BRITISH STANDARD

BS EN ISO 10707:1998
BS 6068-5.16: 1995

Incorporating Amendment No. 1 to BS 6068-5.16:1995 (renumbers the BS as BS EN ISO 10707:1998)

Water quality —

Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of biochemical oxygen demand (closed bottle test)

The European Standard EN ISO 10707:1997 has the status of a British Standard

Confirmed July 2008

ICS 13.060.01

Committees responsible for this British Standard

The preparation of this British Standard was entrusted to Technical Committee EPC/44, Water quality, upon which the following bodies were represented:

Association of Consulting Scientists
British Association for Chemical Specialists
British Gas plc
Chemical Industries Association
Convention of Scottish Local Authorities
Department of the Environment (Water Directorate)
Department of the Environment for Northern Ireland
Department of Trade and Industry (Laboratory of the Government Chemist)
Electricity Association
Industrial Water Society
Institute of Gas Engineers
Institution of Water Officers
Institution of Water and Environmental Management
National Rivers Authority
Royal Institute of Health and Hygiene
Royal Society of Chemistry
Scottish Association of Directors of Water and Sewerage Services
Soap and Detergent Industry Association
Water Companies Association
Water Research Centre
Water Services Association of England and Wales

The following bodies were also represented in the drafting of the standard, through subcommittees and panels:

BLWA Ltd. (The Association of the Laboratory Supply Industry)
Department of Economic Development (Northern Ireland)
Department of Health
Freshwater Biological Association
Scottish National Heritage

This British Standard, having been prepared under the direction of the Health and Environment Sector Board (H/-), was published under the authority of the Standards Board and comes into effect on 15 March 1995

The following BSI references relate to the work on this standard:
Committee reference EPC/44
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Amendments issued since publication

Amd. No.	Date	Comments
9903	March 1998	see national foreword

Contents

National foreword

This British Standard is the English language version of EN ISO 10707:1997. It is identical with ISO 10707:1994.

The UK participation in its preparation was entrusted by Technical Committee EH/3, Water quality, to Subcommittee EH/3/5, Biological methods, which has the responsibility to:

— aid enquirers to understand the text;

— present to the responsible international committee any enquiries on interpretation, or proposals for change, and keep UK interests informed;

— monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this subcommittee can be obtained on request to its secretary.

BS EN ISO 10707 is one of a series of standards on water quality, others of which have been, or will be, published as Sections of BS 6068. This standard has therefore been given the secondary identifier BS 6068-5.16. The various Sections of BS 6068 are comprised within Parts 1 to 7, which, together with Part 0, are listed below.

— *Part 0: Introduction;*

— *Part 1: Glossary;*

— *Part 2: Physical, chemical and biochemical methods;*

— *Part 3: Radiological methods;*

— *Part 4: Microbiological methods;*

— *Part 5: Biological methods;*

— *Part 6: Sampling;*

— *Part 7: Precision and accuracy.*

Amendment No. 1

The contents page and national foreword have been amended to reflect the implementation of EN ISO 10707:1997 and the EN title page, EN foreword and Annex ZA have been introduced.

Cross-references

Attention is drawn to the fact that CEN and CENELEC Standards normally include an annex which lists normative references to international publications with their corresponding European publications. The British Standards which implement these international or European publications may be found in the BSI Standards catalogue under the section entitled “International Standards Correspondence Index”, or using the “Find” facility of the BSI Standards Electronic Catalogue.

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the EN ISO title page, pages 2 to 10 and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 10707

November 1997

ICS 13.060.40

Descriptors: See ISO document

English version

Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of biochemical oxygen demand (closed bottle test)

(ISO 10707:1994)

Qualité de l'eau — Evaluation en milieu aqueux de la biodégradabilité aérobie "ultime" des composés organiques — Méthode par analyse de la demande biochimique en oxygène (essai en fiole fermée
(ISO 10707:1994)

Wasserbeschaffenheit — Bestimmung der vollständigen aeroben biologischen Abbaubarkeit organischer Stoffe in einem wäßrigen Medium — Verfahren mittels Bestimmung des biochemischen Sauerstoffbedarfs (Geschlossener Flaschentest)
(ISO 10707:1994)

This European Standard was approved by CEN on 30 October 1997.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

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.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart 36, B-1050 Brussels

Ref. No. EN ISO 10707:1997 E

Foreword

The text of the International Standard from Technical Committee ISO/TC 147, Water quality, of the International Organization for Standardization (ISO) has been taken over as a European Standard 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 May 1998, and conflicting national standards shall be withdrawn at the latest by May 1998.

