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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.

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[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.

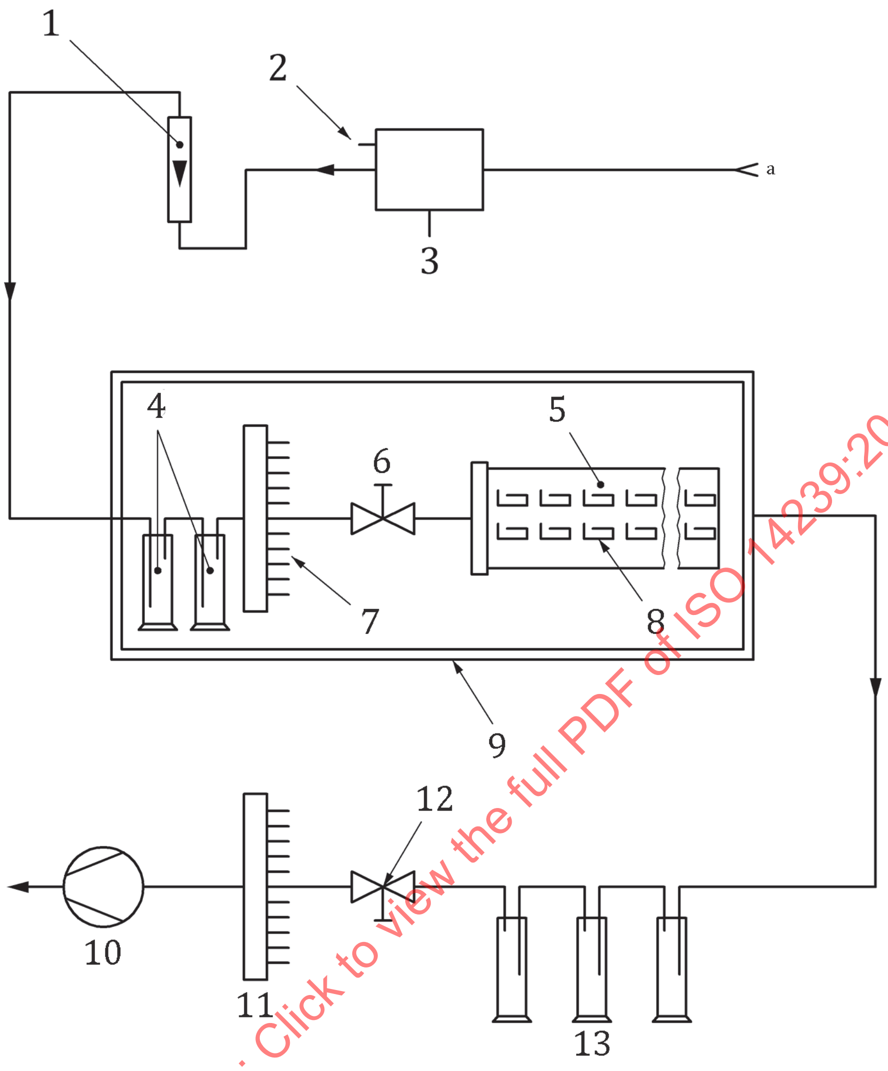
Table 1 *(continued)*

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

4.3.2.3 Polyurethane foam trap, density 16 kg/m^3 for absorption of organic volatiles.

4.3.2.4 Sulfuric acid, $c(H_2SO_4)$ = 0,5 mol/l, for absorption of alkaline volatiles.

4.3.2.5 Sodium or potassium hydroxide solution, c(KOH) [or (NaOH)] = 0,1 mol/l to 0,5 mol/l for absorption of nonradioactive CO_2; or scintillation cocktail for absorption of $^{14}CO_2$[2].

WARNING — If the scintillation cocktail is used as a trap, volatile organic amines and solvents can accumulate in toxic concentrations and there is danger of explosion. Therefore it is essential that the work area is well ventilated.

