



# SLOVENSKI STANDARD SIST EN ISO 11733:2005

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SIST EN ISO 11733:2000

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**Kakovost vode – Ugotavljanje odstranjanja in biorazgradljivosti organskih snovi v vodi – Simulacijski preskus z aktivnim blatom (ISO 11733:2004)**

Water quality - Determination of the elimination and biodegradability of organic compounds in an aqueous medium - Activated sludge simulation test (ISO 11733:2004)

Wasserbeschaffenheit - Bestimmung der Elimination und der biologischen Abbaubarkeit organischer Verbindungen in einem wässrigen Medium - Belebtschlamm-Simulationstest (ISO 11733:2004)

Qualité de l'eau - Détermination de l'élimination et de la biodégradabilité des composés organiques en milieu aqueux - Essai de simulation des boues activées (ISO 11733:2004)

**Ta slovenski standard je istoveten z: EN ISO 11733:2004**

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**ICS:**

13.060.70      Preiskava bioloških lastnosti vode      Examination of biological properties of water

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EUROPEAN STANDARD  
NORME EUROPÉENNE  
EUROPÄISCHE NORM

**EN ISO 11733**

August 2004

ICS 13.060.70

Supersedes EN ISO 11733:1998

English version

**Water quality - Determination of the elimination and  
biodegradability of organic compounds in an aqueous medium -  
Activated sludge simulation test (ISO 11733:2004)**

Qualité de l'eau - Détermination de l'élimination et de la  
biodégradabilité des composés organiques en milieu  
aqueux - Essai de simulation des boues activées (ISO  
11733:2004)

This European Standard was approved by CEN on 29 July 2004.

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EUROPÄISCHES KOMITEE FÜR NORMUNG

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**EN ISO 11733:2004 (E)****Foreword**

This document (EN ISO 11733:2004) has been prepared by Technical Committee ISO/TC 147 "Water quality" in collaboration with Technical Committee CEN/TC 230 "Water analysis", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2005, and conflicting national standards shall be withdrawn at the latest by February 2005.

This document supersedes EN ISO 11733:1998.

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**Endorsement notice**

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# INTERNATIONAL STANDARD

# ISO 11733

Second edition  
2004-08-01

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## Water quality — Determination of the elimination and biodegradability of organic compounds in an aqueous medium — Activated sludge simulation test

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*Qualité de l'eau — Détermination de l'élimination et de la  
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Reference number  
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**ISO 11733:2004(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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

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.

ISO 11733 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 11733:1995), which has been technically revised.

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# Water quality — Determination of the elimination and biodegradability of organic compounds in an aqueous medium — Activated sludge simulation test

**WARNING AND SAFETY PRECAUTIONS** — Activated sludge and sewage 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 determination of the elimination and the biodegradability of organic compounds by aerobic micro-organisms. The conditions described simulate a waste-water treatment plant. Two test systems can be used: activated sludge plants or porous pots. The tests can optionally be performed under conditions of nitrification and denitrification (Annex A) and coupling of the units (Annex B).

The method applies to organic compounds which, under the conditions of the test, are

- a) soluble in tap water at the test concentration and not expected to be transformed to insoluble metabolites if biodegradation, in addition to elimination, is determined;
- b) poorly water-soluble, but which are satisfactorily dispersible in water and allow detection with suitable analytical means (e.g. organic carbon measurements);
- c) non-volatile, or which have a negligible vapour pressure under the test conditions;
- d) not inhibitory to the test micro-organisms at the concentration chosen for the test. Inhibitory effects can be determined by using a suitable test method (e.g. ISO 8192<sup>[15]</sup> or ISO 15522<sup>[27]</sup>). Compounds inhibitory at concentrations used in this test may be tested at concentrations less than their EC<sub>20</sub> value, followed by higher practical concentrations after a period of acclimatization.

The method can also be used to measure the biodegradation and elimination of dissolved organic compounds in waste water (also called “test compound” in the method).

If more or different information is required to predict the behaviour of test compounds or waste water in a treatment plant, other degradation tests may be performed. For appropriate use of this method and for alternative biodegradation methods, see ISO/TR 15462 and for general information on biotesting, see ISO 5667-16.

## 2 Normative references

The following referenced documents are indispensable for the application 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 5667-16, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

ISO 10634, *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*

ISO/TR 15462, *Water quality — Selection of tests for biodegradability*

## ISO 11733:2004(E)

### 3 Terms and definitions

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

#### 3.1

##### **accelerating removal phase**

(activated sludge simulation test) time from the end of the lag phase until the plateau phase is reached, during which the biodegradation of a compound or organic matter increases

NOTE Accelerating removal phase is expressed in days.

#### 3.2

##### **activated sludge**

biomass and inert matter produced in the aerobic treatment of wastewater by the growth of bacteria and other micro-organisms in the presence of dissolved oxygen

#### 3.3

##### **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 COD is expressed, in this case, as milligrams of oxygen consumed per milligram or per gram of test compound

#### 3.4

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

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

#### 3.5

##### **degree of elimination biodegradation**

(activated sludge simulation test) mean eliminated (biodegraded) amount of a chemical compound or organic matter, calculated from the measured concentrations in the inlet and the outlet of the system

NOTE The degree of elimination (biodegradation) is determined when no further elimination can be measured and is expressed as a percentage.