According to CEN/CENELEC Internal Regulations, 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.

NOTE Normative references to International Standards are listed in Annex ZA (normative).

WARNING — SAFETY PRECAUTIONS — Activated sludge and sewage may contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling them. Toxic test compounds and those whose properties are unknown should be handled with care.

1 Scope

This International Standard specifies a method, by analysis of biochemical oxygen demand, for the evaluation in an aqueous medium of the "ultimate" biodegradability of organic compounds at a given concentration by aerobic microorganisms.

The conditions described in this International Standard do not necessarily always correspond to the optimal conditions for allowing the maximum value of bio-degradation to occur.

The method applies to all organic compounds which are sufficiently water soluble to prepare a stock solution or poorly water soluble when using special dosing techniques.

Due to the low concentration of test compound at the beginning of the test, normally no special precautions for the toxicity of the test compound to the microorganisms of the inoculum is necessary; if required a parallel inhibition test can be performed.

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 5814:1990, *Water quality — Determination of dissolved oxygen — Electrochemical probe method.*

ISO 6060:1989, *Water quality — Determination of the chemical oxygen demand.*

ISO 9887:1992, *Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Semi-continuous activated sludge method (SCAS).*

ISO 9888:1991, *Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Static test (Zahn-Wellens method).*

ISO 10304-2:—[1)], *Water quality — Determination of dissolved anions by liquid chromatography of ions — Part 2: Determination of bromide, chloride, nitrate, nitrite, orthophosphate and sulfite in waste water.*

ISO 10634 —[1)], *Water quality — Guidance for the evaluation in an aqueous medium of the "ultimate" biodegradability of poorly soluble organic compounds.*

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1
ultimate biodegradation

the level of degradation achieved when the test compound is totally utilized by microorganisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituents (biomass)

3.2
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 and is expressed in this case as milligrams of oxygen uptake per milligram or gram of test compound

3.3
chemical oxygen demand (COD)

the amount of oxygen consumed during oxidation of a test compound with hot, acidic dichromate. It provides a measure of the amount of oxidizable matter present and is expressed in this case as milligrams of oxygen consumed per milligram or gram of test compound

3.4
theoretical oxygen demand (ThOD)

the total amount of oxygen required to oxidize a chemical completely. It is calculated from the molecular formula and is expressed in this case as milligrams of oxygen required per milligram or gram of test compound

[1)] To be published.

3.5
pre-exposure (or pre-adaptation)

the pre-incubation of an inoculum in the presence of the test compound, with the aim of enhancing the ability of the inoculum to degrade the test compound. If the aim is achieved, the inoculum is said to be adapted

3.6
pre-conditioning (or pre-acclimatization)

the pre-incubation of an inoculum under the conditions of the test in the absence of the test compound, to improve the performance of the test

4 Principle

A solution of the organic test compound in a mineral medium as the sole source of carbon and energy is inoculated with a relatively small number of micro-organisms from a mixed population and kept in completely full, closed bottles in the dark at a constant temperature. Biodegradation is followed by analysis of dissolved oxygen over a period of 28 d. The amount of oxygen taken up by the test chemical (BOD), corrected for uptake by the blank inoculum run in parallel, is expressed as percentage of ThOD or COD.

5 Test environment

Incubation shall take place in the dark in an enclosure which is maintained at a constant temperature (within ± 1 °C) between 20 °C and 25 °C.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 *Water*

Distilled or deionized water free from inhibitory concentrations of toxic substances containing less than 10 % of the initial DOC content introduced by the compounds to be tested. For each series of tests, use only one batch of water.

