INTERNATIONAL STANDARD

ISO 14592-2

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Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations —

Part 2: Continuous flow river model with attached biomass

Qualité de l'eau — Évaluation de la biodégradabilité aérobie des composés organiques présents en faibles concentrations —

Partie 2: Modèle de cours d'eau à courant continu avec biomasse associée

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

The main task of technical committees is to prepare International Standards. 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.

Attention is drawn to the possibility that some of the elements of this part of ISO 14592 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 14592-2 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

ISO 14592 consists of the following parts, under the general title *Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations*:

— *Part 1: Shake-flask batch test with surface water or surface water/sediment suspensions*

— *Part 2: Continuous flow river model with attached biomass*

This corrected version of ISO 14592-2:2002 incorporates corrections to

— the term numbers 3.1.10 and 3.1.11;

— the reference given in the last line of 5.2;

— the reference given in the second line of 6.1.3;

— the reference given in the second line of 6.1.5;

— the reference given in the first line of 8.1.1;

— the reference given in the second line of 8.1.2;

— the reference given in the second line of the second paragraph of 8.2;

— the reference given in the third line of the second paragraph of 8.3

— the reference given in the fifth line of the first paragraph of 8.4;

— the reference given in the fourth line of the second paragraph of 8.4;

— the reference given in the second line of the first paragraph of 9.1;

— the reference given in the third line of the note under 9.2.

Introduction

This International Standard consists of two parts. Part 1 describes a die-away batch test for either surface water with or without added sediment in suspension simulating either a pelagic aquatic environment or a water-to-sediment interface. Part 2 describes a continuous flow system simulating a river with biomass attached to stationary surfaces.

The test has been specifically designed to provide information on the biodegradation behaviour and kinetics for test compounds at low concentrations, i.e. sufficiently low to simulate the biodegradation kinetics expected to occur in natural environmental systems.

This method is designed to determine the primary biodegradation in a continuously operating test system simulating a river. Before conducting this test, it is necessary to have information on the biodegradability behaviour of the test compound (e.g. at usual test concentrations in standard biodegradation tests) and, if possible, on abiotic degradability or elimination (e.g. photolysis, adsorption or stripping) under conditions which are comparable to those of the river model and relevant physico-chemical data (e.g. water-solubility, adsorption coefficient K_{oc}) so as to properly plan the experiment and interpret the results.

Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations —

Part 2:
Continuous flow river model with attached biomass

WARNING AND SAFETY PRECAUTIONS — Activated sludge, sewage and effluent contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling them. Toxic and dangerous test compounds and those whose properties are unknown should be handled with care. Radiolabelled compounds, if used, should be handled respecting existing rules and legislation.

1 Scope

This part of ISO 14592 specifies a method for evaluating the biodegradability of organic test compounds by aerobic microorganisms in natural waters by means of a continuous flow river model with attached biomass.

This part of ISO 14592 is applicable to organic test compounds present in lower concentrations than those of natural carbon substrates also present in the system. Under these conditions, the test compounds serve as a secondary substrate and the kinetics for biodegradation would be expected to be first order ("non-growth" kinetics).

This part of ISO 14592 is applicable to organic test compounds, which under the conditions of the test and at the chosen test concentration, are:

— water soluble;

— quantitatively detectable with appropriate analytical methods or available in radiolabelled form;

— non-volatile from aqueous solution (e.g. Henry's law constant < 1 $Pa \cdot m^3$/mole);

— not significantly adsorbed;

— not photolyzed;

— not inhibitory to the microorganisms of the test system.

The test is not recommended for use as proof of ultimate biodegradability (mineralization) which is better assessed using other standardized tests (see ISO/TR 15462).

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this part of ISO 14592. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 14592 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO/TR 15462, *Water quality — Selection of tests for biodegradability*

3 Terms, definitions and symbols

3.1 Terms and definitions

For the purposes of this part of ISO 14592, the following terms and definitions apply.

3.1.1
ultimate aerobic biodegradation
breakdown of a chemical compound or organic matter by microorganisms, in the presence of oxygen, to carbon dioxide, water and mineral salts of any other elements present (mineralization) and the production of new biomass

NOTE Total mineralization may be different from ultimate aerobic biodegradation in that total mineralization includes secondary mineralization of biosynthesis products. The kinetics may therefore deviate from first-order kinetics in particular towards the end of the experiment. In this part of ISO 14592, primary aerobic biodegradation is determined when using substance specific analysis and total mineralization when using radiolabelled compounds.

3.1.2
primary biodegradation
structural change (transformation) of a chemical compound by microorganisms resulting in the loss of a specific property of that compound

3.1.3
biochemical oxygen demand
BOD
mass concentration of dissolved oxygen consumed under specified conditions by the aerobic biological oxidation of a chemical compound or organic matter in water

NOTE It is expressed in this case as milligrams of oxygen uptake per milligram or gram of test compound.