#### 3.6

##### **denitrification**

reduction of nitrate and nitrite to the end product nitrogen (in the form of the gas) by the action of bacteria

#### 3.7

##### **dissolved organic carbon**

##### **DOC**

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

NOTE Phase separation may be obtained, for example, by centrifugation of the water sample at 40 000 m/s<sup>2</sup> for 15 min or by membrane-filtration using membranes with a pore size of 0,45 µm.

#### 3.8

##### **lag phase**

(activated sludge simulation test) time from the start of a test until a significant elimination (biodegradation) of a compound or organic matter can be measured (the beginning of the accelerated removal phase)

NOTE The lag phase is expressed in days.

**3.9****nitrification**

oxidation of ammonium salts by bacteria where usually the intermediate product is nitrite and the end product nitrate

**3.10****plateau phase**

(activated sludge simulation test) time from the end of the accelerating removal phase until the end of a test in which the biodegradation of a compound or organic matter is in a steady state

NOTE The plateau phase is expressed in days.

**3.11****pre-exposure**

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

**3.12****pre-conditioning**

pre-incubation of an inoculum under the conditions of the subsequent test in the absence of the test compound and other organic matter, with the aim of improving the performance of the test by acclimatization of the micro-organisms to the test conditions

**3.13****primary biodegradation**

structural change (transformation) of a chemical compound by micro-organisms resulting in the loss of a specific property

**3.14****total organic carbon  
TOC**

all the carbon present in organic matter which is dissolved and suspended in the water

**3.15****ultimate aerobic biodegradation**

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

**4 Principle**

This method is designed to determine the elimination and, if possible, the primary or ultimate biodegradation of water-soluble organic compounds from water by aerobic micro-organisms in a continuously operating test system simulating the activated-sludge process. An easily biodegradable organic medium and the organic test compound are the sources of carbon and energy for the micro-organisms.

Two test units (activated sludge plants or porous pots) are run in parallel under identical conditions, normally with a mean hydraulic retention time, HRT, of 6 h (8.3.1) and a mean sludge retention time, SRT (sludge age), of 6 d to 10 d (8.3.3).

NOTE 1 HTR is the mean period of retention of waste water in the aeration vessel. It is calculated by dividing the volume of sludge, expressed in litres, by the rate of flow of waste water, expressed in litres per day.

NOTE 2 SRT is the mean period of retention of activated sludge in the aeration vessel. It is calculated by dividing the volume or weight of sludge in the aeration vessel by the volume or weight of sludge discarded per day. If a period of 8 days is chosen, remove 1/8 of the volume of the activated sludge of the aeration vessel each working day and discard it.

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The test compound is added together with the organic medium, usually at a concentration equivalent to a DOC between 10 mg/l and 20 mg/l, to the influent of only one of the test units. The second unit is used as control unit to determine the degree of biodegradation of the organic medium when the analysis is based on DOC or COD.

Samples of the effluents taken at regular intervals are analyzed for DOC or COD. The difference between values in the effluent of the test and the control unit compared with the influent concentration of the test compound is used to determine the degree of elimination of the test compound. Depending on the elimination characteristics and other available information, e.g. from other tests, ultimate biodegradability can be stated.

If required, the primary biodegradation of the test compound can be determined by substance-specific analysis. Optionally, the units may be operated under denitrifying conditions (see Annex A) or be coupled (see Annex B).

## 5 Test environment

The test shall take place in diffused light or in the dark, in an enclosure which is free from vapours toxic to micro-organisms and at a controlled temperature in the range of 20 °C to 25 °C. For special purposes, it is permissible to use a test temperature in another range.

## 6 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

**6.1 Tap water**, containing less than 3 mg/l DOC.

**6.2 Deionized water**, containing less than 1 mg/l DOC.

**6.3 Organic media.** <https://standards.iteh.ai/catalog/standards/sist/249f4938-f252-4a9c-9b43-7d05d9fdd0d7/sist-en-iso-11733-2005>

**6.3.1 General.**

Synthetic sewage, domestic sewage or a mixture of both are permissible as an organic medium. Measure the DOC (e.g. ISO 8245<sup>[16]</sup>) or COD (e.g. ISO 6060<sup>[14]</sup>) concentration in each new batch of organic medium and determine the alkalinity, if required and not already known.

Experience has shown that the so-called OECD medium<sup>[29]</sup> (6.3.2) might not be suitable in some cases. Therefore, two more synthetic media which have successfully been tested in laboratories are described in this International Standard. Domestic sewage (6.3.5) may also be used. Its use is recommended, as a continuous inoculation takes place and a vastly greater number of nutrients is available to improve the biodegradation potential of the test.

**6.3.2 Synthetic sewage 1 (OECD medium)**, which gives a mean DOC concentration of about 100 mg/l and a COD of about 300 mg/l in the influent.

It is composed of the following:

— peptone	160 mg
— meat extract	110 mg
— urea	30 mg
— anhydrous potassium monohydrogenphosphate (K <sub>2</sub> HPO <sub>4</sub> )	28 mg
— sodium chloride (NaCl)	7 mg