

# Novel Total Nitrogen Analysis in Wastewaters

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**N**ITROGEN IS AN ESSENTIAL NUTRIENT FOR MANY natural processes, however, too much nitrogen in a water body can have negative impacts, such as increased eutrophication. Nitrogen comes in many forms with both organic and inorganic components. The total sum of all forms of nitrogen (ammonia, nitrate, nitrite, and organic nitrogen) is known as total nitrogen (TN). All forms of nitrogen are interconvertible, and all can be found in natural waters and wastewater effluents. Ammonia has been primarily a concern for wastewater dischargers, because high levels of ammonia are toxic to aquatic life. Because of this, ammonia has long been regulated under the US Environmental Protection Agency (USEPA) National Pollutant Discharge Elimination System (NPDES) permitting program (USEPA, 1972). In recent years, due to increased concerns of harmful algae (cyanobacteria), regulators are studying the contributions of other forms of nitrogen as well, specifically nitrate/nitrite, TN, and likely dissolved and organic contributions in the near future.

The USEPA does not have approved methods for TN, therefore municipal water systems typically use the Total Kjeldahl Nitrogen (TKN) digestion method and then analyze with any one of a variety of analytical methods. TKN digestion is one of the oldest analytical methods still in use (Jones, 1991). It requires sample digestion in hot concentrated sulfuric acid with the addition of potassium permanganate and either a mercury or copper/titanium based catalyst. The digestion converts the organic nitrogen in the sample to ammonia and typically takes an hour to complete. After analyzing TKN, the analyst must determine the amount of ammonia in the undigested sample to calculate the amount of organic nitrogen present in the sample by the difference of the two. Finally, many analytical methods used to quantitate ammonia require the ammonia to first be distilled from the analytically interfering high concentration of sulfuric acid in the digest. The TKN method does not measure nitrate in the sample. In fact, when the nitrate in the sample is high, nitrate can interfere with the TKN method because it reacts with ammonia at the high temperatures of digestion to produce nitrous oxide, giving a false low value for ammonia in the sample (Schleuter, 1977).

This article provides a discussion on a simpler digestion method using a basic pH peroxydisulfate digest, which converts all nitrogen in the sample to nitrate, combined with an automated analysis of the digested sample for nitrate with an ammonia/nitrate analyzer (Timberline TL 2800 Ammonia/Nitrate Analyzer, Timberline Instruments, Boulder, Colorado) and autosampler (Cetac

ASX-260). The instrument is based on membrane diffusion of ammonia from the sample stream into a buffer stream and conductivity detection of ammonium ion. Since the analyzer measures ammonia, the nitrate is first quantitatively reduced to ammonia by treating the sample, online, with an activated zinc cartridge. The peroxydisulfate digestion method is faster (30-minute digestion), requires less sample, and has significantly less hazardous operating conditions compared with TKN digestion. The sample is ready to analyze for the resulting nitrate when the digestion is complete without the need for distillation or any other sample preparation except in some cases a simple dilution.

## Digestion method

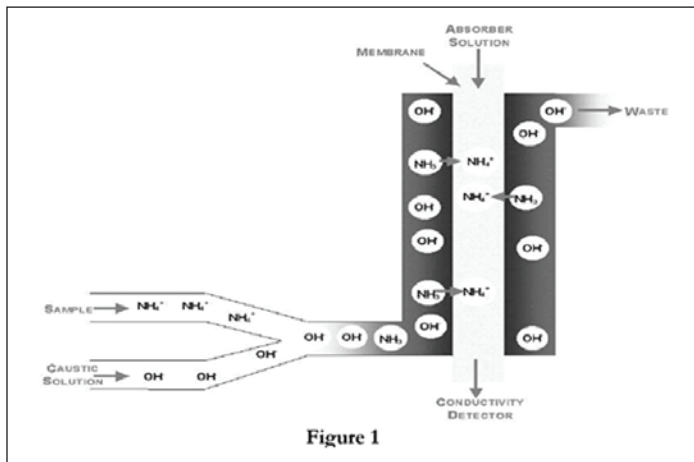
The persulfate digestion method for TN was first reported as an alternative to TKN digestion in 1976 (Grasshoff). D'Elia et al also published a comparison of TKN to persulfate digestion of TN in 1977. Patton and Kryskalla, with the USGS, published an extensive report in 2003 on "The Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water." The digestion method we are reporting on is tailored after the method outlined in the Patton and Kryskalla paper and the APHA 4500-N-C Persulfate Method for Total Nitrogen in Standard Methods (2017), for the determination of nitrogen from organic nitrogen compounds. Method 4500-N-C is also approved for use under Colorado Regulation 85 by the Colorado Department of Public Health and Environment (CDPHE, 2016).

