

Application report AT₄ Respirometric Activity

Biodegradability of the dry residue of the original substance

Note

The topic of the present report is AT₄ determination. This is the respirometric activity of soil samples or fractions from MBA plants (mechanical biological waste treatment plants) over a period of four days.

This report was made by using the OxiTop[®] Control system. It can also be conducted with the OxiTop[®]-IDS system without any problems.

Measuring method Determination of biological oxygen demand

Legal note

The legal basis is the German Federal Soil Protection Law [1] and the Ordinance on Environmentally Compatible Storage of Waste from Human Settlements [2]. The purpose of the Federal Soil Protection Law is to effectively secure or restore the functioning of the soil. It determines the limits for self-checked measurements in the field of existing waste deposits monitoring. The Ordinance on Storage of Waste from Human Settlements specifies detailed requirements on chemical analysis and defines the allocation of landfill sites to classes. The allocation values specified there enable to monitor the rotting process. Microbial soil breathing is also subject of DIN 19737, its succeeding standard, ISO 16072 or its German version, DIN ISO 16072 [3].

Measuring equipment

The measuring systems of the OxiTop Control series are suitable for the analysis of soil samples.

with:

Controller

Software for data transmission from the controller to a PC

Cable to connect the controller to the PC

OxiTop-C measuring heads

Resp. corresponding OxiTop[®]-IDS heads and Multi 3630 IDS 3620 IDS

BOD

AR_BOD_AT4_lab_01_E



a xylem brand

Accessories

Depending on the measurement problem:

sample containers, 500 ml, 1 l or 2,5 l

lid adapter with stand and ring seal

retaining clips

rubber tubular

measuring beaker: $V = 50 \text{ ml}$

Reagents

soda lime absorber with CO_2 indicator

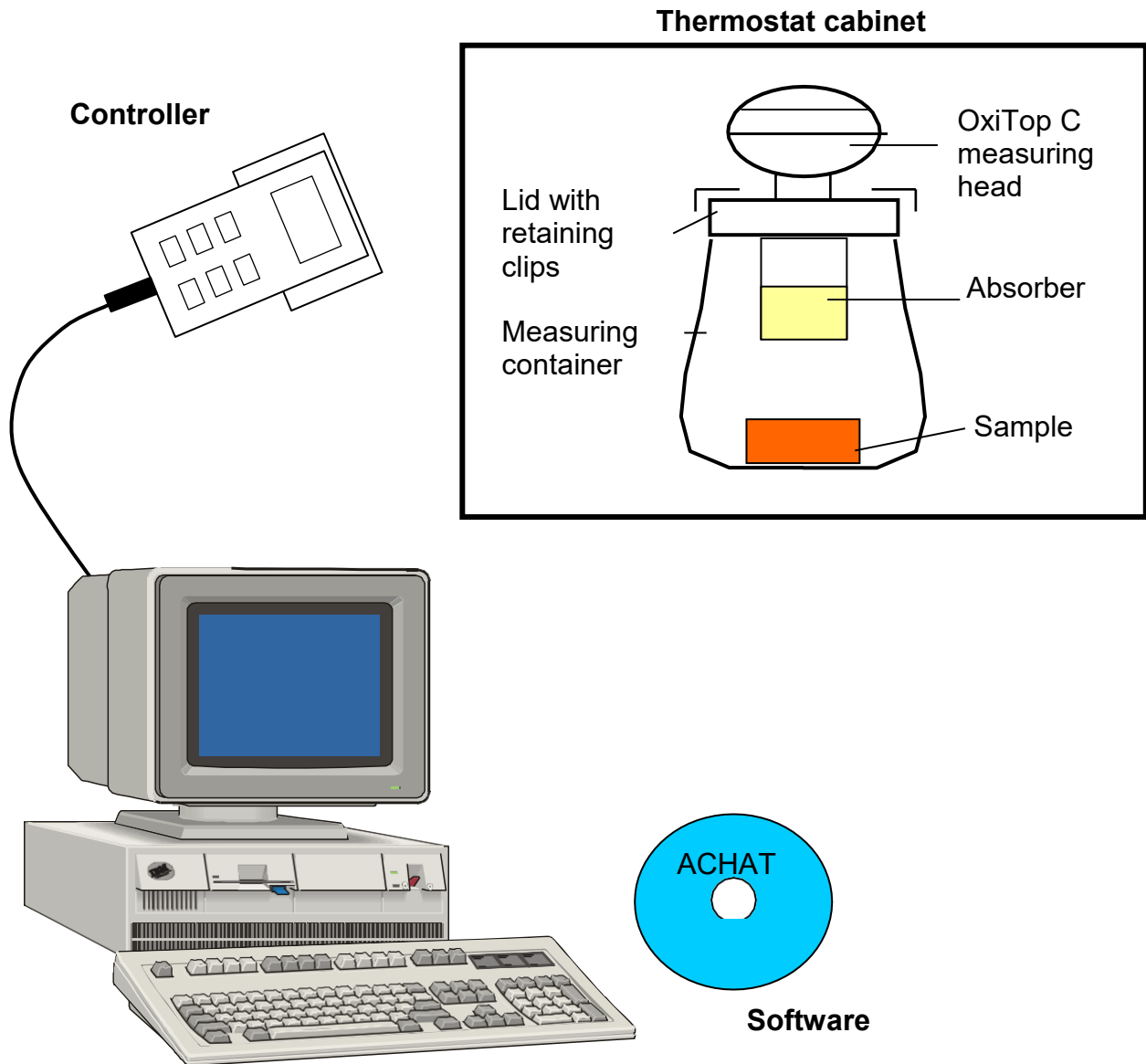
alternative: liquid absorber (NaOH or KOH solution)

