147

INTERNATIONAL STANDARD

ISO 9888

First edition
1991-10-15

Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Static test (Zahn-Wellens method)

Qualité de l'eau — Évaluation, en milieu aqueux, de la biodégradabilité aérobie des composés organiques — Essai statique (Méthode Zahn-Wellens)

Reference number
ISO 9888:1991(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 voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 9888 was prepared by Technical Committee ISO/TC 147, *Water quality*, Sub-Committee SC 5, *Biological methods*.

Annex A of this International Standard is for information only.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

Water quality — Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium — Static test (Zahn-Wellens method)

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 with unknown properties should be handled with care.

1 Scope

This International Standard specifies a method for the evaluation of the elimination and the "ultimate" biodegradability of organic compounds at a given concentration by aerobic micro-organisms.

The conditions described in this International Standard normally correspond to optimal conditions for allowing the maximum value of biodegradation to occur with the chosen inoculum in the test time.

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 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.

The method can also be used to measure the elimination and biodegradation of organic compounds in waste water (also called "test compound" in the method).

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

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 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 at 105 °C at constant weight.

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

4 Principle

Determination of the biodegradation and elimination from the aqueous phase of water soluble organic compounds by aerobic micro-organisms.

The organic compounds are the sole source of carbon and energy in the medium other than the sludge. The concentration of the compounds used is such that the initial concentration of dissolved organic carbon (DOC) is normally between 50 mg/l and 400 mg/l, or of chemical oxygen demand (COD) is between 100 mg/l et 1 000 mg/l.

NOTE 1 The chosen initial concentration of the test compound depends on its solubility and on its toxicity to the bacteria of the inoculum.

Mesurement of the DOC or COD at the beginning and end of the test (normally 28 days) and at three regular intermediate time intervals at least. Determination of the percentage removal of DOC or COD at each of these intervals. Evaluation of the biodegradability of the compound used on the basis of these data.

Specific analysis may give additional information on primary biodegradation.

5 Test environment

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

6 Reagents

Use only reagents of recognized analytical grade.

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

6.2 Test medium.

6.2.1 Components

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
Water (6.1): quantity necessary to make up to	1 000 ml

The pH of this solution should be about 7,4.

6.2.1.2 Solution (b).

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

6.2.1.3 Solution (c).

Dissolve 27,5 g of anhydrous calcium chloride ($CaCl_2$) in 1 000 ml of the water (6.1).

6.2.1.4 Solution (d).

Dissolve 0,25 g of iron(III) chloride hexyhydrate ($FeCl_3.6H_2O$) in 1 000 ml of the water (6.1). Prepare this solution just before use.

NOTE 2 It is not necessary to prepare this solution just before use if a drop of concentrated hydrochloric acid (HCl) or 0,4 g/l of ethylenediaminetetraacetic acid, disodium salt (EDTA) is added.

6.2.2 Preparation

For 1 litre of test medium, add to about 500 ml of the water (6.1)

— 10 ml of solution (a);

— 1 ml of each of the solutions (b) to (d).

Make up to 1 000 ml with the water (6.1).

6.3 Mercury chloride solution.

Dissolve 10 g of mercury chloride ($HgCl_2$) in 1 000 ml of the water (6.1).

7 Apparatus

Ordinary laboratory equipment and the following should be used.

7.1 Glass vessels, with a volume of 1 litre to 5 litres equipped with

- an agitator with a glass or metal stirrer; rotate to ensure adequate mixing.
- glass tubes of 2 mm to 4 mm inner diameter or glass frits to introduce air which shall be free from organic carbon and toxic vapours and shall be presaturated with water vapour to reduce losses by evaporation.

The glassware shall be carefully cleaned and, in particular, free from traces of organic or toxic matter.

7.2 Measuring equipment, of sufficient sensitivity for the measurement of dissolved organic carbon (see ISO 8245) or suitable for the measurement of chemical oxygen demand (see ISO 6060).

7.3 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.

7.4 Centrifuge.

7.5 pH-meter.

8 Procedure

8.1 Preparation of the test solutions

Prepare the following solutions.

8.1.1 Solution of the test compound in water (6.1), with a suitable concentration (e.g. 3 000 mg/l).

Waste water may be used directly or diluted.

