INTERNATIONAL STANDARD

ISO 16221

First edition
2001-02-01

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

Reference number
ISO 16221:2001(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.ch
Web www.iso.ch

Printed in Switzerland

Contents

Page

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 $\pm$ 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

6.2.2.2 Solution b)

Dissolve 0,25 g iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in water (6.1), quantity necessary to make up to 1000 ml. Prepare this solution freshly before use or add a drop of concentrated hydrochloric acid (HCl) to avoid precipitation.

6.3 Artificial seawater

Use any available artificial seawater, or prepare an aqueous solution with the following composition. For 1000 ml of test medium, add to about 800 ml of solution g) 1 ml each of solutions h) through j) and make up to 1000 ml with solution g).

When artificial seawater is used as test medium, an inoculation is required to obtain sufficient biodegradative activity. Use as inoculum any material of marine origin, e.g. filtered seawater, suspension of marine sediment, bacteria from the filters of a marine aquarium. Take a suitable sample of the inoculum and add it to the artificial seawater to obtain a sufficient bacteria concentration in the test (see 6.2.1). Check the activity of the inoculated artificial seawater by means of the reference compound. For any required reduction of DOC in the inoculum and pre-adaptation, see 6.2.1.

6.3.1 Solution a)

Dissolve

sodium chloride (NaCl)	47,8 g
sodium sulfate (Na_2SO_4)	8,0 g
potassium chloride (KCl)	1,4 g
sodium hydrogencarbonate ($NaHCO_3$)	0,04 g
potassium bromide (KBr)	0,2 g
boric acid (H_3BO_3)	0,06 g
sodium fluoride (NaF)	0,006 g
in water (6.1), quantity necessary to make up to	1000 ml

6.3.2 Solution b)

Dissolve 203,3 g magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) in water (6.1), quantity necessary to make up to 1000 ml.

6.3.3 Solution c)

Dissolve 147 g calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in water (6.1), quantity necessary to make up to 1000 ml.

6.3.4 Solution d)

Dissolve 26,61 g strontium chloride hexahydrate ($SrCl_2 \cdot 6H_2O$) in water (6.1), quantity necessary to make up to 1000 ml.

6.3.5 Solution e)

Dissolve 136,1 g anhydrous dipotassium hydrogenphosphate (K_2HPO_4) in water (6.1), quantity necessary to make up to 1000 ml.

6.3.6 Solution f)

Dissolve 26,74 g ammonium chloride (NH_4Cl) in water (6.1), quantity necessary to make up to 1000 ml.

6.3.7 Solution g)

To obtain solution g), mix 500 ml of solution a), 53,3 ml of solution b), 10,3 ml of solution c), 0,9 ml of solution d),1,25 ml of solution e) and 1 ml of solution f) and make up to 1000 ml with water (6.1). Solution g) has a salinity of about 35 %.

6.3.8 Solution h)

Dissolve 15 mg yeast extract in 100 ml water (6.1) just before use.

6.3.9 Solution i)

Dissolve

manganese sulfate monohydrate ($MnSO_4 \cdot H_2O$)	60,46 mg
zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$)	85,6 mg
ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$)	73,7 mg
in water (6.1), quantity necessary to make up to	1000 ml

6.3.10 Solution j)

Dissolve 89 mg iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in water (6.1), quantity necessary to make up to 1000 ml. Prepare this solution freshly before use or add a drop of concentrated hydrochloric acid (HCl) to avoid precipitation.

6.4 Test solutions

6.4.1 Test compound

Prepare a stock solution of a sufficiently water-soluble test compound in water (6.1) and add a suitable amount of this solution to the test vessels to obtain the final concentration of test compound as indicated in the basic standard methods. Add volatile compounds or those of low water solubility directly into the test vessels of the methods suitable for this purpose (see Table 2). Determine the added amount exactly.

NOTE For more details on handling poorly water-soluble compounds, see ISO 10634.

6.4.2 Reference compound

Use as a reference compound an organic compound of known biodegradability, such as aniline or sodium benzoate, which has a degradation degree > 60 % for BOD and CO_2, > 70 % for DOC and 80 % for specific substance analyses, and which would be expected to show a typical biodegradation curve. Prepare a stock solution of the reference compound in water (6.1) in the same way as with a water-soluble test compound (6.4.1), in order to obtain the final concentration of the reference compound as indicated in the basic standard methods.

6.4.3 Solution to check inhibition

If required (when e.g. no information on the toxicity of test compound is available), prepare a solution containing, in the water (6.1), both the test compound (6.4.1) and the reference compound (6.4.2) in appropriate amounts to obtain the same concentrations as foreseen in the test.

7 Apparatus

Use the apparatus as indicated in the basic standard methods.

