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) ≈ 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.

8.2 Preparation of test solutions

Bring the sample (or pretreated sample) to a temperature of (20 ± 2) °C and if necessary (depending on the origin of the sample) shake in a half-filled vessel so as to eliminate any possible supersaturation with oxygen.

Place a known volume of the sample (or pretreated sample), the test portion, in the dilution vessel (6.6), add 2 ml of allylthiourea solution (5.10) per litre of diluted sample and fill to the mark with seeded dilution water (5.5). If the dilution factor to be used is greater than 100, carry out serial dilutions in two or more steps.

Mix gently to avoid entrapment of air bubbles.

The oxygen consumption should be at least 2 mg/l and the oxygen concentration after incubation at least 2 mg/l extent of dilution should be such that, after incubation, the residual dissolved oxygen concentration will be between one-third and two-thirds of the initial concentration.

In view of the difficulty of selecting the right degree of dilution, several different dilutions are recommended, varying according to the dilution factor and encompassing the dilution corresponding to the expected BOD_n (see Table 1).

Determinations of the total organic carbon (TOC) (see ISO 8245), the permanganate index (see ISO 8467), or the chemical oxygen demand (COD) (see ISO 6060) can give useful information in this respect.

Table 2 shows typical intervals for R, the ratio of BOD_n to TOC, permanganate index or COD, dependent on the sample type.

Table 2 — Typical values of ratio R

	Total organic carbon BOD/TOC (see ISO 8245)	**Permanganate index** BOD/Index (see ISO 8467)	**Chemical oxygen demand** BOD/COD (see ISO 6060)
Untreated wastewater	1,2 to 2,8	1,2 to 1,5	0,35 to 0,65
Biologically treated wastewater	0,3 to 1,0	0,5 to 1,2	0,20 to 0,35

An appropriate R-value should be selected from Table 2, to calculate the expected BOD_n value:

$$BOD_n = R \cdot y$$

where y is the chemical oxygen demand, permanganate index or the TOC value.

Care should be taken that the test samples are representative.

If the presence of substances toxic to microorganisms is suspected, several different dilutions of the sample should be made. If the BOD result depends on the dilution, results can only be reported if a dilution range is found at which there is no dependence on dilution. Multitesting (see Annex B) may be applied in this situation.

NOTE 1 In some samples an inhibition by chlorine is seen even after removal, due to chlorine products that are not removed.

NOTE 2 The suppression of nitrification is not achievable in all cases. A significantly increased addition of ATU above 2 mg/l can affect the Winkler titration (see ISO 5813).

8.3 Blank test

Carry out a blank test in parallel with the determination, using the seeded dilution water (5.5) including 2 mg of ATU solution (5.10) per litre.

8.4 Procedure

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

Using each dilution (8.2), fill two incubation bottles (6.1), allowing them to overflow slightly. During filling, precautions shall be taken to prevent changing the oxygen content 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 dilution and at least one bottle of blank solution (8.3).

Put the first series of bottles with diluted test solutions (8.2) in the incubator (6.4) and leave in darkness for n days $\pm$ 4 h.

In the second series of bottles with diluted test solutions, measure the dissolved oxygen concentration at time zero, 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 bottles, using the method specified in ISO 5813.

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

Using each dilution (8.2), fill an incubation bottle (6.2) allowing it to overflow slightly. Precautions shall be taken to prevent changing the oxygen content 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 with diluted test solutions (8.2) in the incubator (6.3) and leave in darkness for n days $\pm$ 4 h.

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

8.5 Control analysis

To check the seeded dilution water, the seeding water and the technique of the analyst, carry out a control in each batch of samples by placing 20,00 ml of the glucose-glutamic acid control solution (5.9) in the dilution vessel, adding 2 ml of ATU solution (5.10) followed by dilution to 1 000 ml with the seeded dilution water (5.5) and proceed as described in 8.4.

The BOD_n obtained should be within the range (210 $\pm$ 40) mg/l of oxygen for BOD_5 and within the range (225 $\pm$ 40) mg/l of oxygen for the BOD_7, corresponding to the range of mean value $\pm$ 2 $\times$ standard deviation determined from the interlaboratory data (see Clause 10). The precise control limits for each laboratory shall be established by performing a minimum of 25 determinations over a period of at least several weeks. The mean and the standard deviations can then be used to calculate control limits for quality control checks. If not, check the seeding water and, if necessary, the technique of the analyst.

The blank test (8.3) shall not exceed 1,5 mg/l of oxygen; if so, check possible sources of contamination.

9 Calculation and expression of results

9.1 Examination for valid oxygen consumption during test

BOD_n is calculated for the test solutions, where the following condition is fulfilled.

$$\frac{\rho_1}{3} \leqslant (\rho_1 - \rho_2) \leqslant \frac{2\rho_1}{3} \tag{3}$$

where

ρ_1 is the dissolved oxygen concentration of one of the test solutions at time zero, in milligrams per litre;

ρ_2 is the dissolved oxygen concentration of this same test solution after n days, in milligrams per litre.

