
**Water quality — Selection of tests
for biodegradability**

Qualité de l'eau — Sélection des essais de biodégradabilité



Foreword

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- type 1, when the required support cannot be obtained for the publication of an International Standard, despite repeated efforts;
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Technical Reports of types 1 and 2 are subject to review within three years of publication, to decide whether they can be transformed into International Standards. Technical Reports of type 3 do not necessarily have to be reviewed until the data they provide are considered to be no longer valid or useful.

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Water quality — Selection of tests for biodegradability

1 Scope

The biodegradability of substances and wastewater ingredients depends not only on the molecular structures of the test material, but also on important additional factors such as the

- aquatic or terrestrial test environment;
- aerobic or anaerobic test conditions;
- source and concentration of the microorganisms of the inoculum;
- acclimatisation and adaptation of the inoculum;
- concentration of the test material;
- availability of inorganic nutrients and additional organic material for co-metabolic processes;
- possible toxic effects of the test material under the test conditions;
- physical and chemical properties and bioavailability of the test material (e.g. volatility, water solubility, adsorption);
- conditions and physical and chemical properties of the test system (volume of test vessels, static, dynamic, closed, CO₂ removal, temperature, mixing, shaking, oxygen supply);
- test duration;
- analytical parameters used (summary parameters such as DOC, BOD, CO₂ or substance specific analysis).

As so many factors can influence the test results it is not possible to define a "true" or "reference" method. The reproducibility of the test results using different methods or conditions or even using identical test methods can be low and differing test results can be obtained. Normally a test material which is either easily biodegradable or poorly biodegradable will produce similar test results. So-called "grey" substances, which are moderately biodegradable and need, for example, special bacteria or long adaptation periods, will often produce disparate results.

Biodegradability tests are used to establish the biodegradability of chemical structures and to predict the biodegradation behaviour of a test material in a natural or technical environment. The aim of each test method should be to simulate, to a certain degree, such an environment. Therefore, as so many factors can influence or even exclude certain methods, it is necessary to have a sufficient number of different standardized test methods to allow the choice of the best one for the specific purpose. It is also to be remembered that it was ISO's intention to standardize the biodegradation tests of the OECD Guidelines for Testing of Chemicals (see annex B).

2 References

- ISO 7827:1994 *Water quality - Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds - Method by analysis of dissolved organic carbon (DOC)*
- ISO 8192:1986 *Water quality - Test for inhibition of oxygen consumption by activated sludge*
- ISO 9408:1991 *Water quality - Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds - Method by determining the oxygen demand in a closed respirometer*
- ISO 9439:1990 *Water quality - Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds - Method by analysis of released carbon dioxide*
- ISO 9887:1992 *Water quality - Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium - Semi-continuous activated sludge method (SCAS)*
- ISO 9888:1991 *Water quality - Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium - Static test (Zahn-Wellens method)*
- ISO 10634:1995 *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*
- ISO 10707:1994 *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 10708:1997 *Water quality - Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds - Method by determining the biochemical oxygen demand in a two-phase closed bottle test*
- ISO 10712:1995 *Water quality - Pseudomonas putida growth inhibition test (Pseudomonas cell multiplication inhibition test)*
- ISO 11733:1995 *Water quality - Evaluation of the elimination and the biodegradability of organic compounds in an aqueous medium - Activated sludge simulation test*
- ISO 11734:1995 *Water quality - Evaluation of the ultimate anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of the biogas production.*
- ISO 14592-1 and ISO 14592-2 *Water quality - Evaluation of aerobic biodegradability of organic compounds at low concentrations in water - Part 1: Shake flask method; Part 2: River simulation test (in preparation)*
- ISO 14593 *Water quality - Evaluation in aqueous medium of ultimate aerobic biodegradability of organic compounds - Determination of released inorganic carbon in sealed vessels (in preparation)*
- ISO 15522 *Water quality - Determination of inhibitory effect of water constituents on growth of sludge microorganisms (in preparation)*

