International Standard 8192

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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

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Foreword

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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.

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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.

For method A (clause A.1), measure the oxygen concentration in each mixture with an oxygen electrode at suitable intervals (at least five times over the 3 h test period).

For method B (clause A.2), measure the rate of decrease in concentration of dissolved oxygen in samples of each mixture with an oxygen electrode, after aeration periods of 30 min and, if required, 3 h.

Take care to ensure that there is no supersaturation of oxygen in the mixtures.

NOTE — The choice of 3 h is arbitrary. Normally the activated sludge should still be actively respiring on the synthetic sewage after this time.

8 Expression of results

Calculate the oxygen consumption rates of the test mixtures from the linear part of the graphs of oxygen concentration versus time. Express the oxygen consumption rates in milligrams per litre per hour or milligrams per gram per hour (for more details see the annex).

The percentage inhibition of oxygen consumption, I, at each concentration is given by the equation

$$I = \frac{R_B - (R_T - R_{PC})}{R_B} \times 100$$

where

R_T is the oxygen consumption rate by the test mixture, F_T;

R_B is the oxygen consumption rate by the blank control, F_B;

R_{PC} is the oxygen uptake rate by the physico-chemical control, F_{PC}.

Plot the percentage inhibition of oxygen consumption against the logarithm of the test material concentration (inhibition curve). In method B (clause A.2), inhibition curves are plotted for each aeration period, for example after 30 and 180 min. Calculate or interpolate from the graph the concentration which inhibits the oxygen consumption by 50 % (EC 50).

If suitable data are available, the 95 % confidence limit of the EC 50, the slope of the curve and suitable values to mark the beginning of inhibition (for example EC 10 or EC 20) and the end of the inhibition range (for example EC 80 or EC 90) can be calculated or interpolated.

In view of the variability often observed in the results, it may in many cases be sufficient that the results be expressed in orders of magnitude, for example

EC 50 < 1 mg/l

EC 50 1 — 10 mg/l

EC 50 10 — 100 mg/l

EC 50 > 100 mg/l

9 Validity of the results

Where possible, check the sensitivity of the activated sludge by means of a reference substance.

In a ring test using activated sludge from domestic sewage, the EC 50 of 3,5-dichlorophenol was found to lie in a range of 5 to 30 mg/l.

If the EC 50 of the reference substance does not lie in the expected range, repeate the test with activated sludge from another source.

10 Test report

The test report shall contain the following information:

a) a reference to this International Standard;

b) a reference to the method used (method A or method B);

c) name, specifications and properties of the test material;

d) source, concentration and any pretreatment of the activated sludge;

e) test temperature;

f) name of the reference substance and result of the inhibition measurement with this substance (EC 50);

g) abiotic oxygen uptake in the physico-chemical control;

h) the test results, especially the EC 50 and if possible other statistical data (see clause 8);

i) all measured data and the inhibition curve (see clause 8);

j) all observations and deviations from the standard procedure which could have influenced the result.

Annex

Test procedures

(This annex forms part of the standard.)

A.1 Method A — Using a low concentration of activated sludge

The activated sludge concentration in the test mixture is low (about 100 to 200 mg of suspended solids per litre). The test mixtures are aerated only at the beginning of the test.

Prepare the preliminary test mixtures according to table 3.

An example of a measuring unit for method A is given in figure 1.

Where possible, keep all solutions at, and perform the test at, a constant temperature of 20 ± 2 °C. The solutions shall be as near as possible saturated with air before the mixtures are prepared.

Prepare the mixtures by adding about two-thirds of the water, test material (except F_B), and synthetic medium to the test vessels.

Add the activated sludge, aerated and thoroughly mixed, to each test vessel (except F_{PC}) in turn at an interval of time of about 5 min. Fill the test vessels completely with water, seal each with a stopper and start the magnetic stirrer.

After 30 min stop the stirrer in the first test vessel and remove the stopper. Insert the adapter with the oxygen electrode and restart the stirrer immediately. Wait for equilibrium and measure the concentration of dissolved oxygen. Then stop the stirrer again, replace the oxygen electrode with the original stopper without introducing an air bubble and restart the stirrer.

Repeat this procedure for the rest of the test vessels in turn, after 30 min have elapsed from the addition of the activated sludge.

Continue this procedure at 30 min intervals for 3 h or until the concentration of dissolved oxygen has reached 1 mg/l.

NOTE — The amount of sludge used should be that which will result in a decrease of dissolved oxygen in the blank control from the saturated value of about 9 mg/l to 1 mg/l in about 3 h. This can be checked beforehand and the final concentration of sludge adjusted to give the required activity.

Plot for each test vessel a graph of dissolved oxygen concentration against time. The respiration rate, R, expressed in milligrams per litre per hour, from the linear part of the graph, is given by the equation

$$R = \frac{\varrho_1 - \varrho_2}{\Delta t} \times 60$$

where

ϱ_1 is the first measurement on the linear part of the graph, of the concentration, expressed in milligrams per litre, of dissolved oxygen;

ϱ_2 is the last measurement on the linear part of the graph, of the concentration, expressed in milligrams per litre, of dissolved oxygen;

Δt is the time interval, in minutes, between these two measurements.

The definitive test is carried out in the same way as the preliminary test. It is necessary to use at least five concentrations in a logarithmic series. For example for a substance which gives complete inhibition at 100 mg/l and no inhibition at 1 mg/l in the preliminary test, a suitable series may be 3,2; 5,6; 18; 32 and 56 mg/l.

Depending on experience it may also be possible to use the data of the preliminary test together with those of the definitive test to calculate or interpolate the test results.

The total available series for the calculation is then 1; 3,2; 5,6; 10; 18; 32; 56 and 100 mg/l.

