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Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Semi-continuous activated sludge method (SCAS)

Qualité de l'eau — Évaluation, en milieu aqueux, de la biodégradabilité aérobie des composés organiques — Méthode semi-continue par boues activées (Méthode SCAS)

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.

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.

International Standard ISO 9887 was prepared by Technical Committee ISO/TC 147, *Water quality*, Sub-Committee SC 5, *Biological methods*.

Annexes A and B of this International Standard are for information only.

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Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Semi-continuous activated sludge method (SCAS)

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 for the evaluation of the biodegradability ("ultimate" or "primary") of organic compounds. The conditions described in this International Standard are much more favorable for biodegradation than those specified in the methods for biodegradability described in ISO 7827, ISO 9408 and ISO 9439.

The method applies to organic compounds which are

a) soluble at the concentration used under the test conditions;

b) non-volatile, or which have a negligible vapour pressure under the test conditions;

c) not lost by foaming from the test solution;

d) not significantly adsorbable on glass and activated sludge;

e) not inhibitory to the test micro-organisms at the concentration chosen for the test. Inhibitory effects can be determined by using a suitable test method (e.g. see ISO 8192). If the test compound is toxic, the test concentration has to be lower or a pre-exposed inoculum can be used.

NOTE 1 Additionally, or alternatively, the semi-continuous activated sludge (SCAS) units may be used to provide sludge exposed to the test compound, in order to see whether the sludge becomes adapted, to be used as inocula in other biodegradation tests.

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 7827:1984, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of dissolved organic carbon (DOC).*

ISO 8192:1986, *Water quality — Test for inhibition of oxygen consumption by activated sludge.*

ISO 8245:1987, *Water quality — Guidelines for the determination of total organic carbon (TOC).*

ISO 9408:1991, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by determining the oxygen demand in a closed respirometer.*

ISO 9439:1990, *Water quality — Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds — Method by analysis of released carbon dioxide.*

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 micro-organisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituents (biomass).

3.2 primary biodegradation: The level of degradation achieved when the test compound undergoes any structural change, other than complete mineralization, as the result of microbial action.

3.3 suspended solids (of an activated sludge): The amount of solids obtained by filtration or centrifuging of a known volume of sludge under specified conditions and drying to 105 °C at constant mass.

4 Principle

The concentration of dissolved organic carbon (DOC) in the effluent from a semi-continuous activated sludge unit, which is being dosed at daily intervals on a fill-and-draw basis with sewage and a known concentration of test compound, is compared with the concentration of DOC in the effluent from a control unit dosed with sewage alone. Any difference between the concentration of DOC in the effluents is assumed to be due to the residual test substance, and the percentage degradation/elimination is calculated from this difference and the concentration of test substance (as DOC) added to the sewage.

Specific analysis may give additional information on primary biodegradation.

The length of the test is indeterminate, but experience suggests that this is from 12 weeks to 26 weeks.

A high concentration of aerobic micro-organisms is used (initially 1 g/l to 4 g/l of suspended solids) and the effective detention period of sewage is 36 h. Since no sludge is deliberately wasted, the retention time of the sludge is high. The carbonaceous material in the sewage feed is normally almost completely oxidized within 8 h after the start of each aeration cycle. Thereafter, the sludge respires endogenously for the remainder of the aeration period, during which time the only available substrate is the test compound (unless this is also readily metabolized). These features, combined with daily re-inoculation when domestic sewage is used as the medium, provide highly favourable conditions for both adaptation and extensive biodegradation.

5 Test environment

Incubation shall take place in the dark or in diffused light, in an enclosure which is maintained between 20 °C and 25 °C and which is free from vapours which are toxic to micro-organisms.

6 Reagents

6.1 Tap water, containing less than 2 mg/l of DOC.

6.2 Distilled or **de-ionized water**, containing less than 2 mg/l of DOC.

6.3 Sewage

6.3.1 Synthetic sewage

Dissolve in 1 litre of tap water (6.1).

