07

Series of the funding programme "Biomass energy use"

Collection of Methods for Biogas

Methods to determine parameters for analysis purposes and parameters that describe processes in the biogas sector

Biomass
energy use

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VOLUME 7

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Methods to determine parameters for analysis purposes and parameters that describe processes in the biogas sector

Edited by Jan Liebetrau, Diana Pfeiffer, Daniela Thrän

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Table of abbreviations and symbols

Without SI-units

Abbreviation/ Symbol	Explanation	Unit of Measurement
3D	Three-dimensional	
5-HMF	5-(hydroxymethyl)furfural	
AbfAblV	German Ordinance on Environmentally Compatible Storage of Waste from Human Settlements and on Biological Waste-Treatment Facilities [Abfallblagerungsverordnung]	
ADF	Acid detergent fibre	[$\%_{TS}$]
ADL	Acid detergent lignin	[$\%_{TS}$]
A_S	Projected area	[m^2]
bLS	Backward-Lagrangian Stochastic	
BMWi	Federal Ministry for the Economic Affairs and Energy	
C	Constant	
CA	Crude ash	[g/kg TS]
C_C	Concentration of the compound of the biogas sample to be tested, relative to specific conditions	[mg/m^3 (STP)]
CCA	Constant current anemometry	
CCD	Cross-correlation development cameras	
c_{CH4}	Measured concentration of methane	[m^3/m^3]
c_{CO2}	Measured concentration of carbon dioxide	[m^3/m^3]
CFD	Computational fluid dynamics	
CFI	Crude fibre	
CFU	Colony-forming units	
CH_4-C	Methane carbon	
CHP	Combined heat and power plant	
C_L	End-effect correction factor	
C_{lam}	Constant of the stirrer used	
C_{MO}	Metzner-Otto constant	
$CO_{2\text{-eq}}$	Carbon dioxide equivalent	
COD	Chemical oxygen demand	[mg_{COD}/L]
C_{SR}	Constant of the measuring system for recalculation of the rotation frequency in the shear rate	[min/s]
C_{SS}	Constant of the measuring system for recalculation of the torque in the shear stress	[Pa/Nm]
CTA	Constant temperature anemometry	
d	Diameter	[m]
DA	Daily averages	
DAD	Diode array detector	
DAS	Data aquisition system	
DC	Direct current	
DGGE	Denaturing gradient gel electrophoresis	
dist	Distilled	

Abbreviation/ Symbol	Explanation	Unit of Measurement
DNS	Dinitrosalicylic acid	
d_s	Sensor diameter	[m]
E(t)	Retention time density function	
ECD	Electron capture detector	
EEG	Renewable Energy Sources Act [Erneubare-Energien-Gesetz]	
EI	Evaluationindex	
EMT	Effective mineralisation time	
ERT	Electrical resistance tomography	
F	System factor	
F(t)	Retention time sum function	
FAL	Bundesforschungsanstalt für Landwirtschaft	
FET	Field effect transistor	
FID	Flame-ionisation detector	
FISH	Fluorescence in situ hybridisation	
F_{N2}	Gas flow velocity	[mL/min]
FVS	Fermentable organic volatile solids (also referred to as fermentable organic dry matter)	[kg FVS/kg TS]
f_W	Stoichiometric water incooperation	[kg water/kg FVS]
f_X	Microbial biomass formation	[kg biomass/ kg FVS]
GB 21	Gas generation after 21 days	[L (STP)/kg_{VS}]
GC	Gas chromatograph	
GC-MS	Gas chromatograph with mass spectrometer	
GWP (value)	Global warming potential	
HAc	Acetic acid	
h_{foam}	Height of the generated foam	[mm]
HHAV	Half-hour average values	
$H_{i,CH4}$	Interior calorific value (also referred to as lower heating value) of the biogas (STP)	[kWh/m^3 (STP)]
HPLC	High-performance liquid chromatography	
HRT	Hydraulic retention time	[d]
IC	Ion chromatography	
ICP-OES	Inductively coupled plasma optical emission spectrometry	
ID	Inner diameter	[mm]
ioTS	Inorganic total solids (also referred to as inorganic dry matter)	[kg ioTS/kg TS]
IR	Infrared	
ISO	International organization for standardisation	
ISTD	Internal standard	
K	Ostwald factor	[Pas^n]
k	First-order reaction constant	[1/d]
K^*	Consistency factor of the flow curve based on the rotational frequency	[$mPa{\cdot}s^m$]
k‘, k‘‘	Metzner/Reed flow factor	[$Pa{\cdot}s^n$]

Abbreviation/ Symbol	Explanation	Unit of Measurement
$K_{1/s}$	Consistency factor of a definied range of the shear rate (consitency factor for the apparent viscosity at a shear rate of $\dot{\gamma} = 1\ s^{-1}$)	[mPa·s^n]
KrWG	Closed Substance Cycle Waste Management Act [Kreislaufwirtschafts- und Abfallgesetz]	
K_{VP}	Factor for the recalculation of the regarding rotational frequency of the stirrer in the relevant shear rates	
L	Characteristic length	[m]
L2F	Laser-2-focus anemometry	
LBP	Linear back projection	
LDA	Laser Doppler anemometry	
LEL	Lower explosive limit	[%]
L_O	Obukhov-length	[m]
LOD	Limit of detection	
LOQ	Limit of quantification	
LTR	From left to right	
M	Torque	[Nm]
MCF	Methyl chloroformate	
$mg_{COD/L}$	Milligrams of COD (chemical oxygen demand) per litre	
MGRT	Minimum guaranteed retention time	
MID	Magnetic flow meter	
MS	Mass spectrometer	
MYA	Malt yeast agar	
N	Rotational frequency	[1/s]
n	Flow exponent	
n'	Metzner/Reed index	[-]
n''	Herschel/Bulkley index	[-]
n-N	non-Newtonian	
Nd:YAG	Neodymium yttrium aluminium garnet double pulse laser	
NDF	Neutral detergent fibre	[$\%_{TS}$]
Ne	Newton number (power indicator)	
NGS	Next generation sequencing	
NH_4-N	Total ammonia nitrogen (TAN)	
NMVOC	Non-methane [volatile] organic compounds	
NO_2-N	Nitrite nitrogen	
NO_3-N	Nitrate nitrogen	
ORGA-Test	Oberhausen-Rostock-Göttinger activity test	
OLR	Organic loading rate	[$kg_{VS}/(m^3 \cdot d)$]
p	Pressure	[Pa]
P	Power input (stirrer power)	[W]
p.a.	Pro analysi (analytical grade)	

Abbreviation/ Symbol	Explanation	Unit of Measurement
P_{el}	Electrical power of the CHP	[kW]
P_{RTI}	Power total rated input	[kW]
PIV	Particle image velocimetry	
ppb	Parts per billion	
ppm	Parts per million	
ppm*m	Parts per million times metre(s)	
ppmV	Parts per million by volume	
PTB	Physikalisch-Technische Bundesanstalt (Physical Technical Federal Institute	
Q_0	Number distribution	[%]
$q_0(x_{EQPC})$	Number density distribution (of the type of quantity "number" 0) of the coextensive circle diameter	[1/µm], [%/µm]
$Q_0(x_{EQPC})$	Cumulative distribution (of the type of quantity "number" 0) of the coextensive circle diameter	[-], [%]
$q_3(x_{EQPV})$	Volume density distribution (of the type of quantity "number" 3) of the coextensive sphere diameter	[1/µm], [%/µm]
$Q_3(x_{EQPV})$	Volume cumulative distribution (of the type of quantity "number" 3) of the coextensive sphere diameter	[%]
qPCR	Quantitative polymerase chain reaction	
r	Radius	[mm], [m]
R_1	Resistance	[Ω]
R_2	Resistance	[Ω]
R_{CR}	Control resistance	[Ω]
Re	Reynolds number	
RenFe [NaWaRo]	Renewable feedstock [Nachwachsende Rohstoffe]	
RGP	Residual gas potential (also referred to as fermentable organic dry matter)	[%]
r_i	Inner radius	[mm]
RID	Refractive index detector	
r_o	Outer radius	[mm]
rRNA	Ribosomal ribonucleic acid	
R_S	Sensor resistance	
STD	Standard/standardised	
STP	Dry and at standard temperature and pressure	
SWOT	S-strength, W-weakness, O-opportunities, T-threats	
T	Actual temperature of the biogas sample	[°C]
TA Luft	Technical Instructions on Air Quality Control [Technische Anleitung zur Reinhaltung der Luft]	
TAN	Total ammonia nitrogen (NH_4-N)	
TC	Total carbon	
TD	Thermal desorption	

Abbreviation/ Symbol	Explanation	Unit of Measurement
TDLAS	Tunable diode laser absorption spectrometer	
T_f	Fluid temperature	[°C]
TGB	Trypton glucose bouillon	
TIC	Total inorganic carbonate buffer	
TN	Total nitrogen	
TOC	Total organic carbon	
T_S	Sensor temperature	[°C]
TS	Total solids (also referred to as dry matter)	[%]
TS_D	Total solids of the digestate	[kg TS/kg WW]
TS_{md}	Total solids, dried and milled	[%]
TS_S	Total solids of the substrate mix	[kg TS/kg WW]
TS_{KF}	Total solids content – Karl-Fischer-method	[g/kg], [g/L], [%]
U	Heater voltage	[V]
u*	Friction rate	[m/s]
U_B	Bridge voltage	[V]
UEL	Upper explosive limit	[%]
V	Volume of the sample	mL
v	Velocity	[m/s]
v_{avg}	Average velocity	[m/s]
VDLUFA	Association of German Agricultural Analytic and Research Institutes [Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten]	
V_{foam}	Volume of the generated foam	[mL]
V_{GP}	Volume of the gas phase in the reactor	[mL]
VOA	Volatile organic acids	[mg/L]
VOC	Volatile organic compounds	
VS	Volatile solids (also referred to as organic dry matter)	[% TS], [kg VS/kg TS]
VS_D	Volatile solids (also referred to as organic dry matter) of the digestate	[kg VS/kg TS]
VS_S	Volatile solids (also referred to as organic dry matter) of the substrate mix	[kg VS/kg TS]
V_{tot}	Total volume	[mL]
W	Wall	
WC	Water content	
WW	Wet weight	
x_{EQPC}	Area diameter	[µm]
x_{EQPV}	Volume sphere diameter	[µm]
x_{max}	Fibre length	[mm]

Abbreviation/ Symbol	Explanation	Unit of Measurement
Y_{FVS}	Biogas (formation) potential of fermentable organic volatile solids (STP)	[m³ (STP)/kg FVS]
Y_D	Specific residual gas potential (STP)	[m³ (STP)/kg WW]
z_0	Roughness length	[m]
z_{up}	Upstream length	[m]

Special characters	Explanation	Unit of measurement
$\dot{\gamma}$	Shear rate	[1/s]
$\dot{\gamma}^*$	Shear rate calculated according to manufacturer's instructions	[1/s]
$\dot{\gamma}_{rep}$	Representative shear rate	[1/s]
δ	Radius ratio	
η	Dynamic viscosity	[kg/ms]
η	Conversion of FVS	[kg FVS/kg FVS]
η_{eff}	Effective viscosity	[kg/ms]
η_{el}	Electrical efficiency of the CHP	[kW/kW]
η_{n-N}	Vicosity for non-Newtonian fluids	[N/m²]. [Pas]
η_S	Apparent viscosity	[mPa·s], [Pa·s]
$\dot{m}$	Shear stress	[N/m²]. [Pas]
$\dot{m}_D$	Mass flow of the digestate	[kg/d]
$\dot{m}_S$	Mass flow of the substrate mix	[kg/d]
ρ	Density (STP)	[kg/m³], [kg/m³ (STP)]
ρ_B	Density of the biogas (STP)	[kg/m³ (STP)]
$\sigma_{p(x,y)}$	Electrical conductivity of the pixels	[mS/cm]
τ	Shear stress	[N/m²], [Pa·s]
τ_w	Shear stress near the wall	[N/m²], [Pa·s]
$\bar{t}$	Average retention time	[s]
$\dot{V}_B$	Volume flow (rate) of the biogas (STP)	[m³ (STP)/d]
ω	Angular velocity	[1/s]

1 Introduction

With the German research programme "Biomass energy use" projects with the aim of the development and optimisation of climate-friendly and energy-efficient technologies for the energetic use of biomass have been funded since April 2009.

In this programme and in general, anaerobic digestion (AD) plants represent a very successful and promising option for the energetic use of biomass. For years, the number of plants has been increasing continuously in Germany. With approx. 8,000 plants (2015), the biogas technology can be referred to as established. Nevertheless, a significant optimisation potential exists with respect to the efficiency of the conversion and utilisation processes at biogas plants. With the optimisation of the biogas production from different biogenous materials, the share of renewable energies of the supply of electricity can be increased and can further contribute especially to the utilisation of biogenic residues.

Regarding this, presently approx. half of the projects (103) in the programme "Biomass energy use" are primarily researching the effective utilisation of residues through the development of innovative combinations of processes, the technological optimisation of the fermentation process, and the expansion of the options for use of the biogas generated. The aspect of sustainability, in particular the reduction of greenhouse gas emissions, plays a particular role in this.

In addition to the individual research activities and objectives of the projects, the idea of compiling the several methods applied in the individual projects was developed jointly across the projects, which are part of the "Biogas" working group of the funding programme "Biomass energy use".

Despite the considerable number of industrial-scale biogas plants and systems the process-accompanying analytics and the scientific methods of analysis are faced with a multitude of challenges. These relate to the requirements that result from the very special characteristics of the substrates and the technical specifics of the process. The measurement methods used were incorporated from applications in other fields of science (e.g. waste management) and had to be – in the great majority of cases – subjected to considerable modifications in order to be applicable to practical requirements. Several methods presented here are applied after a standardised methodological approach with some modification, all the way to completely new developments (e.g. VOA/TIC) that have not yet been standardised. As a result, variants and modifications of methods were created that often make a sensible comparison of the results impossible.

To counter this fact, it is initially necessary to compile the methods used in an overview, to assess them, and to – long-term – enter into a discussion about a harmonisation with the institutions that use them. From this approach, the idea of the "Collection of Methods for Biogas" arose. In the first step, the essential methods used in biogas projects of the funding programme are to be presented. This presentation shall be further developed through the duration of the funding programme, and the methods contained therein be discussed and assessed. Perspectively, an integration of institutions outside of the circle of participants in the funding programme "Biomass energy use" is strived for, in order to be able to provide a broader foundation for the methods presented.

The "Collection of Methods for Biogas" therefore, for the first time, provides the opportunity to give an overview of the methods used in the biogas sector and to perform comparisons with respect to the suitability for specific applications. Furthermore, this collection provides the reader with the opportunity to identify institutions which deal with the methods presented and to exchange experiences with them as well as to develop the methods further.

In the first edition of the "Collection of Methods for Biogas" available in English and presented here, the methods applied at institutions participating in the programme have been compiled. The great variability of the content of the projects involved also results in a great variability of the methods that present virtually the whole spectrum of biogas research.

In the few cases in which different methods for the same measured parameter were used these were presented equivalently.

With the continuation of the funding programme, new projects and methodological approaches will also be added that shall contribute to the further development of this collection and to an intense discussion regarding the methods also beyond the programme.

The methods introduced here are utilised in technical processes and procedures that serve for the conversion of biomass to biogas and its utilisation. In this, both waste treatment plants and agricultural plants are being reviewed.

The methods delineated in this collection refer to a complex process that is being realised, in practice, in a multitude of variations.

The methods presented in the chapters on fundamental parameters, as well as chemical, physical and biological parameters describe the material characteristics of the initial substrates, the digester content, as well as the products. These methods are used in order to describe the quality of the initial substrates and end products as well as process states in the fermentation process. They serve as initial parameters for superordinated investigations such as process assessments, mass and energy balances. This superordinated context is also being

served by the assessment of the emissions. For this, it is necessary to determine the quality and quantity of the emitted substances.
In the chapter on calculation and assessment methods, superordinated methods such as the SWOT analysis, mass balancing, or the determination of process indexes are presented. These are utilised to assess the overall process or sub-processes with respect to their technical suitability or the energetic efficiency.

If you would like to contribute additional methods to the "Collection of Methods for Biogas", even above and beyond the funding programme, or if you have comments and/or suggestions for adjustments regarding the current edition, please contact :

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2 Definitions

Term	Explanation
Activated sewage sludge (inoculum)	Microbial biomass that is utilised to start or accelerate a →fermentation; in accordance with DIN 384148, →digested sludge, or, if deviating therefrom, in exactly documented form.
Amount of biogas produced	e.g. in L (STP) →Biogas generated in unit of volume. The amount of biogas produced is the volume of crude biogas yield converted to standard conditions in accordance with DIN 1343 (0 % relative humidity; 0 °C gas temperature; 1013.25 mbar ambient pressure).
Anaerobic degradability	Degree of the microbial conversion of →substrates or →co-substrates, generally expressed as →degree of degradation.
Anaerobic digestion plant (AD plant)	→biogas plant
Anaerobic treatment	Biotechnological process under exclusion of air (oxygen) with the objective of the decomposition of organic matter while generating →biogas.
Batch test	Discontinuous test in which organic →substrates or →co-substrates are subjected to a →fermentation under defined anaerobic conditions and in which insights regarding the fermentability and →gas yield can be gained.
Biogas	Gaseous product of →fermentation that mainly consists of methane and carbon dioxide and which, depending on the →substrate, may also contain ammonia, hydrogen sulphide, steam and other gaseous or evaporable components.
Biogas (formation) potential	e.g. in L (STP)/kg_{VS} Maximum possible →biogas yield that can be generated from a defined amount of substrate.
Biogas methane content	The biogas methane content is the volume share of methane contained in one unit of volume of →biogas.
Biogas plant (also referred to as anaerobic digestion plant)	Structural unit for the production of biogas from the materials supplied, consisting of at least one or more →digesters as well as the piping and cabling required for this. Generally, a biogas plant also includes stockpiling and feed facilities for the fermentation substrates, gas purification and gas utilisation systems (e.g. CHP) as well as storage and occasionally also processing options for the →digestates and the →biogas in natural gas quality with the objective of feeding it into the natural gas grid. A differentiation is made between →single-stage biogas plants and →multi-stage biogas plants. (in accordance with VDI 3475 Sheet 4)
Biogas processing	The biogas processing includes all technically required facilities for the cleaning of →biogas contaminated with foreign material and for methane enrichment. →gas purification
Biogas rate	e.g. in L (STP)/d →Amount of biogas produced per unit of time.
Biogas rate, specific (biogas productivity)	in L (STP)/(L · d) Relationship of the →biogas rate to the active working volume of the →digester.

Term	Explanation
Biogas yield	e.g. in L (STP)/kg_{VS} or L (STP)/kg_{WW} Amount of biogas produced per amount of substrate used. L (STP) – litre dry and at standard temperature and pressure WW – wet weight VS – volatile solids
Biomass	Biomass is living organic matter consisting of phytomass and zoomass. This also includes secondary products and by-products resulting from phytomass and zoomass, residues and wastes, the energy content of which originates from phytomass and zoomass.
Biowaste	Wastes of animal or plant origin for processing that can be decomposed by microorganisms, soil-borne organisms or enzymes; this includes, in particular, the wastes listed in Appendix 1 No. 1; soil materials without significant shares of biowastes are not considered biowastes; Plant residues that occur on silviculturally or agriculturally used areas and which remain on these areas are not biowastes (BioAbfV 1998 [2007]).
Blank test (zero test)	Fermentation test with pure →activated sewage sludge without the addition of →substrate.
Chemical oxygen demand (COD)	in mg_{COD}/L Metric for the share of oxidisable compounds in the substrate.
Co-fermentation	(here) Anaerobic biotechnological process in which a (main)→substrate is fermented jointly with one or more additional →substrates (→co-substrates).
Co-substrate	Raw material for a →fermentation/→digestion which, however, is not the raw material with the highest percentage share in the overall material flow to be fermented.
Collection tank	A collection tank refers to a storage container/vessel with feeding technology for solid and liquid →substrates and →fermentation aids.
Composite sample	A sample that was created by combining and mixing →individual samples from a →basic quantity.
Content of volatile solids (VS)	in g_{VS}/kg_{WW} and/or g_{VS}/L_{WW} The weight loss (volatile solids burn loss) of a sample relative to the untreated original sample (→wet weight) or the initial volume that is turned to ash, subsequent to prior drying, at a temperature of 550 °C until a constant weight is reached. The weight loss is primarily, but not exclusively, caused by organic contents. Volatile organic substances that escape during the drying at 105 °C are not captured with this method and have to be determined separately.
Cumulative sample	See →composite sample.
Daily load	e.g. in kg_{VS}/d or kg_{TS}/d or kg_{COD}/d Amount of →substrate fed into the fermentation system per day.
Degree of degration, in %	Reduction of mass of the organic substance due to anaerobic degradation relative to the initial amount of →substrate.
Degree of desulphurisation	The degree of desulphurisation describes the degree of the elimination of sulphur compounds in the →biogas by means of biological, chemical or physical desulphurisation processes.
Digestate (also referred to as fermenter residues)	Once the fermentation mix is leaving the →digester, it is referred to as digester residue. Digester residue is quite often utilised as →inoculum.
Digestate processing	Facilities and plant components for the process of →digestates.

Term	Explanation
Digestate storage unit	Vessel or earthen basin in which →digestate is stored unheated prior to further utilisation and processing. Digestate storage units are fed by →digesters. A digestate storage unit is not primarily intended to generate methane. In this, the fill level is subject to severe fluctuations over the course of the year.
Digested sludge	Digested sewage sludge (see also →activated sewage sludge).
Digester (also referred to as fermenter)	Vessel that serves for the targeted conversion (fermentation) of the →fermentation mix by microorganisms. Digesters for biogas production are characterised by the fact that the digester medium (→fermentation mix) contained therein is being actively heated, an active transport of material is maintained (e.g. through stirring or percolation), and the biological process is actively controlled. Digesters are furthermore characterised by the fact that the →biogas generated is captured and made available for use. In general, a differentiation can be made between →pre-digesters, →main digesters, and →post-digesters.
Digester volume (also referred to as fermenter volume)	Portion of the volume of the →digester (vessel) in which the →fermentation takes place.
Digestion (also referred to as fermentation)	Anaerobic process in which a product, here, a →biogas containing methane, is generated through the activity of microorganisms or the effect of their enzymes on a product. Expression for →fermentation (this term is also often used in wastewater treatment).
Discharge concentration	e.g. in kg_{VS}/m^3 or in kg_{TS}/m^3 Concentration of a substance in the discharge (e.g. content of volatile solids in kg_{VS}/m^3). VS – Volatile solids; TS – Total solids
Discharge load	e.g. in kg_{VS}/d or in kg_{TS}/d Amount of mass discharged from a fermentation plant per unit of time.
Dual-phase methane fermentation	The microbial sub-steps of hydrolysis and acid fermentation take place with spatial separation from the methane production.
Energy crops	Crops that are cultivated for the sole purpose of producing energy.
Feeding	The addtion of substrate to a →digester is called feeding.
Feedstock from renewable resources (RenFe)	Crops that are cultivated for the purpose of utilisation for energy and/or material.
Fermentation	Biotechnological process for generating product. →digestion
Fermentation aids	All materials and/or working media fed to the →digester for promoting the micro-biological decomposition processes that are →substrate. The fermentation aids themselves do not have any →biogas (formation) potential and/or it is negligibly low. Fermentation aids can be of organic or inorganic composition (e.g. algae preparations, trace elements for the supply of the microorganisms, enzymes for the hydrolysis).
Fermentation mixture (digester/fermenter content)	→Substrate, including →fermentation aids, recirculates and biocenosis in a →digester.
Fermentation product	The products in solid, liquid and gaseous form generated through fermentation, in the case of agricultural →biogas plants: →biogas and →digester residue.
Fermenter	→Digester

Term	Explanation
Fermenter volume	→Digester volume
Floating sludge layer (scum layer)	Components of the →fermentation mix that float up that may build a layer or cover.
Foam	Gas bubbles building on top of the fermentation mix surface separated by lamellas of liquid whose structure can stabilise itself through media contents (e.g. proteins). Foams can occur due to contents in the →substrate, specific intermediate metabolites, or in the case of stress situations for the anaerobic biocenosis (overloading, population dynamics in the case of a change of substrate, deficiency symptoms) due to exo-enzymes released.
Gas production (GB 21)	e.g. in L (STP)/kg_{VS} (in 21 d) →Gas yield in a specific →batch test after a finite, defined period of time (e.g. gas production GB 21 after 21 days; see Ordinance on Environmentally Compatible Storage of Waste from Human Settlements and Biological Waste-Treatment Facilities [AbfAblV]).
Gas purification	Facilities to remove components from biogas that are not utilisable or pose a problem in the utilisation. Most often, gas purification is limited to desulphurisation and dehumidification for utilisation in CHPs. →Biogas processing
Gas yield	See →biogas yield.
Homogeneity/ inhomogeneity	Degree of even/uneven distribution of a characteristic value/material in a quantity of material; a material may be homogeneous with respect to an analyte or a characteristic, but inhomogeneous with respect to another one.
Hydraulic retention time (HRT)	e.g. in d Average retention time of the →substrate in the →digester (The frequently used quotient of the working volume to the daily fed-in substrate volume is applicable only under the assumption of a volume-constant reaction). $\mathrm{HRT} = \frac{V_{reactor}}{\dot{V}}$
Hydrolysis gas	→Fermentation product of the biochemical hydrolytic substrate breaking-down in the case of the realisation of a separate hydrolysis stage prior to the methanisation. In essence, it consists of carbon dioxide and hydrogen with low shares of methane, hydrogen sulphide, as well as other volatile organic compounds.
Impurities	Substances that interfere with the process, the technology, or the product quality (e.g. plastic, glass or metal particles, and sand).
Inhibition	Hindering of →fermentation through damaging of the effective microorganisms or reduction of the efficiency (activity) of enzymes.
Inlet concentration	e.g. in kg_{VS}/m^3 or in kg_{TS}/m^3 Concentration of a substance in the inlet.
Inorganic total solids (ioTS)	Inorganic total solids (also referred to as anorganic dry matter); ash, calcination residue, is generated in accordance with VDLUFA at 550 °C from the → total solids (also referred to as dry matter) (TS); represents the inert share of the sample of the →substrate
Input	Feeding into the system (system boundary).

Term	Explanation
Mass balance	All mass flows entering via the balance/system boundaries during a standardised period under review Δt_0 are supplied (fed) mass flows, all exiting ones are discharged mass flows.
Methane yield	The methane yield is the product of the →biogas yield and the →biogas methane content.
Methane gas potential of the digestate (residual gas potential)	The methane gas potential of the digestate is determined in a laboratory test under defined conditions and – like the methane yield – is put in relation to the →wet weight or →organic dry matter (volatile solids) of the →digestate, under specification of the temperature and duration selected for the test.
Methane productivity, specific	in L (STP) $CH_4/(L \cdot d)$ Relationship of the amount of methane generated per unit of time to the active working volume of the →digester.
Multi-stage biogas plant	A multi-stage biogas plant is characterised by the fact that the same phases occur in →digesters connected in series. A multi-stage biogas plant can, for example, consist of a →pre-digester, a →main digester and a →post-digester that are connected in series.
Organic loading rate (OLR)	in $kg_{VS}/(m^3 \cdot d)$ Relationship of the →daily load to the digester volume. $\mathrm{OLR} = \frac{\dot{m} \cdot c}{V \cdot 100}$
Output	Export from a balance space.
Phase	The phase refers to the microbial-biochemical process. A differentiation is made between a single-phase methane fermentation and a two-phase methane fermentation.
Pollutants	Substances that inhibit the fermentation process (→inhibition) or negatively affect the usability of the →fermentation product.
Process temperature	The process temperature is the average temperature in the →digester as the mean in the case of the utilisation of multiple measurement sites.
Recirculate	→Fermentation mix or →digestate that is fed back into a →digester in part (e.g. only the liquid phase after separation) or in whole after having left it.
Reference substrate/ reference sample	→Substrate with known biogas potential (e.g. microcrystalline cellulose).
Representative sample	Sample whose characteristics correspond, for the most part, to the average characteristics of the basic quantity of the entire lot.
Sample preparation	Establishing of the sample characteristics required for a representative sample/the fermentation process via separating, comminuting, classifying, etc.
Sampling	Type of the extraction and preparation of portions of the →substrate or of the digester content in order to obtain relevant and representative information regarding the chemical or biological parameters of the overall amount.
Sediment	Deposits of solids in →digesters and storage vessels/containers.
Single-phase methane fermentation	The microbial sub-steps of hydrolysis, acidic fermentation and methane production take place without spatial separation.

Term	Explanation
Single sample	Sample amount that is extracted in a single sampling process; temporally and location-wise it is tightly limited to a single extraction site.
Single-stage biogas plant	A single-stage biogas plant is characterised by the fact that all →phases up to the →biogas take place in one or multiple →digesters connected in parallel.
Sludge load	in $kg_{VS}/(kg_{VS} \cdot d)$ Relationship of the →daily load (kg_{VS}/d) to the volatile solids in the →digester.
Specific stirrer power	The specific stirrer power is the average power demand of the stirrer systems used for mixing the digester, determined as electrical effetive power, relative to the respective digester volume used.
Storage of sample(s)	Type of bridging the time between →sampling, →sample preparation and utilisation of the sample in chemical analyses or biological test.
Substrate	Raw material for a →fermentation, here →digestion.
Total ammonia nitrogen content (TAN) (NH_4-N-content)	The total ammonia nitrogen content (TAN) is the sum of nitrogen compounds of each process stage present in the form of NH_4^+ ions and undissociated NH_3.
Total nitrogen content (Kjeldahl nitrogen, TKN)	The total nitrogen content is the sum of the nitrogen contained in inorganic and organic nitrogen compounds in the →input (see DIN EN 25663).
Total solids content (TS)	In g/kg, in the case of high water content g/L, or % of the total amount (→wet weight) ($\%_{ww}$). Share of substances that remain upon thermal removal of water (in the case of drying for 24 hours at 105 °C and/or until a constant weight is achieved). In addition to water, other volatile components (e.g. volatile organic acids) are also driven out, where applicable.
Total solids content – Karl-Fischer-method (TS_{KF})	In g/kg, in the case of high water content g/L, or % of the total amount (→wet weight) ($\%_{ww}$). Determination according to Karl-Fischer or by means of azeotropic distillation (xylol or toluol method). Here, the pure water content (WC) is determined.
Total solids content milled (TS_{md})	Unlike →TS, TS_{md} refers to a sample that is dried, milled and again dried, as the sample gains water during the milling.
Trace gas concentration	The trace gas concentration is the share of gaseous accompanying substances in the →biogas prior to the gas utilisation (e.g. hydrogen sulphide, ammonia, siloxanes).
Van-Soest carbohydrate analysis	The Van-Soest carbohydrate analysis splits up the cell wall components in the skeleton carbohydrates cellulose, hemicellulose and lignin. In comparison to the →Weender feed analysis, the fermentation characteristics of the carbohydrates can be described more accurately.

Term	Explanation
VOA/TIC value	The VOA/TIC value is the quotient of the amount of →volatile organic acids (VOAs) determined by means of titration with 0.1 N sulphuric acid, expressed as mg/L acetic acid (HAc) and the acid consumption of the same titration up to pH = 5 (TIC) expressed as mg $CaCO_3$/L. The VOA/TIC value is of purely empirical nature and constitutes an early warning parameter for assessing process stability. For a stable operation, a limit value of < 0.3 is considered safe. In the case of pure feestock from renewable resources, a stable operation is still achieved at VOA/TIC values between 0.4 and 0.6. $\frac{\left((\text{consumption B} \cdot 166) - 0{,}15\right) \cdot 500\ [\text{mg/L HAc}]}{\text{consumption A} \cdot 500\ [\text{mg/L } CaCO_3]}$
Volatile organic acids (VOAs)	These are steam-volatile fatty acids (C_1 to C_5). The total share of volatile organic acids is calculated as acetic acid equivalent at each process stage.
Waste	Moveable item that is contained in Appendix 1 of the Closed Substance Cycle Waste Management Act (KrWG) and which the owner wants to get rid of.
Weender feed analysis	The Weender feed analysis serves for the determination of the content of crude ash, crude fibre, crude protein, crude fat and the nitrogen-free extracts of fermentation substrates. From the nutrient contents determined and the corresponding digestibility quotients from feed(ing) value tables, in combination with the specific methane yields of the digestible contents (i.e., the carbohydrates, crude protein and crude fat) the approximate methane yields of plant →substrates can be calculated.
Wet weight (WW)	Mass of a substance or →substrate in the original state with the natural water content.
Wobbe index	The Wobbe index is an indicator for the assessment of the combustion characteristics of a gas. The upper Wobbe index is the quotient of the calorific value and the square root of the relative gas density; the lower Wobbe index is the quotient of the heating value and the square root of the relative gas density. It is generally put in relation to the standard condition.

3 Methods for the determination of fundamental parameters

3.1 Determination of total solids (dry matter) and volatile solids (organic dry matter)

Katrin Strach, DBFZ

Status	Is being used in routine operation.
Standard	The determination of total solids and of volatile solids is modelled after DIN 12880 and/or DIN 12879 (German standard 2001a), (German standard 2001b)
Area of application	Substrates and digestates in which only a small share of volatile components is to be expected.
Disadvantage	In addition to water, other volatile components (e.g. volatile organic acids) are also driven out.

Devices and chemicals

- Muffle furnace
- Drying cabinet
- Precision scale
- Desiccator

Execution method

To determine the total solids (TS) of liquid samples, e.g. digestates, the empty weight of the crucible is recorded first. Then, approx. 5 g of the sample are filled into the crucible and the weight of the filled crucible is once again entered into the log. For drying, the filled crucibles are placed in the drying cabinet at 105 °C. The crucibles are left to dry until a constant weight is achieved. The constant weight of the crucible with the dried sample is recorded. Subsequently, the samples are calcinied in the muffle furnace at first for 30 min at 220 °C, and then for 2 h at 550 °C. After the calcination, the hot crucibles are cooled down in desiccators. After the cooling down of the crucibles, these are weighed once again.

Calculation of the total solids content

$$TS = 100 \cdot \frac{m_3 - m_1}{m_2 - m_1} \quad (1)$$

TS	Total solids content (also referred as dry matter)	(%)
m_1	Mass of the empty crucible	(g)
m_2	Mass of the crucible after the sample was added	(g)
m_3	Mass of the crucible after drying	(g)

Calculation of the volatile solids

$$VS = 100 \cdot \frac{m_3 - m_4}{m_2 - m_1} \quad (2)$$

VS	Volatile solids content	(% $_{TS}$)
m_1	Mass of the empty crucible	(g)
m_2	Mass of the crucible after the sample was added	(g)
m_3	Mass of the crucible after drying	(g)
m_4	Mass of the crucible after calcination	(g)

To determine the total solids of inhomogeneous substances such as silages, grass or manure, a larger weighed-in quantity of 200 to 250 g, in shallow pans, is used. It will be dried and the masses recorded, as described above. In order to determine the volatile solids, the dried sample is ground to ≤ 1 mm. Subsequently, a representative sample is taken. Based on this sample, the TS/VS determination is carried out, as described.

3.2 Total solids content correction according to Weissbach & Strubelt

Britt Schumacher, DBFZ

Status	The method for the correction of the total solids content of silages by volatile organic acids and alcohols was suggested by Weissbach & Strubelt (2008a, 2008b, 2008c), based on earlier research tests in the area of feed(stuffs) evaluation (Weissbach & Kuhla 1995) as well as current studies for the biogas sector. Research tests by (Mukengele & Oechsner 2007) showed an overestimation of the specific methane yield by up to 10 % for maize silage, if no correction for volatile substances was carried out. In the currently applicable version of VDI Guideline 4630 (2006), which is currently undergoing a revision, a correction for the volatile acids is being recommended (determination in accordance with (DIN 38414-19 1999)), wherein 10,000 mg/L acetic acid equivalent concentration corresponds to an VS increase by 1 % absolute relative to wet mass. Alcohols are not being taken into consideration, here. The concentrations of volatile components required for the correction can be determined by means of GC (gas chromatography) and additional lactic acid analytics or HPLC (high-pressure liquid chromatography), c.f. Ch. 4.1 and 4.2. It is important that the methods for the testing of green crop silages be adjusted to the substance concentrations which are considerably higher, here, than in the digester content and/or in digestate. To be determined are the lower fatty acids of carbon chain length C_2 to C_6 (including the iso acids), the alcohols of carbon chain length

	C_1 to C_4 (including 1,2-Propanediol and 2,3-Butanediol) as well as the lactic acid (Weissbach 2011). Since the fermentation acid patterns and alcohol patterns are strongly dependent on the silaging conditions, a determination of the concentration must be performed for each dry solid content correction (Bahnemann 2012).
Associated standard	None known.
Area of application	In the case of the method introduced in 3.1. "Determination of total solids and of organic dry matter", volatile substances such as organic acids and alcohols that may, for example, be contained in silages in not insignificant amounts, are not taken into consideration. This can lead to an underestimation of the total solids content and thereby to an overestimation of the biogas yield and/or the biogas (formation) potential (VDI Guideline 4630 2006) and may make the comparison of substrates amongst one another more difficult. For this reason, Weissbach & Strubelt published an article in "Landtechnik" (Agricultural Engineering) magazine regarding the correction of the total solids content of maize silages (Weissbach & Strubelt 2008b), grass silages (Weissbach & Strubelt 2008a) and sugar-beet silages (Weissbach & Strubelt 2008c). The authors Berg & Weissbach (1976) investigated the concentrations of potentially volatile substances in a representative collection of samples from maize, grass and sugar-beet silages as well as in the drying residues generated therefrom upon determination of the TS content. From the comparison of the concentrations measured in the fresh and the dried sample, conclusions were drawn regarding the rate of volatility of the respective compound. Only in the case of lactic acid, this approach could not be used due to the condensation reactions (lactone formation) which the lactate is subject to during the drying. Here, the rate of steam evaporation volatility of lactic acid determined in earlier tests by means of dry distillation of silage samples was incorporated (Berg & Weissbach 1976). For the tests, water extracts were used that corresponded to a ratio of 50 g of fresh silage per 200 mL of water, each. In these extracts, both the lower fatty acids as well as the alcohols were determined through gas chromatography with a GC/FID device system (Shimadzu) with capillary columns and internal standards (*iso*-caproic acid for the acids, and pentanol for the alcohols). To determine the lower fatty acids, formic acid was added to the extract to release them. Prior to the determination of the alcohols, the fermentation acids in the extracts were neutralised with sodium hydroxide. In parallel to the GC, the pH values and the lactic acid contents in the extracts were measured.

	The determination of the lactic acid was performed by means of the colorimetric method according to Barker & Summerson (1941) with *4-biphenylol*, modelled after the version described by Haacker, Block & Weissbach (1983). All these methods are in-house methods of the "Analytics Laboratory for Agriculture and Environment" (Analytiklabors für Landwirtschaft und Umwelt) Blgg Deutschland GmbH (Lübzer Chaussee 12, D-19370 Parchim) and conform to the standard of certified laboratories of this subject area (Strubelt 2013). An overview of the range of the total solids contents as well as of the shares of potentially volatile substances overall (sum of lower fatty acids, lactic acid and alcohols) in silages that were tested by the authors and utilised to derive the volatility factors, is provided in Table 1. The information shows that in the case of silages – in comparison to the source material from which they were manufactured – a portion of the TS and/or the VS consists of potentially volatile fermentation products that are not captured in the typical determination of TS and VS (Ch. 3.1). The share of these fermentation products may differ widely, depending on the type of crop, the variety, the location, the weather, and the diligence upon ensiling. It may reach a significant scope. The individual substances are volatile to different degrees. Therefore, determining them and including them in the assessment of the substrates by means of a correction of the TS content is an urgent necessity.
Need for research	The coefficients for acids and alcohols suggested by Weissbach and Strubelt can be understood as volatility factors upon drying at 105 °C. The degree of volatility depends on the temperature and on the vapour pressure of the substances. Since no systematic investigation of the volatility is apparent from the publications to date, a verification of the suggested factors should be performed, in particular with respect to different drying temperatures and durations and the different drying behaviour resulting therefrom (Banemann 2012). In addition to silages, it is also conceivable that residues from food production or from other branches of industry may contain volatile substances and that therefore a total solids content correction would be sensible here, too. This would need to be researched separately for each substrate, based on a representative sample selection in accordance with the approach described by Weissbach & Strubelt. Alternatively, in the case of substrates with only a low water content and a high share of volatile substances, the Karl Fischer method could be utilised to determine the water content of a

substrate and thereby its total solids content including volatile components. From this, the ash content can be subtracted to determine a corrected VS content. In the opinion of (Weissbach 2011), the Karl Fischer method provide values for the TS content then calculated as difference that are too inaccurate due to its comparatively high water content.

