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**Water quality — Determination of  
biochemical oxygen demand after  $n$  days  
(BOD <sub>$n$</sub> ) —**

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

*Qualité de l'eau — Détermination de la demande biochimique en  
oxygène après  $n$  jours (DBO <sub>$n$</sub> ) —*

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



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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<sub>5</sub>) — 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<sub>n</sub> 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

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

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

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

is omitted.

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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

**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 <sub>$n$</sub>**

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.