

INTERNATIONAL STANDARD

ISO
9439

First edition
1990-12-01

**Water quality — Evaluation in an aqueous
medium of the “ultimate” aerobic
biodegradability of organic compounds —
Method by analysis of released carbon dioxide**

iTeh STANDARD PREVIEW
(standards.iteh.ai)

*Qualité de l'eau — Évaluation, en milieu aqueux, de la biodégradabilité
aérobie “ultime” des composés organiques — Méthode par dosage du
dioxyde de carbone dégagé*

[https://standards.iteh.ai/catalog/standards/sist/05577dc4-d1f5-4441-9dd9-
e57796755176/iso-9439-1990](https://standards.iteh.ai/catalog/standards/sist/05577dc4-d1f5-4441-9dd9-e57796755176/iso-9439-1990)



Reference number
ISO 9439:1990(E)

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.

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.

International Standard ISO 9439 was prepared by Technical Committee ISO/TC 147, *Water quality*.

Annexes A, B and C of this International Standard are for information only.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

<https://standards.iteh.ai/catalog/standards/sist/05577dc4-d1f5-4441-9dd9-e57796755176/iso-9439-1990>

© ISO 1990

All rights reserved. No part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from the publisher.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

Water quality — Evaluation in an aqueous medium of the “ultimate” aerobic biodegradability of organic compounds — Method by analysis of released carbon dioxide

1 Scope

This International Standard specifies a method, by analysis of released carbon dioxide, for the evaluation in an aqueous medium of the “ultimate” biodegradability of organic compounds at a given concentration by aerobic micro-organisms.

The method applies to organic compounds which are

- a) soluble in the test conditions;
- b) insoluble in the test conditions, in which case special measures may be necessary to achieve good dispersion of the compound;
- c) non-volatile or which have a negligible vapour pressure under the conditions of the test;
- d) not inhibitory to the test micro-organisms at the concentration chosen for the test. The presence of an inhibitory effect can be determined as specified in 8.3, or by using any other method for determining the inhibitory effect of a compound on bacteria (see, for example, ISO 8192).

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 7827:1984, *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*.

3 Definitions

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

3.1 ultimate biodegradation: The level of degradation achieved when the test compound is totally utilized by micro-organisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituents (biomass).

3.2 suspended solids (of an activated sludge): Solids removed by filtration or centrifuging of a known volume of sludge under specified conditions, and, for the purpose of this International Standard, drying at about 100 °C.

3.3 pre-exposure (or pre-adaptation): The pre-incubation of an inoculum in the presence of the test compound, with the aim of enhancing the ability of the inoculum to degrade the test compound.

3.4 pre-conditioning (or pre-acclimatization): The pre-incubation of an inoculum under the conditions of the test in the absence of the test compound, to improve the performance of the test.

4 Principle

Determination of the biodegradation of organic compounds by aerobic micro-organisms, using a test medium.

The organic compound is the sole source of carbon and energy in the medium. The level of biodegradation is determined indirectly by measurement of the released carbon dioxide during the test time (generally 28 days). The concentration of the compounds used is such that the initial organic carbon content of the medium is normally between 10 mg/l and 40 mg/l. If required, more than 40 mg/l may be used to give additional information.

For sufficiently water-soluble compounds, DOC removal at the end of the test may be determined.

5 Test environment

Incubation shall take place in the dark or in diffused light, in an enclosure which is maintained at a constant temperature (within at least ± 1 °C) between 20 °C and 25 °C and which is free from toxic vapours.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 Distilled or de-ionized water.

Containing less than 10 % of the initial DOC content introduced by the organic compound to be tested.

6.2 Test medium

6.2.1 Composition

6.2.1.1 Solution a).

Anhydrous potassium dihydrogenphosphate (KH_2PO_4)	8,5 g
Anhydrous dipotassium hydrogenphosphate (K_2HPO_4)	21,75 g
Disodium hydrogenphosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$)	33,4 g
Ammonium chloride (NH_4Cl)	0,5 g
Water (6.1) (quantity necessary to make up to 1000 ml)	

The pH of this solution should be about 7,4.

