APPLICATION NOTE
E-RES--001-2022/A1

# Determination of Microbial Soil Respiration with Respirometric Sensor System for Soil Analysis

Reference: **ISO 16072:2011** Soil quality — Laboratory methods for determination of microbial soil respiration.

Tested with **RESPIROMETRIC Sensor System for soil analysis code SA102B0**


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## Introduction

Microbial soil respiration results from the mineralization of organic substances. In this process, organic substances are oxidized to the end products as carbon dioxide and water, with concurrent uptake of $O_2$ for aerobic microorganisms.

- Soil respiration is measured by determining $O_2$ consumption and/or $CO_2$ release.
- Respiration, instead, is a measure of soil microorganisms' overall activity.

Soil respiration reflects the soil capacity to support the soil life including crops, plant growth, soil fauna, and microorganisms. It is an important indicator of the soil health: it indicates the level of biological activity, soil organic matter, and decomposition. In particular, during the decomposition process, organic nutrients contained in organic matter (e.g., organic phosphorus, nitrogen, and sulfur) are converted into inorganic forms available for the plant uptake.

In the laboratory, soil respiration is used to estimate soil microbial biomass. Indeed, soil respiration indicates soil ability to sustain plant growth. Excessive respiration and organic matter decomposition usually occur after tillage due to the destruction of soil aggregates that previously protected organic matter and increased soil aeration. Depleted organic matter, reduced soil aggregation, and limited nutrient availability for plants and microorganisms can result in reduced crop production in additional inputs absence. The threshold between accumulation and loss of organic matter is difficult to predict without knowing the carbon added amount [(1)].

Thanks to the **RESPIROMETRIC Sensor** it is possible to measure the soil respiration to:

- determine the soil microbial activity;
- establish the effect of additives (nutrients, pollutants, soil improvers, etc.) on the metabolic performance of microorganisms;
- determine the microbial biomass.

(1) USDA Natural Resources Conservation Service

## Sample

Bio-Organic soil conditioner ID EN99

## Reagents required

Absorption of carbon dioxide:
Potassium Hydroxide (KOH) in flakes, commercial grade or non-deliquescent soda lime, 1.0-1.7 mm granules.

## Sample Preparation

Adjust the moisture content in the soil.
A compost must be moist to allow microorganisms activation.

- If compost is too dry, no microbial activity is possible: compost transformation process (*composting*) is stopped.
- If compost is too wet, unwanted microbial processes occur in anaerobic conditions (= *absence of oxygen*) and the compost may eventually have a bad smell and contain phyto-toxic acids.

A simple test to check compost moisture content is called 'punch test'. After taking a handful of compost, squeeze it hard and then open your hand.

- If the compost is too dry, it will then fall apart. If the moisture content is normal, the compost remains compact.
- If the compost is too wet, the water will splash out of the hand when you squeeze the compost.

Depending on the situation, necessary measures, such as adding water or covering the compost, can be taken.


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Once the soil has been prepared with the correct humidity, it is necessary to determine the dry mass fraction.

Finally, the volume occupied by the soil *(after humidity adjustments*) must be determined as follows:

In a 200/250 ml cylinder add 100 ml of deionized water and add 50/100g of medium.

After mixing, the final volume, subtracted from the volume of the water, is the volume occupied by the soil.

## Analysis Procedure

1. Set the incubator temperature to the desired value, 20-25°C. *We set the tests at 25°±1°C*.
2. Connect the Wireless DataBox™ and fill in the RESPIROSoft ™ software database.
3. Weigh 50 - 200g of well-mixed soil in a beaker and transfer it to a 1-liter flask.
4. Introduce about 6 pellets of KOH into the alkali collector.
5. Screw the RESPIROMETRIC Sensors onto each bottle and tighten.
6. Place the system in the incubator at the test temperature.
7. Wait at least 3 hours to reach the correct test temperature and to develop the typical initial pressure increase.
    - This growth is due to vapor pressure and air flask heating.
    - To remove the additional pressure, we recommend venting test bottles to the atmospheric pressure (*by briefly loosening the ring nut*).
8. Press START and run the test for at least 15 days.

