INTERNATIONAL STANDARD

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Water quality — Determination of biochemical oxygen demand after n days (BOD_n) —

Part 1: Dilution and seeding method with allylthiourea addition

Qualité de l'eau — Détermination de la demande biochimique en oxygène après n jours (DBO_n) —

Partie 1: Méthode par dilution et ensemencement avec apport d'allylthiourée

Reference number
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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 5815-1 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

This first edition of ISO 5815-1, together with ISO 5815-2, cancels and replaces ISO 5815:1989, which has been technically revised.

ISO 5815 consists of the following parts, under the general title *Water quality — Determination of biochemical oxygen demand after n days (BOD_n)*:

— *Part 1: Dilution and seeding method with allylthiourea addition*

— *Part 2: Method for undiluted samples*

ISO 5815-1 is the equivalent of European Standard EN 1899-1.

Introduction

This part of ISO 5815 is a modified version of ISO 5815:1989, *Water quality — Determination of biochemical oxygen demand after 5 days (BOD_5) — Dilution and seeding method*.

The times of incubation specified in this part of ISO 5815 are 5 days, as in ISO 5815:1989 and as applied in many European countries, or 7 days, as applied in several Nordic countries for many years. The 7-day incubation typically gives higher BOD results than 5 days incubation time.

With an incubation period of 5 days, weekend work can only be avoided if samples are collected Wednesdays, Thursdays or Fridays. With an incubation period of 7 days, samples collected on the first five weekdays can be analysed without implying weekend work. For this reason, a 7-day incubation period can be considered more convenient than the conventional 5-day incubation.

A new, modified 7-day incubation period is described in Annex A. Early investigations indicate that BOD results obtained by this modified method are identical to results obtained by the 5-day method described in the main text of this part of ISO 5815. It is hoped that more comparative data on these two incubation methods will be obtained during the coming years, so that the modified 7-day incubation method can be included fully at the time of review and revision of this part of ISO 5815.

For the determination of BOD_n of water samples, the respirometric method described in ISO 9408 may also be used.

In this part of ISO 5815, the limit of determination, D_L, is defined as

$$D_L = t_{0,95(f)} \cdot 2 \cdot s_B \cdot \sqrt{1+\frac{1}{n}} \qquad (1)$$

where

s_B is the within-series standard deviation;

$t_{0,95(f)}$ is the Student t-value;

f is the degrees of freedom for the determination of s_B;

n is the number of analyses for determination of the blank in an analytical series;

s_B is calculated from determinations of real samples with a BOD content near the estimated D_L.

In cases where the analytical method does not require any blank correction, the term

$$\sqrt{1+\frac{1}{n}} \qquad (2)$$

is omitted.

Water quality — Determination of biochemical oxygen demand after n days (BOD_n) —

Part 1:
Dilution and seeding method with allylthiourea addition

WARNING — Persons using this part of ISO 5815 should be familiar with normal laboratory practice. This part of ISO 5815 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.

1 Scope

This part of ISO 5815 specifies a determination of the biochemical oxygen demand of waters by dilution and seeding with suppression of nitrification.

This part of ISO 5815 is applicable to all waters having biochemical oxygen demands greater than or equal to 3 mg/l of oxygen (the limit of determination) and not exceeding 6 000 mg/l of oxygen. For biochemical oxygen demands greater than 6 000 mg/l of oxygen, the method is still applicable, but the errors caused by the necessary dilutions can influence the analytical quality of the test method and the results are to be interpreted with circumspection.

The results obtained are the product of a combination of biochemical and chemical reactions. They do not have the rigorous and unambiguous character of those resulting from, for example, a single, well-defined, chemical process. Nevertheless, they provide an indication from which the quality of waters can be estimated.

The test can be influenced by the presence of various substances. Those which are toxic to microorganisms, for example bactericides, toxic metals or free chlorine, will inhibit biochemical oxidation. The presence of algae or nitrifying microorganisms can produce artificially high results.

Annex A describes alternative incubation periods.

Annex B describes multitesting, which can be used to obtain enhanced precision or to demonstrate the presence of substances toxic to microorganisms.

Annex C provides precision data.

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 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 5813:1983, *Water quality — Determination of dissolved oxygen — Iodometric method*

ISO 5814:1990, *Water quality — Determination of dissolved oxygen — Electrochemical probe method*

ISO 6060:1989, *Water quality — Determination of chemical oxygen demand*

ISO 8245:1999, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*

ISO 8467:1993, *Water quality — Determination of permanganate index*

3 Terms and definitions

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

3.1
biochemical oxygen demand after n days
BOD_n
mass concentration of dissolved oxygen consumed under specified conditions by the biochemical oxidation of organic and/or inorganic matter in water, where n is the incubation time equal to 5 days or 7 days

NOTE 1 Adapted from ISO 6107-2.

NOTE 2 For the purposes of this part of ISO 5815, “biochemical oxidation” is taken to mean “biological oxidation”.