8.1.2 Solution of the reference compound.

Dissolve 3 000 mg of a known water-soluble organic compound (e.g. diethyleneglycol, ethyleneglycol or aniline) in the water (6.1).

8.2 Preparation of the inoculum

Take a sample of activated sludge from the aeration tank of a biological waste water treament plant. (See notes 3 and 4).

Mix the sample well and wash the activated sludge by repeatedly adding tap water or test medium (6.2), centrifuging or settling, and decanting of the supernatant. Before use, determine the concentration of suspended solids (3.2). If necessary, concentrate the sludge by settling so that the volume of sludge added to obtain the desired concentration (see 8.3) of suspended solids is minimal.

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

NOTES

3 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.

4 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 = y % 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.).

8.3 Test

Set up at least two test vessels (7.1) (symbolized F_T) containing at least 500 ml of the test medium (6.2). Add a sufficient amount of solution of the test compound (8.1.1) or of the waste water to be tested to obtain a DOC concentration of 50 mg/l to 400 mg/l or a COD concentration of 100 mg/l to 1 000 mg/l in the final mixture.

Measure the pH value and adjust if necessary to pH 7 $\pm$ 0,5 with an inorganic acid of alkaline solution. Add activated sludge (see 8.2) in an amount corresponding to 0,2 g/l to 1,0 g/l suspended solids in the final mixture.

NOTE 5 Adjust the sludge concentration to the initial concentration of the test compound. For 400 mg/l DOC and 50 mg/l DOC, use respectively about 1 g/l and 0,2 g/l of suspended solids.

Make up with test medium (6.2) to a total volume of 1 litre to 5 litres and mix the content of the vessels. The total volume to be chosen is dependent on the number of samples to be taken for DOC or COD de-

termination and the volumes necessary for the analytical procedure.

Set up at least one blank vessel (7.1) (symbolized F_B) to operate in parallel with each test series. F_B contains only activated sludge (8.2) with the same concentration of suspended solids as the test vessel. Make up the blank vessel with test medium (6.2) to the same total volume as that contained in the test vessel.

In order to check the activity of the inoculum, set up at least one control vessel (7.1) (symbolized F_C) to operate in parallel with each test series. Treat the control in the same way as the test compound but use the solution of the reference compound (8.1.2).

If the test compound can be eliminated by abiotic processes, especially by air stripping, prepare a vessel for abiotic elimination control (symbolized F_S). Mix the test medium (6.2) and the test compound (8.1.1) as for vessel F_T. Do not add any inoculum. However, add a toxic substance of a concentration which is suitable to prevent microbial activity. This could be, for example, 10 ml of the mercury II chloride solution (6.3) per litre.

To start the test, agitate the vessels (e.g. using stirrers) and aerate. Ensure that the sludge is well aerated and does not settle out.

During the test, maintain the vessels in a stirred state and at a temperature of 20 °C to 25 °C. Check the pH value at regular intervals (e.g. if a sample for DOC/COD analysis is taken) and adjust to pH 7,0 ± 0,5 if necessary.

In order to compensate for water losses by evaporation, check the volume of the medium in the vessels before each sampling and, if necessary, make up with water (6.1) to the volume measured after the preceding sampling.

At 3 h ± 0,5 h after starting the test, at the end of the test (normally day 27 and day 28 or, if the test is stopped earlier or later, during the last 2 days of the test) and at least at three intermediate time intervals (e.g. 7 days, 14 days and 21 days), take a minimum volume for DOC or COD measurement from vessels F_T, F_B, F_C and F_S (if included).

Filter these portions through a carefully washed paper filter. If the filtrate is not clear, filter the samples through membrane filters. If filtering is difficult, centrifuge the samples instead of filtering.

Measure the DOC or COD concentrations for each vessel at least in duplicate. If primary biodegradation is to be followed, use specific analysis, e.g. U.V. spectroscopy in addition to DOC or COD measurements.

Perform all the analyses as soon as possible.

NOTE 6 When measurements have to be postponed 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 either 20 ml/l of the mercury chloride solution (6.3) or another inorganic toxic substance to prevent microbial activity and store at 4 °C. If chloride ions are added, the COD and DOC measurements at low concentrations have to be performed with special care. Instead of adding a toxic substance, store the samples at − 18 °C.