6.2.1.2 Solution b).

Dissolve 22,5 g of magnesium sulphate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) in 1000 ml of the water (6.1).

6.2.1.3 Solution c).

Dissolve 27,5 g of anhydrous calcium chloride (CaCl_2) or 36,4 g of calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) in 1000 ml of the water (6.1).

6.2.1.4 Solution d).

Dissolve 0,25 g of iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in 1000 ml of the water (6.1). Prepare this solution just before use.

NOTE 1 It is not necessary to prepare this solution just before use if a drop of concentrated hydrochloric acid (HCl) or 0,4 g/l of ethylenediamine-tetraacetic acid (EDTA) is added.

6.2.2 Preparation of the test medium.

For 1 litre of test medium, add just before use to 800 ml of the water (6.1)

- 10 ml of solution a);
- and then 1 ml of each solution b), c) and d), (to avoid the formation of turbidity in the final medium).

Make up to 1000 ml with the water (6.1).

7 Apparatus

Ensure that all glassware is thoroughly cleaned and, in particular, free from organic or toxic matter.

Usual laboratory equipment, and

7.1 Glass vessels, Erlenmeyer flasks or bottles allowing gas purge and magnetic stirring (see 8.3).

7.2 CO₂-free air production system, capable of supplying each test flask at a flow rate between 50 ml/min and 100 ml/min, held constant within 10 % (see example of assembly in annex A).

Check the absence of carbon dioxide in the air production system.

7.3 Apparatus for the determination of carbon dioxide, carbon dioxide analyser or device for titrimetric determination after complete absorption in a basic solution (see the principle of a test system with released carbon dioxide in annex A).

7.4 Device for filtration, with membrane filters of suitable porosity (nominal aperture diameter between 0,2 μm and 0,45 μm) which adsorb organic compounds or release organic carbon to a minimum degree.

7.5 Instrument for the determination of dissolved organic carbon concentration

7.6 Centrifuge

7.7 pH-meter.

8 Procedure

8.1 Preparation of the test solutions

8.1.1 Solution of the test compound

Prepare a stock solution of the test compound in the test medium (6.2). Dilute a suitable amount of this solution in the previously aerated test medium (see 8.3) in order to obtain a final organic carbon concentration of between 10 mg/l and 40 mg/l.

NOTE 2 Compounds of low water solubility may be added directly, in solid or liquid form to the medium in the appropriate flask; an ISO International Standard will be prepared for guidance.

8.1.2 Solution of the reference compound

Prepare a stock solution of the reference compound (an organic compound of known biodegradability such as sodium acetate, sodium benzoate, aniline) in the test medium (6.2) in the same way as in 8.1.1, in order to obtain a final organic carbon concentration of 20 mg/l.

8.1.3 Solution to check inhibition

If necessary, prepare the solution containing, in the test medium (6.2), the test compound and the reference compound in the respective concentrations used for the preparation of solutions in 8.1.1 and 8.1.2.

8.2 Preparation of the inoculum

Prepare the inoculum using the following sources or using a mixture of these sources to obtain a microbial population that offers sufficient biodegradation activity. Check this activity by means of the reference compound (8.1.2).

The carbon dioxide production of the blank solution should be in the range of 30 mg/l to 40 mg/l but not

greater than 70 mg/l. To reduce the influence of the blank, it may be helpful to precondition the sludge by aerating it up to one week before it is used [see clause 10, item b)].

The quantity of dissolved organic carbon provided by the inoculum shall be less than 10 % of the initial concentration of organic carbon introduced by the test compound.

NOTE 3 In certain circumstances, pre-exposed inocula may be used, provided that this is clearly stated in the test results (e.g. per cent biodegradation = x %, using pre-exposed inocula) and the method of pre-exposure detailed in the test report.

