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Water quality — Growth inhibition test with the marine and brackish water macroalga *Ceramium tenuicorne*

Qualité de l'eau — Essai d'inhibition de croissance sur la macro algue d'eaux marine et saumâtre Ceramium tenuicorne

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Foreword

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The main task of technical committees is to prepare International Standards. 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 10710 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Introduction

The red macroalga *Ceramium tenuicorne* belongs to Ceramiaceae, Rhodophyta. The species can be used as a model organism for the near coastal ecosystem. This species is found in temperate marine waters in both the northern and southern hemispheres and is thus relevant for large areas. As primary producers, they are a food source for many invertebrates and serve as living habitat for bacteria, invertebrates, and juvenile fish. They also serve as substrate for many oviparous fish species.

Water quality — Growth inhibition test with the marine and brackish water macroalga *Ceramium tenuicorne*

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International 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 safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this International Standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for the determination of the inhibition of growth of the macroalga *Ceramium tenuicorne* by substances and mixtures contained in seawater or by waste water with salinities between 4S and 32S. This method is applicable to substances that are easily soluble in water.

NOTE With modifications as described in ISO 14442[4] and ISO 5667-16[2], the inhibitory effects of poorly soluble organic and inorganic materials, volatile compounds, metals, waste water, marine water samples, and elutriates of sediments can be tested.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
algal length
length from the first division to the most distant tip of the plant

NOTE The algal length is expressed in millimetres.

See Figure 1.

2.2
control medium
combination of dilution water and/or nutrient medium used in the test

[ISO 20079:2005[5], 3.6]

2.3
control batch
control medium including organisms used for testing

[ISO 20079:2005[5], 3.5]

2.4
effective concentration
$\mathbf{E_rC_x}$
concentration of test sample at which an effect of x % is measured, if compared to the control

[ISO 20079:2005[5], 3.9]

2.5
growth medium
mixture of natural seawater and nutrients in which algal plants are cultivated, which is used for pre-cultures

NOTE Adapted from ISO 8692:2004[3], 3.3.

2.6
growth rate
μ
⟨water quality⟩ proportional rate of increase in algal length per day

NOTE See Clause 9.

2.7
salinity
practical salinity
S
⟨seawater⟩ ratio K_{15} of the electrical conductivity of the seawater sample, at the temperature of 15 °C and a pressure of one standard atmosphere, to that of a potassium chloride solutions in which the mass fraction of KCl is 32,435 6 × 10^{-3}, at the same temperature and pressure

NOTE Adapted from Reference [14], p. 12.

2.8
test medium
mixture of seawater, nutrients and test sample

NOTE Adapted from ISO 8692:2004[3], 3.5.

2.9
test sample
⟨water quality⟩ aqueous sample, e.g. chemical substance, mixture of chemicals or waste water, for which the inhibitory effects on the growth of algae are determined

NOTE Adapted from ISO 8692:2004[3], 3.4.

3 Principle

Algal tips from monocultures of ceramium female gametophytes are grown in defined test conditions and in a defined medium containing a range of concentrations of the test sample. The test solutions are incubated for a period of 7 d after which the increase in length is measured and the growth rate is calculated. The growth inhibition is determined as a reduction in growth rate, relative to control cultures grown under identical conditions.

When toxicity of samples is to be compared to the toxicity of other chemicals or waste waters, tests can be performed in artificial seawater. If the purpose of the testing is to assess and to predict effects in a specific receiving water body, the tests can be conducted with algae adapted to the salinity in the receiving water body. In this case, natural seawater from an uncontaminated site of the same properties is used.

4 Test organisms, nutrients, media, and materials

Unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

4.1 Test organisms

Use either of the following marine algal clones:

a) *Ceramium tenuicorne* Kützing Waern (7*S* clone originating from the Baltic Sea);

b) *C. tenuicorne* Kützing Waern (20*S* and 30*S* clone originating from the Oslo fjord).

This alga is a widely distributed macroalgae species (phylum Rhodophyta) in estuarine and coastal areas. The strains recommended are available in unialgal, non-axenic cultures[1)].

NOTE 1 This growth inhibition test is based on two clones, which were formerly regarded as two different species. These species were the marine *Ceramium strictum* Harvey sensu Kylin and the brackish water species *C. tenuicorne* Kützing Waern. Complete interfertility (References [11][12]) and DNA data (Reference [10]) have shown that the two entities belong to the same species, with *C. tenuicorne* as the valid name. The marine clone (former *C. strictum*) used in this test was isolated in 1973 and originates from the Oslo fjord (20*S* to 25*S*). It has been maintained as a laboratory culture for over 30 years. The brackish water clone was isolated in 1995 and originates from the Baltic Sea, 20 km south of the Askö laboratory in northern Baltic proper (6*S* to 7*S*). Cultures can be maintained in the medium specified in Clause 5. Regular subculturing is necessary.

NOTE 2 Among the red algae, changes occur between haploid and diploid generations. In the growth inhibition test, the female gametophytic generation is used since it has an even dichotomous growth pattern and the fastest growth rate. In nature it is difficult to distinguish between male and female plants. This can be done in the laboratory where spermatangia are found on the branches of the male plants and trichogynes can be seen on the tips of the claws on the female plants.

NOTE 3 The Baltic Sea clone can be adapted and used in tests in salinities between 4*S* and 12*S*. The marine clone can be used as test organism in salinities between 12*S* and 32*S*.

4.2 Natural and artificial seawater

4.2.1 General

Natural seawater is used for the cultivation of the algae and either natural or artificial seawater should be used for testing. The type of seawater to be used depends on the objective of the test. When natural seawater is used, care shall be taken to ensure that it is not polluted. 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.

4.2.2 Artificial seawater

Prepare the stock solutions for artificial seawater according to Table 1.