6.2 *Test medium*

6.2.1 *Composition*

6.2.1.1 *Solution a)*

Anhydrous potassium dihydrogenphosphate (KH_2PO_4)	8,5 g
Anhydrous dipotassium hydrogenphosphate (K_2HPO_4)	21,75 g
Disodium hydrogenphosphate dihydrate ($Na_2HPO_4.2H_2O$)	33,4 g
Ammonium chloride (NH_4Cl)	0,5 g

Dissolve the ingredients in water (**6.1**) and make up to 1 000 ml.

NOTE 1 The correct composition of the medium can be checked by the measurement of the pH-value, which should be **7.4**.

6.2.1.2 *Solution b)*

Dissolve 22,5 g of magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) in water (**6.1**) and dilute to 1 000 ml.

6.2.1.3 *Solution c)*

Dissolve 27,5 g of anhydrous calcium chloride ($CaCl_2$) or 36,4 g of calcium chloride dihydrate ($CaCl_2.2H_2O$) in water (**6.1**) and dilute to 1 000 ml.

6.2.1.4 *Solution d)*

Dissolve 0,25 g of iron(III) chloride hexahydrate ($FeCl_3.6H_2O$) in water (**6.1**) and dilute to 1 000 ml.

In order to avoid having to prepare this solution immediately before use, add one drop of concentrated hydrochloric acid (HCl) or 0,4 g of ethylenediaminetetraacetic acid (EDTA) (disodium salt) per litre.

6.2.2 *Preparation of the test medium*

For 1 litre of test medium, add 1 ml of each of the solutions a) to d) (**6.2.1**) to about 500 ml of water (**6.1**) and adjust the volume to 1 000 ml.

Strongly aerate the test medium for at least 20 min. Carry out each series of tests with test medium derived from the same batch. Generally, the medium is ready for use after standing for 20 h at the test temperature. Determine the concentration of dissolved oxygen for control purposes; the value should be about 9 mg/l at 20 °C. Conduct all transfer and filling operations of the air-saturated medium bubble-free, for example, by the use of siphons.

7 Apparatus

Usual laboratory equipment, and

7.1 *BOD bottles with glass stoppers,* of capacity 250 ml to 300 ml. The bottles can be made airtight by greasing. In this case, only grease which is free of organic carbon, for example silicon grease, shall be used.

7.2 *Water bath or incubator,* for keeping bottles at a constant test temperature with the exclusion of light.

7.3 *Large glass bottles,* of capacity 2 litres to 5 litres, for the preparation of media and for filling the BOD bottles.

7.4 *Oxygen electrode and meter,* or equipment for iodometric oxygen determination.

7.5 *pH-meter*.

8 Procedure

8.1 Preparation of test compound solution

Prepare a stock solution of the test compound in water (**6.1**) or test medium (**6.2.2**) (e.g. 1 g/l). Add a sufficient amount of the stock solution to the large bottles (**7.3**) containing a known volume of test medium (**6.2.2**) so that the final concentration of the chemical is normally 2 mg/l. This concentration is in general suitable to ensure that the concentration of oxygen does not fall below 0,5 mg/l during the test and the inoculum activity is not limited. For poorly biodegradable compounds and those with a low ThOD, up to 10 mg/l may be used.

In some cases, for example if a poor or partial degradation is expected, it would be advisable to run parallel series of test chemical at two different concentrations, for example 2 mg/l and 5 mg/l. Normally, calculate the ThOD on the basis of formation of ammonium salts but, if nitrification is expected or known to occur, calculate the ThOD on the basis of the formation of nitrate. However, if nitrification is not complete but does occur, correct for the changes in concentration of nitrite and nitrate, determined by analysis (see Annex C).

In the case of test compounds that are very poorly soluble in water for which no stock solution can be prepared, add the test compound in the required quantity directly to the BOD bottles (**7.1**). Use the large bottles (**7.3**) only to add inoculated test medium (**6.2.2**). Avoid loss of test medium and test compound when stoppering the bottles. For alternatives or more details see ISO 10634.

