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A Simple, Inexpensive Method for Cleaning Diatoms¹

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Abstract. Diatom frustules are rapidly and inexpensively cleared of protoplasts using commercially available bleach (5.25% sodium hypochlorite). A comparison of periphyton subsamples cleaned by the bleach method described, and the standard nitric acid/potassium dichromate method, showed little difference in the extent to which frustules are disjoined (hypotheca from epitheca). A community analysis of subsamples showed no differences in taxa or disappearance of small forms when comparing the two methods. No sample heating, subsequent treatment, or fume hoods are required for this procedure.

Traditionally, diatom cleaning techniques have involved the use of strong acids, alone or in combination with chemical agents, to dissolve protoplast material. Van Heurck (1896) reviewed methods used by nineteenth century diatomists; his own procedures involved use of either boiling nitric acid, or boiling sulfuric acid with added potassium chlorate. He cautioned that the nitric acid emits harmful fumes, but with the latter method there is risk of explosion.

In the more recent literature, hot sulfuric acid with added potassium dichromate (Patrick & Reimer, 1966) and boiling nitric acid with potassium dichromate (American Public Health Association, 1981) are recommended to clear diatom frustules. Heating of either acid requires use of a fume hood or other ventilating device. The technique using hydrogen peroxide and potassium dichromate or potassium permanganate (Werff, 1955) was proposed as an alternative to the acid cleaning methods. This method, however, involves a sometimes violent reaction. In addition, hydrogen peroxide is relatively expensive to use when numerous samples are involved. A more thorough review of modern diatom cleaning techniques is given by Ma & Jeffery (1978).

Commercially available bleach (5.25% sodium hypochlorite) has been used by others to clear aquatic plant leaves to enable direct microscopic viewing of periphytic assemblages (Carter, 1982) and to clear zooplankton for identification of their gut contents (Infante, 1978). In this paper, we describe a one-step cleaning method to clear diatom frustules using bleach, followed by a succession of rinsings and decantations, which produces material equivalent to

¹ This method was developed for use in a study of Missouri River periphyton supported by U.S. Army Corps of Engineers Contract DACW45-83-C-0206 to G. L. Hergenrader. Verification of diatom taxa at the Diatom Herbarium of the Academy of Natural Sciences of Philadelphia was made possible through McHenry Funds awarded to J. Carr by the Academy. Assistance and encouragement by Dr. Charles W. Reimer of the Academy of Natural Sciences and G. A. Cunningham is gratefully acknowledged.

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TABLE I

Mean percentage of unseparated frustules from replicate subsamples, based on 1,000 frustules counted per subsample^a

Cleaning method	Percent unseparated
1-h bleach (replicates A, B)	98.8 (0.30)
2-h bleach (replicates A, B)	97.4 (0.75)
3-h bleach (replicates A, B)	97.3 (0.06)
4-h bleach (replicates A, B)	95.3 (0.86)
Nitric acid (replicates A, B, C, D)	95.2 (0.36)

^a Values in parentheses indicate standard error (SE).

that from other, more tedious procedures. Bleach has the advantage of being readily available, does not require heating, is inexpensive to use, and does not necessitate the use of accessory equipment (i.e., fume hoods).

MATERIALS AND METHODS

The diatom sample to be cleaned was placed in a small beaker to which a few ml of distilled water was added. If the sample contained preservative, it was rinsed once by filling the beaker with distilled water, and allowed to settle, after which most of the water was decanted carefully prior to the addition of bleach.

Commercially available bleach (5.25% sodium hypochlorite) was added to the beaker containing the sample. One part bleach to one part of sample with water worked best for periphyton samples in which considerable quantities of organic matter were present. The beaker was covered with a watchglass to prevent contaminants from entering the sample.

The sample was allowed to stand for 1–2 h (depending on the quantity of organic matter present), and was agitated periodically during that time. Following the cleaning procedure, the beaker was filled with distilled water and covered with a watchglass until the sample had settled.

The diatom sample then was decanted and rinsed six or more times with distilled water until the supernatant water had cleared, and allowed to settle between rinsings. (Rinsing the samples a minimum of six times was found to return the pH to approximately 7.) After the final rinsing, the excess water was poured off and the sample transferred to its storage container. Diatoms were dried onto microscope coverglasses and mounted using standard techniques.

In order to compare the standard boiling nitric acid with added potassium dichromate method to our bleach method, a series of subsamples of a Missouri River periphyton collection were cleaned. Duplicate subsamples were cleaned in bleach for 1, 2, 3, and 4 h each at a concentration of one part bleach to one part sample with water (1–2 ml sample plus 3–4 ml distilled water to which 5 ml of 5.25% sodium hypochlorite was added). Four subsamples from the same collection were cleaned by boiling 2 ml of subsample in 20 ml nitric acid with a few crystals of potassium dichromate for 2 h. Both the bleach-cleaned

TABLE II

SIMI values for pooled replicates of subsample assemblages compared for each cleaning method

	Cleaning method			
	2-h bleach	3-h bleach	4-h bleach	Nitric acid
1-h bleach	0.996	0.972	0.975	0.981
2-h bleach	—	0.976	0.989	0.988
3-h bleach	—	—	0.972	0.991
4-h bleach	—	—	—	0.975
All bleach subsamples				0.991

and acid-cleaned subsamples were rinsed as described herein, dried onto cover-glasses, and mounted in Hyrax. One thousand valves from each subsample were identified using a Wild M-20 phase-contrast microscope at 1,000 \times . Taxonomic keys consulted were Hustedt (1930) and Patrick & Reimer (1966, 1975).

