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Water quality — Determination of biochemical oxygen demand after 5 days (BOD_5) — Dilution and seeding method

Qualité de l'eau — Détermination de la demande biochimique en oxygène après 5 jours (DBO_5) — Méthode par dilution et ensemencement

Reference number
ISO 5815 : 1989 (E)

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.

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. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 5815 was prepared by Technical Committee ISO/TC 147, *Water quality*.

This second edition cancels and replaces the first edition (ISO 5815 : 1983), of which it constitutes a minor revision.

Annex A of this International Standard is for information only.

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Water quality — Determination of biochemical oxygen demand after 5 days (BOD_5) — Dilution and seeding method

1 Scope

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.

Annex A gives information on alternative incubation periods and temperatures.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 5813 : 1983, *Water quality — Determination of dissolved oxygen — Iodometric method.*

ISO 5814 : 1984, *Water quality — Determination of dissolved oxygen — Electrochemical probe method.*

ISO 6107-2 : 1981, *Water quality — Vocabulary — Part 2.*

ISO 7393-1 : 1985, *Water quality — Determination of free chlorine and total chlorine — Part 1 : Titrimetric method using* N,N-*diethyl-1,4-phenylenediamine.*

ISO 7393-2 : 1985, *Water quality — Determination of free chlorine and total chlorine — Part 2 : Colorimetric method using* N,N-*diethyl-1,4-phenylenediamine, for routine control purposes.*

3 Definition

For the purposes of this International Standard, the following definition applies.

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, 5 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) Settled effluent from a waste water treatment plant.

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 of 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.

NOTE — 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.

Use this solution within 24 h of preparation and discard any remaining solution at the end of the working period.

5.4 Seeded dilution water.

Add, according to its source 5 ml to 20 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. Prepare immediately before use and discard any remaining solution at the end of the working day.

The oxygen depletion over 5 days, at 20 °C of the seeded dilution water (5.4), which is the blank value (8.3), shall preferably not exceed 0,5 mg of oxygen per litre.

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

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

5.7 Sodium sulfite (Na_2SO_3), solution, approximately 0,5 mol/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 the solution immediately before use and discard any remaining solution at the end of the working day.

5.9 Allylthiourea (ATU) ($C_4H_8N_2S$), solution.

Dissolve 1,00 g of allylthiourea in water, dilute to 1 000 ml and mix. The solution is stable for at least 2 weeks.

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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.

Table 1 — Recommended dilutions for determination of BOD_5

Expected BOD_5 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.

6 Apparatus

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

Ordinary laboratory apparatus and

6.1 Incubation flasks, narrow-mouthed, of capacity between 130 ml 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 °C ± 1 °C.

6.3 Equipment for determining dissolved oxygen concentration.[1)]

6.4 Means of refrigeration (0 °C 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.

7 Storage of the sample

Store the sample at a temperature between 0 °C 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.

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.

ISO standards for free and combined chlorine have been published (ISO 7393-1 and ISO 7393-2).

8.2 Preparation of test solutions

8.2.1 BOD determination without suppression of nitrification

Bring the test sample to a temperature of about 20 °C and shake 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 add seeded dilution water (5.4) to the mark. Mix gently to avoid entrapment of air bubbles.

If the dilution factor to be used is greater than 100 carry out serial dilutions in two or more steps.

1) 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).

8.2.2 BOD determination with suppression of nitrification

Bring the test sample to a temperature of about 20 °C and shake 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), add 2 ml of allylthiourea solution (5.9) per litre of diluted sample and fill to the mark with seeded dilution water (5.4).

Mix gently to avoid entrapment of air bubbles.

NOTES

1 Alternatively use as suppressant 2-chloro-6-trichloromethylpyridine (TCMP) ($Cl\text{-}C_5H_3N\text{-}CCl_3$) fixed on solid sodium chloride. Add it such that the TCMP concentration in the diluted sample is 0,5 mg/l.

2 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_5 (see table 1).

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

3 Take care that representative samples are withdrawn.

4 The suppression of nitrification according to 8.2.2 is not achievable in all cases.

A significant increased addition of ATU over that used in 8.2.2 may affect the Winkler titration.

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).

Put one series of flasks in the incubator (6.2) and leave in darkness for 5 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 incubation, 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 obtained shall fall between 180 mg/l 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:

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

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 5 days.

9.2 The biochemical oxygen demand after 5 days (BOD_5), expressed in milligrams of oxygen per litre, is given by the equation

$$BOD_5 = \left[(C_1 - C_2) - \frac{V_t - V_e}{V_t} (C_3 - C_4) \right] \frac{V_t}{V_e}$$

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 5 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) 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 (5);

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.

Annex A
(informative)

Alternative incubation periods and temperatures

The rate of carbonaceous oxidation during the first stage of the BOD test may be expressed by Phelps' law:

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

where

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

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

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 and 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 incubation at 20 °C. Over the years a vast amount of data have been accumulated and, because of this, other more rapid tests, which have been developed to measure organic pollution, are usually correlated with the 5 day BOD.

One of the drawbacks associated with the test has always been the 5 day delay before a result is obtained. Attempts have been made to produce the same results within a shorter period (3 days or 2,5 days) by using higher temperatures (27 °C or 35 °C respectively).

In some countries with very hot climates, the 3 day test may be a more practical procedure, not to save time, but because ambient temperatures are so much higher and the various microorganisms responsible for degradation and oxidation of organic matter are exposed and acclimatized to temperatures between 25 °C and 30 °C. However, most countries with hot climates use the classic 5 day test, with cooling of the sample.

A 3 day test has been used by some workers and numerous comparisons and correlations with the 5 day test have been compiled. One study of comparisons showed that the two tests rarely differed by more that ± 5 %.

Another approach has been to introduce a 7 day BOD at the standard temperature of 20 °C. No change is thus required in the procedure and samples necessarily requiring the standard 5 day test can be analysed at the same time. Correlations between the two tests are also easier to obtain. The 7 day test has been used in Sweden for several years. A 7 day test at 18 °C has also been studied.

An important consideration in all these modifications is the part played by nitrifying organisms. All the foregoing comparisons and correlations refer to total BOD results, without addition of ATU, and therefore no acount can be taken of the potential contribution of nitrification to the measured BOD when using alternative incubation periods and temperatures.

Both enhanced incubation periods and enhanced temperature will greatly increase the likelihood of nitrification occurring. This in turn leads to the likelihood of still greater concentrations of ATU being required to inhibit nitrification, possibly significantly greater than the concentration of 2,0 mg/l given in this International Standard.

It is unlikely that universal conversion factors can be established for tests using non-standard incubation periods and temperatures, and to correlate them with standard BOD test results, particularly where a range of different sample types is analysed.

This situation is common to most empirical tests including the other common oxygen demand tests, COD and permanganate value. It may be possible to establish conversion factors for a very narrow band or type of sample. For example, work demonstrated that provided sufficient ATU was present to inhibit nitrification, 7 day BOD (ATU) for primary settled sewage and settled final effluents were higher by factors of 1,09 and 1,29 respectively.

Unless acceptable evidence can be established, it is recommended that the actual results found using alternative incubation periods and temperatures should be quoted, identifying the test conditions, without any attempt to convert them to "standard test" BOD results.

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