8.2 Preparation of reference compound solution

Prepare a stock solution of a known biodegradable organic substance, for example sodium acetate, sodium benzoate or aniline, in the test medium (**6.2.2**). In the same way as with the test compound, add a sufficient amount of the stock solution to the large bottles (**7.3**) to give a test concentration of 2 mg/l.

8.3 Preparation of inhibition control

If the toxicity of the test compound is to be investigated (for example in the case of a previous low bio-degradability value having been found), another series of bottles is necessary. Prepare another large bottle (**7.3**) containing aerated mineral medium plus test compound and reference compound at final concentrations that are the same as those in the other large bottles. Add the mixture to the BOD bottles (**7.1**).

8.4 Preparation of the inoculum

In this test use an inoculum without sludge flocs. It should be derived from the secondary effluent of a treatment plant or laboratory-scale unit receiving pre-dominantly domestic sewage or from surface water. Mixtures from these different sources may also be used.

Collect a fresh sample and keep it aerobic during transport. If suspended solids are present, allow to settle for 1 h or filter through a coarse filter paper and keep the sample under aerobic conditions until it is required.

Use a suitable volume of these samples, ranging from one drop (about 0,05 ml) up to 5 ml per litre, to inoculate the large bottles. Trials may be needed to discover the optimum volume. If necessary, concentrate the inoculum by filtration or centrifugation.

NOTE 2 Suitable volume means:

— sufficient to give a population which offers enough bio-degradation activity,

— degrades the reference compound by the stipulated percentage,

— gives between 10^3 to 10^6 active cells/ml.

NOTE 3 If the oxygen consumption in the blank bottles without test compound is too high (> 1,5 mg/l at the end of the test), a preconditioning by aeration of the inoculum between 1 d and 7 d is recommended. This may help to reduce the oxygen consumption of the microorganisms in the blank.

NOTE 4 Pre-exposed inocula may be used in certain circumstances. When such inocula are used, this should be clearly stated in the test results (e.g. percentage biodegradation = *x* % using pre-exposed inoculum) and the method of pre-exposure detailed in the test report. Pre-exposed inocula can be obtained from laboratory biodegradation tests run under a variety of conditions as appropriate [e.g. Zahn-Wellens test (ISO 9888) or SCAS test (ISO 9887)] or from samples collected from locations where relevant environmental conditions exist (e.g. treatment plants dealing with similar compounds or contaminated areas).

8.5 Preparation of test bottles

Prepare parallel groups of BOD bottles (**7.1**) for the determination involving the test and reference compound, the inhibition control and the blank values in simultaneous experimental series. Assemble a sufficient number of BOD bottles to allow at least duplicate measurements of oxygen consumption to be made at the desired test intervals, generally at least after 0 d, 7 d, 14 d, 21 d and 28 d.

In a typical run the following bottles are used:

— at least 10 bottles containing test compound and inoculum (FT);

— at least 10 bottles containing reference compound and inoculum (FC);

— at least 10 bottles containing only inoculum (blank) (FB);

and if necessary

— at least 6 bottles containing test compound, reference compound and inoculum (inhibition control) (FI).

Add fully aerated mineral medium (**6.2.2**) to large bottles (**7.3**) so that they are about one-third full. Then add a sufficient amount of the stock solutions of the test compound and reference compound to separate large bottles to reach the desired final concentration of the chemicals. Do not add any chemicals to the blank control medium contained in another large bottle. Inoculate the large bottles with a suitable volume of inoculum (**8.4**), dilute the solutions to volume with aerated test medium and mix well.

Dispense each prepared solution immediately into the respective group of BOD bottles from the lower quarter (not the bottom) of the appropriate large bottle, for example by siphoning, so that all the BOD bottles are completely filled. Tap gently to remove any air bubbles.

8.6 Performance of the test

Analyse the zero-time bottles (see **8.5**) immediately for dissolved oxygen using an electrode (see ISO 5814).

NOTE 5 Alternatively the Winkler method can be used to measure dissolved oxygen (see ISO 5813). In this case, the contents of the bottles can be preserved for subsequent analysis by adding manganese(II) sulfate and sodium hydroxide. The carefully stoppered bottles should be stored in the dark at 10 °C to 20 °C for no longer than 24 h before proceeding with the remaining steps of the Winkler method.