If a constant level of degradation is attained (above about 80 %) before the end of the 28-day test period, consider that the test is finished. If biodegradation occurs in the final days of the test period, prolong the test period until biodegradation has terminated. If more detailed knowledge of the behaviour of an activated sludge which has adapted to the test compound is required, the same activated sludge can be exposed again to the same test compound.

In this case, concentrate the activated sludge by settling and centrifuging and wash with test solution (see 6.2). Use the remaining sludge, which can also be mixed with fresh sludge to get the original concentration of suspended solids, to repeat the test.

9 Calculation and expression of results

9.1 Calculation

Determine the biodegradation, as a percentage of DOC or COD removal, of the test compound using the following equation:

$$D_t = \left[1 - \frac{\varrho_t - \varrho_{Bl,t}}{\varrho_x - \varrho_{Bl,x}} \right] \times 100$$

where

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

ϱ_t is the average DOC or COD, in milligrams of DOC or COD per litre, at time t in test vessel F_T;

ϱ_x is the DOC or COD, in milligrams of DOC or COD per litre, in test vessel F_T, measured after 3 h ± 0,5 h of incubation;

$\varrho_{Bl,t}$ is the average DOC or COD, in milligrams of DOC or COD per litre, in blank vessel F_B at time t;

$\varrho_{Bl,x}$ is the DOC or COD, in milligrams of DOC or COD per litre, in blank vessel F_B, measured after 3 h ± 0,5 h of incubation.

The measured value after 3 h ± 0,5 h gives information about the adsorption of the test compound onto the activated sludge. The total DOC or COD

elimination D_t of the test compound should be calculated as additional information, or in cases of significant adsorption (greater than 20 %).

For this purpose, use the same formula with ϱ_0 as the initial DOC or COD in test vessel F_T, calculated from the values, in milligrams of DOC or COD per litre, in the test solution or measured before addition of the inoculum.

NOTE 7 Depending on the test compound, it may be necessary to verify the calculated initial concentration with a measured value before the inoculum is added. If there are significant differences, both results should be indicated.

Round percentage results to the nearest whole number.

Carry out the same calculation for the reference compound in vessel F_C and, without taking into account $\varrho_{BI,t}$, for vessel F_S (if included).

9.2 Expression of results

Plot the percentage elimination D_t, and if possible $D_{t'}$, for each flask against time.

Draw an average curve if comparable results in the parallel test flasks are obtained.

From this curve, some parameters for the degradation may be determined. In particular, if sufficient data are available, the lag time and the degradation time can be calculated as described in 9.2.2 and 9.2.4 (see figure A.1).

9.2.1 Adsorption

If the result of analysis of the first sample (after 3 h ± 0,5 h) is significantly different from the theoretical value, report the amount of deficient DOC or COD as "adsorbed by the activated sludge in the static test".

9.2.2 Lag time t_1

In most of the degradation curves a so-called lag time can be observed. This is defined as the time from inoculation until the degradation percentage has increased to at least 10 % of the starting DOC or COD content.

This lag time is often highly variable and poorly reproducible.

The lag time should be noted in days.

9.2.3 Maximum level of degradation

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

9.2.4 Degradation time t_2

The degradation time t_2 is defined as the time from the end of the lag time t_1 till the time that about 90 % of the maximum level of degradation has been reached.

The degradation time should be noted in days.

9.2.5 Indication for biodegradation

If the adsorption is low (e.g. less than 20 % of the test concentration), no significant abiotic elimination has taken place (less than 20 % elimination of the test concentration in vessel F_S) and a typical biodegradation curve with lag and degradation phase is obtained, assign the measured elimination of the test compound D_t to biodegradation. If high initial adsorption takes place, the static test cannot in all cases differentiate between biological and abiotic elimination processes.

NOTE 8 To obtain definitive information on biodegradability in such a case, it is recommended to perform a respirometric test (ISO 9408) or a test which measures carbon dioxide production (ISO 9439) with the use of the adapted inoculum of the static test.

10 Validity of the test

If, in the test with one of the proposed reference compounds, the percentage degradation after 14 days is less than 70 %, the test results are invalid and the series of tests should be repeated.

