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EUROPEAN STANDARD
 NORME EUROPÉENNE
 EUROPÄISCHE NORM

EN ISO 11733

July 1998

ICS 13.060.40

Descriptors: see ISO document

English version

Water quality - Evaluation of the elimination and biodegradability
 of organic compounds in an aqueous medium - Activated sludge
 simulation test (ISO 11733:1995)

Qualité de l'eau - Evaluation de l'élimination et de la
 biodégradabilité des composés organiques en milieu
 aqueux - Essai de simulation des boues activées (ISO
 11733:1995)

Wasserbeschaffenheit - Untersuchung der Elimination und
 der biologischen Abbaubarkeit organischer Verbindungen
 in einem aquatischen Medium - Belebtschlamm-
 Simulationstest (ISO 11733:1995)

This European Standard was approved by CEN on 21 June 1998.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
 COMITÉ EUROPÉEN DE NORMALISATION
 EUROPÄISCHES KOMITEE FÜR NORMUNG

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

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Foreword

The text of the International Standard from Technical Committee ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO) has been taken over as an European Standard by 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of an identical text or by endorsement, at the latest by January 1999, and conflicting national standards shall be withdrawn at the latest by January 1999.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 11733:1995 has been approved by CEN as a European Standard without any modification.

NOTE: Normative references to International Standards are listed in annex ZA (normative).

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Annex ZA (normative)
Normative references to international publications
with their relevant European publications

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

<u>Publication</u>	<u>Year</u>	<u>Title</u>	<u>EN</u>	<u>Year</u>
ISO 8192	1986	Water quality – Test for inhibition of oxygen consumption by activated sludge	EN ISO 8192	1995
ISO 9408	1991	Water quality – Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds – Method by determining the oxygen demand in a closed respirometer	EN 29408	1993
ISO 9439	1990	Water quality – Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds – Method by analysis of released carbon dioxide	EN 29439	1993
ISO 9888	1991	Water quality – Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium – Static test (Zahn-Wellens method)	EN 29888	1993
ISO 10304-2	1995	Water quality – Determination of dissolved anions by liquid chromatography of ions – Part 2: Determination of bromide, chloride, nitrate, nitrite, orthophosphate and sulfate in waste water	EN ISO 10304-2	1996
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	EN ISO 10634	1995
ISO 11732	1997	Water quality – Determination of ammonium nitrogen by flow analysis (CAF and FIA) and spectrometric detection	EN ISO 11732	1997

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INTERNATIONAL STANDARD

ISO
11733

First edition
1995-12-15

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

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*Qualité de l'eau — Évaluation de l'élimination et de la biodégradabilité des
composés organiques en milieu aqueux — Essai de simulation des boues
activées*
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Reference number
ISO 11733:1995(E)

ISO 11733:1995(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 11733 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

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

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Water quality — Evaluation of the elimination and biodegradability of organic compounds in an aqueous medium — Activated sludge simulation 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 elimination and biodegradability of organic compounds at a given concentration by aerobic microorganisms. The conditions described simulate a waste-water treatment plant.

The method applies to organic compounds that under the test conditions are

- water-soluble at the chosen test concentration;
- satisfactorily dispersible in water and allow dissolved organic carbon (DOC) measurements;
- non-volatile, or have a negligible vapour pressure;
- not inhibitory to the microorganisms of the inoculum at the test concentration.

Inhibition can be determined by using a suitable test method (e.g. 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 6060:1989, *Water quality — Determination of the chemical oxygen demand.*

ISO 8192:1986, *Water quality — Test for inhibition of oxygen consumption by activated sludge.*

ISO 8245:1987, *Water quality — Guidelines for the determination of total organic carbon (TOC).*

ISO 9408:1991, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by determining the oxygen demand in a closed respirometer.*

ISO 9439:1990, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of released carbon dioxide.*

ISO 9888:1991, *Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Static test (Zahn-Wellens method).*

ISO 10304-2:1995, *Water quality — Determination of dissolved anions by liquid chromatography of ions — Part 2: Determination of bromide, chloride, nitrate, nitrite, orthophosphate and sulfate in waste water.*

ISO 10634:1995, *Water quality — Guidance for the preparation and treatment of poorly water-soluble or-*

ganic compounds for the subsequent evaluation of their biodegradability in an aqueous medium.

ISO 11732:—¹⁾, *Water quality — Determination of ammonium nitrogen by flow analysis and spectrometric detection.*

ISO 11923:—¹⁾, *Water quality — Determination of suspended solids by filtration through glass-fibre filters.*

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 primary biodegradation: The level of degradation achieved when the test compound undergoes any structural change, other than mineralization, as the result of microbial action.

3.3 concentration of suspended solids: The amount of solids obtained by filtration or centrifugation of a known volume of sludge under specified conditions and drying at 105 °C to constant mass.

3.4 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.

4 Principle

This method is designed to determine the elimination and, under some circumstances, the primary or ultimate biodegradation of water-soluble organic compounds by aerobic microorganisms in a continuously operated test system simulating the activated sludge process. An easily biodegradable organic medium and the organic test compound are the source of carbon and energy for the microorganisms.