Table 1: Bandwidth of the uncorrected total solids as well as the sum of the potentially volatile fermentation acids and alcohols (Weissbach & Strubelt 2008a, 2008b, 2008c)

Substrate	Uncorrected total solids content in g kg^{-1}_{ww}			Fermentation acids & alcohols in g kg^{-1}_{ww}		
	Minimum value	Maximum value	Average value	Minimum value	Maximum value	Average value
Maize silage	224	492	337	12	49	33
Grass silage	179	597	428	7	61	30
Sugar-beet silage	88	207	154	6	124	94

Description of method

Weissbach & Strubelt suggest improved correction equations based on the determination of the total solids content (also referred to as dry matter), the lower fatty acids (Ch. 3.1 and 3.2), the lactic acid (Ch. 3.2), the alcohols, and – in part – also the pH value. In deviation from chapter 3.1, the determination of the total solids content is performed according to Weissbach. As is typical in the case of feed tests, first, a pre-drying at 60 to 65 °C takes place and subsequently a final drying of exactly three hours at 105 °C to determine the total solids content (TS content).

(Weissbach & Strubelt 2008b) recommend the following correction of the TS content for **maize silages**, wherein all data have to be filled-in in g per kg_{ww}:

$$TS_c = TS_n + 0.95\ LFA + 0.08\ LA + 0.77\ PD + 1.00\ OA\ [g\ kg^{-1}_{ww}] \quad (3)$$

TS_c Corrected TS content of maize silages
TS_n TS content of maize silages
LFA Sum total of the contents of lower fatty acids (C_2–C_6)
LA Lactic acid content
PD 1,2-propandiol content
OA Sum total of the contents of other alcohols (C_2–C_4, including 2,3-butandiol)

All information that is relative to TS_n, such as the ash content, must be corrected after TS_c is calculated through multiplication with the quotient of TS_n/TS_c (Weissbach & Strubelt 2008a). The amount of the volatile solids content (VS) is the result of the difference between the corrected total solids content and the corrected ash content. The correction formula for maize may also be applied in the case of sorghum and grain crop silages as an approximated solution (Weissbach 2011).

Based on tests of their own, (Weissbach & Strubelt 2008a) specify the following formula for the correction of the TS of **grass silage**:

$$TS_c = TS_n + (1.05 - 0.059\ pH)\ LFA + 0.08\ LA + 0.77\ PD + 0.87\ BD + 1.00\ OA\ [g\ kg^{-1}_{ww}] \quad (4)$$

TS_c Corrected TS content of grass silages
TS_n TS content of grass silages
pH pH value
LFA Sum total of the contents of lower fatty acids (C_2–C_6)
LA Lactic acid content
PD 1,2-propandiol content
BD 2,3-butandiol content
OA Sum total of the contents of other alcohols (C_2–C_4)

The correction formula for grass silage may also be used for clover, grass ley, alfalfa and green grain silages (Weissbach 2011).

Another correction formula was developed for **sugar-beet silages** (Weissbach & Strubelt 2008c):

$$TS_c = TS_n + 0.95\ LFA + 0.08\ LA + 1.00\ AL\ [g\ kg^{-1}_{ww}] \quad (5)$$

TS_c Corrected TS content of sugar-beet silages
TS_n TS content of sugar-beet silages
LFA Sum total of the contents of lower fatty acids (C_2–C_6)
LA Lactic acid content
AL Sum total of the contents of all alcohols (C_1–C_4, including the diols)

Here, the volatility rate was only estimated based on the results for other silages and was not measured since the relatively high content of soluble pectin substances would make the GC measurements in the drying residue impossible. According to (Weissbach 2011), the application of the correction formula for sugar-beet silages with higher TS contents than those of the samples tested by him is possible without a problem; this also applies to silage effluent. In the case of grass and sugar-beet silage, the correction of the ash content and the VS is carried out analogously to the approach for maize silage (Weissbach & Strubelt 2008a, 2008b, 2008c).

3.3 Determination of the VOA value (according to Kapp) and of the VOA/TIC value (in accordance with FAL)

Katrin Strach, Michael-Dittrich Zechendorf, DBFZ

Status	Are being used in routine operation.
Standard	VOA according to Kapp (Buchauer 1998)
	VOA/TIC in accordance with FAL (Burchard et al. 2001)
Area of application	Can be used for digestates and liquid substrates in which the liquid phase can be separated off by means of centrifugation. Prerequisite is a pH value > 5.
Disadvantage	Substances with pH values below 5 cannot be analysed. Solids have a disruptive impact on the pH measurement.
Advantage	In comparison to cuvette tests, this method is very cost-efficient.
Need for research	In order to be able to, for instance, also measure digestates from hydrolysis containers, it must be possible to also make a determination for sample with pH values below pH = 5. It has to be researched whether it is possible to raise the pH value prior to titration or whether, for example, steam distillation would constitute a suitable method.

The determination of the VOC according to Kapp and of the VOA/TIC in accordance with FAL are, from a technical point of view, two different methods which can be carried out in a single, joint work step. Fundamentally, in both cases, the clear phase of a centrifuged sample is titrated in stages to certain pH values by means of sulphuric acid. Both processes are carried out in a single work step and the titration is performed for all individual stages; subsequently, the amounts of sulphuric acid used are calculated, depending on the method.

By means of the **method according to Kapp**, the concentration of the volatile organic acids (VOAs) is determined through titration. The clear phase of a sample is titrated with the automatic titration machine Mettler Toledo type Rondo 60/T90 with 0.2 N sulphuric acid in stages up to the pH values 5, 4.3, and 4.0. With the acid consumption achieved, the VOA value can be calculated (Buchauer 1998).

Devices and chemicals

- Titrator or burette with pH-meter
- Centrifuge
- Beaker
- Pipette

The calculation of the concentration of the acids is carried out in accordance with the following formula:

$$VOA = 131\,340 \cdot (V_{pH4.00} - V_{pH5.00}) \cdot \frac{N_{H_2SO_4}}{V_{sample}} - 3.08 \cdot \overbrace{V_{pH4.30} \cdot \frac{N_{H_2SO_4}}{V_{sample}} \cdot 1000}^{Ks_{4.3}\ [mmol/L]} - 10.9 \quad (6)$$

VOA	Concentration of the volatile organic acids according to Kapp	(mg/L)
$V_{pH4.00}$	Volume of acid titrated in up to pH = 4.00	(mL)
$V_{pH4.30}$	Volume of acid titrated in up to pH = 4.30	(mL)
$V_{pH5.00}$	Volume of acid titrated in up to pH = 5.00	(mL)
V_{sample}	Volume of centrifuged sample submitted	(mL)
N_{H2SO4}	Normality of the acid (molar concentration of hydronium-ions of the acid)	(mol/L)
$Ks_{4.3}$	Alkalinity 4.3 [mmol/L] (DIN 38409-7 2005)	(mmol/L)

Area of validity

Acids from 0 to 70 mmol/L(0 to 4,203 mg_{HAc}/L)
NH_4^+–N from 400 to 10,000 mg/L
The determination of the **VOA/TIC** in accordance with FAL is carried out through titration of the clear phase of a sample with the pH values up to 5.0 and 4.4.
Subsequently an assessment is performed via the following equation:

$$VOA/TIC = \frac{\left((V_{pH4.4} - V_{pH5.0}) \cdot \frac{20}{V_{sample}} \cdot \frac{N_{acid}}{0.1} \cdot 1.66 - 0.5\right) \cdot 500 \cdot V_{sample}}{0.5 \cdot N_{acid} \cdot V_{pH5.0} \cdot M_{CaCO_3} \cdot 1000} \quad (7)$$

VOA/TIC	Relationship of volatile organic acids and the reactor buffer capability relative to calcium carbonate	(g_{VOA}/g_{CaCO3})
$V_{pH4.4}$	Volume of acid titrated in up to pH = 4.40	(mL)
$V_{pH5.0}$	Volume of acid titrated in up to pH = 5.00	(mL)
V_{sample}	Volume of centrifuged sample submitted	(mL)
N_{acid}	Normality of the acid (molar concentration of hydronium-ions of the acid)	(mol/L)
M_{CaCO3}	Molar mass of calcium carbonate with 100 g/mol	

Execution method

The sample is centrifuged at 10,000 × g, 10 °C for 10 min. For analysis, 10 mL of the clear phase created this way is pipetted off and transferred to the automatic titration machine by means of a sample beaker. Depending on the result to be expected, the drop size of the amount of 0.2 N sulphuric acid added must be set such that the respective pH values are not exceeded. Then, the titration is started and a titration up to the pH values of 5.0, 4.4, 4.3, and 4.0 is performed one after another. The respective acid consumptions are recorded. The calculation of the respective VOA according to Kapp and/or the VOA/TIC in accordance with FAL is performed as described above.

Comment: There is no direct link between VOA/TIC in accordance with FAL and VOA according to Kapp. Due to the different VOA approaches, a calculation of the VOA/TIC by means of VOA according to Kapp is not possible.

3.4 Determination of the ammonia nitrogen content

Katrin Strach, DBFZ

Status	Is being used in routine operation.
Standard	HACH, DR 2000 spectrophotometer handbook
Area of application	Can be used for digestates and liquid substrates in which a liquid phase is created by means of centrifugation.
Disadvantage	For samples with strong inherent colouring, a photometric determination is not always possible. For samples with a pH value < 6, the pH value must be raised to pH value 6–7. Waste is generated that must be disposed of separately. The use of the reagents of the Hach Lange GmbH company is mandatory.
Advantage	This is a quick and easy method.

The determination of the total ammonia nitrogen content (TAN) is performed according to the principle of Nessler. In this, the Nessler's reagent alkaline potassium tetraiodomercurate(II), $K_2[HgI_4]$ is being utilised. With ammonia, it builds a reddish-brown colour complex $[Hg_2N]I$, the iodide of the cation of the Millon's base. With the help of this complex, the ammonia can be determined photometrically.

Devices and chemicals

- Photometer Hach DR 2000 or Hach DR 3900
- Clear phase (centrifugate) of the sample after centrifugation
- Mineral stabiliser (HACH LANGE GmbH)
- Polyvinyl alcohol (HACH LANGE GmbH)
- Nessler's reagent (HACH LANGE GmbH)

Execution method

Prior to the determination of the ammonia nitrogen, the sample must be centrifuged for 10 min at 10 °C at 10,000 × g. From the centrifugate (clear phase), a dilution corresponding to the measuring range of the photometer is prepared (differs from system to system [most often 1:1,000 or 1:2,000]). Subsequently, 25 mL of the dilution are placed in a cuvette. In addition to the preparation of the sample, a reference (25 mL aqua dist.) must be prepared. Next, three drops of mineral stabiliser and three drops of polyvinyl alcohol are added. Shortly before the measurement, 1 mL of Nessler's reagent is added. An intermixing is achieved by carefully swirling the samples around. After a reaction time of the Nessler's reagent of one minute, the sample can be measured. Once the Nessler's reagent has been added, the samples must be measured within 5 min.

4 Methods for the determination of chemical parameters

4.1 Determination of aliphatic, organic acids and benzaldehyde with headspace GC

Martin Apelt, DBFZ

The method described serves for the determination of the following organic acids: Acetic acid, propionic acid, isobutyric acid, butanoic acid, isovaleric acid, valerianic acid and hexanoic acid as well as benzaldehyde.
The headspace GC utilised is particularly suitable for the determination of the content of volatile substances in samples with a complex matrix. To address the impact of the different matrixes, the addition of 2-ethyl-butyric acid as an internal standard is performed. Through the addition of phosphoric acid to the sample, the acids are transitioned into their undissociated form and put into a highly volatile state. This way, the GC determination of the content of the aforementioned acids is possible.
With the help of a GC-MS, it was possible to unambiguously identify benzaldehyde as a component in a lot of samples, whereupon a routine detection of the substance was implemented.

Status	This is a not yet validated in-house method of the DBFZ. Varying and changing matrixes require a constant adjustment of the temperature gradient.
Associated standards	(Wang et. al 2009); (Görtz & Meissauer 2003); (Cruwys et. al 2002); GC Application ID No.: 15883 Phenomenex
Area of application of the method	Due to the determination by means of headspace GC, it is possible to test a lot of different matrixes. At the DBFZ, the determination of the volatile organic acids C_2–C_6 is performed in order to monitor the different fermentation procedures in the area of biogas research and in order to monitor biogas plants already in operation.
Substrates/ materials	Testing of digestates from the area of biogas research
Limitations of the method	The device is calibrated by manufacturing different calibration solutions for the ranges of concentration (c.f. Table 2). Due to the large calibrating/measurement range, it is possible to analyse virtually all samples without dilution. Since the measurement methods is not linear over the whole calibration range, two calibration functions for different

	ranges of concentration are prepared which overlap in their concentration ranges. This way, an exact determination is achieved for the calibration ranges stated in Table 2. In the lower limit of detction range, larger fluctuations of the results may occur due to inhomogeneity of samples and matrix effects.
Advantages	Easy preparation of samples and analytical measurement, which can be applied well for in-process controls with a high throughput of samples. A low use of additional chemicals for the preparation of samples has a positive effect on the cost efficiency of this method. Due to the short time required for analysis for the method described here, a close monitoring of research tests is ensured and is quite universally usable.
Need for research	To identify process disruptions in biogas plants and fermentation tests more quickly, it will be necessary in the future to identify and quantify additional analytes in the digestates. While important indicators for the progress of a fermentation are determined through the determination of the volatile organic acids C_2–C_6, an expansion of the spectrum of analytes would be helpful in order to increase the biogas yield and to identify problems early on. Worth mentioning as progress in the recent past is the identifi cation of a recurring peak, which was identified as benzaldehyde. Now it´s possible to detect them routinely in addition to the acid spectrum with the same method. To what extent an impact on the different fermentation procedures exists here still needs to be researched.

Reagents

- Internal standard (ISTD): 184 mg/L
- H_3PO_4 (diluted 1:4)

Devices and aids

- 20 mL Headspace vial
- Caps
- (Electric) crimping tool
- 5 mL pipette
- 1 mL pipette

Sample preparation

The GC analysis is carried out as a triplicate determination. Therefore, 3 Headspace vials are prepared per sample. Prior to the determination, the sample must be centrifuged for 10 min at 10 °C and 10,000 rpm.

If necessary, subsequent to centrifuging, the sample is strained through a sieve (mesh width approx. 1 mm) in order to remove coarse matrix components.
In principle, a dilution of the samples is possible, however, attention always has to be paid to the fact that the concentrations of the analytes to be determined be within the calibrated ranges of the measurement method.
Of the sample now at hand, 5 mL, each, are pipetted into a Headspace vial. Then, 1 mL ISTD and 1 mL H_3PO_4 (diluted 1:4) are added. Once the phosphoric acid has been added, the vials must be closed immediately with suitable caps and an electric crimper.

Calibration

The device is calibrated by manufacturing different calibration solutions in the following ranges of concentration:

Table 2: The calibration utilised

Analyte	Calibration range [mg/L]	Retention time [min]	LOD [mg/L]	LOQ [mg/L]
Acetic acid	5.223–15669.000	6.0	2.819	8.405
Propionic acid	1.980–5940.000	7.2	1.07	3.189
Isobutyric acid	0.948–2844.000	7.7	0.372	1.176
Butanoic acid	1.920–5760.000	9.0	0.845	2.489
Isovaleric acid	0.930–2790.000	10.1	0.389	1.218
Valerianic acid	0.940–2820.000	12.6	0.626	2.018
Hexanoic acid	0.465–1395.000	15.5	0.205	0.62
2-ethyl-butyric acid (ISTD)	–	13.5	–	–

Analysis

For the calculation of the actual concentrations of the substances to be investigated, the internal standard is referenced and analysed via calibration lines.

Device parameters

Table 3: Description of the gas chromatograph (Agilent 7980A)

Injector	–	Split/splitless		
Detector	–	FID		
Carrier gas	–	Nitrogen		
Column designation	–	ZB-FFAP (Phenomenex) or equivalent		
Column length	m	30		
Column diameter	mm	0.32		
Film thickness	µm	0.25		
Flow Constant flow	mL/min	Total flow: 8.5 Septum purge flow: 3 Split flow: 0.5		
Column temperature programme		Rate [°C/min]	Target temperature [°C]	Duration of stay [min]
		Start 10 30 120	40 100 150 240	0 8 1 2
Measuring time	min	19.417		
Split ratio	–	0.1:1		
Injector temperature	°C	220 °C		
Detector settings	Heating H_2 flow Air flow Make up flow Signal	260 °C 45 mL/min 400 mL/min 25 mL/min 10 Hz/0.02 min		

Table 4: Description of the headspace sample injector (PerkinElmer Turbo Matrix 110)

Temperatures [°C]	Needle Transfer line Oven	95 110 85
Pressures [psi]	Carrier gas flow Vial	32.0 32.0
Times [min]	Pressure build-up Injection Stay Thermostat Cycle PII	4.0 0.10 0.5 32.0 22.0 24.0
Additional settings	High pressure injection Vial vent Shaker Injection method Operating method Injections	On On On Time Constant 1
Transfer line	–	Deactivated ID: 0.32 mm

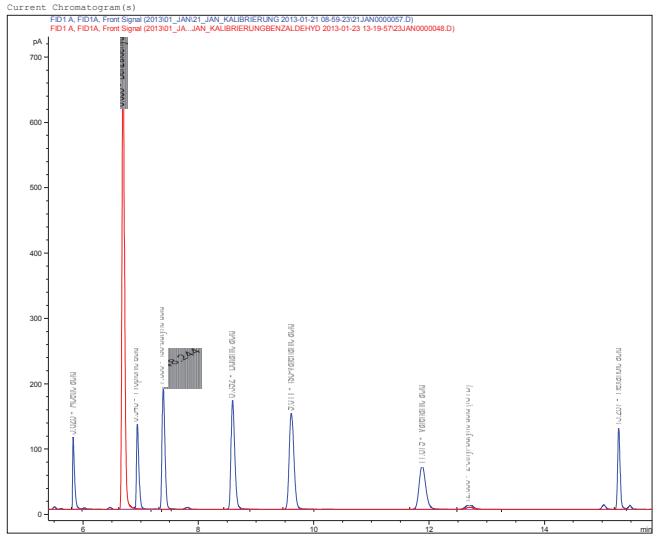


Figure 1: Sample chromatogram of a standard solution mixture (C_2–C_6) and a single standard of benzaldehyde

4.2 Determination of organic acids

Lucie Moeller, Kati Görsch, UFZ; Dietmar Ramhold, ISF Schaumann Forschung mbH; Erich Kielhorn, TU Berlin

Status	The determination of organic acids via ion chromatography and HPLC corresponds to the general standard.
Associated standards	GC
Area of application of the method	Substrates/materials: no restriction
Measuring range	1–1,000 mg/L
Disadvantages	The sample must be pretreated so that it is free from suspended and humic matter. Pretreatment with the help of the Carrez clarification may be necessary.
Need for research	For these methods, there is no need for research.

Whether or not a disruption of the biocenosis in the biogas reactor exists can be determined through the determination of the volatile organic acids (acetic acid, propionic acid, butyric

acid, valeric acid, etc.). These compounds occur upon the decomposition of organic matter and are created as intermediate products during methane production, wherein they are immediately converted to methane in the case of an undisrupted methanogenesis. Their enrichment in the fermentation fluid therefore is indicative of a disruption in the decomposition chain (Ross & Ellis 1992). The proof of volatile organic acids is performed via GC; the utilisation of both ion chromatography (IC) and high-pressure liquid chromatography (HPLC) is possible.

Processing of sample

The sample is centrifuged in 50 mL centrifuge tubes for 20 min at 5.300 rpm and 20 °C (device: Avanti 30 centrifuge, Beckman company). The supernatant is first strained through a sieve (mesh width: 0.75 mm) and then filtered with the help of a pressure filtration unit (device: SM 16 249, Sartorius company), in order to free the solution from disruptive fibres and proteins. The filtration unit is equipped with a screening plate, perforated sheet metal, and a nylon membrane filter (pore size: 0.45 µm, diameter: 47 mm, Whatman company or Pall) that is held by a silicone sealing ring. After closing the unit, a pressure of 5 bar is applied and a container is placed below the unit to catch the filtrate. Once the filtration of 7–8 mL of centrifuge supernatant has been completed, approx. 5 mL of the clear filtrate is located in the receiving container. This filtrate is diluted with bi-distilled water in accordance with the expected/assumed acid concentrations and analysed by means of HPLC and/or IC.

Alternative:

In order to avoid problems due to contamination of the chromatographic column, the so-called Carrez clarification of the samples can be performed. For this, 1 mL of the centrifugate is mixed with 200 µL of Carrez solution I (15 g potassium hexacyanoferrate $K_4(Fe(CN_6)) \times 3\ H_2O$ in 100 mL distilled water) and intensely shaken (Vortex). After two to five minutes, 200 µL of Carrez solution II, consisting of 23 g zinc chloride in 100 mL distilled water (tip: the velocity of dissolution is improved in a water bath at 70 °C) is added and once again intensely shaken. The mixture is subsequently centrifuged for 10 minutes at 10,000 × g. The centrifugate is then filtered through a 0.2 µm filter and diluted accordingly prior to the analysis.

A zinc sulphate solution may also be used as the Carrez solution II. This active substance is, however, less active than zinc chloride so that more solution is required for the clarification.

Process of the Analysis

Ion chromatography with suppressed conductivity detection

The IC system DX600 of the Dionex company consists of the quaternary gradient pump GP 50 2, the eluent generator EG 40, the auto sampler AS 50 (for 1.5 mL vials), the conductivity detector CD 25a (with auto-regenerating suppressor) and the analytical separation column IonPac® AS 11 HC (with guard column AG 11 HC, both 4 mm diameter). Chromeleon 6.5 is used as software.

The recommendations of the Dionex company regarding the separation and detection of oxocarboxylic acids (and select anions) can be adopted as operating parameters of the system (c.f. Table 5). A sample chromatogram is depicted in Figure 2.

Table 5: Separation parameters in ion chromatography

Column	IonPac® AS 11-HC	
Flow rate	1.5 mL/min	
Temperature	30 °C	
Injection volume	10 µL	
Eluent	NaOH	
	0–8 min: 8–28 min: 28–38 min: 38–39 min: 39–40 min:	Isocratic 1 mM Linear to 30 mM Linear to 60 mM Isocratic 60 mM Linear to 1 mM

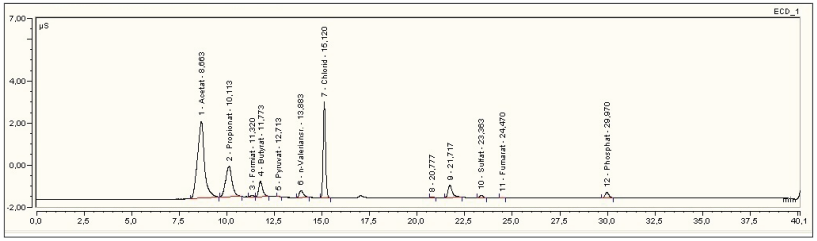


Figure 2: Sample chromatogram for the determination of the volatile organic acids with the help of an IC

High-pressure liquid chromatography (HPLC)

The Shimadzu HPLC system consists of the degasser DGU14A, the pump LC10AT, the auto injector SIL10A, the oven CTO10AC and the detector RID10A; the controlling of the individual components is carried out via communications module CBM10A. The column VA 300/7.8 Nucleogel Ion 300 OA (Macherey-Nagel company, dimensions: 4 × 250 mm; guard column: REF 719537) is heated in the oven to 70 °C. The mobile solvent 0.01 N H_2SO_4 moves the injected sample (10 µL) at a rate of 400 µL/min through the system and the detection is performed through a measurement of the refractive index (device: RID10A, Shimadzu company). The CLASS-LC10 is being utilised as software.

Solutions of the corresponding salts with concentrations of 28.5–285 mg/L are utilised as external standards. The separation of acids is performed under the conditions presented in Table 6. A sample chromatogram is depicted in Figure 3.

Table 6: Separation parameters in high-pressure liquid chromatography (HPLC)

Column	VA 300/7.8 Nucleogel Ion 300 OA
Flow rate	400 µL/min
Injection volume	10 µL
Eluent	0.01 N H_2SO_4 (isocratic)

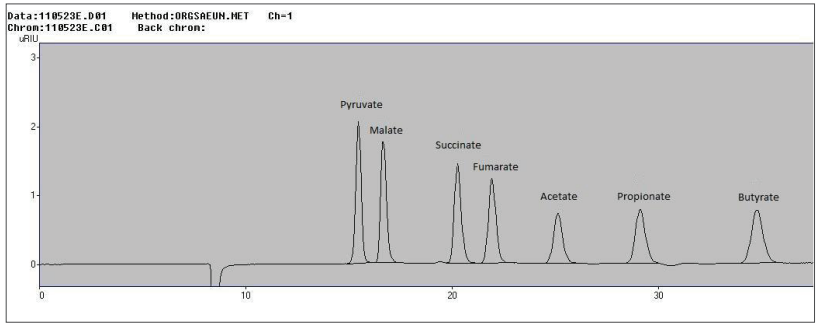


Figure 3: Sample chromatogram for the determination of the volatile organic acids with the help of an HPLC (Source: UFZ)

4.3 Determination of aldehydes, alcohols, ketones, volatile fatty acids

Erich Kielhorn, Peter Neubauer, Stefan Junne, TU Berlin

Status	The methodology presented describes the processing and GC analysis of biogas samples.
Area of application of the method	Liquid samples without solids, i.e., typically the centrifugate or filtrate of the samples are utilised, since with this method the extra-cellular metabolites, meaning those dissolved in the liquid, are determined.
Advantage	Metabolite concentrations can be detected starting at approximately 1 mg/L. The labour input for the preparation and analysis is comparatively low. At the same time, the preparation of the sample ensures a degree of purity that reduces column performance loss. A major advantage is the high sensitivity and separation efficiency of the method so that even very small amounts of metabolites can be quantified reproducibly.
Need for research	It was not possible to unambiguously identify all substances or metabolites that were separated in the chromatogram. Thus, there is a need for additional research in order to determine whether or not these substances are relevant to making a statement regarding the process state.

Sample preparation

In order to obtain water-free samples for the GC-MS analysis, the metabolites are extracted from the biogas liquid with chloroform and are derivatised with methyl chloroformate (MCF) prior to transfer into the solvent phase. For better separation of the phases, methanol and a sodium hydrogen carbonate solution ($NaHCO_3$) are added. As internal standard, 1-propanol can be utilised.

Samples are first centrifuged for 10 min at 4 °C and 9,500 × g. 200 µL supernatant are transferred into an Eppendorf centrifuge tube and mixed with 10 µL of 1-propanol solution diluted with distilled water (1:50) as internal standard. Then, 167 µL methanol and 34 mL pyridine are added. The derivatisation is started by adding 20 µL MCF. The mixture is strongly mixed for 30 s (Vortex mixer). Thereafter, another 20 µL MCF are added and once again mixed for 30 s.

Now, 400 µL chloroform are added in order to extract the metabolites and their derivatives from the reagent mixture. The emulsion is mixed for 10 s. For better phase separation, 400 µL 50 mM $NaHCO_3$ solution are added, followed by 10 s of mixing.

The resulting chloroform phase (bottom phase) is carefully transferred with a pasteur pipette into a micro centrifuge tube (1.5 mL). With a molecular sieve bead that is placed in the tube, any residues of water are removed.

After a 1 min waiting period, the sample is transferred into GC bottles. Until the measurement, the samples are stored at -20 °C.

Materials and devices

For the sample preparation

- 1-propanol ≥ 99.7 % (Sigma-Aldrich) #279544
- Methanol ≥ 99.9 % (Carl Roth) #AE01.1
- Pyridine ≥ 99 % (Carl Roth GmbH) #CP07.1
- Methyl chloroformate (MCF, Aldrich) #M35304
- Chloroform (Aldrich) #528730
- Sodium hydrogen carbonate ≥ 99.5 % ($NaHCO_3$, Carl Roth GmbH) #6885.1
- Molecular sieve 5 Å (Carl Roth) #8475.1
- Threaded brown GC bottles 1.5 mL (Fischer) #1072-8684
- Micro-inserts 50 µL for 1.5 mL threaded bottles (Fischer), #1024-4612
- Springs for micro-inserts (Fischer) #320 55 76
- 8 mm silicone-coated rubber septums (Fischer) #3146116
- Screw tops for threaded bottles, (Carl Roth) #161.1
- Pasteur pipettes (Carl Roth GmbH) #4518

For the analysis

GC/MS system (Agilent Technologies, Waldbronn, Germany (c.f. Figure 4)), consisting of:

- Gas chromatograph GC 7890A
- Mass spectrometer MSD 5975G

Analytical separation column: FactorFour VF-624ms (Agilent Technologies, Waldbronn) #CP9104

- Coating: 6 % cyanopropyl/phenyl, 94 % dimethylpolysiloxane
- Length: 30 m, ID: 0.32 mm
- Film: 1.8 µm,
- Temperature limits: -40 °C–300 °C

Carrier gas: Helium

Figure 4: GC/MS system (Agilent Technologies, Waldbronn, Germany)

Execution method

The temperature of the injection chamber is 150 °C; that of the detection chamber is 280 °C. At the start of the analysis, the column has a temperature of 40 °C. Once the sample has been injected, the temperature is increased by 4 °C per minute, up to 150 °C, and maintained for 15 min. Subsequently, an increase in 5 °C/min steps is performed, up to the final temperature of 180 °C. Said temperature is maintained for an additional 5 min. 1 µL sample is injected with a 1:20 split (final sample volume 0.05 µL).

Analysis of the results

The identification of the substances is performed with the help of a substance database (e.g. the "NIST library"); the quantification is performed with the help of a previously prepared calibration line. The amount of the substances contained can be determined via the peak areas of the chromatogram obtained (c.f. Figure 5).

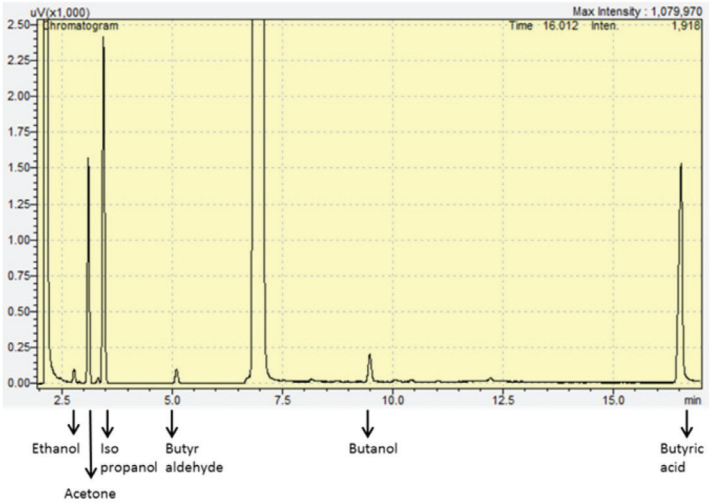


Figure 5: Sample chromatogram, analysis of aldehydes, alcohols, ketones, and volatile fatty acids

4.4 Examination of samples of solids (substrates) and digestates with HPLC for aliphatic and aromatic acids, alcohols and aldehydes

Martin Apelt, DBFZ

With the help of the method described here, it is possible to test a wide range of analytes in a single sample, which are listed in Table 7:

Table 7: List of components

Aliphatic acids	Aromatic acids	Alcohols	Aldehydes
Lactic acid Acetic acid Propionic acid *Iso*butyric acid Butanoic acid *Iso*valeric acid Valerianic acid	Phenyl acetic acid Benzoic acid	Ethanol 1-propanol 1,2-propandiol	Furfural 5-(hydroxymethyl)furfural (5-HMF)

Status:	This is a not yet validated in-house method of the DBFZ.
Associated standards	(Hecht 2010); HPLC Application ID No.: SI-01153 Agilent; (VDLUFA 1988)
Area of application of the method	Since no interference of the multitude of matrixes has an impact on the analysis results, a wide range of the most diverse matrixes can be tested. At the DBFZ, the determination of the aforementioned components is carried out as in-process control of different fermentation procedures in the area of biogas research and in order to monitor biogas plants already in operation. Furthermore, the testing of the most diverse substrates, that are utilised in the fermentation process, is possible.
Substrates/ materials	Testing of digestates from the area of biogas research. Different samples of solids (substrates) that are used in the individual processes.
Limitations of the method	The limitations of the method are imposed by the different calibration ranges (c.f. Table 8). Due to the large calibrating/ measurement range, it is possible to analyse virtually all samples without dilution.

	Since the measurement method – except for phenyl acetic acid and benzoic acid – is not linear over the whole calibration range, two calibration functions are prepared for all other analytes that overlap in their concentration ranges. This way, an exact determination is achieved for the aforementioned calibration range. For the determination of phenyl acetic acid, an additional analysis by means of headspace GC is required, since phenyl acetic acid co-eluates together with the hexanoic acid. If the concentration of hexanoic acid at the headspace GC is below the limit of detection (LOD) of HPLC, the corresponding peak can be quantified as phenyl acetic acid. If, however, in the determination at the headspace GC a concentration of hexanoic acid above the LOD of HPLC is determined, this requires a dilution of the sample. For this, the dilution must be selected such that the concentration of hexanoic acid then achieved will be below the LOD of HPLC.
Advantages	The method is characterised by easy preparation of samples and analytical measurement, which can be applied well for in-process controls with a high throughput of samples. The particular advantage of this method is the determination of lactic acid, aromatic acids, lower alcohols and aldehydes. A low use of additional chemicals for the preparation of samples has a positive effect on the cost effectiveness of this method.
Need for research	To identify process disruptions in biogas plants and fermentation tests more quickly, it will be necessary in the future to identify and quantify additional analytes in the digestates. While important indicators for the progress of a fermentation are determined through the determination of the analytes described, an expansion of the spectrum of analytes would be helpful in order to increase the biogas yield and to identify problems early on. A further optimisation of the method should make it possible to test various saccharides and their decomposition products, and to separate hexanoic acid and phenyl acetic acid.

Reagents

- 5 mM sulphuric acid
- Distilled water

Devices and aids

- 1.5 mL HPLC vial with screw cap and 0.5 mL microlitre insert
- 1.5 mL plastic centrifuge tubes
- 1.5 mL plastic centrifuge tubes with 0.2 µm filter insert
- HPLC with refractive index detector (RID) and diode array detector (DAD)
- Shaker
- Ultrasonic bath

Sample preparation

At a minimum, a double determination is carried out for all samples. In the case of substrates, a minimum of two complete eluates must be prepared for this.

a) For solid samples (substrates):
weigh-in 5 g of substrate and eluate with 50 mL distilled water for 24 h on a shaker. Fill eluate through a sieve into a small plastic tube with screw cap.

b) For digestate samples (e.g. reactor samples):
Prior to the determination, it may be necessary to centrifuge the sample for 10 min at 10 °C and 10,000 rpm. If necessary, subsequent to centrifuging, the sample is strained through a sieve (mesh width approx. 1 mm) in order to remove coarse matrix components.

500 µL of the supernatant or of the filtrate from a) or b) are pipetted as double determination into one Eppendorf tube each with a 0.20 µm filter attachment and centrifuged for 10 min at 10 °C and 15,000 rpm. The filtrate from the Eppendorf tube with filter attachment is pipetted into a 1.5 mL HPLC vial and sealed. If this is not possible, corresponding microlitre inserts for HPLC vials must be used.

Calibration

A separate calibration solution must be prepared for each group of substances. This way, it is possible, in the case of the aliphatic acids, to include hexanoic acid for the determination of phenyl acetic acid. For the calibration of lactic acid, sodium lactate is weighed in and a conversion calculation to lactic acid is performed accordingly. Below, please find the calibration ranges of the individual analytes:

Analysis

A quantitative analysis of the individual analytes is carried out via external standard calibration. For all acids and alcohols, the analysis is performed based on chromatograms from the RID.

Depending on concentration, the aldehyde must be analysed with the RID or DAD at 280 nm. With the RID, very high concentrations of aldehydes can be measured, but the detector features a bad detection sensitivity. Therefore, the analysis in the lower concentration range should be performed with the DAD. In the case of the DAD, please bear in mind that it, in turn, evidences an overload in the case of high concentrations. The respective limits can be found in Table 8.

Table 8: The calibration utilised

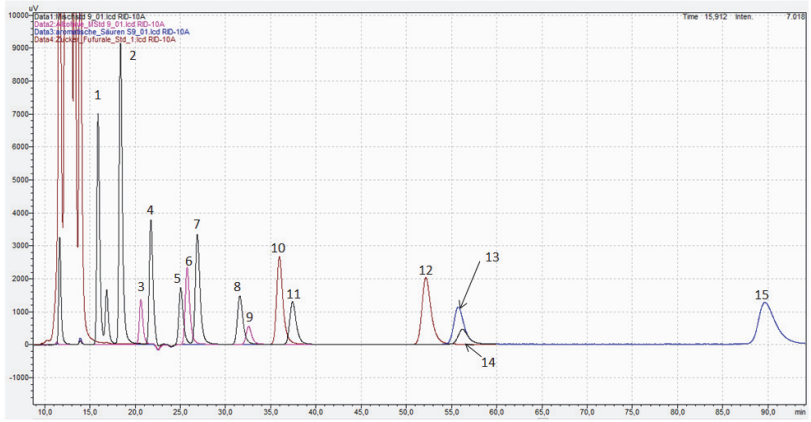
Labelling in Fig. 6	Analyte	Detector	Calibration range [mg/L]	Retention time [min]	LOD[1] [mg/mL]	LOQ[2] [mg/mL]
1	Lactic acid	RID	2.35–7064.44	15.9	0.47	1.55
2	Acetic acid	RID	5.22–15669.00	18.4	1.46	4.72
3	1,2-propan-diol	RID	5.18–1554.00	20.7	1.80	6.19
4	Propionic acid	RID	1.98–5940.00	21.8	0.64	2.04
5	*I*sobutyric acid	RID	0.95–2844.00	25.1	0.41	1.28
6	Ethanol	RID	1.97–5920.50	25.9	1.11	3.37
7	Butanoic acid	RID	1.92–5760.00	26.9	0.39	1.28
8	*I*sovaleric acid	RID	1.86–2790.00	31.6	1.12	2.84
9	1-propanol	RID	4.02–1205.25	32.7	2.65	9.15
	5-HMF	DAD 280 nm	2.20–220.00	35.7	1.48	5.19
10	5-HMF	RID	11.00–2200.00	36.0	9.76	34.41
11	Valerianic acid	RID	1.88–2820.00	37.5	0.73	2.02
	Furfural	DAD 280 nm	2.75–275.00	52.0	2.71	10.90
12	Furfural	RID	13.75–2750.00	52.2	10.81	37.72
13	Phenyl acetic acid	RID	14.24–1424.00	55.8	0.11	0.36
14	Hexanoic acid	RID	only for identification	56.3	2.78	9.65
15	Benzoic acid	RID	25.04–2504.00	89.9	4.08	14.24

[1] Limit of detection [2] Limit of quantification

Device parameters

Table 9: HPLC settings (Shimadzu)

Eluent	Isocratic with 5 mM sulphuric acid	**Column designation**	Hiplex H
Detector	RID DAD (for 5-HMF and furfural at 280 nm)	**Column dimension**	300 × 7.7 mm 8 µm
Flow	0.6 mL/min	**Guard column**	50 × 7.7 mm
Oven temperature	60 °C	**Measuring time**	95 min
Auto sampler	4 °C	**Injection volume**	20 µL



1. Lactic acid	4. Propionic acid	7. Butanoic acid	10. 5-HMF	13. Phenyl acetic acid
2. Acetic acid	5. Isobutyric acid	8. Iso-Valerianic acid	11. Valerianic acid	14. Hexanoic acid
3. 1,2-propandiol	6. Ethanol	9. 1-propanol	12. Furfural	15. Benzoic acid

Figure 6: Sample chromatogram of all standard mix solutions used on the RID

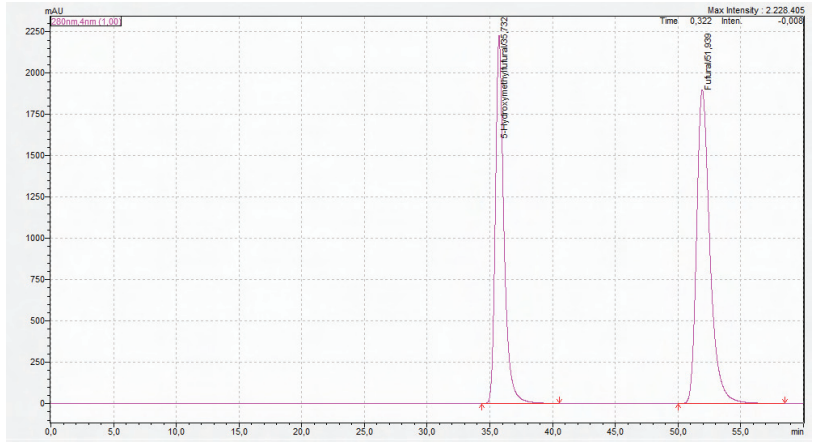


Figure 7: Sample chromatogram of 5-HMF and furfural at 280 nm

4.5 Determination of sugars and glucose degradation products

Jana Mühlenberg, DBFZ

One parameter that, in addition to others, is characteristic of the fermentability of substrates, is the sugar content. The method frequently used for the determination of reducing sugars with dinitrosalicylic acid (DNA) in some samples produces significantly higher total sugar values than the sum of all the individual sugars that were determined by means of high-pressure liquid chromatography (HPLC). This is caused by the unspecific reaction of DNA with all molecules that feature reducing functional groups (e.g. various aldehydes). The method described here allows the determination of monosaccharides (D-(+)-glucose, D-(+)-xylose, L-(–)-galactose, L-(+)-arabinose, mannose, D-(–)-fructose), a disaccharide (sucrose), sugar alcohols (glycerol, xylitol, D-(–)-sorbitol) and that of potential decomposition products which provide for positive results in the DNA test even though they are no sugars.

