Total Nitrogen by alkaline persulfate digestion and enzymatic nitrate analysis

NEMC 2020 Virtual Presentation

Ellen R Campbell, WH Campbell, AH Marceau

NECi Superior Enzymes™

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Clean Water. Fertile Soil. Serious Science.

Green, accurate, affordable Total Nitrogen

NECi is based on the premise that better nutrient management requires new tools.

Enzymes catalyze difficult chemical reactions under gentle conditions in complex matrices. Numerous common lab methods can be done with nonhazardous reagents and low cost instrumentation when the methods are enzyme-based.

Reduced sample prep, low cost instrumentation, reduced training.

This method puts TN in reach of Citizen Scientists and other users with limited lab facilities.

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Total Nitrogen: Why Alkaline Persulfate?

- **This alternative to Kjehldal has been around for a while.**
- J.J. Ameel, R.P. Axler, C.J. Owen, Persulfate digestion for determination of total nitrogen and phosphorus in low-nutrient waters, American Environmental Laboratory. 1 (1993) 10–11.
- **Kjehldal (TKN) drives all N to Ammonia by refluxing samples in concentrated sulfuric acid.**
- **The alternate approach involves heating samples in an aqueous solution to drive all N to nitrate.**
- The EPA and many regulatory associations are now accepting Alkaline persulfate as a valid alternative to Kjehldal.

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Why Enzymes?
They're great for analytical chemistry

- **Selectivity**
  "Find" target in complex mixtures
- **Sensitivity**
  Low detection limits in complex mixtures
- **Specificity**
  False negatives ***and*** false positives are rare
- **Safety**
  For shipping, storage, handling, and disposal.

Reagent Grade Enzymes are accurate, reliable, and environmentally benign.

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Nitrate by Nitrate Reductase Enzyme

$NO_3 + NADH + H^+ \xrightarrow[\text{Nitrate Reductase}]{} NO_2 + NAD^+ + H_2O$

NO_2 + Sulfanilic Acid ⟶ Diazonium Salt

Diazonium Salt + NED ⟶ Pink dye for measuring A 540 ± 20nm

Samples are measured against a standard nitrate curve to determine results in ppm Nitrate-N

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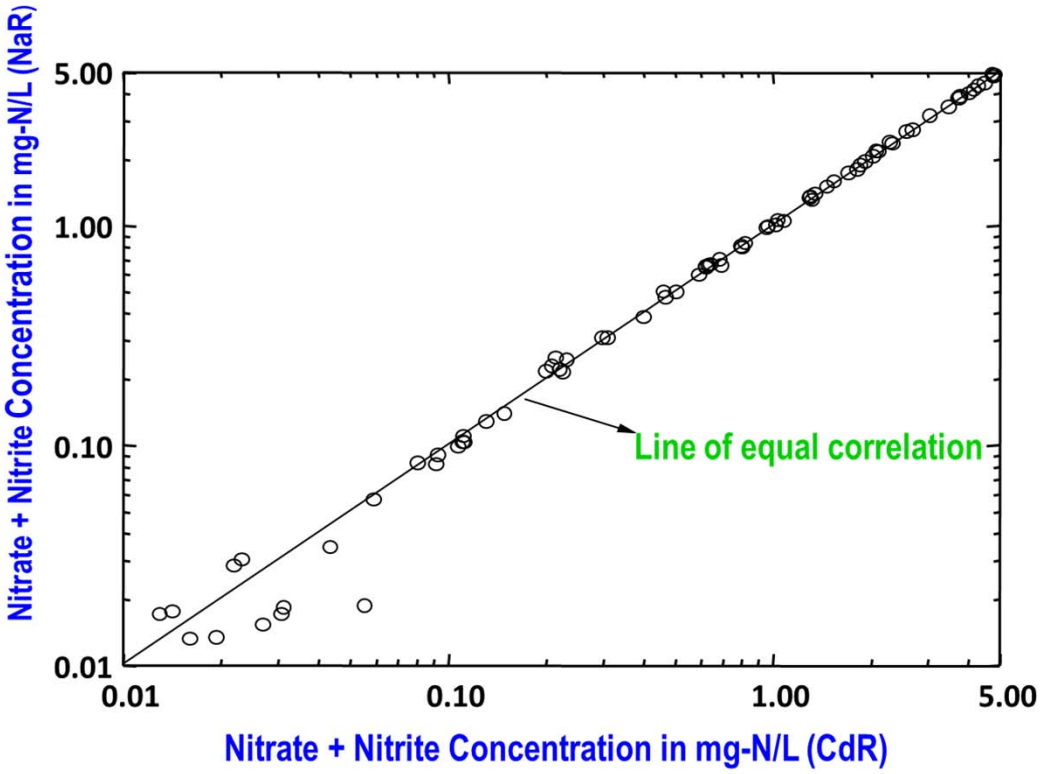
Method Validation: Enzyme vs Cadmium