9.2 Calculation of biochemical oxygen demand after n days (BOD_n)

Calculate the biochemical oxygen demand (BOD_n), expressed in milligrams per litre of oxygen, using the equation:

$$BOD_n = \left[(\rho_1 - \rho_2) - \frac{V_t - V_s}{V_t} \cdot (\rho_3 - \rho_4) \right] \cdot \frac{V_t}{V_{sam}} \tag{4}$$

where

ρ_1 and ρ_2 see 9.1;

ρ_3 is the dissolved oxygen concentration of the blank solution at time zero, in milligrams per litre;

ρ_4 is the dissolved oxygen concentration of the blank solution after n days, in milligrams per litre;

V_{sam} is the volume of sample used for the preparation of the test solution concerned, in millilitres;

V_t is the total volume, in millilitres, of this test solution.

If several dilutions fall within the required range, calculate the average of the results obtained for these dilutions.

Results shall be expressed in milligrams of oxygen per litre. Results less than 10 mg/l of oxygen shall be reported to the nearest mg/l. Results between 10 mg/l of oxygen and 1 000 mg/l of oxygen shall be reported to two significant figures.

Results above 1 000 mg/l shall be reported to three significant figures, e.g. 1 240 mg/l of oxygen.

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

10 Special cases

If the time between sampling and start of analysis cannot be kept to less than 24 h, due to time of transportation, as a result of geographical circumstances, freezing of samples is permitted. Frozen samples shall be homogenized after thawing, and seeding water shall be used in all cases. It is recommended that, wherever possible, local laboratory facilities shall be found to limit the time of transportation.

11 Test report

The test report shall include the following information:

a) a reference to this part of ISO 5815, i.e. ISO 5815-1;

b) specification that the test was carried out with suppression of nitrification;

c) the number of days of incubation (n);

d) the result, in milligrams per litre of oxygen (reported as described in 9.2);

e) for results below the working range, a documentation for an adequate detection limit;

f) any special details which may have been noted during the test;

g) details of any operations not specified in this part of ISO 5815, or regarded as optional, such as filtration (8.1.4), freezing and homogenization (see Clause 10), alternative incubation (BOD_{2+5}) (see Annex A), and multitesting (see Annex B).

Annex A
(informative)

Alternative incubation methods

The rate of oxidation of carbon during the first stage of the BOD test is expressed by Phelps' law:

$$\log_{10} \frac{L}{L-x} = kt$$

where

L is the ultimate BOD at infinite time, in milligrams per litre of oxygen;

x is the BOD at time t, in milligrams per litre of oxygen;

t is the time, in days;

k is the rate constant, expressed as the reciprocal day.

For a given type of organic matter and microbial seed, the effect of temperature on the rate constant k and on the value of L can be predicted to a first approximation. This may be useful when considering the use of the BOD test in warm climates, or in studies of long rivers which traverse a number of climatic regions. It is essential that such relationships, however, are used with caution.

The standard BOD result is obtained after a 5-day or 7-day incubation at 20 °C.

By incubating for 2 days at 0 °C to 4 °C followed by 5 days at 20 °C, a BOD_{2+5} result is obtained. It has been observed[3] that there is no significant difference between BOD_5 and BOD_{2+5} after sample dilution.

This was also investigated in an European interlaboratory comparison performed in 1992 with 95 participants from 11 countries. The correlation between BOD_5 and BOD_7 determinations and BOD_5 and BOD_{2+5} determinations was measured. The results of the latter are shown in Table A.1.

Table A.1 — Interlaboratory comparison of BOD_5 and BOD_{2+5}

	Sample type	Glucose/glutamic acid solution		Mechanically treated wastewater		Biologically treated wastewater	
		A	B	C	D	E	F
BOD_5	Median value, mg/l of oxygen	203	184	58	46	18,2	17,2
BOD_{2+5}	Median value, mg/l of oxygen	201	180	58	46	18,1	17,2
	Significant difference from BOD_5 [a]	No	No	No	No	No	No
	Number of laboratories	91	85	89	86	89	87

[a] Level of significance $\alpha = 0{,}05$.

In practice there is no difference between BOD_5 and BOD_{2+5} results.

When determining BOD_{2+5}, add 8.4.1 with alteration of paragraph 4 as follows:

"Put the first series of bottles with diluted test solutions (8.2) in darkness at (0 to 4) °C for 2 days ± 2 h [1)] and then put them in the incubator (6.4) and leave in darkness with the temperature of the dilution equilibrated at (20 ± 1) °C for 5 days ± 2 h [1)]".

and add 8.4.2 with alteration of paragraph 5 as follows:

"Put the bottles with diluted test solutions (8.2) in darkness at (0 to 4) °C for 2 d ± 2 h [1)] and then put them in the incubator (6.4) and leave in darkness with the temperature of the dilutions equilibrated at (20 ± 1) °C for 5 d ± 2 h [1)]."

When BOD_5 determinations are substituted by BOD_{2+5} determinations, it is necessary for the laboratory to verify that their procedure for BOD_{2+5} determinations gives equivalent results to BOD_5 determinations.

1) When the same incubator is used for storage at both temperature levels, a fan-assisted incubator may be necessary to ensure the change in incubation temperature within the required time interval of up to 2 h.