Status	The method has not yet passed final validation and is still being adjusted with respect to influences by changing matrixes.
Associated standards	Sample preparation from VDLUFA Book of Methods, Volume III; HPLC application of Agilent; addition literature regarding HPLC parameters: (Jourdier et al. 2012; Korakli et al. 2000)
Area of application of the method	The method is suitable for the determination of monosaccharides, disaccharides and sugar alcohols in various substrates. Similarly, biogas reactor contents can be inspected for decomposition products.
Substrates/ materials	Testing of substrates from the area of biogas research, but also of samples from other biomass utilisation processes (e.g. hydrothermal liquefaction of biomasses)
Limitations of the method	Depending on the matrix of the sample, overlaps with other substances may occur. The method is not very flexible since the HPLC column used can only be operated with water as eluent and since the column temperature is limited to 80 °C. Mannose and Fructose are not baseline-separated, but can be analysed.
Advantages	Sugars and decomposition products can be determined in a single analysis. Sample preparation is limited to dilution and filtration and is therefore quick and easy. Water as eluent is not only environmentally friendly but also inexpensive in comparison to other eluents.

Need for research	It may be possible to integrate other decomposition products relevant to the biogas process into the method in order to receive more information from a single analysis. With 90 min, the duration of the analysis is relatively long. New development in column materials in chromatography may, in the future, allow for a shortening of the analysis period. A sample preparation that is geared towards the reduction of matrix effects may also contribute to an improvement of the method. Furthermore, the establishing of an internal standard should be strived for.

Reagents

Millipore water

Devices and aids

- 1.5 mL HPLC vial with screw cap and 0.5 mL microlitre insert
- 1.5 mL plastic centrifuge tubes with 0.2 µm filter insert
- 1.5 mL plastic centrifuge tubes

Device parameters

An HPLC of the Agilent company was used for the analyses. The 1200 series is equipped with a degasser (G1379B), a binary pump (G1312A), an autosampler (G1329A), an autosampler thermostats (G1330B), a column oven (G1316A), a diode array detector (DAD) (G1315D) and a refractive index detector (RID) (G1362A). The individual parameters are compiled in Table 10.

Table 10: Overview of the parameters for HPLC

Eluent	Ultrapure water, isocratic	**Measuring time**	90 min
Detector	RID DAD (for 5-HMF and furfural at 280 nm)	**Auto sampler**	80 °C
Flow	0,35 mL/min	**Auto sampler**	15 °C
Columns	MetaCarb 87P 300 x 7,8 mm MetaCarb 87P (pre-column) 50 x 4,6 mm	**Injection volume**	10 µL

Sample preparation

At a minimum, a double determination is carried out for all samples. In the case of substrates, a minimum of two complete eluates must be prepared for this.

a) For solid samples (substrates):
   Weigh-in 5 g of substrate and elute with 50 mL distilled water for 24 h on a shaker. Fill eluate through a sieve into a small plastic tube with screw cap.

b) For digestate samples (e.g. reactor samples):
Prior to the determination, the sample is centrifuged for 10 min at 10 °C and 10,000 rpm. If necessary, subsequent to centrifugation, the sample is strained through a sieve (mesh width approx. 1 mm) in order to remove coarse matrix components. 500 µL of the supernatant and/or the filtrate from a) or b) are pipetted as double determination into an Eppendorf tube with 0.20 µm filter insert and centrifuged for 10 min at 10 °C and 15,000 rpm. The filtrate from the Eppendorf tube with filter insert is pipetted into a 1.5 mL HLPC vial and sealed. If only little filtrate is present, corresponding microlitre inserts for HPLC vials must be used.

Calibration

A combined calibration solution is prepared for the monosaccharides (except for mannose). Mannose was calibrated separate due to the overlap with fructose. Similarly, separate standards were manufactured for the decomposition products acetaldehyde and hydroxyacetone as well as 5-(hydroxymethyl)furfural (5-HMF) and furfural. The retention times, calibration ranges, detectors used, as well as limits of detection and quantification (LOD and LOQ) are compiled in Table 11.

Table 11: Overview of substances with retention time, calibration range, LOD, LOQ, and detector

Analyte	Detector[a)]	Calibration range [mg/L]	Retention time [min]	LOD [mg/L]	LOQ [mg/L]
Sucrose	RID	2.48–990.00	19.38	3.77	13.32
D-(+)-Glucose	RID	1.00–1000.00	23.27	6.47	12.58
D-(+)-Xylose	RID	5.04–1008.00	25.13	12.25	42.93
L-(–)-Galactose	RID	4.03–806.00	27.74	8.53	30.94
L-(+)-Arabinose	RID	2.45–980.00	30.42	15.32	53.25
L-(–)-Mannose	RID	1.10–1096.50	32.22	4.00	15.55
D-(–)-Fructose	RID	2.57–1336.00	33.83	6.04	21.30
Glycerol	RID	2.59–1035.00	36.29	6.51	22.94
Xylitol	RID	4.97–993.00	70.11	9.15	32.14
D-(–)-sorbitol	RID	2.45–978.00	79.54	6.39	22.89
Acetaldehyde	RID	1.85–370.00	29.20	3.20	12.66
Hydroxyacetone	RID	2.30–460.00	45.04	4.98	21.95
5-HMF	DAD 280 nm	3.50–87.50	62.65	6.15	22.12
	RID	87.50–1750.00	63.04		
Furfural	DAD 280 nm	4.16–104.05	81.75	8.52	30.49
	RID	104.05–2081.00	82.23		

[a)] RID – refractive index detector; DAD – diode array detector

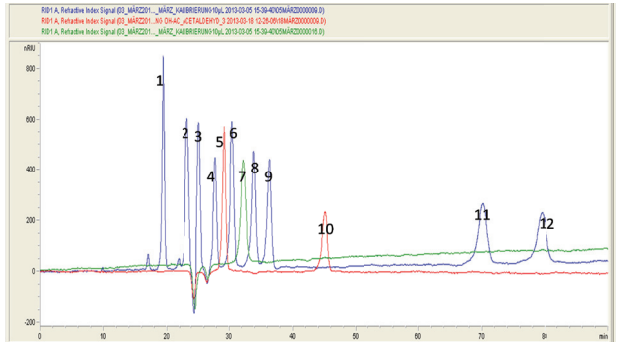
Analysis

For the quantitative analysis, the external calibration is used. In this, sugar and sugar alcohols are analysed via the refractive index detector (RID). The determination of furfural and 5-HMF is carried out in the lower calibration range via the diode array detector (DAD) at 280 nm. From this, the limits of detection and quantification for furfural and 5-HMF were determined, too. Values above 100 mg/L have to be analysed by the RID. If both D-(–)-sorbitol and furfural are present in the sample, there is a slight overlap of the signals in the RID, starting at an amount of approx. 60 mg/L furfural. Via a back calculation it is then possible, after analysis of the furfural, to deduct this area share and calculate the amount of D-(–)-sorbitol. If the share of D-(–)-sorbitol is significantly higher than that of furfural, it is also possible to dilute the sample in order to obtain a "furfural-free" RID signal for D-(–)-sorbitol.

Sample chromatogram

Using the aforementioned parameters, chromatograms as depicted in Figure 8 result.

a)



b)

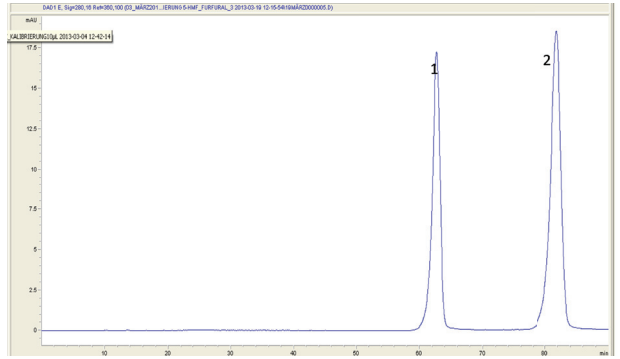


Figure 8: Affiliation of the substances; (a) RID: 1) sucrose; 2) D-(+)-glucose; 3) D-(+)-xylose; 4) L-(–)-galactose; 5) acetaldehyde; 6) arabinose; 7) mannose; 8) D-(+)-fructose; 9) glycerol; 10) hydroxyacetone; 11) xylitol; 12) D-(–)-sorbitol; (b) DAD (280 nm): 1) 5-(hydroxymethyl)furfural (5-HMF); 2) furfural

4.6 Determination of sugars based on GC-MS analytics

Erich Kielhorn, Peter Neubauer, Stefan Junne; TU Berlin

Status	The method presented is based on a 1986 publication regarding sugar analysis. In this publication, mixtures of pure monosaccharide standards were analysed. In late 2011, the method was adjusted for the analysis of sugars in liquid samples from biogas plants. The main principles of the analysis presented is published (Li & Andrews 1986).
Associated standards	Nitrogen determination according to Kjeldahl
Substrates	Utilised as substrate are liquid samples without solids, i.e., typically the centrifugate or filtrate of the samples. Extra-cellular sugars, meaning those dissolved in the liquid, are determined with this method.
Limitations of the method	Sugar concentrations can be detected starting at approx. 1 mg/L. One disadvantage is that the samples must be inactivated directly on-site in order to suppress any further metabolic activity. This is presently done by adding KOH (addition of 2 vol.-% of a 30 % KOH solution). Furthermore, an immediate cooling of the samples is desirable, which can be performed on-site with little effort. The drying of the samples takes several hours so that the analysis of the sample(s) typically takes two days.
Advantages	Other than that, the input of labour for the preparation and analysis is comparatively low. A major advantage is the high sensitivity and separation efficiency of the method so that even small amounts of sugars can be detected and quantified reproducibly.
Need for research	Even though the centrifugate is being analysed, after drying the samples residues remain that cannot be dissolved with the solvent. To that extent, it remains to be determined whether or not the sugars contained are transferred into the solvent completely or only in part. Furthermore, it was not possible to identify all split substances unambiguously. Thus, there is a need for additional research in order to determine whether these may be metabolites that can provide information regarding the state of the process.

Sample/data preparation

Since sugars are low-volatility compounds, a two-stage derivatisation of the sugars is performed. In the first step, the aldehyde groups of the sugars are converted into oximes by hydroxylamine. In the second step, the silylation with hexamethyldisilazane is carried out. As internal standard, phenyl-β-D-glucopyranoside is utilised.

The samples are first centrifuged for 10 min at 4 °C and 9,500 × g. 1 mL of the supernatant is placed in a GC glass bottle and dried for ≥ 8 h in the rotary evaporator under vacuum and at room temperature. The gentle evaporation of the sample serves for removing the water that would interfere with the analysis. Subsequent to the complete evaporation of the water, the remaining total solids are resuspended in 0.5 mL pyridine solution (prepared earlier -25 mg/mL hydroxylamine hydrochloride for oxime formation and 1 mg/mL phenyl-β-D-glucopyranoside as internal standard dissolved in pyridine) and sealed and incubated in the water bath at 75 °C for 30 min. After the cooling down, 0.5 mL hexamethyldisilazane and 15 µL trifluoroacetic acid are added. The precipitate that forms is separated by centrifugation at 9,500 × g (room temperature) for 10 min. The supernatant is transferred into GC bottles. The samples are stored at -20 °C prior to analysis.

Materials and devices

For the sample preparation

- Pyridine stock solution, consisting of:
  Pyridine ≥ 99 % (Carl Roth GmbH) #CP07.1
  Hydroxylamine hydrochloride ≥ 98 % (Sigma-Aldrich) #255580
  Phenyl-β-D-glucopyranoside (Fluka) #78554
- Hexamethyldisilazane ≥ 98 % (Carl Roth GmbH) #3840.1
- Trifluoroacetic acid ≥ 99 % (Carl Roth GmbH) #P088.1
- Micro centrifuge tubes 2 mL (Carl Roth GmbH) #CK06.1
- Transparent GC sample glass bottles 2 mL (Carl Roth GmbH) #159.1
- Glass inserts 100 µL for 2 mL glass bottles (CarlRoth GmbH), #C516.1
- 8 mm silicone-coated rubber septums (Carl Roth GmbH) #164.1
- Screw caps for glass bottles, (Carl Roth GmbH) #161.1
- Centrifuge CT15RE® (himac laboratory centrifuge)
- Vacuum centrifuge/"Speedvac" (Bachofer)
- Vortex mixer (neoLab®) and Water bath (GFL)

For the analysis

- GC/MS system (Agilent Technologies, Waldbronn, Germany), consisting of:
  Autosampler AS G26 14A | Injector 76 83B | Gas chromatograph GC 7890A | Mass spectrometer detector MSD 5975G | Software G 1701 EA
- Analytical separation column: Agilent J&W DB-5MS (Agilent Technologies, Wald bronn, Germany) #122-5523
  Coating: Phenyl-Arylene polymer, comparable to (5 %-phenyl) methyl polysiloxane
  Length: 30 m, ID: 0.25 mm, Film: 0.25 µm, Temperature limits: -60 to 325/350 °C
- Carrier gas: Helium
- Isopropanol

Execution method

For the analysis of the sugars, a gas chromatograph GC 7890A (Agilent) with attached mass spectrometer is utilised. The separation of the sugars is achieved via a quartz glass column (coated with 5 % phenyl 95 % dimethylarylenesiloxane, through which the carrier gas helium is flowing. The addition of sample corresponds to 0.2 µL. 2 µL sample are injected with a 1:10 split (final sample volume 0.2 µL). The temperature of the injection chamber is 155 °C; that of the detection chamber is 300 °C. At the start of the analysis, the column has a temperature of 155 °C. Once the sample has been injected, the temperature is increased by 4.5 °C per minute, up to a final temperature of 280 °C, and maintained constantly for another 10 min.

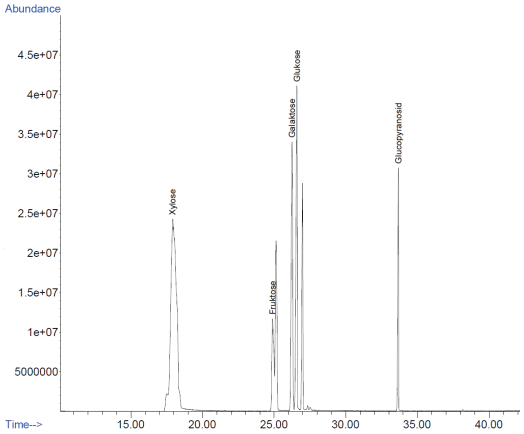


Figure 9: Sample chromatogram, analysis of sugar standards

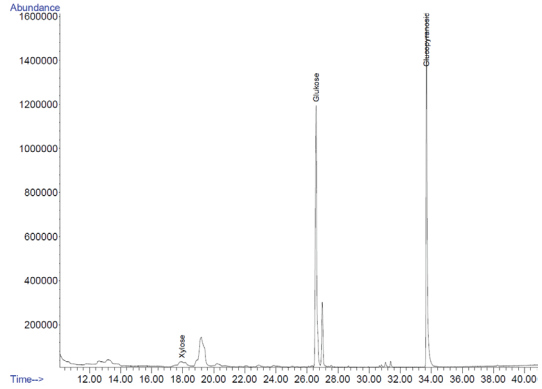


Figure 10: Sample diagram, liquid sample from the hydrolysis tank of a biogas plant

Analysis

The analysis is performed with the "GCMSD Data Analysis" software programme. Both the identification of an unknown substance as well as its quantification are possible. Based on the mass fraction of a substance determined by the mass spectrometer, the unknown molecule can be identified via a pre-installed substance library ("NIST 08"). Subsequent to the identification, the quantification is performed by first analysing a standard with a defined concentration and then creating a calibration line. Via the peak areas of the chromatogram obtained, the amount of the substances contained can be determined.

4.7 Determination of total Kjeldahl nitrogen and crude protein

Michael Dittrich-Zechendorf, DBFZ

Status	The method is an in-house method that is carried out by modelling it after the prescribed method of VDLUFA, Book of Methods III, Testing of Feedstuffs, Determination of Crude Protein, Official Method, Hamburg 1988 (VDLUFA 1988).
Associated standards	Nitrogen determination according to Kjeldahl
Area of application of the method	Determination of the crude protein content of feedstuffs based on determined nitrogen contents (according to Kjeldahl)
Disadvantages	Lengthy. May be potentially falsified due to, for example, melamine (or other nitrogen sources) (non-specific method) The fixed factor must be adjusted, depending on the sample, where applicable.
Advantages	Nitro, nitroso and azo compounds are not being detected.
Need for research	For this method, there is no need for research.

Through an acidic thermal decomposition under catalyst involvement, protein(s) and other compounds containing nitrogen are split into ammonia. Ammonia is released by means of alkaline water vapour distillation and captured in boric acid. Subsequently, a quantitative determination of the ammonia takes place by means of sulphuric acid titration. The share of ammonia determined allows for conclusions regarding the nitrogen bound in the protein. For this, the factor 6.25 is used for the conversion of the nitrogen content to the crude protein content. The method is carried out according to the determination according to Kjeldahl.

Devices and chemicals

- Devices: Turbosog, Turbotherm, Kjeldatherm, Vapodest 50sc
- Decomposition vessels and accessories
- 1.5 L beaker
- 250 mL wide-neck Erlenmeyer flask
- Drying cabinet
- Precision scale
- Crucible
- Desiccator
- Boric acid 2 %
- Sulphuric acid (nitrogen-free) 0.025 mol/L (normality: 0.05 mol/L)
- Soda lye (nitrogen-free) 32 %
- Ammonia sulphate $(NH_4)_2SO_4$ at least 99,5 % (p. a.)
- Catalyst tablets ($CuSO_4 \times 5\ H_2O$, Na_2SO_4, Se)
- Aqua dist.

Preparation of the analysis

The analysis is carried out on sample wet weight. Both liquid and solid samples can be analysed. At a minimum, a double determination is carried out for each sample.

Execution of the analysis

Thermal decomposition

For the analysis, approximately 0.3 g_{TS} (m_1) are weighed-in with an accuracy of 0.1 mg. Distilled water is used as blank reading, and ammonia sulphate [$(NH_4)_2SO_4$] as standard substance. Two catalyst tablets are placed in each decomposition vessel, covered with 20 mL 98 % sulphuric acid, and placed in the glass on a heating block. The suction extraction facility is placed on top of this. The samples are decomposed for 55 min at 230 °C and subsequently for 1:15 h at 390 °C, wherein the solution should have taken on a clear green colouring. Once the decomposition has ended, let it cool down for approx. 20 min. Subsequently, approx. 90 mL boiling water (aqua dist.) are layered underneath, running down the side of the glass.
To prevent the crystallising out of the sulphate, the samples are once again placed on the still warm heating block.

Alkaline water vapour distillation

The decomposition vessels are placed in the distillation device, and subsequent to addition of 66 mL soda lye, distilled for 5 min at 100 % steam output. Within the process, the distillate is transferred into 60 mL boric acid. Then the boric acid is titrated with 0.1 N sulphuric acid until pH = 5.

Calculation of the total Kjeldahl nitrogen content

$$TKN = \frac{(V_1 - V_0) \cdot c \cdot f \cdot 0.014}{m \cdot 100} \quad (8)$$

TKN	Total Kjeldahl nitrogen content	($\%_{ww}$)
V_1	Volume of the sulphuric acid consumed when titrating the sample	(mL)
V_0	Volume of the sulphuric acid consumed when titrating the blank reading	(mL)
c	Normality of the acid	(mol/L)
f	Factor of the acid	
m	Mass of the sample	(g)

Calculation of the protein content

$$CP = TKN - \left(NH_4^+\text{-}N \cdot \left(\frac{100 - TS}{1000}\right)\right) \cdot 6.25 \quad (9)$$

CP	Protein content	($\%_{ww}$)
TKN	Total Kjeldahl nitrogen content	($\%_{ww}$)
NH_4^+-N	TAN (total ammonia nitrogen)	(g/L)
TS	Total solids content of the sample	(%)

For all samples, the dry matter must be determined in order to be able to put the result in relation to the total solids. In addition, the ammonia nitrogen content (TAN) must be measured in order to calculate the protein content.

4.8 Determination of the protein content

Lucie Moeller, Kati Görsch, UFZ

Status	This method was developed after the method for the determination of the crude protein content according to Dumas.
Associated standards	Determination of crude protein according to Dumas.
Substrates/ materials	This method is suitable for samples with total solids contents of up to approx. 7 %.
Measuring range	0.1–100 mg/L TN (total nitrogen)
Disadvantages	A TOC/TN analyser is necessary in order to determine the nitrogen content.
Advantages	The method is quick and easy to execute.
Need for research	For this method, there is no need for research.

For the description of the properties of the foams generated during biogas production, a protein determination is required. Due to the characteristics of samples originating from biogas plants, the utilisation of spectrophotometric methods for protein determination (e.g. Bradford, Lowry) is difficult and leads to measuring inaccuracies. For this reason, an analysis for the determination of the content of N-protein (Dumas, 1831) was developed.

The total protein content of a sample is calculated based on the following formula:

$$\text{Total protein} = 6.25 \cdot (\{TN\} - \{NH_4N\} - \{NO_3N\} - \{NO_2N\}) \quad (10)$$

{TN}	Total nitrogen from the homogenized sample	(mg/L)
{NH_4-N}	Total ammonia nitrogen (TAN) from the filtrate	(mg/L)
{NO_3-N}	Nitrate nitrogen from the filtrate	(mg/L)
{NO_2-N}	Nitrite nitrogen from the filtrate	(mg/L)

For the determination of the total, ammonium, nitrate, and nitrite nitrogen, several sub-steps are required. Nevertheless, in comparison to other sample determination methods for intensely coloured samples (e.g. Kjeldahl), this method is relatively quick to execute. A comparison of the results from this method with those of the conventional, more laborious method according to Kjeldahl (DIN EN 25 663) showed a deviation of 5 %.

Determination of the TN content from the homogenized sample

Processing of sample
The sample is homogenised with the help of a conventional immersion blender. Of this, two times 5 mL are filled in measuring cylinders (in the case of very liquid samples with solids contents of less than 5 %, the utilisation of a 5 mL pipette is possible). The homogenic sample is transferred into 50 mL volumetric flasks and filled up with distilled water to the calibration mark. Of these 1:10 dilutions once again two times 5 mL each are filled into volumetric flasks and filled up to 50 mL, so that four samples (each with a 1:100 dilution) can be measured. These dilutions are subsequently filtered through a 250 µm mesh sieve in order to free the solutions of interfering fibres.
The samples are measured for TN by means of a TOC analyser.

Measuring process
For the determination of the TN content, the TOC-$V_{CSH/CSN}$ device with a TN unit (Shimadzu company) was used. The measuring principle of the device is based on a combustion of the sample at 720 °C, wherein the nitrogen present in the sample is converted into nitrogen monoxide which is detected by chemoluminescence.
Due to the difficulty of measuring 5 mL homogenized sample, a relative error of ± 15 % should be taken into consideration.

Determination of the ammonia nitrogen concentration of the filtrate

Processing of sample
The sample is centrifuged for 20 min at 5,300 rpm and 20 °C in 50 mL centrifuge tubes (device: Avanti 30 centrifuge, Beckman company). The supernatant is strained through a sieve (mesh width: 720 µm) and transferred into a pressure filtration unit (device: SM 16 249, Sartorius company) with nylon membrane filter (pore size: 0.45 µm, Whatman company or Pall). The exact description of the device is provided in Chapter 4.2 "Determination of organic acids". Depending on the anticipated ammonia nitrogen concentration, the filtrate is diluted with distilled water (based on experience, at least 1:1,000).

Measuring process
For the determination of the ammonia nitrogen (TAN) content, a photometric test of the Merck company is utilised (Spectroquant, in accordance with DIN 38406 E5, measuring range: 0.01–3 mg/L NH_4-N). The photometric measurement is carried out in a quartz cuvette (10 mm side length) with the Multilab P5 device (WTW company).

Determination of the ammonium and nitrite nitrogen concentrations from the filtrate
For nitrate, the sample is treated in accordance with the provisions of the Spectroquant nitrate test (Merck company, in accordance with DIN 38405 D9, measuring range: 1.0–25.0 mg/L NO_3-N). The photometric measurement against ta blank is carried out in single- use cuvettes (10 mm side length) with the Cadas 200 device (Dr. Lange company).
The presence of nitrite in the sample can be checked with the help of a test strip (Merckoquant nitrite test, Merck company, measuring range: 0.5–10 mg/L).

4.9 Determination of crude fat

Michael Dittrich-Zechendorf, DBFZ

Status	The method is an in-house method that is carried out modelled after the prescribed method of VDLUFA, Book of Methods III, Testing of Feedstuffs, Determination of Crude Fat, Chapter 5.1.1, Official Method, Procedure B, Hamburg 1988 (VDLUFA 1988).
Associated standards	Determination of crude fat, official method
Area of application of the method	Determination of crude fat in feedstuffs. Not suitable for oilseeds.

The sample is heated with hydrochloric acid in order to open up (decompose) proteins and release bound lipids. The decomposition solution is filtered and, after drying, the fat remaining in the filter is extracted with hexane. The solvent is distilled off and the dried residue is weighed. The fat content is calculated from the difference between the weighing-in and weighing-out.

Devices and chemicals

- Soxtherm extraction unit Makro and Multistat device
- Precision scale
- Hydrolysis automaton "Hydrotherm"
- Pleated filter with an average pore diameter of approx. 5 µm
- Drying cabinet
- Desiccator
- Weighing paper, fat-free
- Crucible
- pH indicator paper
- Wadding, chemically pure and degreased
- Extraction beaker(s)
- Extraction sleeves
- Sleeve holder(s)
- Compressor at least 4.5 bar
- Water supply at least 0.5 bar
- Hydrochloric acid 3 mol/L
- Hexane
- Aqua dist.
- Where applicable, liquid N_2
- Where applicable, dry ice

Preparation of the analysis

Prior to the analysis, the fresh samples are ground to ≤ 1 mm; where applicable, they are embrittled for this by means of liquid nitrogen and solid CO_2 (dry ice). A double determination is carried out. The dry matter of the dried sample must be determined in order to be able to put the result in relation to the total solids.

Execution of the analysis

Hydrolysis

Approximately 2.5 g of a fresh sample – accurate to 0.1 mg – are placed on the weighing paper, which is then folded together. The paper, together with the sample, is put into a hydrolysis beaker to prevent baking onto the beaker's bottom while heating it up. Subsequent to the addition of 100 mL 3 mol/L hydrochloric acid, an automatic heating to boiling temperature takes place and is held for 1 h at mild simmering. It has proven advantageous to continue the simmering process until the complete decomposition of the substrate. Where applicable, rinse the border that occurred into the glass with some HCl and continue the simmering process. Subsequent to the completion of the hydrolysis, the decomposition mixture is drained into the prepared pleated filter and rinsed with hot distilled water. The pleated filters are rinsed 16 times with 40 mL distilled water, each. The filters should be pH-neutral (testing by means of Unitest paper). The filters are then placed on watch glasses and dried over night in the drying cabinet at 50 °C. Depending on the number of samples, the extraction beakers are dried with three boiling stones, each, for at least 1 h in the drying cabinet at 105 °C, or – preferably – over night at 50 °C.

Table 12: Programme of the extraction unit

Programme step	Programme parameter(s)	Comment
T category	135 = < 200–300 °C	
Hot plate temperature	150 °C	
Lowering interval	4 min	
Lowering impulse	3 s	
Boiling phase	30 min	
Removal by distillation A	4 intervals	Subsequent to A the solvent level should be at least 10 mm below the sleeve
Extraction time	1 h	
Removal by distillation B	4 intervals	Subsequent to B the solvent level should be at least 10 mm below the sleeve
Removal by distillation C	2 min	

Extraction

Subsequent to the cooling down in the desiccator, the extraction beakers are weighed accurate to 0.1 mg and the mass (a) is recorded. Subsequent to the cooling down in the desiccator, the dried filters are transferred into an extraction sleeve and covered with fat-free wadding. The prepared sleeve is placed in the appropriate holder and [then] placed into an extraction glass. Into this glass hold with round-nose pliers, 140 mL of fresh hexane are added. The glass is immediately placed in the ready-to-operate extraction unit. The extraction takes place according the programme described in Table 12.

After the completion of the programme, the extraction beaker is removed from the extraction unit and the extraction sleeves with the corresponding holders are removed and disposed of (and/or reused). The extraction beakers are dried in horizontal position for 2 h at 50 °C in the drying cabinet. After cooling down to room temperature in the desiccator, a weighing accurate to 0.1 mg is carried out and the mass (b) is recorded. Drying and weighing must take place immediately one after the other.

Calculation of the fat content

$$CF = \frac{b - a}{(0.01 \cdot TS_{md} \cdot m) \cdot 100} \quad (11)$$

CF	Crude fat content	($\%_{TS}$)
a	Mass of the empty extraction vessel	(g)
b	Mass of the extraction vessel after the extraction	(g)
TS_{md}	Total solids of the dried and milled sample	(%)
m	Mass of the dried and milled ample	(g)

4.10 Determination of crude fibre

Michael Dittrich-Zechendorf, DBFZ

Status	The method is an in-house method that is carried out modelled after the prescribed method of VDLUFA, Book of Methods III, 2nd Supplement, Hamburg 1988 (VDLUFA 1988).
Associated standard	Determination of crude fibre, official method
Area of application of the method	This method determines the acid-insoluble and alkali-insoluble, fat-free, organic share in feedstuffs.
Disadvantages	Non-specific method, no indications regarding the individual fibre fractions.
Need for research	For this method, there is no need for research.

The dried sample is treated by boiling in H_2SO_4 and KOH. The undissolved residue is weighed out after drying and then turned to ash. The difference between the ash content and the undissolved residue is referred to as crude fibre. These skeletal substances essentially include: cellulose, hemicellulose, pentosans, lignin, cutin and pectin.

Devices and chemicals

- Fibretherm FT 12 device
- Fibrebag & accessories
- Drying cabinet
- Muffle furnace
- Precision scale
- Crucible & desiccator
- Sulphuric acid 0.13 mol/L
- Potash lye 0.23 mol/L
- Hexane
- Aqua dist.
- Boiling stones

Preparation of the analysis

The samples must be dried in the drying cabinet at 105 °C for approx. 24 h and subsequently ground to ≤ 1 mm. Furthermore, for each sample a crucible must be calcinied empty at 500 °C for 2 h. A double determination is carried out. In addition, corresponding to the number of samples, Fibrebags must be dried in the drying cabinet at 105 °C for 1 h.

Execution of the analysis

Subsequent to the drying, the empty weight of the Fibrebags is determined. Then, approx. 1 g of dried sample must be weighed, accurate to 0.1 mg. A glass spacer is carefully inserted into the Fibrebags and together are placed in the sample carousel. All Fibrebags are thoroughly rinsed with a spray bottle filled with hexane. This way, excess fat is eluated

Table 13: Method for the determination of crude fibre

1	Dosage	H_2SO_4	1 L
2	Heating	45 %	0 h 30 min
3	Suctioning off		2 min/30 s
4	Washing cycle 1/2		
5	Washing cycle 2/2		
6	Dosage	KOH	1 L
7	Heating	40 %	0 h 30 min
8	Cooling	91 > 85 °C	
9	Suctioning off		2 min/30 s
10	Washing cycle 1/2		
11	Washing cycle 2/2		
12	Dosage	H_2O wash	1 L
13	Heating	50 %	0 h 5 min
14	Cooling	90 > 60 °C	
15	Method completed		

from the samples. The sample carousel should be dried in the drying cabinet (105 °C) for approx. 5 min and be subsequently placed in the boiling container.

To determine the dried mass of the Fibrebags, first, the empty weight of an empty crucible calcined at 500 °C is determined. After removal of the spacer, the Fibrebag is placed in the crucible rolled up. The crucibles are dried for approx. 24 h at 105 °C, cooled down in the desiccator, and weighed. The ashing of the Fibrebags is carried out at 500 °C for at least 2 h. After cooling down, the samples are weighed. In addition, the dry matter of the analysis sample must be determined in order to be able to put the result in relation to the total solids.

Result calculation

$$CFC = \frac{(m_4 - m_1) - (m_5 - (m_6 - m_3))}{((m_2 - m_1) \cdot TS_{md}) \cdot 100 \cdot 100} \quad (12)$$

CFC	Crude fibre content	($\%_{TS}$)
m_1	Mass of the empty dried Fibrebag	(g)
m_2	Mass of the dried Fibrebag with sample	(g)
m_3	Mass of the empty crucible of the blank reading	(g)
m_4	Mass of the crucible & Fibrebag & sample after drying	(g)
m_5	Mass of the crucible & Fibrebag & sample after calcination	(g)
m_6	Mass of the crucible & Fibrebag after calcinationof the blank reading	(g)
TS_{md}	Total solids of the dried and milled sample	(%)

4.11 Process specification for the determination of ADF and ADL

Michael Dittrich-Zechendorf, DBFZ

Status	The method is an in-house method that is carried out modelled after the prescribed method of VDLUFA, Book of Methods III, 2nd Supplement, Hamburg 1988 (VDLUFA 1988).
Associated standards	Determination of ADF and ADL, official method
Area of application of the method	This method determines the acid-insoluble components and the crude lignin of a sample.
Need for research	For this method, there is no need for research.

By boiling the dried samples in acidic ADF solution, cellulose, lignin and lignin-*N*-compounds are not eluated from the feedstuff. This undissolved residue is weighed out after drying. The residue remaining in the filter crucible in the determination of the ADF is treated at room temperature for 3 h with 72 % sulphuric acid. Subsequently it is rinsed with hot water to the neutral point, dried, and weighed. After ashing the organic substance, the substance is weighed again; the calcination loss corresponds to the "crude lignin".

Devices and chemicals

- Fibretherm FT 12 device, Fibrebag (ADF) & accessories
- Drying cabinet and Muffle furnace
- Precision scale
- 5 L beaker
- Crucible & desiccator
- Acidic ADF solution
- Hexane
- 72 % sulphuric acid
- Aqua dist.

Preparation of the analysis

The samples must be dried in the drying cabinet at 105 °C for approx. 24 h. Furthermore, for each sample two crucibles must be calcinied empty at 500 °C for 2 h. The dried samples are ground with a mill to ≤ 1 mm. A double determination is carried out. The Fibrebags must be dried in the drying cabinet at 105 °C for 1 h.

Manufacturing of detergents for the ADF determination

Devices and chemicals

- 5 L volumetric flask
- 250 L beaker
- 50 L volumetric bulb pipette

- Piston pipette
- Top unit scale
- Small weighing bowl(s)
- Glass funnel
- Aqua dist.
- Sulphuric acid (H_2SO_4) 98 %
- *N*-cetyl-*N*,*N*,*N*-trimethyl ammonium bromide

Manufacturing of the ADF solution

In a 5 L volumetric flask, approx. 2 L distilled water are placed and 136 mL concentrated sulphuric acid are pipetted in. In addition, 100 g *N*-cetyl-*N*,*N*,*N*-trimethyl ammonium bromide are transferred into the volumetric flask. Subsequent to intermixture and cooling down, it is filled up with distilled water up to the calibration mark. The solution is stored in the dark at 18–20 °C.

Execution of the ADF analysis

Subsequent to the drying at 105 °C, the empty weight of the Fibrebags is determined and recorded (m_1). Then, approximately 1 g of dried sample must be weighed in accurate to 0.1 mg. The mass of the sample in the Fibrebag must be recorded (m_2). A glass spacer is carefully inserted into the Fibrebags and both are placed in the sample carousel. All Fibrebags are thoroughly rinsed with hexane. This way, excess fat is eluated from the samples. The duration and sequence of the process steps of the Fibretherm FT 12 can be found in Table 14. After completion of the method, the Fibrebags must be dried in the drying cabinet over night at 105 °C and the mass must be recorded (m_4).
If ADL (crude lignin) is to be determined, the "Execution of ADL analysis" must be carried out thereafter. If lignin does not need to be determined, at this point the ashing in the muffle furnace is carried out at 500 °C for at least 2 h. Subsequent to cooling down of the sample in the desiccator, the sample is weighed and the weight is recorded (m_5). The ash determination obtained here is, for the most part, identical to the ash determination from the TS/VS determination (Ch. 3.1).

Execution of the ADL analysis

In preparation, dry crucibles and Fibrebags at 105 °C for 24 h.
For the ADL determination, additionally the Fibrebags weighed for the determination of the ADF (prior to the ashing!) are hung in a sample carousel and secured. Subsequently, the sample carousel with the Fibrebags is place in a 5 L beaker and covered at room temperature with 72 % sulphuric acid. The sulphuric acid is stirred every hour and during this period is kept for 3 h at a temperature of 20–23 °C. Subsequently, it is rinsed with hot water to the neutral point and dried for 24 h at 105 °C (m_7).
The ashing of the Fibrebags is carried out at 500 °C for at least 2 h in the muffle furnace. Subsequent to cooling down in the desiccator, the samples are weighed out and the mass is recorded (m_5).
In addition, the dry matter of the analysis sample must be determined in order to be able to put the result in relation to the total solids.

Table 14: Method for the determination of ADF

1	Dosage	ADF solution	1.3 L
2	Heating	34 % (device-dependent)	1 h
3	Suctioning off		2 min/30 s
4	Washing cycle 1/2		
5	Washing cycle 2/2		
6	Dosage	H_2O wash	1.3 L
7	Heating	50 % (device-dependent)	0 h 5 min
8	Cooling	90 > 60 °C	
9	Suctioning off		2.5 L
10	Dosage	H_2O wash	1.3 L
13	Heating	55 % (device-dependent)	0 h 2 min
14	Cooling	90 > 60 °C	
15	Method completed		

Result calculation

$$ADF = \frac{(m_4 - m_1) - (m_5 - (m_6 - m_3))}{((m_2 - m_1) \cdot TS_{md}) \cdot 100 \cdot 100} \quad (13)$$

ADF	Share of acid detergent fibre	($\%_{TS}$)
m_1	Mass of the empty dried Fibrebag	(g)
m_2	Mass of the dried Fibrebag with sample	(g)
m_3	Mass of the empty crucible of the blank reading	(g)
m_4	Mass of the crucible & Fibrebag & sample after drying	(g)
m_5	Mass of the crucible & Fibrebag & sample after calcination	(g)
m_6	Mass of the crucible & Fibrebag after calcination of the blank reading	(g)
TS_{md}	Total solids of the dried and milled sample	(%)

$$ADL = \frac{(m_7 - m_1) - (m_5 - (m_6 - m_3))}{((m_2 - m_1) \cdot TS_{md}) \cdot 100 \cdot 100} \quad (14)$$

ADL	Share of acid detergent lignin	($\%_{TS}$)
m_7	Mass of the ADL-crucible & Fibrebag after drying	(g)

4.12 Determination of Neutral Detergent Fibre (NDF)

Michael Dittrich-Zechendorf, DBFZ

Status	The method is an in-house method that is carried out modelled after the prescribed method of VDLUFA, Book of Methods III, 2nd Supplement, Hamburg 1988 (VDLUFA 1988).
Associated standard	Determination of NDF, official method
Area of application of the method	For the determination of components insoluble in neutral detergent solution

By boiling the dried samples in neutral NDF solution, hemicellulose, cellulose, lignin and lignin-N-compounds are not eluated from the feedstuff. This undissolved residue is weighed out after drying and turned to ash. The difference between the ash content and the undissolved residue is referred to as neutral detergent fibre (NDF). Particular attention must be paid to the adherence to the pH value.