## Free Gas Volume calculation

Calculate the free gas volume according to equation: $\boldsymbol{V_{fg} = V_{tot} - V_{av} - V_{am} - V_{sm}}$

Where:
- $\boldsymbol{V_{fg}}$ is the free gas volume, in liters;
- $\boldsymbol{V_{tot}}$ is the total volume of the measuring vessel without soil, adsorption vessel and absorption material, in liters;
- $\boldsymbol{V_{av}}$ is the absorption vessel volume, in liters;
- $\boldsymbol{V_{am}}$ is the absorption material volume, in liters;
- $\boldsymbol{V_{sm}}$ is the moist soil volume, in liters.

## Dry Soil Mass calculation

Calculate the dry soil mass according to equation: $\boldsymbol{m_{sd} = m_{sm} \cdot W_{sd}}$

Where:
- $\boldsymbol{m_{sd}}$ is the dry soil mass, in kilograms;
- $\boldsymbol{m_{sm}}$ is the moist soil mass, in kilograms;
- $\boldsymbol{W_{sd}}$ is the dry mass fraction of the moist soil sample.

## O2 Consumption Rate calculation

Calculate the $O_2$ consumption rate according to equation: $\boldsymbol{RO_2 = 32000 \cdot V_{fg} \cdot \frac{\Delta P}{t \cdot R \cdot T \cdot m_{sd}}}$

Where:
- $\boldsymbol{RO_2}$ is the $O_2$ consumption rate on a soil dry mass basis (mg $O_2$·g–1·h–1);
- 32000 is the molar mass of oxygen (mg/mol);
- $\boldsymbol{V_{fg}}$ is the free gas volume, in liters;
- $\boldsymbol{R}$ is the gas constant (83,14 hPa l·mol$^{-1}$·K$^{-1}$);
- $\boldsymbol{T}$ is the measuring temperature, in kelvin;
- $\boldsymbol{m_{sd}}$ is the dry soil mass, in grams;
- $\boldsymbol{\Delta P}$ is the measured pressure reduction, in hectopascals;
- $\boldsymbol{t}$ is the elapsed time, in hours.


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## Results obtained

VELP's **RESPIROSoft**TM software automatically determines pressure values during the sampling time and reports them in a graph (Figure1).

