
Water quality — Preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium

Qualité de l'eau — Préparation et traitement des composés organiques peu solubles dans l'eau en vue de l'évaluation de leur biodégradabilité en milieu aqueux

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

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

This second edition cancels and replaces the first edition (ISO 10634:1995), which has been technically revised to take into account user feedback, new technologies and available reagents.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The standardizing work carried out by ISO/TC 147/SC 5 has shown that the development of a single method for evaluating the biodegradability of organic compounds with a low solubility in water (i.e. < 100 mg/l^{[1][2][3]}) cannot be envisaged in the immediate future. In fact, the selection of the most suitable working method to obtain a satisfactory emulsion or dispersion of these compounds in the test media depends particularly on their physicochemical properties. Consequently, the selection of the most suitable method has to be left to the judgement of laboratories responsible for the tests based on their experience and the product information supplied by the applicant. For this reason, this document describes various techniques for treating poorly water-soluble organic compounds before they are investigated for biodegradability tests. The objective is to reach a stage where, for any given technique, the same working method is used by all laboratories, thus making it easier to compare results. Specificities of the selected protocol should be kept in mind for the evaluation and interpretation of the results of the biodegradation test.

The techniques described in this document will not necessarily produce the same biodegradability results of the test compound if they are used in parallel. The use of solvents and dispersing or emulsifying techniques can be additional sources of uncertainty and can lead to test results which differ from those obtained without using these techniques. Furthermore, dispersions or emulsions can be produced that would not exist as such in nature. It is recommended to perform biodegradability tests with the direct addition of a test compound and using dispersion techniques in parallel because activity of inoculum used should be comparable. The presence of microorganisms with potential to degrade the test compound is assumed to be identical. The composition and activity might change when the tests are conducted subsequently.

According to current standards for testing biodegradability, only pure or compounds containing a low amount of impurities should be tested. Biodegradability tests are not recommended for heterogeneous mixtures or multicomponent compounds as the results of such tests are difficult to interpret, especially when the degradation is partial. Moreover, the use of solvents and dispersion techniques can lead to unrepresentative heterogeneous distributions and to misleading test results in the subsequent biodegradability tests.

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WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified staff.

1 Scope

This document specifies techniques for preparing poorly water-soluble organic compounds (i.e. liquid and solid compounds) with a solubility in water of less than approximately 100 mg/l and introducing them into test vessels for a subsequent biodegradability test in an aqueous medium using standard methods.

The subsequent tests on biodegradability are primarily methods using the analysis of the released carbon dioxide described in ISO 9439 and the determination of the oxygen described in ISO 9408 and following the usual precautions for ISO 10707. Thus, one can notice that the methods measuring the removal of dissolved organic carbon (DOC) are not appropriate.

This document does not specify the biodegradation test methods. It is restricted to describing techniques for introducing the test compounds into the test medium and to keeping them in a dispersed state^[4]. These techniques are implemented while observing the experimental conditions described in the standardized methods for evaluating biodegradability. ISO 9439, based on CO₂ evolution, is not suitable for testing volatile compounds.

Some of the preparation methods described in this document might not be accepted by regulators for making conclusions on the ready biodegradability of tested compounds.

Examples of biodegradability curves are given in [Annex A](#).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 9408, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer*

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)*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

4 Presentation of suitable preparation and analytical methods

4.1 Preparation methods

In this document, several techniques for introducing the test compounds into the test medium are described. The preparation methods are as follows:

- direct addition: this technique is recommended for poorly soluble compounds instead of the preparation of a stock solution;
- ultrasonic dispersion: this technique can be applied to non-volatile liquid and solid compounds;
- adsorption or weighing on an inert support;
- dispersion or solubilization with additive;
- combination of methods listed above.

NOTE Regarding the combination of methods, the techniques are generally run individually in parallel (i.e. simultaneously by the same method and with the same inoculum) to gain insight into whether one technique is dominant or whether both are contributing to enhance bioavailability and biodegradation.

4.2 Analytical methods

The test compound concentration shall fulfil the requirements of ISO 9439, ISO 9408 and ISO 10707.

When the test compound is introduced directly or on an inert support in the test vessel, it is not necessary to confirm the tested concentration.

When the preparation method uses a stock solution of the tested compound, it is necessary to confirm the concentration tested. For this purpose:

- a specific analytical method is required if the support or additive is an organic chemical (for example, surfactant);
- the total organic carbon (TOC) analysis is acceptable if the support or additive is an inorganic compound (for example, silica gel) or if a homogeneous dispersion is obtained by physical treatment (for example, ultrasonic treatment).

