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Method Summary of
CAChE-NACF SOP-006, Rev. 10
Prepared for Battelle NEON program

Determination of Total Nitrogen in Water

Effective date 09/18/2023

Prepared by

Center for Aquatic Chemistry and Environment - Nutrient Analysis Core Facility

(CAChE-NACF)

1) **Summary of Method**

   a) CAChE-NACF follows the ASTM D5176-91(2003) - "Standard Test Method for Total Chemically Bound Nitrogen in Water by Pyrolysis and Chemiluminescence Detection" (the procedure is a modification of the classical Dumas (1831) method of determining nitrogen by a combustion technique with the addition of chemiluminescence). The method involves converting all forms of nitrogen into nitric oxide (NO) upon combustion of a sample with oxygen at a temperature of 1050°C. The NO is reacted with ozone (O_3) to form a metastable form of nitrogen dioxide (NO_2^*). As the metastable form of nitrogen dioxide decays, a quanta of light is emitted in an amount directly proportional to the amount of nitrogen in the sample. The chemiluminescent emission is detected by a photomultiplier tube.

   $$R\text{-}N + O_2 \text{------}> NO + H_2O + \text{Oxides}$$
   $$NO + O_3 \text{------}> NO_2^* + O_2 \text{------}> NO_2 + \mathit{hv}$$
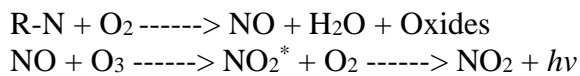
   An ANTEK Instrument, Inc. Model 9000 Nitrogen Analyzer is used to determine TN of a 5 μL injection from a prepared water sample. The instrument is run according to the Installation/Operation/Service Manual provided by ANTEK Instruments, Inc., except that Oxygen gas is used as a carrier gas instead of Argon/Oxygen to promote complete combustion and oxidation of the nitrogen in the water samples. Total nitrogen is determined on unfiltered samples, while total dissolved nitrogen is determined on filtered samples. An auto-sampler is used to inject the samples into the analyzer.

   Alternatively, a Trace S/N Cube from Elementar, Inc. is available to determine TN/DN of a 40 μl injection from a preserved water sample. The chemistry and principles of the Trace S/N Cube is the same as the ANTEK-9000, the only difference is an Argon/Oxygen mixture is used as the carrier gas as suggested by the manufacturer.

2) **Sample Storage**

   a) Sample bottles are stored in refrigerators at 2-6 ℃. Samples have a holding time of 28 days from sample collection time. Storage of samples is in refrigerators designated for sample storage only.

3) **Sample Preparation**

   i) Approximately 1.5 mL of sample is transferred into a glass auto-analyzer sample vials in sample trays. Then they will be acidified with 10 μl of 3 N HCl. The vial is sealed with a teflon/silicone lined cap and stored at room temperature until analyzed. When preserved by acid, samples have a shelf life of **90 days** without deterioration; however, sample analysis within 28 days is recommended. Sample preparation is the same for all standards, samples, controls, MB and DIW vials (DIW is used at different points in the run to rinse the equipment and to monitor instrument baseline)

4) **Analysis**

Each analytical batch (20 samples) include:

- One or two DIW - wash (run in)

- Calibration curve standards when needed (daily calibration curve is not required if QC requirements are met)

- Initial calibration verification standard (ICV), quality control standard (secondary source, QC), method blank (MB), matrix spike and duplicate (UMS, UMSD, MS, MSD)

- First set of 10 samples

- Samples are bracketed by continuing calibration verification standards (CCVs). Each bracket includes no more than 10 samples plus one analytical replicate at the end of each bracket and one MB. CCVs and RBs are run every 10 samples to monitor baseline and intra-run calibration drifts.

5) **Quality Control/Assurance System**

Quality checks are evaluated as described in the NEON SOW