Be aware that the salinity and the temperature will influence oxygen measurement in seawater. Use only oxygen measurement apparatus whose results may be corrected for salinity.

8 Test procedure

Set up a sufficient number of test vessels as described in the basic standard methods in order to have

— at least two test vessels (symbol F_T) for the test compound (6.4.1) in inoculated test medium (6.2 or 6.3);

— at least two blank vessels (symbol F_B) containing only inoculated test medium (6.2 or 6.3);

— at least one vessel, for checking the procedure (symbol F_C) containing the reference compound (6.4.2) and inoculated test medium (6.2 or 6.3);

— if needed, at least one vessel for checking a possible inhibitory effect of the test compound (symbol F_I) containing solution (6.4.3) and inoculated test medium (6.2 or 6.3);

— if needed, at least one vessel for checking a possible abiotic elimination (symbol F_S) containing the test compound (6.4.1) and uninoculated test medium (6.2 or 6.3). Add no inoculum and sterilize by addition of a suitable inorganic toxic compound to prevent microbial activity. Use, for example, 1 ml per litre of a solution containing 10 g/l of mercury(II) chloride ($HgCl_2$). Add the same amount of toxic substance again, two weeks after the test has begun.

NOTE 1 Mercury chloride is the only inorganic toxic compound which has shown its suitability in such biodegradation tests. As it is used only in very small amounts this poses no threat of harm to the environment.

NOTE 2 To obtain a biodegradation curve, it is necessary to prepare sufficient vessels for those methods in which only single determinations can be made and the vessels have to be sacrificed for measurements (ISO 10707 and ISO 14593). The number of test vessels depends directly on the number of intended measurements.

Add appropriate amounts of the test medium (6.2 or 6.3), the test (6.4.1) and the reference compounds (6.4.2) to the respective vessels according to Table 1 to obtain the desired test concentrations and final test volumes as indicated in the basic method standards (Table 2).

Table 1 — Final distribution of test and reference compounds

Vessel	Test medium	Test compound	Reference compound	Inoculum
F_T Test compound	+	+	–	+
F_T Test compound	+	+	–	+
F_B Blank	+	–	–	+
F_B Blank	+	–	–	+
F_C Inoculum check	+	–	+	+
F_I Inhibition control (optional)	+	+	+	+
F_S Abiotic elimination check (optional)	+	+	–	–

Table 2 — Test specifications

Basic method standard	Concentration of test, compound mg/l	Analytical parameters	Test duration days	Suitable for compounds of low water solubility	Suitable for volatile compounds
ISO 7827	5 to 40 (DOC)	DOC	60	no	no
ISO 10707	2 to 10 (substance)	BOD	60	yes	yes
ISO 10708	100 (ThOD)	BOD	60	yes	no
ISO 9439	20 (TOC)	CO_2	60	yes	no
ISO 14593	20 to 40 (TOC)	TIC	60	yes	yes

NOTE 3 DOC removal may be due to biodegradation but also to abiotic processes such as adsorption on the inoculum or the vessel wall or, in the case of volatile test compounds, stripping and adsorption on the tubing and is therefore not in any case an unequivocal proof of biodegradation.

Measure the pH and adjust if necessary to a value between 7 and 8. Use the given pH in the case of natural seawater. Place all test vessels in a water-bath or constant temperature room, allow them to reach the desired temperature (see clause 5), seal the vessels, make any necessary connections, and start the incubation. Take samples or make the readings of the measured parameters as indicated in the basic standards (see Table 2). Measure the analytical parameters at the beginning (time 0), the end (time t) of the test period and at suitable intermediates to obtain biodegradation curves. When primary degradation is being monitored, determine the concentration of the test compound using specific analysis in vessel F_T and F_S at the end of the test (time t). In the case of using radiolabelled substances individual test and evaluating techniques are required (see e.g. ISO 14592).

If a nearly constant level of biodegradation is attained (plateau phase) and no further biodegradation is expected, the test is considered to be completed. The test conditions of marine environments are generally less favourable than those of limnic test systems. Therefore the test duration should be extended compared to the usual time of 28 days in aquatic batch tests. The maximum test duration is 60 days. On the last day of the test, carry out all necessary work as indicated in the basic standards. If the test compound contains nitrogen, determine in the case of BOD measurements the final concentrations of nitrate and nitrite, and consider any nitrification as indicated in the basic methods.

9 Calculation and expression of results

Express the measured values and calculate the ultimate and (optionally) primary biodegradation of the test compound as indicated in the respective basic standards.

