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Application report Respiration activity of soils (basic principles, process characteristics)



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Matrix: Soils and solids
Analytical applications
no. 1

**Measurement of the
respiration activity of
soils with the
OxiTop® Control
measurement system**

**Basic principles and
process characteristics**

1st edition: July 1999

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Abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BOD	Biochemical oxygen demand [mg/L]
COD	Chemical oxygen demand [mg/L]
M	Mass [g]
MR	Relative molecular mass [g/mol]
N	Amount of substance [mol]
NADP	Nicotinamide adenine dinucleotide phosphate, oxidized form
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
P	Pressure [Pa] or [bar]; 1 bar = 1000 hPa = $10^5 \text{ Nm}^{-2} = 10^5 \text{ kgm}^{-1}\text{s}^{-2} = 100 \text{ JL}^{-1}$
R'_{CO_2}	Special gas constant of CO_2 at 273 K and 1013 hPa [$8,2502 \text{ Jmol}^{-1}\text{K}^{-1}$] from [10]
R'_{O_2}	Special gas constant of O_2 at 273 K and 1013 hPa [$8.301 \text{ Jmol}^{-1}\text{K}^{-1}$] from [10]
R	General gas constant (at 273.15 K and 1013 hPa): $8.3144 \text{ Jmol}^{-1}\text{K}^{-1}$
T	Absolute temperature [K]
ThOD	Theoretical oxygen demand [mg/g]
DS	Dry substance [%]
V	Volume [L]

Note: This report was made by using OxiTop® Control. All measuring procedures can easily be transferred to the OxiTop®-IDS system.

Introduction

Although biological activity has already been used for decades as the measure in routine measuring processes (BOD, spontaneous oxygen consumption [1, 2]) in the examination of water to analyse the effects of hazardous substances, comparable procedures in soil examination have not achieved any great significance up to now although they have been known for quite some time [3]. One of the reasons for this was that no easy-to-use measurement processes were available: They were either too time-consuming or challenging from an instrument perspective – and hence cost-intensive (for an overview, see [4]). Because of growing demand in the field of inherited waste for biological land recovery processes and also issues of biological waste treatment, an increasing need arose for simple biological measurement processes for solid materials and proposals for measurement processes were made [5,6]. The following shows the basic data of a measurement process in which measurements of the biological activity in soils, waste materials, composts and other solid matters can be carried out in a simple and reproducible way.

Material

OxiTop®-C measuring heads (WTW, Weilheim, Germany)
OxiTop® OC110 Controller (WTW, Weilheim, Germany)
ACHAT OC PC communication software (WTW, Weilheim, Germany)
Data transmission cable, type AK 540/B for RS 232 (WTW, Weilheim, Germany)
Measuring vessels MG 1.0 and 1.5 with lid-locking device DV/MG (WTW, Weilheim, Germany)
Temperature-controllable room or thermostat cabinet in variants TS606/2....TS606-G4/Var (WTW, Weilheim, Germany)
Personal computer, minimum requirements: 80486 processor, 16 MB RAM, RS232 interface
Windows 3.1 or 3.11 operating system (Microsoft Corporation, USA)
EXCEL 5.0 spreadsheet program (Microsoft Corporation, USA).
Preserving jar 0.55 L volume (Weck, Wöhr-Öflingen, Germany), vaseline
Laboratory scales (reading accuracy: min. 0.1 g) for weighing out soil
Laboratory scales (reading accuracy: min. 0.1 g) for reagent preparation

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Glass beakers (50 mL)
 Volumetric pipette, 50 mL
 Measuring cylinder, 50 mL
 Caustic soda solution (1 mol/L), caustic soda solution (2 mol/L)
 Sodium hydroxide (pellets), soda lime
 Deionised water

