SLOVENSKI STANDARD
SIST EN ISO 10253:2000

01-januar-2000

Kakovost vode – Preskus zaviranja rasti morskih alg s Skeletonema costatum in Phaeodactylum tricornutum (ISO 10253:1995)

Water quality - Marine algal growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum (ISO 10253:1995)

Wasserbeschaffenheit - Wachstumshemmtest mit marinen Algen Skeletonema costatum und Phaeodactylum tricornutum (ISO 10253: 1995)

Qualité de l'eau - Essai d'inhibition de la croissance des algues marines avec Skeletonema costatum et Phaeodactylum tricornutum (ISO 10253:1995)

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English version

Water quality - Marine algal growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum (ISO 10253:1995)

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

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

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Foreword

The text of the International Standard from Technical Committee ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO) has been taken over as an European Standard 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of an identical text or by endorsement, at the latest by July 1998, and conflicting national standards shall be withdrawn at the latest by July 1998.

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, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 10253:1995 has been approved by CEN as a European Standard without any modification.

INTERNATIONAL STANDARD

ISO 10253

First edition
1995-10-01

Water quality — Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*

Qualité de l'eau — Essai d'inhibition de la croissance des algues marines avec Skeletonema costatum *et* Phaeodactylum tricornutum

Reference number
ISO 10253:1995(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 10253 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Annex A of this International Standard is for information only.

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Water quality — Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*

1 Scope

This International Standard specifies a method for the determination of the toxic effects of chemical compounds on the growth of marine algae.

The method can be used for testing substances which are readily soluble in water and are not significantly degraded or eliminated from the test.

NOTE 1 With minor changes, the method can also be used to determine the inhibitory effects of effluents. See however the note to table 2.

2 Principle

Monospecific algal cells are cultured for several generations in a defined medium containing a range of concentrations of the test substance, prepared by mixing appropriate quantities of nutrient concentrate, seawater, stock solutions of the test substance, and an inoculum of exponentially growing algal cells. The test solutions are incubated for a minimum period of 72 h, during which the cell density in each is measured at intervals of at least every 24 h. Inhibition is measured as a reduction in growth, or growth rate, relative to control cultures grown under identical conditions.

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 cell density: Number of cells per unit volume.

3.2 growth: Increase in cell density.

3.3 growth rate: Expression of rate of increase in cell density with respect to time.

See 8.2.2.

3.4 test solution: Mixture of seawater, nutrients and test substance in which algal cells are incubated.

3.5 control: Mixture of seawater, nutrients and algal cells without test substance.

3.6 effective concentration, EC10 or EC50: The concentration of test substance which results in respectively a 10 % or 50 % reduction in either growth or growth rate relative to the controls.

3.7 no observed effect concentration, NOEC: The highest concentration tested at which there is no statistically significant reduction of growth or growth rate relative to the controls.

4 Materials

4.1 Test organisms

Use either of the following marine algae.

a) *Skeletonema costatum* (Greville) Cleve (CCAP 1077/1C, NIVA BAC 1, ISTPM P4 — Bouin).

or

b) *Phaeodactylum tricornutum* Bohlin (CCAP 1052/1A — Oban, 1090/1A Göttingen, NIVA BAC 2, ISTPM P1).

These algae are important and widely distributed planktonic phytoplankton species (phylum *Bacillariophyta*) in estuarine and coastal areas.

The strains recommended are available in unialgal, non-axenic cultures from the following sources:

NIVA: Norwegian Institute for Water Research
P.O. Box 173 Kjelsås
N-0411 Oslo
Norway

ISTPM P1
ISTPM P4 —
Bouin: INERIS
9, rue de Rocroy
75010 Paris
France

CCAP: Dunstaffnage Marine Laboratory
P.O Box 3 Oban
Argyll PA34 4AD
United Kingdom

Göttingen: Collection of Algal Cultures
Institute of Plant Physiology
University of Göttingen
Nikolausberger Weg 18
D-3400 Göttingen
Germany

NOTE 2 Stock cultures may be maintained in the medium (see 4.3 and 6.1). Regular subculturing is necessary. Weekly intervals may be necessary for *Skeletonema*, every two or three weeks may be sufficient for *Phaeodactylum*.

4.2 Water

All water used in the preparation of the synthetic seawater, nutrient medium and test substance solutions shall be deionized or of equivalent purity. Take special care to avoid contamination of the water by inorganic or organic substances during preparation and storage. Equipment made of copper shall not be used.

4.3 Seawater

For culturing and testing *Phaeodactylum*, the medium (6.1) is made up by adding nutrients to either natural or synthetic seawater. For *Skeletonema*, the use of natural seawater is necessary for the long-term maintenance of cultures, and may also be necessary for the test medium because a synthetic seawater medium may not always support sufficient growth to meet the test quality criteria. If natural seawater [of salinity 30 ‰ (m/m) ± 5 ‰ (m/m)] is used, care shall be taken to ensure that it is not polluted.

Prepare synthetic seawater with the composition given in table 1.

All the chemicals used shall be of analytical grade.

Table 1 — Synthetic seawater

Salt	Concentration of salt in synthetic seawater g/l
NaCl	22
$MgCl_2 \cdot 6H_2O$	9,7
Na_2SO_4 (anhydrous)	3,7
$CaCl_2$ (anhydrous)	1,0
KCl	0,65
$NaHCO_3$	0,20
Salts of H_3BO_3	0,023

Sterilize the seawater by membrane filtration (5.4).

