

1. Procedure summary

Procedure for using the Sievers Innovox TOC Laboratory Analyzer to obtain the total organic carbon from algae and evaporation pond samples in parts per million (ppm) using the TOC or NPOC protocols.

Related procedures

NA

Procedure impacts and concerns

Safety	Safety glasses, gloves and a lab coat should be worn at all times while performing this procedure. Please read all applicable MSDS forms prior to performing this procedure. Make sure TOC waste container contains sodium bicarbonate and activated charcoal in order to neutralize TOC waste and avoid buildup of toxic fumes. Dispose of used reagents in approved disposal container labeled TOC waste (55 gallon drum behind Culture Room).
Quality	All samples, algae and standards, are run in 40 mL vials and must be within 6.0% and 10.0% RSD, respectively. Factors that may result in high % RSD include: excessive flocculation, instrument maintenance and calibration and errors in sample preparation. All data generated from TOC analysis of raceway samples must fall within 6.0% RSD. NPOC data should fall within 15% RSD. Standards run at the same time as pond samples must be within 10.0% RSD and their calculated values must be within $\pm 10.0\%$ of their expected values. Data are not reportable if values from analysis exceed the above QC guidelines.
Delivery	TOC data is a measure of the organic carbon present in a sample. Data are quality controlled and reported as soon as data analysis is complete. Typically sample analysis turnaround is 1 -3 days.
Environmental	Local policies and procedures should be followed as determined by the site leadership.
Cost	GE Analytical 40 ml TOC vials @ \$391.00 for case of 72 vials.
Compliance	Compliance with OSHA's Hazardous Waste Operations and Response, and Hazardous Communication Standard in addition to the Sapphire Energy, Inc. Chemical Hygiene Plan is required (see 29 CFR 1910.120 and 1200).

Responsibilities and owners

Document Owner	Manage content and distribution	Ronald Treminio
Process Owner	Responsible for content and process validation	Tonia Lane
Plant Manager	Responsible for implementation and conformance	Becky Ryan (LCTS)

2. Process

2.1 Process description

Total organic carbon (TOC) refers to the total amount of carbon bound in an organic compound. TOC analysis using the Innovox analyzer requires measurement of both the total carbon and inorganic carbon present in a sample. Inorganic carbon represents the amount of dissolved carbon dioxide and carbonic acid salts. Subtracting the inorganic carbon from the total carbon yields TOC. In NPOC mode, the Innovox analyzer will acidify and gas-off the inorganic carbon as CO₂. The remaining carbon is then converted to CO₂ via a heated persulfate reaction, measured by NDIR and reported as nonpurgeable organic carbon (NPOC).

2.2 Process diagram: Work Instruction



2.3 Equipment and Supplies

- Sievers Innovox TOC Laboratory Analyzer
- 40 ml I-Chem glass vials (Thermo Scientific #05-719-106)
- Tissue Homogenizer
- Squirt bottle with Nanopure (18.2 mΩ Millipore water) water
- 100 ppm TOC standard (GE instruments #CSTD 68310-01)
- 21 position glass vial rack
- Centrifuge
- 50 ml conical tubes (Fisher Scientific #14-432-22)
- Conical tube tray
- Persulfate (GE instruments #CAPK-68050-01)
- 6M Phosphoric acid (Sigma Aldrich #695017-2.5L)
- 2 L Nanopure water (18.2 mega-ohm ionic purity)
- Hazardous waste bottles
- Stir Bars
- Sodium Carbonate for 100 ppm IC standard¹(Sigma Aldrich #S7795-500G)

Note 1:

Inorganic carbon standards are not sold by GE. Thus, IC standards are made with Na₂CO₃. A 100 ppm C solution is made by first drying overnight some Na₂CO₄ then dissolving 0.8825 g Na₂CO₄ in 1 liter of nanopure water. The 100 ppm C should be remade after 2 months.

2.4 Process Steps

2.4.1 Preparation of samples algae

1. Label 50 mL conical tubes with appropriate pond ID, date, and sample submission form number. Aliquot 45 mL of sample into conical tube. Repeat as necessary to acquire all samples.
2. Once all samples have been collected place the conical tube into a centrifuge. Centrifuge at 4500 rpm's for 15 minutes.
3. After centrifugation, discard the supernatant trying very carefully to not disturb the pelleted algae.
4. Add enough nanopure water to each pellet to fill the conical tube up to the 45 mL mark. Break up pellet by vortexing for 20 seconds.
5. After vortexing, use the tissue homogenizer to homogenize algae sample. Homogenize each sample for 30 seconds. Samples are ready to be transferred to glass TOC vials.

Note 2. PDU

sump samples should also be prepared in the same manner as evaporation pond samples.

6. Label each glass vial with appropriate ID for each sample (see Step 1). Place 40 mL of each sample into individually labeled glass vials. Then, place one 1-mm stir bar into each glass vial. Close glass vial and place in rack for analysis.