Basic principles of the procedure

1 Oxygen consumption and formation of carbon dioxide by aerobic organisms

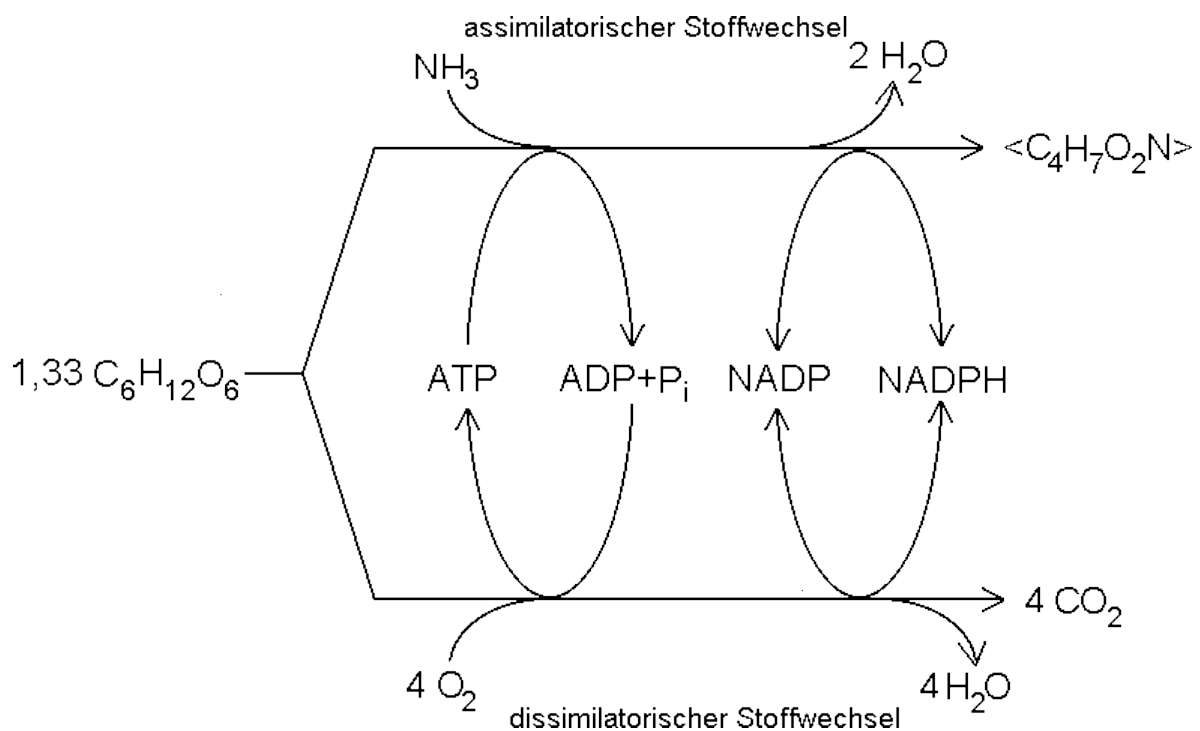


Figure 1:

Aerobic decomposition of an organic substance (an example of glucose is shown for simplicity). Organic substances are mineralised into CO_2 and H_2O on the one hand, where energy is conserved in the form of ATP (dissimilatory metabolism) and converted into cell substance (" $C_4H_7O_2N$ ") on the other hand, where ATP is again consumed as an energy source (catabolic metabolism). Both branches of metabolism are also still linked via NADP that provides or removes electrons ("bonded hydrogen") as required. The consequence of the "split" metabolism path is that the decomposition of a specific amount of substrate consumes only part of the theoretically determined oxygen (ThOD) (normally between 40 and 60%). The stoichiometry referred to in the drawing describes an idealised correlation. There are often deviations from this under real conditions depending on the efficiency factor with which the metabolism of an organism operates.

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As Figure 1 shows, biodegradable organic materials are broken down into carbon dioxide by the consumption of oxygen in aerobic conditions. Equation 1 makes this clear again using the example of glucose; equations can be compiled in the same way for all organic materials.



The "theoretical oxygen demand" (ThOD) for the complete oxidation of a substance can be calculated from the stoichiometry. For the reaction referred to in Equation 1, this value is 6 mol O₂/mol glucose or – based on mass – 1066 mg O₂/g. In the ideal case if the examined material is completely oxidised by the chemical oxidising agent, the ThOD (rated value) is identical to the COD (measured value).

If organisms use a chemical substance as a source of energy or material, they oxidise only one part of the substrate during the consumption of oxygen. The other part is used for the construction of cell substance (see Fig. 1). For this reason, less oxygen is consumed for the complete biological elimination of an organic material from an environmental aspect (soil, water, etc.) than the ThOD would indicate.

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2 Basic principles of manometric oxygen measurement

The manometric oxygen consumption measurement is a procedure practiced since the 20s for the characterization of respiratory processes ("Warburg procedure"; see [11]) and a proven procedure in the analysis of water. Mercury manometers have been used from the outset as measuring instruments and are now being replaced by new developments in the field of sensor engineering [7]. For official measurements in the context of wastewater monitoring, however, this has not received approval as yet.

The principle of manometric oxygen determination is based on the fact that oxygen is consumed by the organisms and carbon dioxide is formed at the same time. The carbon dioxide is bound by an adsorbent and thus does not appear as a free gas which means the change in pressure is only due to the consumption of oxygen. So that the oxygen consumption can be measured manometrically, the following conditions must be fulfilled:

-

The (biologically active) sample must be enclosed in a gas-tight container.

-

Above the sample there must be a sufficiently large gas space with air which provides limitless oxygen for the biological decomposition processes.

-

An adsorbent for carbon dioxide must be placed in the measuring vessel without the sample coming into contact with it.

-

A suitable pressure measuring instrument must be mounted on the reaction vessel.

-

The reaction vessel must be positioned in a location with a constant temperature during the measurement. Temperature changes lead to pressure fluctuations which make measurement of the oxygen consumption impossible (see Section 6). The biological processes lead to a consumption of oxygen with the simultaneous formation of approximately equimolar amounts of carbon dioxide which are bound by an alkaline adsorbent (e.g. sodium hydroxide, sodium hydroxide solution, soda lime or potassium hydroxide solution). Equation 2 shows an example of the reaction of carbon dioxide with sodium hydroxide.



The minimum stoichiometric amount of adsorbent which must be present in a reaction vessel for the complete measuring range to be exhausted can be calculated from Equation 2 (Table 1). An insufficient amount of adsorbent would lead to results that are too low. As will be shown later, a multiple excess of adsorbent must actually be present to ensure the absorption is sufficiently fast and complete.

