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National Renewable Energy Laboratory

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National Renewable Energy Laboratory
15013 Denver West Parkway
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1. Introduction

1.1 This laboratory analytical procedure (LAP) describes the quantitative determination of total organic carbon (TOC) and total inorganic carbon (TIC) in whole suspended biological samples and in the cell-free supernatant by combustion and carbon dioxide (CO_2) detection with a non-dispersive infrared detector. This allows for distinct reporting of soluble and insoluble organic and inorganic carbon. In addition, this procedure covers the quantitative determination of soluble nitrogen in the cell-free supernatant.

1.2 Total carbon (TC) content can be divided into two major categories: inorganic carbon and organic carbon. Inorganic carbon is primarily composed of carbonate, bicarbonate, and dissolved CO_2. Organic carbon includes all carbon atoms covalently bound in organic molecules and can further be divided into the following: dissolved organic carbon (DOC), the fraction of organic carbon that passes through a 0.45-µm filter; purgeable organic carbon (POC) or volatile organic carbon (VOC), the fraction of TOC removed from an aqueous solution by purging with VOC free gas under specified conditions; and non-purgeable organic carbon (NPOC) or non-volatile organic carbon (NVOC), the fraction of TOC not removed by purging with VOC free gas [1].

1.3 TOC can be measured by analyzing a sample for TC and TIC and then subtracting the IC to obtain a calculated value for TOC. This method is applicable when TOC concentrations are a significant fraction of the TC concentration; otherwise, calculated TOC results may be negative or inaccurate due to error propagation [1,2]. Alternatively, TOC can be measured as NPOC by acidifying the sample and purging with VOC free gas to remove any volatile carbon before analyzing. NPOC results are often equivalent to TOC due to relatively low amounts of POC. The method described in this LAP focuses on the calculated value of TOC using the subtraction of IC from TC.

1.4 Total nitrogen (TN) is the measurement of all dissolved nitrogen in a liquid sample, which is converted through a series of reactions to nitrogen monoxide and ultimately nitrous oxide. The detector signal from this is used to determine the TN concentration of the sample [9,11].

1.5 Biological cultures (e.g., algae cultures) provide a unique challenge for measuring carbon because samples may remain biologically active after sampling, potentially affecting carbon allocation. Preliminary data suggest that heat-treating samples immediately following sampling halts biological activity and stabilizes carbon (both TOC and TIC) results.

1.6 Understanding the partitioning of TC, TIC, and TOC in biological cultures is complex and requires the measurement of each in both the whole sample (cells and media) and the supernatant (media without cells). To calculate TOC for the cells alone, the TOC result from the supernatant is subtracted from the TOC result of the whole sample.

2. Scope

2.1 This procedure is intended to quantify TC, TOC, and TIC in biological culture samples (e.g., algae cultures), fermentation samples, and chemically treated samples such as hydrolysate after acid treatment.

2.2 A portion of this procedure is intended to quantify TN in liquid samples that are free of solids and cells.

2.3 Portions of this procedure are specific to a Shimadzu TOC-LCPH/TNM-L analyzer with corresponding software (TOC-Control L) using a combustion method and suspended solids kit and may vary depending on laboratory-specific setup.

2.4 This procedure is **not** intended for the analysis of solid or sludge samples, only liquid samples with suspended solid particles less than 0.8 mm in diameter (TC/TIC/TOC analysis only).

3. Terminology

3.1 *Total Carbon (TC):* The sum of organic and inorganic carbon including elemental carbon.

3.2 *Total Inorganic Carbon (TIC):* All carbon not covalently bound in organic molecules. Predominately composed of carbonate, bicarbonate, and dissolved carbon dioxide [1].

3.3 *Total Organic Carbon (TOC):* The sum of carbon atoms covalently bonded in organic molecules [1].

3.4 *Dissolved Organic Carbon (DOC):* Organic carbon remaining in the sample after it has been filtered through a 0.45-µm filter [1,3].

3.5 *Volatile Organic Carbon (VOC) or Purgeable Organic Carbon (POC):* The sum of organic carbon that is converted to CO_2 and removed from solution by purging with VOC free gas (e.g., zero air) under specified conditions [1].

3.6 *Non-Volatile Organic Carbon (NVOC) or Non-Purgeable Organic Carbon (NPOC):* The sum of carbon that is not converted to CO_2 and removed from solution when purged with VOC free gas [1,4].

3.7 *Total Nitrogen (TN)*: The sum of organic and inorganic nitrogen in water [9].

3.8 *Calibration Standard*: A set of standards, each at a known concentration, used to determine a detector response. The calibration standards must include the analytes of interest and be run in series with a sample set. The detector response can then be used to predict the concentration of an analyte in a sample.

3.9 *Calibration Verification Standard (CVS)*: A standard made from the same parent standard as the calibration for each analyte (TIC, TOC, or TN) and analyzed every 10 samples to monitor instrument functionality during the run.

