TECHNICAL REPORT

ISO/TR 15462

Second edition
2006-05-15

Water quality — Selection of tests for biodegradability

Qualité de l'eau — Sélection d'essais de biodégradabilité

Reference number
ISO/TR 15462:2006(E)

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Published in Switzerland

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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 2.

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.

In exceptional circumstances, when a technical committee has collected data of a different kind from that which is normally published as an International Standard ("state of the art", for example), it may decide by a simple majority vote of its participating members to publish a Technical Report. A Technical Report is entirely informative in nature and does not have to be reviewed until the data it provides are considered to be no longer valid or useful.

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

ISO/TR 15462 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This second edition cancels and replaces the first edition (ISO/TR 15462:1997), which has been technically revised.

Introduction

The biodegradation of substances and wastewater ingredients depends not only on the molecular structures of the test material, but also on important additional factors, such as the

— aquatic or terrestrial test environments;

— aerobic or anaerobic test conditions;

— source and concentration of the microorganisms of the inoculum;

— acclimatization and adaptation of the inoculum;

— concentration of the test material;

— presence of other organic substrate;

— possible toxic effects of the test material under the test conditions;

— physical and chemical properties and bioavailability of the test material (e.g. volatility, water solubility, adsorption on surfaces);

— physical and chemical properties of the test system (e.g. volume of test mixture and test vessels, CO_2 removal and oxygen concentration, temperature);

— test conditions (e.g. mixing, shaking, mode of aeration, batch or dynamic, closed or open test vessels);

— test duration;

— analytical parameters used (sum parameters, such as DOC, BOD, CO_2 or substance specific analysis).

As so many factors can influence the test results, it is not possible to define a “true” or “reference” method. The reproducibility of the test results using different methods or conditions or even using identical test methods can be low and differing test results can be obtained. Usually, a test material, which is either easily or poorly biodegradable, will produce similar test results in replicates and on repetition. Substances, which are partly or moderately biodegradable and need special consortia of bacteria or long adaptation periods, will often produce disparate results.

The biodegradation tests listed in this Technical Report are designed to determine the biodegradability of chemical substances or wastewaters under standardized conditions. The test results are required to predict the biodegradation behaviour of the test materials in natural or technical aquatic environments, for example, in rivers, lakes, ponds, sea, wastewater treatment plants, digesters. To improve their predictive value, the test methods should simulate, to a certain degree, such environments. As the conditions in these environments are often very different, sometimes even diametrically opposed, the standard methods reflect these differences. Therefore, it is necessary to provide a sufficient number of different standardized test methods to allow the choice of the best one for a specific purpose.

Water quality — Selection of tests for biodegradability

1 Scope

This Technical Report gives an overview of biodegradation tests for the aquatic environment standardized by ISO and provides recommendations on their use. In Annex A, the biodegradation guidelines for the aquatic medium of the OECD are included, because these methods are sometimes identical to ISO standards or are useful supplements. In addition, inhibitory tests with bacteria and mixed bacterial inocula are included in this Technical Report because a possible toxicity on the inoculum is important information for the choice and performance of biodegradation tests. It is very helpful to determine bacteria toxicity in advance using the same inoculum as the planned biodegradation test before starting biodegradation testing.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
activated sludge
biomass and inert matter produced in the aerobic treatment of wastewater by the growth of bacteria and other microorganisms in the presence of dissolved oxygen

2.2
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 For the purpose of this Technical Report, it is expressed as milligrams of oxygen uptake per milligram or gram of test compound.

2.3
biodegradation phase
time from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached

NOTE It is expressed in days.

2.4
biogas
carbon dioxide and methane produced by anaerobic bacteria

2.5
chemical oxygen demand
COD
mass concentration of oxygen equivalent to the amount of a specified oxidant consumed by a chemical compound or organic matter when a water sample is treated with that oxidant under defined conditions

NOTE It is expressed as milligrams oxygen uptake per milligram or gram test compound.

2.6
concentration of suspended solids of an activated sludge
amount of solids obtained by filtration or centrifugation at known conditions of a known volume of activated sludge and drying at about 105 °C to constant mass

NOTE Mixed liquor suspended solids is also often used.

2.7
degree of adsorption on activated sludge
percentage of a test compound eliminated by any processes but biodegradation under the conditions of a specific aqueous batch test with activated sludge, determined by comparing the concentration at the beginning with that at the end of the test

2.8
digested sludge
mixture of the settled phases of sewage and activated sludge, which have been incubated in an anaerobic digester at about 35 °C to reduce biomass and odour problems and to improve the dewaterability of the sludge, and which consists of a consortium of anaerobic fermentative and methanogenic bacteria producing carbon dioxide and methane

2.9
dissolved inorganic carbon
DIC
part of the inorganic carbon in 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.

2.10
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 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.

2.11
lag phase
time from the start of a test until adaptation and/or selection of the degrading microorganisms are achieved and the biodegradation degree of a chemical compound or organic matter has increased to about 10 % of the maximum level of biodegradation

NOTE It is expressed in days.

