BRITISH STANDARD

BS EN 13946:2003
BS 6068-5.33:2003

Water quality — Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers

The European Standard EN 13946:2003 has the status of a British Standard

ICS 13.060.70

BSI British Standards

National foreword

This British Standard is the official English language version of EN 13946:2003.

The UK participation in its preparation was entrusted by Technical Committee EH/3, Water quality, to Subcommittee EH/3/5, Biological methods, which has the responsibility to:

— aid enquirers to understand the text;

— present to the responsible international/European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;

— monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this subcommittee can be obtained on request to its secretary.

This is one of a series of standards on water quality, others of which have been, or will be, published as Sections of BS 6068. The various Sections of BS 6068 are comprised within Parts 1 to 7, which are listed below.

Part 1 *Glossary*

Part 2 *Physical, chemical and biochemical methods*

Part 3 *Radiological methods*

Part 4 *Microbiological methods*

Part 5 *Biological methods*

Part 6 *Sampling*

Part 7 *Precision and accuracy*

NOTE The tests described in this British Standard should only be carried out by suitably qualified persons with an appropriate level of biological expertise. Standard biological procedures should be followed throughout.

Cross-references

The British Standards which implement international or European publications referred to in this document may be found in the *BSI Catalogue* under the section entitled "International Standards Correspondence Index", or by using the "Search" facility of the *BSI Electronic Catalogue* or of British Standards Online.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

This British Standard, was published under the authority of the Standards Policy and Strategy Committee on 8 August 2003

Summary of pages

This document comprises a front cover, an inside front cover, the EN title page, pages 2 to 14, an inside back cover and a back cover.

The BSI copyright notice displayed in this document indicates when the document was last issued.

Amendments issued since publication

Amd. No.	Date	Comments

ISBN 0 580 41960 6

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 13946

May 2003

ICS 13.060.70

English version

Water quality - Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers

Qualité de l'eau - Guide pour l'échantillonnage en routine et le prétraitement des diatomées benthiques de rivières

Wasserbeschaffenheit - Leitfaden zur Probenahme und Probenaufbereitung von benthischen Kieselalgen in Fließgewässern

This European Standard was approved by CEN on 21 February 2003.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

Ref. No. EN 13946:2003 E

Contents

page

Foreword **3**

Introduction **3**

1 Scope **4**

2 Principle **4**

3 Terms and definitions **4**

4 Equipment **5**

5 Reagents **5**

6 Procedure **6**

Annex A (informative) **Methods for cleaning diatoms for microscopic examination** **10**

Bibliography **14**

Foreword

This document (EN 13946:2003) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2003, and conflicting national standards shall be withdrawn at the latest by November 2003.

Annex A is informative.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and the United Kingdom.

Introduction

WARNING — Persons using this European Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory conditions.

Diatoms are an important component of aquatic ecosystems and constitute a water quality monitoring tool where the primary objective is either a measure of general water quality or of specific components of water quality (e.g. eutrophication, acidification). The requirement for the monitoring of such processes is inherent in the Water Framework Directive (2000/60/EC) and Urban Waste Water Treatment Directive (91/271/EEC) in addition to other EU Directives and international agreements. This European Standard covers aspects of sampling and pre-treatment relevant to assessment of water quality using benthic diatoms. Some aspects may also be relevant to measures of ecological integrity. These sampling instructions will result in samples suitable for quantifying relative numbers of benthic diatom taxa present. If it is necessary to quantify absolute numbers of taxa, or fresh weight per unit area, modifications to the method are required, which are not within the scope of this standard.

The use of diatoms as indicators of river water quality is widely accepted both in Europe and the USA. The methodology is based on the fact that all diatom species have tolerance limits and optima with respect to their preference for environmental conditions such as nutrients, organic pollution and acidity. Polluted waters will tend to support an increased abundance of those species whose optima correspond with the levels of the pollutant in question. Conversely, certain species are intolerant of elevated levels of one or more pollutants, whilst others can occur in a wide range of water qualities.

Methods using diatoms to assess water quality have been developed in several European countries (recent work is summarized in the proceedings of three symposia [1 to 3]. The methodologies for evaluating the diatom data vary but the sampling and pre-treatment processes are similar [4].

According to the precise usage to which this standard is to be put it is essential for specifiers and users to agree on any necessary variations or optional procedural details prior to use.

All numerical values given in this standard are approximate.