3.10 *Low-Level Calibration Verification Standard (LLCVS):* A standard made from the same parent standard as the calibration for each analyte (TIC, TOC, or TN) at the lowest calibration point to measure long-term variability at the low end of the curve.

3.11 *Second Source Standard*: A standard, independent of the calibration source, that is analyzed following the calibration to validate the primary source standards.

4. Significance and Use

4.1 The procedure described here is used to determine the appropriate preservation, storage guidelines, and hold times for quantification of TC, TOC, TIC, and TN in biologically active and inactive samples.

4.2 In this procedure, we describe the quantification of TC, TIC, TOC, and TN in biologically active and inactive samples using a specific instrument setup as is described herein.

4.3 This procedure may be used in conjunction with other methods to characterize and track the biochemical composition of the cell pellets. Refer to the following NREL laboratory analytical procedures: *Determination of Total Solids and Ash in Algal Biomass* [5], *Determination of Total Carbohydrates in Algal Biomass* [6], and *Determination of Total Lipids as Fatty Acid Methyl Esters (FAME) by* in situ *Transesterification* [7].

5. Interferences

5.1 High concentrations of TIC relative to TOC may result in increased error in the calculated TOC measurement when the TOC result is determined by subtracting TIC from TC. Reporting results with a calculated error is recommended when using this analysis method. Refer to Section 11.6 for the related equation [2].

5.2 High concentrations of TIC may result in incomplete purging due to the buffering capacity of bicarbonate and carbonate ions in solution, possibly leading to erroneously high TOC results. High concentrations of salts associated with high alkalinity may also damage the combustion tube after prolonged use due to the accumulation of deposits on the combustion catalyst [3]. Care should be taken to minimize the amount of IC introduced to the combustion tube. A rigorous preventative maintenance schedule will help preserve the instrument and component lifetime [1].

5.3 During TN analysis, the detector response may vary based on the nitrogen species present, resulting in unexpectedly low or high TN results. Most major forms of nitrogen, including nitrates, nitrites, and ammonia, have been shown to have high rates of detection compared to other nitrogenous compounds such as hydrazines, pyrazolones, and azide compounds [9]. Calibration standards should be prepared to best represent the nitrogen species (or combination of species) present in samples, and it is recommended that recovery experiments be performed before running samples to determine the optimum calibration mix.

5.4 Biologically active samples to be analyzed for TC, TIC, or TOC should be heat-treated to halt biological activity and limit losses of volatile carbon. For specifics, refer to Section 10.2.

5.5 Certain instruments are equipped with a suspended solids kit, which consists of an autosampler equipped with a stir plate to homogenize the sample during injection analysis. Suspended solids kits keep particles suspended and therefore increase the accuracy of the measurement. These systems are also equipped with larger diameter tubing, which allow larger particle sizes to be analyzed. This procedure is applicable only to homogeneous samples with suspended solid particle sizes less than instrument specifications. Particle sizes larger than the tubing will be excluded and will not be counted as part of the TC content. Large particles may also block sample lines and damage the instrument.

5.6 Elemental carbon may not be oxidized at lower combustion temperatures (<680°C); however, this is generally not present in biological samples [1].

5.7 TN must be analyzed at a furnace temperature of 720°C.

6. Apparatus

6.1 Analytical balance, accurate to 0.1 mg.

6.2 Water bath, set to 75°C–80°C.

6.3 Centrifuge (Thermo Scientific Sorvall ST 16R or equivalent) capable of reaching 4,100 relative centrifugal force (RCF).

6.4 Magnetic stir plate.

6.5 TOC/TN analysis instrument (e.g., Shimadzu TOC-LCPH/TNM-L or equivalent) and all associated parts, including required chemical reagents and VOC free gas. If biological cultures are to be analyzed, the system must be equipped with a suspended solids kit.

7. Reagents and Materials

7.1 Reagents:

7.1.1 Hydrochloric acid (HCl), concentrated ACS reagent grade, 36.5%–38% (CAS # 7647-01-0).

7.1.2 Phosphoric acid (H_3PO_4), ACS reagent grade, ≥85 wt % in H_2O (CAS # 7664-38-2). NOTE: *This reagent is specific to the Shimadzu TOC-LCPH/TNM-L system and may not be needed for other laboratory setups.*

7.1.3 Sulfuric acid (H_2SO_4), concentrated ACS reagent grade, 95.0%–98.0% (CAS # 7664-93-9) *(specific for TN analysis).*

7.1.4 Water, 18.2 megaohm (MΩ)-cm.

7.1.5 Potassium hydrogen phthalate (KHP), ≥99.95% (CAS # 877-24-7) *(specific for TC/TOC analysis)*.

7.1.6 Sodium bicarbonate ($NaHCO_3$), ACS reagent, ≥99.7% (CAS # 144-55-8) *(specific for TIC/TOC analysis)*.

7.1.7 Sodium carbonate (Na_2CO_3), anhydrous, ACS reagent, ≥99.5% (CAS # 497-19-8) *(specific for TIC/TOC analysis)*.

7.1.8 Second source organic carbon standard (recommended 20 ppm or mid-range standard) (KHP or equivalent) *(specific for TC/TOC analysis)*.