Measuring
vessels with
measuring heads



Basic design of the measuring system

The measuring containers filled with the samples are incubated in an environment that is precisely temperature controlled (climatized room, thermostat cabinet etc.) and monitored with the controller (see Practical Instructions, *Temperature influence* on page 11).

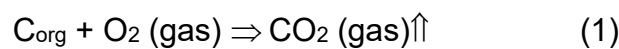


During measurement, measurement data of all measuring stations can be transmitted to the controller by means of the infrared interface any number of times. For monitoring purposes, the controller provides a direct graphic display of the measurement data. Via the serial interface, all stored data can be transmitted to a computer with the Achat communication program and the data cable.

The soil breathing or AT₄ value respectively is calculated with a suitable spreadsheet program (e.g. Microsoft Excel) or another specific software program.

Measuring principle and evaluation

The biological processes that take place in the sample are mainly based on the reaction of the existing carbon. According to Bundesgesetzblatt (German Federal Law Gazette) G 5702 and Abfallablagerungsverordnung (Ordinance on Environmentally Compatible Storage of Waste from Human Settlements), the evaluation of waste samples can be based on aerobic processes (AT₄ determination) or anaerobic processes (ferment test GB₂₁). In the absence of oxygen, anaerobic processes take place while developing biogas (essentially CH₄ + CO₂). For AT₄ determination, enough oxygen must be present so the aerobic process can develop carbon dioxide (CO₂).



To be able to measure the organic substance degradation, the reaction product, CO₂ that has formed in equivalent amounts has to be chemically bound by an absorber and thus removed from the head space. Only then does the pressure difference that has formed (i.e. the pressure change from the start of the measurement) allow to draw a direct conclusion on the consumption of oxygen. Suitable are liquid absorbers (NaOH or KOH solution) with the corresponding molarity or granulate soda lime. Maintaining a constant incubation temperature is especially important. On one hand, it provides a defined framework for microbial reactions and on the other hand, it enables to assign pressure changes to substance degradation by means of the gas law.

There is an interrelationship between the breathing activity and the fermentation test. In the present report, only the aerobic degradation is described due to the lower amount of work required and the considerably shorter measuring duration. In principle, the OxiTop Control system can also measure and record pressure increasing anaerobic processes that develop biogas up to 1350 hPa.

In the "Pressure p" measuring mode, the development of the pressure difference in the head space is determined. It is then available as a parameter for the calculation of soil breathing according to the following formula (see also [3]):

$$BA = \frac{M_{O_2}}{R \cdot T} \cdot \frac{V_{fr}}{m_{Bt}} \cdot |\Delta p| \quad (2)$$

where BA Soil breathing
M_{O₂} Molecular weight O₂ (31998 mg/mol)
R General gas constant (83.14 L·hPa/(K·mol))
T Measuring temperature (in Kelvin)
V_{fr} Free gas volume (in liter)

m_{Bt} Mass of soil dry matter (in kg)
 $|\Delta p|$ Value of pressure change (in hPa)

$$\text{wherein } V_{fr} = V_{ges} - V_{AM} - V_{Bf} \quad (3)$$

where V_{ges} Total volume spanned by the measuring container
 V_{AM} Volume of the absorption agent and auxiliary devices
 V_{Bf} Volume of the damp soil

The volumes can be determined by measuring or, if sufficient, by guessing. The dry matter can be determined according to DIN 38414-10.

