
**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**

*Qualité de l'eau — Évaluation en milieu aqueux de la biodégradabilité
aérobie ultime des composés organiques — Détermination de la demande
biochimique en oxygène en fiole fermée à deux phases*



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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.

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 10708 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Annexes A to E 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 — Determination of biochemical oxygen demand in a two-phase 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 for the evaluation of the ultimate biodegradability by aerobic microorganisms of organic compounds, in particular poorly water-soluble compounds, at a given concentration.

NOTE 1 The conditions described in this International Standard do not necessarily always correspond to the optimal conditions allowing the maximum degree of biodegradation to occur.

The method applies to organic compounds which

- are water soluble or poorly water soluble at the concentration used under the test conditions;
- do not adsorb onto or have any effect on the oxygen electrode (see 8.1.2 and 8.3.4);
- are not inhibitory to the test microorganisms at the concentration chosen for the test.

NOTE 2 For special measures to introduce poorly water-soluble test compounds into the test vessels, see ISO 10634.

NOTE 3 Inhibitory effects can be determined as described in 8.1.4 and 8.3.1, or by using any other method for determining the inhibitory effect on bacteria of a substance (e.g. ISO 8192).

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was 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 edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

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*.

3 Definitions

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

3.1 ultimate biodegradation

breakdown of an organic chemical compound by microorganisms in the presence of oxygen to yield carbon dioxide, water and mineral salts of any other elements present (mineralization) and new biomass

3.2 primary biodegradation

structural change (transformation) of an organic chemical compound by microorganisms resulting in the loss of a specific property

3.3 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 water, expressed in this case as milligrams of oxygen uptake per milligram or gram of test compound

3.4 theoretical oxygen demand (ThOD)

theoretical maximum amount of oxygen required to oxidize a chemical compound completely, calculated from the molecular formula, expressed in this case as milligrams of oxygen required per milligram or gram of test compound

3.5 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, expressed in this case as milligrams of oxygen consumed per milligram or gram of test compound

3.6 dissolved organic carbon (DOC)

that part of the organic carbon in water which cannot be removed by specified phase separation, for example by centrifugation at $40\,000\text{ m}\cdot\text{s}^{-2}$ for 15 min or by membrane filtration using membranes with pores of $0,2\text{ }\mu\text{m}$ to $0,45\text{ }\mu\text{m}$ diameter

3.7 concentration of suspended solids of an activated sludge

amount of solids obtained by filtration or centrifugation of a known volume of activated sludge after filtration or centrifugation and drying at about $105\text{ }^{\circ}\text{C}$ to constant mass

3.8 lag phase

time from the start of a test until adaptation and selection of the degrading microorganisms are achieved and the biodegradation degree of a chemical compound or organic matter has increased to about 10 % of the theoretical maximum biodegradation; it is expressed in days

3.9 maximum level of biodegradation

maximum biodegradation degree of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test; it is expressed as a percentage

3.10 degradation phase

time from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached; it is expressed in days

3.11 plateau phase

time from the end of the biodegradation phase when the maximum level of biodegradation is reached until the end of the test

3.12 pre-exposure

pre-incubation of an inoculum in the presence of the test compound, with the aim of enhancing the ability of an inoculum to biodegrade the test compound by adaptation and selection of the microorganisms

3.13 pre-conditioning

pre-incubation of an inoculum under the conditions of the test in the absence of the test compound, with the aim of improving the performance of the test by acclimatization of the microorganisms to the test conditions

4 Principle

Biodegradation of organic compounds by aerobic microorganisms is determined in an aquatic medium. The organic compound is the sole source of carbon and energy in the medium. The inoculated medium is shaken or stirred in closed bottles, containing known volumes of medium and air, to assure steady-state oxygen partitioning between liquid and gas phases. The degradation is followed by regular measurements of the dissolved oxygen concentration in the aqueous phase for up to 28 days. The total oxygen uptake in the test flasks is calculated from the difference in the measured dissolved oxygen concentrations in the blank and test flasks divided by the oxygen saturation value under normal conditions and multiplied by the total oxygen content originally present in the liquid and gaseous phases. Biodegradability is calculated as the total oxygen uptake divided by the theoretical oxygen demand (ThOD) or chemical oxygen demand (COD), expressed as a percentage.

5 Test environment

Incubation shall take place in the dark or in diffused light in an enclosure which is maintained at a constant temperature ($\pm 0,5\text{ }^{\circ}\text{C}$) in a range between $20\text{ }^{\circ}\text{C}$ and $25\text{ }^{\circ}\text{C}$.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 Water

Distilled or deionized water containing less than 2 mg/l of DOC and/or less than 10 % of the initial organic carbon content introduced by the test compound.

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) in a quantity necessary to make up to	1 litre

NOTE – The correct composition of the medium can be checked by measuring the pH value, which should be 7,4.

6.2.1.2 Solution B

Dissolve 22,5 g of magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) in 1 000 ml of water (6.1).

6.2.1.3 Solution C

Dissolve 27,5 g of anhydrous calcium chloride (CaCl_2) in 1 000 ml of 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 1 000 ml of water (6.1). Prepare this solution freshly before use.

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

6.2.2 Preparation

For 1 litre of test medium, add to about 500 ml of water (6.1)

- 10 ml of solution A;
- 1 ml of each of the solutions B to D.

Make up to 1 000 ml with water (6.1).

6.3 Sodium hydroxide solution

Dissolve sodium hydroxide (NaOH) in water (6.1) to obtain a solution of 0,1 mol/l to 0,5 mol/l.

6.4 Hydrochloric acid solution

Dissolve hydrochloric acid (HCl) in water (6.1) to obtain a solution of 0,1 mol/l to 0,5 mol/l.

7 Apparatus

Ensure that all glassware is thoroughly cleaned and, in particular, is free from organic or toxic matter. Use usual laboratory equipment and the following items.

7.1 Incubation bottles, gas-tight, e.g. narrow-neck flasks with volumes of 200 ml to 300 ml with suitable stoppers (e.g. ground-glass stoppers, screw-caps or butyl rubber stoppers), shielded from light (e.g. made of brown glass).

It is recommended to use stopper clamps. Mark each bottle with waterproof markings. If the oxygen electrode used has no mounted stirrer, provide each bottle with a magnetic stirrer-bar coated with polytetrafluorethylene. Either pre-select bottles of standard volume such that the standard deviation about the mean volume of the batch of bottles is less than 1 ml, or measure and record the volumes of individual, numbered bottles to an accuracy of 1 ml. Carefully grease the stoppers of the bottles with inert silicone grease to assure proper closing and easy removal.

7.2 Oxygen electrode to measure over a range of 0 mg/l to 10 mg/l with a precision of 1 %.

Steady-state shall be reached within about 2 min. Mount the electrode for example in an inert stopper which makes a leak-proof fit in the ground glass neck of the incubation bottle. Preferably use electrodes with mounted stirrers.

7.3 Magnetic stirrer with speed regulation, if required.

Stirrer-rods attached to the oxygen electrode shall be of such a material that no ingredients of any plastic coatings 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.

7.4 Shaking device, if required.

7.5 Water bath, or other device to guarantee an accurate temperature control of $\pm 0,5$ °C in a range of 20 °C to 25 °C.

7.6 pH-meter.