As a comparison of the degree of similarity of subsample assemblages, a similarity index, SIMI, was employed to determine if bleach treatment in some way altered assemblage composition. SIMI is analogous to a correlation coefficient and is computed using the equation.

$$\text{SIMI}(h, j) = \frac{\sum_{i=1}^n P_{hi} \times P_{ji}}{\sum_{i=1}^n P_{hi}^2 \sum_{i=1}^n P_{ji}^2}$$

where: P_{hi} and P_{ji} are the proportions of individuals represented by the i^{th} taxon in assemblages h and j , and n is the total number of taxa in each assemblage (McIntire & Moore, 1977). SIMI values range from 0 to 1 and represent the probability of drawing frustules of the same taxon from both of the compared assemblages.

RESULTS AND DISCUSSION

Percent frustule separation (epitheca from hypotheca) was compared for each subsample (Table I). Although a progressively greater percentage of frus-

TABLE III

SIMI values for all possible comparisons of individual subsample assemblages of 4-h bleach and nitric acid treatments

	Cleaning method				
	4-h bleach (rep. A)	4-h bleach (rep. B)	Nitric acid (rep. A)	Nitric acid (rep. B)	Nitric acid (rep. C)
4-h bleach (replicate B)	0.991	—	—	—	—
Nitric acid (replicate A)	0.980	0.969	—	—	—
Nitric acid (replicate B)	0.992	0.999	0.968	—	—
Nitric acid (replicate C)	0.936	0.941	0.981	0.936	—
Nitric acid (replicate D)	0.912	0.906	0.958	0.898	0.973

tules was separated with increasing contact time in bleach (cf. 98.8% unseparated after 1 h in bleach with 95.3% left unseparated after 4 h), the bleach treatment compared favorably, even after 4 h, with the nitric acid treatment which left an average of 95.2% unseparated.

Relative abundance of each taxon was determined for subsample replicates for the cleaning methods, and data from replicates were pooled to calculate SIMI values (Table II). Comparisons of bleach-cleaned subsamples vs. bleach-cleaned subsamples yielded a range of SIMI values (0.972–0.996) with a mean of 0.980. SIMI values of bleach-cleaned subsamples vs. nitric acid-cleaned subsamples yielded a range (0.975–0.991) with a mean of 0.984. Pooling all bleach-cleaned subsample data and comparing those with pooled nitric acid-cleaned subsample data produced a SIMI value of 0.991.