Stopper the remaining bottles while ensuring that no air bubbles are enclosed, and incubate at the test temperature in the dark. Withdraw at least the duplicate bottles of all series for dissolved oxygen analysis after at least 7 d, 14 d, 21 d and, at the end of the test, after 28 d. Measure the oxygen concentration in the same way as in the zero-time bottles.

For test compounds containing nitrogen, make corrections for uptake of oxygen by any nitrification (see Annex C). To do this, withdraw a sample from the large bottle (**7.3**) at the beginning of the test and use it for analysis of nitrite and nitrate, for example according to ISO 10304-2. Make the same determination with a sample from a BOD bottle at the end of the test. If the Winkler method (see ISO 5813) is used, prepare an additional bottle. From the change in concentration of nitrite and nitrate, calculate the oxygen used for nitrification.

9 Calculation and expression of results

9.1 Calculation

First calculate the oxygen consumption after each time period by subtracting the oxygen concentration of the inoculum blank (mean value of replicates) from that exhibited by the test compound (for each test bottle). Divide this corrected depletion by the concentration of the test compound, to obtain the specific BOD, expressed as milligrams of oxygen per milligram of test compound. Calculate the percentage biodegradability by dividing the specific BOD by the specific ThOD (see Annex A). If the ThOD cannot be determined, or as additional information, use the measured COD value (see Annex B). These calculation steps are combined in equation (1). It should be noted that these two methods do not necessarily give the same values. COD is often less than ThOD, so that the percentage biodegradation, of the test compound at time t (D_t) using COD can be higher than when ThOD is used. Finally calculate the mean values from the percentages of parallel assays.

$$D_t = \frac{(\rho_O - \rho_{O,t}) - (\rho_{O,b} - \rho_{O,t,b})}{ThOD \times \rho_c} \qquad ...(1)$$

where

D_t is the percentage biodegradation of the test compound at time t;

ρ_O is the oxygen concentration, in milligrams per litre, at time zero in the test bottles;

$\rho_{O,t}$ is the oxygen concentration, in milligrams per litre, at time t in the test bottles;

$\rho_{O,b}$ is the mean oxygen concentration, in milligrams per litre, at time zero in the blank bottles;

$\rho_{O,t,b}$ is the mean oxygen concentration, in milligrams per litre, at time t in the blank bottles;

ThOD is the theoretical oxygen demand, expressed in milligrams per milligram of test compound;

ρ_c is the concentration, in milligrams per litre, of test compound in the test bottles.

Round the percentage results to the nearest whole number.

Perform the same calculation for the reference compound and, if used, the inhibition control.

For test compounds containing nitrogen, use the appropriate ThOD according to what is known or expected about the occurrence of nitrification. If nitrification occurs but is not complete, calculate a correction for the oxygen consumed by nitrification from the changes in concentration of nitrite and nitrate during the test (see Annex C).

9.2 Expression of results

Plot the percentage average biodegradation (D_t) versus time (biodegradation curve). From this curve, determine parameters to describe the biodegradation; in particular the lag time, the degradation time and the maximum level of degradation.

NOTE 6 In most of the degradation curves a so-called lag time can be observed. This is defined as the time from the beginning of inoculation until the degradation percentage has increased to about 10 % of the theoretical maximum degradation (ThOD or COD). The lag time is often highly variable and poorly reproducible. It should be noted in days.

The maximum level of degradation should be defined as the approximate level above which no further degradation takes place during the test.

The degradation time should be defined as the time from the end of the lag time till the time that about 90 % of the maximum level of degradation has been reached. Note the degradation time in days.

NOTE 7 Due to the restricted amount of measured values in the closed bottle test, the lag and the degradation time may often be only estimated.

10 Validity of the test

Oxygen depletion in the inoculum blank control shall not exceed 1,5 mg/l after 28 d. Values higher than this require investigation of the experimental techniques and the used inoculum. Preconditioning by aeration of the inoculum during 1 d to 7 d can be helpful to reduce the blank value.