7.1.9 Potassium nitrate (KNO_3), ACS reagent, ≥99.0% (CAS # 7757-79-1) *(specific for TN analysis)*.

7.1.10 Ammonium chloride (NH_4Cl), ACS reagent, ≥99.5% (CAS # 12125-02-9) *(specific for TN analysis)*.

7.1.11 Urea (CH_4N_2O), ACS reagent, 99.0%–100.5% (CAS # 57-13-6), or other second source nitrogen standard *(specific for TN analysis)*.

7.2 Materials *(all are needed for TC/TIC/TOC/TN analyses)*:

7.2.1 TOC vials, clear, 40 mL (ESS # TOC040-0300 or equivalent, certified to <10-ppb TOC).

7.2.2 pH paper, range 0–13.

7.2.3 Pasteur pipets.

7.2.4 Calibrated adjustable pipettes, covering ranges from 0.5–40 mL.

7.2.5 Stir bars, size 4.5 × 12 mm (for TOC vials) (Fisherbrand Octagon Spinbar #14-513-57 or equivalent).

7.2.6 100-mL volumetric flasks, glass (Class A), cleaned with hot water and rinsed with 18.2-MΩ-cm water. It is best to have dedicated glassware for TC/IC/TOC and TN analysis, free of contaminants.

7.2.7 Stir bars (for standards preparation).

8. Environmental Safety and Health Considerations and Hazards

8.1 Hydrochloric acid is toxic and corrosive and should be handled with care.

8.2 Phosphoric acid is corrosive and should be handled with care.

8.3 Sulfuric acid is toxic and corrosive and should be handled with care.

8.4 Potassium hydrogen phthalate is an irritant and may be harmful if absorbed through the skin.

8.5 Potassium nitrate is an irritant, an oxidizing solid, and has specific target organ toxicity.

8.6 Ammonium chloride is an irritant and has acute oral toxicity.

8.7 Wear appropriate personal protective equipment (PPE) and follow all applicable chemical and biohazard handling procedures.

9. Sampling, Test Specimens, and Test Units

9.1 Ensure that a representative sample of the biological system is taken for dilution and analysis. When sampling growing or biologically active cultures, it is recommended to follow a consistent sampling protocol. Sampling depth and distance from the side of the vessel, as well as homogeneity of the culture, should be considered when developing a protocol.

9.2 Samples must be prepared in unused, 40-mL, glass TOC vials (also for TN analysis) with unpunctured septa. Standards must be prepared in clean volumetric glassware and stored in a refrigerator in airtight containers.

9.3 A stir bar (4.5 × 12 mm) must be added to TOC sample vials that contain any suspended particles to ensure a representative aliquot is injected into the analyzer for TC/TIC/TOC analysis. *This applies to a Shimadzu TOC-LCPH/TNM-L system equipped with a suspended solids kit and may not be applicable in other laboratory setups.*

9.4 Time should be limited between sampling of live biological samples and analysis or heat treatment. Live samples are considered biologically active, and TC/TIC/TOC values may change over time between sample collection and analysis on the instrument. A procedure to halt biological activity is included in Section 10.2 as one suggested example.

9.5 Sample preservation, storage guidelines, and hold times for each of the analyses included in this procedure are summarized in Sections 14.7 and 14.8.

