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Soil quality — Laboratory methods for determination of microbial soil respiration

Qualité du sol — Méthodes de laboratoire pour la détermination de la respiration microbienne du sol

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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.

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ISO 16072 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

This International Standard is derived from the German standard DIN 19737 (see [1]). It describes methods for the determination of microbial soil respiration in the laboratory.

Microbial soil respiration results from the mineralization of organic substances. In this process, organic substances are oxidized to the end products carbon dioxide and water, with concurrent uptake of O_2 for aerobic microorganisms. The soil respiration is measured by the determination of O_2 consumption and/or by CO_2 release. Respiration is a measure of the overall activity of soil microorganisms.

Soil quality — Laboratory methods for determination of microbial soil respiration

1 Scope

This International Standard describes methods for the determination of soil microbial respiration of aerobic, unsaturated soils. The methods are suitable for the determination of O_2 uptake or CO_2 release, either after addition of a substrate (substrate-induced respiration), or without substrate addition (basal respiration).

This International Standard is applicable to the measurement of soil respiration in order to:

— determine the microbial activity in soil (see [3]);

— establish the effect of additives (nutrients, pollutants, soil improvers, etc.) on the metabolic performance of microorganisms;

— determine the microbial biomass (see [4]);

— determine the metabolic quotient qCO_2.

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

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

ISO 11465:1993, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

3 Terms and definitions

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

3.1
basal respiration
microbial soil respiration without addition of nutrients

3.2
substrate-induced respiration
SIR
microbial soil respiration after addition of nutrients

NOTE Glucose is an example of an added nutrient.

3.3
microbial activity
metabolic performance of microorganisms

NOTE It can be measured, for example, as O_2 uptake or CO_2 release.

3.4
metabolic quotient
qCO_2
specific metabolic activity of soil microorganisms, which can be calculated as the quotient basal respiration: microbial biomass

NOTE Metabolic quotient is usually expressed as milligrams of CO_2 carbon released per hour per gram of microbial biomass carbon.

3.5
rate of CO_2 formation [O_2 consumption]
R_{CO_2} [R_{O_2}]
amount of CO_2 released [O_2 consumed] per time unit from a mass unit of soil

NOTE 1 Soil respiration is usually measured as the rate of CO_2 formation or O_2 consumption.

NOTE 2 It is usually expressed as milligrams CO_2 [or O_2] per gram per hour (mg CO_2 [or O_2]·g^{-1}·h^{-1}).

3.6
microbial biomass
mass of intact microbial cells in a given soil

NOTE This is usually estimated from the measurement of carbon or nitrogen content of these cells.

4 Procedure

4.1 General conditions

4.1.1 Soil sampling and storage

Sample, store and pre-incubate soils in accordance with ISO 10381-6, independently of the choice of the procedure and the respiration parameter to be measured (basal respiration, SIR).

4.1.2 Measuring and incubation conditions

Soil respiration is strongly influenced by water content and temperature. Therefore these parameters should be recorded in the final report. At suction pressures > 0,03 MPa, the soil respiration will decrease considerably. The water content of the test soil is optimal when it corresponds with a pore water pressure of – 0,01 MPa to – 0,03 MPa (measured with an accuracy of 5 %, in accordance with ISO 11274) or 40 % to 60 % of the maximum water-holding capacity, respectively. A stable temperature should be used. Incubation temperatures between 20 °C and 30 °C are generally recommended, but other temperatures may be used if required. In the description of the methods, examples of incubation temperatures are given as well as the accuracy of temperature maintenance and measurement.

If a method is used for the determination of soil microbial biomass, a temperature of 22 °C is recommended because biomass calculations have been calibrated to this temperature.

When soil samples are compared with respect to soil respiration, they should have the same moisture status (pore water pressure or percentage of maximum water-holding capacity).

4.2 Choice of the measuring system

Each measurement method has its own advantages and disadvantages. Care is needed, because the results obtained by O_2 uptake and by CO_2 release are not strictly compatible. It is the responsibility of the investigator to decide which of these methods is to be used.

One of the systems described in Clause 5 should be used.

Systems for measuring CO_2 do not distinguish between CO_2 released from microbial activities and CO_2 resulting from abiotic processes. For alkaline soils and soils with a high organic matter content, which can release considerable amounts of abiotically released CO_2, methods using O_2 uptake are recommended.