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Samples tested for Validation Studies

Sample Type	Filtered	Acid
Denver area treatment plant Influent wastewater	Yes	Yes
Denver area treatment plant Wastewater effluent #1	Yes	Yes
Denver area treatment plant Wastewater effluent #2	Yes	Yes
Michigan paper mill waste stream effluent	Yes	Yes
Denver area metal finisher waste stream effluent	Yes	Yes
Denver area Commercial laundry waste stream effluent	Yes	Yes
Environmental Resources Associates #507 Hardness WasteWatR reference material	Yes	Yes
Michigan Confined Animal Feeding Operation (CAFO) effluent from tiled field	Yes	Yes
Low-nutrient seawater (collected offshore Hawaii)	Yes	No
ERA # 608 Reference Standard	Yes	Yes
USGS PE N-116 (low nutrient-fortified river water)	Yes	No
USGS PE N-115 (high nutrient-fortified river water)	Yes	No
Tap water at each lab		
Tap water plus added Chlorine		

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Sample Types

- NECi has customers evaluating nitrate in many sample types
- Agriculture: soil and plant extracts, nitrate toxicity in forage
  - Nitrate range from ppb to percent
- Medical R&D: Biological fluids and extracts, tissue culture fluids
  - Nitric oxide as ppb, nitrate as ppm
- Environmental: Drinking water, waste water, ambient water, natural water, industrial effluents, process water
- Anything aqueous: Milk powder, pulverized concrete
- And now they can do TN

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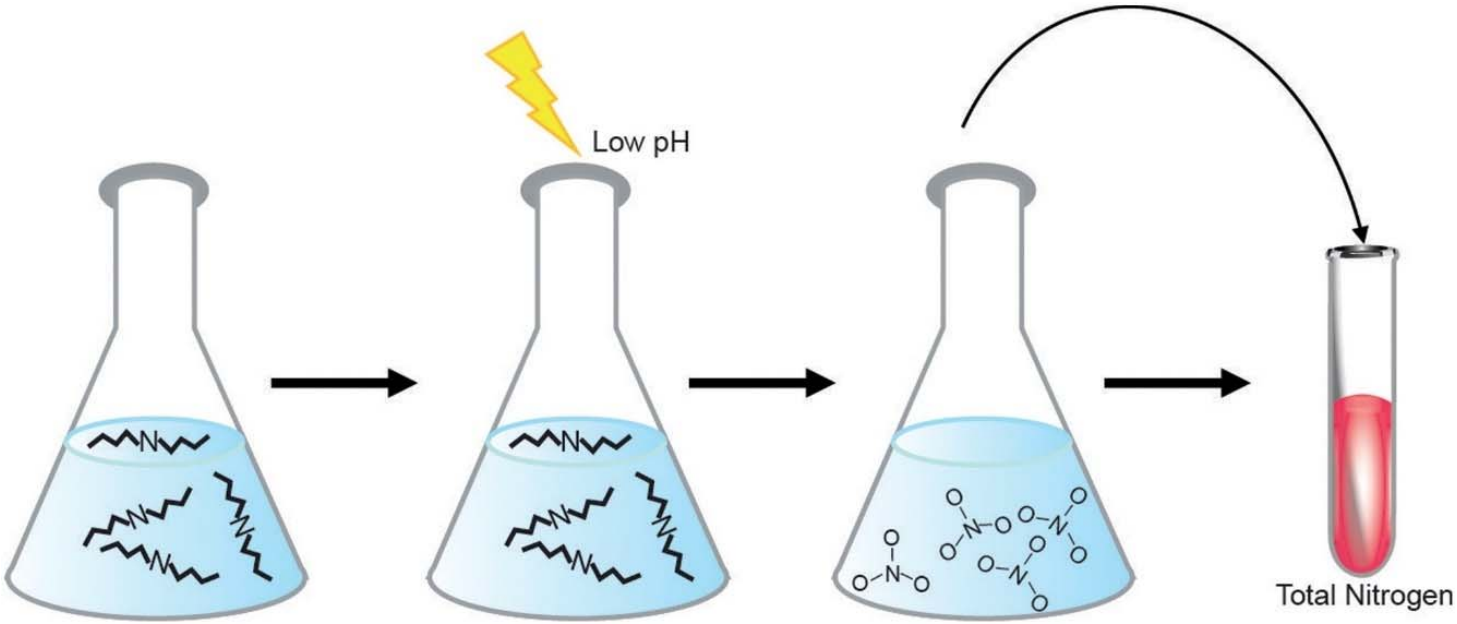
Total N by Alkaline Persulfate Digestion