The correlation between a change in pressure and the consumption of oxygen follows from Equations 3 and 4. The general gas equation shows that, with a constant vessel volume and constant temperature, a change in pressure can be attributed to a change in the amount of substance:

$$\Delta p = \Delta n \cdot R' \cdot T \cdot V^{-1} \quad [\text{Equation 3}]$$

Substance amount n results from the quotient m/M and, hence, the equation takes the following form:

$$\Delta p = \Delta m \cdot R' \cdot T \cdot V^{-1} \cdot MR^{-1} \quad [\text{Equation 4}]$$

Corresponding to the change in pressure, correlating amounts of oxygen as a function of vessel size, temperature and bulk density are specified in Table 2.

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Table 1:

Stoichiometric amounts of NaOH for the absorption of the maximum amount of CO₂ formed in the measuring vessels. The basis of the calculation is that one molecule of carbon dioxide is formed for each molecule of oxygen consumed. In fact, this only applies for substances with an average redox state of carbon (± 0). In highly oxidized substances such as, e.g. formic acid, two mol of carbon dioxide are formed for each mol of oxygen consumed whereas in heavily reduced substances (such as methane), the carbon dioxide formation is lower for the same oxygen consumption.

Volume of the reaction vessel [L]	Oxygen content in the reaction vessel ¹⁾ [mL]	Amount of substance of the carbon dioxide formed ²⁾ [mol]	Equimolar amount of sodium hydroxide for the complete absorption of CO ₂ ³⁾ [g]
0.55	115	0.00479	0.191
(MG1.0) 0.96	202	0.00834	0.334
(MG1.5) 1.50	315	0.01303	0.521

Note: The free gas volume is calculated from the difference of all the components contained in the reaction vessel/ volume of the total volume.

¹⁾ Volume of the reaction vessel x 0.21 (partial pressure of oxygen in air)

²⁾ $n = p \cdot V / R \cdot T$ with $p = 0.21 \text{ bar} = 21000 \text{ Nm}^{-2}$; $V = \text{value in column 1} \times 0.001 \text{ m}^3/\text{L}$, $R_{\text{CO}_2} = 8.2502 \text{ Jmol}^{-1}\text{K}^{-1}$ (special gas constant for CO₂ under normal conditions); $T = 293 \text{ K}$

³⁾ 2 mol NaOH binds 1 mol CO₂; $M_R (\text{NaOH}) = 40 \text{ g mol}^{-1}$; $m = n \cdot M_R$; see also the comment in the legend of the table

3 A measuring vessel from tried-and-tested "vacuum technology": the preserving jar

The demand for the development of a measuring system that is as economical as possible for soil respiration means resorting to commercial vessels and measurement instruments whenever possible. For the measuring vessels, the choice fell on preserving jars [12, 13, 14] which have been used since the introduction of the titrimetric method (Isermeyer, 1952 [3]) for corresponding measurements.

- Everyday use shows that these jars can be reliably sealed so they are gas-tight with a suitable cover and rubber seal.
- They are economical and should be purchased in standardised forms and vessel sizes.
- Commercial sizes are available with standard shaped lids
- The opening is large enough for "solid samples" to be easily placed in the vessel
- Because of the size of the opening it is easily possible, for example, to conduct mixtures by hand which is particularly advantageous for the subsequent addition of additives to the soil.
- It is easy to clean the vessel after use. These advantages led to the development of a special sealing cap for preserving jars that fulfils several requirements:
 - Adapter for attaching the OxiTop®/OxiTop® -C measuring head
 - Gas-tight sealing of the measuring vessel
 - Easy-to-remove mounting plate for the CO₂ adsorbent
- As well as this development, another system was developed which uses wide-necked screw-cap bottles whose particular advantage is the possibility of working in a sterile environment [8]. The application possibilities of this measuring system are described in other application reports (e.g. in [8])

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4 Description of the DV/MG lid-locking device

The DV/MG lid-locking device used in measuring vessels of the type, MG1.0 / MG1.5, is shown below in fig. 2.

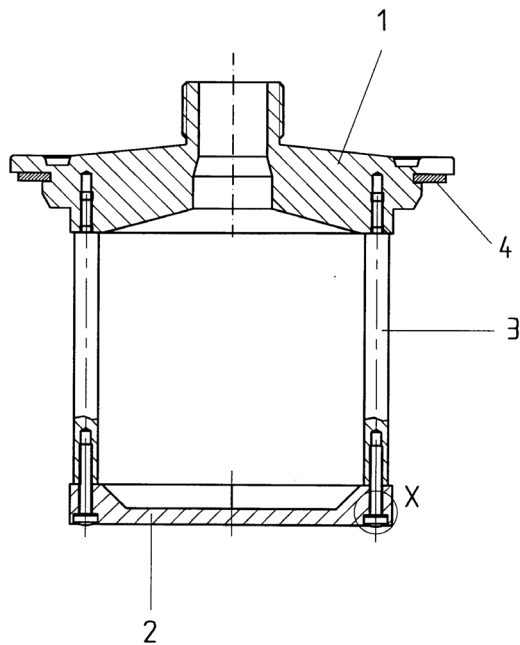


Figure 2:

DV/MG lid-locking device

1. Lid with screw connector for OxiTop® / OxiTop®-C measuring head
2. Base for CO₂ absorption vessel
3. Fixing bolts for the base
4. DV/MG/DI lid seal

The fixing bolts with the base are simple to remove.

