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Soil quality — Determination of dehydrogenase activity in soils —

Part 2: Method using iodotetrazolium chloride (INT)

Qualité du sol — Détermination de l'activité des déshydrogénases dans les sols —
Partie 2: Méthode au chlorure de iodotétrazolium (CIT)

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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.

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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 document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 23753-2 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

ISO 23753 consists of the following parts, under the general title *Soil quality — Determination of dehydrogenase activity in soils*:

— *Part 1: Method using triphenyltetrazolium chloride (TTC)*

— *Part 2: Method using iodotetrazolium chloride (INT)*

Introduction

The soil microflora is responsible for the decomposition and conversion of organic substances, aggregation stability and the carbon, nitrogen, sulfur and phosphorus cycles. Dehydrogenases, as respiratory chain enzymes, play a major role in the energy production by organisms. They oxidize organic compounds by transferring two hydrogen atoms. Dehydrogenases are essential components of the enzyme system of microorganisms. Dehydrogenase activity can therefore be used as an indicator of biological redox systems and as a measure of microbial activity in the soil.

Soil quality — Determination of dehydrogenase activity in soils —

Part 2: Method using iodotetrazolium chloride (INT)

1 Scope

This part of ISO 23753 specifies a method for determining soil dehydrogenase activity using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT). As the INT reduction is less sensitive to O_2, the method is more reproducible than the TTC-method described in ISO 23753-1.

It is not applicable for determining the dehydrogenase activity in upper layers (L, F, H horizons) of forest humus forms with low microbial activity (e.g. mor), or in soils showing reducing properties (e.g. waterlogged soils).

2 Normative references

The following referenced documents are indispensable for the application 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 10381-6, *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, *Soil quality — Determination of pH*

ISO 11259, *Soil quality — Simplified soil description*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

3 Principle

INT solution is added to a soil sample and the mixture is incubated at 30 °C for 18 h. The iodonitrotetrazolium formazan (INTF) released is extracted with acetone (in the case of humic soil) and determined by photometry at a wavelength of 485 nm.

NOTE 1 The method is based on a modified version of the method reported in Reference [1].

NOTE 2 Acetone is used as extractant and samples are not extracted to completion.

In the case of soil having reducing characteristics (e.g. waterlogged soil), dehydrogenase activity should not be used as a measure of the biological activity in the soil [3]. Abiotic components, such as iron(II) compounds or sulfides can reduce INT.

4 Reagents and materials

4.1 Soil

Take and prepare soil samples as specified in ISO 10381-6. If samples which have been sieved in the fresh state are not analysed immediately, they may be kept for up to three months at 4 °C. Determine the dry matter content of the sample in accordance with ISO 11465.

The storage can affect the enzyme activity and hence dehydrogenase activity of samples with different storage times should not be compared.

4.2 Hydrochloric acid, $c(\text{HCl})$ = 1 mol/l.

4.3 Tris buffer solution, c = 0,1 mol/l.

Dissolve 12,11 g of tris(hydroxymethyl)aminomethane in 600 ml of distilled water and adjust the pH value to

a) 7,7 for soil having a carbonate content exceeding 2 %, or

b) 7,9 for soil having a carbonate content not exceeding 2 %

using 1 mol/l hydrochloric acid and make up to 1 000 ml with distilled water. Determine pH value in accordance with ISO 10390.

4.4 Substrate solution (INT)

Weigh 900 mg of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride into a 100 ml conical flask and wet it on a magnetic stirrer with a portion of 100 ml of tris buffer solution (4.3). Then add the remainder of the tris buffer solution and treat the mixture with an ultrasonic probe for 1 min to 2 min, so that the INT is dissolved as much as possible and a suspension is obtained. Remove portions of the suspension later while stirring to keep it homogeneous. Determine the soil texture in accordance with ISO 11259.

The solubility of INT in pH 7,7 tris buffer solution is at most 5 mg/ml. During incubation there is a slight increase due to dissolution of INT from the suspension to a maximum of 7 mg/ml. Therefore 9 mg/ml should be used.

4.5 Analytical grade acetone

4.6 INTF solutions

4.6.1 INTF stock solution

Weigh 50 mg of iodonitrotetrazolium formazan into a 100 ml-volumetric-flask; dissolve it in acetone; and make up to 100 ml.

4.6.2 INTF calibration solutions

Pipette 0 ml, 0,5 ml, 1,0 ml, 2,0 ml, 3,0 ml and 4,0 ml respectively, of stock solution into a series of 50 ml volumetric flasks, add a little acetone (use the solvent which is also being used as extractant in the samples), add 10 ml of tris buffer solution and make up to the mark with acetone to give concentrations of 0 µg, 5 µg, 10 µg, 20 µg, 30 µg and 40 µg INTF per ml of acetone buffer solution.

Since INT and INTF are light-sensitive, the solutions should be protected from exposure to light throughout the analysis.

5 Apparatus

5.1 Photometer

5.2 pH meter

5.3 Test tubes

For example, 2 cm diameter and at least 30 ml capacity (test portion 5 g; see also Clause 6).

5.4 Incubator, capable of being set to (30 ± 1) °C.

5.5 Suitable conical flasks, volumetric flasks, pipettes and funnels

5.6 Fluted filter, with slow filtering action (90 s to 100 s).

5.7 Ultrasonic probe

5.8 Test tube shaker

6 Procedure

Weigh 2,00 g to 5,00 g portions of naturally moist soil into each four test tubes. Add 2 ml to 5 ml of substrate solution (4.4) to three samples. Pipette 2 ml to 5 ml of tris buffer solution (4.3) instead of the substrate solution (4.4) to the blank sample. Homogenize the samples on the test tube shaker; seal the tubes with rubber stoppers; and incubate them at 30 °C in the dark for 18 h.

In order to extract the formazan formed, add 20 ml of acetone to the samples and allow them to stand for 2 h in the dark. Shake the samples on the shaker after adding the extractant, after 1 h, and again after 2 h. Then filter the samples in semidarkness using a fluted filter.

Within 1 h measure the absorbance of the filtrates against the calibration curve zero by photometry at a wavelength of 485 nm.

7 Calculation

Determine the dehydrogenase activity (based on dry soil) from the calibration curve and the following equation:

$$a = \frac{(\overline{\rho}_{cs} - \overline{\rho}_{bs}) \times V \times 100}{m \times DM \times t}$$

where

a is the dehydrogenase activity in µg/g of dry soil per hour;

$\overline{\rho_{cs}}$ is the complete sample average INTF concentration, in µg/ml;

$\overline{\rho_{bs}}$ is the average INTF concentration of the blank sample, in µg/ml;

V is the solution volume [= volume of substrate or buffer solution (5 ml) + volume of extractant (25 ml)], in ml;

m is the initial soil sample mass, in g;