International Standard 5815

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Water quality — Determination of biochemical oxygen demand after *n* days (BOD_n) — Dilution and seeding method

Qualité de l'eau — Détermination de la demande biochimique en oxygène après n jours (DBO_n) — Méthode par dilution et ensemencement

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 5815 was developed by Technical Committee ISO/TC 147, *Water quality*, and was circulated to the member bodies in September 1982.

It has been approved by the member bodies of the following countries:

Australia	Hungary	Norway
Austria	India	Poland
Belgium	Iran	Romania
Canada	Iraq	South Africa, Rep. of
China	Ireland	Spain
Czechoslovakia	Italy	Sweden
Denmark	Japan	Switzerland
Egypt, Arab Rep. of	Korea, Dem. P. Rep. of	United Kingdom
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No member body expressed disapproval of the document.

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Water quality — Determination of biochemical oxygen demand after n days (BOD_n) — Dilution and seeding method

1 Scope and field of application

This International Standard specifies a method for the empirical and conventional determination of the biochemical oxygen demand of waters by dilution and seeding.

The method is applicable to all waters having biochemical oxygen demands greater than or equal to 3 mg of oxygen per litre and not exceeding 6 000 mg of oxygen per litre. For biochemical oxygen demands greater than 6 000 mg of oxygen per litre, the method is still applicable, but the errors caused by the dilutions necessary require the results to be interpreted with circumspection.

The results obtained are the product of a combination of biochemical and chemical actions. 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 may be influenced by the presence of various substances. Those which are toxic to micro-organisms, for example bactericides, toxic metals or free chlorine, will inhibit biochemical oxidation. The presence of algae or nitrifying micro-organisms may produce artificially high results.

2 References

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

ISO 5814, *Water quality — Determination of dissolved oxygen — Electrochemical probe method.*[1]

ISO 6107/2, *Water quality — Vocabulary — Part 2.*

3 Definition

biochemical oxygen demand (BOD): The mass concentration of dissolved oxygen consumed under specified conditions by the biological oxidation of organic and/or inorganic matter in water. (Definition taken from ISO 6107/2.)

For the purpose of this International Standard, "biological oxidation" is taken to mean "biochemical oxidation".

4 Principle

Neutralization of the sample of water to be analysed and dilution with varying amounts of a dilution water rich in dissolved oxygen and containing a seed of aerobic micro-organisms, with or without suppression of nitrification, as desired.

Incubation at a controlled temperature for a defined period, n 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 water.

Simultaneous performance of a check test on a standard solution of glucose and glutamic acid.

5 Reagents

During the analysis, use only reagents of recognized analytical grade and only distilled water or water of equivalent purity (water distilled in an all-glass apparatus or demineralized water).

The water shall not contain more than 0,01 mg of copper per litre, and shall be free from chlorine, chloramines, caustic alkalinity, organic matter and acids.

5.1 Seeding water.

If the test sample does not contain, by itself, sufficient adapted micro-organisms, seeding water, obtained in one of the following ways, shall be used:

a) Urban waste water, drawn from a main sewer or from a sewer of a residential zone free from marked industrial contamination. This water shall be decanted before use.

b) Add 100 g of garden soil to 1 litre of water. Mix and allow to stand for 10 min. Take 10 ml of the supernatant liquid and make up to 1 litre with water.

c) River or lake water containing urban waste water.

d) Effluent from a waste water treatment plant.

1) At present at the stage of draft.

e) Water taken downstream from the discharge of the water to be analysed or water containing micro-organisms adapted to the water to be analysed and cultivated in the laboratory (case of industrial effluents containing substances which degrade with difficulty).

5.2 Salt solutions.

The following solutions are stable for at least 1 month and should be stored in glass bottles in the dark. They should be discarded at the first sign of precipitation or biological growth.

5.2.1 Phosphate, buffer solution.

Dissolve 8,5 g of potassium dihydrogenphosphate (KH_2PO_4), 21,75 g of dipotassium hydrogenphosphate (K_2HPO_4), 33,4 g disodium hydrogenphosphate 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.

5.2.2 Magnesium sulfate heptahydrate, 22,5 g/l solution.

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

5.2.3 Calcium chloride, 27,5 g/l solution.

Dissolve 27,5 g of anhydrous calcium chloride ($CaCl_2$) (or equivalent, if hydrated calcium chloride is used) in water. Dilute to 1 000 ml and mix.

