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Applications for analysis

Determination of the biological biodegradability of organic substances under anaerobic conditions using the OxiTop® Control measuring system.

Experimental solution examples

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Introduction

For decades, biological activity has been used as the quantity to be measured in the routine measurement procedure for the analysis of water (BOD, depletion) to evaluate the effect of pollutants. However, comparable procedures have achieved no great significance in the determination of anaerobic degradation analyses to date. This lies in the fact that, amongst other factors, the measurement procedures were either time consuming and, thus, cost intensive or not easy to perform. As a result of questions within the field of biological waste material treatment under anaerobic conditions or the further development of anaerobic bacteria toxicity tests, demand for a simple and economic measurement procedure increased. Through the further development of the established OxiTop® Control measuring system in the field of aerobic analysis together with the development of specific new accessories, its application range could also be extended to include anaerobic applications.
This measurement procedure is presented below using a series of examples showing simple and reproducible measurements performed on the biological anaerobic degradation process in digested sludges or digested sludge emulsions, particularly in polymers and fats.

Fundamentals

Fundamental advantages of anaerobic degradation

The anaerobic degradation of pollutants, e.g. chemicals in industrial wastewater, has the following advantages as opposed to aerobic degradation procedures:

- The amount of biological mass that results is considerably lower than in aerobic processes.
- The energy intensive aeration procedure is no longer required.
- This forms biological gas (carbon dioxide (CO_2) and methane (CH_4)) that can be used for the generation of energy.

Measuring principle

Anaerobic degradation causes the formation of biological gas (CH_4 and CO_2) that results in an increase in pressure in the headspace of the gasproof, closed graduated flask.
The test substance is used as a C source and is dissolved in water and diluted with a mineral saline solution. (Test substance >> C compounds in digested sludge). A specified amount of digested sludge from a communal sewage treatment plant is added. Sample solutions are conducted parallel to this **without** any test substances **(blank solutions or control solutions)**. The graduated flasks are gently stirred using magnetic stirrers and inductive stirring systems and incubated in the dark at 35°C. The resulting biological gas pressure is recorded by the OxiTop®-C measuring system and automatically stored throughout the entire time period of the experiment. The data are transmitted remotely (by infrared) to the OxiTop® controller. The entire pressure development or biological gas formation of the sample solution can be displayed graphically on the controller. The data stored in the controller are transmitted to a PC and further processed in Excel. After the subsequent degradation that results in the formation of a plateau (constant biological gas pressure), the resulting CO_2 can be collected or absorbed according to a) or b) described below.

a) CO_2 in the headspace
by injecting a CO_2 absorber (e.g. 1 ml KOH (30% v/v)) through the septum in the stopper of the graduated flask. The absorption process leads to a fall in pressure in the headspace.

b) CO_2 in the sample and headspace
by injecting an expelling solution (e.g. 1 ml HCl 19% v/v) into the experimental solution through the septum. The CO_2 driven out of the liquid phase and into the headspace causes an increase in pressure in the headspace. When the CO_2 has been completely driven out of the solution, the CO_2 contained in the headspace is absorbed as described in a) and, as a

result, leads to a reduction in pressure. The resulting residual pressure in the experimental solution minus the residual pressure in the blank solution results in a differential pressure that corresponds to the amount of CH_4 that is formed. This can be used to make quantitative statements on the anaerobic biological degradability of the organic substances being tested.

Basis of calculation

The determination of the anaerobic biological degradability of organic compounds in digested sludge is described in the DIN EN ISO 11734 (1998) or DEV L47 as a process that involves the measurement of the biological gas production.

For practical reasons, the gas pressure is measured in hectopascal ((1 hPa = 10^2 Pa, 1 Pa = 1 N/m^2); or 1 hPa = 1 mbar), the volume in milliliters and the temperature in °C.
The temperature is specified in Kelvin for the calculation; e.g. 35°C = 308.15 K.

Basis of all following calculations is the ideal gas law:

$$n = \frac{p \cdot V}{R \cdot T}$$

where:
n number of mols of gases formed [mol]*;*
p gas pressure in Pascal [N/m^2];
V gas volume [m^3];
R gas constant [8,314 J/(mol * K)];
T incubation temperature [K].

If the calculation is done with the „normal“ units hPa resp. mL as well as the gas constante *R* and a temperature *T* of 35°C (equivalent to 308,15 K) are introduced as a factor in the equation, then the formula simplifies to:

$$n = p \cdot V \cdot 3{,}903 \cdot 10^{-8}$$

where:
n number of mols of gas formed [mol]*;*
p gas pressure [hPa];
V gas volume [mL];

Carbon content in the gaseous phase

The carbon content in the gaseous phase is represented by the sum of methane and carbon dioxide. The net carbon produced in the gas formed in the gaseous phase (after subtracting the respective blank value) as a result of the substance degradation is calculated according to:

$$n_{CO_2,g/CH_4} = \frac{\Delta p \cdot V_g}{R \cdot T} \cdot 10^{-4}$$

where:
$n_{CO_2,g/CH_4}$ number of mols of gases formed (carbon dioxide and methane) [mol]*;*
Δp difference of the gas pressure in the respective test vessel at the end of the experiment minus the gas pressure at the beginning of the experiment minus the respective difference of the blank values in millibars [hPa];
V_g volume of the gaseous phase in the vessel [mL];
10^{-4} conversion factor for Pa in hPa and cubic meter in milliliter

The curve of the biological degradation is plotted as an accumulated increase in pressure Δp in hPa against time. The lag phase can be read (in minutes or in days) from this curve. The lag phase is the time from which the experiment begins up to the point in time at which significant degradation occurs (see examples in the results section).