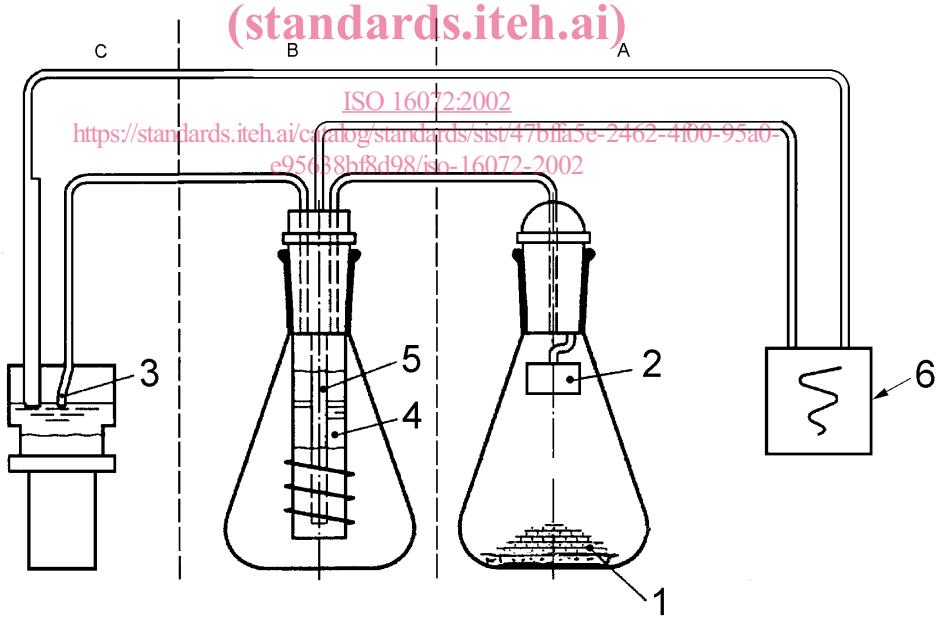
NOTE The advantages and disadvantages are described in the individual descriptions of the methods.

5 Measuring systems

5.1 Determination of O_2 consumption by static incubation in a pressure-compensation system

5.1.1 Principle

The determination is based on the measurement of O_2 consumption during incubation of a soil sample in a closed system. The O_2 in the system is replenished electrochemically. The CO_2 released is absorbed by calcium hydroxide [$Ca(OH)_2$].



Key

A	reaction vessel	1	soil sample	4	electrolyte
B	oxygen generator	2	CO_2 absorbent	5	electrodes
C	pressure indicator	3	pressure cell	6	recorder with display

Figure 1 — Determination of O_2 consumption (showing connection of a measuring unit)

5.1.2 Apparatus

A detailed description of the apparatus can be found in [4]; the essential features are as follows.

The measuring system (see Figure 1) consists of a water bath with temperature control containing measuring units each comprising a reaction vessel (A) in which a CO_2 absorption device (2) is suspended from the stopper, an O_2 generator (B) and a pressure indicator (C). The vessels (A, B, C) of the measuring unit form together a closed system, connected to each other by tubing. In this way fluctuations in atmospheric pressure will not influence the results. The CO_2 released is absorbed by the calcium hydroxide (2). The consumption of O_2 due to respiration results in a negative pressure which activates the pressure indicator (C). This drives the electrolytic O_2 formation as well as the display and graphical registration of measuring values on a recorder (6). The O_2 consumption is shown directly, in milligrams of oxygen (mg O_2) on a digital display.

The system can be obtained commercially[1)] and detailed instructions should be given in the supplier's manual.

5.1.3 Procedure

Use 50 g to 100 g of field-moist, sieved (2 mm) soil for the measurements. The O_2 consumption should not be measured during the first 2 h, the time needed to reach equilibrium in the system.

5.2 Determination of CO_2 release by titration in a static system

5.2.1 Principle

The soil is incubated in a closed vessel and the released CO_2 is absorbed in a solution of sodium hydroxide. After back-titration of the non-consumed sodium hydroxide, the amount of CO_2 released is calculated. The method is suitable for large numbers of samples, and up to 80 respiration measurements per working day are possible.

5.2.2 Reagents

5.2.2.1 CO_2-free water

Boil distilled water and after cooling store it in flasks closed with stoppers provided with absorption tubes containing calcium hydroxide.

5.2.2.2 Sodium hydroxide (NaOH) solution, $c = 0{,}05\ \text{mol·l}^{-1}$.

5.2.2.3 Hydrochloric acid (HCl) solution, $c = 0{,}1\ \text{mol·l}^{-1}$.

The concentrations of NaOH solution (5.2.2.2) and HCl (5.2.2.3) should be chosen so that less than 20 % of the NaOH is neutralized by CO_2. Higher percentages of neutralization will result in less reliable results (see [5]). If other concentrations are used, Equation (1) should be changed accordingly.