10. Procedure

TC/IC/TOC Analysis

10.1 Prepare samples for TC/TIC/TOC analysis.

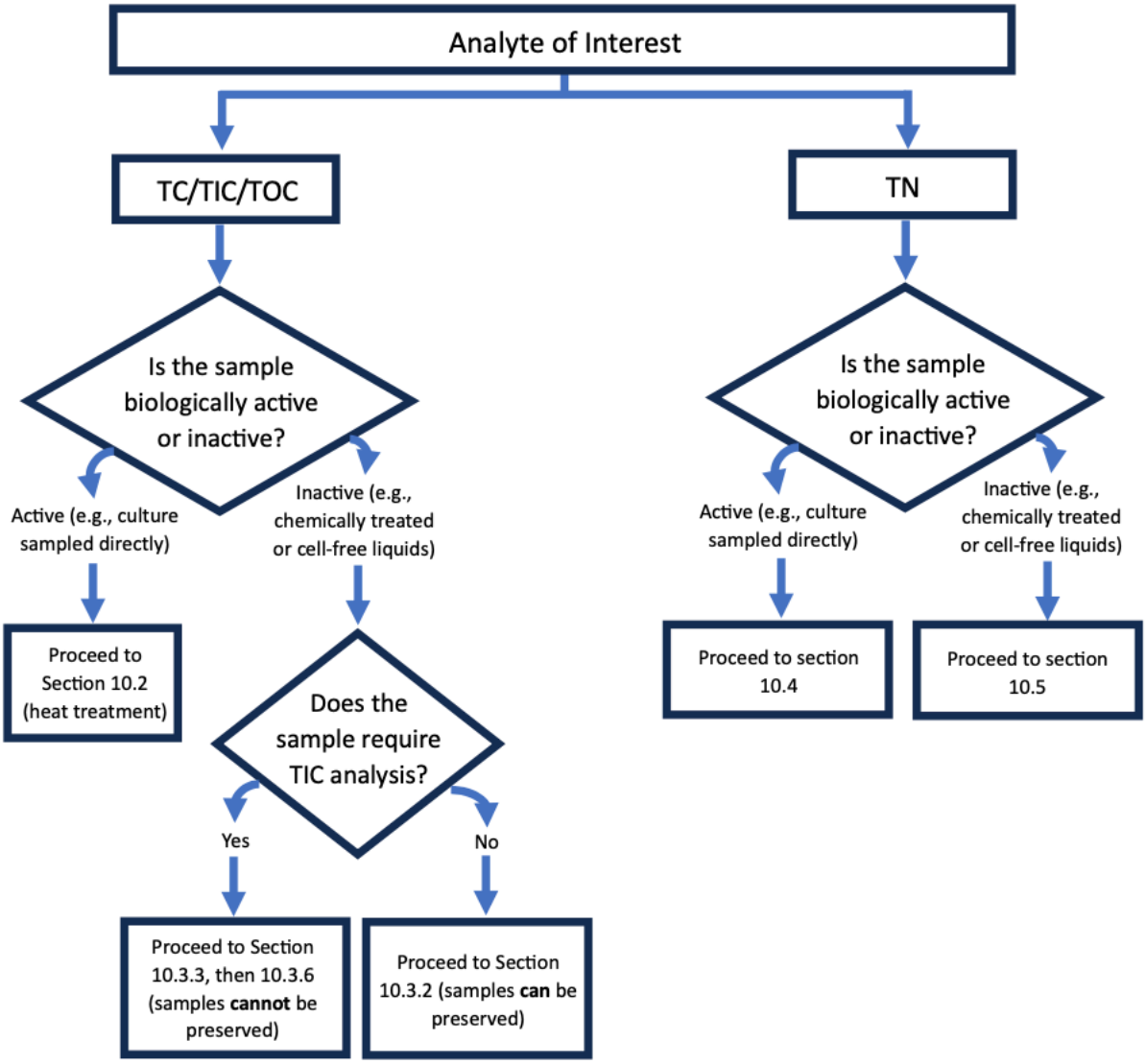

Figure 1. Flow chart for determining sample treatment based on analyte of interest and sample type

10.2 Sample handling for preserving or stabilizing biologically active samples (e.g., live algal cultures) for organic and inorganic carbon (heat treatment):

10.2.1 Based on preliminary data, biologically active samples may be heat-treated immediately following sample collection to effectively minimize potential changes in TOC/TIC concentrations following sampling and to increase the length of time that a sample may be stored until analysis (see Section 10.2.9).

NOTE: Live cultures may be analyzed in two parts: (1) whole sample (sample containing cells and culture medium) and (2) supernatant (cell-free liquid), collected after centrifugation of the whole sample. If only the supernatant component is to be analyzed, skip to 10.2.7.

10.2.2 Fill a water bath with enough water to cover 40-mL TOC vials to the shoulder, and heat the water bath to 75°C–80°C.

10.2.3 Ensure that sufficient volume is available to perform both whole-sample and supernatant component dilutions. Then prepare the whole-sample dilutions directly into 40-mL TOC analysis vials using a calibrated adjustable pipette and 18.2-MΩ-cm water as a diluent.

10.2.4 Add one stir bar (4.5 × 12 mm) to each sample vial prior to capping and heat-treating.

10.2.5 Place each capped 40-mL sample vial containing the diluted whole-sample component into a heat-resistant vial rack and into the preheated 75°C water bath for 15 minutes. Ensure that the water level is no higher than the shoulder of the vial, as heat may deform the plastic cap.

10.2.6 After 15 minutes, remove the vials from the water bath and immediately place them in a 4°C refrigerator until analysis. If the samples are to be analyzed immediately, they may be placed in an ice bath for 2 minutes or until the vials are room temperature, ensuring that the vials are only submerged to the shoulder. **Do not** open TOC sample vial after heat-treating and prior to analysis to avoid offgassing of dissolved gaseous TIC. If supernatant analysis is not of interest, skip to Section 10.2.9.

