SLOVENSKI STANDARD
SIST EN 1899-1:2000

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Kakovost vode – Določevanje biokemijske potrebe po kisiku po n dneh (BPKn) - 1. del: Metoda razredčevanja in cepljenja z dodatkom alilitiosečnine (ISO 5815:1989, spremenjen)

Water quality - Determination of biochemical oxygen demand after n days (BODn) - Part 1: Dilution and seeding method with allylthiourea addition (ISO 5815:1989, modified)

Wasserbeschaffenheit - Bestimmung des biochemischen Sauerstoffbedarfs nach n Tagen (BSBn) - Teil 1: Verdünnungs- und Impfverfahren nach Zugabe von Allylthioharnstoff (ISO 5815: 1989, modifiziert)

Qualité de l'eau - Détermination de la demande biochimique en oxygene apres n jours (DOBn) - Partie 1: Méthode par dilution et ensemencement avec apport d'allylthio-urée (ISO 5815:1989, modifiée)

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English version

Water quality - Determination of biochemical oxygen demand after n days (BOD_n) - Part 1: Dilution and seeding method with allylthiourea addition (ISO 5815:1989, modified)

Qualité de l'eau - Détermination de la demande biochimique en oxygène après n jours (DOB_n) - Partie 1: Méthode par dilution et ensemencement avec apport d'allyl thio-urée (ISO 5815:1989, modifiée)

Wasserbeschaffenheit - Bestimmung des Biochemischen Sauerstoffbedarfs nach n Tagen (BSB_n) - Teil 1: Verdünnungs- und Impfverfahren nach Zugabe von Allylthioharnstoff (ISO 5815:1989, modifiziert)

This European Standard was approved by CEN on 13 February 1998.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Central Secretariat: rue de Stassart, 36 B-1050 Brussels

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Contents

Foreword

This European Standard has been prepared by Technical Committee CEN/TC 230 "Water analysis", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 1998, and conflicting national standards shall be withdrawn at the latest by September 1998.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

This draft European Standard consists of the following parts:

EN 1899-1 Water quality – Determination of biochemical oxygen demand after n days (BOD_n) – Part 1: Dilution and seeding method with allylthiourea addition

EN 1899-2 Water quality – Determination of biochemical oxygen demand after n days (BOD_n) – Part 2: Method for undiluted samples

Annex A, which is normative, concerns alternative incubation periods.

Annex B, which is informative, concerns multitesting, which may be used to obtain enhanced precision, or if the presence of substances toxic to microorganisms is suspected.

Endorsement notice

The text of the International Standard ISO 5815:1989 was approved by CEN as a European Standard with agreed common modifications as given below:

- Change in setup (splitting into two parts)
- Addition of annexes.

Introduction

This European Standard EN 1899-1 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 the proposal are 5 days - as in ISO 5815 and as has been applied in many European countries - or 7 days as has been applied in several Nordic countries through the past many years. The 7 day incubation will typically give higher BOD results than 5 days incubation time.

With an incubation time of 5 days weekend work can only be avoided, if samples are collected Wednesdays, Thursdays to Fridays. With an incubation time of 7 days, samples collected on the first five weekdays can be analysed without implying weekend work. For this reason a 7 day incubation can be considered more convenient than the conventional 5 day incubation.

A new, modified 7 day incubation is described in a normative annex. The first investigations indicate that BOD results obtained by this modified method are identical to results obtained by the 5 day method described in the proposed standard. 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 standard.

1 Scope

This European Standard specifies a determination of the biochemical oxygen demand of waters by dilution and seeding with suppression of nitrification.

This standard is applicable to all waters having biochemical oxygen demands greater than or equal to the limit of determination 3 mg/l of oxygen and not exceeding 6000 mg/l of oxygen. For biochemical oxygen demands greater than 6 000 mg of oxygen/l, 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. In this standard the limit of detection, 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, with f is the degrees of freedom for the determination of s_B and n is the number of analysis 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.

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.

It is absolutely essential that tests conducted according to this standard are carried out by suitably qualified staff.

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.

2 Normative references

This European Standard incorporates by dated or undated reference provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

EN ISO 3696	Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)
EN 25813	Water quality – Determination of dissolved oxygen – Iodometric method (ISO 5813:1983)
EN 25814	Water quality – Determination of dissolved oxygen – Electrochemical probe method (ISO 5814:1990)
EN ISO 8467	Water Quality – Determination of permanganate index (ISO 8467:1993)
ISO 6060:1989	Water quality – Determination of chemical oxygen demand
ISO 6107-2:1997	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.
ISO 8245:1987	Water Quality – Guidelines for the determination of total organic carbon (TOC).