Peptone	160 mg
Meat extract (or 270 mg of commercial peptone-meat extract preparation)	110 mg
Urea	30 mg
Sodium chloride (NaCl)	7 mg
Calcium chloride dihydrate ($CaCl_2.2H_2O$)	4 mg
Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$)	2 mg
Dipotassium hydrogen phosphate (K_2HPO_4)	28 mg

For convenience, a 100 times more concentrated solution may be prepared in distilled water (6.2), which can be stored at 4 °C for up to 1 week, and the synthetic sewage made daily from this by appropriate dilution with tap water (6.1). After dilution, this synthetic sewage contains approximately: 105 mg/l of C, 46 mg/l of N, 5 mg/l of P and the pH is between 7,0 and 7,5.

6.3.2 Domestic sewage

Collect settled sewage, if possible, freshly each day from the outlet of the primary settlement tank of a treatment plant dealing with predominantly domestic sewage. For weekends, sewage may be stored at 4 °C. Sewage for the daily feeding of the test units can be taken from this stored material.

7 Apparatus

Ordinary laboratory equipment and

7.1 Semi-continuous activated sludge (SCAS) units

The aeration units can simply be, for example, measuring cylinders fitted with an aeration tube and glass sinter for supplying compressed air, which shall be free from organic carbon and toxic vapours and shall be presaturated with water vapour to reduce losses by evaporation. Alternatively, the aeration vessel can be, for example, a tube (see figure 1) suitably supported and fitted with an air inlet tube and a tap so that one-third of the total volume of mixed liquor remains in the vessel after draining off settled supernatant. One such apparatus is required for each test substance and one for each control.

NOTE 2 A suitable volume of mixed liquor has been found to be 150 ml contained in a unit of 250 ml to 300 ml. However, if more exposed sludge is required as inocula to follow the course of adaptation by separate die-away tests, larger SCAS units (e.g. 1,5 litres) may be required.

7.2 Wash bottles or **similar vessels**, containing water for saturating air with water.

7.3 Measuring equipment, of sufficient sensitivity for the measurement of dissolved organic carbon (see ISO 8245).

7.4 Device for filtration, with membrane filters of suitable porosity (nominal aperture diameter of 0,2 µm to 0,45 µm) which neither adsorb organic compounds nor release organic carbon significantly.

7.5 Centrifuge.

7.6 pH-meter.

