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Water quality — Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation

Qualité de l'eau — Essai d'inhibition de la consommation d'oxygène par des boues activées pour l'oxydation du carbone et de l'ammonium

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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 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 8192 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 8192:1986), which has been technically revised.

Introduction

Information generated by this method for assessing the potential toxicity of substances, mixtures and waste waters to activated sludge 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 susceptibility of oxygen uptake by different sub-populations of the bacterial communities to inhibition by chemicals and waste waters is not necessarily uniform and selective effects may profoundly influence the outcome of the test.

There are two principal groups of microorganisms contributing to the total oxygen consumption by activated sludge: heterotrophic organisms mainly responsible for the breakdown of carbon-based substrates (carbonaceous oxidation) and autotrophic nitrifying organisms causing the oxidation of ammonium to nitrate (nitrification).

This International Standard may be used to assess the toxicity of substances on total oxygen uptake (i.e. carbonaceous oxidation and nitrification combined) or, by deliberately adding a specific inhibitor of nitrification, also to assess toxicity of substances to the carbonaceous and nitrification components separately.

For the determination of the nitrification inhibition with this method, a sufficiently nitrifying activated sludge is required. Indications of nitrification may be investigated further by application of ISO 9509[4].

The user of this method should be aware that particular problems could require the specification of additional marginal conditions.

The inhibitory effect of a test material may be exerted on both components or it may be exerted predominantly on only one of them. Nitrification is the process more commonly prone to selective inhibition.

Water quality — Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this International Standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for assessing the inhibitory effect of a test material on the oxygen consumption of activated sludge microorganisms.

This method is intended to represent the conditions in biological waste-water treatment plants. It gives information on inhibitory or stimulatory effects after a short exposure (usually 30 min up to 180 min or even more) of the test material on activated sludge microorganisms.

This method is applicable for testing waters, waste waters, pure chemicals and mixtures of chemicals. Concerning the chemicals, the method refers to those which are soluble under the test conditions. Special care is necessary with materials of low water solubility, high volatility and with materials abiotically consuming or producing oxygen.

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 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

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

3.1
activated sludge
accumulated biological mass (floc) produced in the treatment of waste water by the growth of bacteria and other microorganisms in the presence of oxygen

(ISO 6107-1:2004 [3], definition 2)

3.2
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

(ISO 9888:1999 [6], definition 3.4)

3.3
oxygen consumption rate
uptake of oxygen by activated sludge microorganisms per unit volume of sludge, in unit time

NOTE This quantity is expressed in milligrams per litre per hour [mg/(l·h)].

3.4
specific oxygen consumption rate
uptake of oxygen by activated sludge microorganisms per unit mass of dry sludge (suspended solids), in unit time

NOTE This quantity is expressed in milligrams per gram per hour [mg/(g·h)].

3.5
inhibition of oxygen consumption
decrease of the oxygen consumption rate of an activated sludge plus (a) degradable substance(s) in the presence of the test material, compared with that of a similar mixture without test material

NOTE 1 This quantity is expressed as a percentage.

NOTE 2 In the absence of a substrate, some chemicals (e.g. uncouplers of phosphorylation) can increase oxygen uptake.

3.6
toxic range
range of concentration of a test material over which 0 % to 100 % inhibition occurs

3.7
EC_{50}
effective concentration of the test material giving a calculated or interpolated inhibition of oxygen consumption of 50 % compared with a blank control

3.8
nitrification
oxidation of ammonium compounds by bacteria

NOTE Usually the intermediate product is nitrite and the end product is nitrate

[ISO 6107-1:2004 [3], definition 49].

4 Principle

In the presence of easily biodegradable substances, activated sludge consumes oxygen at a higher rate than in their absence, depending on, among other factors, the concentration of microorganisms. Addition of a toxic concentration of a test material results 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 of the rate with that of a control mixture containing no test material.