Drawing: WTW, Weilheim, Germany

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5 Testing the system for tightness

To check the tightness, the lid-locking devices were provided with an additional drilled hole (8 mm) that was closed by a suitably impervious butyl rubber stopper. The rubber seals of the lid were lightly smeared with Vaseline and the lid was clamped with 4 clips to the measuring vessel (1 L) (Caution! Do not use silicone grease as this can cause damage upon contact with the measuring heads). The measuring head was screwed on tight after inserting the rubber sleeve in the thread joint. Do not grease the seal and screw fitting!

After pre-heating to 20°C the measuring heads were started by the controller with a measuring period of 10 days. The measuring vessels remained in the temperature-controlled room at 20°C for the entire measurement duration. Shortly after beginning the measurement the respective rubber plug was pierced with a cannula and 200 ml of air extracted with a syringe. After removing the cannula, the test solution remained in this room for a period of 10 days. During this time the pressure in the measuring vessels remained constant (confirmed by several trials). This confirmed the tightness and suitability of the measuring vessel for manometric measurements.

6 Influence of temperature and light

Unstable temperature conditions lead to strong fluctuations during the measurements. This is because of the dependence of the temperature on the pressure which can be calculated according to Equation 5 (T = absolute temperature):

$$p_2 = p_1 \cdot T_2/T_1 \quad \text{[Equation 5]}$$

With an initial pressure of 1000 hPa and an initial temperature of 20°C (293 K) the pressure increases to 1003 hPa for a rise in temperature of 1°C. With a measuring system resolution of 1 hPa, this is a not insignificant error.

The influence of solar radiation was also examined. In particular, it could be shown here that if moist soil is present in the measurement vessel, the incident light radiation led to a rapid increase in pressure. The reason for this is that the dark soil absorbs light very well causing it to warm up at the same time!

The measuring preparations must therefore be incubated in dark locations at a constant temperature such as are typically offered by thermostat cabinets or air-conditioned rooms.

7 Absorption speed with sodium hydroxide, soda lime and caustic soda solution

During the manometric respiration measurement three processes are superposed:

- a) the consumption of oxygen,
- b) the formation of CO₂ by organisms and
- c) the absorption by the respectively used adsorbent.

Should the CO₂ absorption flow off too slowly and free CO₂ thereby collect in the gas space, the pressure change would be no longer proportional to the oxygen consumption. For this reason, the absorption behaviour of different adsorbents was examined. With the OxiTop®-C measuring system, short-term measurements could be carried out very easily, reliably and almost continuously with a large number of measurement data.

Brief description of the method:

The impact of the sodium hydroxide pellets, caustic soda solution and soda lime adsorbents were examined. To do this, the respective adsorbent was put into the 50 mL measuring beakers and covered with a lid that is non-contact and removable or operable from the outside through the vessel wall (magnetic coupling). The measurement beaker was placed into a 1 L measuring vessel.

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The lid-locking device had a gas-tight butyl septum access. After starting the tightly screwed on OxiTop®-C measuring head, 59 mL of air was removed with a syringe cannula via the septum and the same amount of CO₂ was injected; this procedure was already recorded with the started measuring heads.

After taking the sealing cover off the absorption vessel, the decrease in pressure was measured over 2 to 3 hours via an externally held magnet. All work was carried out with pre-heated material at a temperature of 20°C in a thermostat cabinet.

