

INTERNATIONAL  
STANDARD

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**10707**

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**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)**

*Qualité de l'eau — Évaluation en milieu aqueux de la biodégradabilité  
aérobie «ultime» des composés organiques — Méthode par analyse de  
la demande biochimique en oxygène (essai en fiole fermée)*



Reference number  
ISO 10707:1994(E)

## Foreword

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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 10707 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

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

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# 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)

**WARNING — SAFETY PRECAUTIONS** — Activated sludge and sewage may contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling them. Toxic test compounds and those whose properties are unknown should be handled with care.

## 1 Scope

This International Standard specifies a method, by analysis of biochemical oxygen demand, for the evaluation in an aqueous medium of the “ultimate” biodegradability of organic compounds at a given concentration by aerobic microorganisms.

The conditions described in this International Standard do not necessarily always correspond to the optimal conditions for allowing the maximum value of biodegradation to occur.

The method applies to all organic compounds which are sufficiently water soluble to prepare a stock solution or poorly water soluble when using special dosing techniques.

Due to the low concentration of test compound at the beginning of the test, normally no special precautions for the toxicity of the test compound to the microorganisms of the inoculum is necessary; if required a parallel inhibition test can be performed.

## 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 5813:1983, *Water quality — Determination of dissolved oxygen — Iodometric method.*

ISO 5814:1990, *Water quality — Determination of dissolved oxygen — Electrochemical probe method.*

ISO 6060:1989, *Water quality — Determination of the chemical oxygen demand.*

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 10304-2:—<sup>1)</sup>, *Water quality — Determination of dissolved anions by liquid chromatography of ions — Part 2: Determination of bromide, chloride, nitrate, nitrite, orthophosphate and sulfite in waste water.*

1) To be published.

ISO 10634:—<sup>1)</sup>, *Water quality — Guidance for the evaluation in an aqueous medium of the "ultimate" biodegradability of poorly soluble organic compounds.*

### 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 microorganisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituents (biomass).

**3.2 biochemical oxygen demand (BOD):** The mass concentration of dissolved oxygen consumed under specified conditions by the biological oxidation of organic and/or inorganic matter in water and is expressed in this case as milligrams of oxygen uptake per milligram or gram of test compound.

**3.3 Chemical oxygen demand (COD):** The amount of oxygen consumed during oxidation of a test compound with hot, acidic dichromate. It provides a measure of the amount of oxidizable matter present and is expressed in this case as milligrams of oxygen consumed per milligram or gram of test compound.

**3.4 theoretical oxygen demand (ThOD):** The total amount of oxygen required to oxidize a chemical completely. It is calculated from the molecular formula and is expressed in this case as milligrams of oxygen required per milligram or gram of test compound.

**3.5 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. If the aim is achieved, the inoculum is said to be adapted.

**3.6 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

A solution of the organic test compound in a mineral medium as the sole source of carbon and energy is inoculated with a relatively small number of microorganisms from a mixed population and kept in completely full, closed bottles in the dark at a constant temperature. Biodegradation is followed by analysis

of dissolved oxygen over a period of 28 d. The amount of oxygen taken up by the test chemical (BOD), corrected for uptake by the blank inoculum run in parallel, is expressed as percentage of ThOD or COD.

### 5 Test environment

Incubation shall take place in the dark in an enclosure which is maintained at a constant temperature (within  $\pm 1$  °C) between 20 °C and 25 °C.

### 6 Reagents

Use only reagents of recognized analytical grade.

#### 6.1 Water

Distilled or deionized water free from inhibitory concentrations of toxic substances containing less than 10 % of the initial DOC content introduced by the compounds to be tested. For each series of tests, use only one batch of water.

#### 6.2 Test medium

##### 6.2.1 Composition

##### 6.2.1.1 Solution a)

Anhydrous potassium dihydrogenphosphate (KH <sub>2</sub> PO <sub>4</sub> )	8,5 g
Anhydrous dipotassium hydrogenphosphate (K <sub>2</sub> HPO <sub>4</sub> )	21,75 g
Disodium hydrogenphosphate dihydrate (Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O)	33,4 g
Ammonium chloride (NH <sub>4</sub> Cl)	0,5 g

Dissolve the ingredients in water (6.1) and make up to 1 000 ml.

NOTE 1 The correct composition of the medium can be checked by the measurement of the pH value, which should be 7.4.

##### 6.2.1.2 Solution b)

Dissolve 22,5 g of magnesium sulfate heptahydrate (MgSO<sub>4</sub>·7H<sub>2</sub>O) in water (6.1) and dilute to 1 000 ml.

##### 6.2.1.3 Solution c)

Dissolve 27,5 g of anhydrous calcium chloride (CaCl<sub>2</sub>) or 36,4 g of calcium chloride dihydrate (CaCl<sub>2</sub>·2H<sub>2</sub>O) in water (6.1) and dilute to 1 000 ml.