1 Scope

This guidance European Standard establishes a method for the sampling and laboratory preparation of benthic diatoms for water quality assessments. Data produced by this method are suitable for production of water quality indices based on the relative abundance of taxa. With appropriate modifications the method can be applied to the study of benthic diatoms in lakes.

2 Principle

Benthic diatoms from submerged hard surfaces or submerged macrophytes in rivers or streams are sampled in order to produce representative collections of the diatom assemblage indicative of water quality. Samples are cleaned using strong oxidizing agents in order to prepare diatoms for identification and enumeration.

The data obtained from the microscopic analysis of these samples are suitable for the production of diatom-based water quality indices (see references 1, 2, and 3).

3 Terms and definitions

For the purposes of this European Standard, the following terms and definitions apply:

3.1
artificial substratum
substratum introduced into river by operator specifically for colonisation by diatoms

3.2
benthic diatoms
diatoms living on substrata, rather than suspended in the water column

3.3
boulder
mineral substratum with a diameter > 256 mm

3.4
cobble
mineral substratum with a diameter > 64 mm and ≤ 256 mm

3.5
euphotic zone
the part of the water column in which there is sufficient light for photosynthesis

3.6
frustule
cell wall of diatoms, composed of silica and consisting of two valves linked by two or more girdle bands

3.7
habitat
the specific environment in which an organism lives

3.8
pebble
mineral substratum with a diameter > 16, ≤ 64 mm

3.9
riffle
shallow part of a stream with swift flow, usually with a broken surface

3.10
substratum
natural or non-natural material from which benthic diatoms are sampled

3.11
taxa
taxonomic units, for example families, genera or species

3.12
valve
structural component of the diatom frustule (3.6)

4 Equipment

4.1 Field sampling

— Appropriate water safety equipment;

— Waders;

— Stiff toothbrush (or other similar instrument) or knife (or other suitable blade);

— Plastic tray (approximately 30 cm × 20 cm or larger);

— Sample bottle with a tightly fitting lid;

— Indelible marker pen (or other means of labelling samples). If labels are used, these shall be capable of surviving wet conditions;

— Hoe, with a fine-meshed net attached and a long handle(if vertical hard surfaces are to be sampled);

— A glass-bottomed box or bucket (“Aquascope”) is useful for finding suitable substrata under some circumstances.

4.2 Laboratory

See annex A.

5 Reagents

5.1 General

Reagents used in the preparation of the diatom frustules need not be of analytical grade but should be of a quality appropriate for the digestion process.

5.2 Preservatives

These are required to stop cell division of diatoms and decomposition of organic matter. No preservative is necessary if the sample is to be processed within a few hours of collection, as long as steps are taken to minimize cell division (i.e. by storage in cool, dark place). Lugol’s iodine can be used for short-term storage; however, it is not suitable for long-term storage, due to problems caused by sublimation. Buffered formaldehyde or ethanol are recommended for long-term storage of samples. Samples can also be deep-frozen.

5.2.1 General

5.2.2 Buffered 4% v/v (minimum) formaldehyde (HCHO) solution

Dilute a stock solution of formaldehyde to 4 % in a solution buffered to pH 7. Suitable buffers include HEPES (*N*-2-hydroxymethylpiperazine-*N*-2’-sulfonic acid), borate and hexamethylenetetramine.

A final solution of 1 % to 4 % (v/v) is recommended (the quantity required will depend upon the amount of organic matter present in the sample).

NOTE The buffer is necessary to prevent dissolution of the silica frustules.

5.2.3 Lugol's iodine

Dissolve 2 g potassium iodide and 1 g iodine crystals in 300 ml distilled or demineralised water. The resultant liquid should be straw coloured. It should be stored in an air-tight and light-proof container to minimise sublimation.

Add 1 to 5 drops of Lugol's iodine per 100 ml sample to give a final "straw" colour. More may be necessary if samples are rich in organic matter.

NOTE Some recipes for Lugol's iodine include acetic acid or glutaraldehyde to prevent loss of flagella. These reagents should be omitted when the solution is to be used for diatoms, as they can lead to the dissolution of silica.

70% Ethanol (C_2H_5OH) can also be used for this purpose.

5.3 Reagents for cleaning diatoms

See annex A.

5.4 Reagents for preparing permanent slides

A diatom mountant with a refractive index > 1,6 is required. Proprietary brands include Naphrax and Hyrax.