Compile a table of measured values and the percentages of biodegradation for each measuring interval and each test vessel. Plot a biodegradation curve, in percent, as a function of time. If comparable results are obtained for the duplicate test vessels F_T (< 20 % difference) plot a mean curve, otherwise plot curves for each vessel. Some parameters can be determined and indicated from this curve, in particular (if sufficient data are available) the lag time, the degradation time and the maximum level of degradation.

Determine the mean value of percent biodegradation in the plateau phase or use the highest value, e.g. when the curve starts to decrease from the plateau phase, and indicate this maximum level of biodegradation as "degree of biodegradation of the test compound" in the test report.

If DOC removal was used to determine biodegradation, be aware that elimination from water is primarily measured. If the test substance is not significantly eliminated abiotically (e.g. by adsorption or stripping to the air) and the elimination curve has a typical shape, with lag-, degradation and plateau phases, or if more information on biodegradability e.g. from tests in a limnic environment is available, assign the measured DOC elimination to biodegradation.

Calculate in the same way the biodegradation degree of the reference compound, F_C, and, if included, of the abiotic elimination check F_S and the inhibition control F_I and plot curves.

Information on the toxicity of the test compound may be useful in the interpretation of test results showing a low biodegradation. If in flask F_I the degradation percentage is < 25 % and insufficient degradation of the test compound is observed in flasks F_T, it can be assumed that the test compound is inhibitory. In this case the test should be repeated using a lower test concentration or another inoculum. If in flask F_S (abiotic elimination check if included), a significant amount (> 10 %) of DOC, CO_2 or BOD is observed, abiotic elimination processes may have taken place.

10 Validity of results

The test is considered as valid if the percentage degradation in flask F_C (inoculum check) is greater than 60 % (in the case of DOC measurement, 70%) on the 14th day.

11 Results of ring tests

The methods used in this International Standard, except the DOC removal test, have been checked in a ring test which was organized by OSPARCOM. The results are published in reference [1] (see Bibliography).

Table 3 — Results of the OSPARCOM ring test

Test compound	Biodegradation >60 % after 28 days % of results	Biodegradation >60 % after 60 days % of results	Mean lag time days	Mean time to attain 50 % of maximum biodegradation days
Sodium benzoate	A:100 B:100 C:91 D:100	A:100 B:100 C:100 D:100	A:1,6 B:0,9 C:2,1 D:2,4	A: 2,1 B: 2,9 C: 4,6 D:2,6
Anco Green B	A:60 B:92 C:75 D:71	A:80 B:100 C:83 D:85	A:2,4 B1,3 C:3,0 D:2,1	A: 3,9 B: 2,5 C: 5,9 D:6,1
Aquamul BII	A:0 B:0 C:0 D:0	A:0 B:0 C:0 D:0	A-D up to 60	-
Pentaerythritol	A:0 B:0 C:0 D: 0	A:0 B:36 C:18 D:14	A: >60 B: 21-60 C:28-60 D:14-60	-
NOTE A: ISO 9439 B: ISO 10707 C: ISO 10708 D: ISO 14593; Aquamull BII and Anco Green B are drilling fluids.				

The conclusions in the OSPARCOM report are that all four methods are suitable for marine biodegradability testing, delivering comparable results, but each has its own particular advantages and disadvantages. The choice should depend on the equipment of the laboratory and the specific compound parameters.

The DOC removal test (ISO 7827) and the closed bottle test (ISO 10707) have been adopted as OECD Guideline 306. These methods have been checked in ring tests which were organized by the European Commission, and are known and commonly accepted test methods.

12 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard and to the basic standard used;

b) all necessary information for the identification of the test compound;

c) all measured and calculated data (for example in tabular form) obtained and the degradation curve;

d) the concentration of the test and reference compound used;

e) the name of the reference compound used and the degradation obtained with this compound;

f) the source and characteristics of the seawater, and information on any pretreatment;

g) the incubation temperature of the test;

h) if included, information on abiotic elimination and inhibition and a statement on the toxicity of the test compound;

i) the reasons, in the event of rejection of the test;

j) any alteration of the standard procedure or any other circumstance that may have affected the results.

Bibliography

[1] *Biodegradability of chemical substances in seawater* — Results of the four OSPARCOM ring tests — Final report November 1996. Available from ELF Akvamiljo Mekjarvik 12, N-4070 Randaberg.

[2] ISO 6060, *Water quality — Determination of the chemical oxygen demand*.

[3] ISO 8245, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*.

[4] ISO 10634, *Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium.*

[5] ISO/TR 15462, *Water quality — Selection of tests for biodegradability*.

[6] OECD Guidelines for the Testing of Chemicals (1993) — 306, *Biodegradability in Seawater*. Available from the Organization for Economic Cooperation and Development, 2, rue André Pascal, F-75775 Paris Cedex 16.

ICS 13.060.70

Price based on 12 pages