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Soil quality — Contact test for solid samples using the dehydrogenase activity of *Arthrobacter globiformis*

Qualité du sol — Essai ~~de~~ contact pour ~~des~~ échantillons solides ~~en~~ utilisant ~~l'activité~~l'activité déshydrogénase de Arthrobacter globiformis

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Contents

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

This second edition cancels and replaces the first edition (ISO 18187:2016), which has been technically revised.

The main changes are ~~described below~~as follows:

— ~~The~~the scope was amended to include the possibility of applying the contact assay for testing the effect of chemicals (as detailed in Annex C~~Annex C).~~);

— ~~Further~~further details regarding other potential interfering factors (for the testing of plastic and low-density waste materials) on the performance of the contact assay were addressed~~. Adequate~~, and ~~possible~~adequate methodological alternatives ~~are~~were suggested ~~as~~ to reduce uncertainties in the assay outcome~~. The~~;

— validity criteria were updated to include the range of dehydrogenase activity inhibitions expected under three other reference substances (i.e. copper sulfate (II) pentahydrate, 3,5-dichlorophenol, zinc sulfate heptahydrate) that ~~should be~~are spiked into quartz sand~~,~~ and used as alternative positive controls in the quality testing of waste materials~~.~~;

— in Clause B.4~~— The use~~, genome sequencing is proposed to confirm the identity of *Arthrobacter globiformis*, instead of microbiological identification strips ~~for the quality check of *Arthrobacter globiformis* is~~which are no longer recommended.~~The genome sequencing is instead proposed (in Clause B.4) for confirming the identity of the bacterium.~~

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document describes the miniaturized solid contact assay with *Arthrobacter globiformis* that allows the preliminary assessment of solid material (i.e. soil and waste materials) within 6 h. The principle of the assay relies on dehydrogenase activity inhibition of an added test organism, caused by bioavailable toxic substances in soil and waste samples. This is an ecologically relevant assay as far as it uses a ubiquitous soil bacterial species with high affinity to surfaces [1][2][1] [2] which] whose dehydrogenases are involved in different biological mechanisms withstanding bacteria integrity (e.g. respiratory chains). Moreover, it has been noticed that this parameter (dehydrogenase activity inhibition) is quite sensitive to different toxic substances.[3][4][5][6][7 [3] [4] [5] [6] [7].]

Overall, this assay is non-labour-intensive, rapid, cost-effective and sensitive, providing results that improve the physical and chemical assessment of natural samples while allowing a quick indication of their biological effects.

The miniaturized solid contact assay is based on the solid contact assay established by Reference [8 [8].].

This document is also based on Reference [9 [9].].

The results of an interlaboratory trial towards the evaluation of test variability to assess different waste and soil samples, as well as chemicals, are presented in Annex AAnnex A and in Reference [10 [10].].

Soil quality — Contact test for solid samples using the dehydrogenase activity of *Arthrobacter globiformis*

1 Scope

This document specifies a rapid method for assessing solid samples in an aerobic suspension, by determining the inhibition of dehydrogenase activity of *Arthrobacter globiformis* using the redox dye resazurin.

It is applicable for assessing the effect of water-soluble and solid matter bounded non-volatile contaminants in natural samples, such as soils and waste materials. Although not the main purpose, the contact test can additionally be used for testing the effect of chemicals, as described in the Annex C~~Annex C~~.. The test yields a result within 6 h and can therefore be used for screening potentially contaminated test material.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667--15, *Water quality — Sampling — Part 15: Guidance on the preservation and handling of sludge and sediment samples*

ISO 18400-206~~:2018~~, *Soil quality — Sampling — Part 206: Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

CEN/TR 15310--1, *Characterization of waste — Sampling of waste materials — Part 1: Guidance on selection and application of criteria for sampling under various conditions*

EN 14735, *Characterization of waste — Preparation of waste samples for ecotoxicity tests*

3 Terms and definitions

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

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

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

— ~~—~~IEC Electropedia: available at https://www.electropedia.org/

3.1
contact time
exposure period of the bacteria to a suspension of solid matter

3.2
negative control
sample of a *control substrate* (3.6~~(3.6)~~) with a mixture of known solutions [(distilled water, *medium* (3.13~~(3.13)~~) B or *inoculum* (3.12~~(3.12)~~]))

Note 1 to entry: It is used to standardize the analysis.

