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.

6.1.2 Storage vessels, for storing suitable water samples and organic medium (see clause 7) for one or several days and fitted with outlets for tubes, which can be connected to the tray of the cascade.

6.1.3 Pumps, **dispensers** or **diluters**, for dosing test water, organic medium (see clause 7) and stock solution of the test or the reference compound (see 8.1.2) in such a way that the required amounts and concentrations in the system are obtained.

6.1.4 Means of mixing, in a separate mixing vessel, in the tubes or in the first trays.

6.1.5 Fluorescent tubes, in a row installed about 50 cm above the cascades and required if the system is to be artificially lighted (see clause 7).

6.2 Analytical equipment, consisting of the following:

6.2.1 Equipment suitable for specific analyses, for determining primary biodegradation, depending on the characteristics of the test and the reference compound.

6.2.2 Equipment for counting radioactivity (e.g. liquid scintillation counter).

6.2.3 Laboratory carbon analyser, for determining DOC (see e.g. ISO 8245[7]).

6.2.4 Filtration apparatus or **centrifuge**.

6.2.5 Temperature measurement apparatus.

6.2.6 pH meter.

6.2.7 Biochemical oxygen demand (BOD) measurement equipment, using e.g. ISO 5815[3].

6.2.8 Oxygen measurement apparatus, using e.g. ISO 5814[2].

6.2.9 Ammonia concentration measurement equipment, using e.g. ISO 11732[10].

6.2.10 Phosphorous measurement equipment, using e.g. ISO 6878[5].

6.2.11 Water hardness, measurement equipment.

7 Test environment

The test should normally take place at a given room temperature in an enclosure free from vapours toxic to microorganisms and without direct sunlight on the surface of the cascades. If the test is to occur at a certain temperature, the test system has to be set up in a temperature-controlled room.

Algae are present in natural aquatic environments. Therefore, they should also be present in this test system. To allow sufficient algal growth in the test system but to prevent excess growth, it is recommended that the test be performed under controlled illumination. Depending on the locality, either diffuse daylight or illumination using a white light for not more than 8 h per day may be appropriate. The intensity of the light reaching the surface of the trays should be measured and adjusted to be about 2 300 lx and the wavelength of the light should be within the range of 400 nm to 700 nm. Lamps with almost no ultraviolet light should be used to prevent photolysis. Usual daylight fluorescent tubes fastened in a distance of about 1 m to 1,5 m above the trays have shown to be suitable.

8 Procedure

8.1 Preparation of test and reference compound stock solutions

8.1.1 Test compounds

Prepare a stock solution in deionized water (5.1.1) to a suitable concentration. A suitable concentration is one that when used with the dosing apparatus, provides the desired test compound concentration throughout the test. In the case of poorly water-soluble test compounds, a stock solution is usually prepared at the level of solubility. In this case, make sure that the test concentration obtained in the test system can be adequately detected by the chosen analytical means. If this is not the case, the compound cannot be tested. Store the stock solution in storage vessels (6.1.2) of sufficient size throughout the test in a refrigerator to prevent biodegradation.

Determine the concentration of the test compound in the stock solution by specific analysis and compare it with the expected theoretical value so as to ascertain whether the analytical recovery is acceptable (normally > 80 %). Measure the concentration for each new batch of stock solution. Determine the pH of the stock solution. Extreme pH values outside a range of, for example pH 4 to pH 10, indicate that the test compound at higher concentrations may influence the pH of the test water. In this case, neutralize the stock solution [pH ($7 \pm 0{,}5$)] but ensure that the test compound does not precipitate out of solution. If this is unavoidable, even at reduced concentrations, the compound cannot be tested.

The test compound should not be toxic to the organisms in the test system, especially to bacteria that are of most importance for biodegradation processes. Inhibitory effects of the test compound on bacteria can be determined using test methods such as the respiration inhibition test with activated sludge (see ISO 8192[6]) or the cell multiplication inhibition test with a pure culture (Pseudomonas) (see ISO 10712[9]) or with activated sludge microorganisms (see ISO 15522[13]). Low test concentrations, such as the levels used in this test, should not produce effects toxic to bacteria.