The sensitivity of the activated sludge may be checked with a suitable reference substance. The inhibition of the oxygen uptake by all sludge microorganisms, heterotrophic microorganisms and the oxidation of ammonium salts by nitrifying microorganisms may be separately expressed from measurements of the rate of uptake in the absence and presence of *N*-allylthiourea (ATU), a specific inhibitor of the oxidation of ammonium to nitrite by first-stage nitrifiers. The difference between the two oxygen values is due to nitrification and the residual value in the presence of allylthiourea is due to the heterotrophs. Any oxygen consumption due to abiotic processes may be detected by determining the rate in mixtures of the test material, synthetic medium and water, but omitting activated sludge.

Under certain (rare) circumstances, a test substance with strong reducing properties may cause measurable abiotic oxygen consumption. In such cases, abiotic controls are necessary to discriminate between oxygen uptake by the test substance and microbial respiration. Abiotic controls may be prepared either by omitting the inoculum from test mixtures, or by poisoning the inoculum with a solution of mercury(II) chloride.

5 Reagents, media and inoculum

Use only reagents of recognized analytical grade.

5.1 Water, complying with grade 1 as defined in ISO 3696, dissolved respectively distilled or de-ionized water containing less than 1 mg/l dissolved organic carbon (DOC).

5.2 Specific nitrification inhibitor, *N*-allylthiourea (ATU).

Dissolve 2,50 g of *N*-allylthiourea (ATU) in 1 000 ml of water (5.1). The addition of 2,32 ml of this stock solution to a sample of 500 ml results in a final concentration of 11,6 mg/l (10^{-4} mol/l).

5.3 Mercury(II) chloride solution

If required (see Clause 4), prepare a solution of 0,10 g of mercury(II)chloride ($HgCl_2$) in 10 ml of water (5.1).

WARNING — Stringent safety precautions and extraordinary waste disposal measures apply to the use of mercury salts in the laboratory. Routine deployment of abiotic controls poisoned with mercuric chloride is not recommended.

5.4 Antifoam agent, free from silicone.

5.5 Reference substance, stock solution.

Prepare a solution containing 1,00 g of 3,5-dichlorophenol (3,5-DCP) in 1 000 ml of water (5.1). Use warm water and/or ultrasonication to accelerate the dissolution and make the solution up to volume when it has cooled to room temperature.

Alternatively *N*-methylaniline can be used as a reference substance, especially for inhibition of nitrification processes. When using this substance, prepare a solution containing 1,00 g of *N*-methylaniline (NMA) in 1 000 ml of water (5.1).

5.6 Test medium, synthetic sewage 1 (100-fold OECD medium).

Peptone	16 g
Meat extract	11 g
Urea [$CO(NH_2)_2$]	3 g
Sodium chloride (NaCl)	0,7 g
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	0,4 g
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7\ H_2O$)	0,2 g
Anhydrous potassium monohydrogenphosphate (K_2HPO_4)	2,8 g
Water (5.1)	1 l

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

If the prepared synthetic medium is not used immediately, store it in the dark at 0 °C to 4 °C, for no longer than 1 week.

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

5.7 Test material, stock solution.

The test material may be a pure chemical, a mixture of chemicals, a chemical product or a waste water.

Prepare a stock solution of the test material in water (5.1) at a suitable concentration, for example 1 g/l or 10 g/l. Waste waters 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 vessels. Take care to ensure as much homogeneity as possible. For handling insoluble materials, see, for example, ISO 10634 [7].

5.8 Inoculum

For general use, activated sludge should be taken from the exit of the aeration tank (where substrate concentrations are lowest) of a waste-water plant, treating predominantly domestic sewage, and working efficiently. Depending on the purpose of the test, any type of activated sludge, including sludge grown in the laboratory and sludge grown on industrial waste waters, may also be used at a suitable suspended-solids concentration of, for example, 2 g/l to 4 g/l. However, activated sludges from different treatment plants are likely to exhibit different characteristics and sensitivities.