## Analytical method

The analytical method, post digestion, is an automated flow injection analysis (FIA) method using membrane diffusion and conductivity detection, first described by Carlson (1978), for the determination of ammonia in water without the need for sample dilution or filtration. The ammonia/nitrate analyzer uses membrane diffusion of ammonia in the sample from a basic sample stream, into a slightly acidified buffer stream, where the dissolved ammonia gas is converted back to aqueous ammonium ion. The ammonium ions in the acidic buffer stream are then detected using a conductivity detector.

The on-board peristaltic pump directs the sample and caustic streams together to mix. The resulting solution has a pH sufficiently high (pH 11–13) to convert all the ammonium ions present in the sample to dissolved ammonia gas. This basic solution containing the dissolved ammonia gas then flows to the outside of the diffusion membrane tube. The peristaltic pump separately directs a dilute boric acid solution, pH 6.5,

to the internal diameter of the diffusion membrane tube. The sample/caustic solution flows past the outside of the membrane that is permeable to gases but not to liquids or ionic species. The dissolved ammonia gas in the sample/caustic mixture diffuses across the membrane from the high pH caustic solution to the acidic boric acid solution on the inside of the membrane where ammonia gas is re-dissolved in the acidic buffer and converted back to ammonium ions. Finally, the boric acid solution containing the ammonium ions from the sample flows through the conductivity cell and ammonium ions are detected as an increase in conductivity as compared to the lower conductivity of the boric acid solution background (Figure 1).



When an optional zinc reduction cartridge (Carlson, 1986) is placed in line with the basic sample stream, nitrate and nitrite are quantitatively reduced to ammonia prior to analysis. It is possible to determine any of the following: just ammonia, nitrate/nitrite (sometimes referred to as NO<sub>5</sub>), total inorganic nitrogen (TIN; ammonia plus nitrate/nitrite), or, with sample digestion as described in this article, TN. Without using the zinc cartridge in line, the ammonia/nitrate analyzer measures only ammonia. With the zinc cartridge in line the analyzer measures TIN. If the sample is run twice, once with the zinc cartridge and once without, ammonia, and by difference, nitrate/nitrite can both be determined. Finally, in the case

where the sample has been first digested with a basic persulfate digestion, and the sample is treated with the zinc cartridge in line, the result represents TN, which is the goal of this study and what is reported here. The method does not differentiate between nitrate and nitrite. If speciation of nitrate and nitrite is required, individual analysis for nitrite may be needed.

This analytical method is APHA 4500-N-D, Conductometric Determination of Inorganic Nitrogen for the analysis of ammonia and NO<sub>5</sub> (Standard Methods, 2017). The dynamic range of this method is from a limit of quantitation of 10 ppb ammonia-N to 500 ppm ammonia-N and analysis time per sample is 2 minutes or less. It is also approved for use under Colorado Regulation 85 by CDPHE (2016).

### Experimental methods

#### Digestion procedure

Samples were gathered from up to 12 collection sites for four consecutive months in 250 or 500 mL polypropylene or HDPE bottles, stored at 4°C, and digested within 24 hours of collection. In the lab, a 10 mL aliquot of sample is transferred to 16 mL glass sample vial and placed in the autosampler vial rack. Five milliliters of the digestion reagent was then added to each vial. The digestion reagent was prepared by dissolving 10.05 g of low nitrogen potassium peroxydisulfate and 1.5 g sodium hydroxide to 200 mLs deionized (DI) water. The vials were capped, mixed, and placed into the autoclave at 121°C for 30 minutes on the liquids cycle. After digestion, samples were kept capped at room temperature until analyzed. Samples for this study were all analyzed within five days of digestion.

#### Ammonia/nitrate analyzer

Nitrate standards, typically between 0.050 and 20.0 ppm-N, are prepared from NIST traceable 1000 ppm nitrate-N stock standard solution. A blank using the DI water used to prepare standards was included. A 250 ppm boric acid buffer and 5.0 percent w/w sodium hydroxide solution were prepared according to the manufacturer's specifications. A double zinc cartridge was installed, and the instrument was placed in the Run Total N mode, where the zinc cartridge is always in line with the basic sample stream. Buffer and sodium hydroxide

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solutions flowed through the system for 10 minutes until the background is stable. Injection time was 20 seconds. The instrument was then calibrated. Included in each analytical run were samples, matrix duplicates, matrix spikes, matrix spike duplicates, check standards, and digestion check standards.

