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Soil quality — Laboratory incubation systems for measuring the mineralization of organic chemicals in soil under aerobic conditions

Qualité du sol — Systèmes d'incubation de laboratoire destinés à la mesure de la minéralisation de produits chimiques organiques dans le sol en conditions aérobies

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 14239:1997), which has been technically revised. The main changes are the inclusion of two additional incubation systems.

Introduction

This document describes incubation systems for determining the mineralization of organic compounds in soil under aerobic conditions.

Mineralization is only one of the parameters which can be used to assess the biodegradation of organic compounds in soil. If mineralization is not extensive, this does not necessarily mean that the test material is not biodegradable. Material balance studies to assess the production of metabolites, in addition to mineralization studies, provide a comprehensive assessment of biodegradation.

It is essential that this document be used in conjunction with ISO 11266, which gives general guidance on the information needed to assess the potential of an organic compound to be degraded in soil.

Depending on the aim of the study, it is feasible to use a range of incubation conditions, described below, and different methods of analysis.

Soil quality — Laboratory incubation systems for measuring the mineralization of organic chemicals in soil under aerobic conditions

WARNING — The methods in this document use several materials of a hazardous nature. Due care is necessary in their handling and disposal. In particular, all pertinent national regulations should be complied with.

1 Scope

This document specifies six suitable incubation systems for measuring the rates and extent of mineralization of organic compounds in soil by measurement of carbon dioxide (CO_2) evolution. All incubation systems are applicable to soluble or insoluble compounds but choice of system depends on the overall purposes of the study.

This document does not apply to the use of such systems for material balance studies, which are often test-substance specific.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 11266, *Soil quality — Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions*

ISO 11269-2:2012, *Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of contaminated soil on the emergence and early growth of higher plants*

ISO 11274, *Soil quality — Determination of the water-retention characteristic — Laboratory methods*

ISO 18400-206,[1] *Soil quality — Sampling — Part 206: Guidance on the collection, handling and storage of soil for the assessment of biological functional and structural endpoints in the laboratory*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at http://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Methods

4.1 General requirements

The following procedures shall be followed, whichever incubation system is selected.

1) Under preparation. Stage at the time of publication: ISO/DIS 18400-206:2017.

4.1.1 Soil collection and characterization

Soil shall be collected and handled in accordance with ISO 18400-206[1]. The soil shall be characterized in accordance with ISO 11266.

4.1.2 Test material

The test material shall be characterized in accordance with ISO 11266.

4.1.3 Incubation conditions

The following conditions shall be used unless there is a specific reason for using different conditions:—

Temperature: (20 ± 2) °C

— Pore water pressure of soil: −0,01 MPa to −0,03 MPa (measured to ±5 %) as determined in accordance with ISO 11274 (or between 40 % and 60 % max. water holding capacity (WHC measured to ±5 %) in accordance with ISO 11269-2:2012, Annex A)

— Incubation: in the dark

The incubation conditions should be reported in the test report. If they differ from those above, the reasons for changing them should also be reported in the test report.

A temperature of (20 ± 2) °C has been chosen as a standard for comparative purposes and because it gives relatively rapid results. Temperatures outside this range can be used if they are more appropriate (for example, because of local conditions, lack of cooling equipment).

4.2 Choice of incubation systems

One of the six systems described in this document shall be used:

— the flow-through system (4.3);

— the soda-lime column system (4.4);

— the biometer system (4.5);

— the radiorespirometer (4.6);

— the microradiorespirometer (4.7);

— the miniaturized respirometer (4.8).

Data on the mineralization of organic chemicals can most reliably be obtained from experiments with radiolabelled compounds.

Recoveries of CO_2 in the six systems can be measured using known quantities of unlabelled or ^{14}C-labelled calcium carbonate and adding sufficient hydrochloric acid to dissolve fully the calcium carbonate.

The main advantages and disadvantages of the systems are described in Table 1 below.

Table 1 — Advantages and disadvantages of the incubation systems

Device	Advantages	Disadvantages
flow-through system	— sufficient oxygen for long-term, aerobic degradation studies; — uses standard laboratory glassware; — allows measurement of unlabelled CO_2 (titration), $^{14}CO_2$ (scintillation counting), and/or ^{14}C-labelled volatile products (scintillation counting).	— difficulties with complete recoveries when volatile ^{14}C-compounds are under investigation; — sensitivity to leaks in the system.
soda-lime column system	— free access of oxygen for long-term degradation studies; — uses standard laboratory glassware; requires little space; — adaptable without changes for use with standing or shaken aerobic sediments, pure cultures of microorganisms, algae or plant cell cultures; — problem-free incubation under various environmental conditions; — full recoveries of applied radioactivity in short- or long-term material balance studies.	— $^{14}CO_2$ trapped in soda lime has to be released and re-adsorbed in liquid for scintillation counting; — water content of soils has to be adjusted at least once per month.
biometer system	— requires little space; — adaptable without changes for use with standing cultures of aerobic sediments; — pure cultures of microorganisms or algae; — problem-free incubation under various environmental conditions; ease of measurement of non-radioactive CO_2 (titration), $^{14}CO_2$ (scintillation counting or ^{14}C-labelled volatile products (scintillation counting).	— not ideal for long-term incubations due to lack of free access of air and reduction of partial pressure of oxygen in chamber during incubation; — requires special glassware.