6 Apparatus

General laboratory equipment, and the following (see Annex A).

6.1 Test vessels: 250 ml to 300 ml biochemical oxygen demand **(BOD) bottles** or **Erlenmeyer flasks** with stoppers are recommended (see Figure A.1). Alternatively, larger test vessels may also be used (see Figure A.2).

When using a BOD bottle for oxygen measurements, a suitable sleeve adapter may be required for sealing the oxygen electrode against the necks of the test vessels (see Figure A.1). To avoid loss of displaced liquid on insertion of the oxygen electrode, it is advisable first to insert a funnel or glass tube through the sleeve, or to use vessels with flared-out rims.

6.2 Device for measuring oxygen concentration: comprising a suitable oxygen electrode, a cell to contain the sample and a recorder (see Figure A.2).

6.3 Magnetic stirrers, covered with an inert material.

6.4 Aeration device

If necessary, pass compressed 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.

6.5 pH-meter

6.6 Centrifuge, general bench-top centrifuge for sludge, capable of 10 000 m/s^2.

6.7 Apparatus for culturing nitrifying activated sludge (see Annex B).

7 Test environment

Perform the test at a temperature within the range of (22 ± 2) °C and in an atmosphere free from dust and toxic vapours.

8 Procedure

8.1 General

An overview of the test procedure is shown in Annex C.

The procedures to be applied to nitrifying sludge differ from those applied to non-nitrifying sludge. Therefore, it is advisable first to check the activated sludge for its nitrification activity (see Annex C).

The use of nitrifying sludge is only necessary when the influence of a test material on nitrification is to be determined. Nitrifying sludge is not required if only heterotrophic respiration is determined.

In order to check the nitrification activity of the sludge, apply the nitrification test (8.8) and calculate the rate of nitrification, if any, according to 9.2.

This preliminary test serves as a range-finder for the following definitive test.

See 8.9 for an outline of this preliminary test.

8.2 Elimination of foam

Difficulties can arise if foaming occurs during the incubation, to the extent that the foam, and the sludge solids carried on it, are expelled from the aeration vessels. Occasionally, foaming may simply result from the presence of the synthetic sewage, but foaming should be anticipated if the test material is, or contains, a surfactant. Loss of sludge solids from the test mixtures will result in artificially lowered respiration rates that could mistakenly be interpreted as a result of inhibition. In addition, aeration of surfactant solutions concentrates the surfactant in the foam layer; loss of foam from the test system will lower the exposure concentrations.

If foaming occurs, add a surfactant-free silicone-emulsion antifoam agent (5.4). If the problem is associated with the presence of the synthetic sewage, modify the sewage concentrate (5.6) by including an antifoam agent (5.4) at a rate of 50 µl/l. If foaming is caused by the test material, determine the quantity (generally a few drops from a Pasteur pipette) needed for abatement at the maximum test concentration, then treat all individual aeration vessels identically (including those, for example, blank controls and reference vessels, where foam is absent).

8.3 Preparation of inoculum

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 homogenized by using a blender for a few seconds. Where necessary, remove coarse particles with a suitable sieve.

The sludge may be washed as follows: first centrifuge (6.6) the sludge for about 10 min at approximately 10 000 m/s^2 and discard the supernatant liquid. Re-suspend the sludge in chlorine-free tap water, remove this by re-centrifuging and then repeat, if necessary, the washing and centrifuging process. Determine the dry mass of a known volume of the sludge. Finally re-suspend the sludge in chlorine-free tap water to obtain the required activated sludge concentration, of about 3 g/l of suspended solids.

Having adjusted the concentration of suspended solids, continuously aerate the activated sludge and, where possible, use it within 24 h of collection. If this is not possible, the activated sludge may be fed for up to one additional day with synthetic medium (see 5.6) at a rate not exceeding 50 ml per litre per day, provided no significant change in its activity results and that nitrification, if initially present, is not lost. Alternatively,