11 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 or the waste water;

c) all the data (D_t and, if necessary, $D_{t'}$) obtained (in tabular form) and the degradation curves of the test compound and reference compound;

d) the amount of elimination and adsorption, expressed as a percentage, of the test compound in vessel F_T and the evaluation of biodegradation;

e) the amount of abiotic elimination in vessel F_S, if it was included;

f) the concentration of the test compound in the test and the DOC or COD content of this concentration;

g) the name of the reference compound used and the degradation percentage obtained with this compound (vessel F_C);

h) the source, the characteristics, the concentration of suspended solids and any pretreatment of the activated sludge;

i) the analytical parameter used (DOC or COD), the method of determination and/or the DOC analyser used;

j) the incubation temperature of the test;

k) in the event of rejection of the test, the reasons (see clause 10);

l) any alteration of the standard procedure, any circumstances that may have affected the results.

Annex A
(informative)

Static test — Typical elimination curve

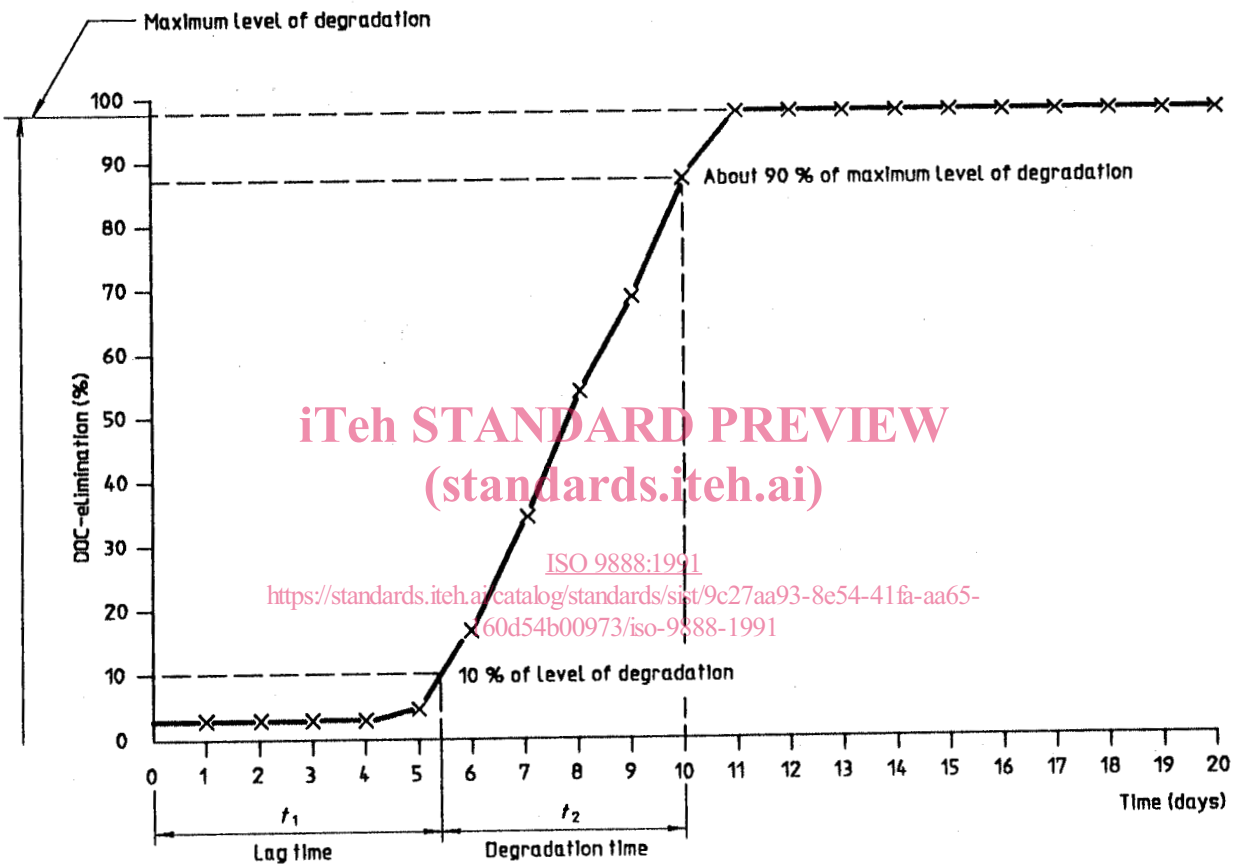

Figure A.1