented during treatment technology certification testing at the GSI Land-Based RDTE Facility.

DATA STORAGE AND ARCHIVING

1. Store and archive data according to *GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests (2011) or GSI/QAQC/QAPP/BS/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Bench-Scale Tests (2010).*

2. Store and archive electronic data (e.g., scanned copies of original, raw data and/or data

entered for analysis) by posting the data distribution files to the LSRI LAN in an organized hierarchical folder system such that the entire LSRI-GSI staff are able to recognize and access the data. Distribute data files via email, when needed, to the applicable GSI team members who do not have access to the LSRI LAN.

3. Archive all hard- and electronic-copies of data and records generated for a period of at least seven years, and ensure that electronic records are retrievable for the length of the required storage time.

REFERENCES AND RELATED DOCUMENTS

Eaton, AD, Clesceri, LS, Rice, EW, and AE Greenberg, Eds. (2005). Standard Methods for the Examination of Water and Wastewater, 21st Edition. American Public Health Association, Washington, DC.

Instruction Manual Total Organic Carbon Analyzer Model TOC-5050A, Shimadzu Corporation, Environmental Instrumental Division.

GSI/QAQC/QMP/1 – Great Ships Initiative Quality Management Plan (2010).

GSI/QAQC/QAPP/BS/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Bench-Scale Tests (2010).

GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests, Revision 2 (2011).

GSI/SOP/G/RA/SC/3- Procedure for Labeling Samples collected at the GSI Land-Based RDTE Facility.

GSI/SOP/G/RA/SC/4 – Procedure for Labeling GSI Bench-Scale Samples.

Great Ships Initiative website: www.greatshipsinitiative.org; Standard Operating Protocols/Procedures: http://www.nemw.org/GSI/protocols.htm.

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