5 Direct addition and addition with inert support

5.1 General

Biodegradability tests should be performed in parallel, with the direct addition of a test compound and using dispersion techniques, because the activity of the inoculum used should be comparable. The presence of microorganisms with the potential to degrade the test compound is assumed to be identical.

The test compound is weighed and directly introduced into the test vessels or weighed onto an inert support and introduced into the test vessels, which are subjected to continuous agitation.

Solid compounds can be grinded (e.g. using a mortar and a pestle) as finely as possible before weighing them. Liquid nitrogen may be used.

NOTE Adding the tested substance adsorbed on inert support can affect the final biodegradation result. The bioavailability of the compound can be limited by the adsorption to the inert support. Therefore, the measured biodegradation result can decrease (e.g. if the adsorption to the surface of the inert material limits the access of the tested substance to the inoculum). In the case of a tested compound that is toxic to microorganisms, the limitation of the bioavailability could limit the toxic effect and increase the biodegradation result.

5.2 Reagents

5.2.1 Inert supports.

Silica gel, fibreglass filters, microscope slides or other non-biodegradable inert supports that do not release organic or inorganic carbon into the aqueous medium can be used.

It should be validated by preliminary work that the support is inert and carbon-free. To avoid or minimize surface area effects, the quantity of the support shall be minimal. The test compound should be adsorbed on the surface.

For example, the following supports are suitable:

- microscopic slide;
- polyethylene slide;
- stainless steel slide;
- silica gel used for thin-layer chromatography (15 µm particle size);
- silica gel used for column chromatography (200 µm to 500 µm particle size).

5.3 Apparatus

5.3.1 Stirrers.

Sufficient stirrers are required to agitate all the test vessels used in a given biodegradability test except for the closed bottle test (see ISO 10707).

Stirrer-rods shall be made of a material such that no ingredient of a plastic coat will contaminate the test medium and no adsorption of test compounds will occur. Heating the test vessels by stirring and raising the test temperature shall be avoided.

5.3.2 Vessels.

It is recommended to use laboratory glass or chemically inert labware for weighing and sample preparations to avoid carbon contamination and adsorption of the test compound.

5.3.3 Mechanical disperser (e.g. Ultra-turrax^{®1}).

5.4 Procedure

5.4.1 Direct addition

Test compounds shall be weighed and directly added to the test vessels.

1) Ultra-turrax[®] is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Non-viscous liquid compounds shall be added with a high precision volumetric syringe, taking into account their relative density.

5.4.2 Addition on inert support

5.4.2.1 Solid test compound

Weigh onto the support (5.2.1) a quantity of the compound corresponding to the concentration of the organic carbon required by the test method to be used.

Introduce a support into each of the test vessels.

Introduce a support without the test compound into each of the control vessels.

5.4.2.2 Liquid test compound

Weigh the liquid, including the viscous compound, without treatment. Prepare the quantity of test compound required by the biodegradability test method to be mixed with the support.

For example, with a final test solution volume of 1 l, add 50 mg of the support (5.2.1) and the amount of the test compound needed for the test flask by direct weighting and emulsify with the mechanical disperser for 1 min. At the same time, carry out the same procedure using only the support in the control vessels. After mixing the inert support with the liquid test compound, the dilution water and inoculum are added.

6 Ultrasonic and physical treatment

6.1 General

An emulsion or dispersion of the compound to be tested is prepared using an ultrasonic probe or an ultrasonic bath and is introduced into the test vessels, which are continuously agitated (see 5.3.1 and 5.3.2).

6.2 Apparatus

6.2.1 **Ultrasonic probe**, capable of producing a frequency of approximately 20 kHz to 35 kHz.

6.2.2 **Ultrasonic bath**, capable of producing a frequency of approximately 20 kHz to 35 kHz.

6.2.3 **Stirrers**, in sufficient numbers to ensure that all the test vessels can be agitated (see 5.3.2).

6.3 Procedure using an ultrasonic probe

6.3.1 Preparation of the test compound

6.3.1.1 Preparation of a stock solution

Add, for example, 1 g or 1 ml of the test compound to a 500 ml beaker containing approximately 400 ml of deionized water.

The test compound shall be present in excess so that a saturated solution is obtained.

6.3.1.2 Preparation with the required quantity

Add the required quantity of the test compound into the test vessels containing mineral medium without inoculum.