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Water quality — Guidance for determination of biodegradability in the marine environment

Qualité de l'eau — Lignes directrices pour la détermination de la biodégradabilité en milieu marin

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 16221 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Introduction

ISO/TC 147 has established International Standards for testing biodegradability of substances and waste water in the aquatic environment. All these methods, which are summarized in ISO 15462, can only be used for the determination and prediction of biodegradability in fresh water. There are, however, many cases, for example, substances used off-shore, where an urgent need exists for testing biodegradability in the marine environment. This International Standard describes biodegradation testing in marine test systems, and is based on an established OECD Guideline and the experience gained by a working group of the Oslo and Paris Commission (OSPARCOM) which has selected suitable standardized ISO methods, adopted for marine conditions and checked in a ring test.

Water quality — Guidance for determination of biodegradability in the marine environment

1 Scope

This International Standard specifies five methods for determining the ultimate aerobic biodegradability of organic compounds in the marine environment by aerobic microorganisms in static aqueous test systems. Standard degradation methods developed for testing in fresh water are modified and adapted to marine conditions. These methods are the DOC die-away test (ISO 7827), the closed bottle test (ISO 10707), the two-phase closed bottle test (ISO 10708), the CO_2 evolution test (ISO 9439) and the CO_2 headspace test (ISO 14593).

The methods apply to organic compounds which

a) are water-soluble under the conditions of the test used;

b) are poorly water-soluble under the conditions of the test used, in which case special measures may be necessary to achieve good dispersion of the compound (see for example, ISO 10634);

c) are volatile, provided that an appropriate test with suitable conditions is used;

d) are not inhibitory to the test microorganisms at the concentration chosen for the tests. The presence of inhibitory effects can be determined as specified in this International Standard.

NOTE The conditions described in this International Standard do not always correspond to the optimal conditions for allowing the maximum degree of biodegradation to occur. For biodegradation methods in fresh water see ISO 14593 and ISO 15462, and for biodegradation at low concentrations see ISO 14592.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 7827, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of dissolved organic carbon (DOC)*.

ISO 9439, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Carbon dioxide evolution test*.

ISO 10707, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of biochemical oxygen demand (closed bottle test)*.

ISO 10708, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Determination of biochemical oxygen demand in a two-phase closed bottle test*.

ISO 14592-1, *Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations — Part 1: Shake-flask batch test with surface water or surface water/sediment suspensions*.

ISO 14592-2, *Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations — Part 2: Continuous flow river model with attached biomass*.

ISO 14593, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)*.

3 Terms and definitions

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

3.1
ultimate aerobic biodegradation
breakdown of a chemical compound or organic matter by microorganisms in the presence of oxygen to carbon dioxide, water and mineral salts of any other elements present (mineralization) and normally the production of new biomass

3.2
primary biodegradation
structural change (transformation) of a chemical compound by microorganisms resulting in the loss of a specific property

3.3
total organic carbon
TOC
all that carbon present in organic matter which is dissolved and suspended in the water sample

3.4
dissolved organic carbon
DOC
that part of the organic carbon in the water sample which cannot be removed by specified phase separation

NOTE Examples of specified phase separation are centrifugation at 40 000 ms^{-2} for 15 min or membrane filtration using membranes with pores of 0,2 μm to 0,45 μm diameter.

3.5
total inorganic carbon
TIC
all that carbon in the water sample deriving from carbon dioxide and carbonate.

3.6
dissolved inorganic carbon
DIC
that part of the carbon in the water sample which cannot be removed by specified phase separation

NOTE Examples of specified phase separation are centrifugation at 40 000 ms^{-2} for 15 min or membrane filtration using membranes with pores of 0,2 μm to 0,45 μm diameter.

3.7
chemical oxygen demand
COD
mass concentration of oxygen equivalent to the amount of a specified oxidant consumed by a chemical compound or organic matter when a water sample is treated with that oxidant under defined conditions

NOTE It is expressed in this case as milligrams oxygen uptake per milligram (or gram) test compound.

3.8
biochemical oxygen demand
BOD
mass concentration of dissolved oxygen consumed under specified conditions by the aerobic biological oxidation of a chemical compound or organic matter in the water sample

NOTE It is expressed in this case as milligrams oxygen uptake per milligram (or gram) test compound.

3.9
theoretical oxygen demand
ThOD
theoretical amount of oxygen required to oxidize a chemical compound completely, calculated from the molecular formula

NOTE It is expressed in this case as milligrams oxygen uptake per milligram (or gram) test compound.

3.10
theoretical amount of formed carbon dioxide
$ThCO_2$
theoretical amount of carbon dioxide formed after oxidizing a chemical compound completely, calculated from the molecular formula

NOTE It is expressed in this case as milligrams oxygen uptake per milligram (or gram) test compound.

3.11
theoretical amount of inorganic carbon
ThIC
theoretical amount of inorganic carbon formed after oxidizing a chemical compound completely, calculated from the molecular formula

NOTE It is expressed in this case as milligrams oxygen uptake per milligram (or gram) test compound.

3.12
lag phase
time from the start of a test until adaptation and/or selection of the degrading microorganisms are achieved and the biodegradation degree of a chemical compound or organic matter has increased to about 10 % biodegradation

NOTE It is expressed in days.

3.13
maximum level of biodegradation
degree of biodegradation of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test

NOTE It is expressed as a percent.

3.14
biodegradation phase
time from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached

NOTE It is expressed in days.

3.15
plateau phase
time from the end of the biodegradation phase until the end of the test

NOTE It is expressed in days.