The used soil dry matter, m_{Bt} can be calculated as follows:

$$m_{Bt} = m_{Bf} \cdot \frac{TS}{100 \%} \quad (4)$$

where m_{Bt} Mass of soil dry matter (kg)
 m_{Bf} Mass of soil moist substance (kg)
 TS Dry matter content in %

The result should be quoted with the incubation temperature and the measuring duration, e.g. soil breathing (20 °C) = 360 mg O₂/kg TS in 4 days (AT₄).

In the "BOD special" measuring mode, it is possible to make an estimate of the soil breathing values and the measuring range to be expected before measuring according to the following derivative and after entering the relevant parameters.

The BOD (BSB) display is calculated according to:

$$BSB = \frac{M_{O_2}}{R \cdot T} \cdot \left(\frac{V_{ges} - V_{Probe}}{V_{Probe}} + \alpha \frac{T}{T_0} \right) \cdot |\Delta p| \quad (5)$$

The following applies to soil breathing:

$$BA = \frac{M_{O_2}}{R \cdot T} \cdot \left(\frac{V_{ges} - V_{Probe}}{V_{Probe}} + \alpha \frac{T}{T_0} \right) \cdot \frac{1}{\rho} \cdot |\Delta p| \quad (6)$$

where BA Soil breathing
 M_{O_2} Molecular weight O₂ (31998 mg/mol)
 R General gas constant (83.14 L·hPa/(K·mol))
 T Measuring temperature (Kelvin)
 T_0 Temperature of the Celsius zero point (273.15 K)
 V_{ges} Total gas volume (in liter)
 V_{Probe} Volume of the sample (in liter)
 α Bunsen absorption coefficient
 ρ Density of the dry matter (kg TS/L)
 $|\Delta p|$ Value of pressure change (in hPa)

Converted according to equation (7) it can be seen that the BOD

display in the "BOD special" measuring mode according to equation (5) corresponds to the product from soil breathing and dry matter density.

$$BA \cdot \rho = \frac{M_{O_2}}{R \cdot T} \left(\frac{V_{ges} - V_{Pr obe}}{V_{Pr obe}} + \alpha \frac{T}{T_0} \right) \cdot |\Delta p| \quad (7)$$

In this mode, the OxiTop Controller OC110 provides a simultaneously calculated BOD measured value in mg O₂/L after free selection of the parameters (gas volume, sample volume, ambient pressure, measuring range, temperature, etc.). To obtain the soil breathing, this BOD value must be divided by the density of the sample. The setting of the corresponding general parameters is described in the operating manual of the controller.

Implementation

Sample preparing

1. The original sample, in its entirety, must be crushed to < 10 mm.
2. If necessary, interfering substances (glass, stones and metals) should be removed prior to crushing. Their weight components must be taken into account in evaluation of the test.
3. All constituents that are not part of the landfill formation such as PE bags should be removed as well.
4. Important data for later evaluation such as sample volume, dry matter density and container volume have to be determined. The dry matter comprises the mineral and organic constituents after complete removal of water by means of drying at 105 °C (see DIN 38414-10 [4]).
5. The optimum water content has to be adjusted (approx. 50 - 60 % of the max. water intake capacity). To do so, 300 g of the sample is moistened with 300 ml of tap water and extracted through a water-jet vacuum using a nutsch with filter plate (P1). Samples that show no absorption of water can be extracted without any addition of water.
6. Sample preparation must be completed, and the test started, within 48 hours. During this period, temperatures over 4°C are permissible for no more than 24 h. If it is not possible to ensure compliance with this procedure, the sample shall be frozen, within 24 h after sampling, at -18 to -20 °C. Freezing of samples shall be documented. Thawing of samples must be gentle, and must last no longer than 24 h; during thawing, the temperature must not exceed 20 °C.

Measurement

1. A sample of 40 g is to be used for the test. The size of the measuring containers should be selected depending upon the load level of the sample. If necessary, consult experience values. The pressure difference should be no more than 100 hPa. If this is not possible with the biggest container, venting is required. Here, the Warning difference pressure function is useful (see Instructions below).
2. Samples are to be tested in three parallel batches for each sampling point.
3. After adding the CO₂ absorber, the measuring containers have to be tightly closed with the lid and measuring head (see instructions below).
4. The measurement results have to be recorded hourly. The relevant settings can be made with the controller.
5. The measuring procedure is started with the controller and the measuring containers are incubated at 20 °C (thermostat cabinet).
6. The evaluation period is four days. It begins after an initial lag phase. The lag phase has ended when the mean oxygen consumption reaches 25% of the value that results in the region of the largest increase in the oxygen consumption within the first 4 days. Each of these values is expressed as a 3-hour mean value.
7. The weight of the oxygen consumed during the lag phase is not taken into account for the final result, i.e. this value is subtracted from the weight of the oxygen consumed throughout the entire test (lag phase + 4 days). It must not be more than 10% of the overall value. If this condition is not fulfilled, the measurement may not be taken into account.