2.4.2 Preparation of evaporation pond samples²

1. Take 100 mL of evaporation pond sample and place in 250 mL beaker with a magnetic stir bar.
2. Place beaker on stir plate and turn dial on stir plate to a moderate speed. Make sure to do within a fume hood.
3. Check the pH of the solution with a wand pH meter.
4. Again make sure to do the following steps within a fume hood. Use hydrochloric or phosphoric acid to drop the pH of the evaporation pond sample below 2.00. At this point, there may be a large amount of effervescence and off-gassing that occurs. Add the acid very slowly and in small volumes (200 uL/liter at a time).
5. After the pH has been dropped and the effervescence has subsided make a 1 to 50 dilution of the pH dropped evaporation sample in a 40mL I-Chem vial. Make sure to add a 1 mm stir bar to the I-Chem vial prior to capping.
6. Label the vial with the appropriate information related to the evaporation sample and place on rack for analysis.

2.4.3 TOC analyzer

1. Open Microsoft internet explorer and type in the appropriate IP address for the TOC analyzer (serial # 0248 <http://192.168.0.100>).
2. Click the enter button on the bottom right of the screen (see Figure 2).
3. Once in the sample input screen (Figure 3.), place the identification of each sample vial into the space provided making sure that each sample vial is in the correct rack position. If more positions are needed, click on the "edit vials" tabs.
4. The first sample rack to be analyzed should contain a rinse vial in the first position and in the last position. All other sample racks should contain a Rinse vial in the last position. Additionally, a rinse vial should be placed before and after a NPOC analysis vial to ensure there are no carry over effects³ on the next algae or evaporation pond sample.
5. Each sample rack should also contain a 100 PPM TOC and a 100 PPM IC standard in the third and second to last position, respectively. This is used as a check to make sure that the calibration of the TOC analyzer is still valid.
6. Also check that the protocol settings are the same for each of the sample vial in the sample rack and that the protocol settings are correct for the samples being analyzed. For example, algae samples should be run under the TOC protocol and evaporation pond samples should be run under the NPOC protocol. See Table 1 below for protocol settings for each analysis type. Please note that both protocols may be used within the same run.
7. Click the "Save as" tab once the ID's for each sample (algal samples, rinses and standards) has been input. Save the name as the date (year, month, day) and the sample submission form number.
8. Now click **RUN** and make sure to click yes when the dialog box asks to start at position 1.

Note 3. Carry over effects are more prominent in NPOC mode due to the high levels of inorganic carbon in sample. However, only evaporation pond and PDU sump samples are to be analyzed in NPOC mode. Thus, it may be necessary to drop the pH of NPOC samples below 2. See 2.4.2 for more details. Also note that there are no repeat criteria when running NPOC mode thus only three measurements are taken and then averaged regardless whether one of the measurements is an outlier.

TOC protocol values													
Sample Type	Range	Measure	Acid	Oxidizer	Conversion factor	Repeat Criteria	Max repeats	Minimum repeats	Repeat Criteria % RSD	Flush	Blank Correction	Manual Dilution	Calibration
Sample	5000	TOC	1	15	None	ON	4	2	3	Dilution/ Sample 1x	OFF	1	Most recent calibration
NPOC protocol values													
Sample Type	Range	Measure	Acid	Oxidizer	Spurge Minutes	Conversion Factor	Repeat Criteria	No. of Repeats	No. of Rejects	Flush	Blank Correction	Manual Dilution	Calibration
Sample	5000	NPOC	2	15	1.5	None	OFF	3	0	Dilution/ Sample 1x	OFF	1	Most recent calibration

Table 1. Protocol values for running TOC or NPOC analysis on the Innovox Laboratory TOC analyzer.

