SLOVENSKI STANDARD
SIST EN ISO 8192:1997

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Kakovost vode - Preskus zaviranja porabe kisika z aktivnim blatom (ISO 8192:1986)

Water quality - Test for the inhibition of oxygen consumption by activated sludge (ISO 8192:1986)

Wasserbeschaffenheit - Bestimmung der Hemmung des Sauerstoffverbrauchs von Belebtschlamm (ISO 8192:1986)

Qualité de l'eau - Essai d'inhibition de la consommation d'oxygene par des boues activées (ISO 8192:1986)

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English version

Water quality - Test for the inhibition of oxygen consumption by activated sludge (ISO 8192:1986)

Qualité de l'eau - Essai d'inhibition de la consommation d'oxygène par des boues activées (ISO 8192:1986)

Wasserbeschaffenheit - Bestimmung der Hemmung des Sauerstoffverbrauchs von Belebtschlamm (ISO 8192:1986)

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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Foreword

This European Standard has been taken over by the Technical Committee CEN/TC 230 "Water analysis" from the work of ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO).

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of an identical text or by endorsement, at the latest by July 1995, and conflicting national standards shall be withdrawn at the latest by July 1995.

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

Endorsement notice

The text of the International Standard ISO 8192:1986 was approved by CEN as a European Standard without any modification.

International Standard 8192

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

Water quality — Test for inhibition of oxygen consumption by activated sludge

Qualité de l'eau — Essai d'inhibition de la consommation d'oxygène par des boues activées

First edition — 1986-07-15

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Descriptors : water, quality, sewage treatment sludges, tests, determination, inhibition, consumption, oxygen, apparatus.

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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 8192 was prepared by Technical Committee ISO/TC 147, *Water quality*.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

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Water quality — Test for inhibition of oxygen consumption by activated sludge

WARNING AND SAFETY PRECAUTIONS — Activated sludge and sewage may contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling them.

Toxic test materials and those whose properties are unknown should be handled with care.

0 Introduction

This International Standard specifies a method for assessing the potential toxicity of substances, mixtures or waste water to activated sludge. Information generated by this method may be helpful in estimating the effect of a test material on mixed bacterial communities in the aquatic environment especially in aerobic biological treatment systems.

The annex forms an integral part of the standard.

1 Scope and field of application

1.1 This International Standard specifies a method for assessing the inhibitory effect of a test material on the oxygen consumption of activated sludge micro-organisms. The inhibitory effect may include the effect on respiration and nitrification.

1.2 This method gives information on inhibitory or stimulatory effects after a short exposure (up to 180 min) of the test material on activated sludge micro-organisms.

1.3 This method is applicable to chemical substances which are soluble under the conditions of the test. Special care has to be taken with those materials of low water solubility and with materials which consume or produce oxygen physico-chemically.

Two examples of the application are given in the annex. Method A is intended to represent the conditions in surface waters, while method B is intended to represent the conditions in biological waste water treatment plants. Results obtained by these two approaches may be different. It is essential that the test report identifies which method was selected.

For volatile materials only the first example may be applicable.

This method can also be used to test waste waters.

NOTES

1 Results with volatile material should be interpreted with caution and are likely to underestimate any inhibitory effects because of the difficulty of maintaining the initial concentration.

2 Results with insoluble materials should similarly be treated with caution and cannot be easily quantified; again, inhibitory effects may be underestimated if the solubility of the compound in the test medium changes.

1.4 The results from this test should be considered only as a guide to the likely toxicity of the test material, since sludges of different sources may differ in bacterial composition and concentration. Also, laboratory tests cannot truly simulate environmental conditions. For example no account is taken of longer-term adaptation of the activated sludge micro-organisms to the test material, or of materials which may adsorb on to the sludge and build up to a toxic concentration over a longer period of time than that allowed in the test.

2 Definitions

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

2.1 activated sludge: Accumulated biological mass (floc) produced in the treatment of waste water by the growth of bacteria and other micro-organisms in the presence of dissolved oxygen. (Definition taken from ISO 6107/1.)