8.1.2 **Reference compound** (optional)

To check the quality of the biomass and the biodegradable potential in the system and as validity criterion (see clause 10) a pre-test or a parallel test with a reference compound should be performed. Preferably use aniline, which has shown to be suitable for the test system developed for this part of ISO 14592. Follow the biodegradation by substance-specific analysis or use radiolabelled substance in the same concentration range as the test compound.

Prepare a suitable stock solution, handle and dose the reference compound as described for the test compound.

8.2 Addition of test or reference compounds to the test water

Assemble the test system and fill it with test water (5.2). Connect the storage vessels to the first trays of the respective cascade. It is recommended that the volumes in the storage vessels be measured at regular intervals to determine the exact amount of test compound or reference compound delivered to the system.

Add the stock solution of the test or reference compound to the first tray of the system so as to obtain the desired test concentration (see 8.1.1). The concentration of the test or reference compounds depends on their chemical and physical properties (e.g. water-solubility, volatility), the analytical tools used and the necessity to test in a concentration range where first order kinetics are expected. To ensure obtaining first order biodegradation kinetics, reduce the test concentration to the lowest possible level for the substance-specific analytical technique used (e.g. headspace gas chromatography or high pressure liquid chromatography). The test mass concentration usually should not exceed 200 µg/l.

Low concentrations may also be achieved if radiolabelled test compounds and detection techniques are used. It is important in such a case that the label be in the appropriate position. For example for aromatic compounds, the benzene ring itself should be labelled with ^{14}C (see annex A, ISO 14592-1:2002[12]) if information on ultimate degradation is required. In the case of primary degradation, the label should be positioned in the part of the molecule that is known to degrade first.

8.3 Operation conditions of the test system

Inoculate the test system by dosing the test water (5.2) through the system usually for a period of about 8 weeks to 12 weeks. To get as many different species of bacteria as possible, it may also be helpful to add inocula from various other sources, for example surface water, soil eluates or effluent from several wastewater treatment plants at the beginning of the water dosage. This may also result in a shorter inoculation phase. Ensure by appropriate means, for example by observation of a distinct green-brown layer or by microscopy of the tray bottom and the glass beads or by measurement of the dry mass and other suitable parameters of the biofilm, that a sufficient amount of biomass is present in the system before a degradation test is started. If necessary, extend the inoculation phase.

The hydraulic retention time in the test system is an important factor for the biodegradation rates. A suitable hydraulic retention time, for the test system (6.1) with seven trays each having a water volume of about 2 l, is obtained if about 15 l test water (5.2) are uniformly pumped over 24 h to the system. The retention time of the system may optionally also be calculated more exactly or determined by suitable methods, for example tracer studies with coloured or radiolabelled compounds, especially if the test system is different from the one described in 6.1.

The composition of the test water (5.2) should enable a continuous minimal inoculation and a minimal supply of organic material and inorganic salts. The illumination of the cascades allows the growth of algae. Make measurements at regular intervals, e.g. once per week, of temperature (6.2.5), pH value (6.2.6), DOC (6.2.3), phosphorus (6.2.10), ammonium nitrogen (6.2.9), and oxygen concentration (6.2.8) preferably in the first and last trays. Keep the DOC mass concentration between 3 mg/l and 5 mg/l, the ammonium nitrogen and phosphorus mass concentration < 1 mg/l and the pH in a range of pH (7 ± 1) to ensure the test system has river model characteristics. Add, if required, DOC-free tap water, organic medium (5.2) or a suitable buffer such as sodium hydrogen carbonate (5.1.2).

The biological quality of the test system should be known and correspond to the biological classification of the rivers for which a prediction of the degradation behaviour of the test compound shall be given. Often this prediction is required for a moderately polluted lowland river. It is helpful to use biological classification systems, but sufficient information on the biological quality may be obtained by analysis of the mentioned chemical parameters in the test water and in addition of biological parameters such as biochemical oxygen demand for five days (BOD_5) or colony-forming units (using e.g. ISO 6222[4]). The chemical parameters of a river with a suitable biological water quality are for instance $BOD_5 < 10$ mg/l, ammonium nitrogen and phosphorus < 1 mg/l and O_2 mass concentration > 4 mg/l.

