Biodegradability of the dry residue of the original material

Application Report: Respirometric activity AT_4

Note:

This report focuses on the AT_4 determination. This refers to the respiratory activity of soil samples or fractions from MBT facilities (mechanical-biological waste treatment facilities) over a 4-day period. The data in this report were determined using the OxiTop®-C. They can be easily transferred to the OxiTop®-IDS system.

Measurement methods:

Determination of the Biological Oxygen Demand

Legal Notice:

The legal basis is provided by the Federal Soil Protection Act [1] and the Ordinance on the Disposal of Municipal Waste [2]. The purpose of the Federal Soil Protection Act is to sustainably safeguard or restore the function of the soil. It provides the framework for self-monitoring measurements in the area of contaminated site monitoring. The Ordinance on the Landfilling of Municipal Waste provides detailed specifications for analysis and defines the classification of landfills. Using the classification values specified therein, it is possible to monitor the process in the compost pile. Microbial soil respiration is also addressed in DIN 19737, its successor standard ISO 16072, or its German version DIN ISO 16072 [3].

Measuring equipment:

The OxiTop® Control and OxiTop®-IDS series measurement systems are suitable for analyzing soil samples and feature:

- Controller or Multi 3620/3630 IDS
- Software for transferring data from the controller to a PC
- Controller/PC Connection Cable
- OxiTop®-C and OxiTop®-IDS measuring heads

Please refer to the user manuals for the respective systems!

Accessories:

Depending on the measurement problem:

- Sample containers: 500 ml, 1 l, or 2.5 l
- Lid adapter, including frame support and O-ring
- Retaining clips
- Rubber quiver
- Measuring cup: V = 50 ml

Reagents:

- Soda lime absorber with CO_2 Indicator
- Alternatively: Liquid absorbent (NaOH or KOH solution)

Basic Structure of the Measurement System

The measuring vessels filled with samples are incubated in a precisely temperature-controlled environment (climate chamber, temperature-controlled cabinet, etc.) and monitored using the controller (see “Practical Notes: Effect of Temperature”) During the measurement, the measurement data from all measurement points can be transmitted to the controller as often as desired via the Bluetooth interface. The controller provides a graphical display of the measurement data for monitoring purposes. The entire data set can be transferred to a computer via Bluetooth. Soil respiration or the AT4 value is calculated using a suitable spreadsheet program (e.g., Microsoft Excel) or specialized software.

OxiTop® **Multi 3620/3630 IDS** **Thermostatic cabinet**

Measurement Principle and Analysis

The biological processes occurring in the sample are primarily based on the conversion of the carbon present. According to Federal Law Gazette G 5702 and the Waste Disposal Ordinance (AbfAblV), aerobic (AT_4 determination) and anaerobic processes (fermentation test GB21) may be used to evaluate waste samples. In the absence of oxygen, anaerobic processes occur, resulting in the formation of biogas (primarily CH_4 + CO_2). In the AT_4 test, however, sufficient oxygen must be present so that carbon dioxide (CO_2) is produced through aerobic respiration.

1 $C_{org} + O_2\ (gas) \rightarrow CO_2\ (gas)\uparrow$

In order to measure the degradation of organic matter, the reaction product CO_2–formed in equivalent quantities—must be chemically absorbed by an absorber and thus removed from the gas chamber. Only in this way can the measured pressure difference be used to directly determine oxygen consumption. Suitable options include liquid absorbers (NaOH or KOH solution) at the appropriate molarity or granulated sodium calcium carbonate. Maintaining a constant incubation temperature is particularly important. This ensures a defined framework for microbial reactions and, based on the gas law, allows pressure changes to be correlated with metabolic turnover. There is a correlation between respiratory activity and the fermentation stage. Due to the lower workload and significantly shorter measurement duration, aerobic degradation is preferred. In principle, however, the OxiTop® IDS system can also measure and record pressure-increasing anaerobic processes involving biogas formation up to 1500 hPa.