10.2.7 Centrifuge the remaining whole sample that was not heat-treated at up to 4,100 RCF until a pellet forms and the supernatant is clear and free of cells, about 5 minutes.

10.2.8 Dilute and heat-treat the supernatant as described in 10.2.2–10.2.6. It is not necessary to include stir bars in the analysis vials for supernatant samples, as there should be no suspended solids.

10.2.9 Following heat treatment, samples should be stored in a 4°C refrigerator and analyzed as soon as possible. Preliminary data indicate that heat-treated algal culture samples are stable in the refrigerator up to about 2 weeks. If heat treatment is not possible, biologically active samples must be diluted directly into 40-mL TOC vials and analyzed within 48 hours of collection.

10.3 Sample handling for biologically inactive samples (e.g., hydrolysates, sterile filtered fermentation samples) for organic and inorganic carbon:

10.3.1 Upon sample receipt, determine if samples can be preserved with hydrochloric acid prior to analysis:

- If TIC analysis is required, samples **cannot** be preserved (complete Section 10.3.3, then proceed directly to 10.3.6).

- If TIC analysis is not required, samples **can** be preserved (proceed to Section 10.3.2).

10.3.2 Preserve samples with concentrated HCl to a pH < 2 by adding acid dropwise. Mix the sample thoroughly after adding each drop and check the pH using a suitable pH strip until the desired pH is reached. Most samples require very little acid for preservation, and therefore the volume added is considered negligible.

NOTE: For samples with a high buffering capacity, concentrated HCl should be added quantitatively, and the analytical results should be corrected for the volume of acid added.

10.3.3 Prepare the sample dilutions directly into 40-mL TOC analysis vials using a calibrated adjustable pipette and 18.2-MΩ-cm water as a diluent. If samples contain particulates, add stir bars (4.5 × 12 mm) prior to capping. For samples that cannot be preserved, proceed to 10.3.6.

10.3.4 For preserved samples only, check the pH of the dilution to ensure a pH < 2.

NOTE: Large dilutions may necessitate a diluent of 18.2-MΩ-cm water that has been acidified using concentrated HCl added dropwise to a pH < 2.

10.3.5 Place preserved TOC vials into a 4°C refrigerator and analyze within 28 days [3].

10.3.6 Per standard method guidelines, unpreserved samples must be analyzed as soon as possible after sample collection [1].

10.4 Sample handling for biologically active samples (e.g., live algal cultures) for TN has not yet been investigated for our method. For the purposes of this method, these samples should be handled as follows:

10.4.1 Prepare the sample dilutions directly into 40-mL TOC analysis vials using a calibrated adjustable pipette and 18.2-MΩ-cm water as a diluent. If samples contain particulates, add stir bars (4.5 × 12 mm) prior to capping.

10.4.2 Samples should be analyzed immediately following sample collection and dilutions.

10.5 Sample handling for biologically inactive samples (e.g., hydrolysates at low pH, sterile filtered fermentation samples, media) for TN:

10.5.1 Preserve samples with concentrated H_2SO_4 to a pH < 2 by adding acid dropwise. Mix the sample thoroughly after adding each drop and check the pH using a suitable pH strip until the desired pH is reached. Most samples require very little acid for preservation, and therefore the volume added is considered negligible [11].

NOTE: For samples with a high buffering capacity, concentrated H_2SO_4 should be added quantitatively, and the analytical results should be corrected for the volume of acid added to account for any sample dilution.

10.5.2 Prepare the sample dilutions directly into 40-mL TOC analysis vials using a calibrated adjustable pipette and 18.2-MΩ-cm water as a diluent. If samples contain particulates, add stir bars (4.5 × 12 mm) prior to capping.

10.5.3 Samples should be stored in a refrigerator at 4°C and analyzed within 28 days [11].

10.6 Preparation of reagents for Shimadzu TOC-LCPH/TNM-L analyzer:

10.6.1 Prepare a 25% (w/w) phosphoric acid solution as a TIC analysis reagent. Dilute 50 mL of 85% phosphoric acid to a total volume of 250 mL with 18.2-MΩ-cm water.

10.6.2 Prepare a 1 M HCl solution for use in regenerating the catalyst as needed. Dilute 14 mL of concentrated HCl in 154 mL of 18.2-MΩ-cm water for a final volume of 168 mL.