6 Procedure

6.1 Choice of substratum

Diatoms can be found growing on most submerged surfaces; however, the composition of the community varies depending upon the substratum chosen. Ideally, a single substratum should be used at all sites included in a survey.

Areas of the river bed with naturally occurring moveable hard surfaces (large pebbles, cobbles and boulders) are recommended wherever possible. If such hard surfaces do not occur naturally, then it is possible to sample vertical faces of man-made structures such as quays and bridge supports (so long as these are not made from wood). Other man-made hard surfaces, such as bricks can also be sampled, if these have been in the river for long enough to ensure that assemblages are in equilibrium with their environment. At least four weeks is recommended but the period depends upon environmental conditions. See also comments in 6.3.3.

In deeper rivers where the underlying substrata are finer silts and sands (and when no hard substrata are available) consideration should be given to the introduction of artificial substrata within the euphotic zone.

Samples of diatoms can also be collected from submerged macrophytes. Where possible, comparative studies in rivers should be based on samples collected from the same macrophyte species (or group of morphologically similar species).

6.2 Sample site selection

A segment of river that has substrata suitable for sampling should be selected. As a general rule, this should be about 10 m in length, but longer lengths may be appropriate, depending upon the physical uniformity of the river and the availability of substrata. "Riffles" are preferred, as these tend to have a good variety of natural hard surfaces (6.1).

A detailed description of the site (location, width, depth, substratum type, percent cover of macrophytes, shade etc.) is required on the first occasion that a sample is collected. A photographic record is also recommended. This information serves as an aid the interpretation of data and to help future samplers locate the site. On subsequent visits, notes may be limited to major changes that have occurred since the previous visit, and any variations in sampling protocol employed.

6.3 Sampling methods

6.3.1 Moveable natural hard surfaces

In general, cobbles are the preferred substratum for sampling, as these balance substratum stability (allowing diatom communities to develop) with manoeuvrability. Pebbles and boulders can also be used. At least five cobbles should be sampled. However, if cobbles are unavailable, then either 5 small boulders or 10 pebbles should be sampled. An area of approximately 10 cm^2 or more should be scraped. If fewer suitable substrata are available, then a note should be made to this effect.

The following microhabitat conditions should be fulfilled:

1) areas of heavy shade should be avoided (if it cannot be avoided, then a note should be made to this effect). Areas very close to the bank should also be avoided;

2) the substrata shall be submerged for long enough to ensure that assemblages are in equilibrium with their environment. At least four weeks is recommended but the period depends upon environmental conditions. The precise depth is unimportant so long as the surfaces have not been exposed to air. All depths that can be easily sampled wearing waders are usually suitable, so long as these remain in the euphotic zone;

3) in general, samples should be collected from within the main flow of the river at the sample site. Zones of very slow current (approx. $\leq$ 20 cm s^{-1}) should be avoided as these allow the build-up of loosely attached diatoms, silt and other debris.

Collect a selection of substrata from a variety of locations within the sample site, which fulfil the microhabitat requirements listed above. Where suitable substrata are very abundant, random or stratified sampling strategies may be appropriate within a defined sample site.

Remove any loosely attached surface contamination (e.g. organic debris) by washing the substratum briefly in the stream water. Place the substrata in a tray, along with approximately 50 ml of river water.

Wash a stiff toothbrush in clean river water and rub it on a clean surface in order to minimise any diatom contamination from previous samples. Brush the upper surface of the substratum vigorously to remove the diatom film, rinsing the toothbrush periodically in the water in order to transfer the diatoms.

A knife or other sharp instrument can also be used to remove the diatom film. This will be more effective at removing firmly-attached diatoms, but will be less efficient at penetrating crevices on rough surfaces, may cause more damage to frustules and may lead to more rock particles being transferred to the sample. However, it is unlikely that there will be any quantitative difference in results. The knife should also be rinsed in river water and cleaned before use.

Alternatively, the diatom film can be removed using a toothbrush or knife and washed directly from the surface of the substratum into a sample bottle. The toothbrush or knife can also be used to remove diatoms from the substratum and then rinsed in some stream water collected directly into a sample bottle, if this is preferred.

If > 75 % of substrata are smothered with filamentous algae, these should be sampled in preference to substrata lacking such growths. Remove as many of the filaments as possible prior to brushing or scraping, as above.

Replace the substratum in the stream, and repeat the process for the other replicate substrata. Transfer the water, which should now be brown and turbid due to the presence of diatoms, from the tray into the sample jar.

