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

*Qualité de l'eau — Évaluation, en milieu aqueux, de la biodégradabilité
aérobie ultime des composés organiques par détermination de la demande
en oxygène dans un respiromètre fermé*



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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 9408 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 9408:1991), which has been technically revised.

Annexes A to D of this International Standard are for information only.

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

WARNING — Activated sludge and sewage contain potentially pathogenic organisms. Take appropriate precautions when handling them. Handle with care toxic test compounds and those whose properties are unknown.

1 Scope

This International Standard specifies a method, by determination of the oxygen demand in a closed respirometer, for the evaluation in aqueous medium of the ultimate biodegradability of organic compounds and waste waters at a given concentration by aerobic microorganisms.

The method applies to organic compounds which

- a) are water-soluble under the conditions of the test;
- b) are poorly water-soluble under the conditions of the test, in which case special measures may be necessary to achieve good dispersion of the compound (see for example, ISO 10634);
- c) do not reach and react with the CO₂ absorbent;
- d) are volatile, provided that a suitable respirometer or suitable conditions (e.g. a smaller ratio of volume head space to volume liquid medium) are used;
- e) are not inhibitory to the test microorganisms at the concentration chosen for the test. The presence of inhibitory effects can be determined as specified in 7.3, or by using any other method for determining the inhibitory effect of a compound on bacteria (see, for example, ISO 8192).

NOTE The conditions described in this International Standard do not always correspond to the optimal conditions for allowing the maximum degree of biodegradation to occur. For alternative biodegradation methods, see ISO 15462.

2 Terms and definitions

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

2.1

ultimate aerobic biodegradation

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

2.2

primary biodegradation

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

2.3**activated sludge**

biomass produced in the aerobic treatment of wastewater by the growth of bacteria and other microorganisms in the presence of dissolved oxygen

2.4**concentration of suspended solids of an activated sludge**

amount of solids obtained by filtration or centrifugation of a known volume of activated sludge and drying at about 105 °C to constant mass

2.5**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

NOTE It is expressed in this case as milligrams oxygen uptake per milligram (or gram) test compound.

2.6**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

NOTE It is expressed in this case as milligrams oxygen consumed per milligram (or gram) test compound.

2.7**theoretical oxygen demand****ThOD**

theoretical maximum amount of oxygen required to oxidize a chemical compound completely, calculated from the molecular formula

NOTE It is expressed in this case as milligrams oxygen required per milligram (or gram) test compound.

2.8**dissolved organic carbon****DOC**

that part of the organic carbon in the water which cannot be removed by specified phase separation

NOTE Examples of specified phase separation are centrifugation at 40 000 m·s⁻² for 15 min or by membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter.

2.9**lag phase**

time from the start of a test until adaptation and/or 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 maximum level of biodegradation

NOTE It is expressed in days.

2.10**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

NOTE It is expressed in percent.

2.11**biodegradation phase**

time from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached

NOTE It is expressed in days.

2.12**plateau phase**

time from the end of the biodegradation phase until the end of the test

NOTE It is expressed in days.

2.13**pre-exposure**

pre-incubation of an inoculum in the presence of the test chemical compound or organic matter, with the aim of enhancing the ability of this inoculum to biodegrade the test material by adaptation and/or selection of the microorganisms

2.14**preconditioning**

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

3 Principle

Determination of the biodegradation of organic compounds by aerobic microorganisms is carried out using a static aqueous test system. Organic compounds in the context of this International Standard include waste waters. The test mixture contains an inorganic medium, the organic compound as the sole source of carbon and energy at a mass concentration of normally 100 mg/l organic carbon [but its theoretical oxygen demand (ThOD) shall be at least 100 mg/l], and a mixed inoculum obtained from a waste-water treatment plant or from another source in the environment.

The mixture is agitated in a closed test vessel and the consumption of oxygen is determined either by measuring the amount of oxygen required to maintain a constant gas volume in the respirometer vessel, or by measuring the change in volume or pressure (or a combination of the two) in the apparatus. The evolved carbon dioxide is absorbed in a suitable substance in the test vessel.

The degradation is followed over a period of 28 d, or longer if necessary, by determining the consumption of oxygen either automatically or manually. The amount of oxygen consumed by the organic compound (after correction by comparison with blank control) is expressed as a percentage of either the theoretical oxygen demand (ThOD), calculated from the formula of the compound, or the chemical oxygen demand (COD).

For sufficiently water-soluble compounds, removal of dissolved organic carbon (DOC) may be determined (optionally) by measuring the concentration of DOC at the beginning and the end of incubation to obtain additional information on the ultimate biodegradability. If a substance-specific analytical method is available, information on the primary degradability may be obtained.

4 Test environment

Incubation shall take place in the dark or in diffused light, at a temperature within the range 20 °C to 25 °C which shall not vary by more than ± 1 °C during the test.

5 Reagents

Use only reagents of recognized analytical grade.

5.1 Water

Distilled or deionized water containing less than 1 mg/l DOC.

5.2 Test medium

5.2.1 Composition

5.2.1.1 Solution a)

Dissolve

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 (5.1), in quantity necessary to make up to	1000 ml

In order to check this buffer solution, it is recommended to measure the pH, which should be at about 7,4. If this is not the case prepare a new solution.

5.2.1.2 Solution b)

Dissolve 22,5 g magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot \text{H}_2\text{O}$) in water (5.1), quantity necessary to make up to 1000 ml.

5.2.1.3 Solution c)

Dissolve 36,4 g calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) in water (5.1), quantity necessary to make up to 1000 ml.

5.2.1.4 Solution d)

Dissolve 0,25 g iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in water (5.1), quantity necessary to make up to 1000 ml. Prepare this solution freshly before use, or add a drop of concentrated hydrochloric acid (HCl) to avoid precipitation

5.2.2 Preparation of the test medium

For 1000 ml of test medium, add to about 800 ml of water (5.1):

- 10 ml of solution a);
- 1 ml of each of the solutions b) to d).

Make up to 1000 ml with the water (5.1). Prepare the test medium freshly before use. The solutions a) to c) may be stored up to 6 months in the dark at room temperature.

5.3 Carbon dioxide absorber

Potassium hydroxide solution (about 10 mol/l), soda lime pellets or other suitable absorbent.