Pre-exposed inocula can be obtained from laboratory biodegradation tests conducted under a variety of conditions (e.g. Zahn-Wellens and SCAS tests) or from samples collected from locations where relevant environmental conditions exist (e.g. treatment plants dealing with similar compounds, contaminated areas, etc.).

8.2.1 Inoculum from a secondary effluent

Take a sample of secondary effluent collected from a treatment plant dealing with predominantly domestic sewage. Keep this sample under aerobic conditions and use on the day of collection.

From this sample, prepare an inoculum as follows:

- let the sample of effluent settle for 1 h;
- take a suitable volume¹⁾ of the supernatant, to be used as inoculum for the test carried out that day

8.2.2 Inoculum from an activated sludge plant

Take a suitable volume¹⁾ of inoculum, for example, a sample of activated sludge from the aeration tank of a sewage works treating predominantly domestic sewage.

Mix well, keep under aerobic conditions and use on the day of collection.

Just before use, determine the concentration of suspended solids¹⁾. If necessary, concentrate the sludge by settling so that the volume of sludge added to obtain 30 mg of dry matter per litre be minimum.

1) "Suitable" volume means

- sufficient to give a population which offers enough biodegradation activity;
- degrades the reference compound(s) by the stipulated percentage;
- gives between 10^3 and 10^5 active cells/ml;
- CO_2 production in the blank controls, must not be greater than 70 mg/l (normally 30 mg/l to 40 mg/l);
- gives not greater than the equivalent of 30 mg activated sludge solids per litre in the final reaction mixture.

8.2.3 Inoculum from a surface water or from the influent system of a domestic treatment station

Take a sample of an appropriate surface water.

This sample, kept under aerobic conditions, shall be used on the day of preparation.

Take a suitable volume (see footnote 1 to 8.2.2) of inoculum from this sample.

If necessary, the inoculum can be concentrated by filtration or centrifugation.

8.3 Test

Provide a number of vessels (7.1) so that the test includes at least the following:

- a) two test vessels for the test compound (symbol F_T);
 - b) two vessels for the blank test (symbol F_B);
 - c) one test vessel for checking the inoculum activity (symbol F_C);
- and, if necessary,
- d) a vessel for checking that the product has not undergone abiotic degradation (symbol F_S);
 - e) a vessel for checking any inhibiting effect of the compound (symbol F_I).

Add appropriate amounts of the test medium (6.2) and the inoculum (8.2) to each of these vessels, as indicated in table 1.

Table 1 — Final distribution of the test medium and the inoculum

Bottle	Solutions				Inoculum ¹⁾ (8.2) ml
	Test medium (6.2)	Solution of the test compound (8.1.1)	Solution of the reference compound (8.1.2)	Inhibition check solution (8.1.3)	
F_T Test	0	2,5	0	0	<i>n</i>
F_T Test	0	2,5	0	0	<i>n</i>
F_B Blank	2,5	0	0	0	<i>n</i>
F_B Blank	2,5	0	0	0	<i>n</i>
F_C Inoculum check	0	0	2,5	0	<i>n</i>
F_S Abiotic degradation check (optional)	0	2,5 ²⁾	0	0	0
F_I Inhibition check (optional)	0	0	0	2,5	<i>n</i>

litres

1) The volume of inoculum (*n* ml) should be compatible with the validity clause for the blank [see clause 10, item b)] and to obtain a sufficient microbial population (see 8.2).

2) The volume of the test solution (8.1.1) in vessel F_S shall be sterilized by the addition of 1 ml of solution containing 10 g/l of mercury chloride ($HgCl_2$), or an other toxic compound capable of preventing microbial activity.

Mix the contents in the vessels and connect them to the CO₂-free air production system (see 7.3 and annex A).

Aerate the vessels with CO₂-free air for 24 h to purge the system of carbon dioxide. After the aeration period, introduce the Ba(OH)₂ or NaOH solution into the CO₂-absorber bottles (see annex A) and connect in series to the air exit of each test vessel, or connect to the CO₂-measuring system.

