



SLOVENSKI STANDARD
SIST ISO 14239:2001
01-september-2001

Kakovost tal - Laboratorijski inkubacijski sistemi za merjenje mineralizacije organskih spojin v tleh pri aerobnih pogojih

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

iTeh STANDARD PREVIEW

Qualité du sol -- Méthodes de mesure de la minéralisation de produits chimiques organiques dans le sol sous conditions aérobies, au moyen de systèmes d'incubation de laboratoire

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ICS:

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INTERNATIONAL STANDARD

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14239

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

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*Qualité du sol — Méthodes de mesure de la minéralisation de produits chimiques
organiques dans le sol sous conditions aérobies, au moyen de systèmes
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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.

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.

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International Standard ISO 14239 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Annex A forms an integral part of this International Standard. Annex B is for information only.

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Introduction

This International Standard 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 International Standard 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.

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

NOTE — Several trade names of products are given as examples of 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.

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

1 Scope

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

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

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

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ISO 10381-6:1993, *Soil quality - Sampling - Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in soil.*

ISO 11266:1994, *Soil quality - Guidance on laboratory testing for biodegradation of organic chemicals in soil.*

ISO 11274:–¹⁾, *Soil quality - Determination of the water retention characteristic - Laboratory methods.*

3 Methods

3.1 General requirements

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

3.1.1 Soil collection and characterization

Soil shall be collected and handled in accordance with ISO 10381-6. The soil shall be characterized in accordance with ISO 11266:1994, 5.1.1.

¹⁾ To be published.

3.1.2 Test material

The test material shall be characterized in accordance with ISO 11266:1994, 5.2.

3.1.3 Incubation conditions

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

Temperature:	$(20 \pm 2)^{\circ}\text{C}$
Pore water pressure of soil:	-0,01 MPa to -0,03 MPa (measured to $\pm 5\%$) as determined in accordance with ISO 11274 [or between 40% and 60% max. <i>WHC</i> (measured to $\pm 5\%$) in accordance with 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 $(20 \pm 2)^{\circ}\text{C}$ 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).

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3.2 Choice of incubation systems

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One of the three systems described in this International Standard, the flow-through system (3.3), the soda-lime column system (3.4) or the biometer system (3.5), shall be used.

Data on the mineralization of organic chemicals can most reliably be obtained from experiments with radiolabelled compounds. Where unlabelled organic chemicals are used, a number of controls are necessary and carbon dioxide evaluation data should be analysed statistically.

Recoveries of carbon dioxide (CO_2) in the three 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.

WARNING The methods in this International Standard 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.

The main advantages and disadvantages of the systems are:

a) Flow-through system:

The main advantages are: sufficient oxygen for long-term, aerobic degradation studies; uses standard laboratory glassware; allows measurement of unlabelled CO_2 (titration), $^{14}\text{CO}_2$ (scintillation counting), and/or ^{14}C -labelled volatile products (scintillation counting).

The main disadvantages are: difficulties with complete recoveries when volatile ^{14}C -compounds are under investigation; sensitivity to leaks in the system.

b) Soda lime column system:

The main advantages are: 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.

The main disadvantages are: $^{14}\text{CO}_2$ 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.

c) Biometer system

The main advantages are: 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), $^{14}\text{CO}_2$ (scintillation counting or ^{14}C -labelled volatile products (scintillation counting).

The main disadvantages are: 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.

3.3 Flow-through system

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3.3.1 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.

3.3.2 Materials and reagents

Reagents of recognized analytical grade shall be used.

3.3.2.1 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.

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

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

3.3.2.4 Sulfuric acid, $c(\text{H}_2\text{SO}_4) = 0,5 \text{ mol/l}$, for absorption of alkaline volatiles.

3.3.2.5 Sodium or potassium hydroxide solution, $c(\text{KOH})$ [or $(\text{NaOH})] = 0,1 \text{ mol/l}$ to $0,5 \text{ mol/l}$ for absorption of nonradioactive CO_2 ; or scintillation cocktail for absorption of $^{14}\text{CO}_2$ ¹⁾.

1) 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.

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.

3.3.2.6 Scintillation cocktails for determination of the $^{14}\text{CO}_2$ in alkali traps¹⁾.

3.3.3 Apparatus and glassware

3.3.3.1 Liquid scintillation counter

3.3.3.2 Scintillation vials

3.3.3.3 Temperature-controlled incubator or room ($\pm 2^\circ\text{C}$)

3.3.3.4 Membrane pump (capacity, approximately $2,8 \text{ m}^3/\text{h}$)

3.3.3.5 Flow meter

3.3.3.6 Flow-restrictor valves

3.3.3.7 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

3.3.3.8 Erlenmeyer flask (250 ml) for system II

3.3.3.9 Gas washing bottles (100 ml) for absorption traps

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

3.3.4 Procedure

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

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3.3.4.1 Incubation system I

Incubation of soil samples shall take place in temperature-controlled incubators or rooms (3.3.3.3). 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 (3.3.3.7) (normally one incubation set consists of 6 sub-samples). Each incubation unit can be aerated separately.