Carbon content in the aqueous phase

The carbon in the aqueous phase is mainly in the form of carbonate resp. hydrogen carbonate, this means inorganic carbon. By injecting hydrochloric acid in the sample the carbonate is transformed in carbon dioxide. This causes an additional increase in pressure, that corresponds to the carbon content of the aqueous phase. It is calculated as follows (The blank value can be calculated similarly):

$$n_{CO_2,l} = \left(\frac{p_2 \cdot (V_g - V_{HCl}) - p_1 \cdot V_g}{R \cdot T} \cdot \right) 10^{-4}$$

where:

$n_{CO_2,l}$ number of mols of gas formed (carbon in the aqueous phase) [mol];
p_1 absolut gas pressure [hPa] before the injection of hydrochloric acid;
p_2 absolut gas pressure [hPa] after the release of carbon dioxide;
V_g gas volume [mL];
V_{HCl} volume of added hydrochloric acid [mL]
R gas constante [8,314 J/(mol * K)];
T incubation temperature [K].

Differentiation between methane and carbon dioxide

By injecting KOH-solution in the rubber sleeve (in the gaseous phase of the bottle!), the formed carbon dioxide is bound. The total amount of carbon dioxide is calculated as follows (The blank value is calculated in the same way):

$$n_{CO_2,l/CO_2,g} = \left(\frac{p_3 \cdot (V_g - V_{HCl} - V_{KOH}) - p_2 \cdot (V_g - V_{HCl})}{R \cdot T} \cdot \right) 10^{-4}$$

where:

$n_{CO_2,l/CO_2,g}$ number of mols of total formed carbon dioxide [mol];
p_2 absolut gas pressure [hPa] before the injection of KOH-solution;
p_3 absolut gas pressure [hPa] after the injection of KOH-solution;
V_g gas volume [mL];
V_{HCl} volume of added hydrochloric acid [mL]
V_{KOH} volume of added KOH-solution [mL]
R gas constante [8,314 J/(mol * K)];
T incubation temperature [K].

remark: If the carbon content of the aqueous phase is not determined, then V_{HCl} does not appear and p_1 replaces p_2 in the equation above.

Total carbon converted to gas

The total carbon converted to gas is calculated according to the equation:

$$n_C = n_{CO_2,g/CH_4} + n_{CO_2,l}$$

where:

n_C number of mols of the total carbon [mol]

Carbon content of the test substance

In order to determine the coefficient of biological degradation it is necessary to make a relation of the experimental values against the actual carbon content of the test substance. The carbon content is calculated with the total formula of test substance:

$$n_{C,theo} = \frac{m_{PS}}{M_{PS}} \cdot x_C$$

where:

$n_{C,theo}$ number of mols of carbon in test substance [mol]

m_{PS} mass of test substance in the bottle [g]

M_{PS} molar weight of test substance [g/mol]

x_C number of C-atoms in the total formula

Example: Glucose $C_6H_{12}O_6$: M_{PS} = 6*12 (g/mol) + 12*1(g/mol) + 6*16 (g/mol) = 180 (g/mol)

x_C = 6 carbon atoms

Coefficient of biological degradation

The coefficient of biological degradation is calculated from the gas concentration in the gaseous phase:

$$D_h = \frac{n_{CO_2,g/CH_4}}{n_{C,theo}} \cdot 100\%$$

The coefficient of total degradation is calculated according to:

$$D_t = \frac{n_{CO_2,g/CH_4} + n_{CO_2,l}}{n_{C,theo}} \cdot 100\%$$

where:

D_h coefficient of biological degradation from the gas formed in the gaseous phase in percent;

D_t coefficient of total biological degradation in percent.

The curve of the degradation process is obtained by plotting the degradation degree D_t against time.

Material, media and instruments

Reagents

- Deionized H2O
- CO_2 expulsion using HCl (19% v/v).
- CO_2 absorber, KOH (30% v/v).

Test substances

- Various degradable polymers and fats were tested in the experiments described below.
- **PVA (polyvinyl alcohol)** is used in the textile industry as a smoothing agent in the processing of threads.
- **CM starch (carboxymethyl starch)** is also used in the textile industry as a smoothing agent.
- **PHB (polyhydroxy butyric acid)** is used, particularly in medicine, as a biologically degradable polymer in implants. PHB is used in the laboratory reactor as a C source, as an electron donor, as an adhesive as well as for the elimination of nitrates in drinking water.
- **Deep frying fat**

The test substances are dissolved in deionized water

Reference substance

As an easily degradable reference substance, **glucose** is used in the experiment. Glucose is normally degraded to > 60%.

The glucose is dissolved in deionized water

Media

- Tap water: pH 7.1
- Saline solution acc. to Baumann A and B:
  Saline solution A:
  5.44 g KH_2PO_4
  6.97 g K_2HPO_4
  10.70 g NH_4Cl ad 1000 ml deionized H_2O, pH 7.0.

  Saline solution B:
  2.19 g $CaCl_2 * 6\ H_2O$
  2.03 g $MgCl_2 * 6\ H_2O$
  0.4 g $FeCl_2 * 4\ H_2O$
  6.3 mg $MnCl_2$
  1.0 mg $ZnCl_2$
  0.6 mg $CuCl_2$
  0.2 mg $Na_2MoO_4 * 2\ H_2O$
  12.2 mg $Co(NO_3)_2 * 6\ H_2O$
  1.0 mg $NiCl_2 * 6\ H_2O$
  1.0 mg Na_2SeO_3 ad 1000 ml deionized H2O

- Solution acc. to TeGeWa:
  85 mg KH_2PO_4
  218 mg K_2HPO_4
  334 mg $Na_2HPO_4 * 2\ H_2O$
  475 mg $(NH_4)_2SO_4$
  33 mg $CaCl_2 * 2\ H_2O$
  23 mg $MgSO_4 * 7\ H_2O$
  0.25 mg $FeCl_3$ ad 1000 ml deionized H2O

Microorganisms

- Digested sludge from a public sewage treatment plant with predominantly communal use;
- Addition of 25 ml (10% v/v).

