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Soil quality — Guidance on laboratory testing for biodegradation of organic chemicals in soil under anaerobic conditions

Qualité du sol — Lignes directrices relatives aux essais en laboratoire pour la biodégradation de produits chimiques organiques dans le sol sous conditions anaérobies

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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.

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.

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

ISO 15473 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

Organic chemicals can be introduced into the soil both intentionally and accidentally, after which they can degrade as a result of biological action. For chemicals which do degrade, the rate of degradation can vary considerably, depending not only on the molecular structure of the chemical, but also on soil conditions such as temperature, water and oxygen availability which influence microbial activity. The activity of microorganisms often plays a major role in degradative processes.

ISO 11266 [3] gives general guidelines for the selection and method of tests to determine the biodegradation of organic chemicals in soils under aerobic conditions.

It is necessary to have laboratory tests available to estimate the rate and extent of biodegradation under anaerobic conditions, and to assess the capability of soil to degrade organic chemicals under these conditions.

This International Standard gives guidance for the method of tests to determine the biodegradation of organic chemicals in soils under anaerobic conditions.

Soil quality — Guidance on laboratory testing for biodegradation of organic chemicals in soil under anaerobic conditions

1 Scope

This International Standard gives guidance on the selection and method of appropriate tests for the determination of biodegradation of organic chemicals in soil samples under anaerobic conditions.

NOTE If the method is intended for tests in the framework of the registration of chemicals, an OECD Guideline on soil degradation [20] gives useful guidance on additional test requirements.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

ISO 10390:1994, *Soil quality — Determination of pH*

ISO 10694:1995, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 11260:1994, *Soil quality — Determination of effective cation exchange capacity and base saturation level using barium chloride solution*

ISO 11261:1995, *Soil quality — Determination of total nitrogen — Modified Kjeldahl method*

ISO 11271, *Soil quality — Determination of redox potential — Field method*

ISO 11274:1998, *Soil quality — Determination of the water retention characteristic — Laboratory methods*

ISO 11277:1998, *Soil quality — Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation*

ISO 14239:1997, *Soil quality — Laboratory incubation systems for measuring the mineralization of organic chemicals in soil under aerobic conditions*

3 Terms and definitions

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

3.1
biodegradation
molecular degradation of an organic substance resulting from the actions of living organisms

[ISO 11266]

3.2
primary biodegradation
the degradation of a substance to an extent sufficient to remove some characteristic property of the parent molecule. In practice this will be determined by analysis as a loss of parent compound or some specific function of the parent compound

[ISO 11266]

3.3
ultimate biodegradation
breakdown of an organic compound to carbon dioxide, methane, water, mineral salts of any other elements present, and products associated with the normal anaerobic processes of microorganisms

3.4
anaerobic transformation
reaction occurring under exclusion of oxygen (reducing conditions)

NOTE Such a reaction generally occurs when the redox potential (E_h) is less than 200 mV [17].

3.5
persistence
residence time of a chemical species in a specifically defined compartment of the environment

[ISO 11266]

3.6
DT-50
disappearance time 50
time taken for the concentration of a given compound to be reduced by 50 % of its original value

[ISO 11266]

3.7
DT-90
disappearance time 90
time taken for the concentration of a given compound to be reduced by 90 % of its original value

[ISO 11266]

3.8
bound residue
non-extractable residue
chemical species in soils originating from, for example, organic molecules that are not extracted by methods which do not significantly change the chemical nature of the residue

NOTE These non-extractable residues are considered to exclude fragments recycled through metabolic pathways leading to natural products [12].

3.9
soil
upper layer of the earth's crust composed of mineral parts, organic substances, water, air and living matter

[ISO 11074-1]

3.10
test substance
chemical substance under investigation added to the test system

3.11
saturated soil
that part of the soil which is completely saturated by water

4 Principle

Two appropriate test methods are described:

a) incubation of a test compound in the soil under methanogenic conditions and monitoring its biodegradation;

b) incubation of a test compound in the soil under water-logged conditions and monitoring its biodegradation.

The latter method simulates conditions under natural anaerobic circumstances, whereas the former method makes use of chemicals to induce a low redox potential in soil, and is the method of choice to measure the potential for degradation in soil under methanogenic conditions. In the water-logged soil method, the establishment of a low redox potential takes more time than under the methanogenic test conditions.