2.12
maximum level of biodegradation
maximum biodegradation degree of a chemical compound or organic matter in a test above which no further biodegradation takes place during the test

NOTE It is expressed in percent.

2.13
mixed liquor suspended solids
MLSS
concentration of solids, expressed in a specified dried form, in the mixed liquor

[ISO 6107-3:1993, 48]

2.14
plateau phase
time from the end of the biodegradation phase to the end of the test

NOTE It is expressed in days.

2.15
pre-conditioning
pre-incubation of an inoculum under the conditions of the test in the absence of the chemical compound and/or organic matter, with the aim of improving the performance of the test by acclimatisation of the microorganisms to the test conditions

2.16
pre-exposure
pre-incubation of an inoculum in the presence of a chemical compound and/or organic matter, with the aim of enhancing the ability of this inoculum to biodegrade the test material by adaptation and selection of the microorganisms

2.17
primary anaerobic biodegradation
level of degradation achieved when a test compound undergoes any structural change, other than complete mineralization, as a result of anaerobic microbial action

2.18
primary biodegradation
structural change (transformation) of a chemical compound by microorganisms resulting in the loss of a specific property

2.19
theoretical oxygen demand
ThOD
theoretical maximum amount of oxygen required to oxidize a chemical compound completely, calculated from the molecular formula

NOTE In this case, it is expressed as milligrams oxygen uptake per milligram or gram test compound.

2.20
total organic carbon
TOC
all carbon present in organic matter which is dissolved and suspended in the water

2.21
ultimate aerobic biodegradation
breakdown of a chemical compound or of 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

2.22
total inorganic carbon
TIC
all that inorganic carbon in the water deriving from carbon dioxide and carbonate

2.23
theoretical amount of formed carbon dioxide
$ThCO_2$
maximum amount of carbon dioxide formed after oxidizing a chemical compound completely, calculated from the molecular formula

NOTE It is expressed in milligrams of carbon dioxide per milligram or gram of test compound.

2.24
theoretical amount of inorganic carbon
ThIC
maximum amount of inorganic carbon formed after oxidizing a chemical compound completely, calculated stoichiometrically from the molecular formula

NOTE It is expressed in milligrams of carbon per milligram or gram of test compound.

2.25
ultimate anaerobic biodegradation
level of degradation achieved when a test compound is utilized by anaerobic microorganisms resulting in the production of carbon dioxide, methane, mineral salts, and new microbial cellular constituents (biomass)

3 Evaluations and recommendations

3.1 Biodegradation test methods

The test methods for aerobic biodegradability are not of equal potential, largely because of the different microbial densities, the concentrations of the test substances and the test durations used. ISO 7827 (DOC removal test), ISO 9439 (CO_2 evolution test), ISO 9408 (oxygen consumption test), ISO 10708 (BOD demand in a two-phased closed bottle test) and ISO 14593 (CO_2 headspace test) are of roughly equal potential. These methods are widely used standards for biodegradation studies in the aquatic environment and correspond in principle to the OECD tests for ready biodegradability. The test duration is 28 d. As inoculum, activated sludge with a concentration of not more than 30 mg/l of dry substance is often used. The test flasks of ISO 10707 (closed bottle test) have a low inoculation, are not stirred or aerated and have therefore a lower degradation potential, but they are especially useful and applicable to volatile and inhibitory test compounds. This test corresponds in principle to the known BOD tests (ISO 5815-1 and ISO 5815-2), which are, however, not recommended for substances because the conditions are very stringent, and the test time (5 d) is very short. Many substances would be classified as not biodegradable and, therefore, discriminated. The BOD_5 is the oldest aquatic biodegradation test and has shown its suitability for wastewaters since many years.

ISO 9887 (SCAS test) and ISO 9888 (Zahn-Wellens test) are tests with a high inoculum concentration. ISO 9887 uses an additional substrate (sewage) and may be extended further than the usual 28 d test duration. Hence, these tests have a high degradation potential and may be used to determine the intrinsic biodegradability of chemicals, which is, in the OECD philosophy, called “inherent biodegradability”. As these tests are open systems based on DOC determination, they cannot be applied directly to volatile or water-insoluble substances. ISO 11733 (activated sludge simulation test) is a continuous dynamic test simulating wastewater treatment plants including nitrification and denitrification techniques. ISO 14592 (shake flask test/river simulation test) is a special test for biodegradation of substances at low environmentally realistic concentrations and is suitable to determine biodegradation kinetics in the aquatic environment. ISO 14592-1 is a batch test simulating standing water bodies like lakes or ponds, ISO 14592-2 is a dynamic system and simulates flowing waters like rivers. ISO 11734 (biogas production measurement) is the only standardized aquatic test for anaerobic biodegradability and is applied independently on tests for aerobic biodegradability. Priority for application should be given to those chemicals which are not sufficiently degraded aerobically and preferentially adsorb onto activated sludge, and which enter in this way anaerobic digesters in wastewater treatment plants. ISO 16221 is a standard for marine biodegradation testing and includes five different tests with different analytical parameters which are based on established fresh water tests adapted for marine conditions.