Dimensions in millimetres

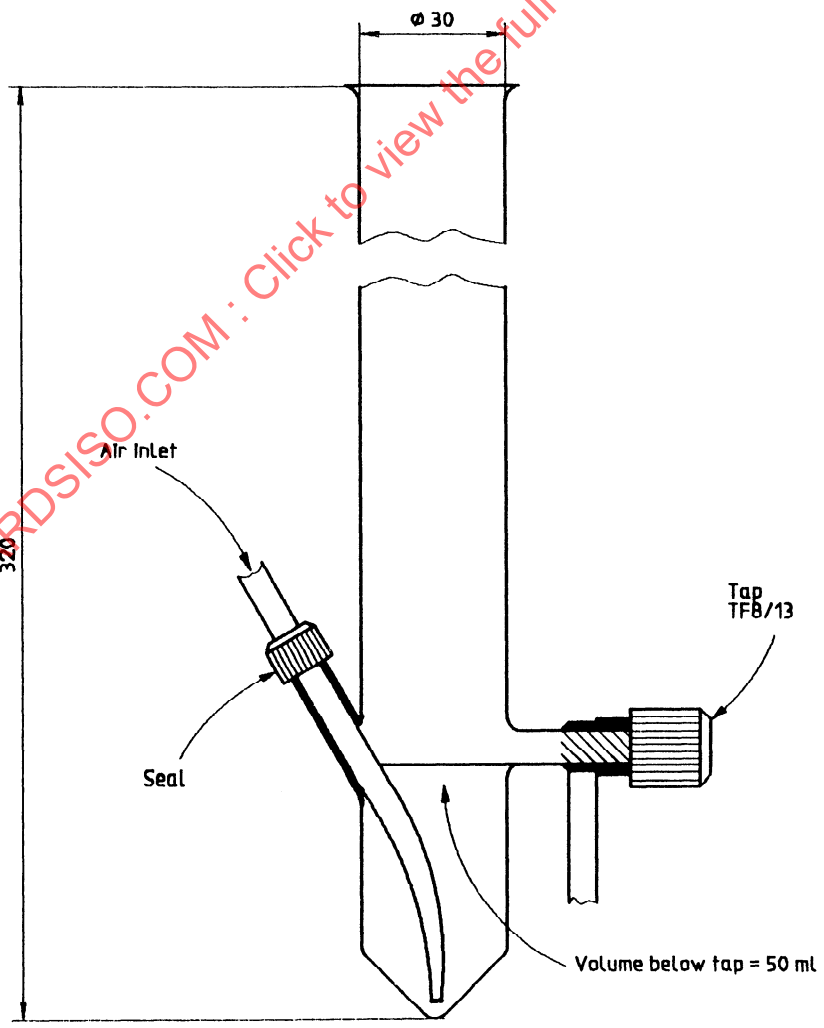

Figure 1 — Example of test apparatus

8 Procedure

8.1 Preparation of the test solutions

Prepare a solution of the test compound (and reference compound if required, see note 3) in the water (6.2) containing not less than 400 mg/l of DOC.

Under the conditions of the test (see 8.3.2), this test solution will give an initial concentration of 20 mg/l of DOC in the SCAS units. If the test compound is not toxic, higher concentrations (e.g. 50 mg/l of DOC) may be used.

Ensure that the pH of the mixture of test solution and sewage is not significantly different from that of the sewage alone.

If necessary, adjust the pH with an inorganic acid or alkaline solution.

NOTE 3 No reference compounds are recommended, but in investigating a new substance it may be useful to test simultaneously one of the compounds (listed in annex A) which have already been evaluated.

8.2 Preparation of the inoculum

Take a sample of activated sludge (containing 1 g/l to 4 g/l of suspended solids) from the aeration tank of a biological waste water treatment plant.

NOTES

4 Depending on the purpose of the test, the waste water treatment plant should receive waste water which is predominantly municipal. To get as many different species or strains of bacteria as possible, it may be preferable in special cases to make a mixture from various sources. Activated sludge may also be taken from a laboratory treatment plant.

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

Keep the inoculum aerated at room temperature until it is to be used.

8.3 Test

8.3.1 Equilibration period

Set up a sufficient number of SCAS units, at least one for each test compound, plus a control. Fill the units to the mark (e.g. 150 ml) with fresh activated sludge (see 8.2) and start aeration. The aeration shall be sufficient to keep the sludge in suspension and the concentration of dissolved oxygen above 2 mg/l.

Before allowing settlement, clean the inner walls of the SCAS units to prevent accumulation of solids at and above the liquid level.

Stop aeration after 23 h and allow the sludge so settle for 30 min, or longer if necessary, to allow the formation of clear supernatant. Open the taps of each vessel to withdraw the volume of supernatant liquor above the tap (i.e. about two-thirds of the total volume). In the case of cylinders, carefully remove the supernatant liquor in order to leave one-third of the total volume of mixed liquor in the cylinder.

Add domestic sewage (6.3.2) or synthetic sewage (6.3.1) to the remaining settled sludges to replace the withdrawn "effluent" (i.e. 100 ml) and start aeration again.

Either centrifuge the effluent at about 40 000 m/s^2 for 5 min or filter it through a membrane filter (7.4), and analyse for organic carbon.

Repeat the fill-and-draw procedure daily and analyse the effluents two or three times per week.

NOTE 6 During this initial period (normally up to 2 weeks) during which no test compound is added, the supernatant liquors become clearer and the concentration of DOC at the end of each aeration cycle approaches a constant value. This value depends largely on the nature of the sewage treated. The degree of DOC elimination is usually about 80 %. When synthetic sewage is used, more than 90 % of the DOC is removed.

At the end of this period, thoroughly mix the individual settled sludges and redistribute equal amounts (i.e. 50 ml) to each vessel.

8.3.2 Test period

Add 95 ml of domestic or synthetic sewage plus 5 ml of water to the control units, and 95 ml of sewage plus 5 ml of the solution of the appropriate test compound to the test units. Then start aeration again.

After 23 h, allow the sludge to settle, as described in the 3rd paragraph of 8.3.1, draw off 100 ml of supernatant and analyse the centrifuged or filtered effluent for DOC or test compound.

Repeat the fill-and-draw procedure daily throughout the test period or, if this is not possible, at least three times a week.

Determine the concentration of DOC in the effluents daily, if the value is changing significantly. Otherwise, carry out the determinations less frequently.

When biodegradation is observed and the concentration of DOC in the test effluents approaches that in the control, continue the analysis until the difference between the concentrations is found to be constant over six consecutive measurements.