4.3.2.6 Scintillation cocktails for determination of the $^{14}CO_2$ in alkali traps[3].

4.3.3 Apparatus and glassware

4.3.3.1 Liquid scintillation counter.

4.3.3.2 Scintillation vials.

4.3.3.3 Temperature-controlled incubator or room (±2° C).

4.3.3.4 Membrane pump (capacity, approximately 2,8 m^3/h).

4.3.3.5 Flow meter.

4.3.3.6 Flow-restrictor valves.

4.3.3.7 Glass dishes for system I, e.g. moist soil (equivalent to 50 g dry mass)

- diameter 5 cm, height 5 cm – for samples equivalent to 50 g air-dried soil
- diameter 9,5 cm, height 5 cm – for samples equivalent to 300 g air-dried soil

4.3.3.8 Erlenmeyer flask (250 ml) for system II.

4.3.3.9 Gas washing bottles (100 ml) for absorption traps.

4.3.3.10 Gas washing bottles (200 ml to 500 ml) for moistening the air.

4.3.4 Procedure

Choose incubation system I or II described in 4.3.4.1 or 4.3.4.2. System I is more applicable when many samples shall be incubated in limited space, system II requires more space but is applicable for small-scale experiments.

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[2] Carbosorb (Canberra Packard) and Oxisolve (Zinsser) are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

[3] Hionic fluor and Optifluor (Canberra Packard) are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

4.3.4.1 Incubation system I

Incubation of soil samples shall take place in temperature controlled incubators or rooms (4.3.3.3.). Set up cylindrical, separately removable incubation units in the chamber (see Figure 1). The incubation units shall contain sets of soil samples in glass dishes (4.3.3.7) (normally one incubation set consists of 6 sub-samples). Each incubation unit can be aerated separately.

In order to ensure aerobic conditions, bring a constant stream of CO_2-free air (4.3.2.1) through each incubation unit using a membrane pump (4.3.3.4).

4.3.4.2 Incubation system II

Incubate the soil sample in a glass flask (e.g. Erlenmeyer flask) (4.3.3.8) in a temperature controlled room or incubator (4.3.3.3). Bring a constant stream of CO_2-free air (4.3.2.1) through the flask.

4.3.4.3 Absorption of volatile products

For both systems, moisten the CO_2-free air passing over the soils by bubbling it through two gas wash bottles (4.3.3.1) about half-filled with acidified, deionized water (approximately 1 ml of concentrated sulfuric acid per litre of water). Distribute the water saturated air to the different incubation units via valves (4.3.3.6).

Establish a constant flow of approximately 0,1 l/min through each incubation unit; use a flow meter (4.3.3.5) to measure the flow rates.

For both systems, bubble the outgoing gas through an absorption system to capture volatilized parent compound, volatile metabolite, and CO_2 for subsequent analyses. All connections shall be made of stainless steel or polytetrafluoroethylene (PTFE) tubing. Quantify any ^{14}C-labelled compounds by liquid scintillation counting, as appropriate.

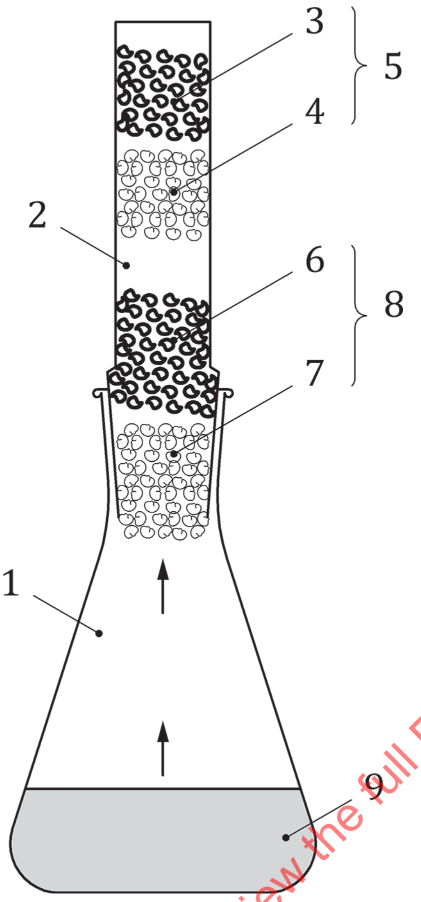
The absorption systems consist of:

