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Soil quality — Determination of abundance and activity of soil microflora using respiration curves

Qualité du sol — Détermination de l'abondance et de l'activité de la microflore du sol à l'aide de courbes de respiration

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Contents

Page

Annex

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.

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.

International Standard ISO 17155 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Annex A of this International Standard is for information only.

Soil quality — Determination of abundance and activity of soil microflora using respiration curves

1 Scope

This International Standard specifies a test method for determining the activity of the active aerobic, heterotrophic microbial biomass in soils. This method is applicable to the monitoring of soil quality and to the evaluation of the ecotoxic potential of soils and soil materials. It is also applicable to soils that are contaminated experimentally in the field or in the laboratory (chemical testing) and for soils sampled along contamination gradients in the field.

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

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

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

ISO 14238:1997, *Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes*

3 Terms and definitions

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

3.1

basal respiration rate

R_B

constant mass of CO_2 released or mass of O_2 consumed per unit mass of soil per unit time without substrate addition

See Figure 1.

3.2
substrate-induced respiration
R_S
constant mass of CO_2 released or mass of O_2 consumed per unit mass of soil per unit time shortly after addition of a carbon substrate

See Figure 1.

NOTE If glucose is used as a carbon substrate, microbial biomass can be determined from the substrate-induced respiration rate (see Reference [1] in the Bibliography).

3.3
lag time
t_{lag}
time from the addition of a growth substrate until exponential growth starts

See Figure 1.

NOTE It reflects the vitality of the growing organisms (see Reference [2] in the Bibliography).

3.4
growth rate
μ
rate constant during exponential increase of the respiration rate

See Figure 1.

3.5
respiratory activation quotient
Q_R
basal respiration rate divided by substrate-induced respiration rate

$$Q_R = R_B/R_S$$

3.6
time to the peak maximum
$t_{peakmax}$
time from addition of growth substrate to the maximum respiration rate

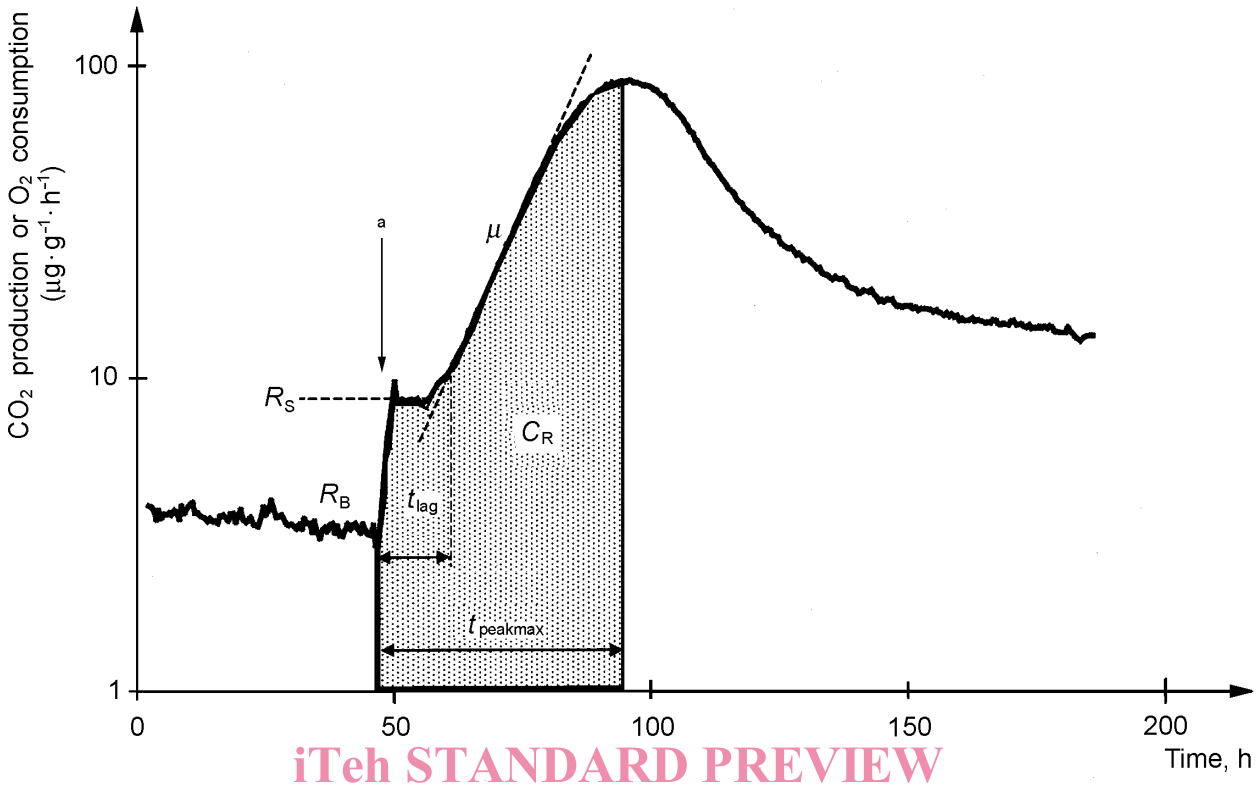
See Figure 1.