The residual concentration of oxygen in the test bottles shall not fall below 0,5 mg/l at any time.

Consider a test valid if the difference of extremes of replicate values at the end of the test is less than 20 %. If 1 of 3 replicates is outside this range, consider it as an outlier and use the remaining values. The percentage degradation of the reference compound shall have reached 60 % after 14 d. If either of these conditions is not met, repeat the test. Because of the extreme stringency of this method, low biodegradation values do not necessarily mean that the test compound is not biodegradable under environmental conditions, but indicates that more work will be necessary to establish biodegradability.

In an inhibition test containing both the test and the reference compound, if less than 25 % based on total ThOD or COD occurred after 14 d, assume that the test compound is inhibitory. Repeat the test series, if possible using a lower concentration of the test compound.

11 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard;

b) all information necessary for the identification of the test compound and the test concentration;

c) all the data obtained (for example in tabular form), the degradation curve and the percentage of degradation of the test compound;

d) the name of the reference compound used, the data obtained, the degradation curve and the degradation percentage;

e) the source, the characteristics, the volume and any pretreatment of the inoculum used, for example pre-exposure or pre-conditioning;

f) the method of oxygen measurement used;

g) the incubation temperature of the test;

h) the percentage of degradation in the inhibition control (if used);

i) in the event of rejection of the test, the reasons;

j) any alteration of the standard procedure or any other circumstances that may have affected the results.

Annex A (informative)
Determination of the theoretical oxygen demand (ThOD)

A.1 Calculation

The ThOD may be calculated if the elemental composition is known or determined.

EXAMPLE

$C_cH_hCl_{cl}N_nNa_{na}O_oP_pS_s$ of relative molecular mass M_r

A.1.1 *Without nitrification*

$$ThOD_{NH_3} = \frac{16\left[2c + \frac{1}{2}(h - cl - 3n) + 3s + \frac{5}{2}p + \frac{1}{2}na - o\right]}{M_r}$$

The following assumptions are made:

H to H_2O, C to CO_2, P to P_2O_5, Na to Na_2O, Cl to HCl and N to NH_3

A.1.2 *With nitrification*

$$ThOD_{NO_3} = \frac{16\left[2c + \frac{1}{2}(h - cl) + 3s + \frac{5}{2}n + \frac{5}{2}p + \frac{1}{2}na - o\right]}{M_r}$$

It is assumed in this case that N is finally transformed to NO_3.

A.2 Example: glucose ($C_6H_{12}O_6$) M_r = 180 g

$$ThOD = \frac{16\left(2 \times 6 + \frac{1}{2} \times 12 - 6\right)}{180} = 1{,}07 \text{ mg } O_2/\text{mg glucose}$$

Annex B (informative)
Determination of the chemical oxygen demand (COD)

The COD of organic substances that are soluble in water is determined by established procedures, for example according to ISO 6060. The COD is often, and especially in the case of poorly soluble substances, determined advantageously using a variant of the above analyses, i.e. in a closed system with a pressure equalizer (Kelkenberg[2)]). In this variant, compounds which are only with difficulty determined by the conventional method, for example acetic acid, may often be successfully quantified. The method may also fail, for example in the case of pyridine. If the potassium dichromate concentration is raised from 0,016 N (0,002 6 mol/l) as prescribed by Kelkenberg to 0,25 N (0,041 6 mol/l), the direct weighing-out of 5 mg to 10 mg of substance is facilitated, which is essential for the COD determination of substances that are poorly soluble in water.

[2)] Kelkenberg, K. *Z. Wasser und Abwasserforschung,* **8**, 146 (1975).

Annex C (informative)
Correction of oxygen uptake for interference by nitrification

Errors due to not considering nitrification in the assessment by oxygen uptake of the biodegradability of test substances not containing nitrogen are marginal, even if oxidation of the ammonium nitrogen in the medium occurs erratically, as is the case between test and blank vessels. However, for test substances containing nitrogen, serious errors can arise.