8.4 Determination of biodegradability

Determine preferably the biodegradation of the reference and the test compounds in parallel cascades. Dose the test water (5.2) and add the appropriate stock solutions (8.1) at the required rates into the uppermost tray of the test system to attain the desired test concentration. Take samples from the inlet of the first tray and the outlet areas of all trays at regular intervals. It is important to take the inlet sample exactly at the place where the test water and the stock solution are added to the water of the first tray because this concentration (ρ_0, see 9.1) is needed for the calculation of the degree of biodegradation. Measure the concentration of the test and the reference compounds in the samples.

If a radiolabelled reference compound and one or more test compounds are to be tested in the same cascade one after another, allow a sufficient break where only test water (5.2) is added to avoid any carryover of traces of radioactivity. If sufficient biodegradation of the reference compound is observed (e.g. > 90 % after two weeks, see clause 10) consider the inoculation to be sufficient.

The frequency of sampling depends on the expected biodegradation of the test compound. Obtain enough valid values for the evaluation of the test results and be sure that a steady state is reached. After addition of the test compound, the test duration normally should not exceed 8 weeks.

Measure the test compound in the test samples with a suitable analytical method (6.2). In the case of radiolabelled compounds, see annex A of ISO 14592-1:2002[12]. Perform all analyses as soon as possible, if they have to be postponed, store the test samples taken for analysis in the dark at about 4 °C in full, tightly closed bottles. If test

samples for analysis have to be stored for more than 48 h, preserve them by deep-freezing or by adding a toxicant, such as mercury(II) chloride (see 5.1.3) or sodium azide (see 5.1.4).

For the ^{14}C radiotracer technique, see annex A of ISO 14592-1:2002 [12] for details. Be aware in particular, that it is normally necessary to strip $^{14}CO_2$ from the water by acidification and aeration before residual concentrations of the labelled compound can be quantified. Bear in mind that the mass transfer of CO_2 at low concentrations from water to air is slow.

9 Calculation

9.1 Degree of biodegradation

Determine the initial mass concentration of the test compound ρ_0 in the inlet of the first tray and the mass concentrations in the outlet of each tray (see 8.4). The initial test sample mass concentration may be confirmed by a calculation from the concentration and the amount of the stock solution added to the test system. Calculate the final mass concentration ρ_n of the test compound as the mean value of the measured mass concentrations in the outlet of the last tray after steady state is reached. Alternatively, the concentration of the test and reference compound may be determined as molar concentration, c, expressed in moles per litre, where c_0 and c_n are determined in the same manner.

Calculate the degree of biodegradation, D_s, expressed as a percentage, using Equation (1).

$$D_s = \frac{\rho_0 - \rho_n}{\rho_0} \times 100 \quad (1)$$

where

ρ_0 is the initial mass concentration, expressed in micrograms per litre, of the test compound;

ρ_n is the final mass concentration, expressed in micrograms per litre, of the test compound;

Use the same procedure to calculate the degree of biodegradation of the reference compound.

9.2 Assumptions and boundary conditions of the river model

Under the conditions of this test system and with the recommended concentrations of the test compound, it may be assumed that biodegradation kinetics follow a first order pattern. The continuous flow river model is regarded as a plug flow reactor with the following assumptions and boundary conditions:

— the cascade with seven trays is a flowing watercourse extending between the co-ordinates $x_1 = 0$ and $x_2 = L$, where $x_1 = 0$ identifies the inlet to the first tray and $x_2 = L$ the outlet from the final tray;

— the ideal plug flow model with complete mixing vertical to the direction of flow;

— the transportation of the primary and secondary substrate in the flowing wave and to the biofilm ensues exclusively by convection;

— the biodegradation of the test compound (secondary substrate) ensues without growth of biomass, for example by means of co-metabolism, and takes place on the surface of the biofilm;

— the biodegradation is substrate-limited but not biomass-limited, the relationship of Equation (2) applies:

$$r_d = k_{eff} \times \rho_s \quad (2)$$

where

r_d is the rate of biodegradation;

k_{eff} is the biodegradation rate constant;

ρ_s is the substrate mass concentration;

— the growth of biofilm achieved with the test compound (secondary substrate) is negligible and is therefore attributable solely to the organic medium of the test water (primary substrate);