Result

1. The data is transmitted from the controller to a computer. There, it is analyzed by means of a suitable program (spreadsheet program such as Microsoft Excel).
2. Basis are the equations 2 and 7 respectively quoted before.
3. The results are displayed (analysis function and 3-hour mean values) in a graphic (oxygen consumption in mg O₂ / g dry matter against the time in hours).
4. The final result should be quoted with 2 significant digits in mg oxygen per g dry matter with mean value and standard deviation. Outliers that differ from the mean value by more than 20 % are to be eliminated.

Practical instructions

OxiTop Controller and OxiTop C measuring heads

Details on the handling of the instruments are given in the respective operating manuals.

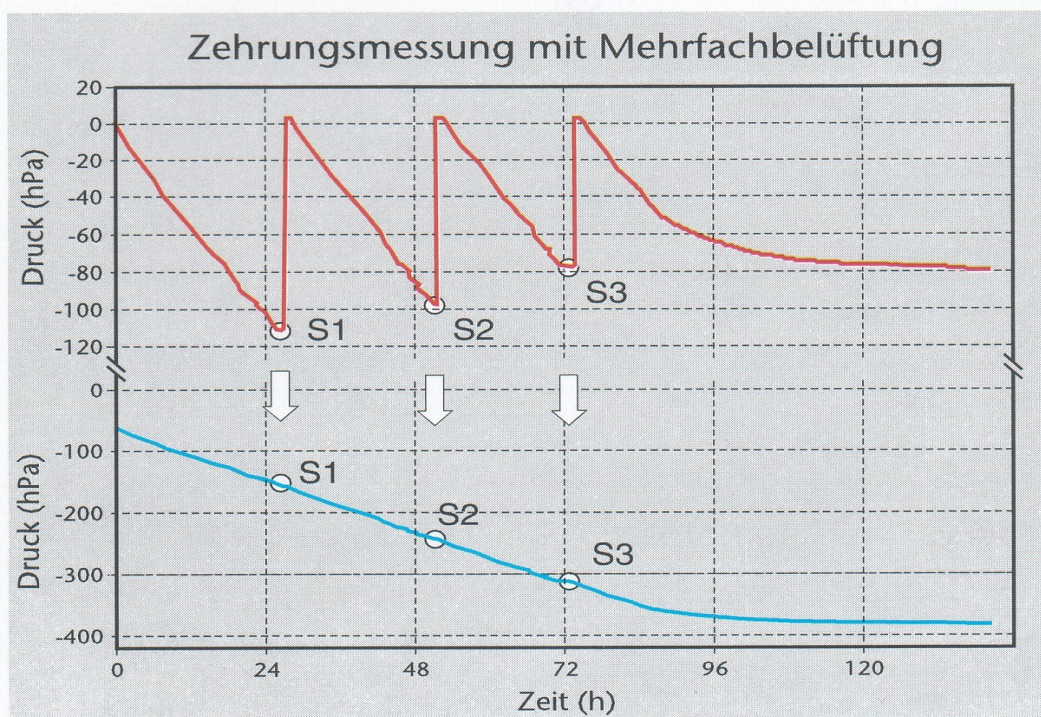
Sample quantity, container size and venting

The statutory sample quantity is 40 g. If the samples are heavily loaded, bigger measuring containers should be used. A reduction of the sample quantity is not intended, as strong variations of the results due to sample inhomogeneities should be avoided. If the samples are heavily loaded, the containers must be vented several times if necessary so the reaction can take place completely without any O₂ limitation. This is the case if the value of the pressure difference is more than 100 hPa. The containers for the B6M and B6M-2,5 systems have a large diameter to enable an optimal exchange of the entire free gas volume with fresh air with 21 % O₂ after the lid is removed. It is useful to hold one's hand into the container shortly in order to displace the gas volume.

If PF45/... sample containers with small openings are used, the entire free head space must be provided with fresh air several times by means of venting actions (e.g. pump with hose). Otherwise there is the danger of O₂ limitation and thus of measured value falsification!

The measuring process with repeated venting is as follows.