5.2.2.4 Barium chloride solution, $c = 0{,}5\ \text{mol·l}^{-1}$.

Dissolve 10,4 g of $BaCl_2$ in 100 ml of CO_2-free distilled water (5.2.2.1).

5.2.2.5 Indicator.

Dissolve 0,1 g of phenolphthalein in 100 ml of aqueous ethanol (volume fraction ethanol 0,6).

5.2.3 Apparatus

5.2.3.1 Wide-mouth flasks (250 ml content) with screw-caps and pour rim, or preserve flasks (1 l content) with rubber rings, covers and 2 universal clips.

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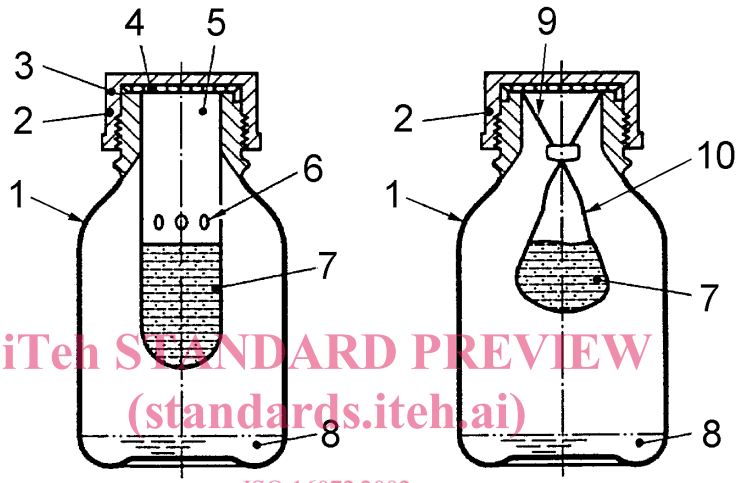
1) Sapromat is the trade name of a product supplied by H+P Labortechnik AG. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

5.2.3.2 Centrifuge tubes or reaction tubes with a rim (e.g. polypropylene, external diameter 29 mm, length 120 mm). Small holes should be drilled in the tubes for gas exchange. Instead of tubes, fine-mesh nylon bags can also be suspended from the neck in the wide-mouth flasks.

5.2.4 Procedure

Weigh 20 g to 25 g of field-moist soil into the centrifuge tubes (5.2.3.2). Suspend the tubes in the wide-mouth flasks (5.2.3.1) (see Figure 2), in which 20 ml solution of sodium hydroxide (5.2.2.2) has been previously pipetted. Close the flasks tightly and incubate for 24 h in a temperature-controlled room at the temperature of choice, e.g. 22 °C ± 1 °C. Before closing the flasks, they should be flushed with clean air with low CO_2 content (e.g. from outdoors). Then remove the tubes. The CO_2 absorbed will precipitate as barium carbonate upon addition of 2 ml of barium chloride solution (5.2.2.4). Titrate the unused sodium hydroxide with hydrochloric acid (5.2.2.3) after addition of 3 or 4 drops of indicator solution (5.2.2.5).



Key

1	wide-mouth flask (250 ml)	6	openings for gas exchange
2	screw-cap	7	soil sample
3	pour rim	8	sodium hydroxide solution
4	closing pad	9	plastic thread
5	suspended centrifuge tubes	10	fine-mesh woven plastic bag

Figure 2 — Incubation flasks for the determination of soil respiration

The determination should be carried out at least in triplicate. Controls (triplicate flasks without soil) should be included.

If the soil respiration is measured in a long-term experiment (> 3 days), then the soil samples should be incubated in flasks in which the sodium hydroxide solution is renewed every 3 days. Also the water content of the soil has to be adjusted every 3 days.

Also suitable for incubation are preserve flasks (1 l content) with rubber rings, covers and 2 universal clips. Weigh the soil samples (up to 200 g) into crystallization disks, which are placed on the bottom of the preserver flasks. Place sodium hydroxide solution in a beaker. On the bottom of the flasks, place 4 ml of CO_2-free water (5.2.2.1) to maintain air moisture.

NOTE When determining basal respiration in the laboratory, an increase in CO_2 release is often observed in the first hours. This can be caused by an increased availability of nutrients due to the moving and mixing of soil particles during sample preparation, but also by the short-term establishment of an equilibrium between gaseous and dissolved CO_2. The incubation time necessary for reaching a steady basal respiration depends in the first instance on the soil's content of easily available carbon compounds. This applies to all methods measuring CO_2 release.