INTERNATIONAL STANDARD

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Water quality — Measurement of biochemical parameters — Spectrometric determination of the chlorophyll-a concentration

Qualité de l'eau — Mesurage des paramètres biochimiques — Dosage spectrométrique de la chlorophylle a

Reference number
ISO 10260:1992(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 10260 was prepared by Technical Committee ISO/TC 147, *Water quality*, Sub-Committee SC 2, *Physical, chemical, biochemical methods*.

Annexes A and B of this International Standard are for information only.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

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Introduction

Chlorophyll-a is the essential photosynthetic pigment present in all green plants. The chlorophyll content of a surface water is an indicator of its trophic state. The determination of the chlorophyll-a concentration provides information concerning the quantity and potential photosynthetic activity of algae. The most important metabolites of chlorophylls are phaeophytines and phaeophorbide. The ratio of chlorophyll to phaeopigments is indicative of the physiological state of the algae.

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Water quality — Measurement of biochemical parameters — Spectrometric determination of the chlorophyll-a concentration

1 Scope

1.1 This International Standard specifies a method for the determination of the chlorophyll-a concentration. The procedure can be applied for phytoplankton in natural surface waters and for testing algal growth in bio-assays. Using appropriate sampling it can also be applied to phytobenthic communities (see annex A).

1.2 Other algal pigments such as chlorophyll-b and chlorophyll-c and some chlorophyll metabolites do not contribute to the determination. Phaeopigments may be determined semiquantitatively, to correct for interference with chlorophyll-a determination and to indicate the portion of inactive algal biomass.

1.3 Chlorophyll is sensitive to light and oxygen, especially when it is extracted. To avoid oxidative and photochemical destruction, the samples shall not be exposed to bright light or air. Homogenization of the sample may in some cases increase the extraction efficiency.

1.4 The extraction procedure with ethanol involves heating to 75 °C for 5 min to inactivate chlorophyllase and accelerate the lysis of pigments. Storage of extracts (except filters containing suspended matter) prior to photometric measurement should be kept to a minimum, but is possible up to 3 d under refrigeration at 4 °C. Storage of extracts at less than − 25 °C is possible for at least 30 d.

1.5 Even though the procedure involves filtration or centrifugation to clarify the final extract, a slight turbidity may remain. The acidification step may also cause turbidity. Therefore, the absorbance measured at 665 nm has to be corrected for turbidity by substracting the absorbance measured at 750 nm.

1.6 The pigment of certain rarely occurring phototrophic bacteria (e.g. *Chlorobium*) interferes with the determination of chlorophyll-a concentration [1]. The contribution of chlorophyll-b and chlorophyll-c to the absorbance at 665 nm is negligible [2].

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes.*

ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques.*

3 Principle

Collection of algae and other suspended matter from a water sample by filtration. Extraction of algal pigments from the filter residue into hot ethanol. Spectrometric determination of the chlorophyll-a concentration in the extract. Evaluation of the chlorophyll-a and phaeopigment concentration from the difference in absorbance at 665 nm prior to and after acidification of the extract [3] [4].

4 Reagents

Use only reagents of recognized analytical grade and only deionized water of equivalent purity.