- one gas washing bottle (4.3.3.9) filled with reagent for absorption of organic volatiles (4.3.2.2 or 4.3.2.3);
- one gas washing bottle (4.3.3.9) filled with reagent for absorption of alkaline volatiles (4.3.2.4) (if necessary);
- one gas washing bottle for absorption of CO_2 (4.3.2.5). If high rates of CO_2 production are expected, a second CO_2 trap is needed.

4.4 Soda-lime column system

4.4.1 Principle

This system allows determination of the dissipation and/or metabolism of ^{14}C-labelled test materials in soil. Soil treated with the ^{14}C-labelled test materials is held in a flask with a ground-glass jointed neck into which a ground-glass jointed glass column has been inserted (see Figure 2).The glass column contains a trap for volatilized ^{14}C-labelled materials and a trap for $^{14}CO_2$. Oxygen and atmospheric gases other than CO_2 move freely into and out of the flask by diffusion.



Key

1	flask with 29/32 neck
2	glass reflux column with 29/32 joint
3	soda lime
4	glass wool plug
5	trap for atmospheric CO_2
6	soda lime
7	oil- soaked glass wool plug
8	trap for $^{14}CO_2$
9	soil plus ^{14}C-supplement

Figure 2 — Incubation vessel for aerobic soil metabolism

NOTE In addition to use with soils, the system has also been used for aerobic degradation studies with standing or shaken sediments, pure cultures of microorganisms, algae and plant cell cultures.

4.4.2 Materials and reagents

Reagents of recognized analytical grade shall be used.

4.4.2.1 Granulated soda lime, grain size 1,5 mm to 3 mm, containing a saturation indicator.

4.4.2.2 Glass wool.

4.4.2.3 Paraffin oil solution in hexane (volume fraction of 2 %), for soaking glass wool plugs with oil.

4.4.2.4 Hydrochloric acid (HCl) (volume fraction of ~18 %), for dissolution of soda lime granules.

4.4.2.5 CO_2-absorbing solution; e.g. 1 mol/l NaOH (see Figure 3) or other suitable CO_2 trapping solutions[4] (see Figure 4).

4.4.2.6 Scintillation cocktail, suitable for mixing with NaOH (if applicable).

4.4.3 Apparatus and glassware

4.4.3.1 Liquid scintillation counter.

4.4.3.2 Scintillation vials.

4.4.3.3 Temperature-controlled incubator or room (±2 °C).

4.4.3.4 Nitrogen gas.

4.4.3.5 Flow meter.

4.4.3.6 Flow restrictor valves, if required for the glassware set-up for CO_2 evolution.

4.4.3.7 Erlenmeyer flask (e.g. 300 ml) with a ground-glass jointed neck (e.g. 24 or 29 standard joint).

4.4.3.8 Open-ended glass tube (reflux column) fitted with a ground-glass standard joint at one end (e.g. 24 or 29); length about 13 cm, diameter about 1,5 cm to 2 cm (see Figure 2).

4.4.3.9 Glassware and equipment for transferring the $^{14}CO_2$ bound by the soda lime (see Figure 3 or Figure 4) to an absorbent (4.4.2.5) that is compatible with the scintillation cocktail (4.4.2.6).

4.4.4 Procedure

4.4.4.1 Preparation of column for trapping ^{14}C-labelled organic materials and $^{14}CO_2$

Prepare a glass column (4.4.3.8) that serves as the extended neck of the incubation flask and which holds, from the base upward, an oil-soaked glass wool plug, (which serves as a trap for volatilized ^{14}C-labelled organic materials), and 8 g to 10 g of soda lime, (which serves as a trap for $^{14}CO_2$) (see Figure 2). Soak the glass wool plugs with oil by dipping them in an oil hexane solution (4.4.2.3) and allowing the hexane to evaporate under a fume hood. For experiments that last for more than 1 month, use an additional plug and layer of soda lime to protect the $^{14}CO_2$ trap from saturation with atmospheric CO_2, see Figure 2.