3.1.4
dissolved organic carbon
DOC

part of the organic carbon in a sample of water which cannot be removed by specified phase separation

NOTE Phase separation may be obtained, for example, by centrifugation of the water sample at 40 000 m/s^2 for 15 min or by membrane-filtration using membranes with pores of 0,45 µm diameter.

3.1.5
lag phase
t_{lag}
⟨continuous flow-through test system⟩ time from the start of a test until significant biodegradation (about 10 % of the maximum level) can be measured

NOTE Lag phase is expressed in days.

3.1.6
degree of biodegradation
⟨continuous flow-through test system⟩ mean biodegraded amount of a test compound, calculated from the measured concentrations in the inlet and the outlet of the system

NOTE The degree of biodegradation is determined when no further degradation can be measured and is expressed as a percentage.

3.1.7
steady state
⟨continuous flow-through test system with constant input⟩ state where the concentration of a test compound remains constant at any place and time

3.1.8
primary substrate
major carbon and energy source which is essential for growth or maintenance of microorganisms

3.1.9
secondary substrate
substrate component present at such low concentrations, that by its degradation, only insignificant amounts of carbon and energy are supplied to the competent microorganisms, as compared to the carbon and energy supplied by their degradation of primary substrates

3.1.10
degradation rate constant
k
rate constant for first order or pseudo first order kinetics which indicates the rate at which degradation processes

NOTE 1 The degradation rate constant is expressed as the inverse of days (d^{-1}).

NOTE 2 For a batch experiment, k is estimated from the initial part of the degradation curve obtained after the end of the lag phase. For a continuously operating test system, k can be estimated from a mass balance for the reactor using data collected under steady-state conditions.

3.1.11
degradation half-life
$T_{1/2}$
characteristic of the rate of a first-order reaction and corresponds to the time interval necessary for the concentration to decrease by a factor of two

NOTE 1 The degradation half-life is expressed in days (d).

NOTE 2 The degradation half-life and the degradation rate constant are related by the following equation:

$$T_{1/2} = \ln 2/k$$

NOTE 3 The degradation half-life $T_{1/2}$ for first-order reactions should not be confused with the half-life time, T_{50}, which is often used to describe the environmental behaviour of pesticides and which is simply the time to reach 50 % of total biodegradation. The half-life time T_{50} may be derived from degradation curves without making assumptions about the kinetics.

3.2 Symbols

Symbol	Description	Units
b	width of a single tray	metres (m)
c[1)]	residual molar concentration of the test compound	micromole per litre (µmol/l)
c_0	initial molar concentration of the test compound in the inlet of tray 1	micromole per litre (µmol/l)
c_n	final molar concentration of the test compound in the outlet of tray n	micromole per litre (µmol/l)
D_s	degree of biodegradation	percentage

1) In ISO 31-8-13:1992, c is defined as the symbol for “molar concentration”, expressed in moles per litre and in ISO 31-8-11.2:1992, ρ is defined as the symbol for “mass concentration”, expressed in kilograms per litre. Note that in ISO 31, "concentration" of the test compound in solution is expressed in two ways:

— “ρ” refers to the mass of the test compound per unit volume;

— “c” is specifically used to mean the number of moles of the test compound per unit volume.

d	depth of the layer of water above the glass beads	metres (m)
r_d	rate of biodegradation	micrograms per litre per day [µg/(l·d)]
k_{eff}	biodegradation rate constant	inverse days (d^{-1})
n	number of the final tray	
S	free flow cross-section of a single tray	square metres (m^2)
$T_{1/2}$	degradation half-life	days (d)
q_V	volume flow rate	cubic metres per day (m^3/d)
v_x	axial flow speed	metres per day (m/d)
x_n	distance between tray 1 and tray n	metres (m)
ρ[1)]	residual mass concentration of the test compound	micrograms per litre (µg/l)
ρ_b	biomass mass concentration	micrograms per litre (µg/l)
ρ_0	initial mass concentration of the test compound in the inlet of tray 1	micrograms per litre (µg/l)
ρ_n	final mass concentration of the test compound in the outlet of tray n	micrograms per litre (µg/l)
ρ_s	substrate mass concentration	micrograms per litre (µg/l)

