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**Water quality — Evaluation of ultimate  
aerobic biodegradability of organic  
compounds in aqueous medium — Carbon  
dioxide evolution test**

*Qualité de l'eau — Évaluation de la biodégradabilité aérobie ultime en  
milieu aqueux des composés organiques — Essai de dégagement de  
dioxyde de carbone*



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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.

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 9439 has been prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 9439:1990), which has been technically revised.

Annexes A to D of this International Standard are for information only.

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## Introduction

The conditions described in this International Standard do not always correspond to the optimal conditions for allowing the maximum degree of biodegradation to occur. With this test system, the microbially derived carbon dioxide (CO<sub>2</sub>) is measured in the traps through which gas exhausted from the test vessels is passed. Some of the CO<sub>2</sub> remains in the medium in the vessels as dissolved inorganic carbon (DIC), the concentration of which may increase as biodegradation proceeds. As the organic carbon approaches complete removal, the concentration of DIC gradually falls and tends to reach zero by the end of incubation. It is thus necessary to acidify the medium at the end of the test to measure the biogenically formed CO<sub>2</sub> completely. The measurement of CO<sub>2</sub> in the external traps may differ from the true production of CO<sub>2</sub> and the kinetic rate may also be lower than a rate based on DOC removal measurement. The consequence may be that the biodegradation curves based on the trapped CO<sub>2</sub> may not fully represent the true microbial kinetic rate. For alternative biodegradation methods, see ISO 15462 and in particular ISO 14593, which is based on CO<sub>2</sub> production as well but does not have this defect.

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# Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Carbon dioxide evolution test

**WARNING** — Activated sludge and sewage may contain potentially pathogenic organisms. Appropriate precautions should be taken when handling them. Toxic test compounds and those whose properties are unknown should be handled with care.

## 1 Scope

This International Standard specifies a method, by determination of carbon dioxide (CO<sub>2</sub>), for the evaluation in an aqueous medium of the ultimate biodegradability of organic compounds at a given concentration by aerobic microorganisms.

The method applies to organic compounds which are:

- a) water-soluble under the conditions of the test, in which case removal of DOC may be determined as additional information (see annex D);
- b) poorly water-soluble under the conditions of the test, in which case special measures may be necessary to achieve good dispersion of the compound (see, for example, ISO 10634);
- c) non-volatile or which have a negligible vapour pressure under the conditions of the test;

NOTE For volatile substances use for example ISO 9408 or ISO 14593.

- d) not inhibitory to the test microorganisms at the concentration chosen for the test.

NOTE The presence of inhibitory effects can be determined as specified in 8.3, or by using any other method for determining the inhibitory effect of a compound on bacteria (see, for example, ISO 8192).

## 2 Terms and definitions

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

### 2.1

#### **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

### 2.2

#### **primary biodegradation**

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

### 2.3

#### **activated sludge**

biomass produced in the aerobic treatment of wastewater by the growth of bacteria and other microorganisms in the presence of dissolved oxygen