4.4.4.2 Incubation of soil treated with ^{14}C-labelled test material

Place soil treated with the ^{14}C-labelled test chemical into the Erlenmeyer flask (4.4.3.7), and close the neck of the flask with the prepared glass column (4.4.4.1). The soda lime on top of the plugs serves as the trap for $^{14}CO_2$. Soda lime can chemically bind up to 20 % of its mass in CO_2, however, if the self-indicating soda lime granules change colour, replace them with fresh soda lime. There is no danger of loss of $^{14}CO_2$ from the soda lime so it can be stored in closed containers until analysis. Incubate the soil samples in the temperature-controlled room or incubator (4.4.3.3).

4.4.4.3 Transfer of $^{14}CO_2$ from soda lime to a scintillation cocktail

Carry out this process in a fume cupboard. Pour the soda lime granules from the $^{14}CO_2$ trap into a vacuum flask and assemble the glassware as shown in Figure 3, Figure 4 or any other equivalent

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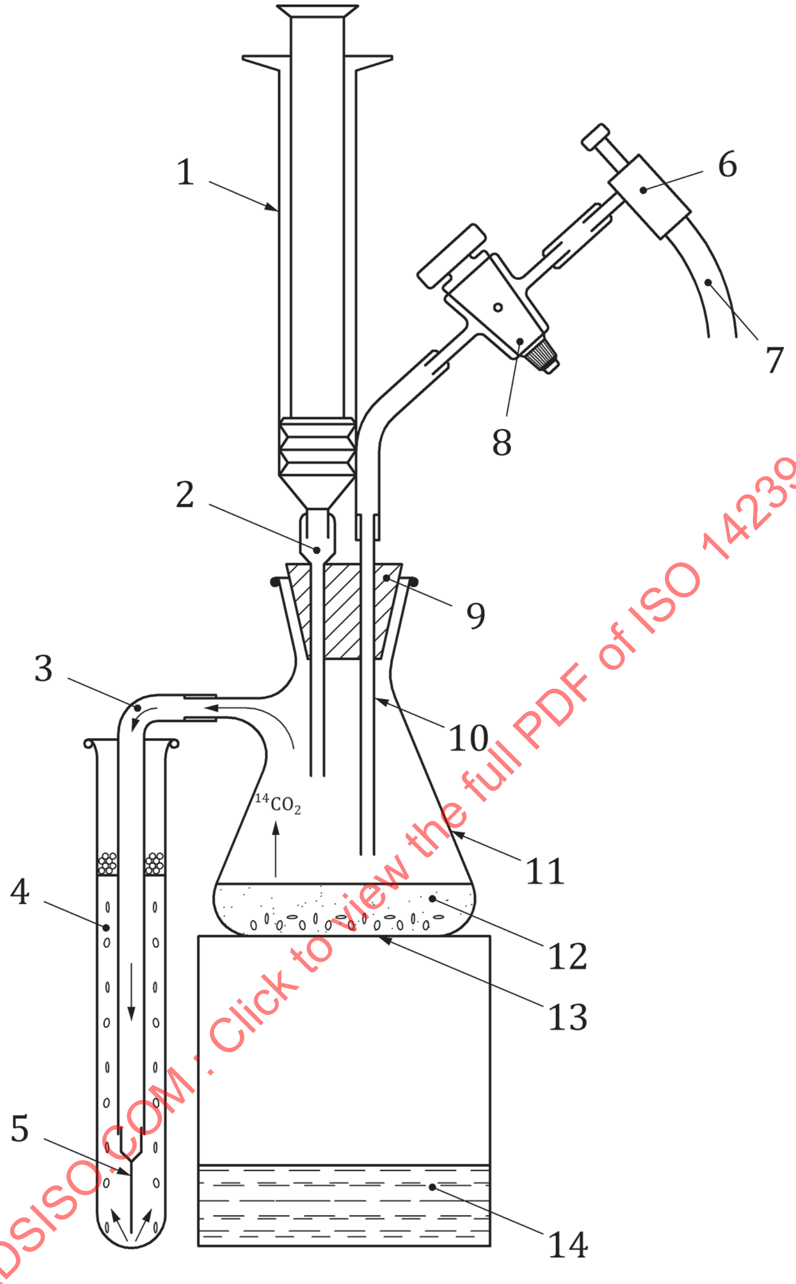
[4] An example of a suitable solution is a volume fraction of 5:4 mixture of Permafluor with Carbosorb (Canberra Packard).This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