Equipment

Customary or general laboratory equipment such as pipettes, beakers, measuring cylinders, etc.

- OxiTop® -Control sensors (WTW, D-82362 Weilheim, FRG).
- OxiTop® OC110 controller (WTW, D-82362 Weilheim, FRG).
- ACHAT OC PC communication software (WTW, Weilheim, FRG), data transmission cable, type AK 540/B for RS 232,
  PC, minimum requirement: 80486 processor, 16 MB RAM, RS 232 interface,
  Windows 3.1 or 3.11 operating system.
- EXCEL®5.0 spreadsheet program (Microsoft).
- MF 45/500 graduated flasks (WTW Weilheim, FRG).
- OxiTop® AD/SK adapter with screw-on stopper (WTW Weilheim, FRG).
- TS 606/2-Var thermostat cabinet (WTW Weilheim, FRG).
- IS 6-Var inductive stirring system (WTW Weilheim, FRG).
- Anaerobic tent (Coy Lab. Prod., USA) with N_2 / H_2 atmosphere and sluice.
- pH meter (readout accuracy 0.01 pH).
- Laboratory balance (min. reading precision 0.1g).

Description of the experiment

Preparation

- Determination of the bottle volume (gas volume) of the MF 45/500 graduated flasks used in the experiment with GK 600, OxiTop® Adapter AD/SK rubber stoppers and an RST 600 magnetic stirrer by:
- Determination of the **dead weight** of the graduated flasks,
- Air bubble-free filling of the graduated flasks with water and determining the weight of the filled bottle.
- Determination of the gas volume by difference formation.
- The average value per bottle is: **617 ml liquid volume**.
- An **experimental volume of 250 ml** per bottle is used
  for each of the following experiments (unless otherwise specified).
- This corresponds to a resulting **headspace of 367ml**.
- Preparation of anoxic media solutions (e.g. by introduction of N_2).

Preparation of the experimental and control solutions

- The filling of the experimental solutions is performed in the anaerobic tent under N_2 atmosphere.
- Place the magnetic stirrer in the graduated flask and fill with media solution (tap water, saline solutions acc. to Baumann, TEGEWA solution) according to the pipetting tables provided for the individual experiments in the results section.
- Inject with (25 ml) digested sludge that is as fresh as possible.
- Then, add the test substances (with the **exception** of the **glucose** that was initially injected **immediately before the start of the experiment**) according to the selected concentration range. Repeat determinations were performed each time.
- Preparation of a control preparation (blank value) without any test substance to determine the intrinsic activity of the digested sludge. Appropriate volumes of deionized H_2O were added.
- Determination of the pH value of the experimental solutions; if necessary, adjust to pH 7 ± 0.2 with diluted alkali or acid.
- Tightly close the openings of the graduated flasks with stoppers and septums and insert an OxiTop® adapter. Loosely attach the GL45 stopper screw fitting and rubber stopper.
- Incubate over night at 25°C (if technically possible, 35°C) in the anaerobic tent in order to ensure an anaerobic atmosphere for the following experiment.
- Preadjust the thermostat cabinet with stirring platforms to 35° C for 2-3 h.
- Before beginning the experiment, inject glucose solution (0.5 ml) into the appropriate bottles.
- Tightly screw on the GL45 stopper screw fitting and the OxiTop® measuring sensor and remove the bottles from the anaerobic tent.
- Select "Pressure p" as the type of experiment on the controller; Enter the duration of the experiment (e.g. 20 days); Set up the warning pressure, e.g. 250 hPa (see also BA OC110); Start the measuring sensors.

Incubation and measuring the gas

- For the selected duration of the experiment, the test solutions are incubated at 35°C in the thermostat cabinet. To obtain a uniform temperature in the samples, the test solutions are stirred at a slow speed by an inductive stirring system.
- Note:
- If necessary, in the case of sensitive bacteria stocks, continuous stirring can or must be avoided. An alternative to this is light movement on a shaking machine (50-100 rpm/min) or manual movement (e.g. 1x per day).
- The formation of biogas during the degradation procedure causes overpressure in the graduated flasks. The pressure values that are measured are automatically stored in the OxiTop® measuring system.
- The transmission of the measured values in the controller is performed by the operator depending on the biogas formation at selected time intervals. The pressure curve is observed and, if necessary, the current value stored.

If the warning pressure is exceeded, selective processing of the sample (e.g. aeration via the septum) must be performed.
At the end of the experiment, the pH value is determined, e.g. by withdrawing some sample. Furthermore, depending on the working method, 1 ml HCl is injected into the individual graduated flasks (see also 3.2). This is used to drive out CO_2 from the aqueous phase. The samples are incubated for a further 4 h at 35°C in the thermostat cabinet.

- 1ml KOH is then injected into the rubber stopper of the sample bottles. This absorbs the CO_2 formed in the headspace.
- The graduated flasks are incubated after the addition of an absorber (KOH) for a further 18-24 h at 35°C. The methane remains as the residual pressure.

- The stored measuring system data are read into the controller and transmitted directly from the controller to the PC using the Achat OC PC communication program. Evaluation of the data is performed using the spreadsheet program, Excel.

Performing the experiments; Results

Preparations with various buffer solutions

To find an optimum medium for performing the experiment, various buffer solutions were tested in the first part of the experiment.