3 Conclusions and recommendations

3.1 Differences between test methods

The test methods for aerobic biodegradability are not of equal potential, largely because of the different microbial densities and test durations used. ISO 7827, ISO 9439, ISO 9408, ISO 10708 and ISO 14593 are of roughly equal potential. ISO 10707 has a lower potential and is especially applicable to volatile and inhibitory test compounds. These methods correspond in principle to the tests on ready biodegradability of OECD (see 3.4). ISO 9887 and ISO 9888 are tests with a high degradation potential and may be used to determine the intrinsic or inherent biodegradability of a chemical. ISO 11733 is a simulation test for wastewater treatment plants and ISO 14592 for river water. ISO 11734 is a test for anaerobic biodegradability. As yet only one method for assessing anaerobic biodegradability has been agreed, and is applied independently of tests for aerobic biodegradability. Priority for applying the test could be given to those chemicals which preferentially adsorb onto solids such as activated sludge and which are not sufficiently degraded aerobically.

Chemicals which degrade readily or easily in the aquatic environment will normally degrade equally well in all of the aerobic ISO tests, while less easily degradable chemicals degrade only in those with a higher potential, but not in any of the tests using lower cell densities. Similarly, some chemicals which degrade in the more powerful batch tests (ISO 9887 and ISO 9888) do not necessarily degrade in the continuously performed activated sludge simulation test, while other chemicals do and there may be of course differences between aerobic and anaerobic biodegradability.

3.2 Analytical parameters

Normally, summary parameters such as dissolved organic carbon, biochemical oxygen demand or biologically released carbon dioxide are used as analytical tools to obtain information on ultimate biodegradability (mineralization). However, the ISO methods also allow the use of substance-specific analytical techniques to investigate the biological transformation of a chemical, the so-called primary biodegradability. A list of test methods is given in annex A.

3.3 Bacterial toxicity

It has also been observed that chemicals which are inhibitory towards bacteria and do not degrade at the normal test concentrations may degrade in tests using lower concentrations of the test compound. If a chemical is suspected of not degrading because of toxicity to the test bacteria, additional vessels may be set up within the test to assess any inhibition. Otherwise specific tests may be used, such as the test for inhibition of oxygen consumption by activated sludge (ISO 8192) or a test on the growth of sewage bacteria (ISO 15522) or *Pseudomonas putida* (ISO 10712).

3.4 Test strategies

As there are differences between the complexity, duration, quality of results and costs of the various tests, these should be considered when choosing which test or test strategy to use when commencing a study. The choice of a test depends normally on the purpose of testing or on legal requirements which have to be fulfilled. One strategy is to apply a simple low-cell density method. If a chemical degrades adequately, further testing is normally unnecessary. A low or zero value for biodegradation may be sufficient for the purpose, otherwise the test could be repeated with an inoculum pre-exposed to the chemical, or a test with a higher cell density and a longer test period could be applied. Another strategy is to start with a high-density method to determine whether biodegradation potential is available at all and to answer the question, "Is the test compound easily biodegradable in environmental compartments?" by using a low-cell density method in the next step. Because of its complexity, the activated sludge simulation test (ISO 11733) is not usually applied until after other tests have first been carried out. Failure to degrade in one of the more powerful tests, ISO 9887 and ISO 9888, makes it often unnecessary to apply a simulation test.

The choice of a method also depends on the physical and chemical properties of the test chemical. Volatile test compounds can only be tested in closed systems such as ISO 10707 or ISO 10708. Insoluble and poorly soluble chemicals cannot be assessed by all the available methods and guidance in preparing such chemicals for testing is given in ISO 10634. In some cases it may be helpful to use an adapted inoculum. Pre-adaptation could be achieved using ISO 9887 or ISO 9888 followed by test methods using the measurement of biochemical oxygen demand (ISO 9408, ISO 10708) or carbon dioxide (ISO 9439 and ISO 14593).