In “Pressure p” measurement mode, the change in pressure difference in the gas chamber is determined and is thus available as a measured variable for calculating soil respiration using the following formula (see also [3]):

2 $$BA = \frac{M_{O2}}{R \times T} \times \frac{V_{fr}}{m_{Bt}} \times |\Delta p|$$

featuring:

BA	*Soil respiration*
M_{02}	*Molecular weight of O_2 (31.998 mg/mol)*
R	*Universal gas constant (83.14 L × hPa/(K × mol))*
T	*Measurement temperature (in Kelvin)*
V_{fr}	*Free gas volume (in liters)*
m_{Bt}	*Mass of dry soil matter (in kg)*
|Δp|	*Magnitude of the pressure change (in hPa)*

Where:

3 $$V_{fr} = V_{ges} - V_{AM} - V_{Bf}$$

featuring:

V_{ges}	*The total volume enclosed by the measuring vessel*
V_{AM}	*Volume of the absorbent and auxiliary equipment*
V_{Bf}	*Volume of the moist soil*

Volumes can be determined by volumetric measurement or - if sufficient - estimated. DIN 38414-10 is recommended for determining dry matter. The calculation of the soil dry matter (m_{Bt}) used is performed according to:

4 $$m_{Bt} = m_{Bf} \times \frac{TS}{100\%}$$

featuring:

m_{Bt}	*Mass of dry soil matter (in kg)*
m_{Bf}	*Mass of soil moisture (in kg)*
TS	*Dry matter content (in %)*

The BOD fee is calculated based on:

5 $$BOD = \frac{M_{O2}}{R \times T} \times \left(\frac{V_{ges} - V_{Probe}}{V_{Probe}} + \alpha \frac{T}{T_0} \right) \frac{1}{\rho} \times |\Delta \rho|$$

The following applies to soil respiration:

6 $$BA = \frac{M_{O2}}{R \times T} \times \left(\frac{V_{ges} - V_{Probe}}{V_{Probe}} + \alpha \frac{T}{T_0} \right) \frac{1}{\rho} \times |\Delta \rho|$$

featuring:

BA	*Soil respiration*
M_{02}	*Molecular weight of O_2 (31.998 g/mol)*
R	*Universal gas constant (83.14 L × hPa/(K × mol))*
T	*Measurement temperature (in Kelvin)*
T_0	*Temperature at the Celsius zero point (273.15 K)*
V_{ges}	*Total gas volume (in liters)*
V_{Probe}	*Volume of the sample (in liters)*
α	*Bunsenscher absorption coefficient*
ρ	*Density of dry matter (in kg/L)*
|Δp|	*Magnitude of the pressure change (in hPa)*

Rearranging Equation (7) shows that, in “BOD Special” measurement mode, the BOD reading corresponds to the product of soil respiration and dry matter density, as given by equation (5).

7 $$BA \times \rho = \frac{M_{O2}}{R \times T} \times \left(\frac{V_{ges} - V_{Probe}}{V_{Probe}} + \alpha \frac{T}{T_0} \right) \times |\Delta \rho|$$

In this mode, the Multi 36x0 IDS provides a simultaneously calculated BOD reading in mg O_2/l based on freely selected parameters (gas volume, sample volume, ambient pressure, measurement range, temperature, etc.). To calculate soil respiration, this BOD value must be divided by the density of the sample. The procedure for setting the relevant parameters is described in the controller’s operating instructions.

Implementation

Sample Preparation

1. The original sample must be ground to a size of less than 10 mm.
2. If necessary, contaminants such as glass, stones, and metals must be removed before comminution. Their mass fractions must be taken into account when evaluating the test results.
3. All items that do not belong in the landfill, such as PE bags, must also be sorted out.
4. The data required for subsequent analysis - such as sample volume, dry matter density, and vessel volume - must be determined. Dry matter comprises the inorganic and organic components after complete water removal by drying at 105°C (see DIN 38414-10 [4]).
5. The optimal moisture content must be adjusted (approximately 50–60 % of the maximum water-holding capacity). To do this, moisten a 300-g sample with 300 ml of tap water and evacuate it for 30 minutes using a Nutsch filter with a filter plate (P1) and a water jet vacuum. Samples that show no net water absorption can be evacuated directly without adding water.
6. Sample preparation must be completed and the test initiated within 48 hours. The sample may be exposed to a temperature above 4°C for a maximum of 24 hours. If this cannot be ensured, it must be frozen at -18°C to -20°C within 24 hours of collection. The freezing process must be documented. Such samples must be thawed gently within 24 hours. During thawing, the temperature must not exceed 20°C.

