INTERNATIONAL STANDARD

ISO 5815-1

Second edition
2019-07

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
ISO 5815-1:2019(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document 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 5815-1:2003), which has been technically revised. The main changes compared to the previous edition are as follows:

— change of working range: 1 mg/l instead of 3 mg/l as lower limit;

— changes in test procedure;

— in 5.2, option to check seeding water suitability in advance with a CGA control analysis batch;

— in 5.3.2, phosphate buffer solution pH-value: requirement for preparation of a new solution if the pH value is out of the range pH 7 and pH 8;

— in 5.5, range for oxygen consumption of seeded dilution water 0,2 mg/l to 1,5 mg/l instead of upper limit 1,5 mg/l;

— in 5.9, allowable range BOD_5 of the CGA control solution changed to (198 ± 40) mg/l and BOD_7 (206 ± 40) mg/l;

— in 6.5, electrochemical probe option to measure the dissolved oxygen concentration added;

— in 8.4, interferences: subclause on presence of peroxides and peroxide compounds added;

— in 9.4, options to determinate the dilutions elaborated;

— in 9.7, control analysis: elaborated description of procedure;

— in 10.3, "approval of results/validity criteria" added;

— Annex A: title changed and “normative” instead of “informative”

— Annex C "Direct seeding of the analysis batches" added;

— new Annex D "Performance data" included.

A list of all parts in the ISO 5815 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The incubation time specified in this document is 5 d or 7 d. The latter corresponds to the practice in several Nordic countries. Annex A describes an incubation time of (2 + 5) d.

ISO 5815-1 specifies the determination of the biochemical oxygen demand (BOD) of waters with an expected BOD in the range 1 mg/l to 6 000 mg/l using the dilution method. A lower limit of working range may result from validation data in the laboratory. For samples with an expected low BOD in the range of 0,5 mg/l to 6 mg/l ISO 5815-2 provides the option of the determination of the (BOD) of waters using undiluted samples.

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 document should be familiar with normal laboratory practice. This document 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.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified staff.

1 Scope

This document specifies the determination of the biochemical oxygen demand of waters by dilution and seeding with suppression of nitrification after 5 d or 7 d incubation time.

It is applicable to all waters having biochemical oxygen demands usually between 1 mg/l and 6 000 mg/l. It applies particularly to waste waters but also suits for the analysis of natural waters. For biochemical oxygen demands greater than 6 000 mg/l of oxygen, the method is still applicable, but special care is needed taking into consideration the representativeness of subsampling for preparation of the dilution steps. The results obtained are the product of a combination of biochemical and chemical reactions in presence of living matter which behaves only with occasional reproducibility. The results do not have the rigorous and unambiguous character of those resulting from, for example, a single, well-defined, chemical process. Nevertheless, the results provide an indication from which the quality of waters can be estimated.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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*

ISO 5667-3, *Water quality — Preservation and handling of water samples*

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

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

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

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

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

ISO 10523, *Water quality — Determination of pH*

ISO 15705, *Water quality — Determination of the chemical oxygen demand index (ST-COD) — Small-scale sealed-tube method*

ISO 17289, *Water quality — Determination of dissolved oxygen — Optical sensor method*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

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 d or 7 d

Note 1 to entry: For the purposes of this document "biochemical oxidation" is taken to mean "biological oxidation".

Note 2 to entry: *n* is either 5 or 7.

3.2
chemical oxygen demand
COD
mass concentration of oxygen equivalent to the amount of dichromate consumed by dissolved and suspended matter when a water sample is treated with that oxidant under defined conditions

[SOURCE: ISO 6060:1989, 3]

3.3
total organic carbon
TOC
sum of organically bound carbon present in water, bonded to dissolved or suspended matter, including cyanate, elemental carbon and thiocyanate

[SOURCE: ISO 8245:1999, 3.3]

3.4
permanganate index (of water)
mass concentration of oxygen equivalent to the amount of permanganate ion consumed when a water sample is treated with that oxidant under defined conditions

[SOURCE: ISO 8467:1993, 3.1]

3.5
seeding water
water with adapted (aerobic) microorganisms via which the oxidation of the water contents occurs

Note 1 to entry: The seeding water is used for producing the seeded dilution water.

3.6
dilution water
water added to the test sample to prepare a series of defined dilutions

[SOURCE: ISO 20079:2005, 3.7]

3.7
seeded dilution water
dilution water to which a definite amount of seeding water is added

3.8
free chlorine
chlorine present in the form of hypochlorous acid, hypochlorite ion or dissolved elemental chlorine

[SOURCE: ISO 7393-1:1985, 2.1]

3.9
combined chlorine
fraction of total chlorine present in the form of chloramines and organic chloramines

[SOURCE: ISO 7393-1:1985, 2.2]

3.10
nitrification
oxidation of ammonium salts by bacteria where usually the intermediate product is nitrite and the end product nitrate

[SOURCE: ISO 11733:2004, 3.9]

4 Principle

The BOD_n, with inhibition of nitrification is determined, using the dilution method. A batch series with different dilutions of a sample is prepared and examined. The dilution water is enriched with oxygen and seeded with adapted aerobic microorganisms.