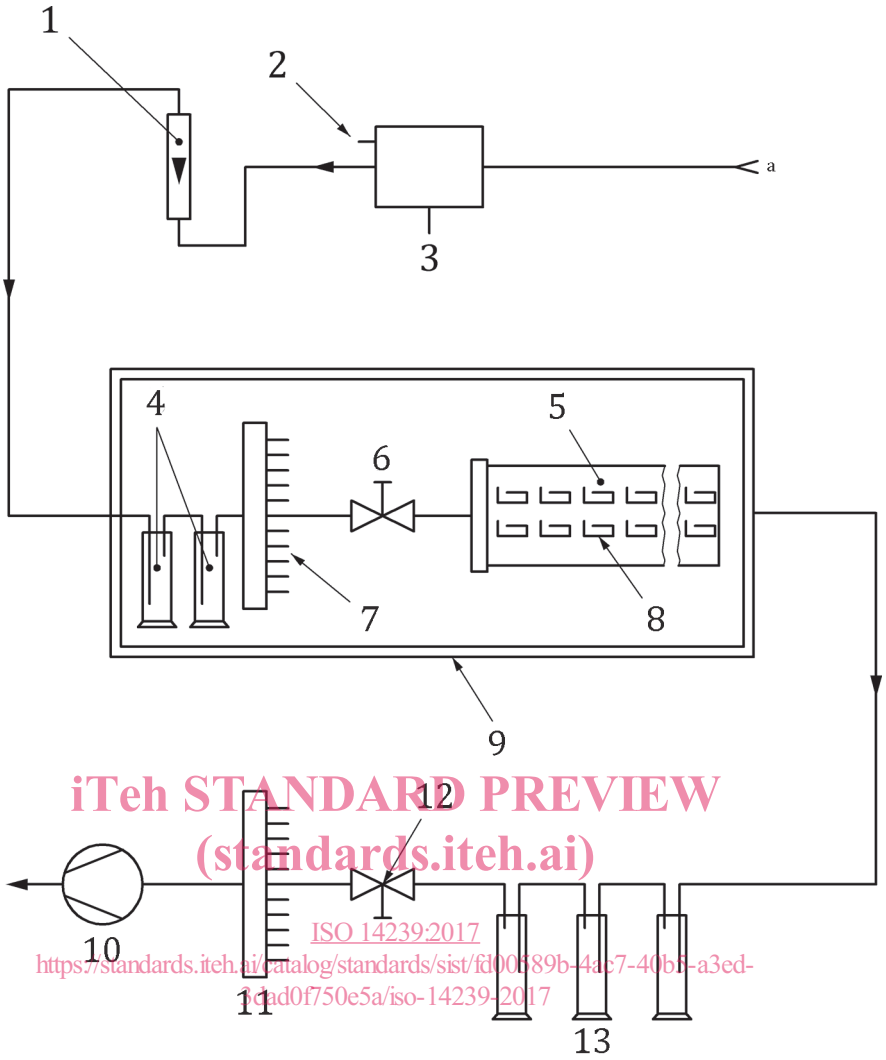
Table 1 *(continued)*

Device	Advantages	Disadvantages
radiorespirometer	— use of standard laboratory glassware; — easy to set up; — requires little space; — adaptable to standing or shaken aerobic sediments or pure cultures of microorganisms; — good recovery of applied radioactivity for mass balance.	— NaOH traps have to be regularly replaced by new ones (to avoid their saturation); — water content of soil has to be adjusted at least once every two weeks.
microradiorespirometer	— use of 24-wells microplate; — easy to set up; — requires very little space; — relatively high throughput analysis.	— not ideal for long term incubation; — not enough soils ^{14}C mass balance; — need to have from five to ten biological repeats to take into account the variability of the measure due to the relatively small amount of soil analyzed; — difficult $^{14}CO_2$ counting using phosphorimager or classical autoradiography.
miniaturized respirometer	— no need for ^{14}C-labeled radiolabeled compound; — suitable to estimate the mineralization of different kinds of ^{13}C-labelled substrates in small soil samples; — allows analysis of functional and molecular characteristics on the same micro-samples.	— need the use of micro-GC to measure $^{13}CO_2$ production and of GC-IRMS to estimate its isotopic signature; — not ideal for long term incubation because of the lack of oxygen due to the incubation of soil in an air-tight device

4.3 Flow-through system

4.3.1 Principle

This method allows determination of the dissipation and/or metabolism of non-radioactive or ^{14}C-labelled test materials in soil. CO_2 free air is drawn through the incubation vessel containing the treated soil samples. The CO_2 and organic volatiles evolved from the soil are trapped in a series of absorption traps (see Figure 1).



Key

1	flow-through monitor	8	sample
2	valve for maintaining a slight pressure	9	incubation chamber
3	reservoir	10	pump
4	wash bottle	11	collector
5	incubation unit	12	valve for flow-through regulation
6	valve	13	absorption traps
7	distribution board	a	Gas supply.

Figure 1 — Example of flow-through incubation system

4.3.2 Materials and reagents

Reagents of recognized analytical grade shall be used.

4.3.2.1 Source of CO_2-free air (e.g. obtained by passing air through an aqueous solution of strong alkali). For studies with ^{14}C-labelled compounds, CO_2 need not be removed from the air unless there is a danger of saturation of the CO_2 traps.

4.3.2.2 Ethylene glycol or ethylene glycol methyl ester, for absorption of organic volatiles.