Experiment 1: Using the following solutions (each in double preparation)

- Tap water
- Mineral saline solution acc. to Baumann A
- Saline solution acc. to TeGeWa

All the experimental solutions were injected with:

- Available digested sludge at a concentration of 6% (v/v).

Test substance for the degradation:

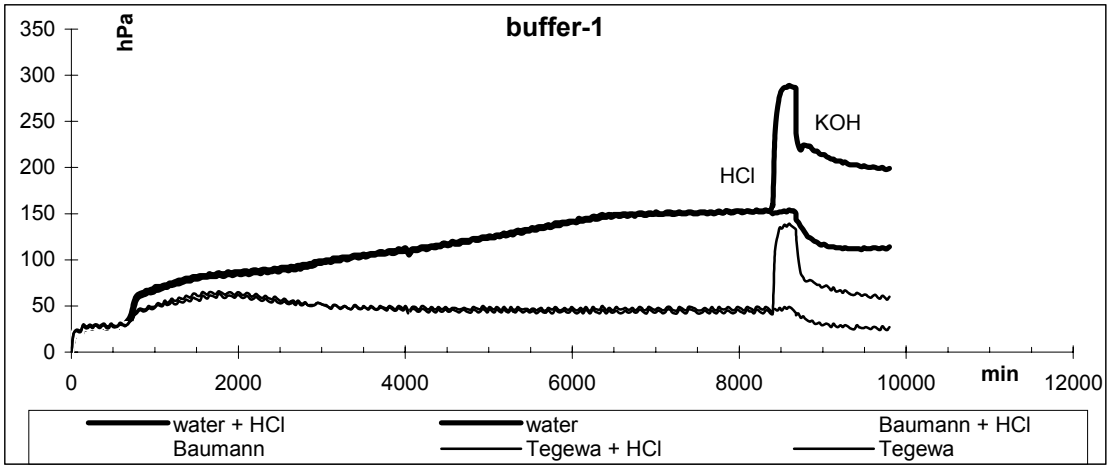
- Glucose; the glucose concentration used was 75 mg (final concentration in a volume of liquid in the graduated flasks of 365 ml)

The liquid volume in the bottles was 365 ml with a headspace of 252 ml.

Pipetting table:

	Tap water		Baumann A		TeGeWa	
Bottle	A	B	C	D	E	F
Digested sludge	19.5ml	19.5ml	19.5ml	19.5ml	19.5ml	19.5ml
Solution	345ml	345ml	345ml	345ml	345ml	345ml
Glucose (150mg/ml)	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
HCl	1ml	-	1ml	-	1ml	-
KOH	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml

Figure 1: Degradation of glucose in various buffer solutions with digested sludge (both with and without the expulsion of CO_2)



The curves displayed show the following experimental solutions:

- **Water + HCl:** the CO_2 was expelled at the end of the experiment with HCl (increase in pressure) and then KOH added as an absorber (pressure drop).
- **Water:** only KOH was added as an absorber at the end of the experiment (pressure drop).
- **Baumann + HCl:** CO_2 was expelled at the end of the experiment by HCl (slight increase in pressure) and KOH was then added as an absorber (pressure drop).
- **Baumann:** KOH was added as an absorber at the end of the experiment (pressure drop).
- **TeGeWa + HCl:** CO_2 was expelled by HCl at the end of the experiment (increase in pressure) and KOH was then added as an absorber (pressure drop).
- **TeGeWa:** KOH was added as an absorber at the end of the experiment (pressure drop).

The time at which the expulsion by the HCl took place, identified as a strong increase in pressure at the end of the experiment, is marked in the graphic. The time when the absorber KOH was added in the rubber sleeve (gaseous phase) that is linked with a drop in pressure is also marked.
Figure 1 shows an onsetting increase in pressure in all the solutions immediately after the start of the experiment that can be traced back to an initial temperature change from 25°C to 35°C. After a lag phase of 12 h, there is an increase in pressure that indicates anaerobic degradation.

- In the solutions that used tap water and TeGeWa solution, the increase in pressure was greater and, in the solutions that used tap water, this gradually turned into a plateau.
- In the TeGeWa solutions, the pressure fell again after reaching a maximum at 28 h and then leveled out to an almost constant value.
- Figure 1 clearly shows that a very good degradation result was achieved for glucose in the solutions that used tap water. In contrast to this, above all, a considerably lower increase in pressure was achieved in the TeGeWa solution and the degradation of glucose was delayed.
- Also, in the mineral saline solution acc. to Baumannn A, only a slight increase in pressure was detected and the plateau of the curve was similar to that obtained when the TeGeWa solution was used.
- After reaching a plateau phase, 1 ml HCl was injected into the respective experimental solutions after 139.5 h (5.8 days) and the CO_2 in the solution was expelled.
- After 144.2 h, the CO_2 now present in the gaseous phase was absorbed by the addition of an absorber (0.5 ml KOH) through the stopper of the graduated flask.
- This resulted in a drop in pressure.

Calculations in experiment 1, Figure 1

The experiment was performed without any control (i.e. without blank value digested sludge) so that the amount of gas formed can only be specified as an absolute value.
The following tables were calculated according to the general gas equation.