Devices and chemicals

- Fibretherm FT 12 device, fibrebag (NDF) & accessories
- Drying cabinet and Muffle furnace
- Precision scale, crucible & desiccator
- NDF solution
- Hexane
- Aqua dist.

Preparation of the analysis

The samples must be ground to ≤ 1 mm and dried in the drying cabinet at 105 °C for approximately 24 h. Furthermore, for each sample a crucible must be calcinied empty at 500 °C for 2 h. A double determination is carried out. In addition, corresponding to the number of samples, Fibrebags must be dried in the drying cabinet at 105 °C for 1 h.

Manufacturing of detergents for the NDF determination

Devices and chemicals

- 5 L volumetric flask
- 5 L & 1.5 L beaker
- Glass funnel
- 50 mL volumetric bulb pipette
- Top unit scale and small weighing bowl(s)
- Magnetic stirrer with magnetic stir bar
- Aqua dist.
- EDTA disodium salt (EDTA disodium salt dihydrate also possible) p. a.
- Disodium tetraborate decahydrate p. a.
- Dodecylsulphate sodium salt p. a.
- Triethylene glycol p. a.

- Sodium dihydrogen phosphate p.a.
- Soda lye/sulphuric acid p.a.
- Antifoaming agent (TANAFOAM 1573)

Manufacturing of the NDF solution

Approximately 2 L distilled water and a magnetic stirrer are placed in a 5 L beaker. 93 g (103 g EDTA disodium salt dihydrate) and 34 g disodium tetraborate decahydrate are transferred into the 5 L beaker. The solution is stirred on the stirring disk until all solids have been dissolved. Subsequently, 150 g dodecylsulphate sodium salt is added into the beaker in the same manner and 50 mL triethylene glycol are pipetted in while stirring.
Approximately 1 L distilled water is placed in a 1.5 L beaker and – while stirring until complete dissolution – 22.8 g sodium dihydrogen phosphate are added into the beaker: Thereafter, this phosphate solution in the 5 L beaker is filled up with distilled water to approximately 4.5 L and 2 mL of antifoaming agent is added. The pH value is measured and adjusted with soda lye/sulphuric acid to be between 6.9 and 7.1. The solution is transferred into the 5 L volumetric flask by means of the glass funnel and filled up to the calibration mark with distilled water. The shelf life of the solution is four weeks.

Execution of the analysis

Subsequent to the drying, the empty weight of the Fibrebags is determined (m_1) and approximately 1 g of dried sample is weighed in accurate to 0.1 mg. The mass of the Fibrebag filled with the sample is recorded (m_2). A glass spacer is carefully inserted into the Fibrebags and both together are placed in the sample carousel. All Fibrebags are thoroughly rinsed with hexane. This way, excess fat is eluated from the samples. After drying for approx. 2 min in the exhaust, the Fibretherm is started with the settings listed in Table 15.
Once the method has been completed, the spacer is removed from each Fibrebag, whereupon care must be taken that none of the samples are discharged. The Fibrebag is placed in the crucible rolled up and dried for approximately 24 h at 105 °C. Subsequent to the drying, it is left to cool down in the desiccator and the mass is determined. The ashing of the Fibrebags is carried out at 500 °C for at least 2 h. After cooling down in the desiccator, the samples are weighed. In addition, the dry matter of the analysis sample must be determined in order to be able to put the result in relation to the total solids.

Result calculation

$$NDF = \frac{(m_4 - m_1) - (m_5 - (m_6 - m_3))}{((m_2 - m_1) \cdot TS_{md}) \cdot 100 \cdot 100} \quad (15)$$

NDF	Share of neutral detergent fibre	($\%_{TS}$)
m_1	Mass of the empty dried Fibrebag	(g)
m_2	Mass of the dried Fibrebag with sample	(g)
m_3	Mass of the empty crucible of the blank reading	(g)
m_4	Mass of the crucible & Fibrebag & sample after drying	(g)
m_5	Mass of the crucible & Fibrebag & sample after calcination	(g)
m_6	Mass of the crucible & Fibrebag after calcination of the blank reading	(g)
TS_{md}	Total solids of the dried and milled sample	(%)

Table 15: Method for the determination of NDF

1	Dosage	NDF solution	1.3 L
2	Heating	35 %	1 h
3	Suctioning off		2 min/30 s
4	Washing cycle 1/2		
5	Washing cycle 2/2		
6	Dosage	H_2O wash	1.3 L
7	Heating	55 %	0 h 5 min
8	Cooling	91 > 60 °C	
9	Dosage	H_2O wash	1.3 L
10	Heating	55 %	0 h 2 min
11	Cooling	90 > 60 °C	
12	Method completed		

4.13 Measurement of organic trace compounds

Jorge Iván Salazar Gómez, Andrea Gerstner, Alisa Jovic, Fraunhofer UMSICHT

Status	The method was tested and good results were achieved, but a validation of the method presented still has to take place.
Associated standards	The sampling and the subsequent measurement of the VOCs is carried out modelled after DIN EN ISO 16017 1 ("Sampling and analysis of volatile organic compounds by sorbent tube/ thermal desorption/capillary gas chromatography - Part 1").
Substrates/ materials	Application in the case of volatile organic trace compounds in gaseous state (biogas sampling generally at room temperature).
Limitations of the method	An exact measuring range has not yet been determined (validation required), potential overloading of the thermal desorption tubes (connection in series required), time-consuming conditioning of the 2-bed and 3-bed thermal desorption tubes before and after the sampling, too high a water vapour content may interfere with adsorption process (condensate trap required).
Advantages	Longer storability of samples (if refrigerated, the samples can be stored for several weeks). Good usability/handling, easy sampling, short sampling durations. No consumption of solvents. Good applicability in case of volatile substances and low substance concentrations in biogas. Special coating of metal surfaces prevents adsorption of reactive compounds. Sharp substance peaks due to high heating rate of the TD tubes in the thermal desorption unit.
Need for research	Validation of the method is still required.

In addition to the main components methane and carbon dioxide, biogas contain a series of volatile organic compounds (VOC). This includes, among others, sulphurous compounds such as methane thiol, organosilicon substances (siloxanes) or terpenes such as limonene and pinene. The quantitative analysis of the VOC can provide important indications regarding the processes in the digester and may help in the conceptualisation of necessary gas purification processes, where applicable. The concentrations of the individual VOCs in biogasses typically amount to only a few ppm. Therefore, the enrichment of the VOCs in the so-called thermal desorption tubes (TD tubes) is sensible in order to improve the limit of detection and limit of quantification. The subsequent measurement of the VOCs is carried out by means of gas chromatograph mass spectrometer (GC-MS). The utilisation of an MS ensures a sufficient sensitivity of the method of analysis. Furthermore, it is possible to quickly identify unknown substances in a complex gas mixture qualitatively.

Sampling

The sampling is carried out with thermal desorption tubes made of stainless steel. These are coated with Silcosteel® in order to prevent reactive compounds from adsorbing on the metal surface and/or decomposing due to the high temperatures of the desorption process. Located in the tubes are a few mg of one or more adsorbents. In the latter case, the materials are layered one after another based on increasing adsorption strengths ("sandwich package"). This ensures that even particularly volatile compounds are retained in the last adsorbent layer of the tube.

The sampling and the subsequent measurement of the VOCs is carried out modelled after DIN EN ISO 160171[1]. Prior to sampling, the TD tubes must be conditioned, i.e., they are heated in the laboratory at temperatures of 20–30 °C above the actual desportion temperature in order to remove any potentially adsorbed compounds. A TD tube conditioned in this manner is connected gas-tight on-site – with hoses made of Tygon® – to both the sampling site at the biogas plant/system and to the sampling pump. Tygon® hoses feature the necessary inertness for sampling. Additionally, a condensate trap can be installed between the sampling site and the tube in order to separate off water vapour from the biogas that could disturb the adsorption process. It must be taken into consideration that polar compounds, such as alcohols, may be separated off with the condensate water. Before the TD tube is connected to the sampling site, all lines carrying gas as well as the condensate trap should be rinsed with biogas for 10 min in order to obtain a representative sample. When sampling, the exact volume flow is adjusted via a flow meter (Figure 11).

Once the necessary flow-through has been set, a sufficient gas volume is suctioned into the tube. The amount of gas should be sized such that the TD tube is not overloaded and that a breach of the VOCs at the outlet of the tube is prevented. To check whether a breach occurred, two TD tubes can be connected in series. If VOCs are detected in the second TD tube, substances have breached. The optimal flow rate and sampling duration depends on the individual gas composition at the respective biogas site and must be determined in preliminary tests, where applicable. Typical sampling durations are between 30 s and 10 min, wherein flow rates are adjusted to between 50 and 100 mL/min.

[1] DIN EN 304:16017-1: "Sampling and analysis of volatile organic compounds by sorbent tube/thermal desorption/capillary gas chromatography - Part 1".

Thermal desorption and GC-MS measurement

The TD tube loaded with the material to be tested is slowly heated in the thermal desorption unit (TD unit) of the GC-MS to the final desorption temperature (300 °C). The desorbed VOCs are enriched in the carrier gas flow (helium) and are adsorbed onto TENAX® in the cold trap of the TD unit and are subsequently once again desorbed at a high heating rate. Through this step (focussing), later narrow, i.e., well analysable substance peaks are achieved in the chromatogram. All transfer lines and valves of the TD unit are also rendered inert with Silcosteel®. At the end, the transfer to the GC, the separation of the VOCs on the capillary film column of the GC and the detection of the individual substances in the MS (Figure 12) take place. For qualitative analysis of the substance peaks, calibration lines are determined with the help of TD tubes that were doped with liquid calibration standards.

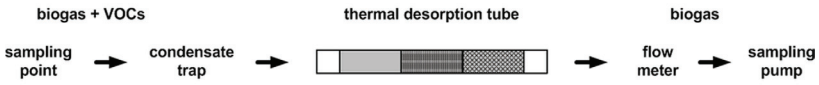


Figure 11: Schematic depiction of the VOC sampling with a 3-bed TD tube (Source: Fraunhofer UMSICHT)

Materials and devices

Calibration standards

Liquid calibration standards are offered by a series of companies, e.g. Supelco or LGC-Standards. Typically, these standards contain multiple VOCs, that are dissolved in a suitable solvent (e.g. methanol). Furthermore, possible, to have custom calibration standards manufactured. All calibration standards feature a certificate so that they can be traced back to primary standards. The calibration standards typically feature concentrations of 1,000–5,000 µg/mL. For the dilution of calibration standards, highly pure solvents are required: e.g. methanol ≥ 99.9 % and/or acetonitrile ≥ 99.8 %. For the calibration of the GC-MS, 5 to 10 standard solutions that cover the anticipated concentration range

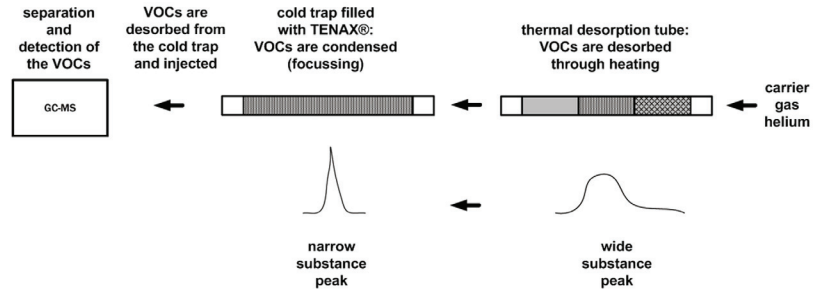


Figure 12: Schematic depiction of the thermal desorption with subsequent analysis (Source: Fraunhofer UMSICHT)

must be manufactured per calibration line via dilution of the calibration standards. For doping with the standard solutions, conditioned TD tubes are affixed in a special facility (Calibration Solution Loading Rig). Then, the required amount (most often, 1 µL) of the respective standard solution is injected into the TD tube. Through flushing with inert gas (helium or nitrogen), the excess solvent is removed from the TD tube. In this, a gas flow of 50–100 mL/min and a flushing duration of 20 s to 1 min has proven to be useful.

TD tube

For the sampling, three different types of TD tubes (e.g. by Markes International) are utilised: TD tubes with Tenax® TA, 2-bed tubes with Tenax® TA and UniCarb™ as well as 3-bed tubes with Tenax® TA, UniCarb™ and Carboxen™-1000. Through the utilisation of multi-bed tubes, very volatile VOCs can be detected. However, since the post-treatment of these tubes is considerably more laborious than that of simple Tenax® tubes, their utilisation is passed over, most of the time.

Measuring conditions and results

VOC samples and TD tubes doped with standard solutions are analysed under the same conditions. Listed below are the parameters of the devices used, the GC-MS QP2010Plus and TD unit TD20 (both by Shimadzu), that have proven to be useful for the measurement of the VOC samples and the doped TD tubes (Table 16). These device-specific parameters must be adjusted and optimised for each GC-MS.

Table 16: Typical measuring conditions GC-MS

Device parameters GC-MS QP2010Plus	Settings
Oven programme	Start temperature 50 °C for 5 min 1. Hold ramp 5 °C/min to 200 °C, 15 min
Analytical separation column	Rxi5MS (Restek) or comparable column Length: 60 m*1.00 µm*0.25 mm Column flow: 2.43 mL/min, pressure 244.2 kPa Interface temperature: 250 °C, split ratio: 1:1 Carrier gas: helium Linear Velocity: 40 cm/s
MS	Ion source temperature: 200 °C, Interface temperature: 250 °C, Detector voltage: 0.9 V Mode: Scan Mass range: 11–500 amu
Device parameters Thermal desorption	**Settings**
TD20	Desorption flow: 60 mL/min Desorption time: 5 min Desorption temperature: 300 °C Temperature of transfer line: 250 °C Temperature of cold trap: -15 °C Desorption temperature of cold trap: 300 °C, Desorption time of cold trap: 5 min

Depicted in a sample chromatogram is the VOC composition of a biogas sample from a waste fermentation plant. A TD tube with Tenax® TA was utilised for sampling. The sampling duration was 10 min and the gas flow as 100 mL/min, so that a gas volume of 1 L was sampled. More than 100 individual substances were identified (Figure 13).
The advantage of the method presented is that even low VOC concentrations in the biogas can be determined with sufficient certainty. As such, for the organosilicon compound octamethylcyclotetrasiloxane (short designation: D4) an absolute amount of 290.98 ng was determined in the aforementioned sample (c.f. Figure 14). This corresponds to a gas phase concentration of 24 ppb D4.

Quantification

The concentration of the compound to be tested in the biogas sample, C_m, in mg/m³, is calculated based on the equation (16):

$$C_m = \frac{m_F - m_B}{V} \quad (16)$$

m_F	Mass of the compound to be tested that was determined by means of GC-MS in the biogas sample (when two TD tubes were placed behind one another during sampling, the sum m_F must be calculated from the two individual values);	(ng)
m_B	Mass of the compound to be tested in the blind sample (if two TD tubes were placed behind one another, the sum m_B must be calculated from the two individual values)	(ng)
V	Volume of the sample	(mL)

Comment: If it is desired that the concentration information be put in relation to specific conditions, e.g. standard conditions 0 °C (273,15 K) and 1013,25 mbar, the following conversion results:

$$C_c = C_m \frac{1013.25}{p} \cdot \frac{T + 273.15}{273.15} \quad (17)$$

C_c	Concentration of the compound of the biogas sample to be tested, relative to specific conditions	(mg/m³(STP))
p	Atmospheric pressure	(mbar)
T	Actual temperature of the biogas sample	(°C)

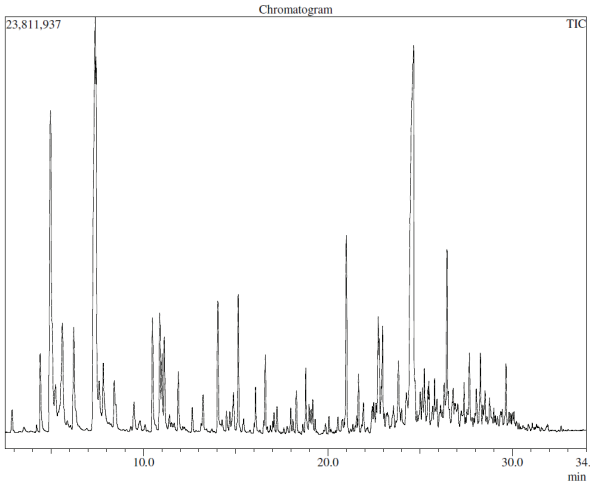


Figure 13: Chromatogram of a biogas sample (waste fermentation plant)
(Source: Fraunhofer UMSICHT)

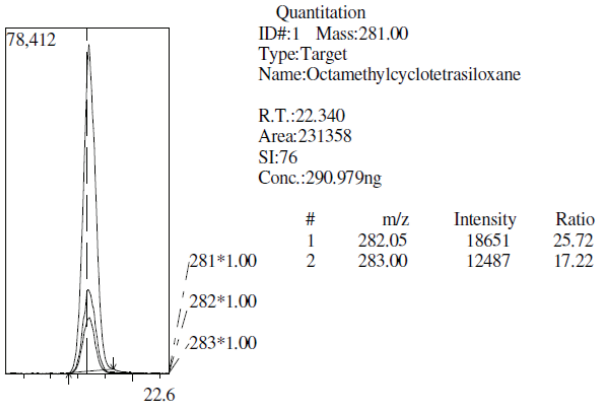


Figure 14: Quantification of octamethylcyclotetrasiloxane (D4)
(Source: Fraunhofer UMSICHT)

4.14 Measurement of H_2 (dissolved - gaseous)

Jens Zosel, KSI

Status	Prototype available
Associated standards	■ Gas-analytical measurement methods ■ Sampling from biological media ■ Extraction of dissolved gases
Area of application of the method	Measurement of the partial pressure of the dissolved hydrogen and other dissolved gases such as oxygen and methane
Substrates/ materials	Liquid and pulpy substrates in anaerobic and aerobic fermentation processes
Limitations of the method/ disadvantages	Lower limit of detection: 1 Vol.-ppm for the aforementioned gases in the extracted gas mixture
Advantages	■ In-situ measurement method ■ Quasi-continuous recording of measuring data ■ Calibration-free detector ■ High long-term stability ■ High selectivity and sensitivity
Need for research	■ Measurement of gas solubility in different substrates ■ Integration of additional volatile components ■ Further automation of the method

Issue at hand

Sensors for the online monitoring of biogas plants and complex control processes for the optimisation of the biogas production from liquid biogenous media are increasingly gaining in importance. For this, robust, long-term stable sensors are required that can be utilised in the liquid phase and gas phase of biogas plants at ambient temperatures of up to 55 °C and pressure of up to 2 bar. In particular, they also have to feature a high sensitivity and selectivity in the case of the presence of highly corrosive components in the liquid phase, e.g. of hydrogen sulphide and organic acids.

Approaches and results

Dissolved hydrogen

The hydrogen dissolved in biogas media is quantified both with membrane-covered amperometric sensors (Zosel et al. 2008) as well as with a novel measuring system in which the hydrogen is extracted from the liquid phase and subsequently determined in the gas phase (Schelter et al. 2011).

Tests with amperometric sensors, depicted in Figure 15, confirm that hydrogen partial pressures in the liquid phase are significantly higher than those in the gas phase.

The measurement of the dissolved hydrogen is therefore an absolutely necessary component of a reliable early warning system for biogas plants. Due to insufficient long-

Figure 15: Amperometric hydrogen sensor (Source: KSI)

term stability, the amperometric sensors have, however, proven to be not suitable for practical use. Biofilms that are formed by numerous microorganisms on the membrane and the solid surfaces have a negative impact on the sensors within a few days. For this reason, the particularly long-term stable measuring system depicted in Figure 16 was developed which is based on the extraction of the dissolved hydrogen (and other gases) from the liquid biogas substrate.

This measuring system consists of an extraction unit that is continuously flushed with an inert gas. Inside this extraction unit, the extraction is carried out via an open interface between the media to be measured and the carrier gas. The gas mixture extracted this way is cleaned, separated chromatographically, and detected coulometrically.

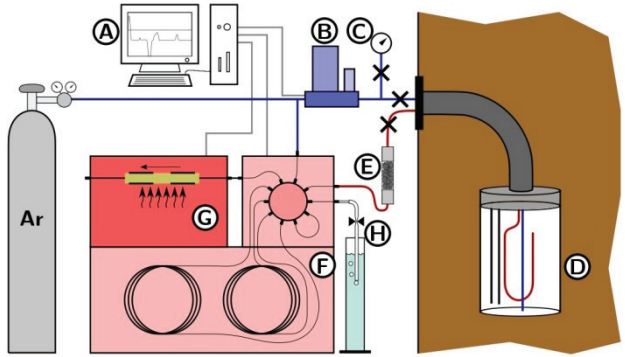


Figure 16: Measuring system for the determination of dissolved gases in biogas media: (A) control unit; (B) mass flow controller; (C) manometer; (D) extraction unit in biogas medium; (E) filter; (F) field gas chromatograph; (G) coulometric detector, and (H) back-pressure regulator (Source: KSI)

From the graphs of the hydrogen and oxygen dissolved in the biogas medium, depicted in Figure 17, it is apparent that the functionality of the measuring system is guaranteed even in the case of prolonged use in a biogas plant.

The hydrogen concentrations in the extraction gas range between 1 and 2 Vol.-ppm, with a noise amplitude of ~ 0.5 Vol.-ppm. Selected measured values are in the range between 1.5 to 4 Vol.-ppm above the aforementioned hydrogen base concentration. No correlation can be derived between the feedings of the digester and the increased hydrogen values found. However, there is a correlation between the feedings of solid dung and the increased occurrence of larger hydrogen spikes.

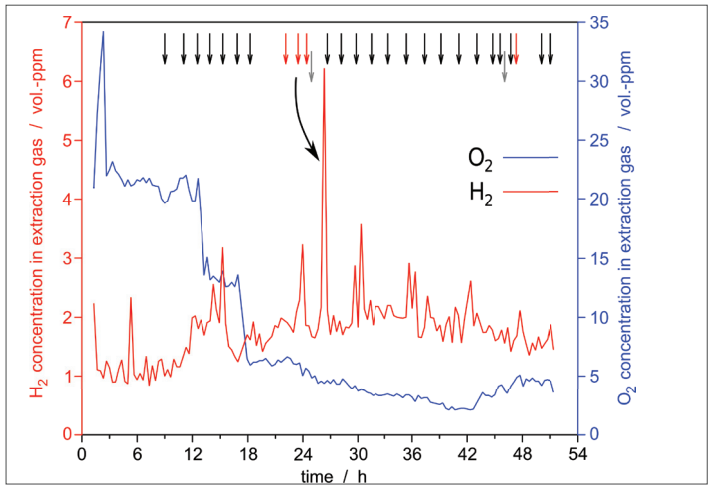


Figure 17: Online measurement of the dissolved hydrogen and the dissolved oxygen in a biogas plant, feeding times marked with arrows, red = solid cattle dung, black = maize silage, grey = cattle manure (Source: KSI)

The partial pressure of dissolved hydrogen can be used not only as a measure for the current process stability, but also as an early warning indicator for developing instabilities, as shown in Figure 18.

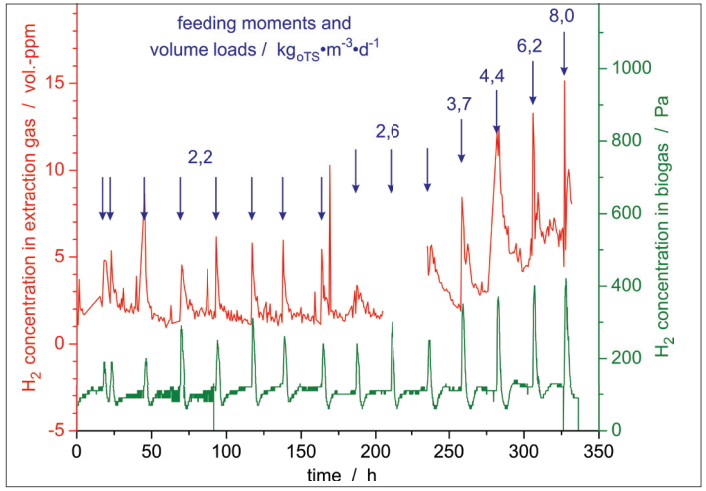


Figure 18: Online measurement of the dissolved hydrogen and the hydrogen in the biogas in a biogas laboratory system whose microbiology is being stressed increasingly by increasing the organic loading rate; feeding times indicated by arrows, feeding amounts specified as organic loading rate (Source: KSI)

Both the dissolved hydrogen as well as the hydrogen in the biogas were measured in a biogas laboratory system over the course of two weeks, during which the microbiology was stressed out from a continuous increase of the organic loading rate. While the base concentration of hydrogen in the biogas is constant, independent of the organic loading rate, the dissolved hydrogen partial pressure increases with increasing organic loading rate. With additional investigations regarding the limits of the process stability, handling instructions can be derived so that operators receive a tool for secure plant management.
Measuring the dissolved hydrogen makes sense particularly during the startup phase of new plants and for the monitoring of substrate change processes. For plants working stable at steady state conditions the measuring system can provide information on load optimisation.

Hydrogen in the biogas
With respect to the aforementioned problem regarding the measuring of the dissolved hydrogen in biogas media, hydrogen measurements, to date, are in practice carried out solely in the gas phase of biogas plants. Quite often, a hydrogen measurement is installed downstream of the desulphurisation of the biogas. In comparison to the measurement in the liquid phase, this approach is associated with the following disadvantages:

- Increased response times due to the large volumes in the headspace of the plants
- Undefined lowering of the hydrogen partial pressure in the biogas due to microbial activities in the headspace or in the desulphurisation and diffusion through sealing materials
- Lower partial pressures due to delayed mass transfer from liquid phase (Pauss et al. 1990).

The measurement of the H_2 partial pressures in the range of 1–1,000 Pa present in biogas is currently carried out usually with electrochemical sensors that are commercially available (e.g. City-Technology Ltd., www.citytech.com). When using these sensors, above all, their relatively high cross-sensitivity to H_2S, which requires a prior separating out of this gas, has to be taken into consideration. Heat conductivity sensors feature a high long-term stability but are often not sufficiently sensitive for the concentrations at hand. Other sensor principles such as FET sensors are in development and cannot be used long-term stable in biogas mixtures yet.

Conclusions
With the newly developed measuring system for long-term stable and selective dissolved gas analysis in biogenous media, a helpful tool for the optimisation of biogas processes was created. Successful tests in different biogas plants have confirmed that with its help hydrogen, oxygen and methane can be detected with high sensitivity, selectivity and long-term stability. The measuring rate of approximately 3–6 measured values per hour is sufficient for an early detection of disruptions in the microbial biogas production. The dissolved hydrogen is a key parameter that can be utilised as a guide value for the process stability and for the optimisation of the plant load.

4.15 Emission measurements on plants for biological waste treatment

Carsten Cuhls, gewitra – Ingenieurgesellschaft für Wissenstransfer mbH; Torsten Reinelt, Jan Liebetrau, DBFZ

Status	Established sampling configuration and measurement methods. The measurement methods are applied in accordance with the applicable VDI guidelines and DIN/EN standards, c.f. associated standards, and are continuously being developed further both process-dependent and with respect to measuring technology.
Associated standards	VDI 3481 Sheet 3, VDI 3481 Sheet 4, DIN EN 13526, DIN EN 12619, DIN EN ISO 21258, DIN EN ISO 25139, VDI 2469 Sheet 1, VDI 3496 Sheet 1
Application range of the method	Monitoring of specific, conducted and diffuse emission sources.
Limitations of the method	Conducted and diffuse emission source: in accordance with the applicable VDI guidelines and DIN/EN standards, c.f. associated standards, e.g. measurement ranges
Advantages	Measurement method for diffuse emission sources: Higher representativeness of the tunnel measurement method (measurement method with wind tunnel) with respect to the sampling due to the larger encapsulated area of the wind tunnel used. Both convectional and diffuse emissions are detected.
Need for research	Due to existing associated standards, no current need for research.

In the analysis of emissions from biowaste treatment plants, conducted and open emission sources have to be fundamentally differentiated. Waste treatment plants are usually equipped with exhaust air collection systems which conduct the exhaust air from the encapsulated areas to an exhaust gas treatment. Afterwards, the cleaned air is released into the atmosphere. The exhaust gas treatment usually consists of a biofilter; at times, an acidic scrubber is installed, as well.
For the analysis of the emissions from the encapsulated emission sources, the exhaust flow of the air collection systems is tested directly, if possible. The volume flows and the concentrations in the corresponding pipe systems are recorded.

Gas sampling systems

Conducted emissions
The gas sampling from encapsulated process components along with exhaust air collection systems are carried out directly in the respective exhaust air duct.

Diffuse emissions

Open biofilter

The gas sampling from open biofilters is carried out in the exhaust gas flow subsequent to passing through the biofilter material. To detect the exhaust gas flow, a thin film is placed on the biofilter material. It is then sealed on the sides with sand bags so that the foil bulges due to the exhaust gas flow. The gas sampling line is installed underneath the foil (c.f. Figure 19).

Open compost heaps

For the emission measurements at open compost heaps and/or non-encapsulated composting plants, a wind tunnel designed by the gewitra GmbH is being utilised. The simulation of the wind is carried out through a mild air flow generated by means of a fan (c.f. VDI Guideline 3475 Sheet 1 and VDI Guideline 4285 Sheet 1).

The emission measurements are performed with an aerated tunnel on the heap surface of the respective sampling site (compost heap). The tunnel covers a surface area with a width of 6 to 8 m and a length of up to 10 m (c.f. Figure 20). Longitudinally and at the entrance area, the tunnel is sealed towards the ground with the use of sand bags. To ensure a free and realistic down gradient of the supplied ambient air, the exit area of the tunnel is not sealed. At the tunnel entrance, two fans are installed that pull in the ambient air from an area with as little preload as possible. At the tunnel exit and/or in the rear internal area of the tunnel, the sampling of the target gases is carried out.

Measured parameters/gaseous substances

Carbon compounds

Total carbon (TC)

The organic substances in the exhaust gas, except for dust-like organic substances, are stated as total carbon (TC). The parameter TC consists of the non-methane volatile organic compounds (NMVOC) and the carbon fraction in methane (CH_4-C). As such, by definition, TC combines the volatile organic compounds (VOC). For the measurement of the organic compounds, the flame ionisation detector (FID) is being utilised with hydrogen as fuel gas and propane as a reference.

Volatile Organic Compounds (VOC)

The volatile organic compounds (VOC) include a multitude of substances that all feature a carbon structure. They can have very diverse impacts on the environment. As formers of photochemical oxidants, they lead – together with nitrogen oxides – to the formation of ozone; furthermore, they are also of importance as carriers of intensely smelly substances and as substances hazardous to health.

Non-methane volatile organic compounds (NMVOC)

The non-methane volatile organic compounds (NMVOC) is the total parameter for organic and carbon-containing substances that evaporate easily or are already present as a gas at low temperatures, wherein the gas methane (CH_4) is excluded.

Figure 19: Gas sampling from open biofilters (Source: gewitra)

Setup of the wind tunnel above the sampling site

Ambient air supplied to the wind tunnel with two separately installed fans and supply air ducts (front view of the wind tunnel)

Exhaust air from the wind tunnel with the sampling hose (rear view of the wind tunnel)

Exhaust air measuring technology with sampling pump and analysers as well as online acquisition of measured values

Figure 20: Emission measurements with the tunnel measurement method (wind tunnel) at open composting plants (Source: gewitra)

The parameter NMVOC is determined from the difference between TC and the carbon fraction in the methane (CH_4-C).
NMVOC are mostly formed as metabolites of both aerobic and anaerobic degradation and conversion processes from organic substances contained in the waste material. They may be contained in traces in the waste material in the form of solvents and solvent-containing products.
Due to their volatile characteristics,NMVOCs enter from the waste material into the exhaust gas and/or environment through stripping processes. The gaseous expulsion is intensified by high temperatures and high flows as a result of the stripping effect.
The NMVOC emissions of biological waste treatment consist of the following components which have a total stake of more than 90 % of the NMVOCs: Sulphur compounds (carbon disulphide, dimethyl sulphide, dimethyl disulphide), nitrogen compounds (basic amines), aldehyde (acetic aldehyde, 3-methylbutanal), ketones (acetone, 2-butanone, 2-pentanone), alcohols (ethanol, 2-propanol, 2-butanol, 2-methylpropanol), carbonic acids (formic acid, acetic acid, propionic acid, valerianic acid), esters (methyl acetate, ethyl acetate), terpenes (mycrene, α-pinene, β-pinene, limonene, α-thujone).

Methane (CH_4)
Methane (CH_4) is the largest organic individual component in the sum parameter TC. Methane is odourless and explosible.
The explosion range of methane/air mixtures exists at an oxygen content greater than 11.6 Vol.-% and a methane content in accordance with IEC 6007920 between 4.4 Vol.-% (100 % LEL) and 16.5 Vol.-% (100 % UEL) and/or a methane content in accordance with PTB, EN 50054 between 5.0 Vol.-% (100 % LEL) and 15.0 Vol.-% (100 % UEL).
Methane is a greenhouse gas. The global warming potential value (GWP value) of methane is 28 (c.f. Section "Carbon dioxide equivalent" at the end of this Chapter).

Nitrogen compounds

Ammonia (NH_3)
Ammonia (NH_3) is generated by the process of ammonification in the decomposition of organic nitrogen compounds. Ammonia (NH_3) is generated in the decomposition of organic nitrogen compounds such as proteins or urea. It is in a pH value-dependent balance with the ammonium ion (NH_4^+). The emissions of ammonia increase in the case of an increase of the pH value > 7, in the case of temperatures > 45 °C or in the case of high aeration rates and drop in the case of comparatively high C/N ratios.

Ammonia has the following characteristics:

- Ammonia is volatile so that a part can enter the atmosphere through evaporation, in particular from highly alkaline materials. Ammonia can be noticed by the penetrating salmiac-like odour
- At an approx. neutral pH value, ammonia exists as ammonium ion (NH_4^+)
- Ammonia dissolves in water while establishing the balance
  $NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$
  which depends on the pH value and shifts to the right with dropping pH value
- Ammonia and/or ammonium ions (NH_4^+) are cationic and are, due to their positive charge, severely absorbed to negatively charged clay minerals.

Nitrous oxide (N_2O)
Nitrous oxide is a greenhouse gas that is generated in presence of ammonia in biological waste treatment and. The GWP value of nitrous oxide is 265 (c.f. Section "Carbon dioxide equivalent" at the end of this Chapter).

Measurement method

Overview
The measurement methods utilised for the emission measurements of the individual substances correspond to the requirements of the respective VDI guidelines and standards in accordance with Table 17.

Table 17: Measurement methods utilised

Substance	Acquisition of measured values	Measurement method	Measuring device, sampling	Guideline, standard
Total carbon	Continuous, online data	FID method	Bernath Atomic 3006	VDI 3481 Sheet 3, VDI 3481 Sheet 4, EN 13526, EN 12619
Methane, nitrous oxide	Continuous, online data	IR method	ABB Advance Optima URAS 14	VDI 2469 Sheet 2
Methane, nitrous oxide	Discontinuous, laboratory analysis	GC method with autosampler	Sampling with evacuated vials	VDI 2469 Sheet 1
Ammonia	Discontinuous, laboratory analysis	Wet-chemical method with sulphuric acid	Sampling with Desaga pump and 2 gas-washing bottles	VDI 3496 Sheet 1

Continuous measurements

Total carbon
The sampling for the continuous determination of TC is carried out by a heated line that leads to an FID.
The analysis values are continuously being recorded during sampling (A/D converter LABCOM 16 and MemoComp software, Breitfuss company).

Methane, nitrous oxide
The sampling for the continuous determination of methane (CH_4) and nitrous oxide (N_2O) is carried out by a sampling line that leads to the gas analyser with online data acquisition. The gas sample passea over a condensate trap and is continuously measured by means of ND infrared spectroscopy (gas pump and gas analyser Advance Optima URAS 14, ABB company). The analysed values are continuously being recorded during sampling (A/D converter LABCOM 16 and MemoComp software, Breitfuss company).

Discontinuous measurements

Methane, nitrous oxide

For the discontinuous determination of methane (CH_4) and nitrous oxide (N_2O), gas samples are directly taken by evacuated headspace vials (20 mL) from the sampling line by means of a double cannula through a butyl septum. Prior to sampling, the headspace vials are evacuated to a residual pressure of 6 mbar with a rotary valve vacuum pump (Vacuubrand, type RE 2). The residual pressure is checked by a digital vacuum meter (Greisinger Electronic, GDH 12 AN).

For the analysis of N_2O and CH_4, a gas chromatograph (SRI 8610 C) is utilised. N_2O is measured by an electron capture detector (ECD), while CH_4 is measured by an FID. The quantification of the sample is carried out with external standards. For the emission measurements, mixed standards with rated concentrations of 0.27 ppmV, 1.5 ppmV and 2.7 ppmV N_2O and/or 1.7 ppmV, 7.5 ppmV and 16.5 ppmV CH_4 (Air Products speciality gases) are utilised. For the calculations of the N_2O concentrations, a linear progression of the calibration function is assumed up to 4 ppmV, while the signal for concentrations > 4 is following a polynomial of the 2nd degree. The signal progression of the FID for CH_4 is linear in the relevant range of concentration. Standards are measured after 20 samples each in order to perform an adjustment of the calibration over the course of time.

Ammonia

The sampling for the determination of ammonia (NH_3) is carried out by a sample line that is passed – without gas refrigeration – over two gas washing bottles filled with sulphuric acid (pump by Desaga company). The sampling of the target gas takes an average of 30 min and is carried out through absorption in sulphuric acid in accordance with VDI Guideline 3496 Sheet 1. Subsequently, the concentration of ammonia nitrogen is determined in the laboratory wet-chemically from the taken sample. The result is a half hour average.

Measurement of the volume flows

The volume that flows within the pipes are detected by a vane anemometer or Pitot tube sensors. For this, 10 individual measurements are carried out from which the arithmetic mean is calculated. The used vane anemometer is of type 1416, with a measurement range of 0.7–20 m/s; the used Pitot tube with manometer is an AIRFLOW Pitot tube with digital manometer model DM30 with the measurement range at -3,000–3,000 Pa. During the emission measurement, the constance of the volume flows is ensured so that a continuous measurement can be omitted.

Evaluation of the measured values

Mass concentrations

In accordance with the Technical Instructions on Air Quality Control, Section 2.5 a) (TA Luft), the mass concentration refers to the mass of emitted substances relative to the volume of exhaust gas at standard temperature and pressure (273.15 K; 101.3 kPa) after the deduction of the water content of water vapour.

The mass concentrations of the substances TC, methane and nitrous oxide are continuously being recorded online with a scanning interval of 1 s and are being logged as average per minute and/or per 30 s. Simultaneously, exhaust gas samples for the determination of CH_4 and N_2O are taken discontinuously from parallel points of measurement. The parameter NMVOC refers to the concentration of total carbon in the measured gas less the carbon concentration of methane in accordance with VDI 3481 Sheet 4.

During the measurement period, the mass concentration of ammonia is taken discontinuously. The sampling of the exhaust gas flow is carried out over a duration of 30 min through absorption in sulphuric acid in accordance with VDI Guideline 3496 Sheet 1 and is subsequently analysed in the laboratory.

The mass concentrations of the measured substances are stated in the unit mg per m^3 of dry air at standard temperature and pressure (mg/m^3 STP) as half hour averages (HHAVs) and as daily averages (ADs).

Mass flows

The mass flow (emissions mass flow), in accordance with the Technical Instructions on Air Quality Control, Section 2.5 b) (TA Luft), refers to the mass of the emitted substances relative to time. The mass flow states the emissions occurring during an hour of operation of the plant as intended under the typically practised operating conditions.

The mass flows are calculated by means of volume flows and occurring concentrations in the corresponding pipe systems.