— due to this behaviour of the primary substrate the biomass mass concentration ρ_b together with the area-related activity of the biofilm are approximately constant locally over the flow length of the cascade x, i.e.

$$\frac{d\rho_b}{dx} = 0;$$

— because of the thin layer of water of depth d above the glass beads (about 1 cm) and the low biochemical oxygen demand (BOD) of the primary and secondary substrate oxygen profiles (ρ_{O_2}) occur neither in the direction of flow or vertical to the direction of flow x, i.e.

$$\frac{d\rho_{O_2}}{dx} = 0;$$

— the void volume between the glass beads is not considered for the calculation of kinetic data because it is almost completely filled up with the biofilm;

— the test system is at steady state during the measuring period.

NOTE The river model described in this part of ISO 14592 is regarded as a plug-flow reactor. This is not entirely correct, it is actually a set of mixed tanks in series. But for the purpose of this test the assumption is acceptable. More knowledge on the hydraulic retention time (t_{hr}) of the test system may be obtained by tracer studies (see 8.3) and the use of specialized literature (see, for example, reference [19] in the Bibliography). If, for example, the average hydraulic retention time form the start of the system to the outflow of tray n ($t_{hr,n}$) has been determined the biodegradation rate constant may simply be calculated by

$$k_{eff} = -\ln\left(\frac{\rho_n}{\rho_0}\right) \times t_{hr,n}$$

9.3 Degradation rate constant

Prepare a three-dimensional plot of the measured test compound mass concentration (y-axis, µg/l) versus flow distance (x-axis, m) and versus time (z-axis, d). Estimate from the plot the duration of the lag-phase, the steady state and the section of maximum degradation of the test compound (see example in annex A). Use for the calculation of the kinetic parameters only the values obtained in the section of maximum degradation.

Calculate the axial flow speed, v_x, expressed in metres per day (m/d), according to Equation (3):

$$v_x = \frac{q_V}{S} \tag{3}$$

where

q_V is the volume flow rate, expressed in cubic metres per day (m^3/d);

S is the free flow cross-section, expressed in square metres (m^2), of a single tray.

Estimate the free flow cross-section, S, of a single tray according to Equation (4):

$$S = b \times d \tag{4}$$

where

b is the width, expressed in metres (m), of a single tray;

d is the depth, expressed in metres (m), of the layer of water above the glass beads.

Calculate the biodegradation rate constant, k_{eff}, expressed in inverse days (d^{-1}), in each tray according to Equation (5):

$$k_{eff} = -\ln\left(\frac{\rho_n}{\rho_0}\right) \times \frac{v_x}{x_n} \quad (5)$$

where

ρ_0 is the initial mass concentration, expressed in micrograms per litre, of the test compound in the inlet of tray 1;

ρ_n is the final mass concentration, expressed in micrograms per litre, of the test compound in the outlet of tray n;

x_n is the distance, expressed in metres (m), between tray 1 and tray n;

v_x axial flow speed, expressed in metres per day (m/d).

9.4 Degradation half-life

Calculate the degradation half-life, $T_{1/2}$, expressed in days (d), of the test and the reference compound according to Equation (6):

$$T_{1/2} = \frac{\ln 2}{k_{eff}} \quad (6)$$

where k_{eff} is the biodegradation rate constant, expressed in inverse days (d^{-1}).

9.5 Expression of results and indication of biodegradation

Plot the measured mass concentrations of the test or reference compound versus time in tabular form and in a three-dimensional diagram (see the example in annex A). Indicate the degree of biodegradation D_s of the test or the reference compounds, expressed as a percentage (%) (9.1), the degradation rate constant k_{eff}, expressed in inverse days (d^{-1}) (9.3) and the degradation half-life $T_{1/2}$, expressed in days (d) (9.4).

Sufficient information on the general biodegradability of the test compound should be available to perform this test. It is recommended to carry out biodegradation tests in advance based on parameters indicating clearly biological processes, such as a respirometric test or a test to measure the production of carbon dioxide. Suitable methods are specified in ISO/TR 15462. Biodegradable chemicals only can usefully be tested in a river simulation test.