The sample is incubated at (20 ± 1) °C for a specified period (*n*), 5 d or 7 d, in the dark, in a completely filled and stoppered bottle. The dissolved oxygen concentration is determined before and after incubation. The mass of consumed oxygen per litre sample is calculated.

5 Reagents

Use only reagents with the degree of purity "for analysis".

5.1 Water, at least grade 3 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, which can be obtained in one of the following ways:

a) municipal waste water, decanted or coarsely filtered;

b) surface water containing municipal waste water;

c) settled effluent from a waste water treatment plant;

d) water taken downstream from the discharge of the water to be analysed, or water containing microorganisms that are adapted to the water to be analysed;

e) commercially available seeding material.

Use seeding water with a COD of about 300 mg/l or a TOC of about 100 mg/l (see 5.5). If the COD or TOC are higher, adapt to these concentrations with dilution water (5.4) before preparing the seeded dilution water (5.5) or use a correspondingly changed volume of the seeding waters for seeding the dilution water (5.4).

If the sample comes from a process that has been subjected to disinfection treatment (chlorination, UV, ozone or other), use inoculum, even when there is no residual disinfectant present.

For commercially available seeding material consider respective application recommendations.

The selected seeding material can be checked in advance by running the procedure with a control analysis (9.7) batch only to prove its suitability for the analysis of samples.

5.3 Salt solutions

5.3.1 General

The following solutions can be kept for at least six months in glass bottles in the dark at (5 ± 3) °C. Discard the solutions at the first signs of precipitation or opaqueness.

5.3.2 Phosphate-buffer solution

Dissolve 8,50 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 1,70 g of ammonium chloride (NH_4Cl), in about 500 ml of water (5.1). Dilute with water (5.1) to 1 000 ml and mix. Measure the pH value. If the pH value is outside the range pH 7 to pH 8, prepare a new solution.

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

Dissolve 22,5 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) in water (5.1). Dilute with water (5.1) to 1 000 ml and mix.

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

Dissolve 27,5 g of anhydrous calcium chloride ($CaCl_2$) (or an equivalent amount, if the hydrate is used (for example 36,4 g $CaCl_2 \cdot 2H_2O$) in water (5.1), dilute with water (5.1) to 1 000 ml and mix.

5.3.5 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 (5.1). Dilute with water (5.1) to 1 000 ml and mix.

5.4 Dilution water

Determine the total volume of dilution water required for the actual test. Pour about half the required volume of water (5.1) into the feed vessel (6.3) for the dilution water and add 1 ml of each of the salt solutions (5.3.2, 5.3.3, 5.3.4 and 5.3.5) for each litre of the total volume. Then fill to the required total volume with water (5.1) and mix by stirring, aeration or shaking. Bring the dilution water obtained in this way to a temperature of (20 ± 2) °C, keep at this temperature and aerate slightly by mixing. If, for example, specially adapted seeding water or seeding material is necessary, the procedure according to Annex C can be followed.

EXAMPLE If 20 l of dilution water are required, prepare 10 l of water (5.1). Stirring continuously, add 20 ml of each of the salt solutions individually and fill up with water (5.1) to 20 l.

5.5 Seeded dilution water

The preparation of a seeded dilution water is needed when the test solutions are prepared according to 9.3 The mass concentration of oxygen consumed over 5 d (or 7 d) at (20 ± 1) °C by the seeded dilution water with the addition of allylthiourea (ATU) solution to inhibit nitrification [blank value (see 9.5)], shall be between 0,2 mg/l and 1,5 mg/l.

The volume increase of the dilution water by seeding water should be as low as possible.

The amount of the seeding water (5.2) needed to attain a hypothetic COD of 0,6 mg/l to 3,0 mg/l, corresponding to the aimed oxygen consumption in the blank values (9.5), is calculated with Formula (1):

$$V_{\text{seeding water}} = \frac{\text{COD}_{\text{target}} \cdot V_{\text{dilution water}}}{\text{COD}_{\text{seeding water}}} \tag{1}$$

where

$V_{\text{seeding water}}$	is the volume of the seeding water (5.2) to be added to the dilution water (5.4) in litres, l;
$\text{COD}_{\text{target}}$	is the hypothetic COD (0,6 mg/l O_2 to 3 mg/l O_2) in the seeded dilution water (5.5) in milligrams per litre of oxygen, mg/l O_2;
$\text{COD}_{\text{seeding water}}$	is the COD of the seeding water (5.2) in milligrams per litre of oxygen, mg/l O_2;
$V_{\text{dilution water}}$	is the calculated amount of the dilution water to be seeded (5.4) in litres, l.