Table 1a: Formation of bio gases

Fermentation	Tap water + glucose		Tap water + glucose	
	Beginnig	End	Beginning	End
time [min]	616	8008	616	8008
p [hPa] (meas. value)	30	153	27	152
p [hPa]	1043.25	1166.25	1040.25	1165.25
Headspace [ml]	252.0	252.0	252.0	252.0
Temp [°C]	35	35	35	35
T [K]	308.15	308.15	308.15	308.15
Gas [mmol]	10.26	11.47	10.23	11.46
Diff. [mmol]		1.21		1.23

==> In tap water, 75mg glucose were converted to: 1.22 mmol carbon in the gas (CO_2g+CH_4)

Duration: 123.2 h

Table 1b: Expulsion using HCl

Expulsion	Tap water + glucose	
	Beginning	End
time [min]	8372	8652
p [hPa] (mea.value)	153	287
p [hPa]	1166.25	1300.25
Headsp.[ml]	252.0	251.0
Temp [°C]	35	35
T [K]	308.15	308.15
Gas [mmol]	11.47	12.74
Diff. [mmol]		1.27

==> In tap water, expelled with 1ml HCl: 1.27 mmol carbon in the gas ($CO_2fl.$)

Duration: 4.7 h

Table 1c: Breakdown of the amount of CO_2 into CO_2fl and CO_2g by KOH absorption

Absorption	Tap water with HCl		Tap water without HCl	
	Beginning	End	Beginning	End
time [min]	8652	9520	8652	9520
p [hPa] (mea.value)	287	201	153	112
p [hPa]	1300.25	1214.25	1166.25	1125.25
Headsp.[ml]	251.0	250.5	252.0	251.5
Temp [°C]	35	35	35	35
T [K]	308.15	308.15	308.15	308.15
Gas [mmol]	12.74	11.87	11.47	11.05
Diff. [mmol]		-0.87		-0.43

==> After HCl was added, 0.5ml KOH absorbed: 0.87 mmol CO_2

Duration: 14.5 h

==> Without HCl expulsion, 0.5ml KOH absorbed: 0.43 mmol CO_2

Duration: 14.5 h

Table 1d: Fermentation of glucose in a solution acc. to Baumann A (with digested sludge)

Fermentation	Baumann A + glucose		Baumann A + glucose	
	Beginning	End	Beginning	End
time [min]	616	8008	616	8008
p [hPa] (mea. value)	27	58	30	72
p [hPa]	1040.25	1071.25	1043.25	1085.25
Headsp. [ml]	252.0	252.0	252.0	252.0
Temp [°C]	35	35	35	35
T [K]	308.15	308.15	308.15	308.15
Gas [mmol]	10.23	10.54	10.26	10.67
Diff. [mmol]		0.30		0.41

==> In the Baumann A solution, 75mg glucose were converted to : 0.36 mmol carbon in the gas (CO_2g+CH_4)

Duration: 123.2 h

Table 1e: Expulsion using HCl

Expulsion	Baumann A + glucose	
	Beginning	End
time [min]	8372	8652
p [hPa] (measured value)	58	79
p [hPa]	1071.25	1092.25
Headspace [ml]	252.0	251.0
Temp [°C]	35	35
T [K]	308.15	308.15
Gas [mmol]	10.54	10.70
Diff. [mmol]		0.16

==> In the Baumann A solution, 1ml HCl expelled: 0.16 mmol carbon in the gas (CO_2fl)

Duration: 4.7 h

Table 1f: Breakdown of the amount of CO_2 into CO_2fl and CO_2g by KOH absorption

Absorption	Baumann A with HCl		Baumann A without HCl	
	Beginning	End	Beginning	End
time [min]	8652	9520	8652	9520
p [hPa] (measured value)	79	57	73	67
p [hPa]	1092.25	1070.25	1086.25	1080.25
Headspace [ml]	251.0	250.5	252.0	251.5
Temp [°C]	35	35	35	35
T [K]	308.15	308.15	308.15	308.15
Gas [mmol]	10.70	10.46	10.68	10.60
Diff. [mmol]		-0.24		-0.08

==> After HCl was added, 0.5ml KOH absorbed: 0.24 mmol CO_2

Duration: 14.5 h

==> Without HCl expulsion, 0.5ml KOH absorbed: 0.08 mmol CO_2

Duration: 14.5 h

Since the increase in pressure (Figure1) in the trial solutions using TeGeWa solution was very slow, the resulting biogas amounts were not calculated.

Table 2: Amounts of gas formed and degree of degradation after a total fermentation period of 123.5 h

		Tap water	solution Baumann A
Gaseous phase after fermentation	mmol C in gaseous phase (CO_2g + CH_4)	1.22	0.36
Gaseous phase after expulsion with HCl	mmol C in gaseous phase (CO_2fl)	1.27	0.16
Total gas	mmol C in gaseous phase (CH_4 + CO_2fl + CO_2g)	2.49	0.52
CO_2 absorption by KOH without HCl expulsion	mmol C in gaseous phase (-CO_2g)	0.43	0.08
CO_2 absorption by KOH after HCl expulsion	mmol C in gaseous phase (-(CO_2g + CO_2fl))	0.87	0.24
Degree of biological degradation, D_t		99.6%	20.8%
Degree of biological degradation, D_h (gas formed in the gaseous phase)		48.8%	14.4%

The amout of gas formed by expulsion with 1 mL HCl couldn't be absorbed completely with 0.5 mL KOH. For the following experiments the amount of KOH solution was increased to 1 mL. A check of the amount of absorption material can be useful for the special case.

