Limnol. Oceanogr., 27(2), 1982, 373–375

Preparation of ^{15}N-labeled phytoplankton samples for optical emission spectrometry[1]

Abstract—A method is described whereby the ^{15}N content of phytoplankton on a filter can be determined in an optical emission spectrometer after Kjeldahl digestion, microdiffusion, and Dumas oxidation. Total N content can be measured in the same sample, and the procedure offers some additional advantages. Values of ^{15}N content obtained in this way were the same, within the limits of analytical error, as those obtained by direct Dumas oxidation of ^{15}N-labeled plankton samples.

Optical emission spectrometry is an attractive alternative to mass spectrometry in ^{15}N investigations of nitrogen uptake by phytoplankton. The costs of installing and operating the equipment are modest, and the amount of time spent on filtering the plankton following an incubation is reduced, since the relative ^{15}N content (in atom %) can be determined in a sample no greater than 3 μg N. Murphy (1980) used emission spectrometry in a study of the Lower Great Lakes, and we have used it in the Oslofjord (Paasche and Kristiansen 1982) and elsewhere.

The isotope analysis requires prior conversion of the sample to N_2 gas. This is conveniently carried out by Dumas oxidation in an evacuated discharge tube that is subsequently placed in the emission spectrometer (Fiedler and Proksch 1975). Plankton on a glass-fiber filter can be put directly into the discharge tube together with the Dumas reagents CuO and CaO (Murphy 1980). We have used Kjeldahl digestion to convert the organic N in the plankton to an NH_4^+ salt before the Dumas oxidation. This has some practical advantages. The total N content can be determined in an aliquot of the sample, eliminating the need for a duplicate filter to be analyzed (e.g. in a CHN elemental analyzer). The N content is needed to calculate the absolute uptake rates and to determine the size of the subsample to be enclosed in the discharge tube (which must be in the range of 3–20 μg N for a discharge tube of 2.5-ml capacity). Other advantages are that there is no glass-fiber filter in the discharge tube and that somewhat smaller amounts of Dumas reagents can be used with the sample in an inorganic form. This makes it easier to outgas adsorbed atmospheric N_2 before sealing the evacuated discharge tube. Finally, the period of heating needed to complete the oxidation of N in the sample to N_2 is reduced from 15 h to 3 h at 550°C. From a theoretical point

[1] This work was supported by The Norwegian Marine Pollution Research and Monitoring Programme.

of view, it is preferable to have the sample in the same chemical form (i.e. an NH_4^+ salt) as the standards used to construct the calibration curve needed for converting the emission-spectrometric readings to true values of atom % ^{15}N. Against these advantages must be weighed the extra effort involved in a Kjeldahl digestion. We describe here the procedure we have used for preparing Kjeldahl-digested plankton for emission spectrometry and compare the results with those produced by direct oxidation of plankton on filters in the discharge tubes.

The successive steps in our procedure were as follows. The plankton was collected on a preignited 25-mm Whatman GF/C glass-fiber filter. After drying, the unstained filter edges were trimmed off, and the central part with the plankton was digested with 0.25 ml of concentrated H_2SO_4 containing 1% $NaHSeO_3$. The digest was neutralized to pH 5–7 with concentrated NaOH, using thymol blue as an indicator, and made up to 3.0 ml. A 0.25-ml subsample was diluted to 25 ml for NH_4^+ analysis by an automated indophenol blue method (Berg and Abdullah 1977), giving the N content of the sample. Blanks for the NH_4^+ analysis were prepared from digests of unused GF/C filters that had been wetted with filtered seawater and then dried. The presence of chemicals used for digestion and neutralization caused an apparent 7% increase in the intensity of the indophenol blue color, which was corrected for by means of internal NH_4^+ standards. A portion of the remaining neutralized digest calculated to contain 10–15 μg N was placed in a Conway microdiffusion vessel, and the NH_3 released upon addition of NaOH was allowed to diffuse overnight into about 10 μl of 0.5 N H_2SO_4 in a 3–4-mm length of 2-mm (i.d.) Pyrex tubing, open at both ends, that had been placed in the center well of the Conway vessel. This is a modification of a procedure described by Blackburn (1979). The recovery of NH_4^+ was better than 50%. According to Blackburn (1979), there is no isotope discrimination during microdiffusion of NH_3 under conditions similar to these. After drying at 80°C, the Pyrex tubing was placed in a discharge tube with about 10 mg CuO and 5 mg of a powdered mixture (1:1) of CaO and Al_2O_3. The discharge tube was evacuated, sealed, heated, and analyzed in the emission spectrometer as described by Fiedler and Proksch (1975). A correction for ^{14}N contamination during the digestion and subsequent handling of the samples was made by carrying a blank filter together with a known $^{15}NH_4Cl$ standard through the same procedure, and measuring the resulting isotope dilution. The mean amount of contaminating ^{14}N was 1.2 μg per filter, which corresponded to about 5% of the algal N collected on a filter in a typical experiment.