For direct seeding of test batches or automated systems which use a direct seeding, see instructions in Annex C.

Add the seeding water (5.2) to the dilution water (5.4) and mix by stirring or shaking. Determine the oxygen content as specified in ISO 5813, ISO 17289 or ISO 5814. Aerate the seeded dilution water up to an oxygen content of preferably a minimum 8 mg/l. The water shall not be supersaturated with oxygen by aeration: let it stand about 1 h in an unstopped container before use. Keep the seeded dilution water at (20 ± 2) °C. The so prepared seeded dilution water can be used immediately for the preparation of the analysis batches.

Throw away the residue of the dilution water at the end of the working day, unless the laboratory experience reveals via the complied-with control analysis (9.7) with the control solution (5.9), and the blank value determination (9.5) that the water is acceptable for a longer time.

5.6 Hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) solution, for example $c(\text{HCl}) \approx 0{,}5$ mol/l or $c(H_2SO_4) \approx 0{,}25$ mol/l.

5.7 Sodium hydroxide (NaOH) solution, for example $c(\text{NaOH}) = 0{,}5$ mol/l, $\rho \approx 20$ g/l.

5.8 Sodium sulfite (Na_2SO_3) solution, for example $\rho(Na_2SO_3) = 50$ g/l.

5.9 Glucose-glutamic acid (GGA), control solution.

Dry about 200 mg to 300 mg of anhydrous D-glucose ($C_6H_{12}O_6$) and 200 mg to 300 mg of anhydrous L-glutamic acid ($C_5H_9NO_4$) at (105 ± 5) °C for 1 h. Weigh (150 ± 1) mg of each substance, dissolve in water (5.1), dilute with water to 1 000 ml, and mix. The theoretical oxygen demand of this solution is 307 mg/l of oxygen for BOD_5 (the empirical BOD_5 is (198 ± 40) mg/l of oxygen and the BOD_7 (based on conversion factor $\text{BOD}_7/\text{BOD}_5 = 1{,}04$ from Table D.3 and previous empirical BOD_5) is (206 ± 40) 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 frozen solution can be kept for a maximum of three months. Use the thawed solution immediately after thawing.

5.10 Allylthiourea (ATU) solution, $\rho = 1{,}0$ g/l.

Dissolve 200 mg of allylthiourea ($C_4H_8N_2S$) in water (5.1), dilute with water (5.1) to 200 ml and mix. Store the solution at (5 ± 3) °C. The solution is stable for at least two weeks.

WARNING — The reagent is toxic and shall therefore be handled according to the safety data sheet.

The nitrification inhibition is not attained in all cases by addition of 2 ml of the ATU-solution (ρ = 1,0 g/l) per litre of analysis batch. The addition of a significantly higher volume than 2 ml of this ATU-solution can disturb the titration according to ISO 5813 (see 9.6.1).

6 Apparatus

Usual laboratory equipment, and in particular the following.

6.1 General

Plastic and glass vessels shall be carefully cleaned and, in particular, made free of absorbed toxic and biodegradable compounds and shall be protected from contamination.

6.2 Incubation bottles, BOD-bottles (Karlsruhe type) with a content between 100 ml and 300 ml or conical shoulder bottles with stoppers and a suitable funnel, or other suitable, bubble-free closing bottles. For the use of automatic systems, it is important to use incubation bottles with a definite volume, as the incubation bottles serve as dilution vessels.

6.3 Feed vessel for the seeded and non-seeded dilution water, made from glass or plastic.

Take measures to ensure that the vessel is kept clean and free from microorganism growths, and protected from light.

6.4 Tempering cabinet, room or incubator, capable of being maintained at (20 ± 1) °C and darkened.

6.5 Equipment for determining dissolved oxygen concentration, as specified in ISO 5813 (iodometric method), or ISO 5814 (electrochemical probe method) using an oxygen probe or ISO 17289 (optical sensor method) using optical oxygen measurement.

6.6 Cooling and freezing device, for transport and storage of the samples.

6.7 Dilution vessel, mixing vessel preferably made from glass, for example volumetric flask or graduated measuring cylinder, with sufficient volume capacity for the dilution batch and the possibility of a thorough mixing.

6.8 Aeration equipment, 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 or reducing materials, or metals. If contamination is suspected, filter and wash the air.

6.9 pH-measuring equipment, which fulfils the requirements for the determination of pH, as specified in ISO 10523.

6.10 Stirrer, to ensure that the sample is homogeneous for the extraction of partial samples and no air is taken in.

6.11 Glass fibre filter GF 6.

7 Sampling and preservation

Closable vessels made from glass or plastic are suitable for sampling. The volume should be large enough to ensure that a proper dilution series can be derived. Fill the sampling vessels completely, close