5.2.4 Iron(III) chloride hexahydrate, 0,25 g/l solution.

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

5.3 Dilution water.

Add to about 500 ml of water 1 ml of each of the salt solutions (5.2.1, 5.2.2, 5.2.3 and 5.2.4). Dilute to 1 000 ml and mix. Bring the solution thus obtained to a temperature of about 20 °C and keep at this temperature; aerate for 1 h, taking every precaution not to contaminate it, in particular by the addition of organic matter, oxidizing or reducing substances, or metals[1] to ensure that the dissolved oxygen concentration is at least 8 mg/l.

The oxygen depletion of this solution should not exceed 0,2 mg/l over 5 days.

Use this solution within 8 h.

5.4 Seeded dilution water.

Add, according to its source and if necessary, 1,0 to 50 ml of the seeding water (5.1) per litre of dilution water (5.3). Store the seeded dilution water thus obtained at about 20 °C and use at the earliest after 8 h.

The oxygen depletion (5 days, 20 °C) of the seeded dilution water should be between 0,3 and 1 mg of oxygen per litre.

5.5 Hydrochloric acid (HCl), solution, for example 0,5 mol/l.

5.6 Sodium hydroxide (NaOH), solution, for example 20 g/l.

5.7 Sodium sulfite (Na_2SO_3), solution, for example 63 g/l.

5.8 Glucose-glutamic acid, standard solution.

Dry some dehydrated glucose ($C_6H_{12}O_6$) and some glutamic acid ($HOOC\text{-}CH_2\text{-}CH_2\text{-}CHNH_2\text{-}COOH$) at 103 °C for 1 h. Weigh 150 ± 1 mg of each, dissolve in water, dilute to 1 000 ml and mix.

Prepare this solution just before use.

6 Apparatus

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

Usual laboratory equipment, and

6.1 Incubation flasks, narrow-mouthed, of capacity between 130 and 350 ml, with ground-glass stoppers, and, preferably, with straight shoulders.

The use of 250 ml flasks is preferred.

6.2 Incubator, capable of being maintained at 20 ± 1 °C.

6.3 Equipment for determining dissolved oxygen concentration.[2]

6.4 Means of refrigeration (0 to 4 °C), for transport and storage of the sample.

6.5 Dilution vessel, a stoppered glass flask graduated to the nearest millilitre, of a capacity dependent on the volume of the diluted sample used.

1) It is recommended that either a bottle of compressed air be used, or a compressor in which the air does not come into contact with any lubricating fluid (compressors using diaphragm pumps). Filter and wash the air before use.

2) The determination of dissolved oxygen may be carried out by means of the iodometric method (see ISO 5813) or by means of the electrochemical probe method (see ISO 5814).

Table — Recommended dilutions for determination of BOD_n

Expected BOD_n mg/l	Dilution factor	Result rounded to	Generally applicable to*
3 to 6	between 1 and 2	0,5	R
4 to 12	2	0,5	R, E
10 to 30	5	0,5	R, E
20 to 60	10	1	E
40 to 120	20	2	S
100 to 300	50	5	S, C
200 to 600	100	10	S, C
400 to 1 200	200	20	I, C
1 000 to 3 000	500	50	I
2 000 to 6 000	1 000	100	I

* R : river water ;
E : biologically purified sewage water ;
S : clarified sewage water or lightly-contaminated industrial effluent ;
C : raw sewage water ;
I : heavily contaminated industrial effluent.

7 Storage of the sample

Store the sample at a temperature between 0 and 4 °C in a filled and hermetically stoppered bottle until the analysis is performed. Begin the determination of the BOD as soon as possible and, whenever feasible, within 24 h of collection of the sample.

Samples may also be stored by deep freezing.

8 Procedure

8.1 Preliminary operations

8.1.1 Neutralization of sample

If the pH of the sample is not between 6 and 8, neutralize it after having determined by a separate test the volume of hydrochloric acid solution (5.5) or of sodium hydroxide solution (5.6) necessary. Ignore any precipitate which may be formed.

8.1.2 Presence of free and/or combined chlorine

Neutralize the free and combined chlorine in the sample by adding the required volume of sodium sulfite solution (5.7). Take care to avoid adding an excess.