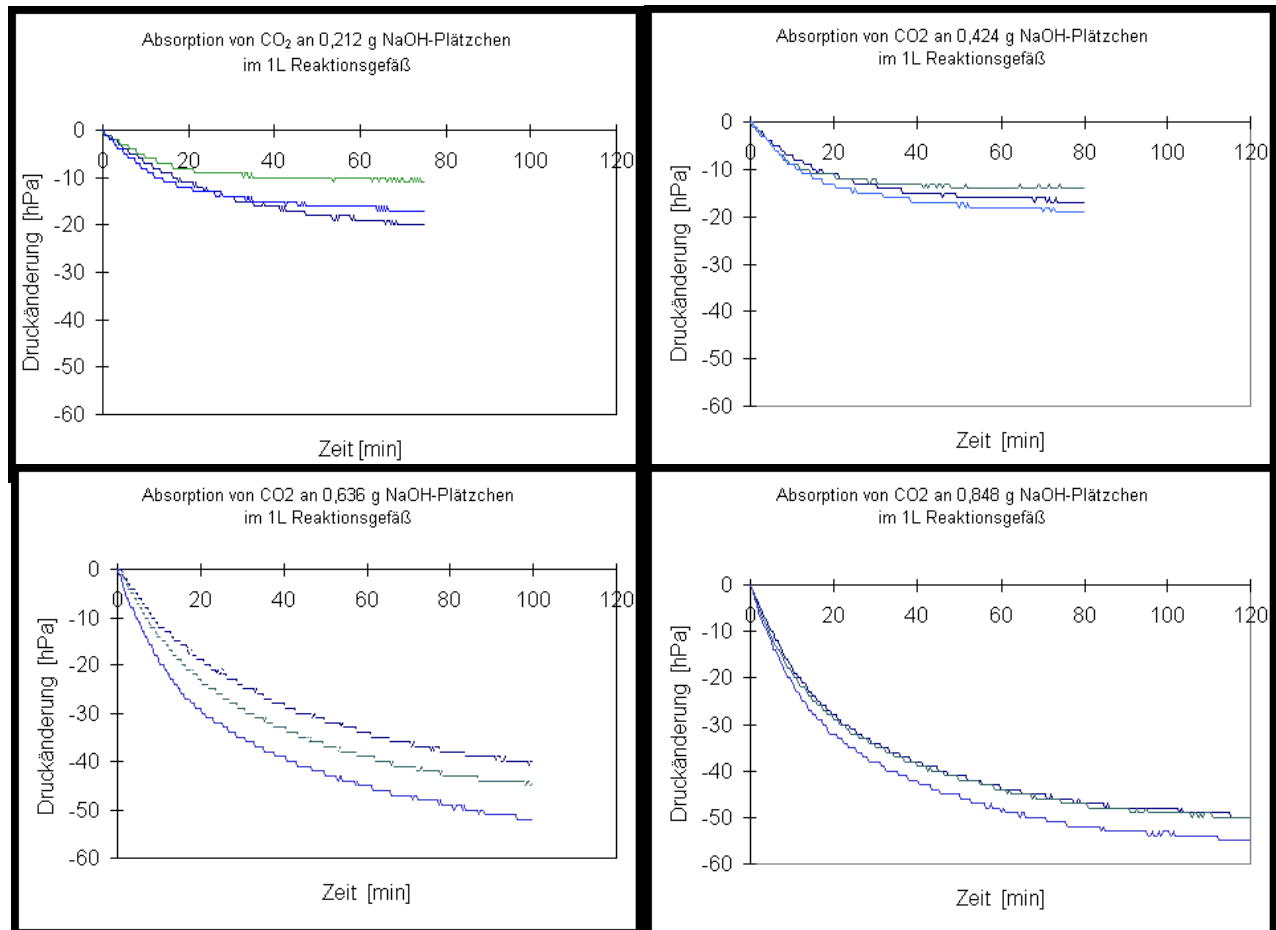


Figure 3:

Absorption of CO₂ by sodium hydroxide pellets at 20°C (the results of multiple determinations are shown each time). Increasing amounts of adsorbent were added to 50 ml measuring beakers in the 1 l measuring vessels (see headings of the partial figures; corresponding to 5.3, 10.6, 15.9 and 21.2 mmol NaOH). After sealing the vessels, 59 ml of air was removed in each case and immediately replenished by 59 ml of CO₂ (= 2.4 mmol). The measuring head was started and the decrease in pressure recorded. The stoichiometric ratio of NaOH to CO₂ appeared in the ratios of approximately 1:1, 1:2, 1:3 and 1:4. The measurements showed that at least a 4x stoichiometric surplus of NaOH pellets must be used in order to achieve complete and rapid absorption of CO₂. The single and double stoichiometric amount of NaOH is not nearly sufficient for a successful measurement.