Multiple ventilation pressure vs. time



The upper curve represents the actual pressure development with the individual venting actions. The lower curve shows the development after the individual parts were put together. Due to air pressure changes, the respective start values may not be on the initial zero line after the venting. For evaluation, the entire pressure changes have to be taken into account, i.e. each value refers to the last starting point after venting but not to the zero line.

Warning difference pressure

In the "Pressure p mode", the OxiTop Control system has the setting function, "Warning difference pressure". Here, e. g., a medium warning difference pressure of 100 hPa (recommended) is set. This value can be selected higher or lower, depending on the sensitivity of the organisms. After the measurement data has been collectively called up, the sample management of the controller only displays those samples where the selected warning difference pressure was exceeded. The test samples concerned can then be selectively treated, e. g. be vented.

Sealing

In order to work correctly, it is important that the leak tightness of the system is maintained for the entire duration of the process. When filling the sample into the measuring container, make sure the seam of the lid and the sealing are not soiled. It is recommended to check the sealing surfaces for damages prior to each measurement.

Clean surfaces, fixing the lid with the retaining clips and tightening the measuring head by hand provide a proper sealing of the container. It is strongly recommended not to grease the sealing surfaces or any other parts as this could cause incorrect measurements and damage the system. An easy check of the sealing function that takes three to six hours can be done in the "Pressure p" mode by filling the measuring container about half with hot water of 45...50 °C. When cooling down to measuring temperature a stable negative pressure must develop.

Absorber

Soda lime Nkl or sodium hydroxide solution is used as an absorber. Soda lime Nkl has the advantage of being a granulated solid substance and thus easily manageable. Besides, it has a large active surface. Especially for measurements of soil breathing and AT₄ determination, it does not much affect the water balance of the sample. Finally, it indicates its CO₂ depletion or absorber capacity by a coloration of the indicator. Thus the user can directly see or definitely exclude a possible CO₂ limitation. Usually, a teaspoon of soda lime is used per measuring container and then discarded. The corresponding absorber volume has to be taken into account for the free head space.

Sodium hydroxide in pellet form as used for BOD determination cannot be used for soil breathing and AT₄ determination because it considerably changes the moisture content of the sample. It can be used in the form of a 2 to 4 molar sodium hydroxide solution. Normally, a quantity of 30 to 40 ml is sufficient. This absorber should also be exchanged when venting in between. Follow the relevant safety instructions when handling sodium hydroxide solution.

Measuring function

The controller is especially suitable to determine the soil breathing and AT₄. It can be operated according to the operating manual in the "Pressure p" or "BOD special" modes.

Determination of volumes

For an exact determination, the individual parameters have to be inserted in the equations as precisely as possible. The volumes are important here. The free gas volume can be determined by measuring the actual container volume and subtracting the parts that remain in the container (carrier, beaker, absorber etc.).

Lag phase and duration of measurement

The actual measuring duration for AT₄ determination is four days. To get it right with respect to the lag phase occurring at the beginning, one normally assumes that the total measuring duration is 6 days.

In order to obtain meaningful data consistent with legal requirements, the specified storing times and temperatures have to be kept.

Temperature influence

As can be seen from the equations 2 and 7, the incubation temperature has considerable effect on the measurement result. On one hand, the high precision and 1hPa resolution of the OxiTop-C measuring heads provide a reproducible result of the pressure measurement. This, however, also considerably depends on the stability of the ambient temperature. According to present knowledge, only very few conditioning chambers meet these requirements. Very good results over the entire OxiTop application range were achieved with the thermostat cabinets with PI controller and air circulation, which were especially designed and developed for this purpose.

Bibliography

- [1] Federal Soil Protection Law; Federal Law Gazette G 5702 Part I of 17 March, 1998
- [2] Ordinance on Environmentally Compatible Storage of Waste from Human Settlements and on Biological Waste Treatment Plants; Federal Law Gazette G 5702 Part I of 27 February, 2001
- [3] DIN 19737 or successive standard DIN ISO 16072, issued 2005/6
- [4] DIN 38414-10 on dry matter determination

Note

The information contained in our application reports is only intended as a basic description of how to proceed when using our measurement systems. In isolated instances or if there are special general conditions on the user side, exceptional properties of the respective sample can, however, lead to a change in the execution of the procedure or require supplementary measures and may, in rare cases, lead to a described procedure being unsuitable for the intended application.

In addition, exceptional properties of the respective sample such as special general conditions can also lead to different measurement results.

The application reports have been prepared with the greatest possible care. Nevertheless, no responsibility can be accepted for the correctness of this information.

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