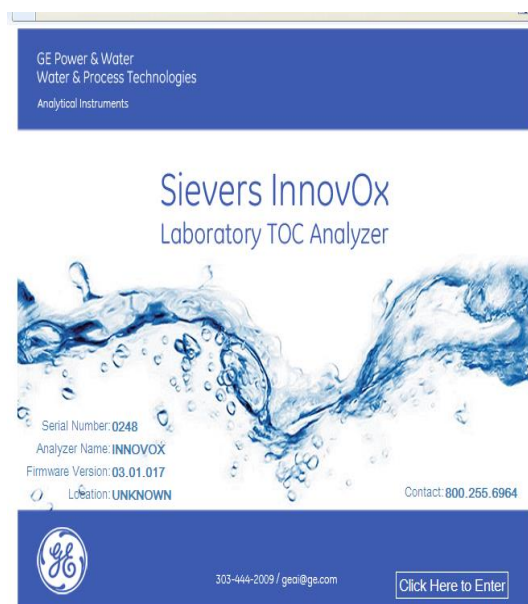
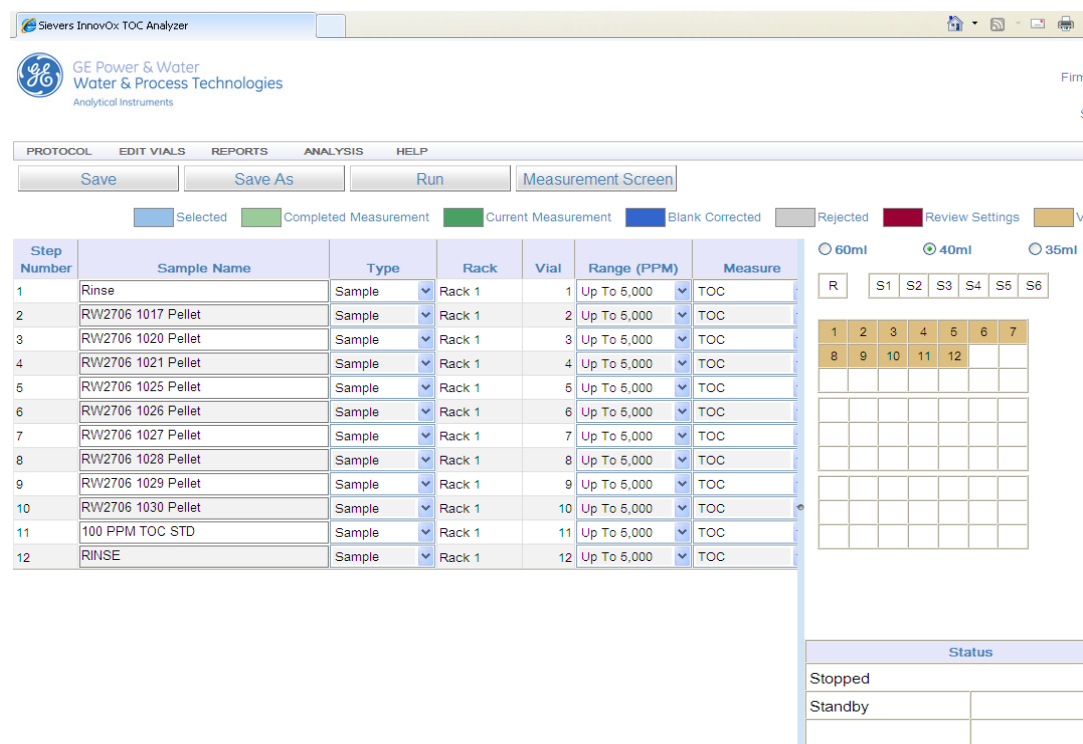


Figure 1. Start Screen for TOC analyzer.



Step Number	Sample Name	Type	Rack	Vial	Range (PPM)	Measure
1	Rinse	Sample	Rack 1	1	Up To 5,000	TOC
2	RW2706 1017 Pellet	Sample	Rack 1	2	Up To 5,000	TOC
3	RW2706 1020 Pellet	Sample	Rack 1	3	Up To 5,000	TOC
4	RW2706 1021 Pellet	Sample	Rack 1	4	Up To 5,000	TOC
5	RW2706 1025 Pellet	Sample	Rack 1	5	Up To 5,000	TOC
6	RW2706 1026 Pellet	Sample	Rack 1	6	Up To 5,000	TOC
7	RW2706 1027 Pellet	Sample	Rack 1	7	Up To 5,000	TOC
8	RW2706 1028 Pellet	Sample	Rack 1	8	Up To 5,000	TOC
9	RW2706 1029 Pellet	Sample	Rack 1	9	Up To 5,000	TOC
10	RW2706 1030 Pellet	Sample	Rack 1	10	Up To 5,000	TOC
11	100 PPM TOC STD	Sample	Rack 1	11	Up To 5,000	TOC
12	RINSE	Sample	Rack 1	12	Up To 5,000	TOC

Figure 3. Sample input screen.

2.4.4 Preparation of Reagents & Solutions

1. Persulfate (2ppt)

Persulfate	1 bag (~600 grams)
Nanopure H ₂ O	2 L

- Dissolve entire content of bag into 2 L container labeled "Persulfate" with 2 L of nanopure water.
- Mix until reagent is completely dissolved.
- Can be stored at room temperature for 1 month.

2. 6M H₃PO₄(Phosphoric acid)

- Making 6M Phosphoric acid should be done in the fume hood.
- Use 2000 mL graduated cylinder; add 1200 ml of nano pure water to the container labeled "ACID".
- Using a 1000 mL graduated cylinder, slowly add 800 ml of 85% Phosphoric acid (by weight in water) to the container labeled "ACID" containing the water from step 2.
- Stir until completely mixed.

2.4.5 Waste Disposal

- Add 200 g sodium bicarbonate and 40 g activated charcoal to the 3L TOC waste container prior to use.
- Dispose of TOC liquid waste in approved disposal container⁴.
- Currently, TOC liquid waste is disposed in the 55 gallon drum behind Culture Room labeled TOC

Note 4. The pH of TOC liquid waste container should be between 6 & 7.

waste.

3. Required documents

Input documents

1. Sample submission form.

Output documents

1. Excel or OSI-PI data spreadsheet

4. Document control

Revision history

R0 – Initial Release – Ron Treminio	10/15/13
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Document approval

Nicole Heaps (SD)	10/8/13
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Document reviewers

Tonia Lane	8/22/13
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5. Risk analysis