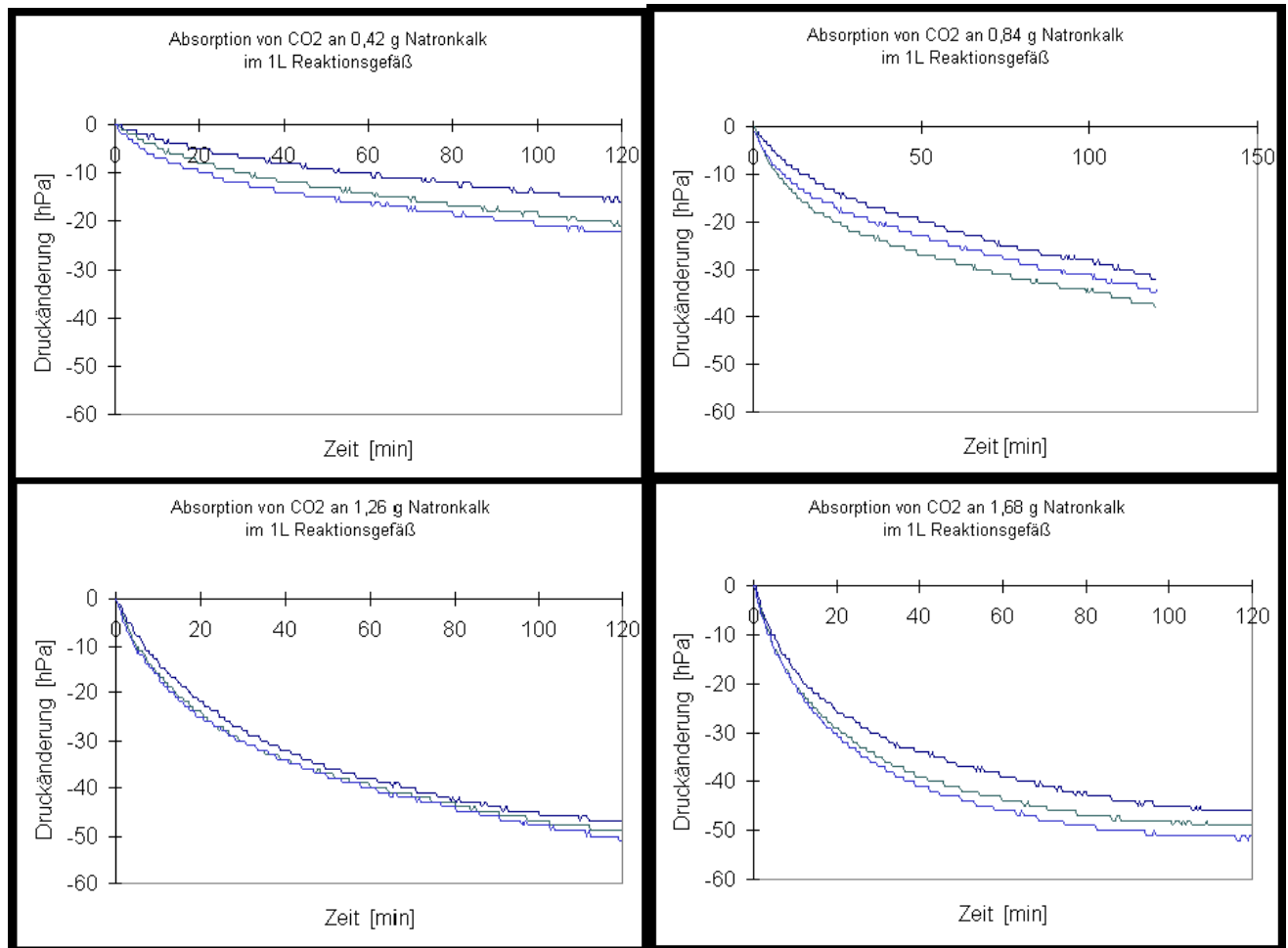
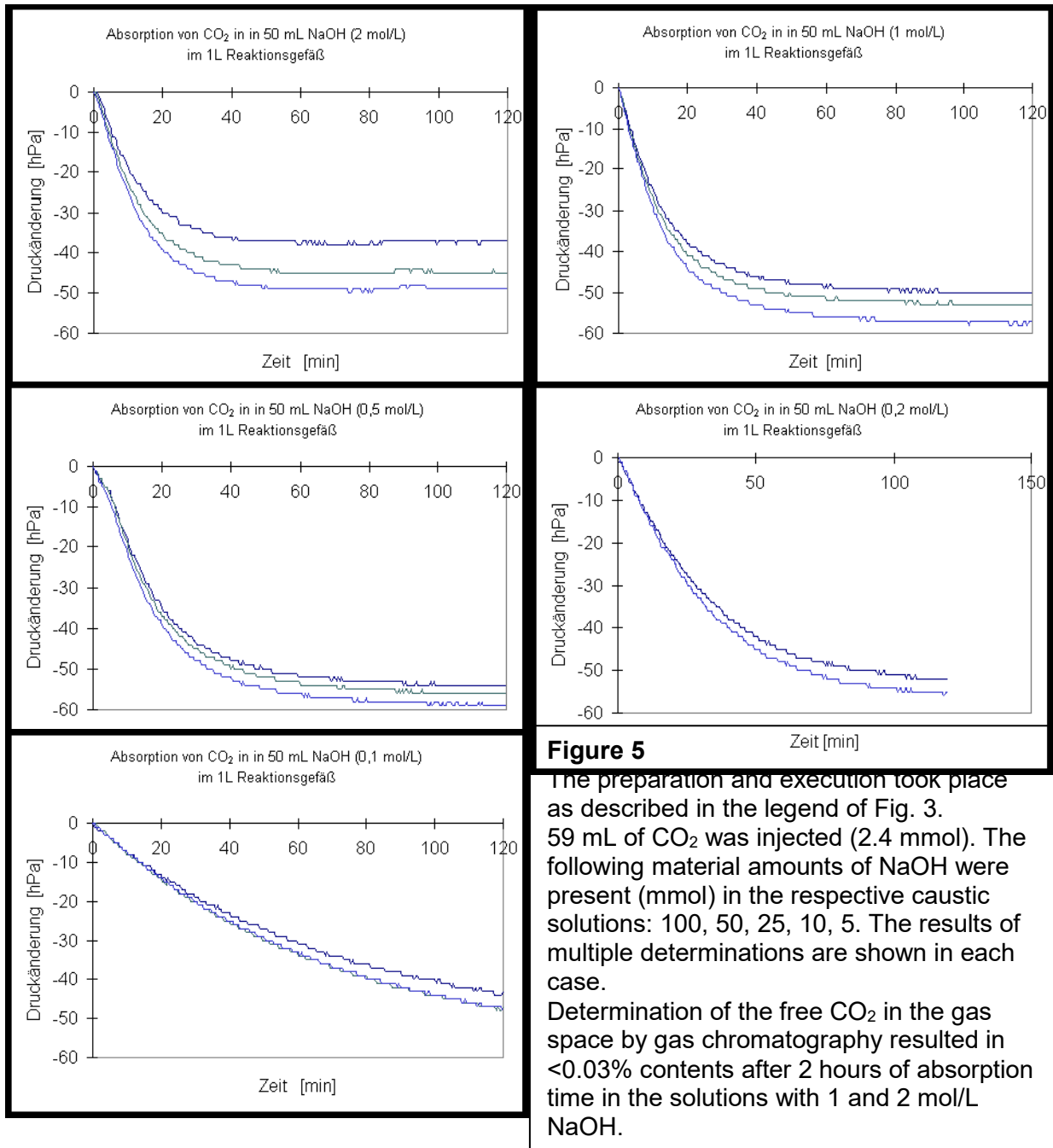


Figure 4:

The measurements and sample preparations were carried out as described in the legend of Fig. 3. Also here 59 mL of CO₂ were injected into each of the vessels (2.4 mmol; 106 mg) for absorption. The results of multiple determinations are shown in each case. The CO₂ absorption capacity of the soda lime was 28%, corresponding to a maximum of 118, 235, 353 and 470 mg). Here it also became apparent that a significant surplus of adsorbent must be present in order to ensure fast and complete absorption of the CO₂.