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TKN

- Refluxing in concentrated acid
  - Requires hoods, glassware, safety considerations
- Product is a gas
  - Gases are hard to control
- Results are analyzed by Gas Chromatography
- Trained technicians and costly equipment required

Alkaline Persulfate

- Heat samples in an aqueous solution.
  - Heating block or autoclave
- Product is nitrate in water
- Results analyzed by any nitrate method.
- For total REACH compliance and max safety, analyze enzymatically.

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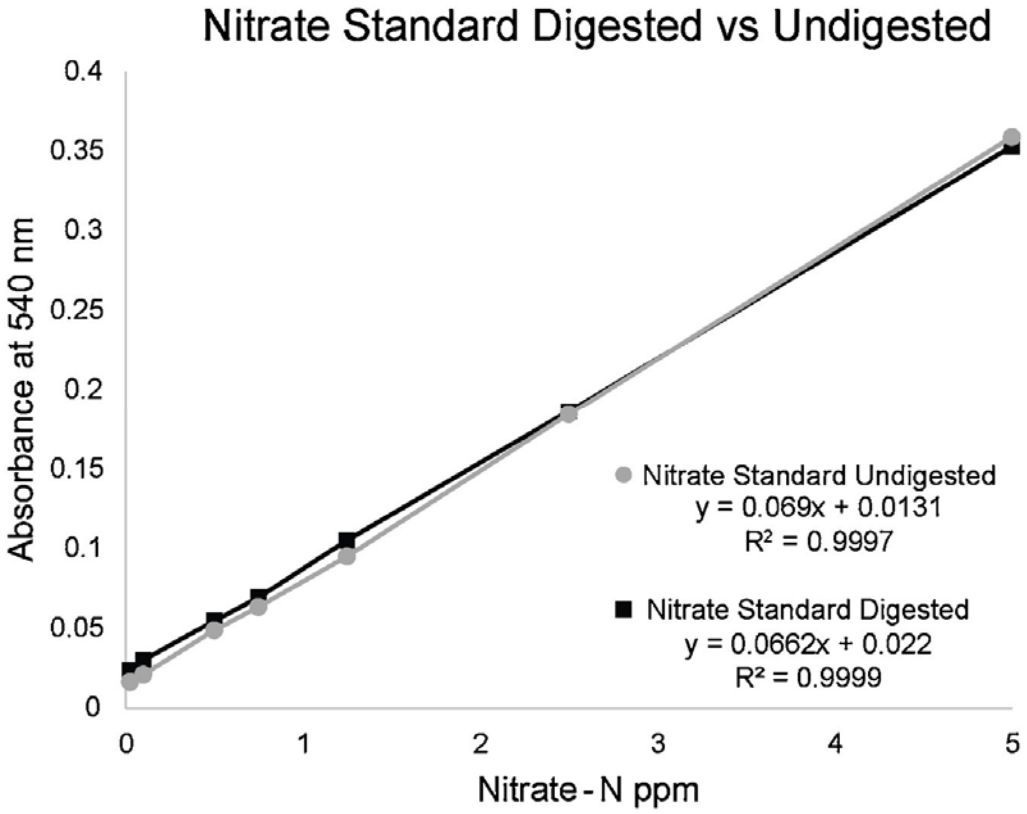
Proof of Concept: Nitrate standards



- Nitrate standards were prepared and either digested by the alkaline persulfate method or left undigested. There is little to no difference between the nitrate standard curves.