Label the sample bottle with details relevant to the sample. Transfer the sample to the laboratory in a cool, dark place. If samples are brought to the laboratory within 24 h and these precautions are followed, it is not necessary to add preservative in the field. If preservatives are necessary, then these should be added immediately after collection, unless there are other reasons (e.g. health and safety) why preservatives cannot be used in the field. All future handlers of preserved samples shall be informed of the nature of any preservatives present.

6.3.2 Method for sampling vertical man-made surfaces *in situ*

The criteria listed above for microhabitat selection should be followed as far as possible.

As this type of sampling is often necessary in lowland, navigable rivers a sample depth of about 30 cm is recommended to allow for fluctuating water levels and wave action.

Agitate the hoe in the water in front of the area to be sampled to dislodge any loosely attached materials.

Scrape the surface with the sharpened blade of the hoe to remove the attached diatoms. An area of approximately 10 cm^2 should be scraped. Then remove the diatom film adhering to the hoe blade and directly place into a sample bottle into which some river water has been placed.

NOTE Specialised apparatus may also be useful under some circumstances. More details are given in reference [4].

This process should be repeated at least three times and the replicates pooled. For details regarding labelling and transfer to the laboratory see 6.3.1.

6.3.3 Use of introduced ("artificial") substrata

Substrata with heterogeneous surfaces (e.g. rough tiles, frayed polypropylene rope) are preferred over substrata with smooth surfaces (e.g. glass slides). These should be left in the river for long enough to ensure that assemblages are in equilibrium with their environment. At least four weeks is recommended, but the period depends upon environmental conditions and longer periods of exposure may be appropriate under some circumstances (i.e. very oligotrophic conditions, low temperatures, heavy shade).

Detailed methods will depend upon the type of substratum chosen. If rough tiles are used, then samples can be collected as described in 6.3.1. For frayed ropes, the final 5 cm is removed with a pair of scissors or brushed with a toothbrush and placed in a sample container. Full details of methods are necessary if results from introduced substrata are to be interpreted correctly.

Care should be taken with the design and deployment of introduced substrata to ensure that they do not interfere with the activities of legitimate river users and to minimise risks of vandalism. Extra, replicate substrata should be deployed, to allow for potential losses due to spates or vandalism.

Where introduced substrata are to be used for comparative studies in the same watercourse, it is important that all substrata are exposed to identical conditions. Both the length of exposure and the start date shall be the same (to allow for the impact of hydrological events upon the developing diatom community).

For details regarding labelling and transfer to the laboratory see 6.3.1.

6.3.4 Sample collection from submerged macrophytes and macroalgae

Sample the entire plant (five replicates) and place into a plastic bag for transfer to the laboratory. Stir or agitate the plants vigorously in some distilled or demineralised water in a large beaker to dislodge attached diatoms. Remove the macrophytes from the beaker, allow the diatoms to settle and pour off the supernatant.

Alternatively, cut some lengths at random from submerged plants using a knife or scissors and put the sections into a sampling bottle. These can be fractionated further in the laboratory, if required, and the macrophyte sections plus attached diatoms placed directly in a flask for cleaning (6.4.2).

In the case of filamentous macroalgae, it is also possible to gently squeeze a handful of material and to collect the expressed suspension (which will contain epiphytic diatoms) in a sample bottle.

Methods described in 6.3.5 may also be appropriate under some circumstances.

6.3.5 Sample collection from emergent macrophytes

In general, samples should be collected from emergent macrophytes only if there are portions that remain permanently submerged but which are not contaminated by the bottom sediments.

Cut stems at water level and invert a plastic sampling bottle or glass jar over the underwater stem. Cut off the stem below the mouth of the bottle, then turn the bottle plus stem back to an upright position and close. In the laboratory remove the diatoms from the stem by stirring, scraping or gentle brushing.

If the diatom film is not delicate, then it may be possible to cut 5 to 6 stems and transfer these directly to a sampling container without the need to invert bottles over the stem.

6.4 Pretreatment prior to microscopic examination

6.4.1 Preservation and preliminary laboratory treatment

Samples should be placed in a cool, dark place on return to the laboratory and allowed to stand for at least 24 h, after which suspended material has settled to the bottom of the jar (the clear supernatant can be poured off carefully). Alternatively, the sample can be centrifuged. Any large substrata or coarse plant material should first be removed by passing the sample through a kitchen sieve. The speed and time required to effect complete sedimentation of all diatoms (including very small taxa) will depend upon the characteristics of the individual centrifuge used. Preliminary tests should be performed to ensure that no diatoms are left in the supernatant at the selected speed and time.