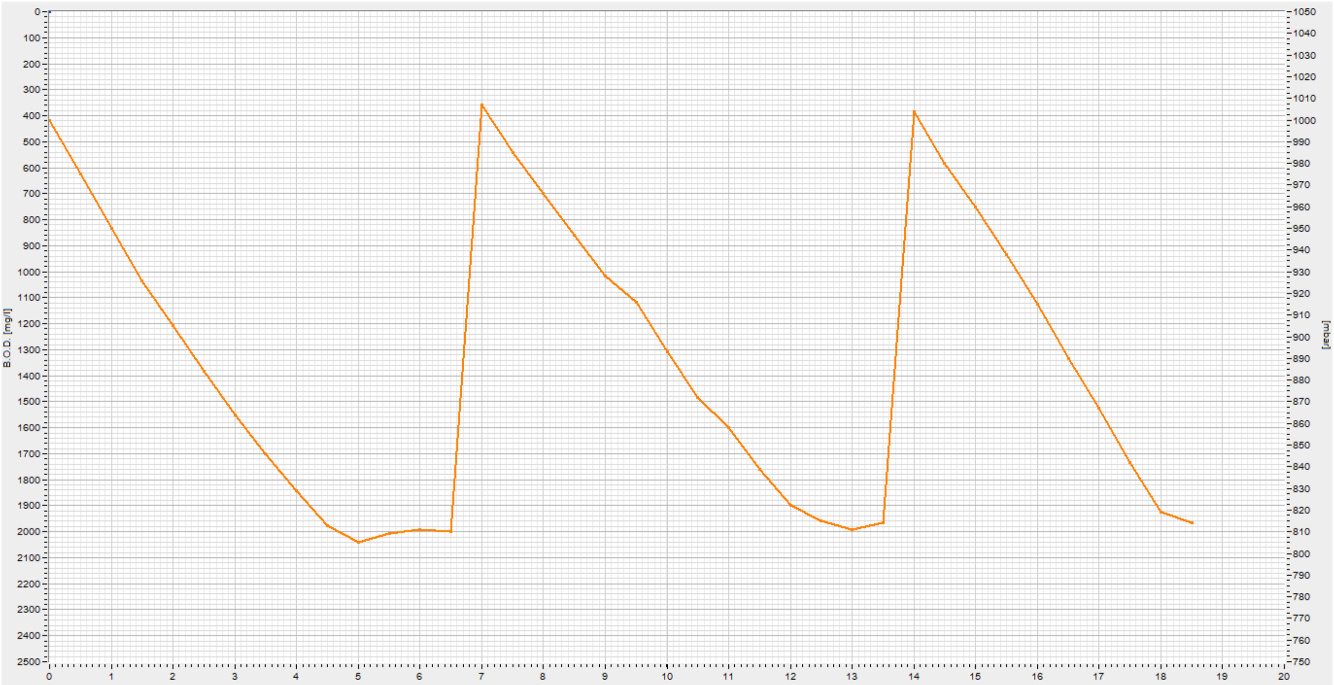

**Figure1:** Graph of 100g of sample 298K test

**NOTE:** when pressure goes around 800mbar all the Oxygen inside the bottle is consumed. Therefore, the system requires to be aerated (*unscrew the sensor and insufflate air inside the bottle*).

Following are shown tests results carried out on the same sample by varying the soil sample weight. The reported analysis has a 20 days duration. This timing was chosen to monitor the variability of the kinetics absorption over time.

**Test 200g of soil:**

*T = 298 K*
*Vfg =0.965 L*
*t= 72 h*

| Sample Name | P Initial (hPa) | P final (hPa) | $RO_2$ [mg $O_2$/ (g*h)] |
|---|---|---|---|
| Organic soil conditioner | 992 | 813 | 0.0261 |
| Organic soil conditioner | 996 | 812 | 0.0268 |
| | | **Average ± SD** | **0.0264 ± 0.0005** |
| | | **RSD%** | **1.95%** |


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**Test 200g of soil:**

*T = 298 K*
*Vfg =1.037 L*
*t= 72 h*

| Sample Name | P Initial (hPa) | P final (hPa) | $RO_2$ [mg $O_2$/ (g*h)] |
|---|---|---|---|
| Organic soil conditioner | 990 | 852 | 0.0439 |
| Organic soil conditioner | 989 | 857 | 0.0439 |
| Organic soil conditioner | 1000 | 864 | 0.0436 |
| | | **Average ± sd** | **0.0438 ± 0.0001** |
| | | **RSD%** | **0.4%** |

**Test 200g of soil:**

*T = 298 K*
*Vfg =1.081 L*
*t= 72 h*

| Sample Name | P Initial (hPa) | P final (hPa) | $RO_2$ [mg $O_2$/ (g*h)] |
|---|---|---|---|
| Organic soil conditioner | 991 | 928 | 0.0428 |
| Organic soil conditioner | 990 | 924 | 0.0448 |
| | | **Average ± sd** | **0.0448 ± 0.0014%** |
| | | **RSD%** | **3.29%** |

## Conclusions

**RESPIROMETRIC Sensor System for Soil Analysis** is a functional and reliable solution for microbial soil respiration determination, as can be seen from the RSD% value (at least 3.29%), which represents appreciable repeatability for these analyses.

As expected, the bio-organic soil conditioner shows a high activity even on the first day of analysis.
Thanks to its innovative wireless technology, the sensor transmits the Pressure Value to the **Wireless Databox**™, based on the data transmission frequency set before starting the analysis.

Then, the intuitive **RespiroSof**t™ interface optimally displays results for data management and result comparison.

Definitely, connect the RESPIROMETRIC Sensor to the exclusive **VELP Ermes Cloud Platform** to improve your laboratory experience.


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