3.3
positive control
sample of a *control substrate* (3.6~~(3.6)~~) with a mixture of known solutions ~~[~~(distilled water, *medium* (3.13~~(3.13)~~) B or *inoculum* (3.12~~(3.12)~~]~~)) and a reference substance

Note 1 to entry: It is used to check the sensitivity of the test organism.

3.4
blank A
blank, which sets the own fluorescence of the substrate after being deactivated

Note 1 to entry: Blank A is not added with bacteria.

3.5
blank B
blank, which sets the natural fluorescence of the substrate without being deactivated

Note 1 to entry: Blank B is not added with bacteria.

3.6
control substrate
reference or standard substrate used as a control and as a dilution substrate for preparing dilution/concentration series with *test substrates* (3.7~~(3.7),~~), a reference substance or a test chemical

EXAMPLE Quartz sand or LUFA standard soil type 2.2.

3.7
test substrate
natural or artificial substrate that is naturally contaminated or spiked with a test chemical

Note 1 to entry: The test substrate is the *test material* (3.8~~(3.8)~~) after being prepared for testing (e.g. sieved) and/or diluted with a *control substrate* (3.6~~(3.6).~~).

3.8
test material
original sample of soil or waste material without any changes (e.g. sieving)

3.9
dehydrogenase activity
activity of hydrogen-abstracting enzymes which are involved in many energy, redox reactions, and biosynthetic metabolic processes (e.g. the respiratory chain), which require cell integrity to be produced

Note 1 to entry: These enzymes can reduce resazurin into resorufin in the extracellular environment[2~~[2],~~].

Note 2 to entry: See Reference [11~~[11].~~].

3.10
EC*x*
effect concentration for *x* % effect
concentration (mass fraction) of a test substance or sample that causes *x* % of an effect on a given endpoint within a given exposure period when compared with a control

EXAMPLE An EC50 is a concentration estimated to cause an effect on a test ~~endpoint~~end point in 50 % of an exposed population over a defined exposure period.

Note 1 to entry: The EC*x* is expressed as a percentage of soil or waste tested per dry mass of soil mixture. When chemicals are tested, the EC*x* is expressed as mass of the test substance per dry mass of substrate, in milligrams per kilogram.

3.11
freeze-dried bacteria
bacterial culture preserved through the water removing of a frozen cell suspension by sublimation under reduced vacuum pressure

Note 1 to entry: The preserved cultures can be stored at (−20 ± 2) °C. The bacteria are active after being reconstituted with sterilized distilled water [20 min to 30 min at (6 ± 2) °C] and ready to be used in the test, see 7.3.4~~7.3.4.~~ b).

3.12
inoculum
suspension of bacteria used to inoculate a nutrient solution

3.13
medium
aqueous nutritive solution required for bacterial growth

3.14
optical density of bacterial inoculum
measurement of the attenuation of a light beam passing through a bacterial suspension at 600 nm (used to determine the cell count indirectly)

Note 1 to entry: In a bacterial test, the absorbance is usually measured as FAU (formazine attenuation units) at 600 nm (see Reference [12~~[12]~~]~~.~~).

3.15
test start
moment when the substrates, reagents and the bacterial *inoculum* (3.12~~(3.12)~~) are prepared immediately before the incubation and reaction period

Note 1 to entry: It is when preparing the *test* (3.7) and *control substrates* (3.6~~(3.6)~~) for incubation (i.e. Table 1~~Table 1~~~~,~~ day 0).

3.16
reaction time
time it takes for the enzyme to react (from the addition of the resazurin solution until the end of the kinetics period)

3.17
slope
quotient of the *relative fluorescence* (3.18~~(3.18)~~) variation along the *reaction time* (3.16~~(3.16)~~) between 15 min and 45 min

Note 1 to entry: The slope (expressed as min~~-~~$^{-1}$) results from fitting a linear regression model to the fluorescence readings over time.