For the evaluation of the test results it is important to distinguish between biological and abiotic degradation or elimination processes. For this purpose additional information such as physico-chemical data of the test compound and the results of suitable pre-tests (e.g. adsorption, stripping or photolysis) should be used. Also the form of the degradation curves gives useful information. If, for example, high elimination is observed in the first tray at the beginning of the test, either the test compound is being eliminated by adsorption (especially onto the biomass) or the compound is being very rapidly degraded. Adsorption can be proven by separate adsorption tests under similar test conditions or by making a substance-specific measurement of the test compound adsorbed onto the biomass. In the case of significantly adsorbing compounds, this test method should not be used to determine biodegradation kinetics.

The test results and especially the kinetic values for biodegradation obtained using this part of ISO 14592 are only directly comparable with data from other tests if similar test systems and test conditions have been used. It is especially not recommended to equate these values with those obtained in batch systems (e.g. ISO 14592-1) or from monitoring studies in rivers. For example, relating these data to the biomass, the surface or the volume of other test systems or to the conditions of natural rivers is without detailed knowledge of these systems and approved correlation factors not recommended.

10 Validity of the test

Consider the test to be valid if the degree of biodegradation of the reference compound is > 90 % after two weeks.

11 Test report

The test report shall contain at least the following information:

a) a reference to this part of ISO 14592, i.e. ISO 14592-2;

b) a description of the test system, for example the number and the volume of trays, and the mean hydraulic retention time;

c) all necessary information for the identification, characterization (e.g. type of labelling) and use of the test compound and of the reference compound;

d) the mass concentration of the stock solutions of the test and reference compound and the test mass concentration used in the test system;

e) all information on the test water used and on the biological quality of the test system;

f) the test temperature, the lighting and the test duration;

g) all information on analytical techniques or the method used for radiochemical measurements;

h) all measured data and calculated values in tabular form and as a diagram;

i) any alteration of the standard procedure and any observations and circumstances that may have affected the results and the reasons in the event of rejection of the test.

Annex A
(informative)

Example of expression of results

Test results

Test compound:	aniline
Analysis:	^{14}C radiolabelled test compound
Initial mass concentration ρ_0 (µg/l):	15
Degree of biodegradation D_s (%):	87
Degradation rate constant k_{eff} (d^{-1}):	9,6
Degradation half-life $T_{1/2}$ (d):	0,07