10.6.3 Prepare a 0.05 M HCl solution for use in the B-type halogen scrubber. Add 10 mL of 1 M HCl to 190 mL of 18.2-MΩ-cm water. *The B-type halogen scrubber is specific to the Shimadzu TOC-LCPH/TNM-L system and may not be required depending on laboratory-specific setup.*

10.7 Preparation of stock standards for organic and inorganic carbon analyses:

10.7.1 Prepare a 1,000-ppm potassium hydrogen phthalate stock standard for TC/TOC analysis (store at 4°C, stable for 1–2 months). Using an analytical balance, weigh *exactly* 2,125.0 mg of potassium hydrogen phthalate into a weigh boat. Then, using a Pasteur pipette, quantitatively transfer the material into a clean, 1-L, Class A, glass volumetric flask. Dilute to volume with 18.2-MΩ-cm water.

10.7.2 Prepare a 1,000-ppm inorganic carbon stock standard for IC/TOC analysis (store at 4°C, stable for 1–2 months). Using an analytical balance, weigh *exactly* 1,748.5 mg of sodium bicarbonate into a weigh boat. In a separate weigh boat, measure *exactly* 2,206.0 mg of sodium carbonate. Then, using a Pasteur pipette, quantitatively transfer the material into a clean, 500-mL, Class A, glass volumetric flask. Dilute to volume with 18.2-MΩ-cm water.

10.8 Preparation of intermediate calibration standards and CVS for organic and inorganic carbon analyses:

10.8.1 Prepare 100-ppm intermediate calibration standards, one each for TC and TIC:

- Using a calibrated pipette, add 10 mL of the 1,000-ppm stock TC standard to a clean, 100-mL, Class A, glass volumetric flask. Dilute to volume using 18.2-MΩ-cm water.

- Using a calibrated pipette, add 10 mL of the 1,000-ppm stock IC standard to a clean, 100-mL, Class A, glass volumetric flask. Dilute to volume using 18.2-MΩ-cm water.

NOTE: TC and IC calibration standards are auto-diluted by the Shimadzu TOC-LCPH/TNM-L analyzer using the manually prepared 100-ppm intermediate standards. Refer to Table 1 for suggested calibration levels.

Table 1. Calibration Standard Concentrations and Dilution Factors From Intermediate Stock Standard Solutions for TIC/TC

Calibration Standard Concentration (ppm)	Dilution Factor (Performed by Instrument)	Diluted From TIC/TC Standard (ppm)
0	1	18.2-MΩ-cm water
2	50	100
5	20	100
10	10	100
25	4	100
50	2	100
100	1	100

10.8.2 Prepare CVS and LLCVS for both organic and inorganic carbon analyses. Standards should be prepared in clean, 100-mL, Class A, glass volumetric glassware. Dilute standards to volume using 18.2-MΩ-cm water. Refer to Table 2 for CVS/LLCVS preparation and suggested concentrations.

Table 2. CVS and LLCVS Preparation Diluted to Volume With 18.2-MΩ-cm Water

CVS/LLCVS Concentration (ppm)	TOC/IC 1,000 ppm Stock Solution (mL)	Final Volume (mL)
2	0.2	100
40	4	100

TN Analysis

10.9 Preparation of stock standard and second source standard for TN analysis:

10.9.1 Prepare a 1,000-ppm mixed nitrogen stock standard for TN analysis (this solution must be prepared fresh daily). Using an analytical balance, weigh *exactly* 3,609.1 mg of potassium nitrate into a weigh boat. In a separate weigh boat, measure *exactly* 1,909.5 mg of ammonium chloride. Then, using a Pasteur pipette, quantitatively transfer the material into a clean, 1-L, Class A, glass volumetric flask. Dilute to volume with 18.2-MΩ-cm water.

10.9.2 Prepare a 1,000-ppm urea second source standard for TN analysis (this solution must be prepared fresh daily). Using an analytical balance, weigh *exactly* 2,143.8

mg of urea into a weigh boat. Then, using a Pasteur pipette, quantitatively transfer the material into a clean, 1-L, Class A, glass volumetric flask. Dilute to volume with 18.2-MΩ-cm water.

10.10 Preparation of intermediate calibration standard, CVS, and second source standard for TN analysis:

10.10.1 Prepare a 100-ppm intermediate calibration standard for TN:

- Using a calibrated pipette, add 10 mL of the 1,000-ppm stock TN standard to a clean, 100-mL, Class A, glass volumetric flask. Dilute to volume using 18.2-MΩ-cm water.

NOTE: TN calibration standards are auto-diluted by the Shimadzu TOC-LCPH/TNM-L analyzer using the manually prepared 100-ppm intermediate standards. Refer to Table 3 for suggested calibration levels.

Table 3. Calibration Standard Concentrations and Dilution Factors From Intermediate Stock Standard Solution for TN

Calibration Standard Concentration (ppm)	Dilution Factor (Performed by Instrument)	Diluted from TN Standard (ppm)
0	1	18.2-MΩ-cm water
1	100	100
2	50	100
5	20	100
10	10	100
25	4	100
50	2	100

10.10.2 Prepare CVS, LLCVS, and second source standards for TN analysis. Standards should be prepared in clean, 100-mL, Class A, glass volumetric glassware. Dilute standards to volume using 18.2-MΩ-cm water. Refer to Table 4 for CVS/LLCVS preparation and suggested concentrations.