It should also be mentioned that test strategies and philosophies exist for testing chemicals, e.g. EU Guidelines for the notification of new chemicals in the European Union or the OECD Chemicals Testing Programme 1979 which can be studied in documents of these organizations. The specific wording of EU and OECD includes strictly defined expressions such as "ready" or "inherent biodegradability" or the so-called 10-days-window which are not used within International Standards. Nevertheless many test methods are identical or at least similar. For a comparison of International Standards with OECD Guidelines see annex B.

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Annex A

Overview on ISO methods

No.	Title	Principle	Scope	Recommendation
ISO 7827	<i>Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds - Method by analysis of dissolved organic carbon (DOC)</i>	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed microorganisms. Measurement of the removal of dissolved organic carbon (DOC) to determine the ultimate biodegradability within 28 days. Evaluation of the test results by comparing the DOC concentration at the beginning and the end of the test. Additionally specific analysis can be used to determine the primary biodegradability of a test compound.	The test can be used for organic compounds which are: - water-soluble at the test concentration (10 - 40 mg/l DOC); - nonvolatile, or having a negligible vapour pressure; - not significantly adsorbable on glass and activated sludge; - not inhibitory to the test microorganisms at the test concentration.	Test method for water-soluble test compounds. Corresponds to OECD 301 A
ISO 9439	<i>Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds - Method by analysis of released carbon dioxide</i>	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed microorganisms. Measurement of the biologically produced carbon dioxide (CO₂) to determine the ultimate biodegradability within 28 days. Evaluation of the test results by comparing the CO₂ with the theoretical amount. For water-soluble test compounds, the DOC removal at the end of the test can be determined to obtain additional information on substance elimination from water.	The test can be used for organic compounds which are: - water-soluble at the test concentration (10-40 mg/l DOC); - water-insoluble under the test conditions; - nonvolatile, or having a negligible vapour pressure; - not inhibitory to the test microorganisms at the test concentration.	Test method for water-soluble and poorly soluble test compounds using an analytical parameter which is normally strictly of biological origin. Corresponds to OECD 301 B

No.	Title	Principle	Scope	Recommendation
ISO 9408	<i>Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds - Method by determining the oxygen demand in a closed respirometer</i>	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed microorganisms. Measurement of the biochemical oxygen demand (BOD) in a closed respirometer to determine the ultimate biodegradability within 28 days. Evaluation of the test results by comparing the BOD with the theoretical oxygen demand (ThOD) or the chemical oxygen demand (COD). For water-soluble test compounds, the DOC removal at the end of the test can be determined to obtain additional information on substance elimination from water or use of specific analysis to determine the primary biodegradability.	The test can be used for organic compounds which: - are water-soluble at the test concentration (100 mg/l); - are water-insoluble under the test conditions; - do not react with the CO₂ absorbant; - are volatile, provided a suitable respirometer is used; - are not inhibitory to the test microorganisms at the test concentration.	Test method for water-soluble and poorly soluble test compounds which requires a rather expensive respirometer producing detailed biodegradation curves, using an analytical parameter which is normally strictly of biological origin. Corresponds to OECD 301 F
ISO 9887	<i>Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium - Semicontinuous activated sludge method (SCAS)</i>	Semi-static, aquatic test system using organic test compounds and easily biodegradable organic medium (sewage) as the source of carbon and energy for a high-density inoculum of aerobic mixed microorganisms (activated sludge). Daily fill-and-draw of the test vessels with sewage and test compound and measurement of the removal of dissolved organic carbon (DOC) in the test and blank control vessels to determine the ultimate biodegradability within the test time of up to 26 weeks. Evaluation of the test results by comparing the DOC concentration before and after the fill-and-draw procedure. Additionally specific analysis can be used to determine the primary biodegradability of a test compound.	The test can be used for organic compounds which are: - water-soluble at the test concentration (20 to about 50 mg/l DOC); - nonvolatile, or having a negligible vapour pressure; - not lost by foaming from the test solution; - not significantly adsorbable on glass and activated sludge; - not inhibitory to the test microorganisms at the test concentration.	Test with high biodegradation potential, especially for compounds not easily degradable and for wastewater including cometabolic degradation. Useful method for the pre-adaptation of an inoculum for soluble and insoluble chemicals. Corresponds to OECD 302 A