If the water-logged ("flooded") conditions are chosen, the soil will establish conditions depending on the nature of the soil. Such conditions can be nitrate-reducing (450 mV to 200 mV, pH 7), Fe-reducing (+ 150 mV to – 100 mV, pH 7), or sulfate-reducing (– 50 mV to – 200 mV, pH 7). If "methanogenic" conditions are chosen, the redox potential will be less than – 200 mV.

The water-logging method is more appropriate for aerobic soils that may be transiently anaerobic. The methanogenic conditions are more appropriate for organic marsh (permanently flooded) surface soils, soils of landfills and sludge-amended soils.

NOTE Organic soils containing easily degradable organic matter may eventually achieve methanogenic conditions under water-logged test conditions.

After addition of the test compound to a selected soil (5.1), biodegradation is measured under anaerobic conditions by following the production of carbon dioxide, methane and other volatiles. If such volatile compounds have to be determined, the use of ^{14}C (radioactive) substances is highly recommended. The disappearance of the test compound can also be followed by substance-specific analysis.

It is also possible to use radio-labelled compounds to determine the rate of disappearance of the test compound and the formation of metabolites and bound non-extractable residues. The metabolites can be identified using appropriate analytical methods.

5 Materials

5.1 Soil

5.1.1 Selection and sampling

If practical, soils selected for testing should come directly from the site where chemical contact is anticipated. However, if it is not possible to obtain samples owing to contamination which has already been introduced, the soil selected should have properties as close as possible to the contaminated soil.

The field history of the soil used should be considered, and recent amendments (e.g. pesticide applications) and tillage practices noted. Precise data should be provided on the sampling site, its location, its status of aeration (e.g. colour, water content, smell), the presence of plants or previous crops, the date of removal of the sample from the field, and the sampling depth.

5.1.2 Soil characteristics

A knowledge of soil characteristics is essential for full interpretation of the results of the study. It is therefore recommended that at least the following tests be performed on the selected soil:

a) physical properties:

   1) particle size analysis in accordance with ISO 11277;

   2) field water content by an appropriate method;

   3) total water-holding capacity and/or water-retention characteristics in accordance with ISO 11274;

b) chemical properties:

   1) pH of the soil in accordance with ISO 10390, or the pH in KCl or $CaCl_2$ solution;

   2) organic matter content in accordance with ISO 10694;

   3) cation exchange capacity (CEC) in accordance with ISO 11260;

   4) nitrogen content in accordance with ISO 11261;

   5) redox potential in accordance with ISO 11271;

c) biological properties:

   It may be useful to determine the microbial biomass of soil. This should be done by an appropriate method, e.g. the substrate-induced respiration method [4]. However, if anaerobic biodegradation prevails in the collected soil, the fumigation method [5] should be used.

5.2 Test substance

Ideally, substances to be tested should be pure compounds (chemical purity > 95 % mass fraction). The influence of any carriers or formulation ingredients should also be considered.

The following data on compounds are important for the interpretation of results:

— name (IUPAC);

— structure;

— relative molecular mass;

— data on purity and the chemical nature of major impurities;

— stability in water and in organic solvents;

— solubility in water;

— vapour pressure;

— octanol/water partition coefficient;

— sorption constant;

— acid dissociation constant;

— for radio-labelled chemicals:

  — the nature and position of the label;

  — specific activity;

  — radiochemical purity.

NOTE The results of studies using radio-labelled materials depend on the position of the radio-label. Therefore the labelling positions within the molecular structure need careful consideration.

5.3 Glassware and apparatus

General laboratory equipment and glassware, in particular the following.

5.3.1 Round-bottomed flask (of about 250 ml and 500 ml).

5.3.2 Ice bath.

5.3.3 Column containing reduced copper.

5.3.4 Gassing line with syringes and gassing needles.

5.3.5 Glass tubes or flasks with butyl rubber stoppers.

5.3.6 Pipettes with PVC tubes of internal diameter 0,5 mm to 1 mm.

5.3.7 Gas-tight syringes (10 ml, 20 ml, 50 ml and 100 ml).

5.3.8 Apparatus and electrodes to measure redox potentials.

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

5.3.9 Scintillation cocktails.

5.3.10 Liquid scintillation counter.

5.3.11 Scintillation vials.

5.4 Reagents

All chemicals used should be of analytical grade.

5.4.1 Oxygen-free **nitrogen, helium (pure)** or **argon**.

5.4.2 Titanium(III) chloride.

5.4.3 Sodium citrate.

5.4.4 Potassium dihydrogenphosphate (KH_2PO_4).

5.4.5 Disodium hydrogenphosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$).

5.4.6 Sodium carbonate.