The results show that the most economical adsorbent when working with soils is caustic soda solution (50 mL, 1 mol/L) followed by soda lime. The use of solid sodium hydroxide is not advisable as this dries out the soils (see Section 8).

- The absorptive capacity is entirely sufficient (sufficient surplus)
- The absorption rate is high and does not slow down even if 200 mL CO₂ have already been absorbed (see fig. 6)
- The soil sample remains practically unchanged with respect to the water content (see fig. 7)
- The caustic soda solution can be titrated with HCl (1 mol/L) and, thus, the amount of CO₂ formed is obtained as additional information (see [3], although for more concentrated NaOH; see also [9]).



8 Absorption speed with increasing saturation of the absorbent

In the preceding examinations the absorption speed was examined with one-time injection of carbon dioxide (59 ml). It is expected that up to 200 ml of CO₂ must be absorbed in a 1 L measurement vessel during the course of a measurement. For this reason, an examination was made of whether the absorption speed changes if the multiple amount of CO₂ is injected. To do this, 50 mL of sodium hydroxide solution was placed in a 1 L measurement vessel (1 mol/L), 50 mL of air removed and then the same amount of CO₂ injected. The decrease in pressure was recorded for approximately one hour and then 59 mL of CO₂ was injected again. This procedure was repeated a total of 6 times so that 354 mL of CO₂ was injected altogether. This corresponds to an amount of material of 14.4 mmol where the sodium hydroxide solution contained a total of 50 mmol NaOH (allowing for the stoichiometry, this is approximately a 1.7x surplus of adsorbent).

The result (Fig. 6) shows that the absorption speed with increasing saturation does not slow down within the concentration range of interest.

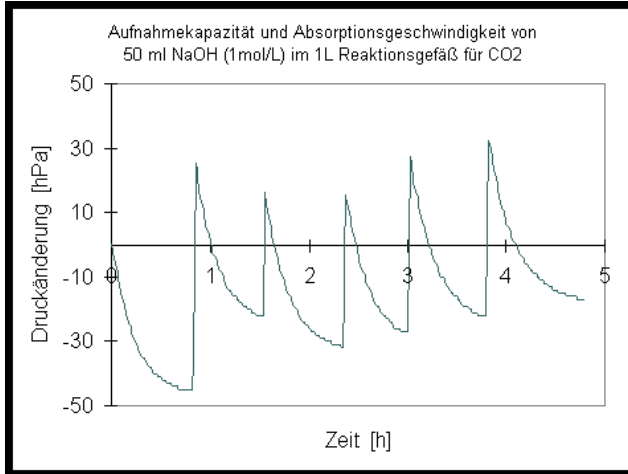


Figure 6: Influence of multiple CO₂ injections (59 mL in each case) on the absorption speed in the 1 L measuring vessel with 50 mL NaOH (1 mol/L) at 20°C.

9 Influence of adsorbents on the water content of soils

Adsorbents also frequently bind water as well as CO₂. This can lead to the soil samples in the vessel drying out. The three adsorbents mentioned above were examined for this effect for this reason.

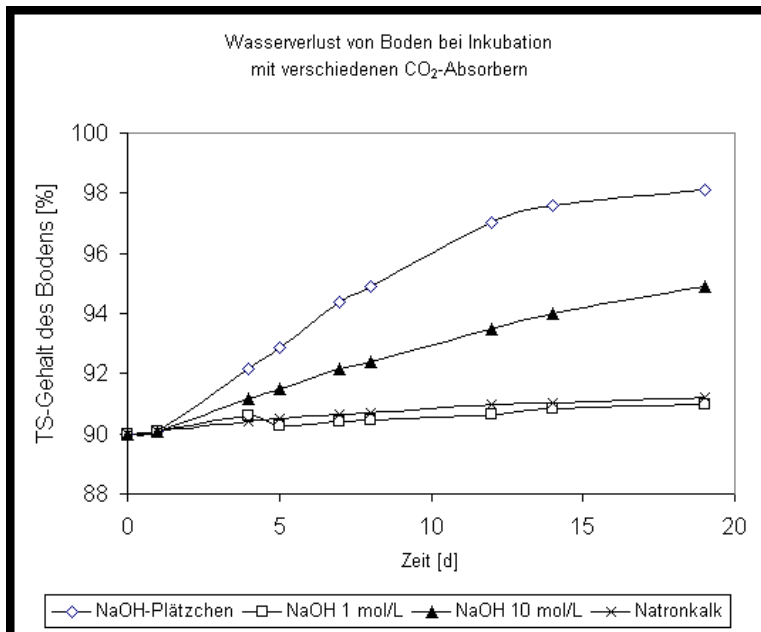


Figure 7

Dependency of the content of dry substance in a soil with incubation using different adsorbents. Four solutions with 100 g each of dry soil substance (damped with 11 g of water) were produced (corresponds to a water content of 10%, or dry substance content of 90%). The following were used as adsorbents: 4 g NaOH pellets, 50 mL NaOH (1 mol/L), 10 mL NaOH (10 mol/L) and 4 g soda lime. The solutions were sealed and incubated at 20°C in the dark. The vessels were opened at regular intervals and the weight increase of the adsorbent measured. The water loss of the soil, and hence the change of the DS (dry substance), were calculated from these values.