NOTE It reflects also the viability of the growing organisms.

3.7
cumulative CO_2 evolution or O_2 consumption
C_R
total area bounded by the soil respiration rate curve to the time axis from time of the addition of substrate to the time

See Figure 1.

3.8
soil material
material composed of excavated soil, dredged materials, manufactured soils, treated soils and fill materials



[a] Addition of substrate

Figure 1 — Soil respiration rate before and after addition of an easily degraded substrate

4 Principle

The CO_2 production or O_2 consumption (respiration rate) from unamended soils as well as the decomposition of an easily degraded substrate (glucose + ammonium + phosphate) is monitored regularly (at least every hour). Using the CO_2 production or O_2 consumption data, the different microbial parameters (basal respiration, substrate-induced respiration, lag time, respiratory activation quotient, $t_{peakmax}$, C_R) can be calculated.

5 Reagents

5.1 Glucose, $C_6H_{12}O_6$.

5.2 Potassium dihydrogen phosphate, KH_2PO_4.

5.3 Diammonium sulfate, $(NH_4)_2SO_4$.

5.4 Substrate, consisting of a mixture of 80 g of glucose (5.1), 13 g of diammonium sulfate (5.3) and 2 g of KH_2PO_4 (5.2) which is thoroughly ground and mixed in a mortar.

6 Apparatus

Ordinary laboratory equipment and

6.1 Respirometer for continuous measurement of CO_2 evolution or O_2 consumption, maintained at a constant temperature (preferably 20 °C). Suitable examples of equipment are given in ISO 16072[3].

7 Sampling

7.1 Sample quantities

Choose the size of the soil samples size with respect to the apparatus (6.1) used and on the organic matter content of the sample (7.1). Samples of organic horizons (e.g. mor layers) should not exceed 1 g of organic matter (see References [4] and [5]) in order to provide an optimal substrate/soil ratio (see 8.1). It is recommended to measure at least three replicates per sample.

7.2 Sampling and storage

The recommendations in ISO 10381-6 for the collection, handling and storage of soil samples shall be followed.

7.3 Soil sample characteristics

Soil samples generating soil respiration curves can be obtained from mineral soils, organic soils, polluted and unpolluted soils. Determine the following characteristics for each soil sample:

— particle size distribution in accordance with ISO 11277;

— water content in accordance with ISO 11465;

— water-holding capacity in accordance with annex A of ISO 14238:1997;

— pH in accordance with ISO 10390;

— organic matter content in accordance with ISO 10694.

8 Procedure

8.1 Test

Pre-incubate moist soil samples (preferably 40 % to 60 % of maximum water holding capacity or 0,01 MPa to 0,03 MPa suction pressure) at 20 °C for 3 d to 4 d before the beginning of the measurement. Measure the basal respiration of the sub-samples first. Measure the respiration rates until constant rates are obtained.