2.2 suspended solids: Solids removed from activated sludge by filtration or centrifuging and dried at about 100 °C to a constant mass.

Determined as dry mass per unit volume, this quantity is expressed in grams per litre or milligrams per litre.

2.3 oxygen consumption rate: Uptake of oxygen by activated sludge micro-organisms per unit volume of sludge, in unit time.

This quantity is expressed in milligrams per litre per hour.

2.4 specific oxygen consumption rate: Uptake of oxygen by activated sludge micro-organisms per unit mass of micro-organisms (suspended solids), in unit time.

This quantity is expressed in milligrams per gram per hour.

2.5 inhibition of oxygen consumption: Decrease of the oxygen consumption rate of an activated sludge in the presence of the test material, compared with that of a similar mixture without test material.

This quantity is expressed as a percentage.

2.6 toxic range: The range of concentration of a test material over which 0 to 100 % inhibition occurs.

2.7 EC 50: Effective concentration of the test material giving a calculated or interpolated inhibition of oxygen consumption of 50 % compared with a blank control.

3 Principle

Activated sludge in the presence of a suitable, easily biodegradable substrate will consume oxygen rapidly at a rate depending on, among other factors, the concentration of micro-organisms. Addition of a toxic concentration of a test material can result in a decrease in the oxygen consumption rate. The rates are measured using an oxygen electrode. The percentage inhibition of the oxygen consumption is estimated by comparison with a control mixture containing no test material.

The sensitivity of the activated sludge can be checked with a suitable reference substance. Any abiotic oxygen consumption due to physico-chemical processes can also be detected.

4 Medium and reagents

4.1 General

The chemical products used for the preparation of the medium and the reagents shall be of recognized analytical grade.

The water used shall be distilled or deionized water, free from substances that might inhibit the growth of micro-organisms under the test conditions.

Measurements of pH shall be made using a pH meter, measurements being referred to the temperature of test.

4.2 Synthetic medium, stock solution (see table 1).

Table 1 — Synthetic medium (100-fold OECD synthetic sewage)

Constituent	Quantity
Peptone	16 g
Meat extract	11 g
Urea	3 g
Sodium chloride (NaCl)	0,7 g
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	0,4 g
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	0,2 g
Dipotassium hydrogen phosphate (K_2HPO_4)	2,8 g
Water	1 000 ml

The pH of this solution shall be 7,5 ± 0,5.

If the prepared medium is not used immediately, it shall be stored in the dark at 0 to 4 °C, for no longer than 1 week, in conditions which do not produce any change in its composition.

NOTE — Alternatively, sterilize the medium prior to storage, or add the peptone and meat extract shortly before carrying out the test. Before use, ensure the medium is mixed thoroughly and adjust the pH as necessary.

4.3 Test material, stock solution.

The test material may be pure chemicals, mixtures of chemicals, chemical products, or waste waters.

Prepare a stock solution of the test material in water at a suitable concentration, for example 1 g/l or 10 g/l. Waste water may be used without dilution.

For insoluble materials a suspension or dispersion may be prepared or the test material may be added directly to the test vessel. Take care to ensure as much homogeneity as possible.

4.4 Reference substance, stock solution.

Dissolve a suitable quantity of the reference substance in water. 1 g of 3,5-dichlorophenol in 1 000 ml of water has been found to be suitable.

4.5 Isotonic solution (see table 2).

Table 2 — Isotonic solution

Constituent	Quantity
Sodium chloride (NaCl)	5 g
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	0,12 g
Water	1 000 ml

5 Inoculum

For general use, activated sludge should be taken from the aeration tank of a waste water plant treating predominantly domestic sewage and working normally. Depending on the purpose of the test, any type of activated sludge at a suitable concentration, for example 2 to 4 g/l, can also be used. However, activated sludges from different treatment plants may have different characteristics and sensitivities.

Where possible, aerate the activated sludge and use it within 24 h of collection. If this is not possible, feed it daily with an appropriate substrate, for example a synthetic medium (see 4.2).

