INTERNATIONAL STANDARD

**ISO 5815-2**

First edition
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# Water quality — Determination of biochemical oxygen demand after $n$ days ($BOD_n$) —

## Part 2: Method for undiluted samples

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

*Partie 2: Méthode pour échantillons non dilués*



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



This first edition of ISO 5815-2, together with ISO 5815-1, 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 allythiourea addition*



— *Part 2: Method for undiluted samples*

ISO 5815-2 is the equivalent of European Standard EN 1899-2.

# Introduction

This part of ISO 5815 is intended for analysis of biochemical oxygen demand (BOD) in waters with a BOD in the range 0,5 mg/l to 6 mg/l of oxygen.

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 the 5-day incubation.

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

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



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.

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

## Part 2: Method for undiluted samples

**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 determination of the biochemical oxygen demand (BOD) of waters of undiluted samples. It is applicable to all waters having biochemical oxygen demands greater than or equal to 0,5 mg/l of oxygen (the limit of determination) and not exceeding 6 mg/l of oxygen.

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, inhibit biochemical oxidation. The presence of algae or nitrifying microorganisms can produce artificially high results. In these situations a modification of the method may be necessary.



Annex A describes alternative incubation periods.

Annex B describes procedures for modification of the method by addition of seeding material, salts, inhibition of nitrification by allylthiourea (ATU) addition, neutralization, homogenization and/or filtration. These modifications may be found necessary for specific evaluations of the water quality of receiving waters.

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*

## 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 equilibrated to 20 °C followed by, if necessary, aeration. Incubation at 20 °C for a defined period, 5 days or 7 days, in the dark, in a completely filled and stoppered bottle. Determination of the dissolved oxygen concentration before and after incubation. Calculation of the mass of oxygen consumed per litre of sample.



## 5 Apparatus

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

**5.1 Incubation bottles**, BOD bottles, with glass 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.

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

**5.3 Equipment for determining dissolved oxygen concentration**, in accordance with ISO 5813 and ISO 5814.

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

**5.5 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.

## 6 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.

## 7 Procedure

### 7.1 Preparation of test solutions

Bring the test sample to a temperature of (20 ± 2) °C and aerate if necessary. In case of aeration, let the sample stand about 15 min. Remove air bubbles and possible supersaturation of oxygen.

### 7.2 Procedure

#### 7.2.1 Measurement of dissolved oxygen using iodometric method (in accordance with ISO 5813)

Using each sample (7.1), fill two incubation bottles (5.1), allowing them to overflow slightly. During filling operations, take precautions to prevent changing the oxygen concentration of the medium.

Allow any air bubbles adhering to the walls to escape. Stopper the bottles, taking care to avoid trapping air bubbles.

Divide the bottles into two series, each containing one bottle of each sample.

Put the first series of bottles in the incubator (5.2) and leave in darkness for $n$ days ± 4 h.

In the second series of bottles, measure the dissolved oxygen concentration in each of the bottles at time zero after 15 min, using the method specified in ISO 5813 with the addition of azide in the alkaline iodide-azide reagent.

After the incubation, determine the dissolved oxygen concentration in each of the first series of bottles, using the method specified in ISO 5813.

#### 7.2.2 Measurement of dissolved oxygen using electrochemical probe (in accordance with ISO 5814)

Using each sample (7.1), fill an incubation bottle (5.1), allowing it to overflow slightly. During filling operations, take precautions to prevent changing the oxygen concentration of the medium.

Allow any air bubbles adhering to the walls to escape.

Measure the dissolved oxygen concentration in each of the bottles at time zero, using the method specified in ISO 5814.

Stopper the bottles, taking care to avoid trapping air bubbles.

Put the bottles in the incubator (5.2) and leave in darkness for $n$ days ± 4 h.

After the incubation, determine the dissolved oxygen concentration in each of the bottles, using the method specified in ISO 5814.

#### 7.2.3 Control analysis

For each series of determinations, include at least one double determination of a sample ($BOD_{n1}$, $BOD_{n2}$).

Plot the relative percentage difference ($r_i$) of each series ($i$) on quality control charts:

$$r_i = \frac{(\mathrm{BOD}_{n1} - \mathrm{BOD}_{n2}) \cdot 100}{0{,}5(\mathrm{BOD}_{n1} + \mathrm{BOD}_{n2})} \ \% \tag{3}$$

where

$\mathrm{BOD}_{n1}$ is the result of the first $\mathrm{BOD}_n$ determination of sample;

$\mathrm{BOD}_{n2}$ is the result of the second $\mathrm{BOD}_n$ determination of sample.

Consider the upper control limit as:

$$3{,}267\ 8 \cdot \bar{r}\ \% \tag{4}$$

where $\bar{r}$ is the average value of $r_i$ values.

The repeatability coefficient of variation (CV) can be calculated as:

$$\mathrm{CV} = \frac{\bar{r}}{1{,}128}\ \% \tag{5}$$

After incubation, the residual dissolved oxygen concentration should be at least 2 mg/l. The oxygen consumption should be at least the limit of determination of the laboratory for BOD measurement.

Care should be taken that representative samples are collected.



## 8 Calculation and expression of results

Calculate the biochemical oxygen demand after $n$ days ($\mathrm{BOD}_n$), expressed in milligrams per litre of oxygen, using the equation



$$\mathrm{BOD}_n = (\rho_1 - \rho_2) \tag{6}$$

where

$\rho_1$ is the dissolved oxygen concentration of the test sample at time zero, in milligrams per litre;

$\rho_2$ is the dissolved oxygen concentration of this same test sample after $n$ days, in milligrams per litre.

The results shall be reported to two significant figures, e.g. 4,5 mg/l of oxygen.

The results of interlaboratory testing on the trueness and precision of results are given in Annex C.

## 9 Test report

The test report shall include the following information:

a) a reference to this part of ISO 5815, i.e. ISO 5815-2;

b) the number of days of incubation ($n$);

c) the results in milligrams per litre of oxygen (reported as described in Clause 8);