When no biodegradation is observed, analyse the effluents two or three times weekly and continue the test for at least 12 weeks but not more than 26 weeks.

Measure the DOC concentration for each vessel at least in duplicate. If primary biodegradation is to be followed, use a specific method of analysis, e.g. UV spectroscopy, in addition to DOC or COD measurements.

Perform all analyses as soon as possible.

NOTE 7 When measurements have to be postponed for up to 48 h, keep the samples at 4 °C in the dark and in tightly stoppered bottles. If the samples have to be stored for more than 48 h, add for example 20 ml/l of a solution containing 10 g/l of mercury chloride ($HgCl_2$) or another inorganic toxic substance to prevent microbial activity and store at 4 °C. If chloride ions are added, the DOC measurements at low concentrations have to be performed with special care. Instead of adding a toxic substance, the samples can be stored at − 18 °C.

9 Calculation and expression of results

Determine the percentage elimination of dissolved organic carbon (or test compound as such) corresponding to each analysis, using either of the following equations:

$$D_d = \frac{\varrho_o - (\varrho_t - \varrho_{Bt})}{\varrho_o} \times 100 \quad \ldots (1)$$

$$D_s = \frac{2\varrho_o - 2(\varrho_t - \varrho_{Bt})}{2\varrho_o + (\varrho_t - \varrho_{Bt})} \times 100 \quad \ldots (2)$$

where

D_d is the percentage removal of the amount of substance as such or as DOC, expressed as a percentage of the amount added daily (as in sewage treatment);

D_s is the percentage removal of the amount of substance as such or as DOC, expressed as a percentage of the amount present at the start of each day;

ϱ_o is the concentration, in milligrams per litre, of test compound as such, or of DOC, added to the sewage at the start of the aeration period;

ϱ_t is the concentration, in milligrams per litre, of test compound as such, or of DOC, found in the supernatant liquor of the test unit at the end of the aeration period;

ϱ_{Bt} is the concentration, in milligrams per litre, of test compound as such (i.e. 0) or of DOC found in the supernatant liquor of the control unit at the end of the aeration period.

Take the average of the last six values as the percentage degradation.

NOTES

8 The concentration of DOC in effluents from control units varies with the type of sewage and the nature of the sludge used. For synthetic sewage (6.3.1), values of 5 mg/l to 8 mg/l of DOC are usually found, while for domestic sewage a range of 8 mg/l to 20 mg/l has been recorded. The normal degree of DOC elimination of sewage in a biological treatment system is about 80 %.

9 Some compounds have been found to persist for up to 3 months before being extensively degrade. Therefore, it is justified to continue the test for at least this time, but it would not be worthwhile to continue beyond six months.

10 The concentration of the test compound used may be inhibitory to the activited sludge, so that the supernatant may become turbid by lysis, the concentration of DOC may rise to unexpectedly high values and sludge may be lost on decanting. In this case, the procedure should be repeated with a lower concentration of test compound, providing that the concentration chosen can be determined with sufficient accuracy and precision.

The toxicity of the test compound should be checked beforehand by a suitable method (e.g. see ISO 8192).

11 Any removal of DOC due solely to biodegradation will normally occur gradually over several days or weeks, except when adaptation is sudden as indicated by an abrupt disappearance, in the course of a day or two, occurring after several weeks (see figure 2).

However, physico-chemical adsorption can sometimes play an important role. This is indicated when there is complete removal, or significant partial removal, of the added DOC at the outset. Subsequent events depend on factors such as the degree of adsorption and the concentration of suspended solids in the discarded effluent. Usually, the difference between the concentration of DOC in the control and test supernatant liquors gradually increases from the low value, as the adsorption sites become saturated, to a more or less constant value, unless adaption takes place.

If a clear distinction is to be drawn between biodegradation (or partial degradation) and adsorption, further tests are necessary. This can be done in a number of ways, but the most convincing is to use the supernatant liquor or exposed sludge as inocula in the respirometric method (see ISO 9408) or carbon dioxide evolution method (see ISO 9439).