**Results and discussion**

Two Colorado municipal water quality labs, the City of Westminster and the City of Northglenn, were involved in the analysis of the samples. Normal monthly wastewater plant and in-stream samples were collected and exchanged, digested as described by each lab, and measured for TN using the ammonia/nitrate analyzer as described, with double zinc reduction cartridges. This process was completed four consecutive months for a total of 46 valid samples. Relative percent difference (RPD) was determined for each valid data point as described in Standard Methods. Typically, an RPD of less than 20 percent is considered acceptable. The data also include duplicate samples, check standards, and a complex

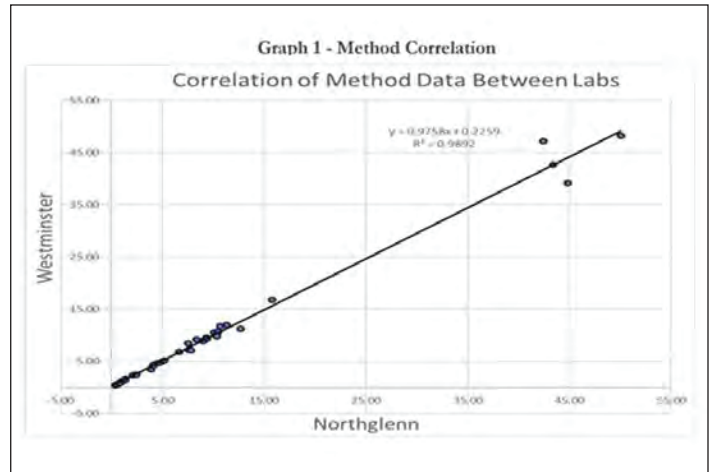
nutrient standard to check the digestion process for both laboratories.

Table 1 (in the left column) shows the results from each lab on the cross-analyzed samples along with RPD and percent recoveries for one of the four months of the study. The RPD average for the sample data over all four months is 5.23 percent with a standard deviation of 4.12 percent, indicating the method is reproducible between labs within satisfactory results. Spikes and check standards for each month are reported with a percent recovery value.

Table 1 – March 2018 Data

Sample Name	TN Conc Northglenn (mg/L-N)	TN Conc Westminster (mg/L-N)	Rel. % Diff (RPD)	% Rec
BDC 0.5	1.21	1.35	11.4	--
BDC 1.0	1.32	1.42	7.07	--
BDC 1.5	2.41	2.47	2.33	--
BDC 2.0	15.7	16.8	6.54	--
BDC 3.0	10.7	11.8	10.0	--
BDC 4.5	11.3	12.0	6.36	--
BDC 5.0	9.31	9.53	2.34	--
BDC 6.0	7.53	8.45	11.5	--
Westminster Influent	42.4	47.2	10.8	--
Westminster Effluent 004	10.1	10.7	3.49	--
Northglenn BDC 004	4.60	4.69	1.94	--
Northglenn UV Effluent	9.06	8.98	0.830	--
Nutrient Digestion Check:			--	
Northglenn-9.43 mg/L-N	9.28			98
Westminster-10.15 mg/L-N		9.88		97
Glutamic Acid 2.90 mg/L-N	2.94	NA	--	101


NA = Not Analyzed



Graph 1 above shows the data correlation between labs for all four months. Perfect correlation would produce a linear trendline with a slope of 1.00. The trendline for data from this study has a slope of 0.975 and a correlation coefficient of 0.9885, indicating very good correlation and suggesting little or no bias toward either lab in the data.

**Conclusion**


This article has presented a cross-lab study between two water quality labs using persulfate digestion of wastewater samples as an alternative to TKN digestion for TN. Four consecutive months of samples were digested, including spiked samples



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and check standards. The digested samples were analyzed using an ammonia/nitrate FIA analyzer that uses diffusion/conductivity detection with a zinc reduction cartridge to reduce the nitrate resulting from the digestion to ammonia. The digestion method used is modeled after Standard Methods' protocol for basic persulfate digestion. The data presented demonstrate this method of digestion and analysis with the ammonia/nitrate analyzer performs well and does not present any significant laboratory bias as indicated by the low average RPDs and excellent correlation between both laboratories' data. This method for TN is approved for use by Colorado Regulation 85.

Note: This paper is an abbreviated version for publication. A complete version with more detail and all four months of data is available from the primary author.

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