glassware. Add 50 ml of hydrochloric acid (4.4.2.4) dropwise to the soda lime while maintaining a slow flow of nitrogen gas (4.4.3.4) (e.g. 2 l/h to 5 l/h) through the system to sweep the liberated $^{14}CO_2$ into the CO_2-absorbing solution. Maintain the temperature of the water bath at 60 °C to 70 °C.

After the soda lime has fully dissolved, sweep the system with a stream of the nitrogen gas for at least 20 min to ensure transfer of all traces of $^{14}CO_2$ from the flask into the CO_2 trapping devices. Take samples (see Figure 3) or the whole trap (see Figure 4) for liquid scintillation counting (4.4.3.1), after mixing with a scintillation cocktail (4.4.2.6) if needed.

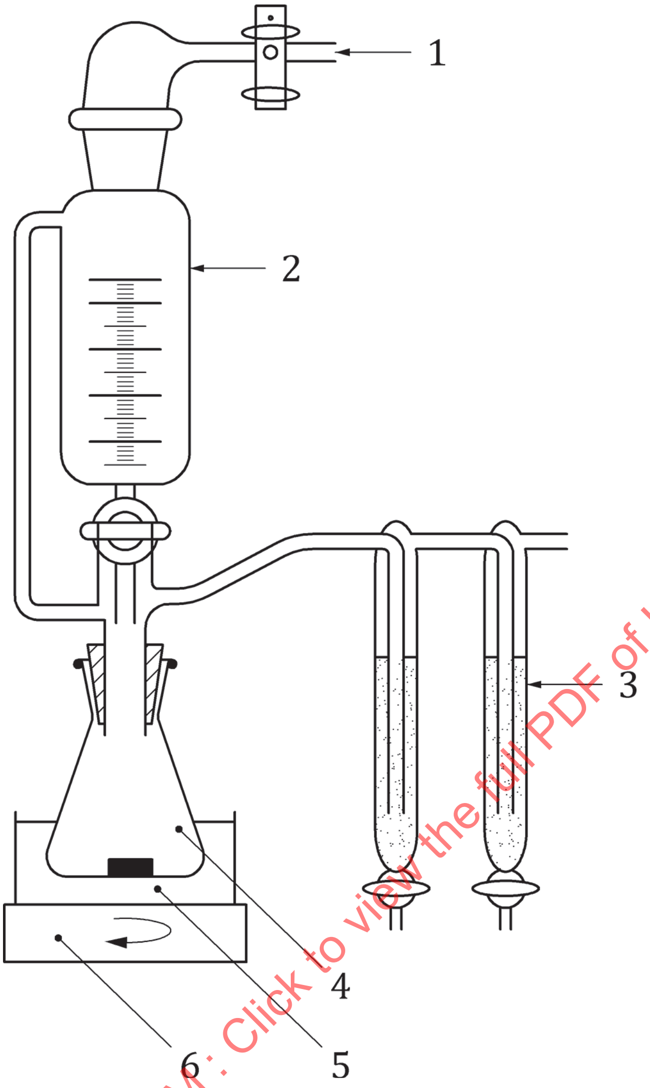
NOTE If NaOH is used to capture $^{14}CO_2$, it is possible to determine whether the radioactivity consists exclusively of $^{14}CO_2$ or a mixture including ^{14}C volatile organics. $^{14}CO_2$ can be eliminated from a sample of the NaOH by slowly acidifying to pH 1. The acidified solution can then be measured for loss of radioactivity. This operation should be carried out under a fume hood.