Experiment 2 using various buffer solutions

In a further experiment using various buffer solutions, several parameters were changed:

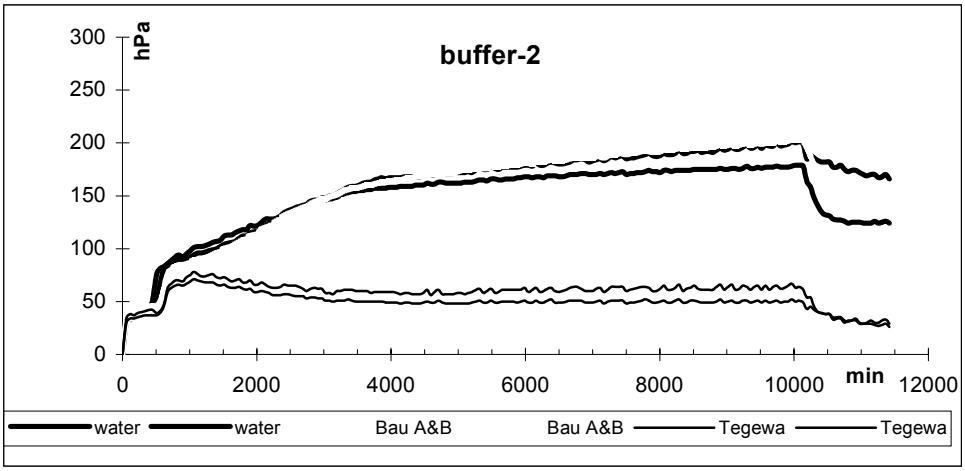
- Amount of **fresh digested sludge**: 25 ml; concentration: 10% (v/v).
- Liquid volume: 250 ml; headspace: 367 ml.

The mineral salt solution acc. to Baumann A was supplemented by the mineral salt and trace element solution acc. to Baumann B.

Pipetting table:

	Tap water		Baumann A + B (H_2O)		TeGeWa	
Bottle	A	B	C	D	E	F
Digested sludge:	25ml	25ml	25ml	25ml	25ml	25ml
Solution	224.5ml	224.5ml	12.5ml A	12.5ml A	224.5ml	224.5ml
			12.5ml B	12.5ml B		
			199.5ml H_2O	199.5ml H_2O		
Glucose	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
KOH	1ml	1ml	1ml	1ml	1ml	1ml

Figure 2: Degradation of glucose in various buffer solutions with digested sludge



For technical reasons, the experiment was performed without a control solution (blank value digested sludge) in the same way as experiment 1 so that the amounts of gas formed can only be specified as absolute values.
Also, at the **end of the experiment, the CO_2 was not expelled using HCl**.
The CO_2 found in the gaseous phase was absorbed by adding KOH in the rubber sleeve.

- After an increase in pressure at the beginning of the experiment, a lag phase was then observed before a steep increase in pressure due to the incipient degradation of the introduced glucose was displayed by all solutions after approx. 10 h.
- The increase in pressure was considerably greater than in experiment 1 and higher maximum values were reached for all solutions. This could be traced back to the use of fresh digested sludge.
- In the TeGeWa solution, a drop in pressure was observed after 35 h.
- This time, the solutions with mineral salt solution acc. to Baumann A and B showed an increase in pressure and plateau formation almost parallel to those of the solutions that used tap water with similarly high maximum pressure values.
- After reaching a plateau in the trial solutions, the CO_2 found in the gaseous phase was absorbed by adding 1ml KOH through the stopper.

The amounts of biogas calculated are listed in the following table.

Table 3: Overview of the amounts of biogas formed

		Tap water	Baumann A&B	TeGeWa
Fermentation	mmol gas (total carbon)	1.8	1.68	0.24
Duration	h	63.5	123.2	123.2
Absorption	mmol CO_2g	0.62	0.67	0.43

Degradation experiments on various polymers

A series of experiments were performed using various easily degradable and badly degradable polymers.

- Digested sludge: concentration used, 10% (v/v)
- Medium: tap water.
- Test substances: PVA, CM starch, PHB, fat.
- Reference substance: glucose.
- Control: preparation with digested sludge without any test substance.

For reasons of capacity, not all substances were tested using repeat preparations.

Degradation experiment using PVA and CM starch

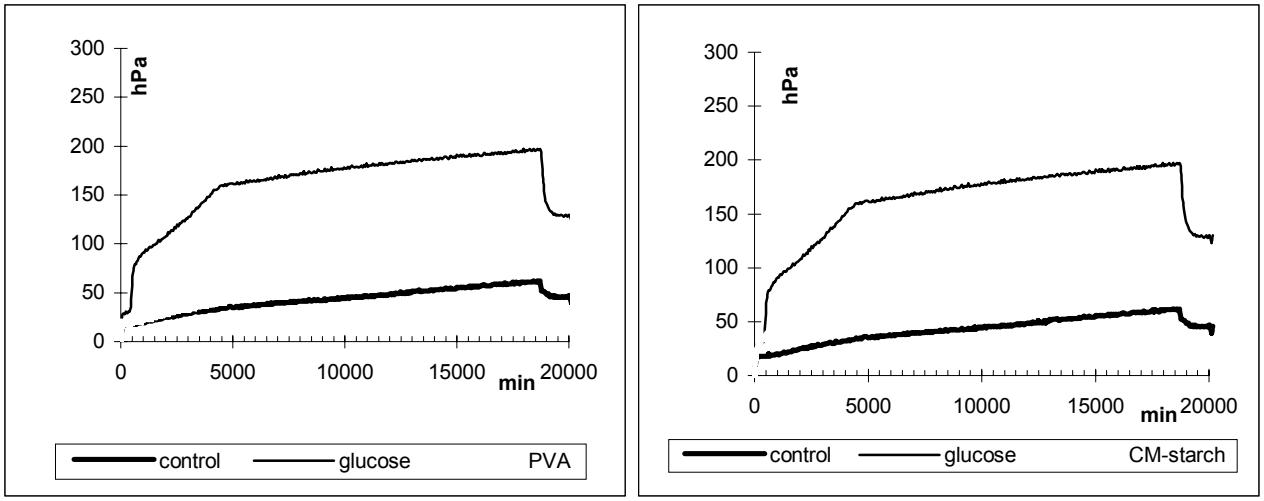
The following experiment (Figure 3) was performed using:

- Digested sludge; concentration, 10% (v/v).
- Test substances: Polyvinyl alcohol (PVA), carboxymethyl starch (CM-S).