The kinetics and the degree of degradation can be variable in different environmental compartments; therefore, results from different test methods can vary for the same test material. It is clear that differences are expected between aerobic and anaerobic biodegradation tests as well as between marine and fresh water test systems. It is also obvious that the potential for degradation increases if the test conditions are favourable. Tests with high inoculum concentrations and which allow even pre-exposure of the inoculum and permit the extension of the test duration until the plateau phase is reached, will more often show biodegradation than tests with less favourable conditions. An important parameter is an optimal test concentration which is neither too high thus avoiding the risk of inhibition nor too low thus making it difficult or impossible to determine the DOC removal, oxygen uptake or carbon dioxide production accurately and precisely. In the extreme, at very low concentrations (e.g. much less than 1 mg/l), biodegradation may not occur at all because the threshold value for a successful degradation is too low.

Chemicals which do not degrade in the rather stringent tests on ready biodegradability may, however, degrade in the powerful inherent tests. Nevertheless, they may fail to degrade in the continuously performed activated sludge simulation test. Tests on ready biodegradability may deliver contradictory results, which cannot be explained just by the different degradation power, as in the case of the rather weak, closed bottle test (ISO 10707). Experience also shows that tests, which are supposed to have the same degradation potential (such as ISO 7827, ISO 9408 and ISO 9439), and which are prepared, stirred and aerated identically and use the same inoculum concentration, may give different results. The reason is the different analytical techniques used. Consumption and hence the measurement of oxygen differs from the production and measurement of carbon dioxide which is the last step of aerobic biodegradation processes. Furthermore, a part of the carbon dioxide will be left dissolved in the test water and its determination lags behind its biological production. The full amount of carbon dioxide is measured only after acidification at the end of the test. This fault of the CO_2 production test is almost completely eliminated when the CO_2 headspace method (ISO 14593) is used. Another improvement is a new analytical development which determines the produced CO_2 by continuous conductivity measurement instead of DIC. The equivalent degrees of biodegradation will take longer than with DOC removal or oxygen uptake and could influence the fulfilling of the 10-days-window (3.5).

Even within one test method, different results may be obtained in parallel vessels if, e.g. the lag-phases vary and the test is finished before the plateau phase is reached. Experience has shown that easily degradable chemicals usually give comparable results as well as poorly degradable substances, which show their relative persistence in nearly all test systems. Chemicals of intermediate ability to biodegrade give more consistent results in tests with higher degradation potential or when pre-exposed inocula are used. Poorly biodegradable substances may, nevertheless, be well eliminated in wastewater treatment plants if they have, for example, a high adsorption potential onto activated sludge. Such adsorption processes do not always take place in parallel to the biodegradation processes. For the determination of this special elimination, ISO 18749 (batch test) is suitable.

3.2 Analytical parameters and expression of test results

In most of the standardized tests, information on ultimate biodegradability is requested, i.e. the complete breakdown of an organic substance to the inorganic catabolites CO_2 and water. To determine the complete aerobic degradation (mineralization), the sum parameters DOC, BOD or CO_2 -evolution are used. BOD and CO_2 always clearly indicate biodegradability, whereas DOC removal may be due to biodegradation as well as to abiotic elimination, such as adsorption onto activated sludge or evaporation. In the case of substances with low water solubility, the DOC cannot be determined because the test substance would be removed from solution by filtration or centrifugation, and therefore cannot be used. In anaerobic tests, the production of biogas (methane and carbon dioxide), measured by changes in pressure, is the usual analytical parameter.

For analytical reasons, sum parameters require rather high concentrations of test substance. If the test concentration should be as close as possible to natural concentrations, e.g. for kinetic reasons, they should be very low in the test. In this case, substance-specific analytical techniques are used to investigate biological transformations of a chemical, the so-called primary biodegradability. If even lower concentrations are required, radio-labelled substances are necessary. There may be also other analytical techniques which are suitable to follow biodegradation, but only the methods mentioned here are used in standardized tests.

In the DOC measurement, the initial concentration is compared with the final concentration. When using BOD and CO_2, the measurements during the test are accumulated and compared at the end with the respective theoretical values ThOD or $ThCO_2$ which are calculated from the molecular formula of the test substance. From the measured values, the percentages of biodegradation are calculated and plotted against the period of incubation to show a degradation curve. Biodegradation curves frequently have

— a marked lag phase, in which the microorganisms of the inoculum adapt to the test substance, followed by

— the actual degradation phase, in which the conversion of the test substance takes place, DOC is removed, oxygen used and CO_2 produced, and

— a plateau phase, in which biodegradation is completed and no further significant biodegradation is measured.