Measurement

1. A 40-gram sample is used for the test. The size of the measuring vessel should be selected based on the sample's load level. If necessary, empirical values should be used. The pressure difference should not exceed 100 hPa. If this cannot be achieved with the largest vessel, the sample must be re-ventilated. The "Warning Differential Pressure" function is useful in this case (see notes below).
2. Three samples must be analyzed in parallel at each sampling location.
3. After inserting the CO_2 absorber, the measuring vessels must be tightly sealed with their lids and measuring heads (see notes below).
4. The measurement results must be recorded hourly. The corresponding settings can be configured via the controller.
5. The measurement process is started using the controller, and the measurement vessels are incubated at 20°C (thermostatic chamber).
6. The evaluation period is 4 days. It begins after an initial lag phase. The lag phase ends when the average O_2 consumption reaches 25 % of the value corresponding to the steepest increase in O_2 consumption during the first 4 days.
7. The mass of oxygen consumed during the lag phase is not included in the final result; that is, this value is subtracted from the total result obtained after the lag phase plus 4 days. The measurement may only be considered valid if the lag phase contribution does not exceed 10 % of the total value.

Result

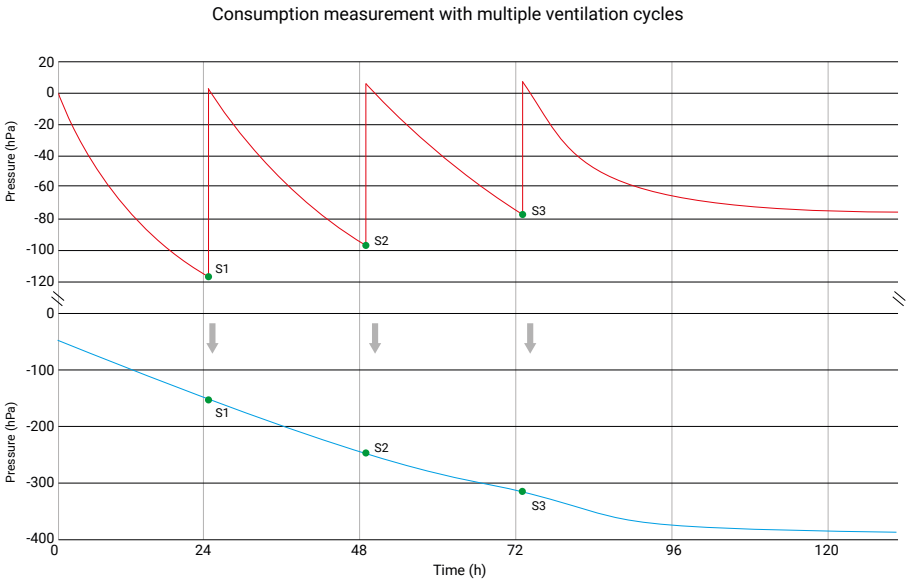
1. The data is transferred from the controller to a computer, where it is analyzed using a suitable program (such as a spreadsheet program, e.g., Microsoft Excel).
2. This is based on equations 2 and 7, respectively, mentioned above.
3. The results (analysis function and 3-hour averages) are presented by plotting oxygen consumption (mg O_2) per gram of dry matter against time in hours.
4. The final result must be reported in mg O_2 per g of dry weight, to 2 significant figures, including the mean and standard deviation. Outliers that deviate by more than 20% from the mean must be excluded.

Practical Tips

OxiTop® Controller, OxiTop® C Sensors, OxiTop® IDS Sensors, and Multi 36x0
For details on how to use the devices, please refer to the corresponding user manuals.

Sample volume, vessel size, and aeration
The sample quantity is legally set at 40 g. If samples contain higher concentrations, correspondingly larger measuring vessels must be used. Reducing the sample quantity is not permitted in order to avoid excessive fluctuations in the results due to sample inhomogeneities. For highly contaminated samples, the vessels may need to be vented multiple times to allow for complete conversion without O_2 limitation. This is the case when the pressure difference exceeds 100 hPa. The vessels for the B6M and B6M2.5 systems have a sufficiently large diameter to ensure, after removing the lid, optimal replacement of the entire free gas volume with fresh, unused air containing 21 % O_2. It is helpful to briefly reach into the vessel with your hand to displace the gas volume. When using PF45/... sample vessels, due to the small vessel opening, the entire free gas space must be replaced with fresh air multiple times during aeration by means of a targeted purging procedure (e.g., a pump with a hose). Otherwise, there is a risk of O_2 limitation and thus of measurement distortion!

The measurement sequence with multiple ventilation looks like this:



The upper curve shows the actual pressure trend during the individual re-venting operations. The lower curve shows the overall trend after the individual segments have been combined. Changes in air pressure can cause the respective initial values after venting to deviate from the initial zero line. For the analysis, the total pressure changes must be taken into account; that is, the values should be referenced to the immediately preceding starting point after venting, not to the zero line.