If nitrification has occurred but is not complete, the observed oxygen uptake by the reaction mixture may be corrected for the amount of oxygen used in oxidizing ammonium to nitrite and nitrate, if the changes in concentration during incubation of nitrite and nitrate are determined, by considering the following equations:

$$2NH_4Cl + 3O_2 \rightleftarrows 2HNO_2 + 2HCl + 2H_2O \quad ...(C.1)$$

$$2HNO_2 + O_2 \rightleftarrows 2HNO_3 \quad ...(C.2)$$

Overall:

$$2NH_4Cl + 4O_2 \rightarrow 2HNO_3 + 2HCl + 2H_2O \quad ...(C.3)$$

From equation (C.1), the oxygen uptake when 28 g of nitrogen contained in ammonium chloride (NH_4Cl) is oxidized to nitrite is 96 g, i.e. a factor of 96/28 = 3,43. In the same way, from equation (C.3), the oxygen uptake when 28 g of nitrogen is oxidized to nitrate is 128 g, i.e. a factor of 128/28 = 4,57.

Since the reactions are sequential, being carried out by distinct and different bacterial species, it is possible for the concentration of nitrite to increase or decrease; in the latter case an equivalent concentration of nitrate would be formed. Thus, the oxygen consumed in the formation of nitrate is 4,57 multiplied by the increase in concentration of nitrate-N, whereas the oxygen associated with the formation of nitrite is 3,43 multiplied by the increase in the concentration of nitrite-N. With the decrease in its concentration, the oxygen "loss" is 3,43 multiplied by the decrease in concentration.

That is:

$$\rho(O_2)_1 = 4{,}57 \times \Delta\rho(NO_3^-) \quad ...(C.4)$$

$$\rho(O_2)_2 = 3{,}43 \times \Delta\rho(NO_2^-) \quad ...(C.5)$$

$$\rho(O_2)_3 = -\,[3{,}43 \times \Delta\rho(NO_2^-)] \quad ...(C.6)$$

where

$\rho(O_2)_1$ is the oxygen consumed in nitrate formation;

$\rho(O_2)_2$ is the oxygen consumed in nitrite formation;

$\rho(O_2)_3$ is the oxygen "lost" in nitrite disappearance;

$\Delta\rho(NO_3^-)$ is the increase in nitrate-N concentration;

$\Delta\rho(NO_2^-)$ is the change in nitrite-N concentration.

So that, using equations (C.4) and C.5) or (C.6):

$$\rho(O_2)_4 = [4{,}57 \times \Delta\rho(NO_3^-)] \pm \pm [3{,}43 \times \Delta\rho(NO_2^-)] \quad \ldots (C.7)$$

and thus

$$\rho(O_2)_5 = \rho(O_2)_6 - \rho(O_2)_4 \quad \ldots (C.8)$$

where

$\rho(O_2)_4$ is the oxygen uptake due to nitrification;

$\rho(O_2)_5$ is the oxygen uptake due to carbon oxidation;

$\rho(O_2)_6$ is the total observed oxygen uptake.

Alternatively if only "total oxidized nitrogen" is determined, the oxygen uptake due to nitrification may be taken to be, as a first approximation, 4,57 times the increase in the concentration of oxidized nitrogen.

The corrected value for oxygen consumption due to oxidation of carbon is then compared with the theoretical oxygen demand $ThOD_{NH_3}$, as calculated in Annex A.

Annex ZA (normative)
Normative references to international publications with their relevant European publications

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.

Publication	Year	Title	EN	Year
ISO 5813	1983	*Water quality — Determination of dissolved oxygen — Iodometric method*	EN 25813	1992
ISO 5814	1990	*Water quality — Determination of dissolved oxygen — Electrochemical probe method*	EN 25814	1992
ISO 9887	1992	*Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Semi-continuous activated sludge method (SCAS)*	EN ISO 9887	1994
ISO 9888	1991	*Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Static test (Zahn-Wellens method)*	EN 29888	1993
ISO 10304-2	1995	*Water quality — Determination of dissolved anions by liquid chromatography of ions — Part 2: Determination of bromide, chloride, nitrate, nitrite, orthophosphate and sulfate in waste water*	EN ISO 10304-2	1996
ISO 10634	1995	*Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium*	EN ISO 10634	1995

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