If necessary the same procedure is performed to check the sensitivity of the activated sludge with the reference substance.

Calculate and express the test results as in clause 8.

Table 3 — Method A — Mixtures for preliminary test

Original concentrations of reagents					
Test material stock solution	1 g/l				
Synthetic medium stock solution	See 4.2				
Activated sludge	3 g of suspended solids per litre				
Components of mixtures	**Test vessel**[1]				
	F_{T1}	F_{T2}	F_{T3}	F_B	F_{PC}
Test material stock solution (ml)	0,3	3	30	0	30
Synthetic medium stock solution (ml)	10	10	10	10	10
Activated sludge (ml)	10	10	10	10	0
Water (ml)	279,7	277	250	280	260
Total volume of mixtures (ml)	300	300	300	300	300
Concentrations in the mixtures					
Test material (mg/l)	1	10	100	0	100
Activated sludge (milligrams of suspended solids per litre)	100	100	100	100	0

1) The same procedure shall be followed for the reference substances, F_{R1}, F_{R2} ....

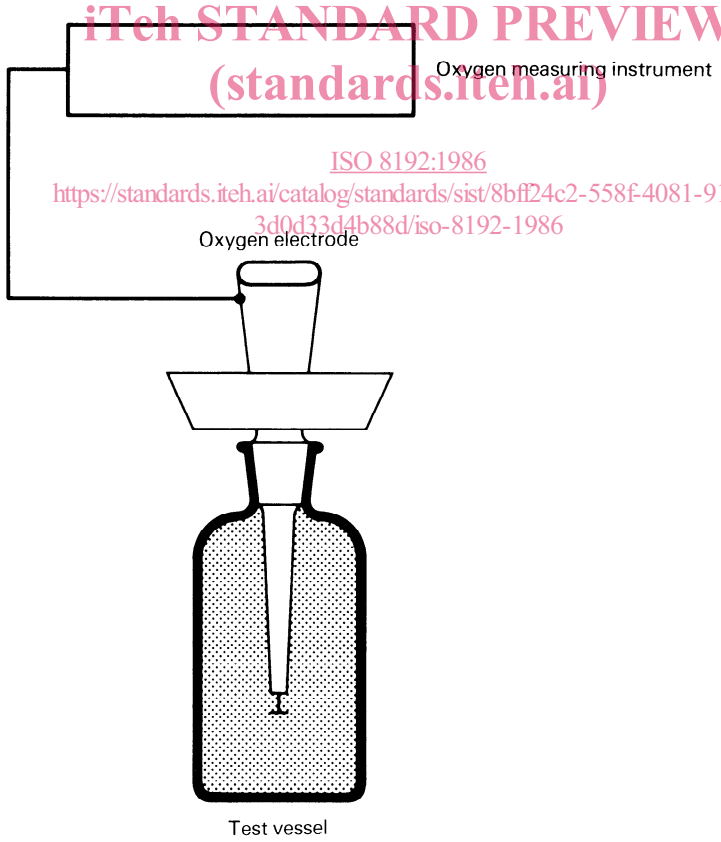

Figure 1 — Example of a measuring unit for method A

A.2 Method B — Using a high concentration of activated sludge

The activated sludge concentration in the test mixture shall be approximately 1 500 mg of suspended solids per litre. The test mixtures are aerated throughout the test. Measurements of oxygen consumption are carried out after 30 min of incubation. If more information after an extended contact time is found necessary, measurements are carried out also after 3 h of incubation.

Prepare the preliminary test mixtures according to table 4 in mixing vessels of about 1 000 ml volume.

All solutions and the place where the test is carried out shall have a constant temperature, for example 20 ± 2 °C.

Prepare the mixtures by adding about two-thirds of the water, test material (except F_B) and synthetic medium to the mixing vessels fitted with magnetic stirrers.

Add the activated sludge to each mixing vessel (except F_{PC}) in turn at convenient intervals of about 10 min. Immediately make up the volume in the mixing vessels to 500 ml with water.

Aerate the contents of the mixing vessels and start the magnetic stirrers.

After 30 min from the preparation of the first mixture start measuring the concentration of dissolved oxygen. Take a sample from the first mixing vessel and measure the rate.

For example use the sample to fill a BOD bottle fitted with a magnetic stirrer. Insert an adapter with an oxygen electrode into the neck of the bottle and start the magnetic stirrer. Measure and record the concentration of dissolved oxygen for about 5 to 10 min or till the oxygen concentration falls below 1 mg/l. Then remove the electrode, return the mixture to the test vessel and continue aerating and stirring. Repeat this procedure with samples from each test vessel in turn to obtain a set of readings taken at 30 min for all test mixtures. If more information after an extended contact time is desired, repeat the procedure after 180 min from the start of incubation have elapsed.

Alternatively, instead of filling a BOD bottle, place a sample in a cylindrical measuring cell of about 20 ml volume which is fitted with an oxygen electrode and a magnetic stirrer. In this case, reduce the volume of the mixtures to about 200 ml. The measured samples are discarded. Before a new measurement starts, the cell must be cleaned with tap water. An example of such a measuring unit is shown in figure 2.

The oxygen consumption rate, R, in milligrams per litre per hour, can be calculated or interpolated from the linear part of the recorded oxygen decrease graph according to the equation

$$R = \frac{\varrho_1 - \varrho_2}{\Delta t} \times 60$$

where

ϱ_1 is the oxygen concentration, expressed in milligrams per litre, at the beginning of the linear phase;

ϱ_2 is the oxygen concentration, expressed in milligrams per litre, at the end of the linear phase;

Δt is the time interval, in minutes, between these two measurements.

Carry out the definitive test in the same way as the preliminary test. For more details on the concentrations recommended, see clause A.1.