Two continuously operating test units (activated sludge plants or porous pots) are run in parallel under identical conditions, with a mean hydraulic retention time of normally 6 h and a mean sludge age (sludge

retention time) of 6 d to 10 d. The test compound is normally added at a concentration between 10 mg/l DOC and 20 mg/l DOC, to the influent (organic medium) of only one of the test units; the second unit is used as a control unit to determine the biodegradation of the organic medium.

In regularly taken samples of the effluents, the DOC or chemical oxygen demand (COD) and/or, if required, the test compound concentration are measured by specific analysis. The difference between the effluent concentrations in the test and control units compared with the influent concentration of the test compound is used to determine the elimination of the test compound. Depending on the elimination characteristics, a biodegradability value can be determined.

5 Test environment

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

6 Reagents and materials

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

6.2 Deionized water, containing less than 2 mg/l of DOC.

6.3 Organic medium

Synthetic sewage, domestic sewage or a mixture of both is permissible as the organic medium. The acidity and alkalinity of the organic medium should be known. Measure the DOC or COD concentration in each new batch of organic medium.

6.3.1 Synthetic sewage

Peptone	160 mg
Meat extract	110 mg
Urea	30 mg
Anhydrous dipotassium hydrogen phosphate (K ₂ HPO ₄)	28 mg
Sodium chloride (NaCl)	7 mg
Calcium chloride dihydrate (CaCl ₂ ·2H ₂ O)	4 mg
Magnesium sulfate heptahydrate (MgSO ₄ ·7H ₂ O)	2 mg
Tap water (6.1)	1 litre

1) To be published.

This synthetic sewage is an example and gives a mean DOC concentration in the influent of about 100 mg/l. Alternatively, use other compositions with about the same DOC concentration, which are closer to real sewage.

NOTE 1 If a less concentrated influent is required, the synthetic sewage (e.g. 1:1) should be diluted with tap water to obtain a DOC concentration of about 50 mg/l. A reduced concentration of synthetic sewage will allow a better growth of nitrifying microorganisms. This modification should be used if the simulation of nitrifying waste water treatment plants is performed.

6.3.2 Domestic sewage

Use fresh, settled and, if necessary, neutralized domestic sewage largely free from coarse particles. The sewage can be stored for several days at about 4 °C if it is proved that the DOC or COD has not significantly (i.e. less than about 20 %) decreased during storage.

6.3.3 Organic medium with improved buffering capacity

Domestic sewage of low acidity or alkalinity, or synthetic sewage prepared from tap water of low acidity or alkalinity, can require the addition of a suitable buffer, for example carbonate or phosphate buffer to maintain a pH of about $7,5 \pm 0,5$ in the aeration vessel during the test. In this case, add, for example, 196 mg of sodium hydrogen carbonate (NaHCO_3) or 1,5 g of potassium dihydrogen phosphate (KH_2PO_4) to 1 litre of organic medium. How much buffer shall be added, and when, has to be decided in each individual case, depending on the acidity or alkalinity of the organic medium and the pH values measured in the aeration tank.

6.4 Test compound

Prepare a solution of a suitable concentration, for example 5 g/l of the test compound in deionized water (6.2).

Determine the DOC and total organic carbon (TOC) of the stock solution and repeat the measurement for each new batch. If the difference between the DOC and TOC is greater than 20 %, check the water solubility of the test compound at the desired test concentration. Compare the DOC or the test compound concentration, measured by specific analysis of the stock solution, with the theoretical value, to ascertain whether the analytical recovery is good enough (normally > 90 % can be expected). Ensure, especially for dispersions, whether or not the DOC can be used

as an analytical parameter or whether only an analytical technique specific for the test substance can be used. Centrifugation of the samples is required for dispersions.

For each new batch, measure the DOC, COD or the test compound concentration with specific analyses.

Determine the pH of the stock solution. Extreme pH values indicate that the compound may have an influence on the pH of the activated sludge in the test system. In this case, neutralize the stock solution to obtain a pH of $7 \pm 0,5$ with small amounts of inorganic acid or base, but avoid precipitation of the test compound.

7 Apparatus

7.1 Test system

The test system for one test compound consists of a test unit and a control unit. One control unit can be used for several test units. In the case of coupling (see 8.2) use one control unit for each test unit. The test system shall be either an activated sludge plant model or a porous pot (see annex A). In both cases, storage vessels of sufficient size for the influent and the effluent are needed, as well as pumps to dose the influent.

Each activated sludge plant unit consists of an aeration vessel with a capacity for about 3 litres of activated sludge and a separator (secondary clarifier) which holds about 1,5 litres. Vessels of different size are permissible if they are operated with comparable hydraulic loads. If it is not possible to keep the test temperature in the test room in the desired range, the use of water-jacketed vessels with temperature-controlled water is recommended. A dosing pump or an airlift pump is used to recycle the activated sludge from the separator to the aeration vessel, either continuously or intermittently.

The porous pot system consists of an inner, porous cylinder with a conical bottom suspended in a slightly larger vessel of the same shape, but made of impervious material. Separation of the sludge from the treated organic medium is effected by differential passage through the porous wall. No settlement occurs and hence there is no sludge return. Porous pots sometimes become blocked and could overflow in the initial stages. In such a case, replace the pot with a clean one, carefully transferring the sludge to it. Clean blocked pots by soaking in dilute sodium hypochlorite solution, then in water, followed by thoroughly rinsing with water.