In order to ensure aerobic conditions, draw a constant stream of CO_2 -free air (3.3.2.1) through each incubation unit using a membrane pump (3.3.3.4).

3.3.4.2 Incubation system II

Incubate the soil sample in a glass flask (e.g. Erlenmeyer flask) (3.3.3.8) in a temperature-controlled room or incubator (3.3.3.3). Draw a constant stream of CO_2 -free air (3.3.2.1) through the flask.

1) 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.

3.3.4.3 Absorption of volatile products

For both systems, moisten the CO₂-free air passing over the soils by bubbling it through 2 gas wash bottles (3.3.3.1) 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 (3.3.3.6).

Establish a constant flow of approximately 0,1 l/min through each incubation unit; use a flow meter (3.3.3.5) to measure the flow rates.

For both systems, bubble the outgoing gas through an absorption system to capture volatilized parent compound, volatile metabolite, and CO₂ for subsequent analyses. All connections shall be made of stainless steel or polytetrafluoroethylene (PTFE) tubing. Quantify any ¹⁴C-labelled compounds by liquid scintillation counting, as appropriate.

The absorption systems consist of:

- one gas washing bottle (3.3.3.9) filled with reagent for absorption of organic volatiles (3.3.2.2 or 3.3.2.3);
- one gas washing bottle (3.3.3.9) filled with reagent for absorption of alkaline volatiles (3.3.2.4) (if necessary);
- one gas washing bottle for absorption of CO₂ (3.3.2.5). If high rates of CO₂-production are expected, a second CO₂ trap is needed.

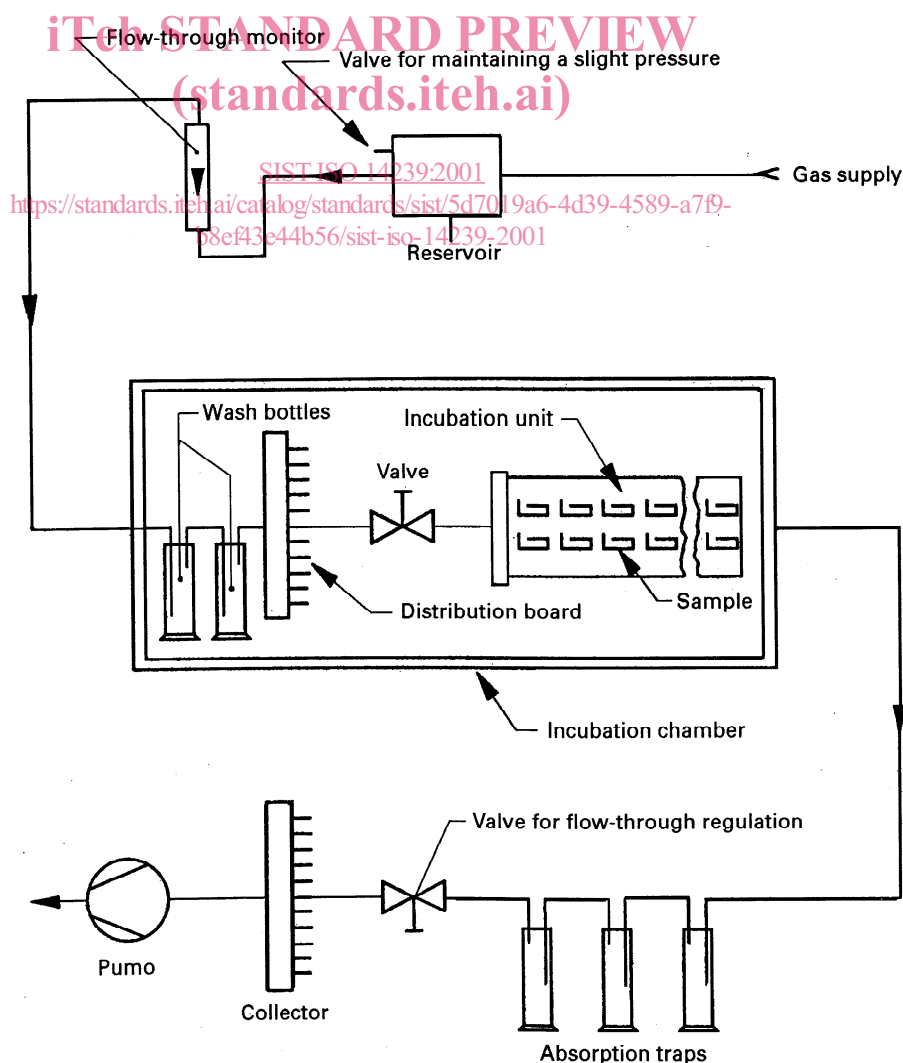


Figure 1 – Flow-through incubation system