Determination of total nitrogen in aqueous samples using persulfate digestion

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Abstract—Determination of total nitrogen in aqueous samples after potassium persulfate digestion compared favorably in both precision and nitrogen recovery with determinations obtained using Kjeldahl digestion.

The determination of total nitrogen (TN = inorganic and organic fixed nitrogen) has largely depended either on acid Kjeldahl digestion (cf. Strickland and Parsons 1972) or on photo-oxidation (Armstrong et al. 1966) of nitrogenous organic compounds. The former procedure is tedious to perform and yields a total Kjeldahl nitrogen (TKN) value that includes only organic N and NH_4^+-N (*not* NO_2^-- and NO_3^--N). The latter is less tedious and does include NO_2^-- and NO_3^--N, but some compounds (e.g. urea and ethylenediaminetetraacetic acid—EDTA) have been reported to be refractory to this type of oxidation (Henriksen 1970; Afghan et al. 1971). In addition, a substantial investment in photo-oxidation equipment is necessary if large numbers of samples are processed.

A persulfate oxidation technique for TN determination has also been developed (Koroleff 1969, 1970). Koroleff found that while there is no single nitrogenous product of nonalkaline persulfate oxidation (presumably NO_3^-, NOCl, and other compounds are produced), under alkaline conditions, NO_3^-, readily reduced to NO_2^- for analysis, is the sole product. Thus, total

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persulfate nitrogen (TPN) should be equivalent to TKN plus NO_2^-- and NO_3^--N. Koroleff's procedure, although apparently in wide use in Scandinavia (Ekedahl et al. 1975), to our knowledge has received little attention elsewhere. Here we report some minor modifications to his procedure and evaluate its efficacy relative to a modified Kjeldahl procedure.

All reagents should be of analytical reagent grade. Nitrogen-free distilled water (NFDW) is prepared by UV oxidation and deionization or by double distillation from acid persulfate and alkaline permanganate. Glassware is prerinsed in dilute HCl and NFDW.

TPN reagents and procedure—

1. Oxidizing reagent: 3.0 g of NaOH and 6.7 g of low N (<0.001%) potassium persulfate (peroxydisulfate), $K_2S_2O_8$, are dissolved in 1 liter of NFDW just before use.

2. 0.3 N HCl: stable for months.

3. Buffer solution: 30.9 g of H_3BO_3 are dissolved in deionized water, 101 ml of 1 M NaOH are added, and the solution made to 1 liter; stable for months.

4. NO_3^- reduction columns and NO_2^- reagents are as given by Strickland and Parsons (1972).

Fifteen milliliters of oxidizing reagent are added to 10.0 ml of sample in 25 × 150-mm (50-ml capacity) borosilicate screwcap culture tubes. A $Mg(OH)_2$ precipitate forms in seawater samples. Blanks for undiluted samples consist of 15.0 ml of oxidizing reagent only. The tubes are capped immediately with size 24 polypropylene screw closures (e.g. Nalgene 2150-0240). Samples are autoclaved at 100°–110°C (Williams 1969) for at least a half hour and slowly brought back to atmospheric pressure. The tubes can then be removed and cooled to room temperature. To each sample are added 1.5 ml of 0.3 N HCl. The samples are mixed with a Vortex mixer until the precipitate dissolves, 2.0 ml of buffer solution is added, and then deionized water to a mark on the tube indicating 50 ml (alternatively, 23.5 ml of a stock solution of 2 parts buffer solution to 21.5 parts of deionized water can be added). Twenty milliliters of each sample are washed through the nitrate reduction column in small aliquots and are discarded. The final 30 ml is passed through the column and analyzed for NO_2^- (cf. Strickland and Parsons 1972). As the borate buffer does not appear to complex Cd^{2+} well, the columns will tend to clog unless rinsed every few samples with about 10 ml of dilute NH_4Cl solution (cf. Strickland and Parsons 1972).