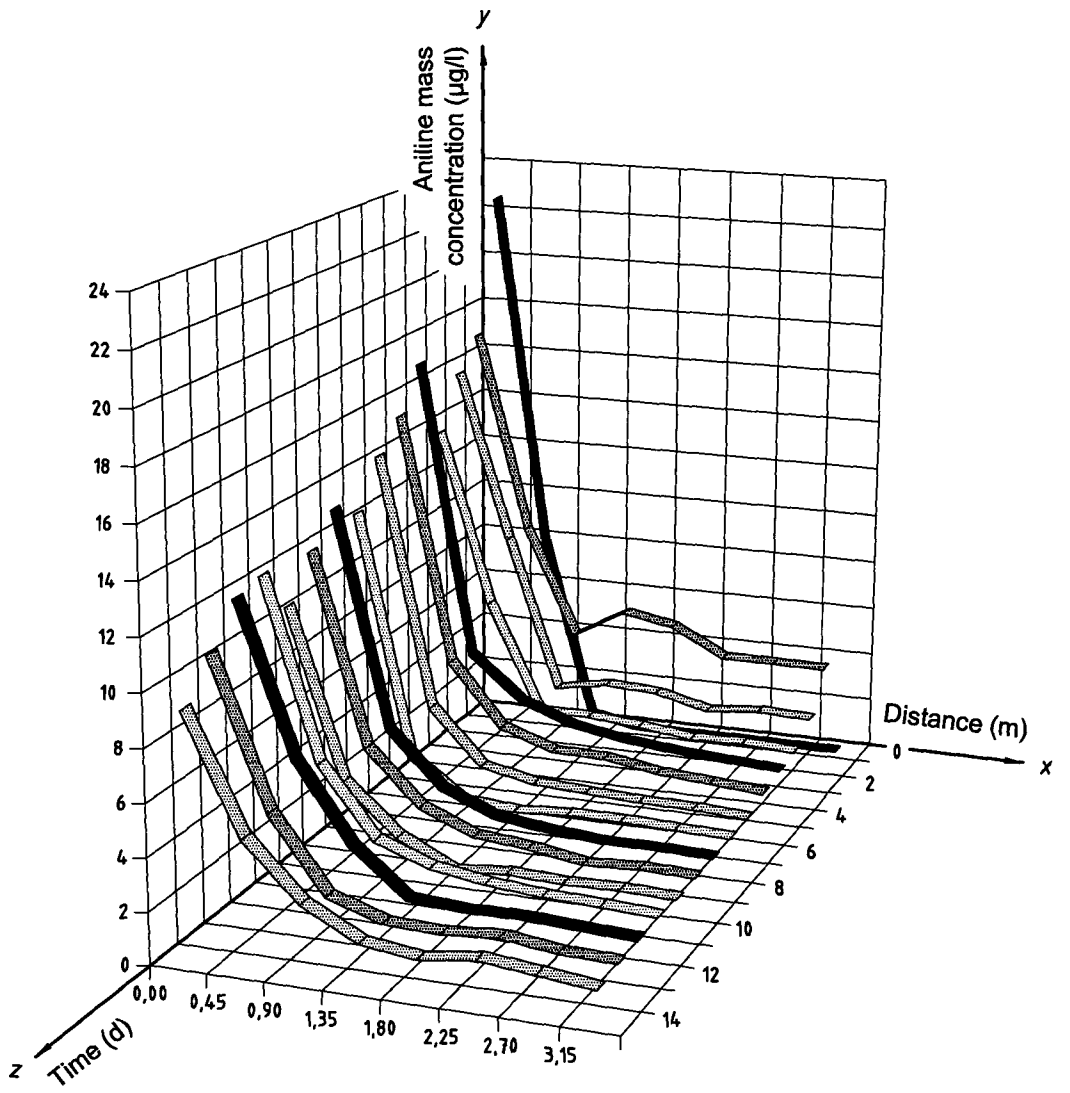

Figure A.1 — Example of degradation curves of the continuous flow river model with attached biomass

Table A.1 — Example of a table with measured mass concentrations of aniline as the test compound

Values expressed in micrograms per litre

Time days	Aniline mass concentration													
	Tray No.													
	1		2		3		4		5		6		7	
	Distance m													
	0,00	0,45	0,46	0,90	0,91	1,35	1,36	1,80	1,81	2,25	2,26	2,70	2,71	3,15
0	22,5 [b]	7,9 [b]	0,4 [b]		0,0 [b]		0,0 [b]		0,0 [b]		0,0 [b]		0,0 [b]	
1	17,1 [b]	9,0 [b]	4,6 [b]		5,8 [b]		5,4 [b]		4,3 [b]		0,4 [b]		4,2 [b]	
2	15,9 [b]	9,1 [b]	2,9 [b]		3,3 [b]		3,1 [b]		2,6 [b]		2,8 [b]		2,8 [b]	
3	13,9 [a]	6,9 [a]	2,5 [a]		2,5 [c]		2,4 [c]		2,1 [c]		2,1 [c]		2,0 [c]	
4	17,1 [a]	5,3 [a]	3,6 [a]		2,8 [c]		2,4 [c]		2,2 [c]		2,1 [c]		2,0 [c]	
5	15,5 [a]	5,6 [a]	3,1 [a]		2,4 [c]		2,4 [c]		2,0 [c]		1,9 [c]		1,7 [c]	
6	14,3 [a]	4,4 [a]	2,2 [a]		1,8 [c]		1,7 [c]		1,6 [c]		1,5 [c]		1,4 [c]	
7	12,6 [a]	3,0 [a]	1,5 [a]		1,3 [c]		1,5 [c]		1,5 [c]		1,4 [c]		1,4 [c]	
8	13,3 [a]	4,8 [a]	2,7 [a]		1,8 [c]		1,5 [c]		1,4 [c]		1,4 [c]		1,3 [c]	
9	12,2 [a]	4,7 [a]	2,7 [a]		1,8 [c]		1,6 [c]		1,3 [c]		1,4 [c]		1,3 [c]	
10	10,6 [a]	4,1 [a]	2,2 [a]		1,2 [c]		1,4 [c]		1,3 [c]		1,3 [c]		1,3 [c]	
11	12,4 [a]	5,5 [a]	2,8 [a]		2,1 [c]		1,7 [c]		1,6 [c]		1,5 [c]		1,4 [c]	
12	12,1 [a]	6,3 [a]	3,4 [a]		1,6 [c]		1,5 [c]		1,6 [c]		1,4 [c]		1,3 [c]	
13	10,7 [a]	5,1 [a]	2,2 [a]		1,6 [c]		1,5 [c]		1,6 [c]		1,4 [c]		1,3 [c]	
14	9,3 [c]	4,9 [c]	2,8 [c]		1,6 [c]		1,4 [c]		1,7 [c]		1,4 [c]		1,2 [c]	