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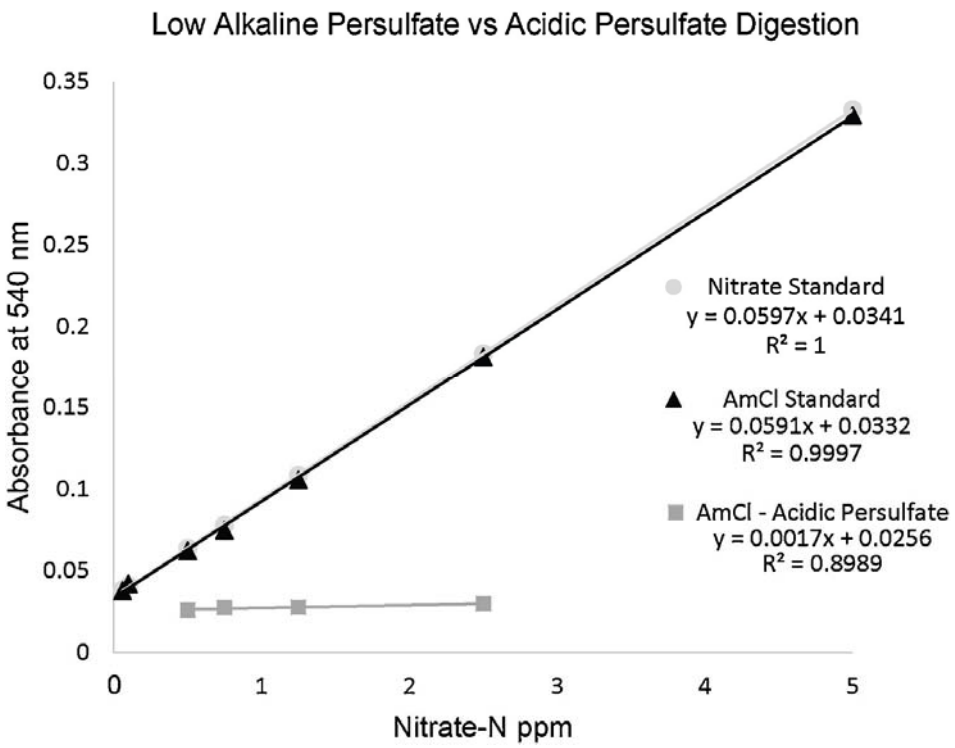
Chlorate does not interfere with Alkaline Persulfate Digestion



Nitrate standard (grey circle) and ammonium chloride (AmCl) standards give very similar trend lines after low alkaline persulfate digestion.

Under acidic persulfate digestion conditions the ammonium chloride standard (grey squares) produced chlorate which interferes with the nitrate reductase assay.

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Standard Method Status: Nitrate Enzyme

- US EPA Clean Water Act
  - MUR 2017, results read on any automated instrument
- US EPA Safe Drinking Water Act
  - Federal Register 2016, Discrete Analyzers specified
- ASTM D7781
  - Discrete Analyzers specified
- Standard Methods for Analysis of Water and Wastewater 4500-NO3J
  - Manual formats: test tube, microplate, all water types

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Nitrate & Total N for Everyone

- Handheld photometer with good optics
  - Lab quality LEDs for increased sensitivity
  - Dual beam
  - Optical bench designed for standard 1cm cuvettes
- Enzymatic reagents in test tube format for labs with minimal equipment but skilled operators
- Onsite test kit formats for nonskilled users with maximized ease of use and safety
- Total N using InstantPot® in place of autoclave for digestion step

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Nitrate and Total N in the Field