Key

1	plastic syringe for injection of HCl	8	one-way stopcock
2	cannula	9	rubber stopper
3	plastic tube	10	glass tube
4	1 mol/l NaOH	11	vacuum flask
5	syringe needle	12	HCl (volume fraction of 18 %)
6	needle valve	13	dissolving granules of soda lime
7	tube to N_2-flask	14	water bath (60 °C)

Figure 3 — Example of apparatus for the release of bound $^{14}CO_2$ from soda lime and its quantitative recapture in 1 mol/l NaOH



Key

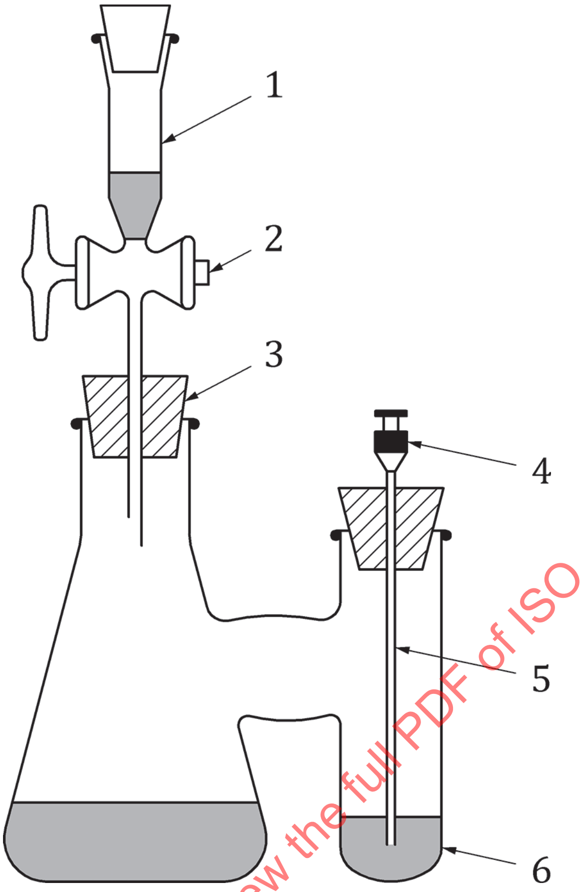
1	nitrogen	4	erlenmeyer flask (250 ml) with 10 g soda lime
2	dropping funnel, with 100 ml of 18 % hydrochloric acid	5	water bath
3	CO_2 absorbent	6	magnetic stirrer

Figure 4 — Example of apparatus for the release of bound $^{14}CO_2$ from soda lime and its quantitative recapture in scintillation liquid containing a CO_2 absorbing liquid

4.5 Biometer system

4.5.1 Principle

Soil treated with the non-radioactive or ^{14}C-labelled test material is poured into the main chamber of the biometer flask (see Figure 5). The CO_2 or $^{14}CO_2$ released during degradation of the chemical is captured in the aqueous alkali in the side-arm of the flask. Quantitative analyses for non-radioactive CO_2 trapped by the alkali are made using classical titration procedures. Analyses for $^{14}CO_2$ are made by liquid scintillation counting.



Key

1	CO_2-absorbing filter assembly (for non ^{14}C-labelled test compounds)
2	stopper and stopcock
3	gas-tight stopper
4	silicon rubber stopper
5	hypodermic needle
6	alkali trap

Figure 5 — Biometer flask

4.5.2 Materials and reagents

4.5.2.1 Potassium or sodium hydroxide solution, c(KOH) [or (NaOH)] = 1 mol/l.

4.5.2.2 For studies with non-radioactive test materials: soda lime or other CO_2-absorbing material[5)]

4.5.2.3 For studies with ^{14}C-labelled test materials: scintillation cocktails for the determination of $^{14}CO_2$ captured in the alkali trap[6)]

[5)] Ascarite (Aldrich Chemical Co. Ltd.) is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

[6)] Hionic fluor and Optifluor (Canberra Packard) are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

4.5.3 Apparatus and glassware

4.5.3.1 250 ml Erlenmeyer flask, with a side-arm made from a 50 ml round-bottom glass tube fused to it (see Figure 5).