Pipetting table:

	Control	Glucose	PVA	CM starch
Bottle	A	B	C	D
Digested sludge	25ml	25ml	25ml	25ml
Water	225ml	224.5ml	175ml	175ml
Substrate	-	75mg / 0.5ml	50mg / 50ml	50mg / 50ml
KOH	1ml	1ml	1ml	1ml

Figure 3: Degradation of various polymers with digested sludge (only CO_2 absorption in the headspace without CO_2 expulsion from the liquid phase)



After a short lag phase, an increase in pressure was determined after 7.5 h for solutions with glucose, after 9.3 h for solutions with PVA and after 2.8 h for solutions with CM-S that then roughly approximated to a plateau as it continued.

Calculations in experiment 3, Figure 3

The calculations were performed as described in detail for experiment 1.
In the calculation of the amount of gas converted, the control value (digested sludge without test substance) was subtracted.

Table 4: Overview of the amounts of biogas formed

		Glucose	PVA	CM starch
Amount	mg	75	50	50
Fermentation	mmol gas (total carbon)	1.53	0.29	0.87
Duration	h	75.6	301.5	215
Absorption	mmol CO_2g	0.73	0.04	0.3

In particular, the following results were achieved:

- After 76 h, the 75 mg glucose that was used was converted into 1.53 mmol gas. The amount of CO_2 formed was 0.73 mmol and the amount of methane 0.8 mmol.
- After 302 h, 50 mg PVA was converted into 0.3 mmol gas. At this point in time, the conversion of the PVA was not yet complete. The amount of CO_2 formed was 0.04 mmol.
- After 215 h, 50 mg CM starch were converted into 0.87 mmol gas. The amount of CO_2 absorbed was 0.3 mmol.

Degradation experiment using PHB and deep-frying fat

The degradation experiment with polymers (Figure 4) was performed using:

- Digested sludge: freshly employed concentration: 10% (v/v)
- Test substances: PHB, deep-frying fat.

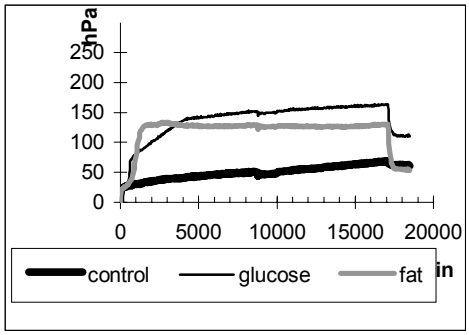
Pipetting table:

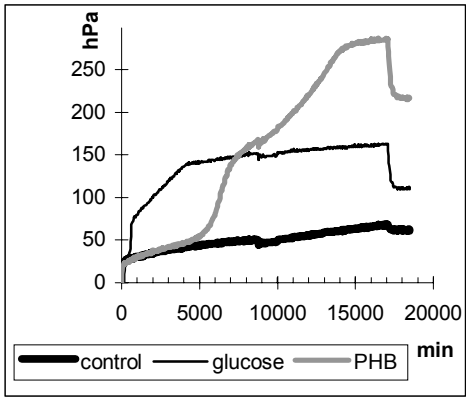
	Control	Glucose	Fat	PHB	PHB
Bottle	A	B	D	E	F
Digested sludge	25ml	25ml	25ml	25ml	25ml
Water	225ml	224.5ml	175ml	175ml	175ml
Substrate	-	75mg / 0.5ml	5ml / 50ml	100mg/ 50ml	100mg/ 50ml
KOH	1ml	1ml	1ml	1ml	1ml

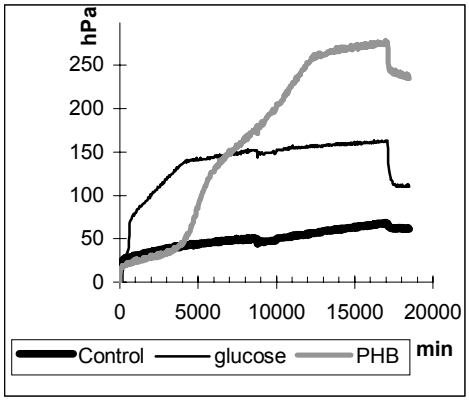
pH values at the beginning and at the end of the experiment:

Bottle	A	B	D	E	F
Beginning	7.94	7.97	8.06	8.05	8.01
End	8.17	8.56	4.95	8.22	7.3
Δ pH	0.23	0.59	-3.11	0.17	-0.71

Figure 4: Degradation of PHB and deep-frying fat in tap water with digested sludge (only CO_2 absorption in the headspace without CO_2 expulsion from the liquid phase)







- In the experimental solution that contained glucose, an increase in pressure was observed after a short lag phase of 8.4 h. After 78 h, a plateau was reached at 142 hPa.
- The preparation with fat required 12 h until an increase in pressure occurred. After 31 h, a plateau was reached at a pressure of 130 hPa.
- The two PHB samples also required a longer lag phase and only showed a continuous increase in pressure after 68 h. After approx. 116 h, a renewed lag phase was detected in both PHB samples. After 144 h, a noticeable increase in pressure occurred that continued up to a maximum of 279 hPa after 244 h.
- At the beginning, the measurement of the pH value resulted in values of approximately pH 8.0 that increased only slightly at the end of the experiment with the exception of the fat sample.
- A PHB sample showed a lower pH value of approximately pH 7.3 at the end of the experiment.
  The fat sample showed a heavily reduced pH value of 5 at the end of the experiment. This can be traced back to an increased formation of acid during the conversion of the fat.