[a] Values for steady state and the part of the curve of maximum degradation.

[b] Values for lag phase.

[c] Values for steady state, but not considered for k_{eff} (d^{-1}) calculation.

Table A.2 — Example of a table with calculated degradation rate constants

Values expressed as inverse days (d^{-1})

Time days	Degradation rate constant k_{eff} at steady state													
	Tray No.													
	1		2		3		4		5		6		7	
	Distance m													
	0	0,45	0,46	0,90	0,91	1,35	1,36	1,80	1,81	2,25	2,26	2,70	2,71	3,15
0	11,3 [b]		21,6 [b]		110,2 [b]		64,1 [b]		18,1 [b]		38,6 [b]		40,2 [b]	
1	6,9 [b]		7,1 [b]		3,9 [b]		3,1 [b]		3,0 [b]		2,5 [b]		2,1 [b]	
2	6,1 [b]		9,2 [b]		5,7 [b]		4,4 [b]		3,9 [b]		3,1 [b]		2,7 [b]	
3	7,5 [a]		9,2 [a]		6,1 [c]		4,8 [c]		4,1 [c]		3,3 [c]		2,9 [c]	
4	12,6 [a]		8,3 [a]		6,5 [c]		5,3 [c]		4,4 [c]		3,8 [c]		3,3 [c]	
5	10,9 [a]		8,6 [a]		6,7 [c]		5,0 [c]		4,4 [c]		3,8 [c]		3,4 [c]	
6	12,8 [a]		10,1 [a]		7,5 [c]		5,8 [c]		4,8 [c]		4,0 [c]		3,6 [c]	
7	15,4 [a]		11,5 [a]		8,0 [c]		5,7 [c]		4,6 [c]		3,9 [c]		3,4 [c]	
8	11,0 [a]		8,6 [a]		7,1 [c]		5,8 [c]		4,9 [c]		4,1 [c]		3,5 [c]	
9	10,1 [a]		8,2 [a]		6,8 [c]		5,4 [c]		4,9 [c]		3,9 [c]		3,4 [c]	
10	10,2 [a]		8,4 [a]		7,9 [c]		5,5 [c]		4,5 [c]		3,7 [c]		3,3 [c]	
11	8,8 [a]		7,9 [a]		6,4 [c]		5,3 [c]		4,4 [c]		3,8 [c]		3,3 [c]	
12	7,0 [a]		6,9 [a]		7,2 [c]		5,6 [c]		4,4 [c]		3,8 [c]		3,4 [c]	
13	8,0 [a]		8,4 [a]		6,9 [c]		5,3 [c]		4,0 [c]		3,7 [c]		3,2 [c]	
14	16,5 [c]		6,5 [c]		6,2 [c]		5,2 [c]		3,7 [c]		3,3 [c]		3,1 [c]	
	10,4 [a]		8,7 [a]											
	9,6 [a]				**Mean of degradation rate constant k_{eff} (d^{-1}) of trays 1 and 2**									
	0,07 [a]		0,08 [a]											
	0,07 [a]				**Mean of half-life $t_{1/2}$(d) of trays 1 and 2**									

[a] Values for steady state and the part of the curve of maximum degradation.

[b] Values for lag phase.

[c] Values for steady state, but not considered for k_{eff} (d^{-1}) calculation.

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