4.5.3.2 25 ml syringes (e.g. Luer-Lock).

4.5.3.3 Syringe needle (15 gauge, 15 cm long).

4.5.3.4 Pipettes and measuring cylinders.

4.5.3.5 Temperature controlled incubator or room (±2 °C).

In addition, for studies with ^{14}C-labelled test materials:

4.5.3.6 Liquid scintillation counter.

4.5.3.7 Scintillation vials.

4.5.4 Procedure

Treat the soil sample with the test chemical, place into the main chamber of the biometer flask (4.5.3.1), and seal with a gas-tight stopper. For tests with non-radioactive test material, insert a CO_2-absorbing filter into the stopper (see Figure 5). This filter shall consist of a tubular funnel, filled with CO_2-absorbing material (4.5.2.2) and fitted with a rubber stopper at the larger end and a stopcock at the narrower end. Partially fill the side-tube of the biometer with 10 ml of alkali (4.5.2.1) and seal it. Incubate the biometer flask and its contents as described in 4.3. Remove the alkali in the side arm at intervals for analysis by titration for non-radioactive CO_2, and by liquid scintillation counting for $^{14}CO_2$.

For tests with non-radioactive test materials, avoid introduction of exogenous CO_2 by removing the alkali using a hypodermic needle (see Figure 5). Close the end of the needle exposed to the atmosphere by an air-tight rubber stopper, and cover the tip of the needle, which is immersed in alkali, with a short length of silicone tubing. During removal of alkali, remove the stopper on the CO_2-absorbing filter and open the stopcock to allow access of CO_2-free air.

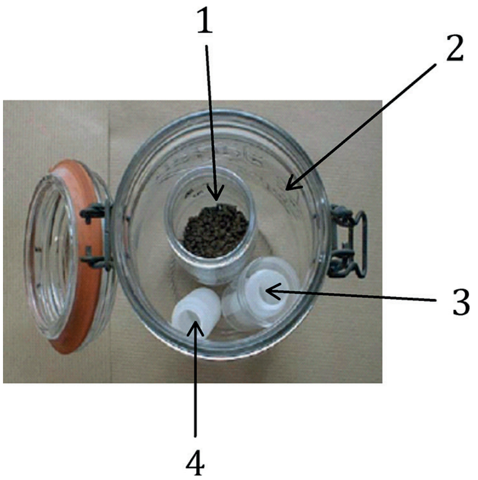
Before introducing fresh alkali into the side-arm, aerate the system, if necessary. This is done by drawing air through the system via the CO_2-absorbing filter.

NOTE To ensure that the radioactivity in the alkali used to capture the $^{14}CO_2$ consists exclusively of $^{14}CO_2$, a sample of the alkali can be carefully and slowly acidified to about pH 1 to remove $^{14}CO_2$ and then checked for the presence of residual radioactivity.

4.6 Radiorespirometer

4.6.1 Principle

Soil treated with the non-radioactive- or ^{14}C-labelled test material is placed into a glass flask placed in the radiorespirometer as well as a scintillation flask containing sodium hydroxide to trap $^{14}CO_2$ and another one containing water to saturate the radiorespirometer with humidity and keep soil moisture constant (see Figure 6). The CO_2 or $^{14}CO_2$ evolved from the chemical trapped in sodium hydroxide is quantified using classical titration procedures or liquid scintillation counting, respectively.



Key

1 soil
2 radiorespirometer
3 flask of water
4 0,2 mol/l NaOH trap

Figure 6 — Example of radiorespirometer device

4.6.2 Materials and reagents

4.6.2.1 Potassium or sodium hydroxide solution, *c*(KOH) [or NaOH] = 1 mol/l, stock solution ready to be diluted to 0,2 mol/l working solution.