Add the stock solutions of the test compound (8.1.1) and the reference compound (8.1.2) to the respective vessels according to table 1 and incubate at a constant temperature (within ± 1 °C) between 20 °C and 25 °C for 28 days.

Check that the air flow rate is the same in all the bottles and is between 50 ml/min and 100 ml/min.

Measure the amount of carbon dioxide released from each bottle at intermediate time intervals (e.g. 1, 7, 14, 21 and 28 days). If necessary, measure at shorter intervals and/or over a period longer than 28 days.

Annex B describes a titrimetric method for the determination of CO₂ when the device shown in annex A is used.

If a constant level of degradation is attained before the end of the 28 day test period, the test is considered to be completed.

On the last day of the test (normally the twenty-eighth day), acidify all the bottles with 1 ml of concentrated hydrochloric acid in order to decompose the carbonates and bicarbonates. Continue aeration for 24 h and then measure the amount of carbon dioxide released from each bottle.

NOTE 4 As the buffering capacity of the medium is comparatively high, it is essential to acidify the test medium at the final sampling date to ensure removal of all dissolved carbon dioxide. If the DOC removal is measured to provide additional information on biodegradation, take a sample at day 0 and at the end of the test (before the acidification of all bottles takes place) from each vessel and determine DOC.

9 Calculation and expression of results

9.1 Calculations

9.1.1 Amount of carbon dioxide resulting from the theoretical degradation of the compound

The theoretical amount of carbon dioxide (ThCO₂), expressed in milligrammes, is given by the formula

$$\text{ThCO}_2 = C \times V \times \frac{44}{12}$$

where

C is the concentration of the test compound in the test solution, measured or calculated from the measured concentration in the stock solution of the test compound (8.1.1) taking into account dilution, expressed in milligrammes of organic carbon per litre;

V is the volume of the test solution, expressed in litres;

44 and 12 are the relative molar and atomic masses of carbon dioxide and carbon, respectively.

9.1.2 Percentage degradation

Determine the percentage degradation *D_t* for the test vessels *F_T*, the inoculum check vessel *F_C* and the inhibition check vessel *F_I* (if it was included), from the amount of carbon dioxide released during each interval, using the following formula:

$$D_t = \frac{(\text{CO}_2)_T - (\text{CO}_2)_B}{\text{ThCO}_2} \times 100$$

where

(CO₂)_T is the average, expressed in milligrammes, of the total amount of carbon dioxide released in vessels *F_T* between the start of the test and time *t*;

(CO₂)_B is the average, expressed in milligrammes, of the total amount of carbon dioxide released in vessels *F_B* between the start of the test and time *t*;

ThCO₂ is the theoretical amount of carbon dioxide, expressed in milligrammes, determined according to 9.1.1.

NOTES

5 The abiotic degradation (vessel *F_S*) is calculated in the same way but without considering (CO₂)_B.

6 The determination of DOC can be used to determine the final percentage degradation *D_t* (see ISO 7827).

9.2 Expression of results

Compile a table of percentage degradation for each determination interval and each test vessel. If comparable results are obtained for the duplicate test vessels, plot a mean degradation curve as a function of time (see example in annex C).

Record the amount of carbon dioxide which may be released in the vessels for checking abiotic degradation (*F_S*) and for checking any inhibitory effect of the compound (*F_I*), if these vessels were included in the test.

10 Validity of results

The test is considered as valid if

- a) the percentage degradation in the vessel F_C for checking the inoculum activity is greater than 50 % on the 14th day;
- b) the amount of CO_2 which has evolved from the blank solution during the test is insignificant: the total CO_2 evolution in the blank solution at the end of the test should be in the range of 30 mg/ml to 40 mg/l and should not exceed 70 mg/l.

If flask F_I (inhibition check) was included in the test, it can be assumed that the test compound is inhibitory if the degradation percentage in this flask is less than 25 % in 28 days. In this case, repeat the test series with lower concentrations of the test material.