The mass flows of the measured substances are determined by multiplying the mass concentration with the exhaust gas volume flow of dry air at standard temperature and pressure in the unit m^3 (STP) per h. The mass flows are specified in the unit g per h.

Emission factors

The emission factor, in accordance with the Technical Instructions on Air Quality Control, Section 2.5 d) (TA Luft), refers to the ratio of the mass of the emitted substances to the mass of the processed substances. Included in the mass ratio are the emissions of the whole plant that occur during operation as intended under the typically practised operating conditions (regular operation).

The emission factors of the measured substances are determined by dividing the sum of the mass flow over the measurement duration in the unit g by the wastes fed during the measurement period into the biological treatment stage in the unit Mg. The mass ratios, and thereby the emission factors, are specified in the unit g per Mg of wet mass.

Carbon dioxide equivalent

The carbon dioxide equivalent (CO_2-eq.) represents a unit of measure for the comparison of emissions of different greenhouse gases, but does not imply the same reaction with respect to a climate change. The equivalent CO_2 emissions are determined by multiplying the emissions of a greenhouse gas for a specified period of time with its GWP. For a mixture of greenhouse gases, the GWP is determined by adding up the equivalent CO_2 emissions for each individual gas. The following also applies to the analyses of the project at hand: The objective of composting of biowastes and green wastes is the decomposition of organic substances and the creation of stable humus forms. The carbon dioxide released as microbial metabolite during the biological stabilisation processes does not originate from a fossil source, but rather from a renewable resource and therefore does not enter the balance as a climate-relevant greenhouse gas, but rather acts as a climate-neutral.

In accordance with the Fourth Assessment Report (AR4), the following GWP values are assumed for a period of 100 years (c.f. Table 18) for the calculation of the carbon dioxide equivalent: **CH_4 = 25; N_2O = 298** (IPCC 2007). The CO_2-eq., determined from the emissions factors of methane and nitrous oxide, is specified in the unit kg per Mg wet mass.

Table 18: Comparison of the GWP values for a period of 100 years in accordance with the Fourth Assessment Report (AR4), Third Assessment Report (TAR) and Second Assessment Report (SAR) of the Intergovernmental Panel on Climate Change (IPCC)

Report	Reference	Methane	Nitrous oxide
Fifth Assessment Report (AR5)	**[IPCC 2013]**	**28**	**265**
Fourth Assessment Report (AR4)	[IPCC 2007]	25	298
Third Assessment Report (TAR)	[IPCC 2001]	23	296
Second Assessment Report (SAR)	[IPCC 1996]	21	310

4.16 Determination of the total emissions from biogas plants by means of optical remote sensing

Tanja Westerkamp, DBFZ

Status	Established measurement method for the detection of emissions from animal husbandry and landfills. The measurement method is applied in accordance with the applicable VDI guideline, c.f. associated standards, and is continuously being developed further both with respect to measuring technology and in terms of the simulation.
Associated standard	VDI 4285
Area of application of the method	Quantitative determination of diffuse and spatially spreaded emission sources
Limitations of the method	No localisation of individual source, lower limit of detection of the measuring devices, dependency on meteorological conditions.
Advantages	Measurement method detects the concentrations of methane and ammonia in the down wind of the source area and allows for the determination of the emission rate of the whole site.
Need for research	Need for research with respect to the limits of the micro-meteorological simulation model.

In addition to stationary emission sources such as the exhaust air from CHPs or gas processing, biogas plants also feature diffuse sources. Those include leakages and diffusion of gas as well as emissions from open storage of substrates and digestate. In order to measure the total emissions of a biogas plant, optical remote sensing represents a suitable method. With the help of a tunable diode laser absorption spectrometer (TDLAS), the concentrations of methane and ammonia in the down wind of the plant can be detected over several hundred meter long measurement paths in the ground level atmosphere. At the same time, the weather conditions at the location of the concentration measurements are recorded, wherein the measurement of the wind by means of 3D sonic anemometer is of decisive importance. The measurement values are entered into the simulation software "Windtrax" for the determination of the emission rates of the plants via inverse dispersion.

Measurement technique

For the measurement of gas concentrations in the atmosphere over long distances, in principle, several measurement methods are suitable. Introduced here is a TDLAS system in combination with a weather station that is equipped with a 3D sonic anemometer. The devices are depicted in Figure 21.

TDLAS

Depending on structure and complexity, molecules feature a certain number of vibrational modes. The vibrations are excited through absorption of light in the middle infra-red range. In addition, harmonic overtones occur in the near infra-red range. Organic molecules in particular feature characteristic absorption spectra in this spectral range. With the help of semiconductor laser diodes it is possible to measure individual absorption lines of certain molecules over a range of a few nanometres in order to determine the concentration of the target substance in the optical path of the laser. In accordance with the Beer-Lambert law, the intensity of the laser beam decreases exponentially dependent on the wave number which is the reciprocal wavelength of the laser light, as follows:

Figure 21a: Weather station (Source: DBFZ)

Figure 21b: To the left: laser spectrometer, to the right: reflector modules (Source: DBFZ)

$$I(\tilde{\nu},L) = I_0(\tilde{\nu}) \exp(-\sigma(\tilde{\nu}) NL) = I_0(\tilde{\nu}) \exp(-S \phi (\tilde{\nu} - \tilde{\nu}_0) NL) \quad (18)$$

I	Transmitted intensity
I_0	Intensity of output
$\tilde{\nu}$	Wave number
σ	Absorption cross-section
N	Particle density
L	Optical path length
S	Line thickness
ϕ	Lineshape function
$\tilde{\nu}_0$	Wave number of the line centre.

In addition to the thickness and shape of the absorption line, the length of the absorption path, meaning the distance between the laser and the detector, and the density of the gas analysed also have an influence on the strength of the absorption. As such, it depends directly on the number of molecules in the beam's paths. In the case of a uniform spatial distribution of the gas to be detected, the resolution therefore improves with the length of the absorption path. Consequently the measuring path should be as long as possible, but should not extend too far outside of the area of the emitted plume in order to obtain an optimal resolution. These facts are schematically depicted in Figure 22.

The resolution of the Open Path TDLAS devices of the manufacturer Boreal Laser Inc. for methane and ammonia is specified with 1 ppm*m. In the case of an optical path length of 10 m, the resolution therefore would be 0.1 ppm, in the case of a 1,000 m measuring path it would be 0.001 ppm. However, with increasing path length, the influence of atmospheric interferences increases. The limit of detection for a path of 200 m length is specified with 0.03 ppm.

The system is designed such that the laser and a photo diode used as detector are located in a shared housing. Central element is the laser diode suitable for the respective target gas. Its laser beam hits a beam separator. The one partial beam exits the housing, crosses through the air to be measured, is reflected by a reflector module at a distance of up to 500 m, and is detected by the photo diode upon re-entry into the housing. The other part of the beam is passed through an internal reference cell. Another photo diode detects this beam that crossed through the reference cell. Subsequently, the measuring signal and the reference signal are compared in order to determine the gas concentration on the measuring path. This way, a continuous calibration is performed. With the help of a software-controlled pan and tilt unit, the lasers can automatically point several reflector units in sequence. A required equipment for the analysis of the measurements is a laser distance meter for distances of up to 500 m.

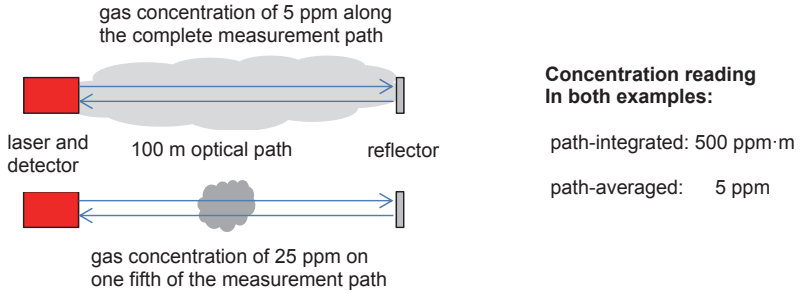


Figure 22: Schematic depiction of path-integrated and path-averaged concentration information (Source: DBFZ)

Weather station

For the determination of emission rates based on measured concentrations, the measurement of the wind conditions near the ground is indispensable. In the case of utilisation of the simulation software Windtrax, the use of a 3D sonic anemometer is a well suited option for this purpose. Furthermore, the ambient temperature and pressure at the measurement site must be recorded.

Measuring set-up

For the estimation of the total emissions at the biomethane plant, long measuring paths are set up in the down wind plume of the plant at a height of approx. one to two meters. Depending on the size of the plant, the distance from the plant, and the wind speed, distances between spectrometer and reflector of approx. 100 m to up to 500 m can be utilised. It must be taken into consideration that the measurements are carried out in sufficient distance from the plant in order to avoid turbulence interference at the place of the concentration measurements. However, the distance should not be selected too large, since the concentration of the gases to be measured decreases with increasing distance. It might be that an interference-free measurement is not possible for all plants and any wind direction since additional constructions or trees may interfere. Furthermore, it must always be clarified to what extent the option exists to be able to set up the measurement devices on neighbouring fields. In the summer months, arable crops that grow tall (e.g. maize) may be potentially an obstruction. Also important are measurements of the background concentrations. Methane, in particular, occurs naturally in the atmosphere. Since the concentration features a diurnal variation, it is recommended that the background should be measured at different times of the day.

Inverse dispersion modelling

Methods of forward and inverse dispersion calculations that are based on Lagrange stochastics models (VDI 3945 BLATT 3 2000; Schürmann 2007; Vesala et al. 2008; Wilson & Sawford 1996) have been evaluated for numerous areas and found application in there. In such models, particles are considered on their path along trajectories, i.e., the flight paths

from the perspective of the particles. The simulation of a multitude of such trajectories allows for the numeric determination of emission flows if influencing meteorological parameters are taken into account. A differentiation must be made between two approaches: The forward model and the backward model.

Forward-calculating Lagrange models determine the dispersion of a substance from a defined point source or area source. This method is, for instance, required by the "Technical Instructions on Air Quality Control" (TA Luft 2002). An exemplary implementation is with the software Austal2000 (Ing.-Büro Janicke engineers' office 2011). However, if one wants to derive the source strengths of diffuse sources from measured concentrations with the help of a forward model, this approach is very CPU-intensive since a multitude of simulated particles will not pass the position of the concentration measurement (Schmid 2002). In order to receive statistically meaningful results, therefore a very large amount of trajectories must be determined, in particular in the case of area sources.

Flesch et al. (1995) therefore developed a method to calculate backwards in time, starting at the points of measured concentrations. The model simulates the flight paths of thousands of air parcels backwards, starting from the site of the concentration measurement, and determines for each parcel at which location it last touched the ground. In this way, a catalogue of the distribution of the touchdown results. When the location and the dimensions of theemission source are given the fraction covered by touchdowns is calculated. By now, this method is established as backward-Lagrangian Stochastic (bLS) model and is implemented in the freeware Windtrax (Thunder Beach Scientific 2011). A big advantage of this method is based on the simple test setup. The concentration measurements in the down wind plume can be carried out at a height of one to two meters above the ground (Harper et al. 2011).

In addition to the measured concentrations as well as location, size and shape of the source area, the following meteorological input data are required for the calculations in Windtrax: Temperature, ambient pressure, wind direction and wind speed in three dimensions. When using a 3D sonic anemometer, all required micro-meteorological parameters for the simulation such as the roughness length z_0, the Obukhov length L_o and the friction rate u^* as well as the standard deviations of the wind speeds are calculated from these data. The roughness length refers to the height at which the wind speed disappears in the ground level atmosphere due to the logarithmic wind profile. It is dependent on the characteristics and plant cover height of the ground. It can be determined from the structure of turbulent fields in proximity to the ground (Foken 2003). The Obukhov length is a measure of the stability of the atmosphere. It specifies the relationship between the dynamic, thermal, and buoyant lift processes. In the case of a stable stratification, i.e., for instance during clear, calm nights, L is positive. In the case of an unstable stratification, for instance on sunny afternoons, L is negative. In the case of a neutral stratification, as it occurs in the case of a cloudy sky and/or strong wind, |1/L| approaches 0. The friction rate is linked with the wind speed and is a measure for the vertical transport of a horizontal movement near the ground.

Numerous experiments were able to show that Windtrax is well suited for the determination of diffuse emissions, taking into consideration certain restrictions. For one, this method was tested in different gas release experiments, c.f., for example, (Crenna et al. 2008; Flesch et al. 2005a; Gao et al. 2010; McGinn et al. 2006). The amounts of gas calculated were subsequently compared to the amount of gas actually released. An overview of the results from 12 such studies provided a mean value of 98 % for the relationship of the calculated emissions to those released. The mean value of the standard deviations is 21 % (Harper et al. 2010). For another, with this method, the emissions of herds of cattle (Laubach & Kelliher 2005; Laubach 2010) and cattle sheds (Flesch et al. 2005b), pig farms with bioethanol production (Harper et al. 2010) as well as of a biogas plant (Flesch et al. 2011) were determined and in part compared to other methods.

For using inverse dispersion modelling by means of Windtrax for the determination of the emissions rates of biogas plants, the following restrictions result (Flesch et al. 2004):

- The mathematical model Windtrax is based on the Monin-Obukhov similarity theory. From this, the restrictions with respect to the applicability of the model do result. Experience has shown that measurements are only usable if $u^* \geq 0.15$ m/s and $|L| \geq 10$ m.
- An idealisation of three-dimensional structure as ground level area sources requires a distance of the concentration measurements from the source of approx. ten times the height of the obstacles. This distance also must be adhered to due to the disturbance of the wind field by the structures.
- The distance between the source and the measurement should be small enough that the concentrations can be measured accurately enough, taking the background into consideration.
- The positioning of the measuring installation should be carried out such that the effective area of influence of the trajectories covers at least 50 % of the source area. This point must in particular be adhered to in the case of changes of the wind direction during the measurements.
- It is recommended that the measurements (concentration and meteorological data) are averaged over 10 to 30 min.

Measurement results

The measured concentration values are specified as path-integrated concentration in the unit ppm*m. In the case of a background concentration of 2 ppm in the air and a path length of 500 m, this results in a path-integrated concentration of 1,000 ppm*m. Assuming good visibility, the measuring accuracy in this example is at a few ppm*m. The measuring of the length has an accuracy of one meter. Deviations from the natural background of more than 10 ppm*m are usually detectable well. For the above example, this means that an average concentration increase of 20 ppb can be detected on the measuring path.

The background of ammonia in the atmosphere near the ground is very low but depends on the ground's plant cover. The values are at the limit of detection of the measuring device.

In order to be able to prove a concentration increase due to the biogas plant, a measured value that is increased by at least 20 ppm*m should be present.
The increase of the measured value through emissions from the system is directly connected to the distance of the measuring path from the plant and to the wind speed. Without a micro-meteorological dispersion model, a direct statement regarding the emissions rate based on the measured concentration is not possible. Windtrax features a graphical user interface in which a schematic of the measuring setup is drawn. Measured concentrations and weather data can be provided via an input file. Recommended are mean values of 10 to 30 min. Depending on the CPU power, the number of simulated particles, and the number of measuring series, the simulation may run from a few minutes to several hours. The results can be written to an output file. As result, an emission rate of the plant is received, for example, in kg/h as well as the corresponding standard deviation. In addition, micro-meteorological indicators are determined that are an additional metric for the quality of the simulation. The emission factors for a respective plant can be derived via the determined emission rate, the density of the measured gas at standard conditions and the production rate of the plant.

5 Methods for the determination of the physical parameters

5.1. Particle size distributions

Marc Lincke, Karin Jobst, Fraunhofer IKTS

Status	Under development/validation
Associated standards	Modelled after ISO 13320 (2009) and ISO 13322-2 (2005).
Area of application	■ Types of substrate: biogenous substrates of any composition ■ TS range: 0 %–100 % ■ Limitations of the method: measuring range of the particle size (0.1 µm–20 mm) ■ Advantages: simple and robust method, wide measuring range
Need for research	Additional investigation, statistical substantiation as well as validation with model substrates of known granulometric composition in combination with corresponding mass balance calculations

Substrates, process media and incidential residues that occur in biogas production constitute very inhomogeneous material systems, which are characterised by irregular particle shapes and fibre structures as well as by a wide distribution of particle sizes. The knowledge and the adjustment of an optimal granulometric state is of great importance for the assessment as well as the optimisation of bioengineering processes that are related to biogas production. Especially for difficult to decompose and persistent substrates, the granulometric state has a direct impact on the biological decomposability as well as the viscosity and therefore it has an immidiate inlfuence on the mixing quality of the fermentation substrate.
At Fraunhofer IKTS it was possible, as a part of a joint project that was financed with funds of the European Union and the Free State of Saxony, to develop a measurement method together with a corresponding measuring guideline for these material systems. The scientific approach is characterised by a combination of different measurement methods and the particular results of each method are merged into a joint distribution graph. Since the material systems used in biogas plants in general feature high distribution ranges from the µm range to the cm range and therefore cannot be detected granulometrically with a single measurement method, this approach becomes inevitable. In a first step, the fractionation into coarse and fine share is carried out at a separating cut of 1 mm. The assessment of the fine share is performed by means of laser diffraction spectroscopy and the assessment of the coarse share with the help of a quantitative image analysis. The approach is explained in more detail below. In Figure 23, the principle of the granulometric assessment in accordance with the measuring guideline of Fraunhofer IKTS is depicted.

The method introduced is currently being validated and investigated at IKTS in order to enable and ensure its widespread establishment.

Sampling and sample preparation

For a meaningful analysis, first and foremost a representative sample, which reflects the properties of the substrate in the silo and/or digester as best as possible, is important. The sampling, conservation and transport are carried out in accordance with the provisions of VDI Guideline 4630.

Materials and devices

- Analysis sieve (1 mm mesh width)
- Beakers
- Scale
- Drying cabinet
- Laser diffraction spectroscopy (Mastersizer S2000)
- Quantitative image analysis system (FibreShape/QicPic)

Execution method

The substrate to be assessed is separated into a fine and a coarse share with the help of a sieve (1 mm mesh width). For this purpose, a defined quantity – depending on the type of substrate – is placed on the sieve and rinsed with water until no particles are visible any more in the residual rinsing water that already passed the sieve. For the analysis of the coarse fraction, a minimum of 3,000 assessable particles are needed. The investigations of Fraunhofer IKTS show that the sample and rinsing water amounts needed can differ

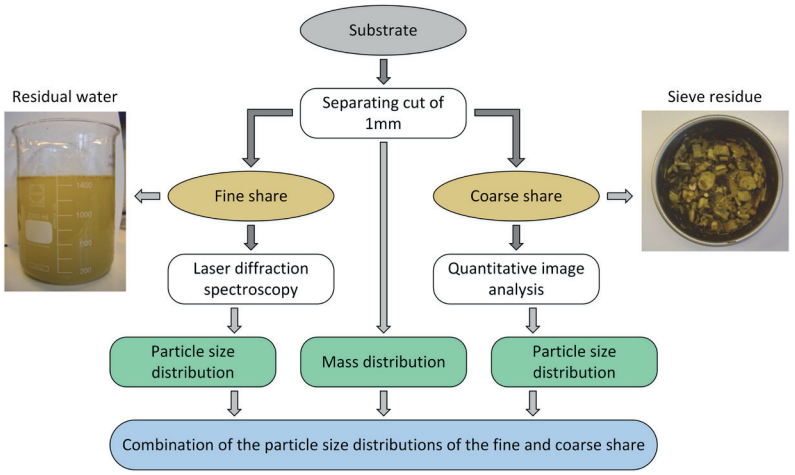


Figure 23: Principle of granulometric assessment (Source: IKTS)

widely, depending on the substrate. The sieve residue is dried in a drying cabinet until a constant weight is achieved.

Irrespective of the granulometric assessment of the fine and coarse fraction, the coarse substance share is calculated with the help of Equation 19 and put in relation to the dry residue content, which is determined in accordance with DIN 38414 Part 2.

$$CS = \frac{(m_{SieveDried} - m_{SieveEmpty}) \cdot 100}{DR \cdot m_{Content}} \quad (19)$$

CS	Coarse substance share	($\%_{DR}$)
$m_{SieveEmpty}$	Mass of empty sieve	(g)
$m_{SieveDried}$	Mass of sieve with dried sample	(g)
DR	Dry residue	(%)
$m_{Content}$	Mass of the content of the sample	(g)

The fine share in the rinsing water is measured directly after the sieving with the help of laser diffraction spectroscopy. In this measurement method, a monochromatic laser beam is diffracted by the particles contained in the suspension and the strength of the diffraction is indirectly proportionally dependent on the particle size. Particles with a larger diameter cause less diffraction of the laser beam. The diffracted light is measured by photo detectors which are mounted at different angles. With the help of the light diffraction theory, the particle size distribution can be calculated from the obtained diffraction image (angle-dependent light intensity distribution).[1] The measurements are carried out in accordance with an internal measuring guideline as well as on the basis of ISO 13320. Based on the evaluated laser diffraction, volume-equivalent particle sizes x_{EQPV} are calculated which can be depicted as density distribution $q_3(x_{EQPV})$ or as cumulative distribution $Q_3(x_{EQPV})$ (quantity type: volume). For further investigations, it is assumed that the sphere diameter x_{EQPV} is identical to the area diameter x_{EQPC}. The investigation of this empirical approach is currently carried out for biogenous substrates in on-going research activities.

The dried coarse fraction is characterised with the help of a quantitative image analysis. The measurement is carried out in accordance with an internal measuring guideline. As a result, a number distribution Q_0 of the particle contour data is available.

Data analysis

A prerequisite for the combination of two particle size distributions is the overlap of the measuring ranges of both methods, i.e., the maximum particle size of the fine share must be equal to or larger than the minimum particle size of the coarse share. Furthermore, both distribution functions must feature standardised types of distributions as well as comparable dispersity parameters and/or particle sizes.

For this reason, a dispersity-size and type-of-quantity conversion of the distribution function of the coarse fraction is required. In the first step, the contour data of each particle is consolidated into a single value, the projection area identical diameter x_{EQPC} (c.f. Figure 24),

[1] http://www.malvern.de/ProcessGer/systems/laser_diffraction/technology/ technology.htm (21 Dec 2011)

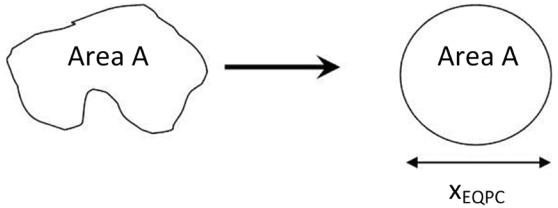


Figure 24: Diameter of the projection area identical circle (x_{EQPC}) (Source: IKTS)

which is available as density distribution $q_0(x_{EQPC})$ and/or cumulative distribution $Q_0(x_{EQPC})$ of the type of quantity "number".
Subsequently, the conversion of the type of quantity from e = 0 (count) in r = 3 (volume) is carried out in accordance with the following equations

$$q_r(x) = \frac{x^{r-e}}{M_{r-e,e}} \cdot q_e(x) \quad (20)$$

$$M_{r-e,e} = \int_{x_{min}}^{x_{max}} x^{r-e} \cdot q_e(x)dx \quad (21)$$

$$Q_r(x) = \int_{x_{min}}^{x} q_r(x)dx \quad (22)$$

x	Particle size
$q_r(x)$	Density distribution
$Q_r(x)$	Cumulative function
$M_{r-e,e}$	Momentum
Index e	Information regarding the existing type of quantity
Index r	Information regarding the sought-after type of quantity

If the particle size distributions of the fine and coarse fractions are subsequently available in the same type of quantity and feature equal dispersity parameters, both the density functions and/or the cumulative functions can be brought together into a single density function and/or cumulative function (c.f. Figure 25) with the help of software tools and taking into consideration the quantity shares of the coarse and fine fractions derived from the parameter *CS* (Equation 19).

Need for research
The methodology requires further investigation and statistical substantiation in the future. This is intended to be achieved through the application of the procedure to model substrates with known granulometric composition in combination with corresponding mass balance calculations.

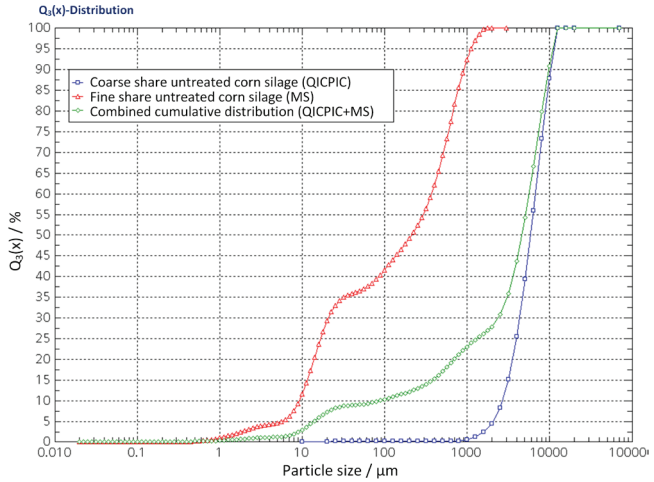


Figure 25: Cumulative distribution $Q_3(x)$ maize silage untreated (Source: IKTS)

5.2 Determination of the surface tension

Lucie Moeller, Kati Görsch, UFZ

Status	This method corresponds to the general standard.
Associated standards	Ring, plate and detachment method
Substrates/ materials	This method is suitable only for samples with total solids contents of up to approximately 6 %.
Measuring range	0.1–100 mN/m
Disadvantages	In the case of two-phase sampels (e.g. oil/water) the mixture may separate in the syringe during the measurement.
Advantages	No wetting problems
	Reduced sample volume (0.25 mL to 5 mL)
Need for research	For this method, there is no need for research.

The surface tension of a liquid is interconnected with the presence of surface-active substances. The lower the surface tension, the more easily the formation of foam may occur. To check the tendency of the fermentation substance to foam, its surface tension is determined with the help of a so-called drop volume method. The measuring principle is based on the formation of a drop at the end of a capillary, wherein the duration from the formation to the falling off of the drop depends on the surface activity of the tested liquid.

Processing of sample

For the determination of the surface tension, the centrifuge supernatant of the sample is being used. For this, the sample is centrifuged in 50 mL centrifuge tubes for 20 min at 5,300 rpm and 20 °C (device: Avanti 30 centrifuge, Beckman company). The supernatant is strained through a sieve (mesh width: 750 µm). To calculate the surface tension, the density of the sample is required. For this, the density of the centrifuge supernatant is determined by weighing out a specific volume (triple determination with building of an average).

Measuring process

As measuring device, a drop volume tensiometer (device: Lauda TV T-1, Lauda Dr. R. Wobser GmbH & Co. KG company) is being utilised. The device is controlled with the help of the Lauda software programme (Version 2.2) (Lauda Dr. R. Wobser GmbH & Co. KG company). Approximately 2 mL of the centrifuge supernatant are pulled up with the syringe, mounted in the device and heated for at least 5 min to 26 °C. To calculate the surface tension, the program needs the density of the sample. For the determination of the surface tension, the following parameters are selected: Mode STD (standard mode with constant drip rate), Red. on (reduction mode: uniform formation of individual drops). During the measurement, the surface tension of a total of eight drops is determined and averaged. Details regarding the measurement can be found in the operating manual of the device.

5.3 Determination of the foaming potential by means of the "bubble test"

Lucie Moeller, Kati Görsch, UFZ

Status	This method was developed for the determination of the foaming tendency of activated sludges.
Substrates/ materials	No restrictions
Limitations of the method	The filter clog and need to be cleaned.
Need for research	For this method, there is no need for research.

Formation of foam in the process of anaerobic digestion is a frequent problem and can lead to serious opertional problems (Moeller & Görsch 2015). However, research regarding foam formation during anaerobic digestion is relatively new. New methods for testing of the foaming tendency of substrates must be developed and/or methods from other disciplines must be tested for their utilisation in the field of anaerobic digestion. One to assess the tendency of a mixture to foam, and to assess the effectiveness of anti-foaming agents is the utilisation of the so-called "bubble test".

Description of the method

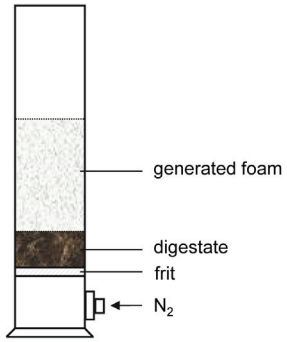


Figure 26: Schematic of the foam generator for the determination of the foaming potential of a liquid mixture (Source: UFZ)

The tendency of a liquid mixture to form foam can be determined with the help of a foam generator. This is a measuring cylinder which, in its bottom area, is equipped with a diffuser stone for the injection of gas (Figure 26).

50 mL of a liquid mixture (fermentation material or model foam with a stability comparable to that of the foam occurring in biogas plants, such as a 0.5 % solution of protein powder in distilled water) are filled into the measuring cylinder. Through injection of nitrogen gas (10 L/h) over a defined period (5 minutes), the formation of foam is triggered in the prepared mixture. The foaming tendency, the foaming potential and the foam stability can be determined based on the foam development (Ganidi 2008).

The **foaming tendency** is determined as the height of the foam after five minutes of gasification, relative to the weight of the total solids in the fermentation material:

$$\text{Foaming tendency} = \frac{h_{foam}\ [mm]}{m_{TS}\ [g]} \quad (23)$$

The **foaming potential** is calculated after a 5 minute generation time as volume of the generated foam (V_{foam}), relative to the gas flow velocity (F_{N2}):

$$\text{Foaming potential} = \frac{V_{foam}\ [mL]}{F_{N_2}\ [mL/min]} \quad (24)$$

The **foam stability** is calculated as the volume of the generated foam ($V_{foam,\ t=1h}$) one hour after the gasification has ended, relative to the gas flow velocity (F_{N2}):

$$\text{Foam stability} = \frac{V_{foam,\ t=1h}\ [mL]}{F_{N_2}\ [mL/min]} \quad (25)$$

With this method, the effectiveness of the anti-foaming agents can be assessed, for instance. Model foam is well-suited for assessing the effectiveness of anti-foaming agents because the tests can be consistently reproduced. With the bubble test, the effects of individual anti-foaming agents can be easily seen (Figure 27). The ratio of the foam-generating (protein powder) solution to the anti-foaming agent should correspond to the ratio commonly used in full-scale biogas plants (e.g. 0.002 Vol.-%). The effect of the anti-foaming agent is then assessed based on the difference between the bubble test with and without the anti-foaming agent. In order to ensure the accuracy of the results, the each test should be repeated at least twice.

Figure 27: Foam generator with model foam

5.4 LEIPZIG FOAM TESTER - Test set for the determination of the tendency of a substrate to foam

Lucie Moeller, Yvonne Köster, Andreas Zehnsdorf, UFZ

Status	The device is available from the Eismann & Stöbe GbR company.
Area of application of the method	Wet fermentation in mesophile and thermophile operation
Substrates/ materials	Fermentation materials should have a TS content of less than 12 %. All substrates can be tested, but in some cases they need to be disintegrated.
Limitations of the method	To date, there are no known disadvantages.
Advantages	Leipzig foam tester enables the detection of critical substrates before the use, the diagnosis of foaming causes in biogas plants as well as the optimization in safe test mode.
Need for research	For this method, there is no need for research.

Foam formation is among the most frequent disruptions in biogas production via anaerobic digestion. Even though the consequences are very well known to plant operations, the actual causes of foam formation are often not understood. Biogas plants that ferment biogenous residues and waste materials are particularly affected by foam formation (Moeller & Görsch 2015). The main reason for this is found primarily in the the constant changing substrate quantity and quality. This method is particularly advantageous in that

Figure 28: LEIPZIG FOAM TESTER (André Künzelmann)

the foaming tendency of a new substrate (previously unknown to the plant operator) can be quickly and easily estimated before it is introduced to the digester.

Description of the method

The LEIPZIG FOAM TESTER (Figure 28, Moeller et al. 2015) is an easy-to-use test kit that can estimate the propensity of a substrate to produce foam during anaerobic digestion.. The advantage of the test kit is that it is simple (complicated analysis is not needed) and was designed to be used on site by plant operators. Upon delivery of an unkown substrate, an aliquot is added to the active fermentation material and incubated at constant temperature for several hours. The test kit enables the operator to directly observe foam development. Based on the result ot the test, plant operators can assess which steps should be taken in order to avoid a severe foam formation in the biogas plant.

Execution of the test

Preparatory measures

A sample of the fermentation material should be extracted from the digester and, if the material appears to be nonhomogeneous, strained through a sieve (mesh width 10 mm). It is also advantageous to know the TS content of the substrate.

Carrying out the test (Figure 29)

2 % (w/w) substrate (relative to the total solids content of the substrate) are weighed into the test bottle and mixed with active fermentation material. The final weight of the test mass (fermentation material) should be approximately 500 g. It is important that the contents of the bottle are thouroughly mixed prior to the start of the test. The test bottle, which is equipped with a lid that features a pressure release, and is closed and incubated in the LEIPZIG FOAM TESTER at either 37 °C (mesophile) or 55 °C (thermophile). The duration of the test is dependent on the activity of the fermentation material and the temperature of the mixture at the start of the test. It is advantageous to let the test run for at least 12 hours, but preferably for 24 hours. After the test ist finished, the bottle can be removed and the intensity of the foam formation can be assessed. Since the existing fermentation material may also produce foam, a control test (without addition of the new substrate) should also be conducted.

The intensity of the foam formation is calculated as follows:

$$\text{Intensity of the foam formation } [\%] = \frac{V_{foam}\ [mL]}{V_{tot}\ [mL]} \cdot 100\ [\%] \quad (26)$$

Protein powder may be used as a reference substrate. But in this case, only 1 % protein powder (w/w) should be used since it causes a very severe foam formation. The use of protein powder enables different fermentation materials to be tested for their foaming propensity.
The method described may also be used to troubleshoot foaming events in biogas plants by retroactively testing problematic substrates. The fermentation material used in this case should feature properties (mesophile/thermophile, substate matrix, TS content) similar to tht of the fermentation material of the foaming plant. The fermentation material of an already foaming plant cannot longer be utilised for the determination of the cause. The viscosity of the source fermentation material plays an important role for the foaming tendency of the mixture with the respective substrate. The higher is the viscosity of the fermentation material, the higher the intensity of the foam formation in combination with the foam-provoking substrate. Since the viscosity cannot be measured by the plant operator, the TS content, which influences viscosity and can be easily measured, should be used as an indicator of viscosity.

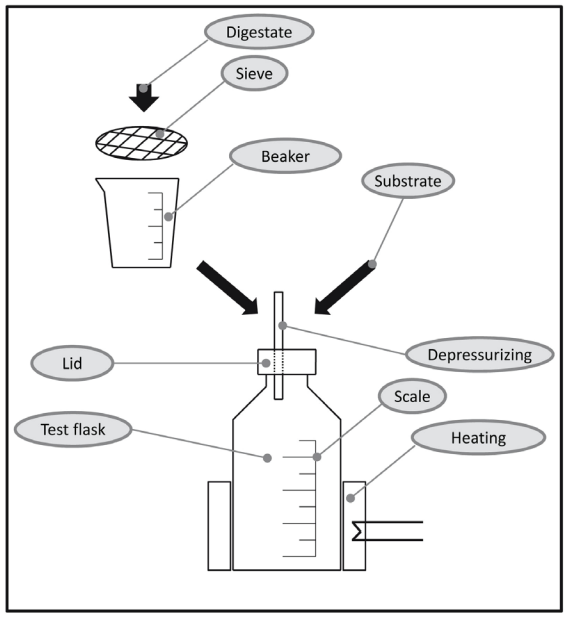


Figure 29: Test setup with labelling of the individual components

5.5 Viscosity

Status	Under development/validation
Type of substrate	Biogenous substrates
Particle sizes	Maximum length = 50 mm (dependent on the measurement method)
Limitations of the method	High dynamic viscosities
Need for research	■ Comparative tests between the systems and standardised measurements must be carried out by means of rotation viscometers ■ Development of stirrer geometries for long-fibre substrates ■ Investigation of the impact of the vortex formation on the torque as well as the mechanical correction for the determination of the apparent viscosity. ■ Transfer of the approach to industrial scale stirrers/pumps as an additional control parameter.

The viscosity and the flow behaviour, respectively, of the substrates utilised and of suspensions brought to fermentation determine, to a great degree, flow-engineering and material transport processes and have a strong impact on the biogas production.
Due to the complex composition of biogenous substrates (highly concentrated, long-fibred), commercial measuring system can be utilised only to a limited extent for the assessment of the flow characteristics of such material systems. To derive practicable measurement methods, systematic tests were carried out by different scientific institutions, whose results are presented in the following chapters. In this, it has to be taken into consideration that the measurement methods pointed out are always linked to defined conditions of use. Furthermore, it has to be noted that these, in part modified methods require further scientific tests and should therefore not be considered to be standardised measurement methods. Progressing insights and results of validation still to be carried out will be published at the appropriate time.

5.5.1 Measurement methods for the rheologic characterisation of fermentation substrates

Manuel Brehmer, Matthias Kraume, TU Berlin

For the rheologic characterisation of fermentation substrates, different devices, measurement methods and processes are available. In this, the correct choice depends on both the particle size and shape as well as the total solids content. However, since only an exact knowledge of the flow behaviour allows for an optimal operation of the mixing technology (Knoch 1997), a recommendation for the selection of the suitable measuring system in dependence on the substrate composition is provided.

Quickly and precisely, the flow characteristics of shear thinning liquids such as fermentation substrates can be determined via rotation tests. For this, rheometers are available that consist of a drive unit and a measuring system. The latter, in turn, consists of two components, the measuring body and the measuring cup. Most of the industrially utilised ***rheometers*** work in accordance with the Searly method, i.e. the measuring body located internally (rotor) is in rotation while the exterior measuring cup is at rest (stator). The advantage of this setup is the option to heat the sample via the exterior, resting cylinder wall. Located in the gap between the rotor and the stator is the sample to be tested. In the case of the sufficiently tight annular gap between the measuring body and the measuring cup, and assuming wall adhesion, a linear velocity gradient in the gap can be assumed (Moshage 2004). Through the measurement of the torque *M* required for the rotation in dependency on the rotational frequency *N* and/or the angular velocity *ω*, the shear stress τ and the shear rate $\dot{\gamma}$ can then be calculated. In order to take into account the different flow characteristics of the fluids, different measuring systems were developed and – for the most part – standardised. A differentiation is made between concentric, cone/plate, plate/plate and relative measuring systems (Mezger 2006).