No.	Title	Principle	Scope	Recommendation
ISO 9888	<i>Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium - Static test (Zahn-Wellens method)</i>	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for a high-density inoculum of aerobic mixed microorganisms (activated sludge). Measurement of the removal of dissolved organic carbon (DOC) or chemical oxygen demand (COD) in the test and blank control vessels to determine the ultimate biodegradability and the elimination from water within 28 days. Evaluation of the test results by comparing the DOC concentration at the beginning and the end of the test. Additionally specific analysis can be used to determine the primary biodegradability of a test compound.	The test can be used for organic compounds which are: - water-soluble at the test concentration (50 - 400 mg/l DOC); - nonvolatile, or having a negligible vapour pressure; - not lost by foaming from the test solution; - not inhibitory to the test microorganisms at the test concentration.	Static method for the evaluation of an elimination degree (biodegradation and abiotic substance removal) of test compounds and wastewater with a predictive value for waste water treatment plants. Corresponds to OECD 302 B
ISO 10634	<i>Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium</i>	Guidance describing several techniques for preparing poorly water-soluble organic compounds and introducing them into test vessels for a subsequent test for biodegradability in an aqueous medium using standard methods.	Guidance for poorly water-soluble test compounds	Guidance for the preparation and handling of poorly water-soluble compounds. No biodegradation test method.
ISO 10707	<i>Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds - Method by analysis of biochemical oxygen demand (closed bottle test)</i>	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for a low concentrated inoculum of aerobic mixed microorganisms. Measurement of the biochemical oxygen demand (BOD) in completely filled closed bottles to determine the ultimate biodegradability within 28 days. Evaluation of the test results by comparing the BOD with the theoretical oxygen demand (ThOD) or the chemical oxygen demand (COD).	The test can be used for organic compounds which: - are water-soluble at the test concentration (2-10 mg/l); - are water-insoluble at the test conditions, provided a suitable dosing technique is used; - are volatile, provided a suitable dosing technique is used; - are not inhibitory to the test microorganisms down to the low test concentrations used.	Simple test method at relatively low test concentrations and with rather low biodegradation potential especially used for volatile and toxic test compounds. Corresponds to OECD 301 D

No.	Title	Principle	Scope	Recommendation
ISO 10708	<i>Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds - Method by determining the biochemical oxygen demand in a two-phase closed bottle test</i>	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed microorganisms. Measurement of the biochemical oxygen demand (BOD) in closed bottles with head-space to determine the ultimate biodegradability within 28 days. Evaluation of the test results by comparing the BOD including the uptake of oxygen from the headspace with the theoretical oxygen demand (ThOD) or the chemical oxygen demand (COD). For water soluble test compounds the DOC removal at the end of the test can be determined to obtain additional information on substance elimination from water or use of specific analysis to determine the primary biodegradability.	The test can be used for organic compounds which: - are water-soluble at the test concentration (100 mg/l ThOD); - are water-insoluble at the test conditions, provided a suitable dosing technique is used; - do not significantly adsorb on or react with the oxygen electrode; - are inhibitory to the test microorganisms at the low test concentrations used.	Test method especially for poorly water-soluble test compounds using simple equipment.
ISO 11733	<i>Evaluation of the elimination and the biodegradability of organic compounds in an aqueous medium - Activated sludge simulation test</i>	Continuously operated, aquatic test system using organic test compounds and easily biodegradable organic medium (sewage) as the source of carbon and energy for an inoculum of aerobic mixed microorganisms (activated sludge). Daily addition of sewage and test compound to the test vessels and measurement of the dissolved organic carbon (DOC) or chemical oxygen demand (COD) in the influent and effluent of test and control units to determine the ultimate biodegradability within the test time of up to 12 weeks. Evaluation of the test results by calculating the DOC removal. Additionally specific analysis can be used to determine the primary biodegradability of a test compound.	The test can be used for organic compounds which are: - water-soluble or satisfactorily dispersible at the test concentration (10-20 mg/l DOC); - non-volatile, or having a negligible vapour pressure; - not inhibitory to the test microorganisms at the test concentration.	Test used if detailed information in waste water treatment plants is required. Corresponds to OECD 303