If preservatives are necessary, but were not added in the field, then these should be added now. All future handlers of preserved samples shall be informed of the nature of any preservatives present.

A preliminary microscopic examination of the sample is recommended. Unusual features (e.g. large numbers of empty frustules) should be noted.

A portion of the sample shall always be retained in case problems are encountered during the preparation process.

6.4.2 Methods for cleaning diatoms

For accurate identification of diatoms, it is necessary to remove all the cell contents and mount the diatoms using a mountant with a high refractive index. This can be effected by exposure to strong oxidizing agents. Hydrogen peroxide is the most commonly used oxidizing agent and is recommended here, but other methods will also give satisfactory results. Some suitable protocols are described in annex A.

Samples rich in organic matter in addition to diatoms need stronger oxidation than clean ones and the optimum ratio of sample to oxidants may need to be tested first. Solid carbonates can cause problems and may influence the type of treatment and the sequence of oxidants used (for example, in carbonate-rich waters the carbonates have to be removed by HCl before using strong sulfuric acid in order to prevent formation of calcium sulphate). Treatment with dilute HCl will also be necessary for samples which are rich in iron.

6.4.3 Preparation of permanent slides

Dilute the cleaned diatom suspension to a suitable concentration. When held up to the light, fine particles should be just visible in the suspension. If the suspension appears very concentrated distilled or demineralised water should be added to reduce their concentration. The density of valves can also be checked quickly by evaporating a drop of the suspension on a coverslip and examining this under medium power objective (i.e. 400 ×). If the suspension is very dilute, either centrifuge the sample again to concentrate the diatoms or allow the diatoms to settle and decant the excess supernatant. If this fails, repeat the preparation procedure.

NOTE Ethanol can also be added to dilute the suspension. This also helps the diatoms to spread evenly on the coverslip.

Shake the vial containing the suspension of cleaned material. Using a clean Pasteur pipette, remove some of the suspension from the central part of the tube. Place a drop onto a new coverslip. Allow the liquid to evaporate, either by leaving the coverslip in a warm, dust-free environment (e.g. in a desiccator) or by heating gently on a hotplate. The result should be a thin grey film over about two-thirds of the coverslip.

Follow manufacturers guidelines on use of the mountant. Ensure that the mountant spreads right to the edge of the coverslip. Allow to cool and check under the microscope. Ideally, the preparation should have between 10 to 15 valves per field at a magnification of 1 000 ×. If the slide has too many valves per field, repeat the procedure with a more dilute sample of digested diatoms.

Label the slide with, as a minimum, details of location and sampling date plus any codes necessary to cross-reference the slide to other information.

Annex A
(informative)

Methods for cleaning diatoms for microscopic examination

A.1 General

Many different methods for cleaning diatoms have been specified in the literature and are suitable for use for studies of water quality. Details of methods are given here, but other methods may be equally suitable. Quantities can be adapted, so long as ratios between reagents are maintained, in order to suit local conditions. The appropriate batch size will also depend upon local conditions.

Care shall be taken to ensure that all apparatus is as clean as possible, in order to minimize the risk of contamination between samples. Stirring rods shall not be used to stir more than one sample so that diatoms are not passed from one to another. Pasteur pipettes shall be used for only one sample and then discarded.

A.2 Method 1: Hot hydrogen peroxide

A.2.1 Apparatus

— Fume cupboard or equivalent system;

— Hot plate, sand bath or water bath;

— Beakers or boiling tubes (one per sample);

— Means of measuring 20 ml volumes of oxidising agents;

— Clean Pasteur pipettes;

— Centrifuge (optional).

NOTE If a centrifuge is not available, samples can be allowed to stand overnight whilst solid material settles, after which the supernatant should be poured off carefully.

— Centrifuge tube (optional). These tubes shall be resistant to attack by the oxidising agents or acids used to clean the diatoms.

A.2.2 Reagents

— 30 % (100 volume) hydrogen peroxide (H_2O_2) solution.

— Dilute (e.g. 1 mol/l) hydrochloric acid (HCl).