4.4 Nutrients

Prepare three nutrient stock solutions in water, with the compositions given in table 2.

Table 2 — Nutrient stock solutions

Nutrient	Concentration in stock solution	Final concentration in test solution
Stock solution 1		
$FeCl_3 \cdot 6H_2O$	48 mg/l	149 µg/l (Fe)
$MnCl_2 \cdot 4H_2O$	144 mg/l	605 µg/l (Mn)
$ZnSO_4 \cdot 7H_2O$	45 mg/l	150 µg/l (Zn)
$CuSO_4 \cdot 5H_2O$	0,157 mg/l	0,6 µg/l (Cu)
$CoCl_2 \cdot 6H_2O$	0,404 mg/l	1,5 µg/l (Co)
H_3BO_3	1 140 mg/l	17,1 µg/l
Na_2EDTA [1]	1 000 mg/l	15,0 µg/l
Stock solution 2		
Thiamin hydrochloride	50 mg/l	25 µg/l
Biotin	0,01 mg/l	0,005 µg/l
Vitamin B_{12} (cyanocobalamin)	0,10 mg/l	0,05 µg/l
Stock solution 3		
K_3PO_4	3,0 g/l	3,0 mg/l
$NaNO_3$	50,0 g/l	50,0 mg/l
$Na_2SiO_3 \cdot 5H_2O$	14,9 g/l	14,9 mg/l

1) Complexing of heavy metals by the relatively high concentration of EDTA present in the nutrient medium may preclude the testing of effluents containing heavy metals.

NOTE 3 These stock solutions will eventually be diluted (see 6.1) to obtain the final nutrient concentrations in the test solutions.

All the chemicals used shall be of analytical grade.

Sterilize stock solutions 1 and 3 by autoclaving at 120 °C for at least 15 min, and stock solution 2 by membrane filtration (5.4).

Store the solutions in the dark at 4 °C.

5 Apparatus

All equipment which will come into contact with the test medium shall be made of glass or a chemically inert material.

Normal laboratory apparatus and

5.1 Temperature-controlled cabinet or room, with a white fluorescent light providing continuous even illumination, suitable for the lighting requirements specified for the test in 6.6.

5.2 Apparatus for measuring algal cell density, preferably a particle counter, or a microscope with a counting chamber. Alternatively, determine the state of growth of the algal cultures by an indirect procedure using a spectrometer, turbidimeter or fluorimeter, when sufficiently sensitive and if shown to be sufficiently well correlated with the cell density. The apparatus used shall be capable of accurately measuring cell densities as low as 10^4 cells per millilitre and to distinguish between algal growth and disturbing effects, for example the presence of particulate matter and colour of the sample.

5.3 Culture flasks, for example conical flasks of capacity 250 ml, with air-permeable stoppers.

5.4 Apparatus for membrane filtration, with filters of mean pore diameter 0,2 µm.

5.5 Autoclave.

5.6 pH-meter.

6 Procedure

6.1 Preparation of culture medium

Add 15 ml of nutrient stock solution 1, 0,5 ml of nutrient stock solution 2 and 1 ml of nutrient stock solution 3 (see table 2) to approximately 900 ml of natural or synthetic seawater (4.3) and then make up to 1 litre with the same seawater.

Adjust the pH to 8,0 ± 0,2 by adding dilute hydrochloric acid or sodium hydroxide solution.

6.2 Preparation of inoculum

The algal inoculum for the test shall be taken from an exponentially growing pre-culture. The pre-culture shall be set up 3 d ± 1 d before the start of the test, as follows.

Add sufficient cells from the algal stock culture to the culture medium (6.1) to obtain an initial cell density of approximately 2×10^3 to 10^4 cells per millilitre. Maintain the pre-culture under the same conditions as those in the test (see 6.6) for 3 d ± 1 d. After this, the pre-culture should be in exponential growth and of sufficient cell density to be used as an inoculum for the test. Measure the cell density in the pre-culture immediately before use (see 6.7), in order to calculate the required inoculum volume.

6.3 Choice of test concentrations

The concentrations of substance to be tested shall normally follow a geometric progression, for example 10 mg/l; 3,2 mg/l; 1,0 mg/l; 0,32 mg/l; ...; 0,01 mg/l.

If possible, the concentrations shall be chosen to obtain several (i.e. 4 or 5) levels of inhibition of growth ranging from less than 10 % to greater than 90 %.

NOTE 4 A suitable concentration range is best determined by carrying out a preliminary range-finding test covering several orders of magnitude of difference between test concentrations. Replication of test concentrations is unnecessary during this preliminary test.

6.4 Preparation of test substance stock solution

Prepare stock solutions of the test substance, where necessary, in the algal growth medium by dilution. The concentration of test substance in the stock solutions shall be such that, when added to the test vessels containing growth medium inoculated with the algae, the intended range of test concentrations is obtained.

Normally, the test shall be carried out without adjusting the pH. However, some substances may exert a toxic effect due to extreme acidity or alkalinity. In order to determine the toxicity of a substance independent of pH, adjust the pH of the master stock solution (before the dilution in series) to that of the culture medium, using either 1 mol/l hydrochloric acid or 1 mol/l sodium hydroxide solution.

6.5 Preparation of test solutions

Prepare the test solutions by mixing the appropriate volumes of test substance stock solutions (6.4), cul-