Table 4. CVS, LLCVS, and Second Source Standard Preparation Diluted to Volume With 18.2-MΩ-cm Water

CVS/LLCVS Concentration (ppm)	TN 1,000-ppm Stock Solution (mL)	Urea 1,000-ppm Stock Solution (mL)	Final Volume (mL)
1	0.1	-	100
25	2.5	-	100
10	-	1.0	100

10.11 Instrument setup:

NOTE: This section is specific to the Shimadzu TOC-LCPH/TNM-L system and software. Instrument and quality control specifications will vary depending on the laboratory specific setup.

10.11.1 If analyzing for TOC/TIC/TOC, skip to 10.11.2. If analyzing for TN, create one calibration curve using the following parameters and hardware settings:

- Injection volume: 150 µL.
- Number of injections: 3 out of 4.
- Calibration: linear regression.
- Furnace temperature: 720°C.

10.11.2 If analyzing for TC/TIC/TOC, create two calibration curves in the instrument software, one each for TC and IC. Use the following parameters and hardware settings:

- Injection volume: 150 µL.
- Number of injections: 3 out of 4.
- Calibration: linear regression.
- Furnace temperature: 680°C.

NOTE: While simultaneous analysis of TC, TIC, and TN is possible using a combination standard, it is not utilized in the method described. Due to sample volume constraints and different dilution factors, it is more practical to analyze TN separately from TC and TIC.

Create a method, selecting “TOC” or “TN” as the preset depending on the desired analysis.

10.11.3 Create sample sequence tables according to laboratory-specific Quality Assurance Plan (QAP) [8]. Analyze CVS at determined frequency (recommended every 10 samples). Multiple wash blanks are recommended prior to calibration.

10.11.4 Perform all instrument maintenance and daily checks in accordance with manufacturer recommendations.

11. Calculations

11.1 Create a calibration curve for TC using linear regression. From this curve, determine the concentration in mg/L of the TC present in the samples.

11.2 Create a calibration curve for TIC using linear regression. From this curve, determine the concentration in mg/L of the TIC present in the samples.

11.3 Create a calibration curve for TN using linear regression. From this curve, determine the concentration in mg/L of the TN present in the samples.

11.4 Calculate the amount of each TC/IC/TN CVS or LLCVS recovery using the following calculation:

$$\%CVS\ recovery = \frac{conc.\,detected\ by\ analyzer\ (mg/L)}{known\ conc.\,of\ standard\ (mg/L)} \times 100$$

11.5 Export the results from the TOC software and calculate TOC/TC/TIC/TN results as mg/L for each sample:

$$Total\ Organic\ Carbon\ = Total\ Carbon - \ Total\ Inorganic\ Carbon$$

11.6 Calculate TOC of the biomass in live biological culture samples:

$$TOC\ in\ biomass\ (mg/L) = TOC\ in\ whole\ (mg/L) - TOC\ in\ supernatant\ (mg/L)$$

11.7 Calculate the uncertainty in the TOC measurement using the following calculation, where percent relative standard deviation (%RSD) is calculated based on data from the three reported replicates for each sample injection [2]:

$$Uncertainty\ in\ TOC\ measurement = \ (TC\ (mg/L)\ *\ \%RSD\ ) + \ (TIC\ (mg/L) * \ \%RSD\ )$$

12. Report Format

12.1 Report data for all samples for TN, TC, TIC, and the calculated values on a mg/L (ppm) basis. Avoid reporting values below the calculated Method Detection Limit (MDL). Results between the limit of quantitation and MDL should be flagged as estimated values.

13. Precision and Bias

13.1 Precision and bias need to be determined by data quality objectives and a laboratory-specific QAP.

14. Quality Control

14.1 *Reported Significant Figures:* Figures need to be determined by data quality objectives and laboratory-specific QAP.

14.2 *Replicates:* The Shimadzu TOC-LCPH/TNM-L analyzer software reports TC, TIC, and TN values for each sample as an average of the most closely aligned three of four replicate injections.

14.3 *Blank:* 18.2-MΩ water—the same water used for standard and sample preparation. This water source must contain less reported TC, TIC, and TN than the lowest-level calibration point.

14.4 *Calibration Verification Standard:* CVS and LLCVS are prepared from the same stock standard as the calibration standards.

14.5 *Second Source Standard*: A source independent of the calibration source that should be analyzed with every calibration.

14.6 *Sample Size:* Dependent on the expected concentration of carbon or nitrogen in each sample, required sample volumes will vary. Samples low in carbon or nitrogen that do not require a dilution will need at least 40 mL for analysis.