4 Principle

It is absolutely essential that tests conducted according to this part of ISO 5815 are carried out by suitably qualified staff.

The sample of water to be analysed is pretreated and diluted with varying amounts of a dilution water rich in dissolved oxygen and containing a seed of aerobic microorganisms, with suppression of nitrification.

The sample is incubated at 20 °C for a defined period, 5 days or 7 days, in the dark, in a completely filled and stoppered bottle. The dissolved oxygen concentration is determined before and after incubation, and the mass of oxygen consumed per litre of sample is calculated.

5 Reagents

Throughout the text, use only reagents of recognized analytical quality.

5.1 Water, grade 3 water in accordance with ISO 3696.

The water shall not contain more than 0,01 mg/l of copper, nor chlorine or chloramines.

5.2 Seeding water, if the test sample itself does not contain sufficient adapted microorganisms.

Seeding water obtained in one of the following ways shall be used:

a) urban wastewater of maximum of COD (chemical oxygen demand measured in accordance with ISO 6060) 300 mg/l or TOC (total organic carbon measured in accordance with ISO 8245) 100 mg/l, collected from a mains sewer or from a sewer of a residential zone free from significant industrial contamination. Decant or filter the water through a coarse filter;

b) river or lake water containing urban wastewater;

c) settled effluent from a wastewater treatment plant;

d) water taken downstream from the discharge of the water to be analysed or water containing microorganisms adapted to the water to be analysed and cultivated in the laboratory (in the case of industrial effluents containing substances which degrade with difficulty);

e) commercially available seeding material.

5.3 Salt solutions, stored in glass bottles at 0 °C to 4 °C in the dark.

The following solutions are stable for 6 months. They shall be discarded at the first sign of precipitation or biological growth.

5.3.1 Phosphate buffer solution, pH 7,2.

Dissolve 8,5 g of potassium dihydrogen phosphate (KH_2PO_4), 21,75 g of dipotassium hydrogen phosphate (K_2HPO_4), 33,4 g of disodium hydrogen phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) and 1,7 g of ammonium chloride (NH_4Cl) in about 500 ml of water. Dilute to 1 000 ml and mix.

The pH of this buffer solution should be 7,2 without further adjustment.

5.3.2 Magnesium sulfate heptahydrate solution, ρ = 22,5 g/l.

Dissolve 22,5 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) in water. Dilute to 1 000 ml and mix.

5.3.3 Calcium chloride solution, ρ = 27,5 g/l.

Dissolve 27,5 g of anhydrous calcium chloride ($CaCl_2$) or equivalent (for example, if hydrated calcium chloride is used: 36,4 g $CaCl_2 \cdot 2H_2O$) in water. Dilute to 1 000 ml and mix.

5.3.4 Iron(III) chloride hexahydrate solution, ρ = 0,25 g/l.

Dissolve 0,25 g of iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in water. Dilute to 1 000 ml and mix.

5.4 Dilution water

Add, to about 500 ml of water, 1 ml of each of the salt solutions (5.3.1, 5.3.2, 5.3.3 and 5.3.4). Dilute to 1 000 ml and mix. Bring the solution thus obtained to a temperature of 20 °C ± 2 °C and keep at this temperature; aerate for at least 1 h using a suitable equipment. Take every precaution not to contaminate it (6.8), in particular by the addition of organic matter, metals, oxidizing or reducing substances, to ensure that the dissolved oxygen concentration is at least 8 mg/l.

The water shall not be supersaturated with oxygen: let it stand 1 h in an unstoppered container before use. Use this solution within 24 h of preparation and discard any remaining solution, unless laboratory experience and/or the control values show that the water is acceptable for a longer time period.

5.5 Seeded dilution water

Add, depending on its source, 5 ml to 20 ml of the seeding water (5.2) per litre of dilution water (5.4). Store the seeded dilution water thus obtained at about 20 °C. Prepare immediately before use and discard any remaining solution at the end of the working day, unless the laboratory experience and/or the control values (8.5) show that the seeded dilution water is acceptable for a longer time period.

The mass concentration of oxygen consumed over n days, at 20 °C, by the seeded dilution water, which is the blank value (8.3), shall not exceed 1,5 mg/l.

5.6 Hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) solution, $c(H_2SO_4) \approx 0{,}25$ mol/l, $c(HCl) \approx 0{,}50$ mol/l, or as appropriate.

5.7 Sodium hydroxide (NaOH) solution, $\rho \approx 20$ g/l or as appropriate.

5.8 Sodium sulfite (Na_2SO_3) solution, $\rho \approx 50$ g/l or as appropriate.

5.9 Glucose-glutamic acid, control solution.

Dry some anhydrous D-glucose ($C_6H_{12}O_6$) and some L-glutamic acid ($C_5H_9NO_4$) at (105 ± 5) °C for 1 h. Weigh (150 ± 1) mg of each, dissolve in water, dilute to 1 000 ml and mix. The theoretical oxygen demand of this solution is 307 mg/l oxygen [the empirical BOD_5 is (210 ± 20) mg/l of oxygen and the BOD_7 is (225 ± 20) mg/l of oxygen].