No.	Title	Principle	Scope	Recommendation
ISO 11734	<i>Evaluation of the ultimate anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of the biogas production.</i>	Static, aquatic test system using organic test compounds as the sole added source of carbon and energy for an inoculum of anaerobic mixed micro-organisms (digested sludge). Measurement of the biogenically produced gas (carbon dioxide and methane) by measurement of pressure and dissolved inorganic carbon (DIC) to determine the ultimate biodegradability within 60 days. Evaluation of the test results by comparing the yield of biogas with the theoretical amount. Additionally specific analysis can be used to determine the primary biodegradability of a test compound.	The test can be used for organic compounds which are: - water-soluble at the test concentration (20 - 100 mg/l organic carbon); - are water-insoluble under the test conditions, provided a suitable dosing technique is used; - are volatile, provided a suitable dosing technique is used (case-by-case decision); - not inhibitory to the test microorganisms at the test concentration.	Test used to evaluate anaerobic biodegradability.
ISO 14592-1 (draft)	<i>Evaluation of the aerobic biodegradability of organic compounds at low concentrations in water</i> - Part 1: Shake flask method - Part 2: River simulation test	Test system simulating the conditions of a static or a dynamic aerobic surface water system. Determination of the primary biodegradability of a water-soluble test substance under realistic environmental conditions especially at low concentrations. Ultimate biodegradability can be determined if the test substance is radio-labelled in the appropriate position(s).	The test can be used for organic compounds which are: - known to be in principle biodegradable; - water-soluble at the test concentration; - nonvolatile and for which a substance-specific analytical technique or radio-labelled substances are available.	Test method to determine biodegradation kinetics under environmentally realistic conditions.
ISO 14593 (draft)	<i>Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds - Method by the analysis of released inorganic carbon in sealed vessels</i>	Static, aquatic test system using organic test compounds as the sole source of carbon and energy for an inoculum of aerobic mixed microorganisms. Gas-tight closed bottles with sufficient oxygen in the head-space. Measurement of the biogenically produced carbon dioxide (CO ₂) after acidification directly in the gas phase or after alkalization in the form of dissolved inorganic carbon (DIC) to determine the ultimate biodegradability within 28 days. Evaluation of the test results by comparing the yield of CO ₂ with the theoretical amount.	The test can be used for organic compounds which are: - water-soluble at the test concentration (20 mg/l DOC); - water-insoluble at the test conditions; - volatile; - not inhibitory to the test microorganisms at the test concentration.	Test method for water-soluble, poorly soluble and volatile test compounds using an analytical parameter which has a strictly biological origin.

Annex B

Comparison of International Standards with OECD Guidelines

Test method	OECD	ISO
DOC die-away test	301 A	7827
CO ₂ evolution test	301 B	9439
Modified MITI test (I)	301 C	-
Closed bottle test	301 D	10707
Modified OECD screening test	301 E	7827
Manometric respirometry test	301 F	9408
Modified SCAS test	302 A	9887
Zahn-Wellens/EMPA test	302 B	9888
Modified MITI test (II)	302 C	-
Aerobic sewage treatment: Coupled units test	303	11733
Inherent biodegradability in soil	304	-
Biodegradability in seawater	306	-
Test guidance for poorly water-soluble substances	-	10634
Anaerobic degradation test	-	11734
Two-phase closed bottle test	-	10708
Biodegradation test at low concentrations	-	14592
CO ₂ test in sealed vessels	-	14593