Start with about one third of the desired volume of water, add the weighed quantities of chemicals in accordance with Table 1 and make up to volume with water.

This stock solution with a salinity of 100*S* (equivalent to 10 % mass per volume) has a durability of at least six months, if stored in darkness at room temperature. Before use, the stock solution should be diluted with water to the desired salinity. Adjust the pH to 8,0 ± 0,2 with 1 mol/l HCl or 1 mol/l NaOH.

The artificial seawater shall be sterilized by autoclaving or sterile filtration (pore size, 0,2 µm) before use. Re-check the pH after sterilization, and adjust if necessary to 8,0 ± 0,2 with 1 mol/l HCl or 1 mol/l NaOH, before use.

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1) Suitable suppliers are: a) ITM, Department of Applied Environmental Research of Science, Stockholm University, S-106 91 Stockholm, Sweden; b) University of Oslo, Department of Biology, P.O. Box 1066 Blindern, N-0316 Oslo, Norway. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these suppliers.

Table 1 — Artificial seawater with a salinity of 10 % mass per volume or $100S$
(adapted from Reference [13])

Substance	Quantity per 1 l medium g/l	Quantity per 5 l medium g/5 l	Quantity per 10 l medium g/10 l
NaCl	70,1	351	702
Na_2SO_4	11,7	58,7	117
KCl	2,03	10,2	20,3
KBr	0,293	1,47	2,93
$Na_2B_4O_7{\cdot}10H_2O$	0,113	0,567	1,13
$MgCl_2{\cdot}6H_2O$	31,7	158	317
$CaCl_2{\cdot}6H_2O$	6,6	33	66
$SrCl_2{\cdot}6H_2O$	0,066	0,334	0,668

4.2.3 Natural seawater

Natural seawater shall be collected from an uncontaminated site. Filter to remove larger particles. Dilute as necessary with water. Salinity should be increased by addition of natural seawater of a higher salinity or with artificial seawater (see Table 1). Check the pH and adjust if necessary to 8,0 ± 0,2 with 1 mol/l HCl or 1 mol/l NaOH.

The natural seawater shall be sterilized by autoclaving or sterile filtration (pore size, 0,2 µm) before use. Re-check the pH after sterilization, and adjust if necessary to 8,0 ± 0,2 with 1 mol/l HCl or 1 mol/l NaOH, before use.

NOTE 1 A paper filter of around 30 µm mesh size is sufficient.

NOTE 2 Natural seawater can be stored frozen at temperatures below –18 °C for several years before use.

4.3 Nutrients

Prepare the six nutrient solutions in water, with the compositions given in Table 2.

Solutions 1, 2, 3, 4 and 6 in Table 2 are prepared in 100 ml one-mark volumetric flasks (6.14). A 1 l one-mark volumetric flask (6.14) is recommended for the preparation of stock solution 5, due to the low masses of the trace element reagents. Precipitation in the trace element solution is avoided by adjustment with NaOH to pH 8. The trace element stock solution (solution 5) shall be diluted 10 times before use in the cultivation media (see Table 3). After this dilution, a freshly prepared iron solution 3 may be added to trace element solution 5 to increase the durability of the iron.

The iron solution shall not be older than one month.

These stock solutions will eventually be diluted according to Table 3 to obtain the final nutrient concentrations in the growth and test media. The final concentrations in the media are given in the two rightmost columns of Table 2.

4.4 Media

Additions of stock solutions to salt water for preparation of media are shown in Table 3. For cultivation, testing in natural seawater, and testing in artificial seawater, additions shall be made in accordance with columns A, B, and C, respectively.

Table 2 — Nutrient stock solutions (adapted from Reference [9])

Reagent	Compound mass concentration	Levels in the medium after additions according to Table 3 as	
		elemental mass concentration	elemental amount of substance concentration
		µg/l	µmol/l
1 — Nitrogen solution			
KNO_3	5 000 mg/100 ml	3 462 (N)	247 (N)
2 — Phosphorus solution			
KH_2PO_4	680 mg/100 ml	775 (P)	25 (P)
3 — Iron solution			
$FeCl_3 \cdot 6H_2O$	100 mg/100 ml	103 (Fe)	1,9 (Fe)
4 — Carbon solution			
$NaHCO_3$	5 760 mg/100 ml	16 480 (C)	1 370 (C)
5 — Trace element solution			
Na_2EDTA	6 000 mg/l		
$MnSO_4 \cdot H_2O$	620 mg/l	10 (Mn)	0,18 (Mn)
$ZnSO_4 \cdot 7H_2O$	250 mg/l	2,84 (Zn)	0,043 (Zn)
$Na_2MoO_4 \cdot 2H_2O$	130 mg/l	2,58 (Mo)	0,027 (Mo)
$CoSO_4 \cdot 7H_2O$	4 mg/l	0,042 (Co)	0,000 7 (Co)
$CuSO_4 \cdot 5H_2O$	4 mg/l	0,05 (Cu)	0,000 8 (Cu)
6 — Vitamins			
Thiamine (B_1)	10 mg/100 ml	50	
Cyanocobalamin (B_{12})	0,1 mg/100 ml	0,5	
Biotin	0,1 mg/100 ml	0,5	

Table 3 — Additions of stock nutrient solutions to seawater for preparation of growth and test medium

Stock solutions	A Growth medium in natural seawater for cultivation	B Test medium in natural seawater	C Test medium in artificial seawater
	ml/l	ml/l	ml/l
1 — Nitrogen solution	0,5	0,5	0,5
2 — Phosphorus solution	0,5	0,5	0,5
3 — Iron solution	0,5	0,5	0,5
4 — Carbon solution	—	—	2
5 — Trace element solution diluted 10 times	0,5	—	—
6 — Vitamin solution	0,5	—	—