14.7 *Sample Storage (TOC):* The Environmental Protection Agency (EPA) recommendation for TOC analysis of drinking water is to preserve samples to a pH < 2 and store between 0°C and 4°C for no more than 28 days before analysis. Standard method guidelines indicate that unpreserved samples should be analyzed immediately following sample collection. Freezing samples is not recommended [1,3]. We recommend that heat-treated samples are stored between 0°C and 4°C for up to 2 weeks.

14.8 *Sample Storage (TN):* Samples should be preserved to a pH < 2 with H_2SO_4 and stored at a refrigerated temperature between 4°C and 6°C. Samples should be analyzed within 28 days of preservation. Unpreserved samples should be analyzed immediately following sampling [11].

14.9 *Standard Preparation:* All calibration and check standards should be prepared using 18.2-MΩ water as described in the procedure.

14.10 *Standard Storage:* TC and TIC stock standards should be stored in a 4°C refrigerator and are stable for 1–2 months [9]. TN standards must be prepared fresh daily. Intermediate standards, including CVS and LLCVS, are stable for up to 1 week [9].

14.11 *Definition of a Batch:* A batch is any number of samples that are analyzed together. The maximum size of a batch will be limited by the equipment constraints or laboratory-specific QAP.

14.12 *Method Detection Limit (MDL):* The MDL is the concentration at which the analyst has >99% confidence that the analyte can be detected but the concentration is too low to quantify the analyte within a specified acceptable recovery. This concentration is determined using the EPA guidelines for MDL calculations and will vary depending on the instrument and laboratory [10].

15. Appendix

Table 5. Quality Control Specifications (May Vary Depending on Laboratory-Specific QAP)

Quality Control	Frequency	Acceptance Criteria
TC calibration	Once per sequence	$r^2 \geq 0.999$
IC calibration	Once per sequence	$r^2 \geq 0.999$
TN calibration	Once per sequence	$r^2 \geq 0.999$
Blank	Every 10 samples	<2 ppm
LLCVS (TOC/IC/TN)	Once, immediately after calibration	±50%
CVS (TOC/IC/TN)	Every 10 samples	±10%
Second source CVS (TOC/IC/TN)	Once, immediately after calibration	±10%

15.1 List of revisions/updates:

- Revision September 13, 2023, update to include TN method by TNM-L, sample preservation and storage recommendation updates, and other miscellaneous corrections.

16. References

[1] American Public Health Association, American Water Works Association, and Water Environmental Federation. 2017. *Standard Methods for the Examination of Water and Wastewater, 23rd ed*. Washington, D.C. 5-23–5-32.

[2] Shimadzu. 2020. "TOC in Daily Practice, TOC – Determination Methods According to EN 1484, SCA-130-501." Accessed December 2020. https://www.shimadzu.fr/sites/shimadzu.seg/files/5_toc_in_daily_practice_17e_0.pdf.

[3] B. B. Potter and J. C. Wimsatt. 2009. *Method 415.3 Measurement of Total Organic Carbon, Dissolved Organic Carbon and Specific UV Absorbance at 254 nm in Source Water and Drinking Water*. Washington, D.C.: EPA. EPA/600/R-09/122, Revision 1.2.

[4] European Committee for Standardization. 1997. *Water Analysis - Guidelines for the Determination of Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC), ISO-CEN EN 1484*.

[5] S. Van Wychen and L.M.L. Laurens. 2023. *Determination of Total Solids and Ash in Algal Biomass*. Golden, CO: National Renewable Energy Laboratory. NREL/TP-2700-87520. https://www.nrel.gov/docs/fy24osti/87520.pdf.

[6] L.M.L. Laurens and S. Van Wychen. 2023. *Determination of Total Carbohydrates in Algal Biomass*. Golden, CO: National Renewable Energy Laboratory. NREL/TP-2700-87500. https://www.nrel.gov/docs/fy24osti/87500.pdf.

[7] L.M.L. Laurens, A. Sowell, S. Van Wychen, and K. Ramirez. 2023. *Determination of Total Lipids as Fatty Acid Methyl Esters*. Golden, CO: National Renewable Energy Laboratory. NREL/TP-2700-87501. https://www.nrel.gov/docs/fy24osti/87501.pdf.

[8] U.S. Environmental Protection Agency. 2020. "EPA Quality Management Tools for Organizations and Programs." Accessed December 2020. https://www.epa.gov/quality/epa-quality-management-tools-organizations-and-programs.

[9] Shimadzu. 2017. *TOC-LCPH/CPN User's Manual.*

[10] U.S. Environmental Protection Agency. 2016. *Definition and Procedure for the Determination of the Method Detection Limit, Revision 2*. EPA 821-R-16-006.

[11] Shimadzu. 2017. "Introducing a New ASTM Method for the Determination of Total Nitrogen, and TKN by Calculation in Water Samples." White paper, first edition.