Among the classic, concentric measuring systems are the cylinder and the double gap measuring systems. Both are wide-spread in both industry and research. In addition to the easy handling and a quick, exact determination of the shear stress and the shear rate, the temperate impact can also be taken into consideration without a lot of effort.
The ***cylinder measuring system*** (c.f. Figure 30) is described in the standards ISO 3219 and DIN 53019. It consists of a measuring body that rotates inside an exterior measuring cup. Both are located along a joint rotationally symmetrical axis. The shear stress and the shear rate can be calculated with the equations listed below.

$$\tau = \frac{1+\delta^2}{2000\cdot\delta^2}\cdot\frac{M}{2\pi L\cdot r_i^2\cdot C_L} \tag{27}$$

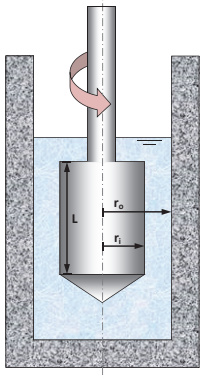


Figure 30: Cylinder measuring system (Source: Brehmer 2011a)

$$\dot{\gamma} = \omega\cdot\frac{1+\delta^2}{\delta^2-1} \tag{28}$$

The constant needed C_L is referred to as end-effect correction factor and takes into consideration effects at the end surface of the measuring system. Said factor is specified by the manufacturer. The maximum permissible ratio of the radius δ is also specified in ISO 3219 and amounts to:

$$\delta = \frac{r_o}{r_i} \leq 1.2 \tag{29}$$

Often, the flow behaviour or laminar flow media is described in the literature not by the shear stress but rather via the dynamic viscosity *η*. It represents the molecular transport coefficient of the impulse (Kraume 2004). In the case of non-Newtonian (n-N) liquids, the following connection exists between both parameters:

$$\tau = \eta_{n-N}\frac{dv_x}{dy} \tag{30}$$

The index of the viscosity provides an indication for the fact it itself depends on the velocity gradient. In this case the viscosity is usually referred to as apparent viscosity.

$$\eta_{n-N} = f\left(\frac{dv_x}{dy}\right) \tag{31}$$

In addition to the setup described, with a single annular space between rotor and stator, there are also concentric measuring systems with dual annular gap, so-called ***double gap measuring systems***. In this, the measuring cylinder is a hollow cylinder. The exact

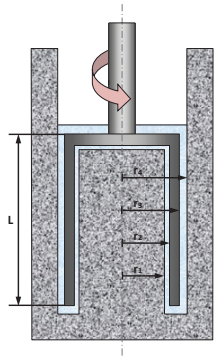


Figure 31: Double slit measuring system (Source: Brehmer 2011b)

characterisation is provided in the DIN 54453 standard. Due to its construction, (c.f. Figure 31) with four wetted surface and a gap width $\delta \leq 1{,}15$ mm, higher shear rates can be generated at lower rotational frequency, and thereby low-viscosity fluids ($\eta < 100$ mPas) can be measured well. Depending on the torque and angular velocity, the shear stress and shear rate can be determined, too, in accordance with "Equations 32 and 34".

$$\tau = \frac{1+\delta^2}{(\delta^2 \cdot r_3^2 + r_2^2)} \cdot \frac{M}{4000 \cdot \pi L C_L} \tag{32}$$

$$\dot{\gamma} = \omega \cdot \frac{1+\delta^2}{\delta^2 - 1} \tag{33}$$

$$\delta = \frac{r_4}{r_3} = \frac{r_2}{r_1} \leq 1.15 \tag{34}$$

However, both of the classic systems introduced are only usable in a very limited range in the case of substrates, such as those found in biogas reactors. The reason is the narrow gap width, as a result of which only the liquid phase of the substrates can be measured. The solids can lead to friction on the cylinder wall which results in a higher torque and thereby in a higher viscosity. As an example, the viscosity graph of the double gap measuring system in Figure 33 may be referenced. They nevertheless provide good results in the case of fermentation substrates such as sewage sludge, fats, and the biogenous residues of wet fermentation (c.f. Figure 32 and Figure 33). That is, substrates with flocs or very low solids contents. Additionally occurring effects such as Taylor vortex(es), sedimentation, or the destruction of flocs and as such the occurrence of thixotropy may additionally influence the measuring result. According to (Mezger 2006), it applies as a rule of thumb that the particle size should not exceed $^1/_{10}$ the gap's dimension.
In addition to the concentric measuring systems, relative measuring systems are available, too, as previously mentioned. In contrast to the former, the latter do not feature clearly defined shear conditions (Mezger 2006). At this point, the ***stirrer*** respectively a vane should be mentioned, here. Similar to the concentric measuring systems, the power input of the agitating mechanism depends, among other things, on the viscosity of the fluid to be stirred. This dependency can be described by the so-called performance characteristic. This is the function connection between the dimensionless power number, also known as Newton number *Ne*, and the Reynolds number *Re*.

$$Ne = \frac{P}{\rho \cdot N^3 \cdot d^5} \tag{35}$$

$$Re = \frac{\rho \cdot N \cdot d^2}{\eta} \tag{36}$$

$$Ne = \frac{C_{lam}}{Re} \tag{37}$$

The power input *P* is the result of

$$P = M \cdot \omega = M \cdot 2\pi N \tag{38}$$

For non-Newtonian fluids, (Metzner & Otto 1957) postulate that the dependency (Equation 37) can also be utilised if the Reynolds number is built with an effective viscosity.

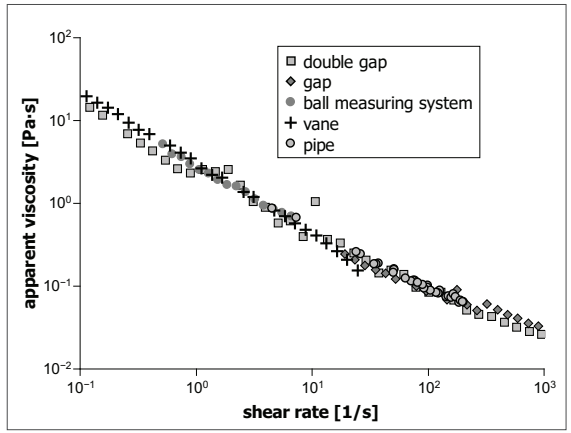


Figure 32: Comparison of different measurement methods using the example of sewage sludge (Source: Brehmer 2011a)

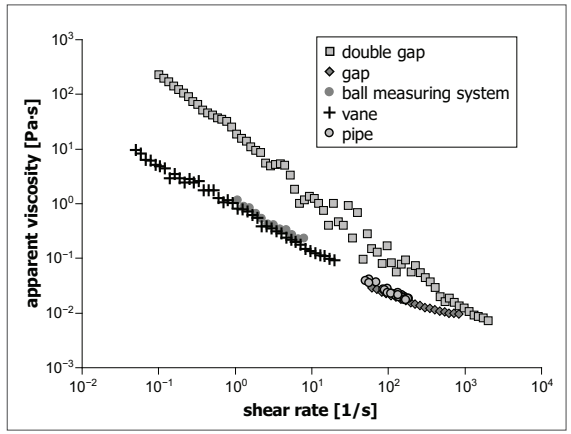


Figure 33: Comparison of different measurement methods using the example of a substrate mixture of sewage sludge, fats, and biogenous residues (Source: Brehmer 2011a)

$$Re = \frac{\rho \cdot N \cdot d^2}{\eta_{eff}} \quad (39)$$

However, in order to be able to calculate the effective viscosity η_{eff} from the Reynolds number, the constant C_{lam} of the stirrer used must be known in dependence on the geometrical conditions. It can be easily determined in advance utilising Newtonian fluids with known viscosity (c.f. Figure 34).

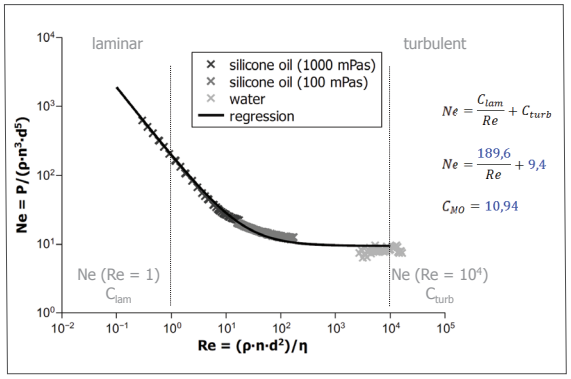


Figure 34: Example for the functional dependency of the power input on the Reynolds number for a vane (Source: Brehmer 2011b)

Finally, with the help of the Metzner-Otto method, (Metzner & Otto 1957), a method for the calculation of the representative shear rate $\dot{\gamma}_{rep}$ is available. According to this, the representative shear rate in the laminar flow region ($Re \leq 10$) is proportional to the stirrer's rotational frequency:

$$\dot{\gamma}_{rep} = C_{MO} \cdot N \quad (40)$$

The proportionality, the Metzner-Otto constant C_{MO}, depends both on the stirrer used and the rheology of the fluid as well as the geometrical conditions, the stirrer diameter and the tank diameter. It can also be determined in advance with a non-Newtonian fluid whose rheology is known or at least easily measurable. A xanthan gum water solution may be referenced here as a potential liquid.
In addition, it must be taken into consideration that the method may lead to imprecise results in the case of substrates with a higher TS content (TS > 6 %). Due to differences in density within the suspension, a separation may occur. The wrapping of long fibre substrate components around the stirrer shaft (c.f. Figure 35) during the measurement also leads to measuring inaccuracies (c.f. Figure 36). However, according to Mezger (2006) a calculation of viscosity and shear rate should be omitted completely and only the torque and the rotational frequency should be specified.

The ***ball measuring system*** was originally developed for the measurement of dispersions with particle sizes of up to 5 mm and is – just as the vane – one of the relative measuring systems (Mezger 2006). In this measuring system, a ball of a precisely defined diameter is moved on a circular path with a defined track through a measuring cup. Since the sphere during the first round only encounters non-sheared sample material, it must be ensured that only one round is carried out. A heating of the substrate analogous to the vane is only possible to a limited extent due to the required sample volume. Due to the torque and the rotational frequency, conclusions regarding the shear stress and the shear rate can be drawn analogous to the other rotation measurement methods and modelled after the Metzner-Otto method.

Figure 35: Wrapping of the vane shaft with long fibre substrate components (Source: Brehmer)

$$\tau = C_{SS} \cdot M \quad (41)$$

$$\dot{\gamma}_{rep} = C_{SR} \cdot N \quad (42)$$

The results shown in Figure 32, Figure 33 and Figure 36 lead to the conclusion that the system is of limied suitability regardingthe rheological characterization of fermentation substrates. A particular disadvantage is the adhesion of soild particulates to the surface

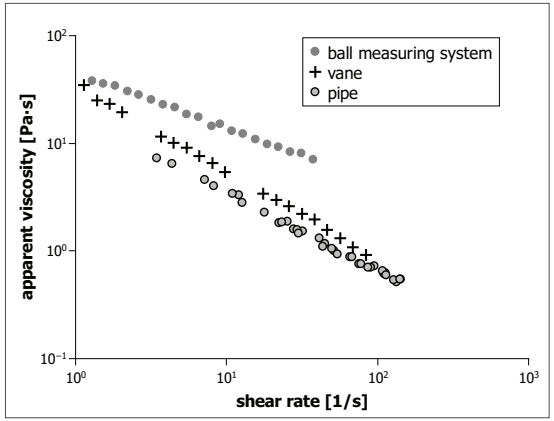


Figure 36: Comparison of different measurement methods using the example of a substrate mixture of maize silage and coarse rye meal (Source: Brehmer 2011a)

Figure 37: Adherence of solids to the surface of the ball (Source: Brehmer)

area of a ball (c.f. Figure 37). This leads to an increase of the surface area of the ball and therefore to an increased torque.

For the exact rheological characterisation of substrates with higher TS contents, another method is available. Through ***pressure loss measurements*** in dependence on the volume flow, the viscosity and the shear rate can also be determined in a horizontal pipe. This does, however, require that the no-slip condition are applicable and that the flow characteristics of the fluid do not display any time dependence. Furthermore, it must be an established, laminar flow with parallel flow lines and constant velocity (Wilkinson 1960), (Malkin & Isayev 2005). The latter can easily be achieved by adherence to the upstream length z_{up}. It is calculated based on the following relationship:

$$\frac{z_{up}}{d} \approx 0.058 \cdot Re \tag{43}$$

For the generation of the volume flow, pumps (Türk 1987) or compressed air (Brehmer 2011a) can be utilised. However, as an example, only the variant utilising compressed air (c.f. Figure 38) will be covered in more detail, here. In this, the fermentation substrate is placed in a tank. Through the generation of a excess pressure in the inside of the tank, different volume flows can be realised. In contrast to the pump, a pulsation is excluded this way. In order to compensate for the drop of the hydrostatic pressure during the measurement, the pressure must be regulated in dependence on the volume flow and the tank capacity.

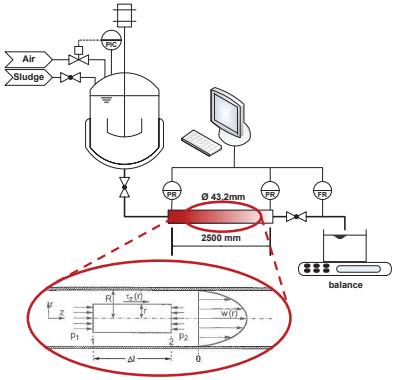


Figure 38: Flow diagram for the laminar flow tube (Source: Brehmer 2011a; Kraume 2004).

Based on the assumptions listed above, the shear stress can be determined via a force balance (Equation 44), and the shear rate can be determined utilising the Metzner-Reed equation (Equation 45).

$$\tau_W = \frac{d \cdot \Delta p}{4 \cdot L} = k' \cdot \left(\frac{8 \cdot v_{avg}}{d}\right)^{n'} \tag{44}$$

$$\left(\frac{dv}{dR}\right)_W = \frac{3n' + 1}{4n'} \cdot \frac{8 \cdot v_{avg}}{d} \tag{45}$$

$$n' = \frac{d \ln\left(\frac{d \cdot \Delta p}{4 \cdot L}\right)}{d \ln\left(\frac{8 \cdot v_{avg}}{d}\right)} \tag{46}$$

The Term $\frac{8 \cdot v_{avg}}{d}$, where v_{avg} represents the average velocity of the fluid in the tube, has the same unit as the shear rate at the wall. For this reason, this term is referred to as apparent shear rate. It should be noted that "Equation 41" is only apparently similar to the Ostwald–de Waele relationship (c.f. Equation 49). In general, *n'* is no constant, but rather dependent on $\frac{8 \cdot v_{avg}}{d}$. For the laminar and time-independent flow of a fluid, the exponent *n'* is defined as in Equation 46. According to (Wilkinson 1960), the exponent *n'* and the factor *k'* for shear thinning fluids can be converted as follows into the flow exponent *n* and the Ostwald factor *K*:

$$k' = K \cdot \left(\frac{3n + 1}{4n}\right)^n \tag{47}$$

$$n = \frac{n'}{1 - \frac{1}{3n'+1}\left(\frac{dn'}{d\ln\tau}\right)} \tag{48}$$

The biggest advantage of the tube viscometer it the possibility, in the case of a sufficient sizing of the system, to be able to still measure substrates with a high solid content and long, fibrous particles accurately. Only the increased time investment and the larger amount of substrate needed are contrasted to this.

In order to be able to compare the measuring points obtained – consisting of shear stress, shear rate, temperature, and measuring duration – with one another and mathematically describe them, different model functions were developed whose number by now, however, exceeds 20. Chen suggests in his work dated 1986 to describe the flow behaviour of fermentation substrates with a dry matter content of less than 4.5 % with the Power-law by Ostwald-de Waele. An approach which is by far used most often for the description.

$$\tau = K \cdot \dot{\gamma}^n \tag{49}$$

For substrates whose dry matter content is above 4.5 %, the approach of Herschel-Bulkley should be applied.

$$\tau = \eta_0 \cdot \dot{\gamma} + k'' \cdot \dot{\gamma}^{n''} \quad (50)$$

Comparison measurements with all five measuring system presented have show that there is no measuring systems that is equally well suited for the rheological characterisation of all substrates. Rather, the selection should be carried out in dependence on the TS content and the particle structure (Brehmer 2011b). Table 19 contains the corresponding information.

Table 19: Suitability of the measuring system for the rheological characterisation in dependence on the particle structure (Brehmer 2011b)

Particle structure	Pipe	Vane	Ball measuring system	Gap	Double gap
None	○	○	–	+	++
Flocs	○	○	–	++	+
Solid particles	+	+	○	○	– –
Fibres (TS < 8 %)	++	○	–	– –	– –

–– not suitable
– less suitable
○ suitable
+ well suitable
++ very well suitable

5.5.2 Modification of measuring systems for the application to flow behaviour determination of fibrous suspensions

Karin Jobst, Marc Lincke, Fraunhofer IKTS

A number of measurement methods and corresponding devices are available for the determination of the viscosity of suspensions. Typically, these methods are based on an assessment of the laminar velocity profile in a gap of known width. The gap width in turn depends on the measuring bob and measuring cup utilised and it determines the maximum particle size of the suspension to be assessed. With the rotational viscometer ViscoTester VT550, for instance, suspensions with maximum particle sizes between 0.1 mm (System NV) and 1.7 mm (System MV3) can be measured, depending on the cylinder measuring system selected. The tested biogenous suspensions typically contain particles with a fibre length of up to 50 mm and larger, which is why these cylinder measuring systems cannot be used. Due to the large number of particles within the gap, solid friction occurs, which distorts the measuring result. Additional measuring errors arise due to phase separations as a result of sedimentation in the cylindrical measuring cup. A separation of the larger particles in preparation of the measurement is not recommended, since these coarse particles have a considerable influence on the flow behaviour of the suspension. Due to these problems concerning the assessment of the flow characteristics of biogenous suspensions, intensive investigations regarding suitable measurement methods for fibrous fluid systems of substances with a maximum fibre length of 20 mm and/or 50 mm have been carried out at Fraunhofer IKTS as part of a collaborative project funded by the Free State of Saxony and the European Union. For the extension of the known standard measurement methods, two different measurement methods for long-fibre material systems have been developed, which are currently undergoing validation (c.f. Figure 39). These two measuring approaches are introduced below.

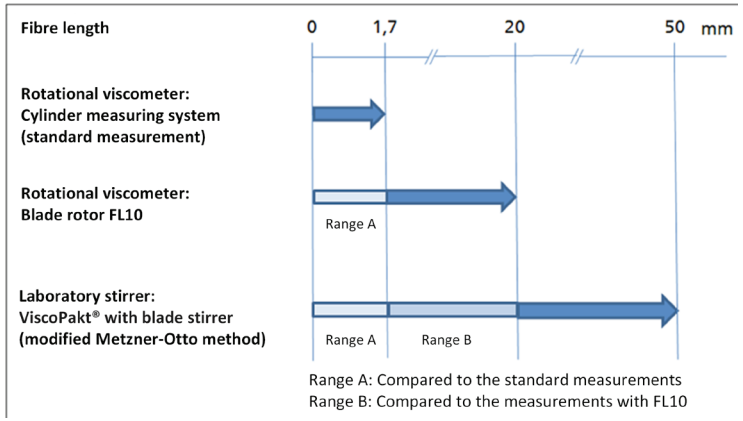


Figure 39: Measurement method(s) for the determination of the viscosity in dependence on the fibre length (Source: IKTS)

Viscosity determination of fluid systems with fibre lengths of with x_{max} < 20 mm

A standard rotational viscometer with a 6-bladed stirrer (blade rotor FL10) is qualified for the assessment of the flow behaviour of fibrous suspensions with a maximum fibre length of 20 mm. For the adaptation of this measuring system to fibrous suspensions, a measuring guideline was developed at Fraunhofer IKTS.
The ViscoTester VT550 device used for this investigations is a Searle-type rotational viscometer in which the flow resistance of the test substance is measured against a specified rotational frequency. The torque required for maintaining the specified rotational frequency is measured via a stiff torsion-spring with a contactless sensor. The VT550 operates in a rotational-speed range of 0.5 to 800 min^{-1}, the range of the torque is between 0.01 to 3 Ncm. The internal control unit of the measuring device stores all data and calculates the values for the shear stress τ, the shear rate $\dot{\gamma}$ and the dynamic viscosity η with the help of the values of the measured torque M, the rotational frequency N as well

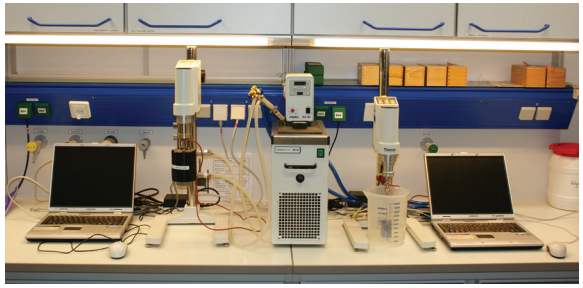


Figure 40: ViscoTester 550 with cylinder measuring device (left) and with blade stirrer FL10 (right) for the measurement of fermentation substrates (Source: IKTS)

as specific system factors (geometry) of the measuring system used. Reference to the apparent viscosity η_S is only made when the viscosity – in the case of the assessment of fermentation substrates – itself is dependent on the velocity profile and therefore does not represent a constant parameter.
The control of the device is exercised via an RS232 interface with the application software RheoWin. The determined data is available as ASCII files for further analysis and post-processing. To derive comparable results between the system selected here and known cylinder measuring systems, defined geometrical conditions regarding the measurement were introduced. In addition to the blade rotor FL10 (D = 20 mm) serving as rotor, a measuring cup with the dimensions of D = 133 mm and H = 177 mm is utilised as stator. Furthermore, the installation conditions of the blade rotor were defined and must be used for all measurements.
For the verification of the suitability of the system VT550-FL10, comparative measurements were carried out with a standardised cylinder measuring device (measuring system MV3) using suspensions with maximum particle sizes of 1.5 mm, whereupon the calculation of the flow curve and/or the viscosity is carried out in accordance with the calculation guidelines of the manufacturer of the measuring device, respectively. The results indicated a systematic error in the case of the measuring setup VT550-FL10. For this reason, another system factor F, which incorporates the geometrical conditions of the measuring setup, was determined for this system with the help of extensive comprehensive tests. This factor was implemented into the analysis, in addition to the system factor specified by the manufacturer, by subsequently converting the shear rates $\dot{\gamma}^*$ determined in accordance with the manufacturer's instruction in accordance with Equation 51 and utilising it for the identification of the apparent viscosity $\eta_S = f(\dot{\gamma})$

$$\dot{\gamma} = \mathrm{F} \cdot \dot{\gamma}^* \tag{51}$$

Sampling and sample preparation
For the viscosity measurement, a sample amount of 10 L is recommended, whereof 1.6 L are required per individual measurement. The sampling is carried out in accordance with VDI Guideline 4630.
Prior to the retrieval of the sample for the measurement, the contents of the transport vessel must be homogenized in order to dissolve floating layers and settling layers.

Materials and devices
- Measuring cup
- Water bath with thermostat
- Heating sleeve for breakers
- Thermometer
- Blade stirrer FL10
- ViscoTester VT550

Execution method
Prior to the measurements, the samples to be inspected are heated to a defined temperature (e.g. process temperature) in a water bath. To reduce evaporation losses of the sample, the containers are covered. Subsequently, 1,600 mL of the heated sample are placed in the measuring cup. A constant temperature must be ensured during the measurement (utilisation of a thermostat and heating sleeves, if necessary). The temperature of the sample is measured before and after the measurement. In order to eliminate a significant temperature impact on the measuring result, the tolerated temperature change should not exceed 2 K (empirical value).
Once the measuring cup and the rotor have been positioned, the shearing range to be assessed and the number of measuring points to be selected are specified. The selection of at least one measuring point per second has proven to be useful. With the help of the rotational frequency, which is thereupon automatically applied by the measuring system, the torque recorded and the system factors stored in the system, the shear stress, the shear rate and the apparent viscosity can be determined. All measurements should be repeated at least three times (triple determination). In Figure 41, the graph of the apparent viscosity is depicted for one fermentation substrate.

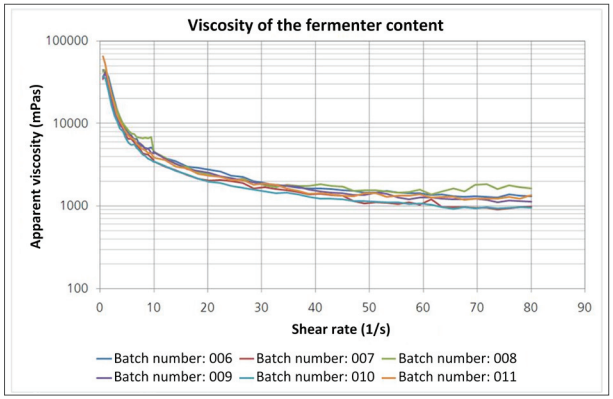


Figure 41: Viscosity curve of the digester content (Source: IKTS)

Since this measuring setup is a stirring system, a vortex formation may occur, depending on the sample's properties. Especially material systems with a low viscosity ($K_{1/s}$ < 2000 mPa·s^m) tend to form severe turbulences in the measuring cup which generate an increase in torque, thereby pretending an increase in viscosity. In the case of a vortex formation in the measuring cup, the measured values are no longer usable. In the case of very fibrous substrates (x_{max} > 20 mm), wrapping around the stirrer and the stirrer shaft may occur, whereby the stirrer geometry is changed and therefore the system framework conditions specified for the assessment are no longer applicable. In Figure 42, both potential error sources are depicted.

Figure 42: Faulty measurements due to vortex formation and wrapping around a stirrer shaft (Source: IKTS)

Need for research

To verify the additionally introduced system factor, further comparative tests have to be carried out between the VT550-FL10 system and standardised measurements by means of a rotational viscometer, taking into consideration applicable conditions of utilisation (c.f. Figure 39). Furthermore, a comparison of the results to measurements of tube viscometers is useful.

Viscosity determination of fibrous material systems with x_{max} < 20 mm

Suspensions with fibre lengths larger than 20 mm cannot be tested with known standard measuring systems. The geometry of the measuring device must be adjusted to the proportions of the particles contained in the suspension. For suspensions with a maximum particle size of up to 50 mm, laboratory stirrers with a torque measurement and recording are suitable. In the view of this background, a method for viscosity determination based on the METZNER-OTTO method was developed at Fraunhofer IKTS as part of a group project funded by the European Union and the Free State of Saxony.

Modified Metzner-Otto method for the determination of the viscosity

The foundation for this modified method is the relationship between the stirrer's rotational frequency *N* and the shear rate $\dot{\gamma}$. Through the utilisation of a suitable stirrer system, the corresponding Newton numbers Ne and Reynolds numbers Re are determined for defined stirrer tip-speeds *u* and average apparent viscosities η_S. Foundation for this are the performance characteristics of the stirrer system to be determined with a Newtonian fluid. By recording the power that a stirrer needs for stirring the medium to be characterised, the Newton number corresponding to the stirring process can be derived and therefrom the effective Reynolds number with the average apparent viscosity η_S (c.f. Figure 43). For the conversion of the relationship of $\eta_S = f(N)$ into the known dependency of the apparent

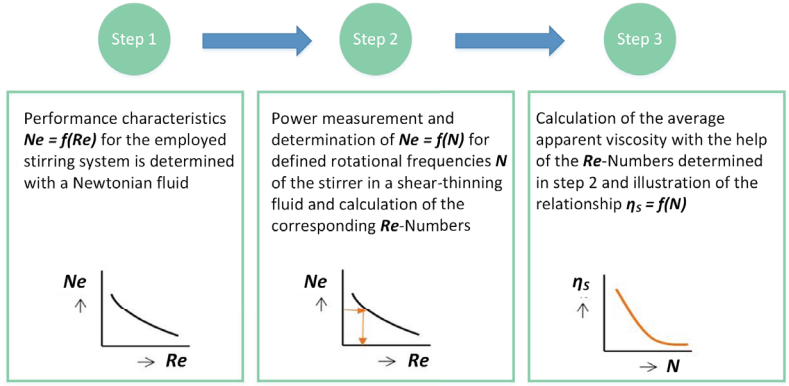


Figure 43: Approach for the determination of the viscosity according to Metzner & Otto

viscosity on the shear rate $\eta_S = f(\dot{\gamma})$, Metzner & Otto utilised proportionality constants, the so-called Metzner-Otto constants k_{MO}, that are dependent on the stirrer used. Newer tests (Pawlowski 2004) discussed this concept rather critically, since the Metzner-Otto constant built as proportionality factor between the stirrer rotational frequency and the shear rate is not only dependent on the stirrer geometry, but also on the rheological behaviour of the fluid.

With the help of systematic tests at Fraunhofer IKTS, the additional influence of the rheological behaviour and therefrom a possibility for the conversion of the relationship $\eta_S = f(N)$ into the form $\eta_S = f(\dot{\gamma})$ was determined.

Measuring system ViscoPakt® laboratory stirrer

For the specific tests, a ViscoPakt®-laboratory-stirrer is used, which is equipped with a simple torque measurement. Changes of the rotational frequency due to load variations are compensated electronically. The stirrer drive is equipped with interfaces for the continuous

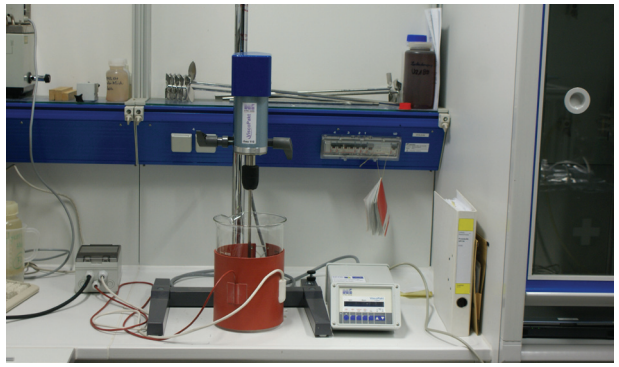


Figure 44: Test bench for the determination of the apparent viscosity in highly concentrated, fibrous suspensions (Source: IKTS)

recording of the rotational frequency and the torque. A maximum torque of 110 Ncm can be measured at a resolution of 0.2 Ncm.
The stirrer system consists of a blade stirrer with a diameter of 70 mm and a SCHOTT laboratory glass 5,000 mL as stirred reactor. The aspect ratio utilised between fill level and reactor diameter is 1.0. The placement of the stirrer was carried out centric and the distance between the stirrer and the bottom of the reactor was held constantly at 28 mm.
For the stirrer system described, the performance characteristics *Ne = f(Re)* depicted in Figure 45 were determined, which constitute the foundation of the viscosity determination via the stirrer torque.
For different rotational frequencies *N* in the range of 30 to 700 min^{-1}, the torque *M* was measured and therefrom the input stirrer power *P* as well as the *Ne* number is determined in accordance with the following equations:

$$P = 2\pi \cdot M \cdot N \tag{52}$$

$$Ne = \frac{P}{\rho \cdot N^3 \cdot d^5} \tag{53}$$

The stirrer diameter is referred to as d and the density of the material system to be assessed as *ρ*.
Utilising the performance characteristics, the apparent viscosity can be determined for highly concentrated suspensions with fibre lengths of up to 50 mm in dependence on the stirrer rotational frequency of the blade stirrer employed, in accordance with steps 2 and 3 depicted in Figure 43.

$$Ne(N) \Longrightarrow Re(N) \Longrightarrow \eta_S(N) = \frac{\rho \cdot N \cdot d^2}{Re} \tag{54}$$

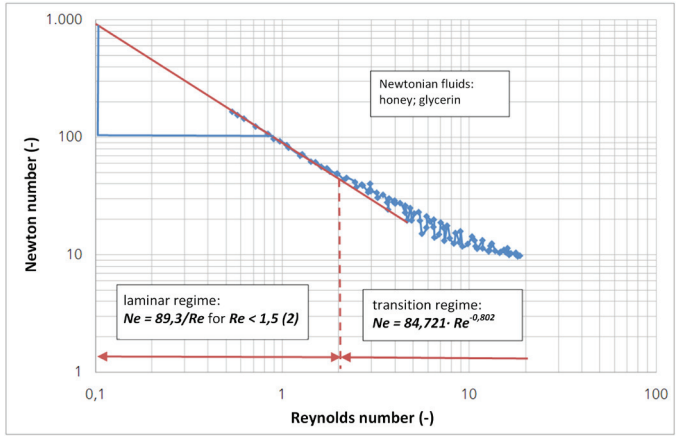


Figure 45: Performance characteristics of the stirrer system utilised for the viscosity measurement (Source: IKTS)

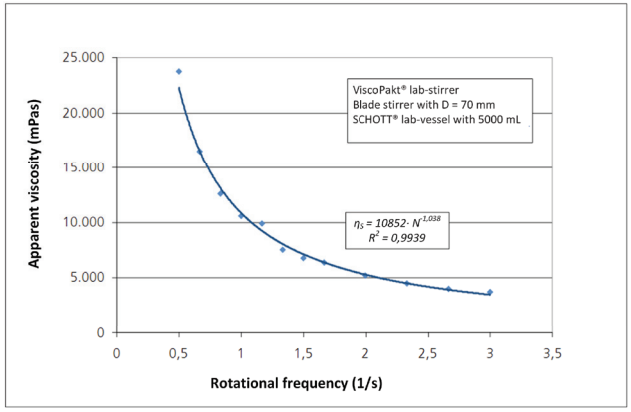


Figure 46: Apparent viscosity in dependence on the rotational frequency for the hydrolysate of a biogas plant (Source: IKTS)

Through the utilisation of the functional relationships mentioned in Figure 45 and Equation 54, the functional relationship between the apparent viscosity and stirrer rotational frequency $\eta_S = f(N)$ was determined for the material system tested. The hydrolysate of a biogas plant can be described with the functional relationship shown in Figure 46, for instance.

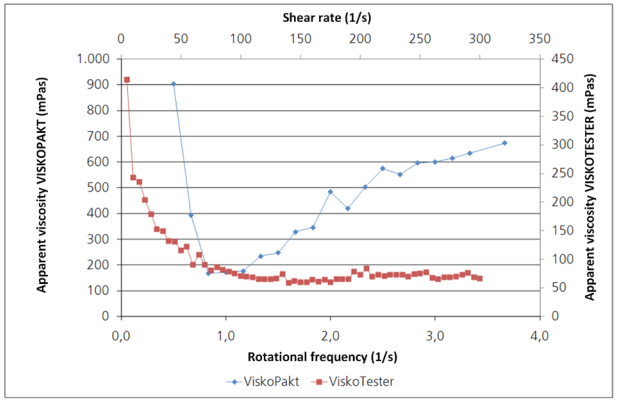


Figure 47: Faulty assessment of the viscosity for low-viscous material systems in the case of utilisation of the ViscoPakt®- laboratory stirrer (Source: IKTS)

In addition to the maximum particle size of approx. 50 mm, the degree of the viscosity must be specified as additional limitation of use for the measuring system depicted. In the case of substances with η_S < 1,000 mPas, the formation of turbulences may already

occur at low stirrer rotational frequencies, which would falsely mimic an increase in viscosity (Figure 47). To determine a potentially existing relationship between $\eta_S = f(N)$ and $\eta_S = f(\dot{\gamma})$, measurements with a rotational viscometer ViscoTester VT550 were carried out in addition to the tests performed by means of the ViscoPakt®- laboratory stirrer. Prerequisite for these comparative measurements was, that no exceeding of the limitations of use existed regarding the particle size and solids concentration of the examined substances. Considering the established statements proposed in literature regarding the fact that the relationship between the stirrer rotational frequency and the shear rate is, in addition to the stirrer geometry, dependent on the rheological properties of the employed substances, great attention was paid to the selection of the material systems. According to the approach of Ostwald-de-Waele, the employed material systems feature significant differences in respect of their flow exponent *n*. So far, the flow exponent has been varied between –0.2 and 0.7.

$$\eta_S = K \cdot \dot{\gamma}^{n-1} \tag{55}$$

A comparison of the flow curves $\eta_S = f(\dot{\gamma})$ determined by the ViskoTester 550 with the measuring results of the ViscoPakt® laboratory stirrer $\eta_S = f(\dot{\gamma})$ showed that both measuring systems describe the viscosity of each of the tested material systems with comparable flow curves. The relationship determined by the ViscoPakt® laboratory stirrer can be described with

$$\eta_S = K^* \cdot N^{n-1} \tag{56},$$

whereby a simple equating of the flow functions $\eta_S = f(\dot{\gamma}) = f(N)$ was made possible. A rearrangement of the equations for $\dot{\gamma}$ provides the factor K_{VP} for each tested material system. With the factor K_{VP}, the employed rotational frequencies of the stirrer can be converted into the corresponding shear rates.

$$\dot{\gamma} = K_{VP} \cdot N \tag{57}$$

Since the geometrical dimensions of the stirrer system remained constant for all tests, it was possible to trace back K_{VP} to the sole dependence on the flow properties in the form of the flow exponent *n*, which therefore can be calculated via the empirically determined relationship.

$$K_{VP} = 8.52 \cdot n + 6.8 \tag{58}$$

The flow exponent *n* is identified via the exponents of the flow function (Equation 55) determined by measurements with the ViscoPakt® laboratory stirrer.

Sampling and sample preparation

A sample amount of 30 L is recommended for the viscosity measurement, wherein 3.5 L are required for each individual measurement (repetition measurements are reconsidered). The sampling is carried out according to VDI Guideline 4630. A special sample preparation for the measurements is not required.

Figure 48: Wrapping around blade stirrer and vortex formation (Source: IKTS)

Materials and devices

- Measuring cup
- Blade stirrer
- Stirrer with torque measurement (ViscoPakt 110)
- Heating sleeve
- Thermostat and thermometer
- Scale and measuring cylinder (determination of density)

Execution method

The samples are heated to the desired temperature in a water bath in a closed container. Once the sample has reached the desired temperature, 3,500 mL are filled into the measuring cup. This measuring cup is equipped with a heating sleeve in order to minimise the heat loss during the measurement. Subsequent to the positioning of the measuring cup and the blade stirrer, the rotational frequency is increased in defined steps (e.g. ΔN = 10 min^{-1}) and the related torque is recorded. With the help of the rotational frequency and the measured torque, the apparent viscosity can be calculated in accordance with the method described above. The temperature of the sample is checked before and after the measurement and should be deviating from the initial value by less than 2 K (empirical value). Depending on the viscosity, the formation of vortices may occur during the measurement, which leads to a disproportionate increase of the torque due to the turbulent flow regime. Analogous to the measurements with the blade rotor (c.f. section "Viscosity determination of fibrous material systems with x_{max} < 20 mm"), this increase leads to an increase of the apparent viscosity at higher rotational frequencies/shear rates in the further calculation. As soon as a macroscopic vortex forms, the measured values are no longer analysable. In the case of fibre lengths larger than 50 mm, wrapping around the stirrer blades and the stirrer shaft may occur, whereby the measurement of the torque is falsified. These measurements must be discarded.

Need for research

- Development of stirrer geometries for long-fibre substrates.
- Investigation of the impact of vortex formation on the measured torque as well as the mathematical correction for the determination of the apparent viscosity.
- Transfer of the approach to industrial scale stirrers and pumps as an additional control parameter.

5.6 Flow analysis

From the point of view of a best possible decomposition of the raw materials used, the efficient mixing of biogas reactor constitutes an important focus. An essential prerequisite in this is to ensure a mixing of the reactor volume that is a complete and as homogeneous as possible. This process engineering state cannot be realised in practical operation despite very high energetic expenditures. The mixing processes require up to 55 % of the electricity generated in-house by the biogas plant. The recording of the mixing state in bioreactors via measuring equipment is therefore even more important.
Flow analyses and/or velocity measurements can be carried out both on industrial-scale biogas reactors as well as on systems at a pilot plant scale. For this, different measuring techniques are available which will be covered in more detail in the following two sections. The deciding factors for the selection of the measuring technique are the measuring range and the accessibility of the systems.
Known commercial measuring systems such as sensors or optical measurement methods allow for both exactly localised as well as global considerations of the mixing state. In this, it must be differentiated between the measuring technique for utilisation at pilot plant scale and that at industrial scale reactors. For the application case mentioned first, measuring systems have to be relied on that can still detect velocitys in the mm/s range. Here, constant temperature anemometry and particle image velocimetry, among other, have proven to be suitable. Both measurement methods require the use of a transparent model medium. The selection of the velocity measuring technique for industrial scale biogas reactors has proven disproportionately more difficult. Due to the high solids content, the use of permanently installed measuring technology is not possible at present. However, with MID measuring sensors or vanes, devices are available for mobile, short-term use.
For process-engineering application, tomographic measurement methods have already been used for quite some time for the measurement of temperature, concentration, and velocity fields. In the area of biogas engineering, i.e. for the investigation and assessment of mixing processes in biogas reactors, the use of this measuring technique is new. In addition to the location-resolved view of the mixing states, the advantages of this method are both a utilisation of model fluids as well as of the opaque fibrous original substrates occurring in the biogas sector.

5.6.1 Utilisation of measuring sensors

Manuel Brehmer, Matthias Kraume, TU Berlin

Flow analyses and/or velocity measurements can be carried out both on industrial-scale biogas reactors as well as on systems at a pilot plant scale For this, different measuring techniques are available which will be covered in more detail in the following two sections. The deciding factors for the selection of the measuring technique are the measuring range and the accessibility of the system.

Measurements at industrial scale systems

For the measurements at industrial scale systems, a vane anemometer developed by Fraunhofer Umsicht (Deerberg et al. 2009) can be relied on. This anemometer consists of a lance on which a vane is mounted at its end (c.f. Figure 49). The setup allows for the measurement also in coarse particular media as they are found in biogas reactors.

Figure 49: Sensor head of the vane anemometer for the measurement of the velocity in industrial scale biogas plants; built by Fraunhofer UMSICHT (Source: TU Berlin)

Figure 50: Example of a lock chamber for rinsing and sealing of the sensor (Source: TU Berlin)

Utilised for this is the magnetic induction. With each passing of the vanes through the magnetic field, a signal is generated. From the frequency of the signalling, the velocity of the fermentation substrate can be determined subsequently. According to the manufacturer, velocity as low as 1.2 mm/s can be detected this way. Prerequisite for the utilisation of this measuring technique is, however, a connecting branches and an adequate sealing of the sensor against exiting of the substrate and/or biogas. Since the sealing towards the liquid phase can be realised significantly easier, it is therefore recommended to utilise an connecting branches below the liquid level. Concomitantly, the risk of a methane emission is also reduced significantly this way. In order to remove particles that get trapped between the vanes and the wall of the sensor, the sensor can be flushed through a nozzle located behind the vane. So that the sensor does not have to be completely removed for each flushing process, the use of a lock chamber is recommended. This way, the necessary sealing can also be achieved concomitantly. In Figure 50, an example of such a lock chamber is depicted. It consists of two pinch valves, a transparent PVC pipe equipped with inlet and outlet, and three round flanges. The latter ensure a centring of the sensor. By introducing an O-ring with the diameter of the sensor, and additional sealing is effected. The pinch valves are controlled by a proportional valve. Due to the transparent PVC pipe, the degree of soiling and the functionality of the vane can be checked and remedied without completely removing it.