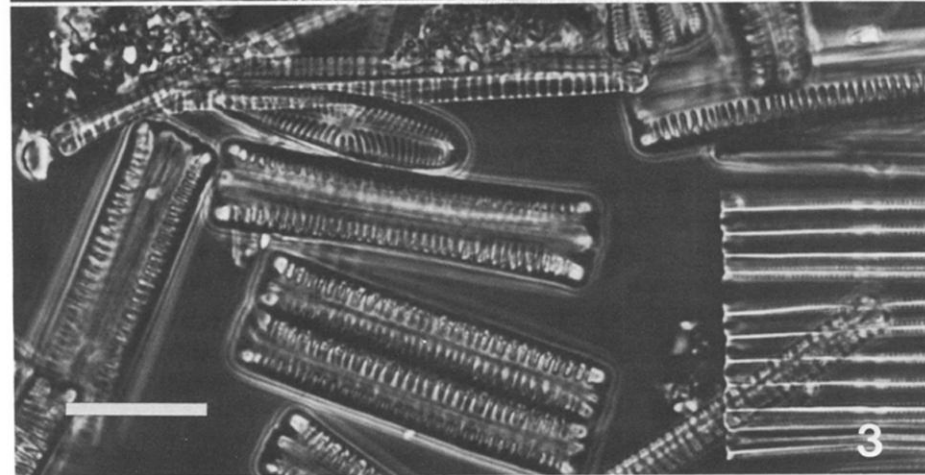
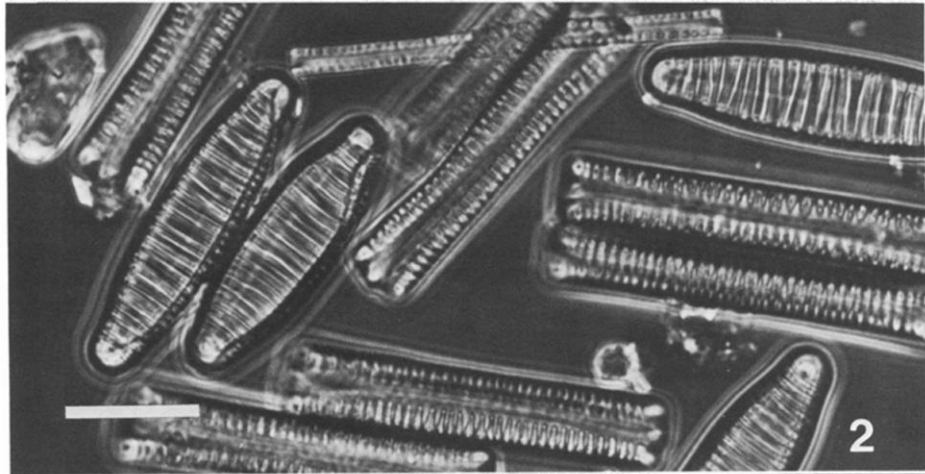
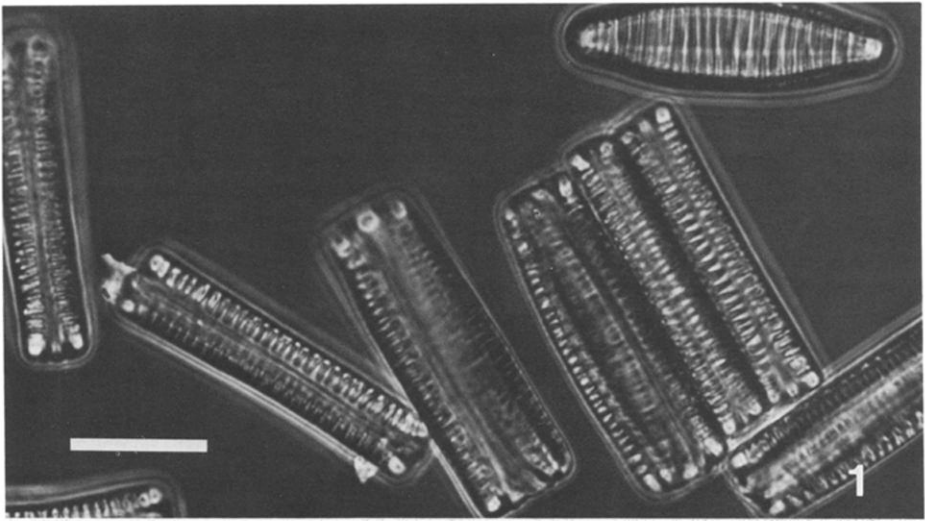
Pooling subsample data can obscure assemblage variability within individual replicates. If bleach treatment were to adversely affect assemblage composition, then longer contact with bleach (i.e., 4 h) should show the greatest difference when comparing with nitric acid-cleaned subsamples. SIMI values for replicate 4-h bleach-cleaned subsamples vs. nitric acid-cleaned subsamples ranged from 0.906–0.999 (Table III) with a mean of 0.954. This value is lower than the SIMI value of 0.975 derived from pooled 4-h bleach-cleaned subsamples vs. pooled nitric acid-cleaned subsamples (Table II), again illustrating the effect of pooling subsample assemblage data. Further, an examination of SIMI values derived from comparing nitric acid-cleaned subsamples with nitric acid-cleaned subsamples (Table III) shows a range of values (0.898–0.981) with a mean of 0.952. This mean value is slightly lower than the 4-h bleach vs. nitric acid comparison value of 0.954. Accordingly, we conclude that subsample assemblage differences, reflected by SIMI values, are caused by inherent variability within the periphyton sample itself and not by bleach treatment. Moreover, visual comparisons of the two cleaning methods (Figs. 1–3) indicate similar optical qualities in the subsamples.

We have used both the nitric acid with potassium dichromate and hydrogen peroxide with potassium dichromate methods in our work with Missouri River periphyton. When large amounts of organic matter were present in our samples, the nitric acid cleaning procedure had to be repeated. The hydrogen peroxide method was unpredictable; either no reaction occurred, or sometimes violent eruptive bubbling caused loss of samples from their containers.

Generally, use of strong bases to clear diatom frustules is not recommended because of resultant silica dissolution. Sodium hypochlorite is a strong base with a pH of 11.5, and care should be exercised in its use to prevent possible loss of samples. Use of higher bleach concentrations or longer contact with bleach than recommended here has been observed to cause etching of the frustules, and, in some cases, separation of girdle bands and alteration of external frustule features.

The bleach method has not been tested with marine diatoms. The reader is cautioned that the rinsing method described here could result in a loss of minute planktonic forms which do not settle out readily.

The diatom cleaning technique described here has proven to be cost-effec-



tive and more efficient than other techniques used previously for Missouri River periphyton samples. The optical properties of the materials so prepared are comparable to those subjected to traditional methods.

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FIGS. 1-3. Cleaned diatom frustules (mainly *Diatoma vulgare* Bory) from a sample collected 17 February 1983 off of rocks below Gavins Point Dam on the Missouri River. Scale bars each indicate 20 μ m. Photomicrographs taken using a Wild M-20 phase-contrast microscope fitted with camera attachment at 400 \times . Fig. 1. Subsample cleaned in bleach for 1 h. Fig. 2. Subsample cleaned in bleach for 2 h. Fig. 3. Nitric acid-cleaned subsample from the same collection.