Where necessary, remove coarse particles by settling for a short period, for example 15 min, and decanting the upper layer of finer solids for use. Alternatively, the sludge may be mixed using a blender for a few seconds.

If inhibitory material is thought to be present, the sludge may be washed as follows: first centrifuge the sludge for about 10 min at approximately 10 000 m/s^2 and discard the supernatant. Resuspend the sludge in chlorine-free tap water or an isotonic washing solution (4.5), remove this by recentrifuging and then repeat if necessary the washing and centrifuging process. Determine the dry mass of a sample of the sludge. Finally resuspend the sludge in chlorine-free tap water or the isotonic solution to obtain a suitable concentration of activated sludge, for example about 3 g/l suspended solids.

In all cases the origin, the concentration and any pretreatment of the activated sludge shall be stated in the test report.

6 Apparatus

Usual laboratory equipment, and

6.1 Test vessels, such as 300 ml BOD bottles or Erlenmeyer flasks with stoppers (see clause A.1) or 1 000 ml beakers (see clause A.2). To measure the oxygen concentration in a BOD bottle a suitable pre-bored stopper as an adapter for the oxygen electrode is required. To avoid loss of displaced liquid on insertion of the oxygen electrode, first insert a funnel or glass tube through the stopper.

6.2 Device for measuring oxygen: a suitable oxygen electrode and a recorder (see ISO 5814, *Water quality — Determination of dissolved oxygen — Electrochemical probe method*).

6.3 Magnetic stirrers.

6.4 Aeration device. If necessary, pass air through an appropriate filter to remove dust and oil and through wash bottles containing water to humidify the air. Aerate the test vessels with Pasteur pipettes, or other aeration devices which do not adsorb chemicals.

7 Procedure

7.1 General

Where possible, perform the test procedure at a constant temperature of 20 ± 2 °C in an atmosphere free from dust and toxic vapours.

7.2 Test mixtures

Prepare in the test vessels (6.1) mixtures, F_T, containing dilution water, synthetic medium and test material, to obtain different known concentrations as required. Adjust the pH to 7,5 ± 0,5, add the inoculum and dilute with water to obtain equal final volumes. If the inhibitory effect of the pH is to be tested, do not adjust the pH.

7.3 Reference mixtures

Prepare mixtures, F_R, with a suitable reference compound in the same way if required (see 7.7).

7.4 Blank control

At least one blank control, F_B, shall be prepared which contains an equal volume of activated sludge and synthetic medium as the test mixture but no test material. Dilute with water to the same volume as the test mixtures.

7.5 Physico-chemical test

If required, prepare mixtures, F_{PC}, to measure the physico-chemical oxygen consumption. They contain test material, synthetic medium and water as the test mixtures, but no activated sludge. If required, add an inhibitor such as mercury chloride to prevent a biological oxygen consumption.

7.6 Preliminary test

To estimate the range of concentrations needed in a definitive test for determining the inhibition of oxygen consumption, a preliminary test is useful.

Carry out the test using at least three concentrations of test material, for example 1; 10; and 100 mg/l, a blank control and, if necessary, a physico-chemical control with the highest concentration of test material. If possible the lowest concentration of test material used shall have no effect on the oxygen consumption.

7.7 Definitive test

Carry out the test using a range of concentration deduced from the preliminary test. It is necessary to use at least five concentrations in a logarithmic series. A blank control shall be included. The physico-chemical control need not be repeated if there is no oxygen uptake in the preliminary test. However, if significant uptake did occur, then controls shall be included for each concentration of test material.

The sensitivity of the sludge may be checked using a reference substance (for example 3,5-dichlorophenol). Where possible, check the sensitivity for each test series or at regular intervals if the same source of inoculum is used.

7.8 Determination

Aerate all mixtures (7.2, 7.3, 7.4 and 7.5) to give as near as possible oxygen saturation.

Stirring is necessary to give good mixing and to allow regular and reproducible oxygen measurement in the incubation and oxygen measuring vessels.

Ensure that all mixtures are at the same temperature (normally 20 ± 2 °C) and that this temperature does not significantly change during the test.