4.6.2.2 For studies with ^{14}C-labelled test materials: liquid scintillation cocktails for the quantification of trapped $^{14}CO_2$ by liquid scintillation counting.

4.6.3 Apparatus, glass- and plastic-ware

4.6.3.1 Glass flask (1 l), equipped with a seal and closing tightly to avoid leakage.

4.6.3.2 Glass flask (150 ml).

4.6.3.3 Polyethylene liquid scintillation flask (20 ml).

4.6.3.4 Bottletop liquid dispenser (to deliver 5 ml of KOH or NaOH solution per trap).

4.6.3.5 Temperature controlled incubator or room (±2°C).

4.6.3.6 Liquid scintillation counter (for studies with ^{14}C-labelled test materials).

4.6.4 Procedure

Put the soil sample in the glass flask (4.6.3.2), treat it with the test chemical, and place it in the radiorespirometer flask (4.6.3.1). Add a scintillation flask (4.6.3.3) containing 0,5 ml of 0,2 mol/l NaOH and another one containing 0,5 ml of distilled water. Close tightly the radiorespirometer to ensure air-tightness and avoid any leakage. Incubate the radiorespirometer and its content under constant temperature in an incubator (4.6.3.5) and in the dark to avoid photodegradation. Remove the sodium hydroxide trap at regular intervals to monitor the evolution of CO_2 or $^{14}CO_2$ from the unlabeled or ^{14}C-labeled test material by titration or liquid scintillation counting (4.6.3.6), respectively.

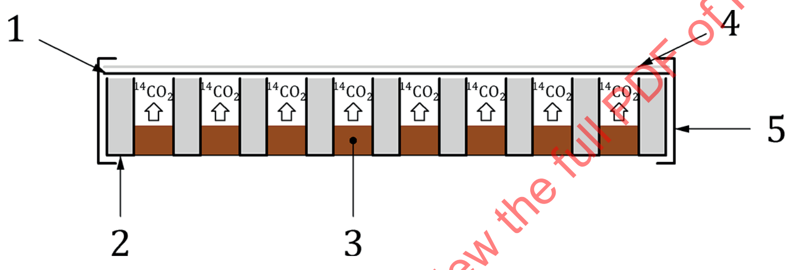
After having removed the NaOH trap and before introducing a new trap, let aerate the system to avoid placing the soil microbial community in anaerobic conditions.

4.7 Microradiorespirometer

4.7.1 Principle

This device is only allowing monitoring the evolution of $^{14}CO_2$ from the ^{14}C-labelled test material.

Soil treated with the ^{14}C-labelled test material is placed into a 24-well plate immediately covered with a filter paper impregnated with barium hydroxide and with a plastic or metal lid tightly clipped to the plate to ensure air-tightness and avoid any leakage (see Figure 7). Covered plate is placed in a zipped plastic bag containing a humidified sheet of adsorbent paper to saturate the atmosphere of the bag with humidity and keep soil moisture constant (see Figure 7). The $^{14}CO_2$ evolved from the ^{14}C-labelled test material trapped on the filter paper is quantified using photostimulated luminescence counter or classical autoradiography.



Key

1	filter paper impregnated with $Ba(OH)_2$
2	24-well plate
3	soil
4	lid
5	clip

Figure 7 — Schematic representation of the microradiorespirometer device

4.7.2 Materials and reagents

4.7.2.1 Barium hydroxide solution, $c(Ba(OH)_2)$ = 1 mol/l, stock solution ready to be diluted to 0,3 mol/l working solution.

4.7.2.2 Filter paper, cut into pieces adapted to 24-well plate (usually 11 cm by 9 cm).

4.7.2.3 Revelator and fixator for autoradiography.

4.7.2.4 Autoradiographic cassette and film (for classical autoradiography).

4.7.2.5 Autoradiographic screen and cassette (for photostimulated luminescence counter analysis).

4.7.3 Apparatus, and plastic-ware.

4.7.3.1 24-well culture plate.