After measuring the basal respiration, add 0,2 g of the substrate (5.4) per gram of organic matter and mix homogeneously with a spatula into the soil samples. If the organic matter content is less than 5 % the substrate-to-soil ratio is 1 % [i.e. 1 g of substrate added per 100 g of soil (dry mass)].

Continue to measure the CO_2 evolution or O_2 consumption until the respiration rates decline (see Figure 1).

8.2 Influence of chemicals

The effect of chemicals on the soil microbial activity can be determined as follows. Using a range-finding test, determine the concentration range that chemicals would likely have an effect on this activity. Test a single, microbiologically active soil at five concentrations in a logarithmic series including a blank control in triplicate (for example 0, 1, 3,2, 10, 32, and 100 times the lowest concentration). Use the test procedure specified in 8.1. Using this simple test design, dose-response relationships can be established.

Before the start of the test, the test chemical may be added to the soil in one of the following manners:

— in an aqueous solution (depending on its solubility in water);

— in an organic solution using a water-miscible solvent (depending on the solubility in the solvent);

— mixed with a solid, e.g. coated on quartz sand (prior to mixing with the soil).

If the test chemical is added in the form of an organic solution, keep the amount of water-miscible solvent to the minimum ($< 1\,\%$) necessary for the application of the compound. Furthermore, take into account the possible toxicity and biodegradability of the solvent used.

NOTE Long-term effects of chemicals can be detected by using different incubation times (weeks or months).

9 Calculation

9.1 Microbial parameters

9.1.1 Basal respiration

Calculate the basal respiration (R_B) as the average of the hourly respiration rate during a stable period.

9.1.2 Substrate-induced respiration

Calculate the substrate-induced respiration (R_S) as the average of the values shortly after the substrate addition when the respiration is fairly constant after substrate addition. A minimum of 3 hourly measurements should be used to calculate the average.

9.1.3 Respiratory activation quotient

Calculate the respiratory activation quotient (Q_R) by dividing the basal respiration rate by the substrate-induced respiration rate.

$$Q_R = R_B/R_S$$

9.1.4 Lag time, growth rate and time to the peak maximum

Calculate the lag time (t_{lag}), growth rate (μ) and time to the peak maximum ($t_{peakmax}$) by making a plot of the logarithm of the respiration rate against time (see Figure 1). The exponential phase of the growth will then appear linear and a regression can be made giving the growth constant, μ, and the intercept with the y-axis. The lag time can be calculated as the time between substrate addition and the beginning of exponential growth (see Figure 1). $t_{peakmax}$ can be calculated as the time between substrate addition and the maximum respiration rate.

NOTE A mathematical equation $\mathrm{d}p/\mathrm{d}t = r\mathrm{e}^{\mu t} + K$ to which the data can be fitted was given by Stenström et al.[2] The respiration rate $\mathrm{d}p/\mathrm{d}t$ (where p is the product and $\mathrm{d}p/\mathrm{d}t$ is the rate of microbial product formation) after substrate addition represents the sum of the respiration rates of growing ($r\mathrm{e}^{\mu t}$) and non-growing micro-organisms (K). According to the equation for substrate-induced respiration (SIR), $\mathrm{SIR} = r + K$ at the time of substrate addition, where r is the initial respiration rate of growing micro-organisms and K the rate of non-growing micro-organisms.

9.1.5 Cumulative CO_2 evolution or O_2 consumption

The effect of a chemical on the lag time and on μ can be combined by determining the cumulative CO_2 evolution or O_2 consumption from the time point of the addition of substrate to the time point of the maximum respiration in the blank control (i.e. $t_{peakmax}$ in Figure 1). It is essentially equivalent with the (linear) shaded surface area in Figure 1.

For each concentration, the cumulative CO_2 evolution or O_2 consumption (C_R) is measured to the $t_{peakmax}$ in the control blank. A plot of C_R versus the logarithmic concentration of the test substance often gives an S-shaped curve from which EC_{10} and EC_{50} can be estimated.

NOTE This test is carried out under conditions stimulating microbial growth. However, a soil respiration inhibition test can also be carried out at low, growth-limiting concentrations of ^{14}C-labelled acetate followed by $^{14}CO_2$ determination (see Reference [9]).