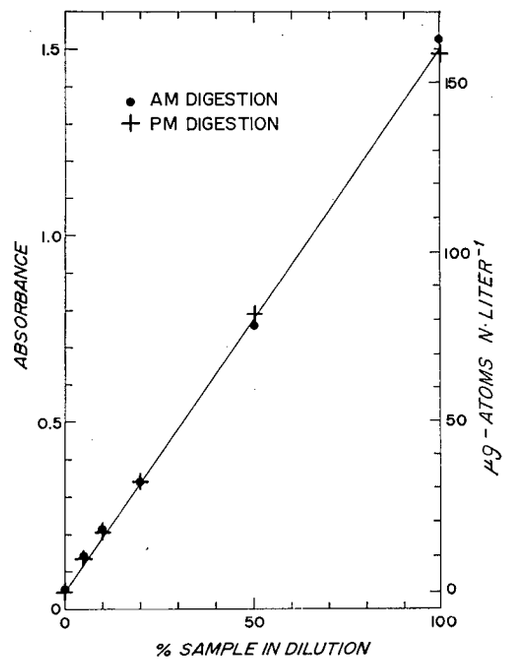

Fig. 1. Recovery of N in various dilutions of an algal culture. Dilutions subjected to persulfate digestion on two successive occasions (morning—AM—and afternoon—PM).

TKN reagents and procedure—

1. Digestion mixture: 50 parts of concentrated H_2SO_4 to 50 parts of NFDW to 5 parts of 5% $CuSO_4$.

2. Ammonium reagents: phenol and alkaline solutions as given by Solórzano (1969); sodium nitroprusside solution—0.25% of sodium nitroprusside; oxidizing solution—0.2 g of sodium dichloroisocyanurate (Liddicoat et al. 1975) per 100 ml of alkaline solution.

Table 1. Precision of total N determinations by persulfate and Kjeldahl methods.

Concentration range*	TPN				TKN + NO_2^- and NO_3^--N§			
	Mean concentration	N (pairs)	2SE†	CV%‡	Mean concentration	N (pairs)	2SE	CV%
<20	14.2	23	1.76	8.7	14.3	12	1.06	5.3
20–40	26.9	14	2.26	5.9	27.1	12	2.62	6.9
40–60	50.7	11	3.14	8.6	47.3	3	2.88	7.3
60–80	70.9	20	5.24	5.2	70.1	3	2.24	2.2
80–100	88.2	12	3.94	3.2				
100–120	110.9	16	5.72	3.7				

*Data produced in our laboratories over the past year were collected at random and for comparison divided arbitrarily into the concentration ranges shown. All concentrations are expressed as μg-atoms N liter^{-1}.

†Two standard errors of the mean of two determinations (95% confidence interval), i.e. $2s/\sqrt{2}$, where s is the standard deviation, calculated by the formula $s = (\Sigma d^2/2N)^{1/2}$, d being the difference between duplicates (Youden 1959).

‡CV% = coefficient of variation = $100s/\bar{X}$, where $\bar{X}$ is the mean.

§TKN (total Kjeldahl nitrogen) does not include NO_2^- and NO_3^--N, hence for comparison with TPN (total persulfate nitrogen), these values must be added to TKN.

Two milliliters of digestion mixture and two glass beads are added to 25.0 ml of sample in a Kjeldahl flask. The flask is heated to volatilize the water and digested until the remaining solution turns clear. After rinsing with a minimum amount of NFDW, the solution is transferred to a beaker, cooled on ice, and the pH is adjusted to 5.0–5.2 with NaOH. An aliquot of the solution is poured into a 50-ml volumetric flask and diluted to volume. Samples can be stored overnight at this point. The reagents for NH_4^+ determination are added as prescribed by Solórzano (1969), and the color developed in the dark (Gravitz and Gleye 1975) for a consistent period (not less than 90 min) at room temperature. The factor (F) relating absorbance to NH_4^+ concentration should be determined by difference in absorbance from NH_4Cl spiked and unspiked seawater. TKN blanks are obtained using NFSW. Absorbances are recorded at 640 nm in a 1-cm cuvette.