For direct Dumas oxidation, a portion of the filter with plankton was sealed into the discharge tube with 10–15 mg of CuO and 15 mg of $CaO\text{-}Al_2O_3$ (1:1) mixture. Contamination of these chemicals with ^{14}N was negligible if they were preheated to 700°C and 1,000°C.

We carried out some tests of the precision of our methods. The emission-spectrometric analysis of NH_4Cl highly (>20 atom %) enriched in ^{15}N gave a relative standard deviation of a single determination of only ±0.7% of the mean (n = 9), as read in a Statron NOI-5 emission spectrometer. The relative standard deviation at low (<1 atom % ^{15}N) enrichments probably is no better than ±3% (Fiedler and Proksch 1975). Starting from cultures of the diatom *Skeletonema costatum* labeled with about 6 atom % ^{15}N, we found a relative standard deviation of ±3.7% of the mean (n = 8) with digested samples. The precision was about the same if filters were oxidized directly in the discharge tubes. Samples at or just above the natural ^{15}N concentration of 0.37 atom % sometimes showed a sloping baseline at the $^{15}N^{14}N$ peak in the emission spectrometer, resulting in erroneously high ^{15}N atom percentages. This difficulty was encountered with Kjeldahl-digested samples as well as with those that were oxidized directly.

Figure 1 shows a comparison between

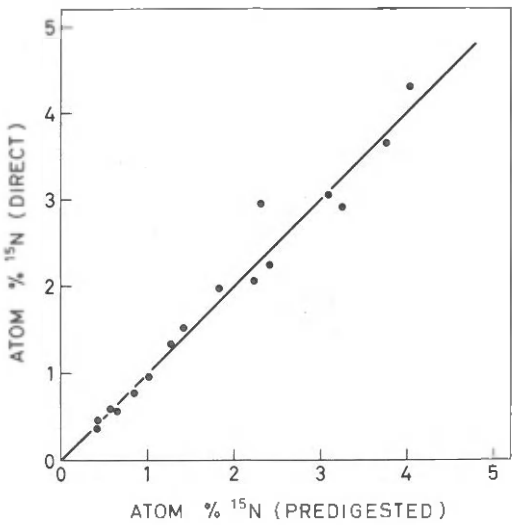

Fig. 1. Comparison of atom % ^{15}N measured in plankton samples processed by Dumas oxidation without (direct: vertical axis) and with (predigested: horizontal axis) Kjeldahl digestion and microdiffusion. Straight line represents a 1:1 relationship.

pairs of measurements based on Dumas oxidation of untreated and of Kjeldahl-digested samples, respectively. Any systematic difference is buried in the scatter arising from the relative lack of precision of both sets of measurements. This comparison suggests that either of the two methods can be used for processing plankton samples for ^{15}N analysis with no risk of large systematic errors.

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Submitted: 13 April 1981
Accepted: 6 July 1981

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