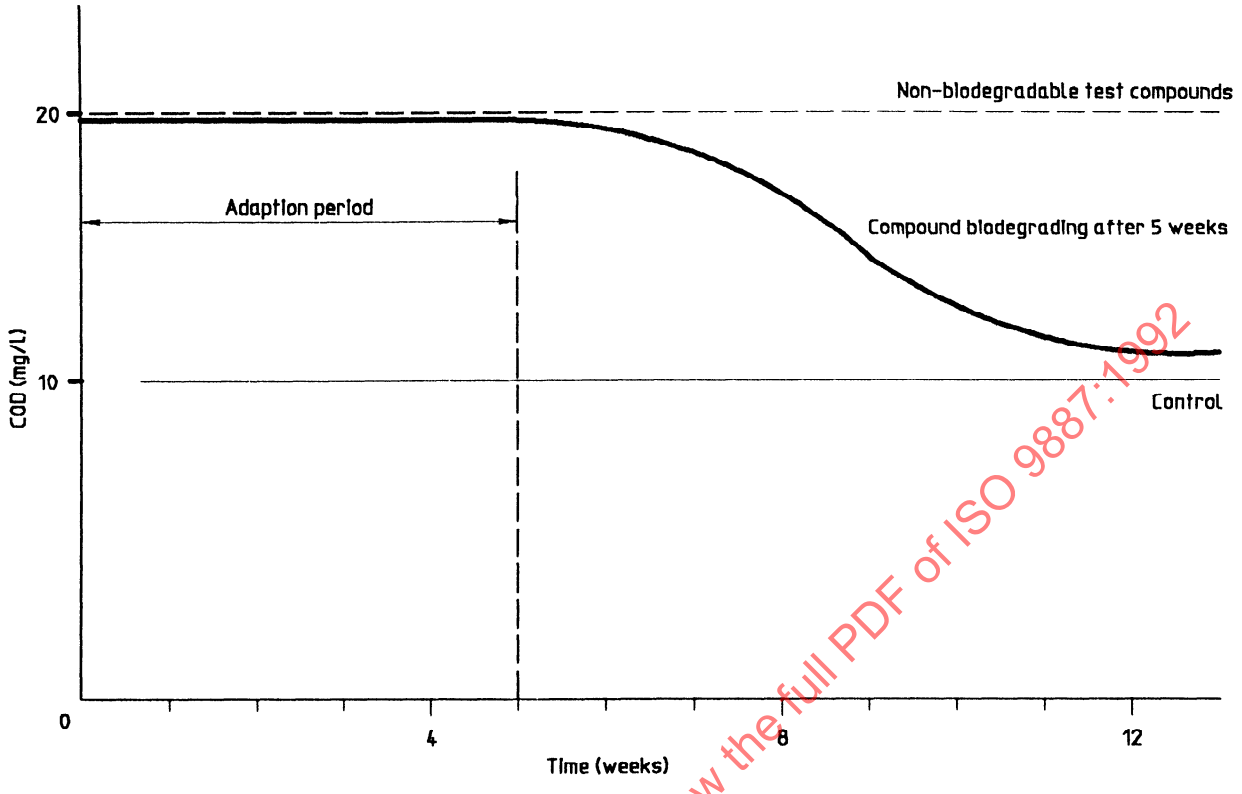

Figure 2 — Graph of removal of compounds (DOC) in SCAS test

10 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard;

b) all information necessary to identify the test compound and, if used, the reference compound;

c) source of the activated sludge;

d) source of the domestic sewage, if used;

e) all the experimental results (test period, adaptation period, maximum percentage removal, graph of concentrations of DOC in effluents from test and control units);

f) in the event of rejection of the test results the reasons;

g) any alteration of the standard procedure or any other circumstance that may have affected the results.

Annex A
(informative)

Examples of results of SCAS test on various compounds

Test compound (CAS number)	ϱ_o mg/l	$\varrho_t - \varrho_{Bt}$ mg/l	Percentage bioeliminated %	Test duration days
Dobanol 45 – 11 EO (68951-67-7)	11,65 [20]	0,30	97,4 (10,7)	50
Plurafac RA30 (EO/PO nonionic) (39316-51-3)	11,45 [20]	1,64	85,7 (14,1)	50
Marlon A (25155-30-0)	12,4 [18]	1,46	88,8 (9,9)	50
Aniline	16,9 [12]	0,7	95,9 (2,6)	40

NOTES

1 Values in square brackets are numbers of determinations.

2 Values in round brackets are standard deviations.