We have found that although the $CuSO_4$ catalyst in the digestion mixture gives a slight blue color after addition of the NH_4^+ reagents (Nicholls 1975), it does not interfere with indophenol blue color formation. We have chosen to retain the catalyst for samples only in the higher TKN ranges (Nicholls 1975). The present procedure affords a range of detection from about 2–50 μg-atoms N liter^{-1}.

During the past year we have randomly used NH_4^+, NO_3^-, glycine, EDTA, and urea as standards to obtain the factor (F) relating 1-cm cuvette absorbance to TPN concentration. Except for NH_4^+ ($0.05 < p < 0.1$; t-test), we have found no significant difference (i.e. $p < 0.1$) in N recovery between NO_3^-, which we considered 100% recoverable, and the other standards. Presumably, the significant difference in NH_4^+-N recovery was due to its dissociation and partial volatilization upon addition of alkaline persulfate reagent as this raises the pH to about 10.8. However, recovery of

NH_4^+-N was excellent (96%) and losses should be inconsequential except in samples containing mostly NH_4^+-N. Note that the quantitative recovery of EDTA-N and urea-N by the TPN procedure is in contrast to the poor recovery by UV oxidation (Afghan et al. 1971).

To test the effect of N concentration on its recovery as TPN, we prepared various dilutions of an algal culture (containing mostly particulate organic N) and subjected them to persulfate digestion on two successive occasions. An equivalent recovery was observed at all dilutions (Fig. 1).

The precision of total N determinations by persulfate and Kjeldahl methods was compared by randomly selecting data from routine seawater samples we have processed (Table 1). The overall coefficient of variation (CV%) for both was about 5%. Although this is better than might be expected using the Strickland and Parsons (1972) procedure for TKN alone, only the less time-consuming TPN procedure lends itself to additional improvements in precision through greater replication. Note, that since TKN-TN is the sum of TKN and NO_2^-- and NO_3^--N, some error is accounted for by the latter analysis. Thus, the determination of TKN alone (i.e. NH_4^+ + organic N) in samples containing a high percentage of NO_2^-- and NO_3^--N is not as precisely determined by difference between TPN and NO_2^-- and NO_3^--N.

TPN recovery was compared to TKN-TN recovery on 21 paired samples containing 20–35 μg-atoms N liter^{-1} and varying ratios of inorganic to organic N. We obtained similar mean values of 27.83 μg-atoms N liter^{-1} for TKN-TN and 26.34 μg-atoms N liter^{-1} for TPN. However, treating the TKN-TN and TPN values as duplicates, we found a higher CV% (ca. 13%) than for duplicates of either method alone (ca. 5%). This suggests that there is some slight difference in what is measured by the two procedures.

We also compared particulate nitrogen (PN) determination using the persulfate technique (by difference in TPN in filtered and unfiltered sample) with PN determination using the Perkin-Elmer model 240 CHN analyzer (on the 550°C precombusted glass filters used above). For the 34 paired comparisons, the mean TPN-PN value of 131.5 μg-atoms N liter^{-1} was reasonably close to the CHN-PN value of 120.0 μg-atoms liter^{-1}. We do not, however, recommend PN determination using persulfate analysis "by difference" as the precision is poor.

We have encountered difficulties in obtaining low N persulfate. Generally, B&A and Baker products have produced the lowest blanks (0.020 to 0.050 absorbance in 1-cm cells). NFDW accounted for only about 0.01 absorbance units. Recrystallization of persulfate may improve its quality. One can expect a factor (F) of about 110 for TPN when using a 1-cm cell. Since day-to-day variation in F with four pairs of blanks and standards has been as great as ±10%, probably due to poor replication rather than a true change in F, it is probably best to establish an overall F value recalculated on a continuous basis, provided that regular checks are made to ensure that the NO_3^--reducing ability of the columns remains constant.

A distinct advantage of the TPN procedure is that it can be used at sea. Samples can either be digested in precombusted ampoules or sealed pyrex tubes while at sea for analysis later on shore or the entire operation completed on board ship. The persulfate method should find wide use in laboratories analyzing aqueous samples for TN.

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