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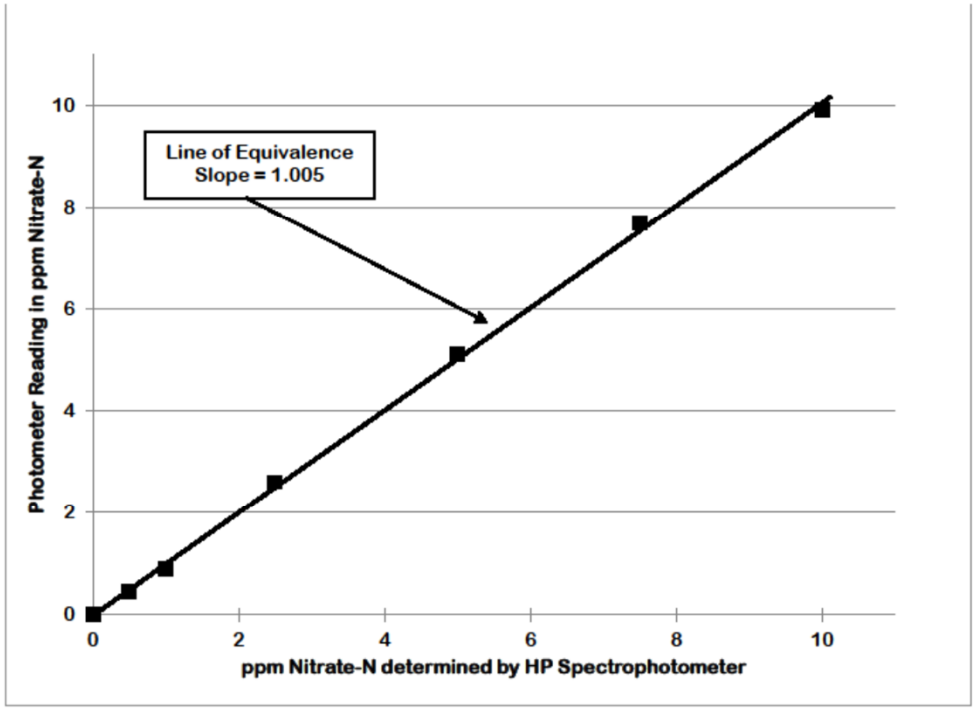
NECi Photometer vs HP UV-Vis 8453: Nitrate



Nitrate-N Standard (ppm)	Photometer Reading (ppm)	HP Spec ABS @ 540 nm
0	0	0
0.5	0.45	0.0311435
1	0.9	0.0664595
2.5	2.6	0.17811
5	5.1	0.371165
7.5	7.7	0.588795
10	9.9	0.77584

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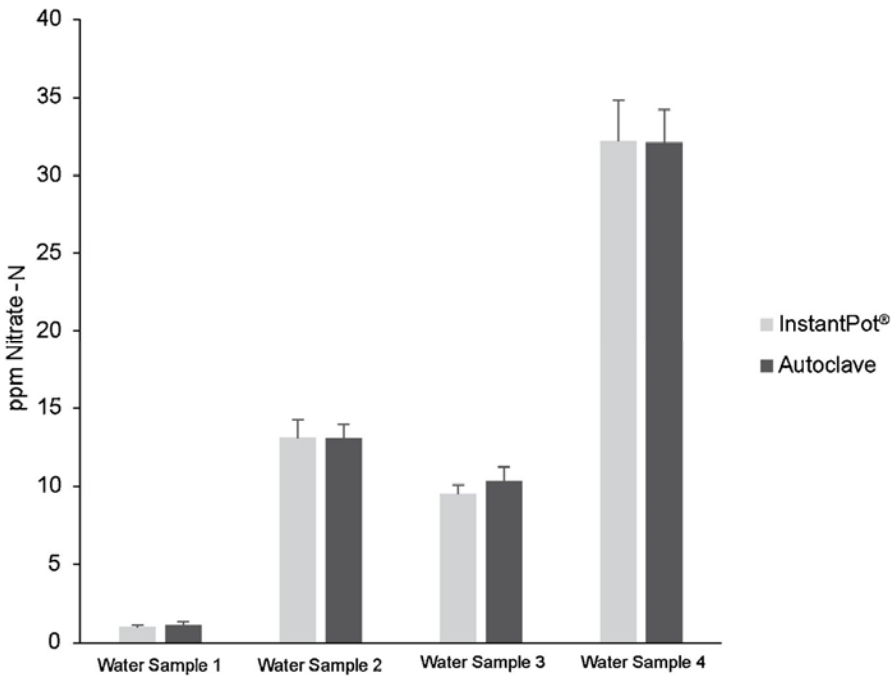
Digestion by Autoclave and InstantPot®



- **Comparison of total nitrogen analysis by alkaline persulfate digestion and nitrate reductase.**
- **3 independent replicates of each water sample digested in either the Instant Pot® (light grey) or autoclave (dark grey) were averaged and plotted by ppm Nitrate – N.**

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Thanks!

- **NECi thanks all the individuals, labs, companies, funding sources, and regulatory agencies who've helped us bring enzyme-based analytical chemistry to the environmental world.**
  - **Dr Charles Patton, USGS**
  - **Dr William Lipps, Shimadzu, ASTM, and StdMethods.org**
  - **SBIR Programs of the USDA, Natl Science Foundation, and NIH**
  - **Economic development organiations: MEDC and KEDA**
  - **Our customers**

- **Contact: ellenr@nitrate.com**
- **1.888 NITRATE**

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