Warning Differential Pressure
In “Pressure p” mode, the OxiTop® Control and IDS system offers the “Warning Differential Pressure” setting function. Here, for example, an average warning differential pressure of 100 hPa (recommended) is set. This setting can be adjusted higher or lower depending on the sensitivity of the biological material. After retrieving all measurement data via a batch retrieval, the controller’s sample management screen displays only those samples for which the set warning differential pressure has been exceeded. The affected samples can then be processed selectively - for example, by aeration.

Waterproofing
To ensure proper operation, it is important that the system remains airtight throughout the entire process. Therefore, when filling the measuring vessel with a sample, care must be taken to ensure that the rim of the lid and the gasket do not become contaminated. It is recommended to check the sealing surfaces for damage before each measurement. Clean surfaces, securing the lid with the retaining clips, and hand-tightening the measuring head ensure that the vessel is properly sealed. It is recommended to lubricate the flat gaskets on the lid rim with the supplied silicone grease to achieve optimal sealing. Do not lubricate the rubber sleeves! A simple check of the sealing function can be performed in “Pressure p” mode for, for example, 3 to 6 hours with the measuring vessel filled to approximately 50% capacity with water at 45 … 50 °C. As the water cools to the measurement temperature, a stable vacuum must be established.

Absorber

Soda lime (NKI) or caustic soda is used as the absorbent. Soda lime (NKI) has the advantage of being easy to handle in granular form as a solid and having a very large active surface area. Furthermore, it does not significantly affect the water balance of the sample, particularly during soil respiration measurements and AT_4 determinations. Finally, its CO_2 depletion - or absorber capacity - is indicated by the discoloration of an indicator. This allows the user to directly identify a potential CO_2 limitation or reliably rule it out. Typically, one teaspoon of soda lime is used per measuring vessel and then discarded. The absorber volume must be taken into account accordingly when accounting for the free gas space. Sodium hydroxide in the form of solid pellets, as used for BOD determination, cannot be used for soil respiration and AT_4 determination because it significantly alters the moisture content of the sample. It can be used in the form of a 2- to 4-molar sodium hydroxide solution. Typically, a volume of 30 to 40 mL is sufficient. This absorbent should also be replaced during intermediate aeration. The appropriate precautions for handling sodium hydroxide solution must be observed.

Measurement Function

The Controller or a Multi 36x0 IDS is particularly well-suited for conducting soil respiration and AT_4 measurements. It can be operated in the “Pressure p” or “BOD Special” measurement modes, as described in the user manual.

Determining Volumes

For an accurate determination, the individual variables must be substituted into the calculation equations as precisely as possible. Volumes play an important role here. The free gas volume can be determined by measuring the actual volume of the vessel and subtracting the volumes of the components inside it (support, beaker, absorber, etc.).

Settling Phase and Measurement Time

The actual measurement period for the AT_4 determination is 4 days. To ensure accurate measurement, taking into account the lag phase that occurs at the beginning, the total measurement period is generally assumed to be 6 days. To obtain meaningful, legally compliant data, the specified storage times and temperatures must be strictly adhered to.

Effect of Temperature

As can be seen from equations 2 and 7, the measurement temperature has a significant impact on the result. The high precision and resolution of the pressure measurement - 1 hPa - provided by the OxiTop®-C and OxiTop® IDS measuring heads guarantees a reproducible result. However, this also depends significantly on the stability of the ambient temperature. Based on current knowledge, very few climate chambers meet these requirements. The best results across the entire OxiTop® application spectrum have been achieved using thermal cabinets with PI controllers and recirculating airflow, which were specifically designed and developed for this purpose.

Bibliography

[1] Federal Soil Protection Act; Federal Law Gazette G 5702, Part I, dated March 17, 1998
[2] Ordinance on the Environmentally Sound Disposal of Municipal Waste and on Biological Waste Treatment Facilities; Federal Law Gazette G 5702, Part I, dated February 27, 2001
[3] DIN 19737 or its successor standard, DIN ISO 16072, 2005/6 edition
[4] DIN 38414-10 on the determination of dry matter

Note

Xylem Analytics Germany Sales GmbH & Co. KG
Am Achalaich 11
82362 Weilheim
Germany

Phone: +49 881 183-0
Info.XAGS@xylem.com
xylemanalytics.com

Quotes and Orders
Phone: +49 881 183-323
Orders.XAGS@xylem.com

Technical Information
Phone: +49 881 183-321
TechInfo.XAGS@xylem.com

Service
Phone: +49 881 183-325
Service.XAGS@xylem.com