4 Principle

The test system consists of one or more test units (cascades) each usually containing seven trays. Each continuously operating cascade is run with a mean hydraulic retention time of the test water of 24 h. The test water containing organic carbon is used as the major carbon and energy source (primary substrate) for the microorganisms. The organic test compound or the reference compound is added to the influent of the cascades as a secondary substrate preferably at the lowest possible concentration after sufficient biomass has been developed. The test mass concentration is dependent on the expected first order kinetics and the analytical tools (substance-specific analyses or radiolabelled test compounds) used and usually should not exceed 200 µg/l. DOC measurements are unsuitable for the determination of biodegradation as the test concentrations necessary are too high. The test water is sampled regularly and the concentration of the test or the reference compound is measured. Under steady state conditions, the difference between the inlet and outlet concentrations of the cascade is used to determine the degree of biodegradation and to plot degradation curves (see annex A). The degradation rate constant and the degradation half-life of the test and the reference compounds in this test system are calculated using the measured data derived under steady-state conditions. These data, the degradation curves and any other available information are used to evaluate the biodegradability of the test compound.

5 Reagents and media

5.1 Reagents

Use only reagents of recognized analytical grade and radiolabelled compounds of high radiochemical purity.

5.1.1 Deionized water, containing less than 1 mg/l DOC

5.1.2 Sodium hydrogen carbonate ($NaHCO_3$) or any other suitable buffer (optional), for buffering the test water. (A mass concentration of 50 mg/l has been shown to be suitable).

5.1.3 Mercury(II) chloride ($HgCl_2$) (optional), mass concentration of 10 g/l, of which 20 ml/l is added to the water sample containing the test or reference compound and used for stopping all biological activity.

5.1.4 Sodium azide (NaN_3) (optional), added to a mass concentration of 10 g/l to 20 g/l in the water sample containing the test or reference compound and used for stopping all biological activity.

5.2 Test water

Collect a sample of tap water or surface water (e.g. from ponds or rivers) and determine the hardness (6.2.11), alkalinity (6.2.6), DOC (6.2.3), ammonium nitrogen (6.2.9) and phosphorus (6.2.10). This test water should have a DOC mass concentration between 3 mg/l and 5 mg/l and mass concentrations of ammonium nitrogen (NH_4-N) and phosphorus (P) $<$ 1 mg/l each. Suitable surface water may be used directly.

In the case of tap water or if the DOC of the surface water is low, it is necessary to add organic medium to reach the required DOC concentration. Obtain organic medium from either an effluent of a municipal wastewater treatment plant or a laboratory treatment plant (for composition see for example ISO 11733[11]). Fill a storage vessel with the effluent of the secondary clarifier of this plant. Add the correct amount of effluent from the storage vessel to the cascades. Do not use effluent that may have been pre-adapted to the test compound (e.g. from an industrial wastewater treatment plant). Measure the DOC of the organic medium at appropriate intervals or with each new batch.

Usually a ratio of water to organic medium between 1:1 and 10:1 is suitable. Use DOC-free tap water for dilution if the DOC of the test water is too high. If the pH (6.2.6) of the test water is outside of the range of pH 6 to pH 9, take suitable means to maintain the pH constant during the test, preferably at a pH of (7±1). For example, water of low alkalinity could require buffering by the addition of sodium hydrogen carbonate (see 5.1.2).

6 Apparatus

6.1 Test system, consisting of at least one test cascade and the required storage vessels and dosing facilities.

Additional cascades are required if several test compounds or concentrations or the reference compound are tested in parallel.

6.1.1 Cascade, each normally consisting of seven trays (6.1.1.1) installed in the form of an aquatic staircase model.

On the short side of each tray (6.1.1.1), in the middle, downstream, is a hole fitted with a small tube (6.1.1.2) for leading the test water containing the test or reference compound from one tray to the next in the cascade. The bottom of each tray is covered with about 1 kg of glass beads (6.1.1.3) as artificial sediment serving as a support for the growth of biofilm in the test system. The hole is fixed in such a way that the depth of the water is about 1 cm above the glass beads and the volume of the water is 2 l ± 0,2 l.

This system of cascades is one type of river model for determining biodegradation kinetics, which has been shown to be suitable during test development. It is also possible to use other test systems (e.g. different size and shape of the trays, other sediments or different surface-volume relations) and other test conditions (e.g. flowrate of water, hydraulic load, illumination, inoculation). In this case, all the relevant parameters of a different test system have to be documented and taken into consideration for the test performance and the calculation of the test results.

6.1.1.1 Trays, shallow and rectangular, of about 3 l capacity, each placed at a vertical distance of about 5 cm higher than the next one, e.g. plastic photographic washing tanks with side lengths of about 45 cm × 31 cm and a water depth of about 2 cm.

On the short side of each tray, in the middle, downstream, is a hole for transferring the test water.

6.1.1.2 Tubes, fitted to each tray for leading the test water from one tray to the next.

6.1.1.3 Glass beads, 5 mm in diameter.