A method for the determination of free and/or combined chlorine will form the subject of a future International Standard.

8.2 Preparation of test solutions

Bring the test sample to a temperature of about 20 °C, and shake it in a half-filled vessel, so as to eliminate any possible supersaturation with oxygen.

Place a known volume of the sample in the dilution vessel (6.5) and dilute it either with dilution water (5.3) or seeded dilution water (5.4) and mix gently; avoid trapping air bubbles.

If the dilution factor to be used is greater than 100, carry out the dilution in two or several steps. If suppression of nitrification is necessary, add ATU or TCMP reagent.[1)]

NOTES

1 The 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 precisely the right degree of dilution, several different dilutions should be made, varying according to a geometric progression and encompassing the dilution corresponding to the probable BOD_n (see the table).

Determinations of the total oxygen demand (TOC) and the chemical oxygen demand (COD) with chromate may give useful information in this respect.

2 Take care that representative samples are withdrawn.

8.3 Blank test

Carry out a blank test, in parallel with the determination, using the seeded dilution water (5.4).

8.4 Determination

Using each dilution (see 8.2), fill two incubation flasks (6.1) by siphoning, allowing them to overflow slightly.

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

Divide the flasks into two series, each containing one flask of each dilution and one flask of blank solution (see 8.3).

1) If it is desired to determine the oxygen consumption due to the decomposition of organic materials alone, it is necessary to avoid nitrification processes by inhibiting the responsible micro-organisms; for this purpose, add 2 ml of a 500 mg/l solution of allylthiourea (ATU) ($C_4H_8N_2S$) per litre of diluted sample or a quantity of 2-chloro-6-trichloromethylpyridine (TCMP) ($Cl\text{-}C_5H_3N\text{-}CCl_3$) fixed on sodium chloride (NaCl) such that the TCMP concentration in the diluted sample is about 0,5 mg/l.

Put one series of flasks in the incubator (6.2) and leave in darkness for the required number of days.

Measure the dissolved oxygen concentration at time zero in each of the dilutions and in the blank solution of the other series of flasks, using the method specified in ISO 5813 or ISO 5814.

After the required incubation time, n,[1] determine the dissolved oxygen concentration in each of the dilutions and in the blank solution of the series of flasks placed in the incubator, using the method specified in ISO 5813 or ISO 5814.

8.5 Check test

To check the seeded dilution water, the seeding water and the technique of the analyst, carry out a check test by diluting 20 ml of the standard glucose-glutamic acid solution (5.8) to 1 000 ml with the seeded dilution water (5.4) and proceed as described in 8.4.

The BOD_5 or the BOD_7 obtained shall fall between 180 and 230 mg/l. If not, check the seeding water and, if necessary, the technique of the analyst.

Carry out the test simultaneously with the test samples.

9 Expression of results

9.1 Determine, among the solutions tested, that for which the following condition is fulfilled:

$$\frac{C_1}{3} < (C_1 - C_2) < \frac{2C_1}{3}$$

where

C_1 is the dissolved oxygen concentration, in milligrams per litre, of one of the test solutions at time zero;

C_2 is the dissolved oxygen concentration, in milligrams per litre, of this same solution after n days.

9.2 The biochemical oxygen demand after n days (BOD_n), expressed in milligrams of oxygen per litre, is given by the equation

$$BOD_n = \left[(C_1 - C_2) - \frac{V_t - V_e}{V_t}(C_3 - C_4)\right]\frac{V_t}{V_e}$$

where

C_1 and C_2 have the same meanings as in 9.1;

C_3 is the dissolved oxygen concentration, in milligrams per litre, of the blank solution at time zero;

C_4 is the dissolved oxygen concentration, in milligrams per litre, of the blank solution after n days;

V_e is the volume, in millilitres, of sample used for the preparation of the test solution concerned;

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.

10 Test report

The test report shall include the following information:

a) reference to this International Standard;

b) the date and hour of sampling;

c) the method of storage of the sample;

d) the date and hour of beginning the determination;

e) the type of seeding water used;

f) indication of the suppression of nitrification, if appropriate;

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

h) the results, and the method of expression used;

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

k) details of any operations not specified in this International Standard, or regarded as optional.

1) n is the incubation time in days; it is generally equal to 5 or 7.