Calculations in experiment 4, Figure 4

The calculations were performed as described in detail in experiment 1.
In the calculation of the amount of gas converted, the control value (digested sludge without test substance) was subtracted.

Table 5: Overview of the amounts of biogas formed

		Glucose	Fat	PHB
Amount	mg	75	5ml	100
Fermentation	mmol gas (total carbon)	1.31	1.22	2.99
Duration	h	69.1	18.7	175.5
Absorption	mmol CO_2g	0.66	0.99	0.68
Biolog. degradation degree of D_h (gas formed in the gaseous phase)	%	-	-	63.5%
CO_2 proportion	%	50.4%	81.1%	22.7%

The following conversion of the substrate to gas were achieved:

- 75 mg glucose were converted to 1.31 mmol gas in 69 h. Of this, 0.66 mmol were CO_2 and 0.65 mmol methane.
- 5 ml fat were converted to 1.22 mmol gas after 18.7 h of which 0.99 mmol were CO_2 and 0.23 mmol methane.
- 100 mg PHB were converted to 0.68 mmol CO_2 and 2.31 mmol methane.

Since no stoichiometric quantities were used, only relative values could be derived from these data.

- PHB and glucose formed the same quantity of CO_2 despite the greater quantities of PHB used.
- In the degradation of PHB, the formation of methane that, in contrast to CO_2, does not dissolve in water is predominant and, as a result, causes the high increase in pressure.
- After the intimated intermediate lag phase in which roughly the same quantity of CO_2 was formed in both the gaseous phase of the glucose and degradation of PHB, the methane production of the methanogenic bacteria begins.

Further replications performed with different buffer solutions and with different polymers confirmed the results achieved to date.
Thus, this confirmed that the measurement of degradation in the TeGeWa solution led to no result (see Fig.1).
At the beginning, in fact, a comparable increase in pressure was observed in one of the other solutions. However, the pressure dropped steeply after 30 h and then remained constant.

Discussion

Interpretation of experiments 1 and 2 using different buffer solutions

- At the beginning of both experiments, a distinct lag phase was observed. The microorganisms present in the digested sludge must adapt to the new environmental conditions before their metabolic activity can begin.
- It could be clearly shown that it is of crucial importance to work with fresh digested sludge as, here, the more active microorganisms can adapt better to the environment and, as a result, considerably better degradation values can be achieved for glucose.
- The significance of the medium in performing the experiment is indicated impressively by the two experiments:
- - The low concentration of minerals and the lack of important trace elements is probably the reason for the worse pressure values in the solutions with saline solution according to Baumann A (experiment 1).
- - On the other hand, the increase in pressure and the rest of the curve in the solutions with mineral salt solution and trace element solution run parallel for the solutions according to Baumann A and B and for the solutions that used tap water.
- - This indicates that the presence of trace elements is of considerable significance for the metabolic activity of the microorganisms in the digested sludge.
- - The buffer capacity of the phosphate buffer in the solutions according to Baumann plays a minor role in these glucose degradation experiments.
- - The most important minerals and trace elements for the metabolism of the microorganisms are present in tap water.

Conclusion from the two experiments:

- For the reasons specified, tap water could also be used as the experimental medium for the digested sludges employed.
- The fact that the defined and safe provision of microorganisms can be ensured even for different sludges speaks for the use of the mineral salt solution and trace element solution according to Baumann.

Interpretation of the experiments regarding the degradation of polymers

- The results of the degradation experiments again showed that it is of great importance to work with fresh digested sludge because it contains active microorganisms that are able to adapt to the experimental conditions.
- After a lag phase, the microorganisms are able to degrade the more easily degradable polymers such as PHB as well as modified polymers such as carboxymethyl starch. Degradation experiments with these two polymers showed a reproducibly typical curve of the increase in pressure.
- In addition, in PHB, after the 1st increase in pressure, a 2nd lag phase appeared that can be traced back to the formation of methane. This indicates that the degradation of the PHB in a second phase is carried out essentially by methanogenic bacteria.
- PVA could only be degraded to a minimum extent within the relevant experimental period. Further experiments will show whether a greater degradation can be achieved over a longer experimental period.
- Experimental solutions with fat resulted in a strong acidification of the experimental solution. As a result, the conditions for the methanogenic bacteria were no longer optimum or were even toxic which limited the formation of biogas.
- Pressure measurement using the OxiTop® control system enabled a precise tracking of the microbial activities, in particular the recognition of multiphase biogas stages.
  Furthermore, it is easy to determine the amount of CO_2 in the gaseous phase and in the liquid phase as well as to determine the amount of methane.

Summary

In contrast to the traditional method of pressure-free gas measurement by the displacement of water with its many opportunities for error and time-consuming documentation, the OxiTop® control system provides anaerobic degradation with continuous storage of the measured values throughout the entire measurement procedure. The development of the biogas can be directly tracked on the controller which, in addition to providing a continuous overview, makes it possible to take rapid decisions on any interventions that may be necessary. For example, it is extremely advantageous to be able to track multistage degradation functions as well as to determine the quantity of CO_2 in both the gaseous phase and in the liquid phase through the simple calculation of the methane gas that results from the degradation process. The data can be realized in an extremely short time, are independent of the operator and are very simple to portray.
A series of experiments showed that it is easy to track the biogas production of the individual substances tested and that it is easy to reproduce within the framework of the variation of biological processes.
Furthermore, it was established that the increase in pressure of the various substances tested when using media solutions with defined mineral salt solutions and trace elements (acc. to Baumann) as in tap water, run parallel to one another.

Accordingly, the test method of anaerobic degradation for the digested sludge used could be simplified by the use of tap water.

Literature

Baumann, U., Schefer, W. Textilveredelung 25 (1990) 7/8, 248-251.