Flow analyses in a pilot scale

For the flow analyses in the pilot plant scale, optical methods of measurement and thermoelectric anemometry are available, among others. With both methods it is possible to exactly determine even low velocities such as they occur in biogas reactors. Commercially available vanes are error-prone due to the shear thinning flow properties of the fluids. General information regarding flow measurement methods can be found in the publications of (Nitsche & Brunn 2006), (Eckelmann 1997), (Herwig 2006) and (Debatin 1997). For more in-depth literature, in particular in the area of thermal anemometry, please see the

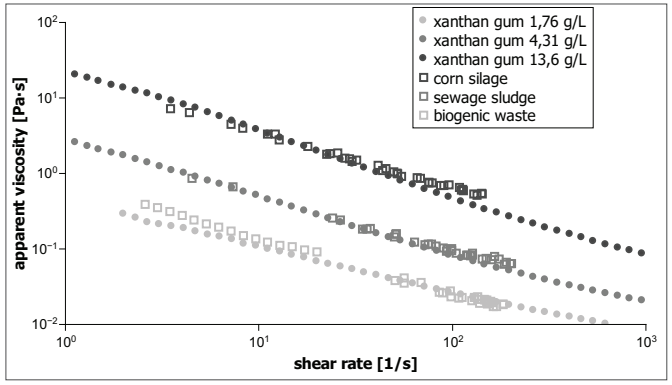


Figure 51: Comparison of the flow characteristics of substrates and substitute media (Source: Brehmer 2011c)

works of (Lomas 1986) and (Tsi 2008). The literature sources mentioned above also serve as foundation for the sections below.

In addition to the previously mentioned decisive capability to be able to exactly determine slow velocities, both the optical measurement methods as well as the thermoelectric anemometry, however, also feature two disadvantages: They are sensitive to particles and especially in the case of the optical velocity measurements, a transparent medium must be utilised. However, since the fermentation substrates of biogas reaction are neither transparent nor particle-free, substitute with identical flow characteristics (shear thinning flow behaviour) must be utilised. One option is the addition of additives to the water. Xanthan gum may be referenced here as an example. Xanthan comes from the foodstuffs industry and is available on the market, among other ways, as a transparent additive (e.g. from the COLLTEC GmbH & CO.KG company) and can be mixed with water at different concentration ratios. As such, it allows for the setting of different viscosities and to address the differing conditions at the biogas plants (c.f. Figure 51). Another option is the use of substances from the cosmetics industry. These are non-perishable and therefore easier to handle. An example to be mentioned here is Merat 550, a basic component of shampoos.

Optical measurement methods

The optical measurement methods use laser light, wherein a differentiation is made between laser-2-focus anemometry (in short: L2F), laser Doppler anemometry (in short: LDA) and particle image velocimetry (in short: PIV). The great advantage, in contrast to thermoelectric anemometry, is that the capturing of measured data does not influence the flow. The disadvantage, however, is the high price of considerably more than 50,000 Euro and the large amount of equipment needed.

In all optical measurement methods, seeding particles, are utilised. They must fulfil two complementary conditions:

- a slip-free movement with the flow,
- a good dispersion of the laser light.

Furthermore, the volume to be measured must be optically accessible. Here, problems arise in particular in the case of round tanks since they cause an additional dispersion of

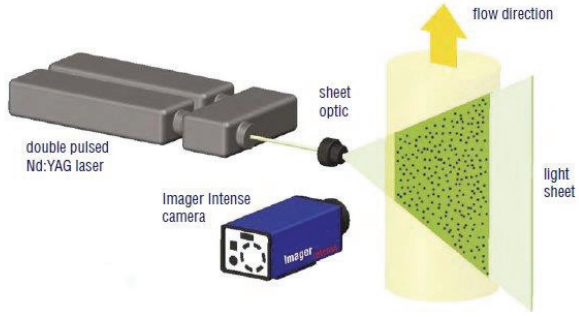


Figure 52: System components of Particle Image Velocimetry (Source: lavision.de 2011)

the laser light. However, this dispersion can be compensated for by the analysis software to a certain degree. Alternatively, the cylinder can be placed in a cubic container and the space in between filled with water or glycerin. The PIV method will be covered in more detail as an example of the optical measurement methods. It is characterised, first and foremost, by the possibility to measure whole velocity fields in a single sectional plane. Via a laser, light of a high intensity is projected onto the sectional plane. It is scattered orthogonally by the particles (c.f. Figure 52). Through the correlation of two captured images, a velocity field can be generated at the computer due to the distance passed by the particles. In the correlation, the captured images are split into analysis fields. The size of the analysis field therefore determines the resolution of the velocity field. It must, however, not be selected too small since the fields are analysed via statistical methods. If too few seeding particles are in a field, no average shift of the particles can be determined in this field. In reverse to L2F anemometry, a time is specified and the distance is measured. To capture the images, digital cross-correlation cameras (CCD) are often utilised. Nd:YAG double pulse lasers are often utilised. The temporal resolution of the PIV depends on the CCD utilised which, nowadays, can capture up to 7,000 images per second.

Thermoelectric anemometry

Thermoelectric anemometry detects the flow velocity via the proportional relationship to convective heat transfer due to a temperature gradient between the measuring sensor and the flow. In this, a differentiation is made between constant current anemometry (CCA), pulse wire anemometry and constant temperature anemometry (CTA). A big advantage of this measurement method is the high resolution of the measured data, which – at up to 400 kHz – also allows for the utilisation for the measurement of turbulences. It is also a very inexpensive instrument, given its accuracy. In comparison to the PIV, the price is only approx. 10,000 Euro. The signal process is in all cases carried out be a Wheatstone bridge (c.f. Figure 53). With the hot-wire sensor and/or hot-film sensor, three different parameters can be determined:

Velocity

$$U \sim v^n \quad \text{for} \quad n < 1;\ p,\ \Delta T = \text{const.} \tag{59}$$

Mass flow

$$U \sim (\rho v)^n \quad \text{for} \quad n < 1;\ p,\ \Delta T = \text{const.} \tag{60}$$

Temperature difference

$$U \sim T \quad \text{for} \quad \rho v = \text{const.} \tag{61}$$

The presently most common method of thermoelectric anemometry is that of the constant temperature anemometer. In the case of a change of the flow velocity, the keeping constant of the sensor resistance leads to a changed voltage which can be directly measured and transformed. The constant temperature and the constant sensor resistance are automatically adjusted via a control circuit.

The exact relationship between velocity and heater voltage then results via

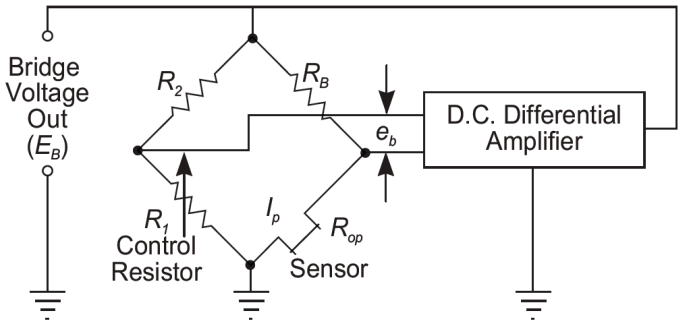


Figure 53: CTA Wheatstone bridge (Source: TSI 2008)

$$U^2 = C_1 + C_2 \cdot v^n \tag{62}$$

The constants C_1 and C_2 must be determined empirically in advance. They depend on the media, the sensor temperature (T_S) and the fluid temperature (T_f), as well as the operating/sensor resistance (R_s), the projected area (A_S) and the diameter of the sensor (d_s).

$$C_1 = f(T_S, T_f, R_S, A_S) \tag{64}$$

$$C_2 = f(T_S, T_f, R_S, A_S, v, d_s) \tag{65}$$

Figure 54: Towing-channel for the calibration and determination of the constants of a film sensor

The exponent *n* depends on the flow state and-or on the Reynolds number. The heater voltage (*U*) of the sensor is directly linked to the bridge voltage (U_B) of the Wheatstone bridge (c.f. Figure 53) that can be measured.

$$U = \frac{U_B \cdot R_{CR}}{R_{CR} + R_2} \qquad (65)$$

For the calibration of each sensor and/or for the determination of the constants, towing-channel can be utilised. One such towing-channel is depicted in Figure 54. In this, during

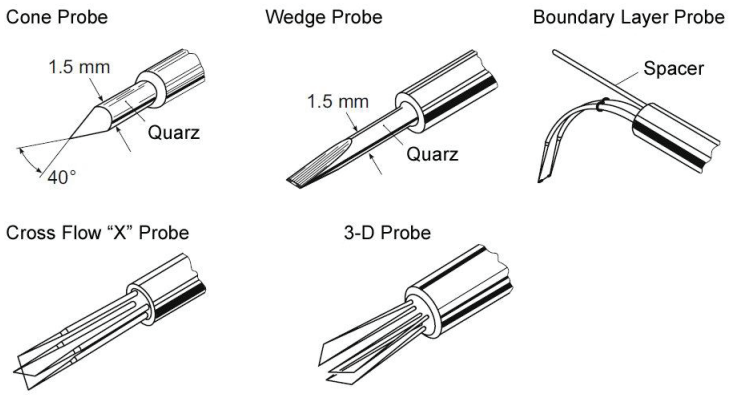


Figure 55: Different sensor variants (Source: Nitsche & Brunn 2006)

the measurement the sensor is pulled through a tube at an exactly controllable velocity. Through multiple repetitions at different velocities, this way the calibration curve for the calculation of the velocity in dependence on the heater voltage (Figure 62) can be developed. The realisation of the exact velocities can, for example, be carried out via utilisation of linear units in combination with DC servo motor and an integrated motion controller. Depending on the medium and area of utilisation, different styles of the sensors were developed (c.f. Figure 55). The differ with respect to their shape, dimensionality and temperature resistance and/or robustness. For liquid media, so-called film sensors are utilised which, in contrast to the hot wire, do not consist of a pure platinum or wolfram wire but rather feature a platinum-coated quartz cylinder. This quartz core makes the wire more robust against mechanical stresses.

5.6.2 Electrical Resistance Tomography (ERT)

Annett Lomtscher, Fraunhofer IKTS

Status	Development completed, ready to be used.
Type of substrate	Model substrates, biogenous substrates of any composition
Limitations of the method	For the operating principle of this measurement method, the substrates to be mixed must feature different conductivities
Advantages	Online measurement; no tracer(s) required; localized consideration of the mixing states; no spot measurement, but rather inclusion of the whole flow volume; taking into consideration of the real circumstances (granulometry, rheology); detection of dead zones, floating layers and settlings as well as badly mixed areas in the reactor
Need for research	Transfer of scale

The experimental evaluation of mixing processes in stirred systems becomes a challenging task, especially when opaque, fibrous and highly concentrated substrates (like digestion residues in biogas plants) are used. The analysis of mixing processes is locally limited by using measurement systems, like sensors, which are currently available on the market.
Electrical Resistance Tomography (ERT) enables a space-resolved consideration of the mixing process, as illustrated in Figure 56. Areas with a high colour concentration in the reactor are presented as red coloured regions in the generated tomograms.
ERT can be used for online measurement and visualization of liquid-liquid, solid-liquid as well as liquid-gaseous systems. Further typical applications, in addition to the mixing processes, are crystallisation, filtration and separation processes, level detection on diffuse interfaces, multi-phase flows of oil, water and gases, as well as analyses regarding pneumatic conveying.

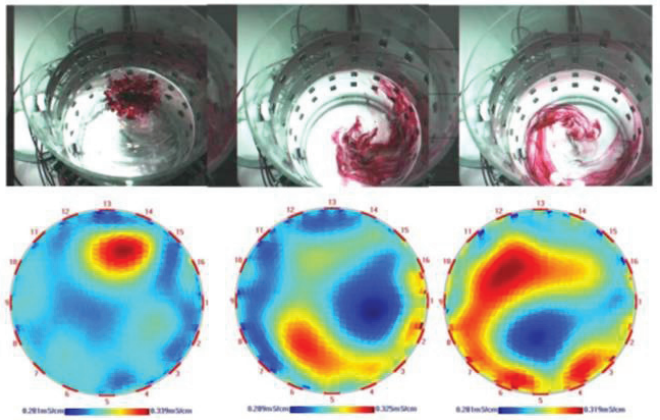


Figure 56: Visual comparison of the mixing of two liquids using ERT (Source: ITS 2012)

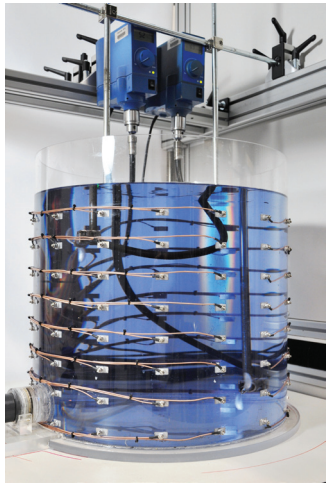


Figure 57: Process tomography (ITS System P2+) at Fraunhofer IKTS

With ERT a powerful technique is provided to allow a comprehensive and non-invasive quantification of mixing processes in biogas plants.
In addition to the evaluation of biogenous suspensions, virtually all types of complex suspensions can be analysed. Investigations in different scales are an essential prerequisite regarding the evaluation and optimization of large-scale mixing processes under consideration of similarity laws. Besides the true-to-scale replica of different reactor systems and the various installed stirring systems, the particles and fibers of the dispersed phase are also scaled. This is an indication for the high practical relevance of the work carried out at Fraunhofer IKTS.
The ERT system used at Fraunhofer IKTS (Figure 57) is a commercial instrument (ITS P2+; Industrial Tomography Systems, Manchester, UK) and comprises a vessel with the sensor system consisting of an arrangement of several measurement electrodes grouped in planes, a Data Acquisition System (DAS) and a PC with control and data processing software. Figure 58 presents the experimental setup for a cylindrical reactor.
Each sensor plane consists of 16 electrodes, equally spaced around the periphery of the reactor. The number of sensing planes depends on the geometry of the mixing vessel. Prerequisite for the evaluation of the mixing process with ERT are differences in the electrical conductivity between the continuous and the dispersed phase.
The adjacent measurement strategy, a pre-defined measurement protocol, is used to inject

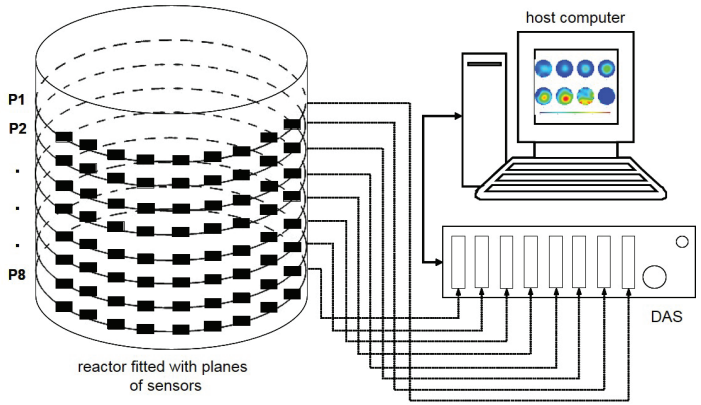


Figure 58: Components of the ERT system at Fraunhofer IKTS by means of the example of a cylindrical reactor with a height to diameter ratio of 1 and eight sensor planes.

a defined current between every pair of electrodes in every sensor plane and measure the resultant voltage difference between the remaining pairs of electrodes, which are temporarily carrying no AC current. Thus, ERT produces a spatial cross-sectional potential field, (Figure 59) which is influenced by the different electrical conductivities of the components of the multiphase system. As a result, the distributions of the electrical conductivities and the volume concentrations of the dispersed phase in the reactor can be determined.
The image reconstruction is realized by using the linear back projection algorithm (LBP). The advantage of LBP is its simplicity and low computational demand. To gather information about the internal conductivity distribution of the multiphase system, the reactor interior cross-section is spatially gridded into squares of equal size (pixels). Each sensor plane consists of such a two dimensional tomogram composed of 316 pixel. During the experiment, the electrical conductivities $\sigma_{P(x,y)}$ can be determined in accordance with Equation 66 for every individual pixel in every sensor plane at any time.

$$\sigma_{P(x,y)} = \frac{\Delta\overline{\sigma}_{(x,y)}}{\sigma} = \frac{\sum_{m=1}^{M}\sum_{n=1}^{N} S_{m,n,x,y} \ln\left[\frac{V'_{(m,n)}}{V_{(m,n)}}\right]}{\sum_{m=1}^{M}\sum_{n=1}^{N} S_{m,n,x,y}} \qquad (66)$$

$\sigma_{P(x,y)}$	Electrical conductivity of the pixel P(x,y)	(mS/cm)
σ	Electrical conductivity	(mS/cm)
$\Delta\overline{\sigma}_{(x,y)}$	Average partial conductivity change	(mS/cm)
m	Electrode pair to which the current is applied	(–)
n	Electrode pair at which the voltage is measured	(–)
$V_{(m,n)}$	Reference voltage measurement	(mV)
$V'_{(m,n)}$	Voltage measurement after indicating the conductivity change	(mV)
S	Sensitivity coefficient	(–)

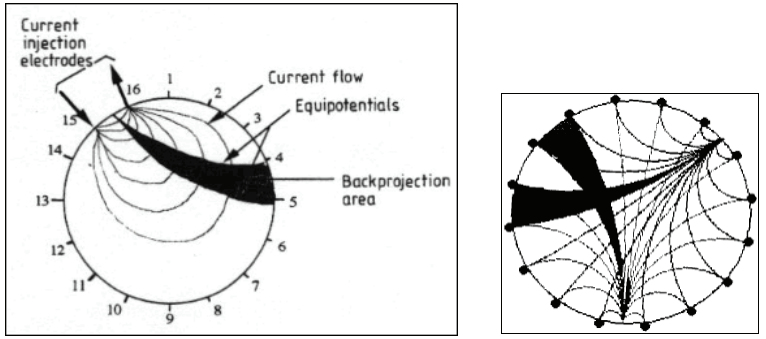


Figure 59: Measuring principle of the ERT system (Source: according to Lee 2009)

For the determination of additional information regarding mixing and flow processes it is necessary, that the calculated pixel conductivities are converted into solid concentrations using Maxwell's equation (ITS 2009):

$$\varphi_{2_i} = \frac{2\sigma_1+\sigma_2-2\sigma_{mc_i}-\frac{\sigma_{mc_i}\sigma_2}{\sigma_1}}{\sigma_{mc_i}-\frac{\sigma_2}{\sigma_1}\sigma_{mc_i}+2(\sigma_1-\sigma_2)} \quad (67)$$

φ_{2i}	Volume concentration of the dispersed phase in sensor plane i	(%)
σ_1	Electrical conductivity of the continuous phase	(mS/cm)
σ_2	Electrical conductivity of the dispersed phase	(mS/cm)
σ_{mci}	Electrical conductivity of the suspension (measured conductivity) in sensor plane i	(mS/cm)

The current state of the mixing process is displayed in two-dimensional tomograms or in three-dimensional illustrations (Figure 60) along the reactor height respectively, which show the distribution of the dispersed phase inside the continuous phase. Sensor plane P1 indicates the upper plane and the sensor plane with the highest number represents the bottom plane in the selected reactor system.

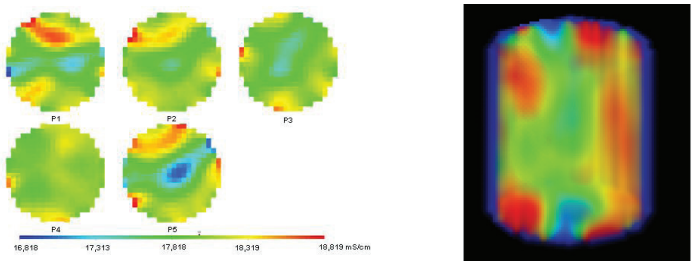


Figure 60: Visualization of the mixing process inside a cylindrical reactor with five planes of sensors: two-dimensional tomograms (to the left) and three-dimensional illustration of the reactor (to the right) (Source: IKTS)

The experimental investigations with ERT show that mixing processes in biogas reactors are significantly influenced by the substrate properties such as rheological behavior and granulometric parameters, reactor geometry (Figure 61) as well as mixing parameters like the selected stirring system.
Combined with advanced cross-correlation techniques, ERT offers the possibility to derive the axial flow velocity profile inside a stirred system. The analysis of the velocity profiles in the reactors is carried out with the commercial software AIMFlow, version 1.0 (MOSAIC Scientific Ltd., Leeds, UK). This software uses cross-correlation between two sensor planes to calculate the axial flow velocity. The determined conductivity maps are the initial source for the velocity calculation. The results are positive (= directed downwards) and negative (= directed upwards) flow velocities.

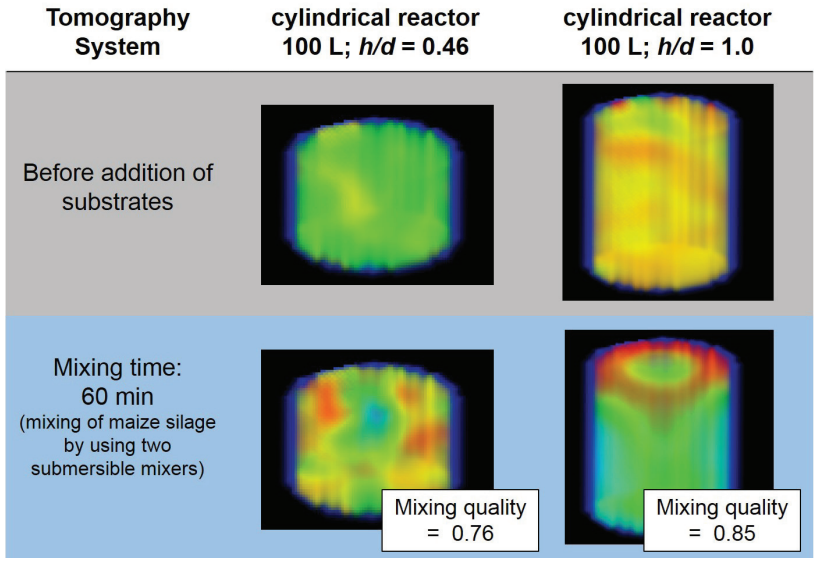


Figure 61: Average mixing quality in dependence on the reactor geometry (Source: IKTS)

Figure 62 shows the velocity profile of a suspension with fibrous particles (wheat straw) by using two submersible mixers.

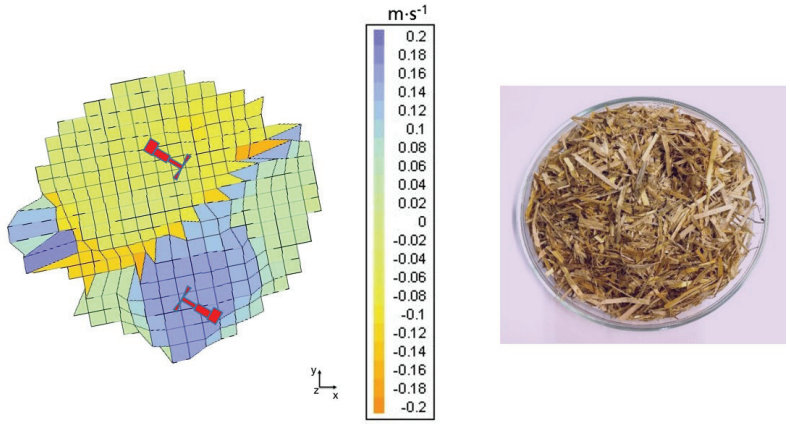


Figure 62: Velocity profile (to the left) of a suspension consist of xanthan gum and wheat straw (to the right) by using two submersible mixers (Source: IKTS)

5.7 Determination of the retention time by means of tracers

Helge Lorenz, Yong-Sung Kim, René Benndorf, Peter Fischer, DBFZ

Status	Development at laboratory and semi-industrial scale completed; ready to be used.
Associated standards	Biowaste Ordinance (BioAbfV 1998, as of April 23 2012), DIN CEN/TS 15290, DIN 38405, DIN 38414 S7, DIN EN 13650, DIN EN 13657, DIN EN ISO 17294-2
Type of substrate	■ Tracer *Bacillus atrophaeus* Suitable for substrates with fluid dynamic behaviour (e.g. fermentation substrate) under anaerobic or anti-germinating (e.g. seawater) conditions; ■ Tracer lithium hydroxide Suitable for substrates with fluid dynamic behaviour (e.g. fermentation substrate)
Limitations of the method	■ Tracer *Bacillus atrophaeus* Detection limit in fermentation substrate at approx. 10^3 spores/g ■ Tracer lithium hydroxide Low natural background level of the substrate must be taken into account; dosage at least 50 mg Li/kg TS fermentation substrate
Advantages	■ Tracer *Bacillus atrophaeus* Advantages of the bacteria spores: High thermal resistance and tenacity, no background concentration in fermentation substrate, epidemiologically safe, biological/ecological tracer ■ Tracer lithium hydroxide Detection by means of elementary analysis, high stability, chemically inert
Need for research	Implementation of tracer analyses at industrial-scale

The retention time has a strong impact on the efficiency of the decomposition processes and is essential to hygienisation. The average retention time is of interest for the process-kinetic evaluation; however, the minimum retention time is of greater importance for the hygienisation.
The definition of the minimum retention time in accordance with the Biowaste Ordinance BioAbfV (1998): "... is the period of time that was determined up to the last sample without findings prior to the first-time detection of the tracer."

The theoretical average retention time of the fermentation substrate in the digester is expressed by the technical or hydraulic retention time (HRT); it is the period of time that a substrate particle remains, on average, in the digester until it is removed. The HRT is a theoretical parameter and serves as basis for describing the real process.
In the case of constant volume-processes, the hydraulic retention time is calculated from the ratio of the useable reactor volume (m^3) to the daily fed amount of substrate fed (m^3/d):

$$HRT = V_R / \dot{V}_{fed} \quad (68)$$

HRT	Hydraulic retention time	(d)
V_R	Reactor volume	(m^3)
$\dot{V}_{fed}$	Amount of substrate fed	(m^3/d)

Reactors that have a defined mixing behaviour can be regarded as ideal reactors. In the biogas sector, two types of reactors are of importance with respect to the continuously operated digesters: the fully-mixed “stirred tank reactor” and the “plug-flow reactor”. In process engineering, these two types are described by idealised assumptions, which will not occur during normal operation. In a real reactor, some individual substrate particles are discharged sooner and others later. Retention time density function, and the retention time sum function are determined from retention time tests. Extrapolating from these allows the real average retention time to be calculated.
The equivalent number of stirred tank reactors calculated from the moments of the retention time distribution is decisive for the mixing of the digester. A „stirred tank“ is indicative that a digester is ideally and completely mixed (minimum retention time theoretically = 0). In the case of an infinite number of stirred tanks, an ideal plug-flow exists. If the real average retention time is below the hydraulic retention time, dead zones exist inside the digester. Dead zones are either poorly mixed or not mixed at all. Such dead zones reduce the real volume of the digester, which causes a shorter retention of the particles. A short-circuit flow exists when large amounts of the substrate are discharged before the calculated hydraulic retention time. This means there is a direct flow from the inlet to the outlet; thereby, the real average retention time is greatly reduced. Dead zones and short-circuit flows are undesirable since they reduce the average retention time and thus – among other things – the biogas yield.
In the case of a continuous reactor feeding, the minimum guaranteed retention time (MGRT) defined in the Biowaste Ordinance (BioAbfV 1998) can also be significantly shorter than the calculated hydraulic retention time due to short-circuit flows. Furthermore, the retention time distribution differs for particles of different size.
If partial fractions of the wastes remain in the reactor only for a short period, the epidemiological and phytohygienic aspects are problematic, and the fermentation substrate digestion is also incomplete. Insufficiently sanitised digestates are considered as a vector for the spreading of pathogenic germs. Therefore, knowledge of the minimum retention time of the substrate particles in the digestion tank is essential for the assessment of the hygienisation. In accordance with the Biowaste Ordinance (BioAbfV 1998), in biogas plants which use biowaste as fermentation substrate, the waste matrix must be treated in such a way "that

over the contiguous period of the minimum retention time the treatment temperature is affecting the whole material in the thermophile range (at least 50 °C)." If these conditions cannot be kept, an alternative hygienisation of the digestates is necessary.
Analyses with tracers (markers, indicators) are suitable for the determination of the minimum retention time of substrate particles in the digestion tank. According to the Biowaste Ordinance BioAbfV (1998), the time span determined from the tracer analysis represents the shortest retention time in the digester for all substrate components (solid and liquid). For this, substrate is marked with a tracer and subsequently the time span from the input until the first detection of the tracer in the outflow is recorded.

Different materials can be used as tracers. In particular, the tracer material must be stable, chemically inert, and have the same fluid-dynamic behaviour as the substrate particles.
It is known that the mixing state of a reactor affects the quality of the fermentation process greatly. Analysing the retention time behaviour is important to carry out in order to test the quality of the process. Comparative tests with different tracers help to characterise the retention time behaviour of real reactors. Weak points in the reactor such as zones that are not or only insufficiently mixed (stagnation zones, dead zones), short-circuit flows, and floating covers (setting and floating layers) can be identified by a combination of experimental retention time tests by means of tracer and tools of CFD (computational fluid dynamics). Depending in which way the tracer is inserted into the reactor (as impulse function [Dirac-pulse: one-time addition of tracer at t = 0] or as jump function [addition of tracer continuously from t = 0 on]), the retention time density function E(t) or the retention time sum function F(t) can be determined.
The marking substances for the experimental determination of the functions E(t) and F(t) should feature the following characteristics:

- Viscosity and density of the tracer must correspond to those of the reaction mass or to the reactor content (chemically inert, no impact on the physical properties of the reaction mass, no adsorption onto reactor parts, easily detectable also in very low concentrations),
- Addition of tracer should be carried out isokinetically so that the flow state is maintained,
- Diffusion coefficient of the tracer should be as low as possible.

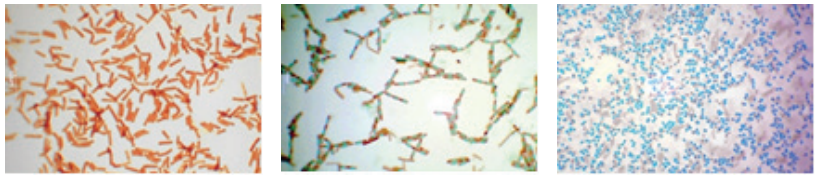


Figure 63: Vegetative cells, endospores and spores of *Bacillus atrophaeus* (1,000 x) (LTR) (Source: DBFZ)

5.7.1 Tracer analysis with bacteria spores for the determination of the minimum retention time

With the aerobic endospore former *Bacillus atrophaeus* (synonym *B. globigii* DSM 675), a microorganism is available that can already be used as a bioindicator in various ways due to the specific tenacity of its spores.
In biogas plants, the bacterial spores (approx. 1 to 2 µm in size, Figure 63) mix very well with the fermentation substrate. In batch tests under anaerobic conditions it was shown that spores were not decomposed over a retention time of at least 37 days (DBFZ study). The spores of the ecologically harmless bacterium are inert in the anaerobic digester. Additional advantages of the bacteria spores are their high thermal resistance, their lack of natural occurrence in the fermentation substrate (no background concentration in the digester), their quality of biological material as well as their epidemiological harmlessness (non-pathogenic). The microorganism can be detected quickly and reliably in the laboratory.

Figure 64: Spore powder of *Bacillus atrophaeus* as final product (Source: DBFZ)

For the analyses with this tracer it is important to know the spore concentration in the initial suspension as well as the detection limit of *Bacillus atrophaeus* in environmental samples (fermentation substrate). The spore concentration mentioned in the revised version of the Biowaste Ordinance (10^6 spores/mL of fermentation substrate) is quantitatively difficult to set in industrial-scale facilities. Therefore, the detection method should be improved to such an extent that even lower spore concentrations (10^3 to 10^4 spores/mL) can be detected in environmental samples

Spore production on surface method (solid state fermentation)
The medium for the preculture is a tryptone glucose broth (TGB). TGB can be inoculated with a non-specific amount of a *Bacillus atrophaeus* sample (e.g. DSM stock culture[3], spore suspension).
It is incubated in an incubator shaker at 37 °C and 120 rpm over night (22 ± 2 h) in order to obtain a culture in the exponential growth level.
Under a sterile bench conditions, approx. 2 to 5 mL of the TGB culture are transferred with a sterile glass pipette to a Petri dish with yeast extract agar (MYA) and tilted several times until the surface of the agar is completely covered with the inoculum. Excess inoculum is removed and can be transferred to the next plate. The Petri dish is incubated at 30 ± 1 °C.

[3] *Bacillus atrophaeus* (DSM No. 675 *Bacillus atrophaeus* Nakamura 1989), Deutsche Stammsammlung für Mikroorganismen und Zellkulturen GmbH [German Collection of Microorganisms and Cell Cultures] (DSMZ Sales, Inhoffenstraße 7 B, D-38124 Braunschweig)

After three to five days of incubation, the state of the culture is assessed under the microscope. The incubation is continued until the maximum sporulation rate is reached and the inoculated vegetative cells are autolysed (eight to ten days).
Under sterile bench conditions, the bacteria spores are removed by means of a sterile glass spatula and suspended in water (washed with approx. 10 mL $aqua_{dist.}$). The supernatant is drawn off by a glass pipette and collected in Schott bottles.
Approx. 50 mL of spore suspension is placed in centrifuge tubes (tare out samples by means of $aqua_{dist.}$). Four consecutive rinsing steps are carried out by centrifugation (each time pipetting off the supernatant carefully, refilling with $aqua_{dist.}$ and centrifuging 20 min, 4 °C, 7,000 rpm). Subsequent to the last step, pipette off again and suspend in $aqua_{dist.}$. The suspension (approx. 100 mL) is transferred into a 500 mL Schott bottle with screw cap and heated for 15 min at 80 ± 1 °C in a water bath in order to kill vegetative cells. The concentration of the *Bacillus atrophaeus* spore suspension should be approx. 10^9 spores/mL and can be stored in a refrigerator for approx. one year at 4 °C.

Spore staining for sporulation control
An air-dried heat-fixated preparation of the bacteria culture is made. Firstly, the sample on the microscope slide is heat-fixated at 90 °C until complete drying. By addition of a 5 % aqueous malachite green solution, the preparation is stained for 1 min at 80 °C. After washing, a 3 % aqueous safranine solution is added and counterstained for approx. 1 min at room temperature. The preparation is checked under a microscope at a thousandfold magnification.

Result of the staining

Spores	Turquoise
Bacteria cell	Red
Cell residue	Diffuse red matrix

Application of spores as tracer
Immediately prior to the addition of the biotracers, at least two single samples are collected at the digester outflow as negative control.
For the determination of the retention time, the biological tracer is mixed homogeneously with the fresh fermentation substrate and added once into the reactor as impulse function (Dirac-pulse). The amount of the spores mixed with fermentation substrate is to calculate in such way, that a certain spore concentration per gram (and/or mL) of digester content can be set (e.g. 10^4 spores/mL of fermentation substrate). To do this, it is necessary that the spore concentration of the spore powder or spore suspension must be determined in advance.

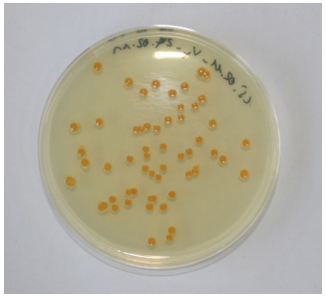


Figure 65: Detection of *Bacillus atrophaeus* in surface method on standard I nutrient agar (Source: DBFZ)

A control of the adjusted concentration of *Bacillus atrophaeus* spores in the feeding charge must be carried out.
After feeding of the marked fermentation substrate in industrial-scale biogas plants, the sampling (single sample of at least 20 g) is carried out in the outflow until the tracer is for the first time detectable in a sample, and – in particular – at least:

- Immediately or 5 min after the addition of the spores in the case of continuous mode of operation,
- each hour, until and including the 24th hour,
- thereafter every 2 hours, until and including the 36th hour,
- thereafter every 4 hours, until and including the 48th hour,
- thereafter every six hours.

Detection of the spores
For a predilution, 20 g from each sample (approx. 20 mL/g of sample) are weighed into 180 mL sodium chloride solution (0.9 % saline solution) and mixed for approx. 20 hours at 4 °C on the shaker (150 rpm).
After homogenisation, 1 mL of each sample is pipetted in geometric series up to dilution level 10^{-8} in respectively 9 mL NaCl solution, and mixed.

Thereafter, respectively 0.1 mL of each dilution level (starting with 10^{-8}) is pipetted and evenly spread on two parallel standard I nutrient agar plates using a sterile glass rod or Drigalski spatula (incubation at 37 °C for 22 ± 2 h).
The suspension and the dilution stages can be stored at 4 °C.
Only such colonies are counted on the agar plates which show a typical orange growth (Figure 65).
Calculation of the bacteria concentration on agar plates (CFU/mL):

$$\text{CFU per mL} = \frac{\sum c}{\sum_{i=1}^{\infty} 10^{-i+1} \cdot n_i} \cdot F_a \cdot F_b \cdot F_c \qquad (69)$$

CFU	Colony-forming units
c	Sum of the CFU counted on the plates
n	Number of plates of each counted dilution level, starting with the lowest level (n_1)
F_a	Factor of the first counted dilution level
F_b	Factor of the predilution
F_c	Multiplication factor of the application volume of 0.1 mL relative to 1 mL

5.7.2 Tracer analysis with lithium for the determination of the minimum retention time

The suitability of a lithium compound (lithium hydroxide monohydrate [$LiOH \times H_2O$]) as tracer has been proven by a DBFZ study. The substance has similar dynamics in the digester as the fermentation substrate and does not react with it. Lithium is elementarily simple to analyse and can be precisely detected.
While determining the lithium in the digestate, a low natural background level of the substrate must be taken into account. The lithium background level must be determined before the analysis. For this, samples are drawn at the digester outflow at least 5 days prior. In a fermentation substrate (digestate, maize silage), the background level was determined to approx. 0.25 to 0.30 mg Li/kg total solids (TS). Depending on the type of the fermentation substrate, the lithium background concentration can be up to 5 mg per kg/TS.

Application of lithium as tracer
For the determination of the retention time, the dissolved chemical tracer is mixed homogeneously with the fermentation substrate and added once into the reactor as impulse function (Dirac-pulse). The amount of the lithium tracer mixed with fermentation substrate should be calculated, that a specific concentration per kilogram of digester content can be set (at least 50 mg Li/kg TS fermentation substrate). The amount and application of the lithium tracer depends on the amount of the reactor as well as on the amount of solids of the fermentation substrate.
After the feeding of the marked fermentation substrate in industrial-scale biogas plants, the sampling (single sample of at least 50 g) is carried out in the outflow until the tracer is first detected above the background concentration in a sample, and – in particular – at least:

- Immediately or 5 min after the addition of the lithium in the case of continuous mode of operation,
- each hour, until and including the 24th hour,
- thereafter every 2 hours, until and including the 36th hour,
- thereafter every 4 hours, until and including the 48th hour,
- thereafter every six hours.

Detection of the lithium
The chemical decomposition of the samples is made by means of aqua regia, and the analysis by means of inductively coupled plasma optical emission spectrometry (ICP-OES) (detections are carried out in accordance with DIN CEN/TS 15290, DIN 38405, DIN 38414 S7, DIN EN 13650, DIN EN 13657, DIN EN ISO 17294-2). A concentration value in mg Li/kg TS is determined.

5.8 Determination of the retention time behaviour with the help of studies of tracers

Anne Kleyböcker, GFZ

Status	Tracer studies contribute to the detection of bypasses, stagnation zones and dead zones in biogas digesters. These affect the biogas production process and lead to economic losses. The addition of uranine as tracer has already been established for various substrates (sewage sludge, biowastes).
Associated standards	In addition to uranine, sodium fluoride, lithium chloride and radioactive isotopes are also utilised as tracers.
Area of application of the method	The tracer uranine can be used for various substrate combinations.
Need for research	The use of uranine as tracer is cost-efficient and can be applied to various substrates. Nevertheless it is necessary to test the tracer for substrates with a high turbidity, and to determine the limits of detection.