Prepare the solution immediately before use and discard any remaining solution at the end of the working day. The solution may also be frozen in small amounts. The thawed solution shall be used immediately after thawing.

5.10 Allylthiourea (ATU) solution, ρ = 1,0 g/l.

Dissolve 200 mg of allylthiourea ($C_4H_8N_2S$) in water, dilute to 200 ml and mix. Store the solution at 4 °C. The solution is stable for at least two weeks. This compound is toxic and should therefore be handled with care.

6 Apparatus

The glassware used shall be clean, i.e. free from adsorbed toxic or biodegradable compounds, and shall be protected from contamination.

6.1 Incubation bottles, BOD bottles, with stoppers, of capacity preferably 250 ml to 300 ml or 100 ml to 125 ml and preferably with straight shoulders, or any equivalent bottles.

It is important that the bottles are thoroughly cleaned before use. If the iodometric method (ISO 5813) for determining dissolved oxygen is used, it is normally sufficient to rinse the bottle several times with tap water, then deionized water. If the electrode method (ISO 5814) is used, a more stringent cleaning procedure, for example as follows, is required. Add to the empty bottle 5 ml to 10 ml of a wash solution (for example 2,5 g of iodine plus 12,5 g of potassium iodide per litre of 1 % (volume fraction) sulfuric acid, shaking well to coat the bottle walls. Let stand for 15 min, pour off the solution and rinse thoroughly with tap water and finally deionized water.

6.2 Dilution water vessel, glass or plastics.

Measures shall be taken to ensure the vessel is kept clean and free from microorganism growths. Check that plastic vessels do not cause elevated blank values (8.3).

6.3 Incubator, capable of being maintained at (20 ± 2) °C.

6.4 Equipment for determining dissolved oxygen concentration, in accordance with ISO 5813 or ISO 5814.

6.5 Means of refrigeration at 0 °C to 4 °C, for transport and storage of the sample.

6.6 Dilution vessel, a stoppered glass flask of a capacity dependent on the volume of the diluted sample used, with graduations of between 2,5 ml and 10 ml, or any appropriate vessel allowing for dilution.

6.7 Aeration equipment, e.g. bottle of compressed air or a compressor.

The air quality shall be such that the aeration does not lead to any contamination, especially by the addition of organic matter, oxidizing of reducing materials, or metals. If contamination is suspected, the air shall be filtered and washed.

7 Storage of the sample

Store the sample at 0 °C to 4 °C in a filled and hermetically stoppered bottle immediately after sample collection and until the analysis is performed. Begin the determination of the BOD_n as soon as possible and within 24 h of completion of sample collection. Regarding freezing of samples, see special cases in Clause 10.

Ensure that the sample bottles do not give rise to elevated blank values.

8 Procedure

8.1 Pretreatment

8.1.1 Neutralization of sample

If the pH of the sample after dilution is not between 6 and 8, neutralize it after having performed any necessary predilution and after having determined, by a separate test, the volume of hydrochloric acid solution (5.6) or of sodium hydroxide solution (5.7) necessary to be added. Ignore any precipitate which is formed.

8.1.2 Presence of free and/or combined chlorine

Remove any free and combined chlorine in the sample by adding the required volume of sodium sulfite solution (5.8). Take care to avoid adding an excess.

NOTE Methods for the determination of free and combined chlorine are given in ISO 7393-1 and ISO 7393-2.

8.1.3 Homogenization

Homogenization by disruption of particles with for example a laboratory blender is not recommended for routine use but consider its use when testing a sample containing large particles and requiring a high dilution factor.

When samples have been frozen (see Clause 10), homogenize after thawing of the samples.

8.1.4 Presence of algae

Consider filtering samples containing algae to avoid producing unusually high results. A filter pore size of 1,6 µm is appropriate. Filtering can change BOD results radically and it shall only be performed if deemed necessary in the evaluation of the quality of the water. If filtration was carried out, the filter pore size shall be recorded in the test report.

Table 1 — Typical dilutions for determination of BOD_n

Expected BOD_n mg/l of oxygen	Dilution factor [a]	Examples of waters [b]
3 to 6	between 1,1 and 2	R
4 to 12	2	R, E
10 to 30	5	R, E
20 to 60	10	E
40 to 120	20	S
100 to 300	50	S, C
200 to 600	100	S, C
400 to 1 200	200	I, C
1 000 to 3 000	500	I
2 000 to 6 000	1 000	I

[a] Volume of diluted sample/volume of the test portion.

[b] R: River water;
E: Biologically purified municipal sewage;
S: Clarified municipal sewage or lightly contaminated industrial effluent;
C: Raw municipal sewage;
I: Heavily contaminated industrial effluent.