3.16
pre-exposure
pre-incubation of an inoculum in the presence of the test chemical compound or organic matter, with the aim of enhancing the ability of this inoculum to biodegrade the test material by adaptation and/or selection of the microorganisms

3.17
preconditioning
pre-incubation of an inoculum under the conditions of the subsequent test in the absence of the test chemical compound or organic matter, with the aim of improving the performance of the test by acclimatization of the microorganisms to the test conditions

4 Principle

This International Standard describes five methods for determining the biodegradability of organic compounds in the marine environment by aerobic microorganisms using static aqueous test systems. Standard degradation methods developed for testing in fresh water are modified, adapted and used for this purpose.

Test mixtures are prepared containing natural or artificial seawater, marine bacteria and the organic compound, at a suitable concentration, as the sole source of carbon and energy. The test mixtures and controls are incubated at the desired temperature. Ultimate biodegradation is followed over a specified period by measuring summary parameters as described in the basic test methods. Biodegradation based on DOC (dissolved organic carbon) removal is determined by comparing the measured concentrations at the start and the end of the test as specified in the DOC die-away test (ISO 7827). BOD (biochemical oxygen demand) is measured and compared with the theoretical oxygen demand (ThOD) or the measured chemical oxygen demand (COD) as specified in the closed bottle test (ISO 10707) and the two-phase closed bottle test (ISO 10708). The evolution of carbon dioxide (CO_2) is determined and compared with the theoretical carbon dioxide evolution ($ThCO_2$) using the CO_2 evolution test (ISO 9439), and TIC (total inorganic carbon) is determined and compared with the theoretical inorganic carbon (ThIC) in accordance with the CO_2 headspace test (ISO 14593).

If required and if a substance-specific analytical method is available, information on primary degradability may be obtained by measuring the loss of the test compound during the test, or biodegradation may be determined at low concentration using radio-labelled (usually ^{14}C) test compounds (ISO 14592).

5 Test environment

Incubation shall take place in the dark or in diffused light, at the desired temperature, usually within the range 15 °C to 25 °C which shall not vary by more than ± 1 °C during the test. In those cases where the objective of the study is to simulate environmental situations, tests may be carried out beyond this normal temperature range.

6 Reagents

Use as test medium natural (6.2) or artificial seawater (6.3). Use only reagents of recognized analytical grade.

6.1 Water, distilled or de-ionized, containing less than 1 mg DOC per litre.

6.2 Natural seawater

6.2.1 Sampling and pretreatment

Use any seawater of natural origin. Collect a sample in a thoroughly cleansed container and transport to the laboratory, preferably within two days. During transport do not allow the temperature of the sample to exceed significantly the range 10 °C to 30 °C.

Provide the following information:

— place and depth of collection,

— pollutional and nutritional status of the sampling site (e.g. concentration of nitrate, ammonium and phosphate) and appearance of the sample,

— date of collection and time between sampling and start of the test,

— temperature at collection,

— salinity and DOC (use e.g. ISO 8245).

When natural seawater is used, normally sufficient microorganisms are available and no additional inoculation is required.

It is recommended to determine the number of colony-forming heterotrophic bacteria in the natural seawater, e.g. by plate count using a marine agar. A suitable bacterial concentration is about 10^5 cells/ml in the test vessels. When the natural seawater has too low a bacterial density, inoculate as described for artificial seawater (6.3). Check the activity of the natural seawater by means of the reference compound.

NOTE 1 Normally the natural seawater and the inoculum should not be pre-exposed to the test compound, to allow a general prediction of the degradation behaviour in the environment. In certain circumstances, depending on the purpose of the test, pre-exposed inocula may be used, provided that this is clearly stated in the test report (e.g. percent biodegradation = x %, using pre-exposed inocula) and the method of pre-exposure is detailed in the test report. Pre-exposed inocula can be obtained from marine laboratory biodegradation tests conducted under a variety of conditions or from samples collected from locations where relevant environmental conditions exist (e.g. contaminated areas).

NOTE 2 The amount of bacteria for the test may be increased, e.g. by centrifugation and re-suspension in a smaller seawater sample.

To reduce the concentration of DOC or BOD in the blank, preconditioning is possible. Incubate the sample in the dark or in diffused light at the test temperature, under aerobic conditions, for up to one week. When the added inoculum contains too much DOC (> 10 % of the organic carbon added by the test compound), remove the surplus by washing with artificial seawater (6.3) and centrifuging. The total inorganic carbon (TIC) content of natural seawater is usually high; if so, this shall be reduced in accordance with ISO 14593. Measure the pH of the seawater sample. Sparge with CO_2-free air for about 1 h while maintaining the pH at 6,5 using concentrated hydrogen chloride (HCl). Finally, restore the pH to its original value with sodium hydroxide (NaOH).

Prior to use, remove coarse particles from the seawater by filtration using e.g. a coarse paper filter or by sedimentation. To obtain sufficient buffering capacity and supply of nutrient to the test solution, add as mineral nutrients (6.2.2) the usual inorganic medium of standard biodegradation tests, excepting the solutions with magnesium sulfate and calcium chloride, as these minerals are in sufficient concentration in any natural sea water.

6.2.2 Mineral nutrients

For 1000 ml of test medium, add to about 800 ml of natural seawater (6.2) 10 ml of solution a) and 1 ml of solution b) below, and make up to 1000 ml with the seawater (6.2).

6.2.2.1 Solution a)

Dissolve

anhydrous potassium dihydrogenphosphate (KH_2PO_4)	8,5 g
anhydrous dipotassium hydrogenphosphate (K_2HPO_4)	21,75 g
disodium hydrogenphosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$)	33,4 g
ammonium chloride (NH_4Cl)	0,5 g
in water (6.1), quantity necessary to make up to	1000 ml