A.2.3 Method

Homogenize the sample by shaking and transfer 5 ml to 10 ml of the suspension to a beaker or boiling tube. Add approximately 20 ml of hydrogen peroxide and heat on a hotplate, sand bath or water bath at about (90 ± 5) ºC until all organic material has been oxidized (typically 1 h to 3 h). Coarse plant material in macrophyte samples can be removed after 30 min. Caution is needed while pouring cold concentrated hydrogen peroxide onto rich organic material and aquatic plants, and also during the heating process.

Remove the beaker or boiling tube from the heat. Add a few drops of hydrochloric acid to remove remaining hydrogen peroxide plus any carbonates and wash down sides of beaker with distilled or demineralised water. Allow to cool in the fume cupboard.

NOTE The addition of hydrochloric acid can be omitted if the sample comes from a region where carbonates are unlikely to be present.

Transfer contents of beaker or boiling tube to centrifuge tube, top up with distilled or demineralised water and centrifuge (or allow to stand – see A.2.1). Decant supernatant and resuspend pellet with distilled or demineralised water and repeat centrifugation.

The washing process should be repeated at least three times, or until all traces of hydrogen peroxide have been removed. When all traces of hydrogen peroxide and acid have been removed, mix the diatom pellet in a small amount of distilled or demineralised water and transfer to a clean, small capacity vial. Add a few drops of 4 % formalin, hydrogen peroxide or ethanol to prevent fungal growth. The sample can then be stored indefinitely.

A.3 Method 2: Cold hydrogen peroxide

A.3.1 Apparatus and reagents

As Method 1, but excluding hotplate, sand bath or water bath.

A.3.2 Method

Follow Method 1 but do not heat the beaker containing the sample. Instead leave the beaker (covered by a watch glass or similar) for at least four days. For acceleration of oxidation put the beakers in the sunlight or under an UV lamp.

Then transfer the contents of the beaker to a centrifuge tube and continue with Method 1.

If this does not result in cleaned frustules, replace the hydrogen peroxide and leave for another period or use another method after washing.

A.4 Method 3: Hot hydrogen peroxide with potassium dichromate

A.4.1 Apparatus

As Method 1

A.4.2 Reagents

As Method 1, with addition of crystalline potassium dichromate (or potassium permanganate).

A.4.3 Method

Homogenize the sample by shaking and transfer 2 ml to 5 ml of the thick suspension to a beaker. Add 50 ml of hydrogen peroxide and heat under a fume cupboard on a hotplate at 90 °C until all organic material has been oxidized (0,5 h to 3 h). The samples may bubble vigorously. Caution is needed while pouring cold concentrated hydrogen peroxide onto rich organic material and aquatic plants, and also during the heating process.

Remove the beaker from the heat. Add a spatula tip of potassium dichromate grain by grain (note that this normally causes additional effervescence). This should result into a clear bluish-green solution after a few minutes.

If it is still turbid, add a few drops of hydrochloric acid to remove remaining hydrogen peroxide plus minor amounts of carbonates and wash down sides of beaker with distilled or demineralised water. If larger quantities of carbonates are present, add 20 ml of concentrated hydrochloric acid and heat gently.

Then transfer the contents of the beaker to a centrifuge tube and continue with Method 1.

When all traces of hydrogen peroxide and acid have been removed, mix the diatom pellet in a small amount of distilled or demineralised water and transfer to a clean, small capacity vial. Add a few drops of 4 % formalin, hydrogen peroxide or ethanol to prevent fungal growth. The sample can then be stored indefinitely.

A.5 Method 4: Cold acid (permanganate) method of cleaning

A.5.1 Apparatus

— Fume cupboard.

Means of measuring 5 ml to 10 ml volumes of acids and oxidising agents.

NOTE 1 Automatic pipettes, if used, should be periodically stripped down and cleaned to prevent corrosion.

— Pasteur pipettes;

— Centrifuge tubes.

NOTE 2 The entire procedure can be performed in large (e.g. 30 ml) centrifuge tubes, as long as these tubes are made of a material that is resistant to the acid mixture used.

— Centrifuge.

A.5.2 Reagents

— Dilute (e.g. 1 mol/l) hydrochloric acid (HCl).

— Concentrated sulfuric acid (H_2SO_4)

Either Potassium permanganate ($KMnO_4$), as crystals (approximately 0,1 g to 0,5 g per sample) or a saturated solution of potassium permanganate (1 ml to 2 ml per sample).