10 Calculation equation for oxygen consumption

The basic calculation of oxygen consumption in aqueous systems is given by the BOD equations (see WTW BOD Primer). [15]

These equations cannot be adopted directly for soils.
Equation 6 applies here for the incubation

Calculation of the soil respiration

$$BA = \frac{M_R(O_2)}{R \cdot T} \cdot \frac{V_{fr}}{m_{Bt}} \cdot \Delta p \quad [\text{Equation 6}]$$

BA = soil respiration [in mgO₂ / kgTS]

M_R (O₂) = molar mass of oxygen : 32000 mg/mol

V_{fr} = free gas volume [in L] (see equation 7)

R = general gas constant: 83,14 L mbar mol⁻¹ K⁻¹

T = measuring temperature [in K]

m_{Bt} = mass of dry soil substance in the measuring preparation

Δp = reduction in pressure of the measuring preparation [in mbar]

Result specifying the measuring period and the incubation temperature

Example: soil respiration (20°C) = 360 mg O₂/kg TS in 4 days

Note:

The use of the general gas constant R instead of the specific gas constant R' for oxygen (20°C) provides sufficiently accurate results (error <0.5%) for most practical applications.

The "free gas volume" for each measurement solution must be known

Calculation of the free gas volume:

$$V_{fr} = V_{ges} - V_{AG} - V_{AM} - V_{Bf} \quad [\text{Equation 7}]$$

V_{fr} = free gas volume [in L]

V_{ges} = total volume of headspace enclosed in the measuring vessel by the lid [in L]
(without soil, without absorption vessel, without absorbing agent)

V_{AG} = characteristic volume of the vessel for the absorbing agent [in L]

V_{AM} = characteristic volume of the absorbing agent [in L]

V_{Bf} = volume of the moist soil [in L]

Calculation of the dry soil substance used:

$$m_{Bt} = m_{Bf} \cdot \frac{TS}{100\%} \quad [\text{Equation 8}]$$

m_{Bt} = mass of dry soil substance [in kg]

m_{Bf} = mass of moist soil substance [in kg]

TS = dry soil substance content [in %]

100% = correction term

Table 2 lists some examples of standard measurement solutions where the amount of oxygen correlates to a corresponding change in pressure. Equation 9 is used for the calculation:

$$\Delta m = \Delta p \cdot V_{fr} \cdot M_R / (R'_{O_2} \cdot T) \quad [\text{Equation 9}]$$

$$\Delta p = 1 \text{ hPa} = 0.1 \text{ JL}^{-1}; T = 293 \text{ K}; M_R = 32 \text{ gmol}^{-1}; R'_{O_2} = 8.301 \text{ Jmol}^{-1}\text{K}^{-1};$$

Table 2:

Derivation of the amount of oxygen which correlates to a pressure change of 1 hPa in different test preparations.

Measuring vessel	Measuring-volume with DV/MG ¹⁾	Net weight of soil	Soil volume ²⁾	Volume of the absorption vessel	Volume of adsorbent	Free gas volume V _{fr} ³⁾	Consumed amount of oxygen with a change in pressure of 1 hPa ³⁾
MG 1.0	960 mL	100g TS +10g H _a O	0.06 L	0.01 L	0.05 L	0.84 L	1.11
MG 1.0	960 mL	200g TS +20g H _a O	0.12 L	0.01 L	0.05 L	0.78 L	1.03
MG 1.0	960 mL	300g TS +30g H _a O	0.18 L	0.01 L	0.05 L	0.72 L	0.95
MG 1.5	1500 mL	100g TS +10g H _a O	0.06 L	0.01 L	0.05 L	1.38 L	1.82
MG 1.5	1500 mL	200g TS +20g H _a O	0.12 L	0.01 L	0.05 L	1.32 L	1.74
MG 1.5	1500 mL	300g TS +30g H _a O	0.18 L	0.01 L	0.05 L	1.26 L	1.66
Preserve jar, 0.5 L	550 mL	100g TS +10g H _a O	0.06 L	0.01 L	0.05 L	0.43 L	0.57

1) The volume of the vessel was measured in liters after the lid was attached (tolerance: ± 2%)

2) For purposes of simplification the volume for dry soil was assumed to be 0.5 mL per g DS in this example table, which is roughly applicable in many cases. This is sufficient for practical considerations; for precise measurements the actual soil volume must be determined experimentally

3) Calculated with equation 9

**Note: A technical change in the construction of the lid resulted in other free gas volumes.

It is recommended to determine the free gas volume through calibration prior to starting the trial.

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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.

BOD



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

The current version of our general terms of business applies.

Any further questions? Please contact our Customer Care Center:

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