3 Definition

For the purposes of this European Standard, the following definition applies:

Biochemical oxygen demand after n days (BOD$_n$): The mass concentration of dissolved oxygen consumed under specified conditions by the biochemical oxidation of organic and/or inorganic matter in water. n is the incubation time; it is equal to 5 or 7.

NOTE: This definition is similar to the definition of ISO 6107-2. For the purpose of this European Standard, "biochemical oxidation" is taken to mean "biological oxidation".

4 Principle

Pre-treatment of the sample of water to be analyzed and dilution with varying amounts of a dilution water rich in dissolved oxygen and containing a seed of aerobic microorganisms, with suppression of nitrification.

Incubation at 20 °C for a defined period, 5 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 Reagents

5.1 General

Throughout the text, use only reagents of recognized analytical quality.

5.2 Water

Use only grade 3 water (in accordance with EN ISO 3696). However, the water shall not contain more than 0,01 mg/l of copper, nor chlorine or chloramines.

5.3 Seeding water

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

a) Urban waste water of maximum 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 main 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 waste water;

c) Settled effluent from a waste water treatment plant;

d) Water taken downstream from the discharge of the water to be analyzed or water containing microorganisms adapted to the water to be analyzed and cultivated in the laboratory (in the case of industrial effluents containing substances which degrade with difficulty);

e) Commercially available seeding material.

5.4 Salt solutions

5.4.1 General

The following solutions are stable for 6 months and shall be stored in glass bottles at 0 °C to 4 °C in the dark. They shall be discarded at the first sign of precipitation or biological growth.

5.4.2 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.

NOTE: The pH of this buffer solution should be 7.2 without further adjustment.

5.4.3 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.

5.4.4 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 (for example 36,4 g $CaCl_2 \cdot 2H_2O$)) in water. Dilute to 1 000 ml and mix.

5.4.5 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.

5.5 Dilution water

Add to about 500 ml of water 1 ml of each of the salt solutions (5.4.2, 5.4.3, 5.4.4 and 5.4.5). 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 (see 6.7), 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.

5.6 Seeded dilution water

Add, according to its source, 5 ml to 20 ml of the seeding water (see 5.3) per litre of dilution water (see 5.5). 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 (see 8.5) show that the seeded dilution water is acceptable for a longer time period.

The oxygen consumed over n days, at 20 °C of the seeded dilution water, which is the blank value (see 8.3), shall not exceed 1,5 mg/l of oxygen.

5.7 Hydrochloric acid (HCl) or sulfuric acid, (H_2SO_4), solution, $c(H_2SO_4) \approx 0,25$ mol/l, $c(HCl) \approx 0,50$ mol/l, or as appropriate

5.8 Sodium hydroxide (NaOH), solution, approximately 20 g/l or as appropriate

5.9 Sodium sulfite (Na_2SO_3), solution, approximately 50 g/l or as appropriate

5.10 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 1000 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.

5.11 Allylthiourea (ATU), solution, 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

6.1 General

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

6.2 Incubation bottles, BOD bottles, with stoppers, for example preferably 250 ml to 300 ml or 100 ml to 125 ml with stoppers and preferably with straight shoulders, or any equivalent bottles.

It is important that the bottles are thoroughly cleaned before use. If the iodometric method (EN 25813) for determining dissolved oxygen is used, it is for example normally sufficient to rinse the bottle several times with tap water then deionised water. If the electrode method EN 25814 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 % (*V/V*) 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 deionised water.

6.3 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 (see 8.3).

6.4 Incubator, capable of being maintained at (20 ± 1) °C

6.5 Equipment for determining dissolved oxygen concentration

In accordance with EN 25813 or EN 25814.

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

6.7 Dilution vessel, a stoppered glass flask of a capacity dependent on the volume of the diluted sample used with graduation of between 2,5 ml and 10 ml or any appropriate vessel allowing for dilution.

6.8 Aeration equipment, a 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 a contamination is suspected, the air shall be filtered and washed.

7 Storage of the sample

Store the sample at a temperature (0 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 11.
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 (5.7) or of sodium hydroxide solution (5.8) 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 (5.9). Take care to avoid adding an excess.

NOTE: For free and combined chlorine see ISO 7393-1 and ISO 7393-2.