Saturated oxalic acid ($C_2H_2O_4$): dissolve approximately 10 g oxalic acid crystals in 100 ml distilled or demineralised water over gentle heat whilst stirring. Allow to cool. Crystals of oxalic acid should precipitate out. If not, add some more oxalic acid and repeat the heating and cooling stages.

A.5.3 Method

Homogenize the sample by shaking and transfer 5 ml to 10 ml of the suspension to a centrifuge tube.

If calcareous material is present (or suspected) in the sample, this should be removed first. Add dilute hydrochloric acid drop wise until effervescence, indicating carbon dioxide release has ceased.

Add distilled or demineralised water and centrifuge. Discard the supernatant.

NOTE This stage can be omitted if it is certain that the sample was not collected from a catchment where calcareous rocks are present.

Carefully add 5 ml of concentrated sulfuric acid.

Add approximately 0,1 g of solid potassium permanganate (or a few drops of saturated potassium permanganate solution) and agitate gently to allow the crystals to dissolve. The suspension will turn purple after this stage. If using potassium permanganate crystals, it is important that these have dissolved completely before proceeding to the next step.

Slowly add 10 ml of saturated oxalic acid to the sample, which will result in strong effervescence. The end-result should be a suspension of bleached particles (mainly diatom valves).

Add distilled or demineralised water and centrifuge. Approximately 3 000 revolutions per minute for 5 min in a bench centrifuge is usually adequate to ensure that all the valves are precipitated. Decant and discard the supernatant.

Add distilled or demineralised water again and stir. Repeat the centrifugation stage at least three times to remove all traces of acidity from the suspension. The pH of the supernatant can be easily checked with indicator paper.

When the sample supernatant is neutral, mix the diatom pellet in a small amount of distilled or demineralised water and transfer to a clean, small capacity vial. Add a few drops of 4 % formalin, hydrogen peroxide or ethanol to prevent fungal growth. The sample can then be stored indefinitely.

Bibliography

[1] Prygiel, J., Whitton, B.A. & Bukowska, J. (1999). *Use of Algae for Monitoring Rivers III.* Proceedings of the 3rd European Workshop, Douai, 1997. Agence de l'Eau Artois-Picardie. In press.

[2] Whitton, B.A. & Rott, E. (1996). *Use of Algae for Monitoring Rivers II.* Proceedings of the 2nd European Workshop, Innsbruck, 1995.,196pp. Universität Innsbruck, Innsbruck.

[3] Whitton, B.A., Rott, E. & Friedrich, G. (eds) (1991). *Use of Algae for Monitoring Rivers*. 193pp. Universität Innsbruck, Innsbruck.

[4] Kelly, M.G., A. Cazaubon, E. Coring, A. Dell'Uomo, L. Ector, B. Goldsmith, H. Guasch, J. Hürlimann, A. Jarlman, B. Kawecka, J. Kwandrans, R. Laugaste, E.-A. Lindstrøm, M. Leitao, P. Marvan, J. Padisák, E. Pipp, J. Prygiel, E. Rott, S. Sabater, H. van Dam, J. Vizinet (1998). Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *Journal of Applied Phycology* 10: 215-224.

blank

BSI — British Standards Institution

BSI is the independent national body responsible for preparing British Standards. It presents the UK view on standards in Europe and at the international level. It is incorporated by Royal Charter.

Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover. Tel: +44 (0)20 8996 9000. Fax: +44 (0)20 8996 7400.

BSI offers members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

Buying standards

Orders for all BSI, international and foreign standards publications should be addressed to Customer Services. Tel: +44 (0)20 8996 9001. Fax: +44 (0)20 8996 7001. Email: orders@bsi-global.com. Standards are also available from the BSI website at http://www.bsi-global.com.

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

Information on standards

BSI provides a wide range of information on national, European and international standards through its Library and its Technical Help to Exporters Service. Various BSI electronic information services are also available which give details on all its products and services. Contact the Information Centre. Tel: +44 (0)20 8996 7111. Fax: +44 (0)20 8996 7048. Email: info@bsi-global.com.

Subscribing members of BSI are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration. Tel: +44 (0)20 8996 7002. Fax: +44 (0)20 8996 7001. Email: membership@bsi-global.com.

Information regarding online access to British Standards via British Standards Online can be found at http://www.bsi-global.com/bsonline.

Further information about BSI is available on the BSI website at http://www.bsi-global.com.

Copyright

BSI
389 Chiswick High Road
London
W4 4AL