11 Test report

The test report shall provide all pertinent information, particularly the following:

- a) a reference to this International Standard;
- b) any information necessary to identify the compound subjected to the test;

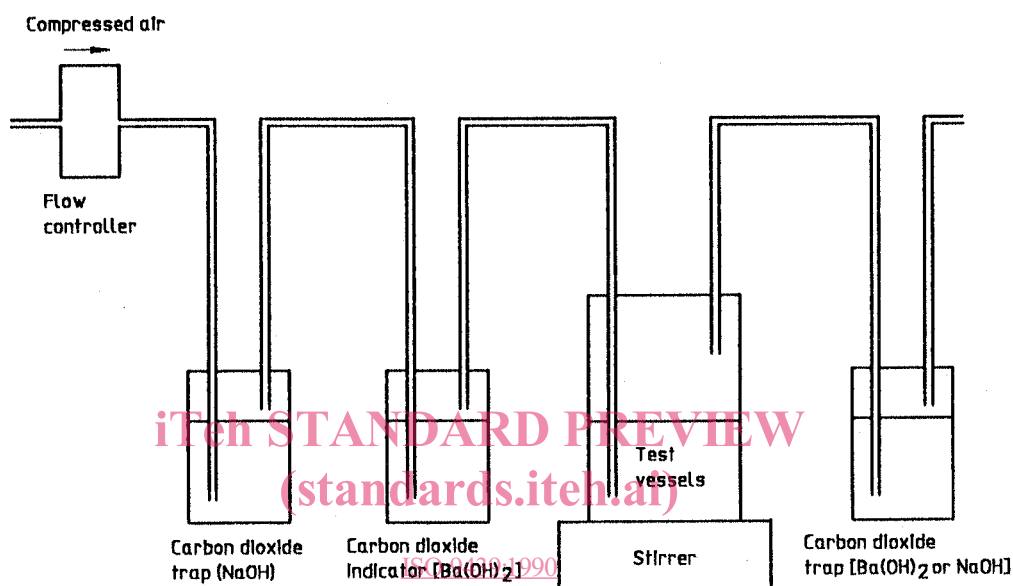
- c) the name and concentration of the reference compound used;
- d) all the results obtained (in tabular form) and the degradation curve, including the results obtained for the inoculum activity check tests (vessel F_C) and for the inhibition check tests (vessel F_I) and abiotic degradation check tests (vessel F_S) if they were included;
- e) the main characteristics of the carbon analyser employed;
- f) the main characteristics of the device used to determine carbon dioxide;
- g) the reasons for any rejection of the test results (see clause 10);
- h) any pre-conditioning or pre-exposure of the inoculum;
- i) the concentration of the test compound used and the DOC content of this concentration;
- j) the source, the characters and the amount of the inoculum used;
- k) any other facts that are relevant to the procedure followed.

ISO 9439:1990

<https://standards.itech.ai/catalog/standards/sist/05577dc4-d1f5-4441-9dd9-e57796755176/iso-9439-1990>

Annex A (informative)

Principle of a test system with released carbon dioxide



<https://standards.iteh.ai/catalog/standards/sist/65577dc4-d115-4441-9dd9-e57796755176/iso-9439-1990>

Figure A.1

Compressed air or synthetic CO₂-free air is supplied at a constant low pressure to the test system.

Carbon dioxide in the air is removed by passing through a sodium hydroxide solution. A second trap containing barium hydroxide solution is used to indicate the total absence of carbon dioxide.

The air is then used to aerate the test mixture in the test vessels. If biodegradation takes places, carbon

dioxide is produced and absorbed in a second carbon dioxide trap containing a solution of either sodium hydroxide or barium hydroxide. In the first case, carbon dioxide is determined as dissolved inorganic carbon in a suitable analyser. In the second case, carbon dioxide is analysed by a titrimetric method as described in annex B and a series of three traps is necessary.