General
The retention time in a digester must be guided primarily by the generation time of the microorganisms. If the retention time is shorter than one generation time, the microorganisms are washed out and the process of the biogas production can no longer take place completely. In this context, the type of digester is of importance. If the biomass – due to growth bodies – remains longer in the reactor than the liquid phase, other guidelines apply. The retention time behaviour strongly depends on the mixing through, which is influenced by the reactor geometry, and the inflow and discharge rates. The better an agitated reactor is mixed through, the better the substrate is distributed in the reactor and the lower the discharge of non-fermented material will be. First and foremost, bypasses, stagnation zones and dead zones should be avoided and, best case, the formation of a floating sludge layer and formation of foam should be fought (Janke 2008). For ecological and economic reasons, the energy demand for the mixing through should be kept as low as possible.
The retention time distribution can be determined with the help of a tracer that can be introduced into the reactor as a shock load and whose concentration in the discharge is measured in dependence on the time (Danckwerts 1953). For the interpretation of the results, standardised concentrations are suitable in order to be able to better compare the results with others (Levenspiel 1962). To date, tracer studies in biogas reactors have primarily been carried out with sodium fluoride, lithium chloride and radioactive isotopes (Anderson et al. 1991; Borroto et al. 2003; Heertjes et al. 1982; Monteith & Stephenson 1981; Tenney & Budzin 1972; White 1974).
Not only can the average retention time $\bar{t}$ – which in the case of continuously stirred tank reactor (CSTR) corresponds to the calculatory hydraulic retention time – be determined via

the retention time distribution, but bypasses and stagnation zones can also be detected this way. The calculatory hydraulic retention time (HRT) is calculated by dividing the volume of the digester by the loading rate of the substrate in accordance with Equation 70 (Janke 2008).

$$HRT = \frac{V_{reactor}}{\dot{V}} \quad (70)$$

The average retention time $\bar{t}$ is determined with Equation 71 via the distribution sum function F(t) (Equation 72, (Kraume 2004)), wherein the distribution sum function is the integral of the distribution density function E(t).

$$\bar{t} = \int \left(1 - F(t)\right) dt \quad (71)$$

$$F(t) = \int E(t) dt \quad (72)$$

According to (Kraume 2004), the distribution density function for an ideally mixed through stirred tank for $n = 1$ and for a cascade of ideal stirred tanks with $n = 2$ stirred tanks is calculated in accordance with Equation 73. In this, it is assumed that each stirred tank is ideally mixed through and that no return transport of material into a tank previously flowed through is possible.

$$E(t) = n e^{(-nt)} \frac{(nt)^{n-1}}{(n-1)!} \quad (73)$$

State of development/area of application of the uranine tracer

Suitable as chemical tracer is in addition to sodium fluoride, lithium chloride and radioactive isotopes also uranine ($Na_2C_{20}H_{10}O_5$), a fluorescing dye. According to Käss (2004), uranine is not degradable in severely organically contaminated groundwaters and sewage sludges and is also not adsorbed. In-house laboratory measurements confirmed these claims and showed that uranine is also suitable for biogas plants. The limit of detection of uranine depends on the substrate utilised and can under the most favourable conditions be in a range below 0.002 µg/L.

One advantage of this method is that uranine is more cost-efficient in comparison to other tracers since uranine can be detected already in low concentrations and therefore very small quantities are sufficient for a tracer study. Moreover, it can be fed to the reactor without any great expenditure because of that. For analyses in biogas reactors that are operated with sewage sludge and biowastes, 1 kg of uranine per 1,000 m³ of digester volume is sufficient, for example. Since only a fibre optic fluorimeter is needed for the determination of the uranine concentration, the determination of the concentration is also cost-efficient.

Description of method

Sample preparation/materials/devices

In order to determine the optimal amount of uranine for a tracer study, it is recommended to prepare a calibration series with corresponding uranine amounts in digestate from the digester to be analysed. In this, it must be ensured that a correspondingly large range of concentrations is covered so that even after multiple hydraulic retention times uranine can still be detected. If the uranine concentrations are proportional to the fluorescence, this indicates the suitability of the uranine for the digestate in question. In untreated sludge, uranine cannot be measured undisturbed. Therefore, the fermentation sludge sample is centrifuged twice at 10,000 rpm for 10 min, each. In between the centrifuging processes, the samples are decanted. Subsequently, the sample is checked – under the exclusion of light – for its fluorescence, using a fibre optic fluorimeter of the Hermes company with a fibre optic sensor.

Carrying out a tracer study

The tracer is introduced into the industrial scale biogas reactors as a shock load. To detect any bypasses, it is important to determine within short time intervals the tracer concentration in the digestate directly after the addition of the tracer. Since stagnation zones frequently occur in digesters, the tracer study should cover at least six hydraulic retention times.

Analysis of the results/data

The concentration graph of the tracer in dependence on the time represents the distribution density function. The integral of the distribution density function results in the distribution sum function (c.f. Section "General").

The average retention time can be calculated from the distribution sum function in accordance with Equation 71. The determination of the retention time behaviour is carried out based on the assessment of the distribution density function. In this, the measured values are mapped with the help of models (ideal stirred tank, cascade of ideal stirred tanks, paralleling of ideal stirred tanks, flow tube, and their combinations). The models provide estimates regarding stagnation zones and bypasses.

5.9 Process specification for the determination of sand

Katrin Strach, DBFZ

Status	To date, the method has been utilised for the determination of the particle size distribution of mineral shares in low-fibre agricultural residues, digestates and biowaste. It was evaluated with sand-free reference biowaste and quartz sand. (Kranert et al. 2002b)
Associated standards	Determination of the particle size distribution of bulk materials in accordance with DIN 66165
Area of application of the method	Digestates, liquid manure, solid dung, dry chicken faeces and biowaste.
Limitations of the method	The method has not yet been utilised for materials with a high fibre content and materials that tend to agglomerate. For wet screening, the bottom limit of usability was determined at a 5 % sand share, an upper limit has not been defined. (Kranert et al. 2002a, 2002b)
Advantages	It is a simple, easy to handle and environmentally friendly method. The particle size distribution can be utilised as basis for the assessment of the damage and settling processes to be expected.
Disadvantages	The approach is time-consuming.
Need for research	The method should be tested with additional substrates and digestates in order to determine its limits.

The screen analysis is a method for the determination of the particle size distribution of bulk materials and is described by DIN 66165. In this, the most common and easiest method for the analyses is the dry screening. But if a substrate is used that tends to agglomerate, wet screening provides for more accurate results. In the case of wet screening, the quantification of the particle sizes is realised with the help of the medium water via a set of screens. The individual fractions are present in a mixture of organic and mineral components and can subsequently be "separated" calculatorily via the determination of the calcination loss. What remains is the whole mineral component which is stated as mass per cent of total solids. Modelled after soil science, the mineral components are categorised in accordance with DIN ISO 11277 in dependence on the particle size as follows:

Table 20: Categorisation of mineral components exclusive of soluble salts in dependence on the particle size

Mineral component	Particle size [µm]
Gravel	63,000–2,000
Sand	2,000–63
Silt	63–2.0
Clay	2.0–0.2

In accordance with the definition in Table 20, for the determination of the inert components in substrates, up to seven analysis screens of 63 to 2,000 µm are utilised and placed in a screening tower wherein each consists of a screening frame and a sieve plate with different mesh width. The screening frequency can be adjusted at the control knob of the machine.

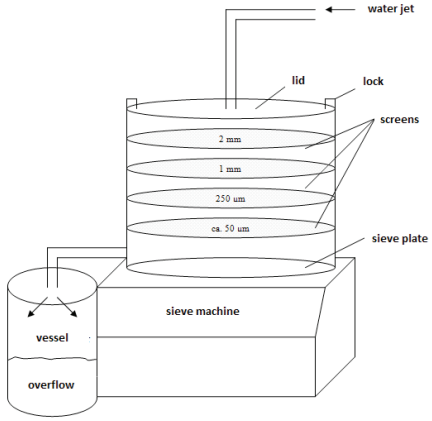


Figure 66: Setup of sieve machine (Source: Schneider 2010)

Wet screening

In wet screening, the material to be tested is transferred into a suspension with water prior to the screening process, homogenised and subsequently placed on the top screen. With the help of a water spray jet, the screen sample can be rinsed through the individual analysis screens. In this, the volume flow must be selected such that the whole screen area is wetted. Furthermore, the optimal frequency of the shaker facility at maximum water throughput can be controlled and set at the overflow. The rinsing and screening is carried out until the suspension carried off below the screening tower is visually clear and does not feature any turbidity.

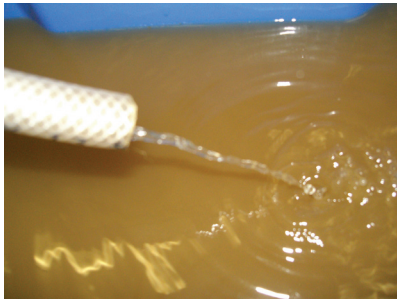


Figure 67: Overflow in the case of completed wet screening (Source: Schneider 2010)

Subsequent to the screening, the whole stack of screens is initially dried for 24 h at 105 ± 5 °C in a drying cabinet, weighed-in and only then through careful beating out of the screens transferred into individual beakers. After the calcination of the samples, the organic components can be determined through weighing and the inert shares can be calculated in accordance with Equations 74 and 75. The suspension of the overflow is caught in a separate vessel and weighed. The inert components are determined analogously on a partial sample.

Dry screening

In the dry screening, the sample to be analysed is first placed for 24 h in a drying cabinet at 105 ± 5 °C and subsequently calcinied in a muffle furnace at 550 ± 5 °C for 3.5 h. The previously carried-out drying has the utmost priority since with the immediate calcination of the wet sample very high temperature can be generated due to the instantaneous evaporation of the water. This can lead to puffing out, encrusting of the sample or even up to the destruction of the crucible. Furthermore, an even distribution of the sample in the crucible must be ensured in order to ensure a complete drying through and calcination of the sample. After the screened material has cooled down, the sample is weighed, homogenised, subsequently introduced to the topmost analysis screen and screened with a suitable frequency for different periods of time. The residues from the individual test screens are subsequently transferred into a beaker by carefully beating out and brushing off the screens and determining the particle size distribution through weighing. This way, the particle size distribution of the mineral share is determined directly; the organic fraction can only be assessed as a sum.

Result calculation

In order to calculate the mineral share of a fraction, the mass of the inorganic share is considered in relation to the mass of the total solids. The impurities of the inorganic share due to organic components are calculatorily adjusted with a reference value of 0.9–0.95. This value depends on the type of the organic material. Here, a representative, mineral-free partial sample should be taken from the sample for determination and calcination.

$$S_{TS} = \frac{m_{ioTS} - (1 - Ref) \cdot m_{VS}}{m_{TS}} \quad (74)$$

S_{TS}	Pure mineral share relative to the total solids	($\%_{TS}$)
m_{iTS}	Mass of inorganic total solids after calcination	(g)
m_{VS}	Mass of volatile solids before calcination	(g)
m_{TS}	Mass of the total solids	(g)
Ref	Calcination loss of the organic share; figure drawn from past experience: 0.9–0.95	

If the specification of the total share of mineral substances is desired, the summation of the individual fractions is carried-out:

$$S_{TS\,tot} = \sum_{1}^{n} S_{TS} \quad (75)$$

S_{TS}	Sand share of the individual fractions	(%)
$S_{TS\,tot}$	Total sand share	(%)

A sample presentation of results is depicted in Figure 68. The substrate and various samples from a digester for biogas production were inspected for mineral components. (Liebetrau et al. 2011)
The Figure shows the shares of the fractions in the overall mineral share of the samples. It is apparent, for example, that all samples inspected have an increased share of clay and silt.

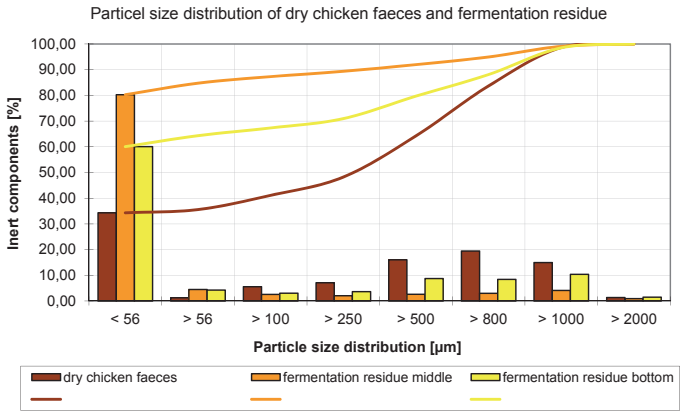


Figure 68: Depiction of results of sand determination (Source: Schneider 2010)

Comparison of the methods

With both screening methods, recovery rates of more than 99 % can be achieved. Due to the easier handling of the dry screening, the error is smaller.
Both methods provide qualitatively very good and comparable results. Starting at a particle size of 500 µm, it was possible to achieve quantitatively comparable measuring results independent of the test duration, while with an increase of the screening duration to 30 min, an approximation of the results could already be observed at 250 µm. The reason for this is the dropping flow rate with decreasing particle size due to the analysis screens. The method of dry screening is therefore suitable for the determination of the inert share with a corresponding time investment, for the quantification of individual shares up to 100 µm.

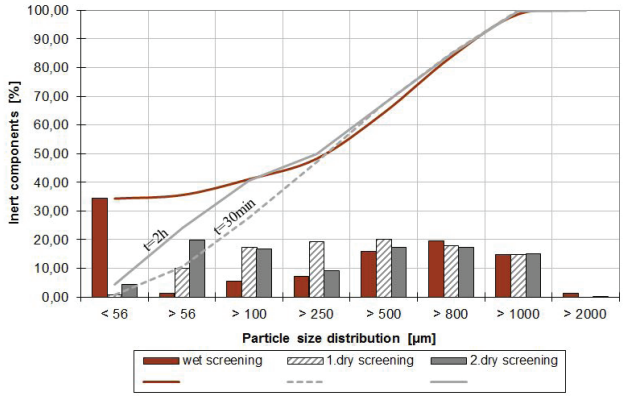


Figure 69: Comparison of the screening methods (Source: Schneider 2010)

6 Methods for the determination of biological parameters

6.1 Description of the experiment for the Oberhausen/Rostock/Göttingen Activity Test (ORGA-Test)

Nils Engler, University of Rostock

Status	The method has been and/or is being applied as part of various research projects. The possibility of its application in full-scale plant operations does not presently exist but is currently the topic of research.
Associated standard	None
Area of application of the method	■ Inspection of digester content for performance of the digester biology ■ Detection of inhibiting effects of substrates or contents on the anaerobic degradation process
Disadvantages	■ Test measuring time (approx. 7 d) ■ Measurement of the composition of the biogas generated is not possible
Advantages	High temporal resolution of the measurement, thereby allows for a detailed inspection of individual decomposition phases.
Need for research	In the case of application as inhibiting substance test: Derivation of actual utilisation limit values of the inhibiting substances for practice.

The method described below was developed with the University of Applied Sciences in Göttingen, Department: NEUTec, and the University of Rostock, Department of Waste and Material Flow Management, as part of a joint research project funded by the AIF under the direction of the Fraunhofer Institute for Environmental, Safety, and Energy Technology UMSICHT. Based on the locations of the three project partners, the method was named Oberhausen/Rostock/Göttinger Activity Test, in short ORGA Test.
The ORGA Test is intended to allow for a comparative quantification of the biological activity of renewable resources biogas digesters. This is realised via a measurement with high temporal resolution of the substrate conversion of a standardised substrate under standardised boundary conditions. From the kinetics of the conversion of a standard substrate, far more differentiated information regarding the condition of the digester biology can be derived than from the biogas potential alone, the way it is determined in classic batch tests. Furthermore, the information is available to the plant operator significantly faster due to the comparatively short test period of 5–7 d.

The instrumental basis for the test method is formed by the *Gas Production System* of the ANKOM company. The method is based on the measurement of the increase in pressure in a constant volume at constant temperature.
Depending on the issue at hand, different standard substrates that have to fulfil the following requirements can be used for the ORGA test:

- Reproducibility: Known and describable composition
- Quantifiability of the results: Known biogas yield
- No impact on the objective of the inspection: Itself free from promoters/inhibitors
- Decomposability: The composition of the essential nutrients corresponds approximately to the substrate of renewable resources biogas plants at concomitant virtually complete decomposability

The ORGA test can be carried out with the following standardised substrates:

- Acetate (acetic acid) is directly available for the methane-producing microorganisms. From the kinetics of the acetate conversion, conclusions regarding the performed especially of the methanogenic phase are possible.
- Maize starch as quickly decomposable substrate for hydrolysing and acid-building microorganisms. It was possible to prove that in the case of utilisation of pure maize starch the individual phases of the methane formation take place temporally one after the other. This makes an analysis of the whole reaction chain of methane fermentations possible, especially the transition from the acidification to the methane building phase.
- As synthetic complex substrate, a mixture made of micro-crystalline cellulose, maize starch, glucose as well as phosphate buffer and urea is utilised, wherein the formulation is adjusted such that the C:N:P ratio approximately corresponds to that of a maize silage. Therewith, a complex, yet long-term reproducible standard substrate is available.

Execution of the test
For the activity test, fermentation vessels with a working volume of 500 mL are utilised. The weighed-in sample quantity is approx. 15 g_{VS}, which corresponds to approx. 200–400 g of digester sample. In order to ensure the stirrability by means of a magnetic stirrer, dilute with clean water, where necessary. Of each digester sample to be analysed, blank test, control (digester sample with standard substrate) and, where applicable, different treatment variations (enzymes, trace elements etc) are each analysed in at least triplicates. The fermentation vessels are heated in the water bath at 38 °C. The temporal resolution of the measured values is 30 min, wherein in terms of the system temporal resolutions in the range of seconds are possible.
The fermentation vessels are mixed through once a day by means of magnetic stirrer. The gas yield is calculated taking into consideration the accompanying blank tests based on kg_{VS} of the substrate used. For the presentation of result, the cumulated gas volume is put in relation to the theoretical maximum biogas yield of the standard substrate according to (Buswell 1952) and presented as standardised time curve. This way, significantly more

information can be obtained in comparison to other methods such as the determination of the biogas potential in the batch test. Additionally, due to the utilisation of selective substrates, a separate analysis and assessment of the individual phases of methane production is possible.
The following criteria serve for quantification of the decomposition performance of the digester biology, and for comparison of different digester samples:

- Acetic acid and synth. complex substrate: t50 as the time that is needed to generate approx. 50 % of the theoretical biogas yield.
- Maize silage: Here, two time criteria, t40 and t60, are utilised which each are required to generate approx. 40 % and/or 60 % of the theoretical biogas yield. Additionally, from the increases s1 (of the hydrolysis) and s2 (of the acetogenesis/methane production, each in [mL/g_{VS} · d]) of the cumulative gas graph, information regarding the condition of the digester biology can be obtained.

Depicted in Figure 70 is a characteristic gas production curve, each for the two substrates acetic acid and maize starch as well as the situation of the assessment criteria.

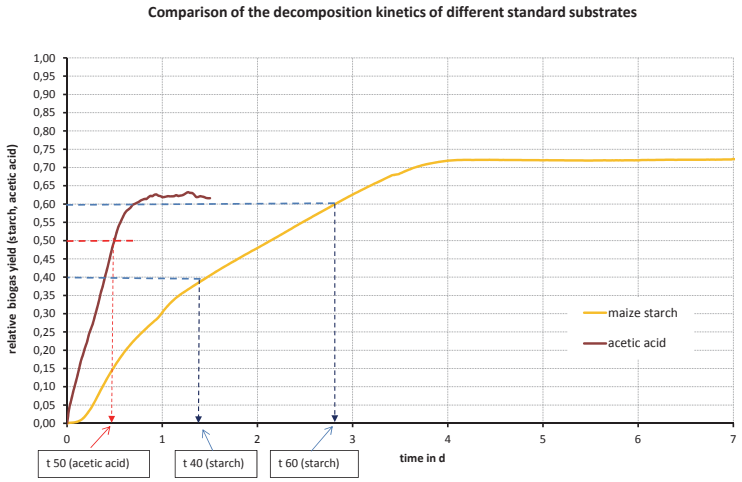


Figure 70: Typical decomposition kinetics of the standard substrates maize starch and acetic acid and assessment criteria (Source: University of Rostock)

State of development and application
The ORGA Test (Engler et al. 2011) is not yet an established method. The objective of the research project was to develop the test methodology and to utilise it for the optimisation of the trace element supply of renewable resources biogas plants.
The method development has been completed and since 2010 the test has been utilised at three participating research sites in accordance with standardised process specifications. As part of the project, samples were taken from approx. 40 biogas plants in Mecklenburg-Western Pomerania, Lower Saxony and North Rhine-Westphalia and analysed with the ORGA Test. Parallel to this, comprehensive analysis of the digester samples were carried out with a particular focus on macro nutrients and trace elements. The results are fed into tests regarding the optimisation of industrial-scale plants. In the IVth quarter of 2011, a large scale test, each, was planned at a industrial-scale plant at each participating research site.
In a modified form, the ORGA Test is also intended to be used for the detection of potential process inhibitors. Preliminary work regarding this has been already conducted.

6.2 Gas yield test (batch)

Marc Lincke, Björn Schwarz; Fraunhofer IKTS

Status	Development completed, applicable.
Associated standards	(VDI Guideline 4630 2006); DIN 38414-8; VDLUFA Book of Methods VII, Environmental Analysis and Gas Yield Measurement 4.1.1
Type of substrate	Biogenous substrates of any composition
TS range	0 %–100 % (of the original sample, max. 10 % of the substrate mixture in the fermenter)
Particle sizes	Maximum length: 5 cm (otherwise pre-shredding is required)
Limitations of the method	None, since any decomposition inhibition by the substrates to be tested is also subject of the analysis
Advantages	Simple and robust method
Need for research	Comparability to results of other methods and inoculum (inter-laboratory comparisons as they have already been carried out by KTBL)

With the help of gas yield tests, statements regarding the anaerobic decomposability, the quantity and quality of the gas yield achievable under optimal conditions and the qualitative assessment of the decomposition velocity can be made. Gas yield tests do not allow statements regarding the process stability of the continuous fermentation of the substrate, since inhibition or adaptation effects only occur after longer feeding cycles.
A VDI guideline (VDI Guideline 4630 2006) for the execution of gas yield tests, discussing potential equipment, test set-ups, methods of characterisation and fundamental calculations very comprehensively, is existent. In the year 2012, the amended version of 2006 was revised. A VDLUFA prescribed method presents the most important steps of the analysis and validates the method through multiple inter-laboratory comparison runs. In the following section, as a practical example for a potential implementation of VDI Guideline 4630, the batch fermentation plant of Fraunhofer IKTS Dresden as well as the concrete approach for the determination of the gas yield will be presented.

Sampling and sample preparation
Complementary to the rules and regulations of VDI Guideline 4630, a sample preparation for solid substrates (in particular cutting or crushing) will only be applied if the untreated sample cannot be transferred into the fermentation apparatus or if the inhomogeneity of the initial sample does not allow a representative partial sample for the fermentation test. In this case, it has to be considered that the result of the biogas yield test does not necessarily correspond to the actual gas yield of the untreated sample as it could have been changed by the sample preparation.

Materials and devices
The test set-up (Figure 71) was modelled after the guidelines of DIN 38414-8 and consists of the following components:

Water bath with thermostat unit:
- 1 litre glass reactor with a useable storage volume of 700 mL
- Gas meter type Milligascounter® MGC-1 V3.0
- Gas bag (diffusion-tight, PP connection)
- Gas-tight hoses are located between the reactors and the Milligascounter® and the gas bags

With the exception of the thermostat unit, all components of the system are the result of comprehensive suitability tests with respect to tightness and measuring accuracy. For newly designed test facilities, such a verification is recommended since experience has shown that even commercially available components may have significant deficits with respect to tightness and measuring accuracy.

Execution method
As inoculum, active digested sludge (from the turnover) of a communal sewage treatment plant is utilised, which is – within a period of time of no more than 4 hours after sampling

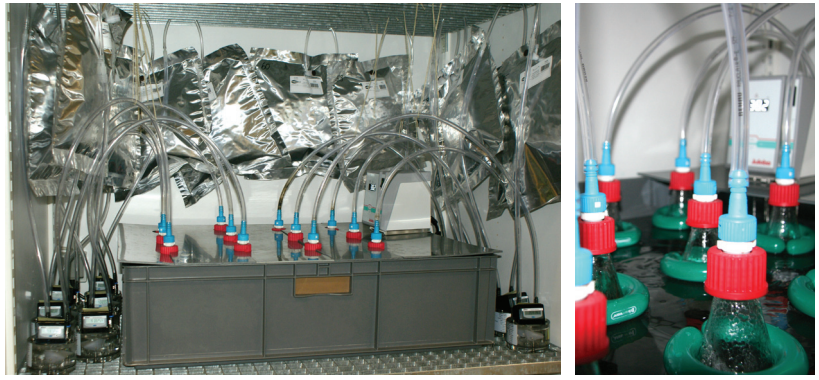


Figure 71: Batch reactors (1 litre scale), water bath, gas meter, gas bag (Source: IKTS)

– filled into the glass reactors (600 g each). At an average TS content of 3.7 % and a VS content of 50 %, the criteria of the VDI guideline (organic share from the inoculum of 1.5 % to 2.0 % in the preparation) are safely adhered to. The inoculum is subjected to a starvation and recovery phase at a temperature of 38 °C for a period of time of approx. seven days. During this phase, a synchronism of the gas production in the individual reactors is ensured. Subsequent to the successful starting-up phase, the reactors are fed with the substrate to be analysed. The calculation of the sample quantities to be used is carried out in accordance with the provisions of VDI Guideline 4630 based on the following equation.

$$\frac{VS_s}{VS_{inoc}} = 0.4 \qquad (76)$$

VS_s	Amount of volatile solids in the substrate	(g)
VS_{inoc}	Amount of volatile solids in the inoculum	(g)

The feeding is carried out by hand with the help of funnels directly into the reactor opening. For the verification of the biological activity of the inoculum, a micro-crystalline cellulose is carried along as a reference substrate with each preparation. For the determination of the own gas potential of the inoculum, at least three reactors are operated as reference without any addition of substrate. A purging of the headspace of the reactor (1 L) with nitrogen is not mandatory, since no differences in gas production were observed so far when the reactor was purged with nitrogen. Once the reactors have been fed, the gas meters are zeroed and empty gas bags are installed. An inspection of the test benches is conducted once a day, in which the following works steps are carried out.

- Measurement of the current air pressure with the help of a digital barometer
- Measurement of the current room temperature with the help of a digital thermometer
- Reading out and logging of the amounts of biogas produced
- Shaking the reactors (manually) (→ is the most effective method for very long-fibred samples [e.g. straw or grass]),
- and checking the fill levels of the water baths.

After a test measuring time of at least 30 days and reaching the abortion criterion of a biogas growth rate per day of less than 1 % of the total biogas volume generated until that point, the tests are aborted.
The quantification of the components of the produced biogas (gas bag) is carried out with a gas analyser Visit 04 (Eheim company), which determines the oxygen and methane content optically and the carbon dioxide as well as hydrogen sulphide content electro-chemically. The average value is determined for the whole amount of biogas produced, but no temporal progression of the gas quality.

Data analysis
The amount of gas determined by means of a gas meter must be converted to standard volume prior to further analysis (Equation 77). Complementary to the specifications of the

VDI guideline, the impact of the gas volume in the headspace on the measured value of the gas meter in the case of changing barometric pressure conditions is also taken into consideration by IKTS. The volume of the gas phase in the reactor (V_{GP}) is 500 mL for the reactors described here. This volume expands in the case of decreasing barometric pressure or is compressed in the case of increasing barometric pressure. This leads to undesirable fluctuations of the measured values towards the end of the tests (in the case of low gas production rates).

$$V_{STD} = \left(V_c - V_{c\text{-}1} - \left(\frac{V_{GP} \cdot P_{c\text{-}1}}{P_c} - V_{GP} \right) \right) \cdot \frac{P_c - P_W + P_L}{P_{STD}} \cdot \frac{T_{STD}}{T_c} + V_{STD\text{-}1} \quad (77)$$

V_{STD}	Standardised gas volume	(mL)
V_c	Current reading of volume	(mL)
$V_{c\text{-}1}$	Volume of previous day (and/or last reading)	(mL)
V_{GP}	Volume of gas phase in the reactor	(mL)
P_c	Current air pressure	(mbar)
$P_{c\text{-}1}$	Air pressure of previous day (and/or last reading)	(mbar)
P_W	Partial pressure of water pressure (26.4 mbar at 22 °C)	(mbar)
P_L	Pressure of the liquid column above the measuring chamber in the gas meter (2 mbar)	(mbar)
P_{STD}	Standard pressure (1013.25 mbar)	(mbar)
T_{STD}	Standard temperature (273.15 K)	(K)
T_c	Current ambient temperature	(K)

To determine the amount of gas that originates solely from the substrate to be analysed, the standardised gas volume of the reference reactors (inoculation substrate only) is deducted from the standardised gas volume of the sample. For the comparability of the biogas yields to other substrates, a standardisation of the specific biogas amount to the amount of volatile solids is carried out in accordance with the following equation.

$$V_S = \frac{V_{STD}}{VS_s} \quad (78)$$

V_S	Specific standardised gas volume, put in relation to the volatile solids	(L (STP)/$kg_{oDRcond}$)
V_{STD}	Standardised gas volume in the sample	(L (STP))
VS_s	Amount of volatile solids in the substrate (input)	(kg)

In Figure 72, the curves of the feed-specific gas production of different substrates are depicted as an example.
The concentration values for methane and carbon dioxide are, in compliance with the VDI guideline, corrected in accordance with Equation 79 in order to eliminate the impact of water vapour, nitrogen and oxygen from the headspace of the reactors.

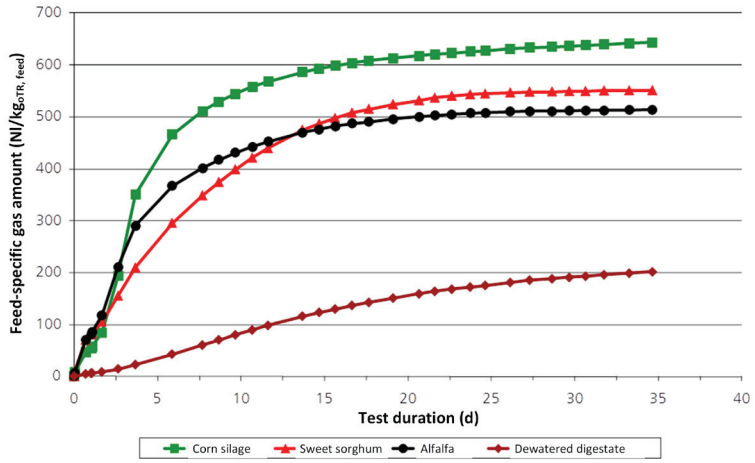


Figure 72: Batch gas production test – feed-specific gas amounts (Source: IKTS)

$$C_{corr} = C_{CH_4 \text{ or } CO_2} \cdot \frac{100}{C_{CH_4} + C_{CO_2}} \quad (79)$$

C_{corr}	Concentration of methane or carbon dioxide after headspace correction	(%)
C_{CH4}	Measured concentration of methane	(%)
C_{CO2}	Measured concentration of carbon dioxide	(%)

If a sealing liquid gets in contact with the produced biogas during the determination of the amount of biogas produced, it must be kept in mind that gas components can migrate into solution there. Even the acidification of the employed liquids does not prevent the purely physical solution of CO_2, for instance. In an extreme case, this can have an impact on both the amount as well as the quality of the gas. When using Milligascounter®s (gas meters with a low amount of sealing fluid), this effect can be significant for small amounts of gas and should be investigated for each test plant in order to introduce correction factors, if necessary.
It is assumed, that no positive or negative additional effects occur in continuous operation and that a complete mixing state of the reactor content is achieved, so that it is possible to convert the results of the batch tests to continuous conditions. Depending on the number of reactors and their hydraulic retention time, an average gas production can be calculated for each individual reactor via process engineering calculations in accordance with the stirred tank theory. For very long retention times, either an estimation of the further progress of the gas production beyond the end of the batch test must be carried out, or a longer test period has to be selected.

6.3 Continuous fermentation tests

Marc Lincke, Björn Schwarz; Fraunhofer IKTS

Status	Development completed, ready to be used.
Associated standard	VDI Guideline 4630 (2006)
Area of application	■ ype of substrates: biogenous substrates of any composition TS range: 0 %–100 %, whereupon – depending on the TS – a mixture-TS of the input of no more than 15 % is established through addition of water or other liquid substrates. ■ Particle sizes: maximum length = 3 cm (precrushing, if necessary)
Limitations of the method	None, since any decomposition inhibition by the substrates to be tested is also subject of the analysis
Need for research	■ Comparability to results of other methods ■ Upscaling to the industrial scale range (advantage IKTS: laboratory and pilot plant [10 m³] usable)

With the help of continuous fermentation tests, statements regarding long-term behaviour and process stability of anaerobic degradation processes can be made with respect to specific gas yields and gas qualities under the following conditions and aspects:

- Impact of organic loading rate and retention time
- Multi-stage and multi-phase process management
- Concentration and impact of inhibitors
- Impact of nutrients and trace substances
- Impact of mixing and feeding intervals

Since the bandwidth of possible test apparatuses and possible test objectives is very high, no standardised test methods for continuous fermentation tests are existent at the moment. Some indications regarding a possible test setup and the execution of continuous tests are provided in the VDI Guideline 4630. Analogous to the descriptions regarding the gas yield test, the execution of quasi-continuous fermentation tests in the laboratory-scale fermentation systems of Fraunhofer IKTS is described subsequently as an example.

Figure 73: Laboratory scale anaerobic digestion plants at the laboratory of Fraunhofer IKTS

Sampling and sample preparation

When taking a sample, a representative sample must be ensured. The recommendations of VDI Guideline 4630 regarding the sampling procedure have to be taken into consideration. In order to keep the substrate at a consistent quality, a suitable storage method is required. This can comprise a dry storage at room temperature (e.g. straw) or a storage at room temperature (e.g. straw) or a storage in a refrigerator (e.g. silages) all the way to frost storage of substrates that spoil easily (e.g. biowaste fractions). Long-fibre substrates (e.g. straw, grass) have to be comminuted (cutting mill, mincer) prior to conservation and/or feeding in order to avoid operational problems such as clogging and wrapping around the stirrer. A maximum fibre length of 3 cm has to be set for the test plants described below.

Materials and devices

The laboratory-scale fermentation systems in essence consist of the following components:

- Stainless steel reactor, gas-tight
- Heating (heating sleeve or a jacketed reactor with water heating system)
- Central stirrer with gas-tight passage through the reactor cover
- Measuring sensors for temperature and pH-value as well as – where applicable – redox-potential
- Feed opening with funnel (sealable, gas-tight)
- Bleeder (for cleaning and emptying)
- Testing opening with immersion pipe
- Gas extraction nozzle
- Quantitative and qualitative gas assessment (Gas meter Milligascounter® MGC-1 V3.0), gas bag, gas analysis device)
- Measuring and control technology

Based on experience, a thorough tightness testing of all system components should be carried out upon commissioning of the plant as well as at regular time intervals. The stirrer ducts in the reactor cover are particularly susceptible to leaks. Massive units consisting of the stirrer and the shaft sealing have been tested and proven at IKTS (c.f. Figure 74).

Figure 74: Continuous laboratory digestion at Fraunhofer IKTS

Method

Depending on the test plan, the reactors are started up with digestate of an industrial scale biogas plant or with the digested sludge of a communal sewage treatment plant. For the verification of a synchronism of the reactors, they are fed with the same input substrate over the course of several days or weeks. Subsequently, the conversion of the substrates is carried out depending on the test plan.

The necessary feeding quantities are calculated based on the intended organic loading rate, in consideration of the substrate characteristics and the reactor size. Additional feedings of water or the liquid phase of the separated reactor-internal digestate have an impact on the hydraulic retention time of the substrates. Taking the reactor size into account, these quantities also have to be selected in such a way that the hydraulic retention time corresponds to the test objective. Furthermore, the input solids content and thereby also the total solids content in the reactor is adjustable via the amount of liquids added. High input solids contents (> 10–12 %TS) have be avoided due to possible problems with the agitation and the resulting formation of a floating sludge layer. This critical value is essentially dependent on the design of the reactors and the stirrers.

By default, a daily feeding of the fermenters should be performed seven days a week. If a feeding on weekends is not possible, the following feed distribution has proven itself for the homogenisation of the gas production of maize silage or similarly decomposable substrates:

Table 21: Distribution of the feeding amounts of continuous fermentation tests

Day of the week	Monday	Tuesday	Wednesday	Thursday	Friday
Feeding amount relative to the average dose per day	160 %	80 %	80 %	90 %	90 %

Otherwise, in the case of equal feeding amounts every workday, severely increasing gas peaks occur over the course of the week since more and more biogas, that is generated by substrates of preceding feeding days, is adding up towards the end of the week. In addition, the reactors are best able to process a larger feeding load after a feeding break on the weekend.

For a default feeding, the substrates required in accordance with the test plan are weighed out in advance and provided in beakers. At the IKTS, the addition of the substrates is performed through manual feeding via a feeding funnel on the cover of the reactors. The extraction of digestate is performed twice a week in order to generate sufficient amounts for the further processing on the one hand, and in order to minimize the workload on the other. The amount extracted is – based on the fill level of the reactors to be measured – specified in such a way that an average fill level is maintained for all comparison reactors. The extraction of a sample from the fermenter is carried out with the help of an immersion pipe. The sample is pushed out of the reactor by positive pressure which is generated with nitrogen in the gas space. Fermentation substrate extracted in excess is returned to the reactor with the input substrates.

All input and output substances are analysed in accordance with the analysis plan immediately afterwards the sampling. With different reactor sizes, even more complex multi-stage procedures, such as a separate upstream hydrolysis or fermenters operated in series, can be replicated, too.

During the operation of the laboratory-scale reactors, pH value, temperature, redox potential and the amount of gas produced are recorded digitally. The biogas produced is captured in gas bags and is discontinuously tested for its composition (CH_4, CO_2, H_2S, O_2). For further analysis, a standardisation of the gas amounts in accordance with Equations 77–79 is carried out.

Pilot scale

The biggest disadvantage of tests at laboratory scale is the limitation of the test setup due to the substrate particle size as well as the substrate quantity. Therefore, the partial substrate amounts that are utilised relative to the type of substrate to be tested are not always representative. Furthermore, it is hardly possible to test the impact of the continuous operation effects of industrial scale devices, for example for the substrate pre-treatment, on the biogas production with reasonable expenditures. With the help of pilot plants for fermentation tests, for instance with a reaction volume of 10 m³, these weak spots in the significance of the laboratory tests can be compensated. The test results achieved are – in comparison to laboratory tests – significantly more practice-oriented with respect to the representativeness of the samples, the functionality of peripheral preparation technologies as well as to the assessment of the mixing behaviour in the reactor.

As a connecting link between systematic laboratory tests with a large variation potential and the industrial-scale application, a pilot plant represents an important stage for process developments.

Figure 75 shows, as an example, the pilot fermentation plant of Fraunhofer IKTS. The pilot plant consists of the following essential components.

- Substrate pre-treatment container with collection tank, screw extrusion press and bioextruder
- Mixing tank/separate hydrolysis stage
- Feeding screw with solids collection tank
- Technology container with fermentation substrate distribution, heating system and control system
- Two heated digesters with a digester volume of a total of 10 m³
- Dual-configured gas path with two gas reservoirs and condensate trap
- Gas purification (activated carbon)

Figure 75: Pilot biogas plant Fraunhofer IKTS

6.4 Continuous fermentation tests

Katrin Strach,DBFZ

Status	Development mostly completed, usable.
Associated standards	Modelled after VDI Guideline 4630 (2006)
Area of application	All biogenous substrates in the TS range of 0–100 %; the TS of the substrate mixture should not exceed 35 %.
Limitations of the method	The maximum particle size of the input is dependent on the digester geometry, the maximum organic loading rate on the mode of feeding.
Need for research	Up-scaling into industrial-scale operation; comparability to other methods (e.g. batch test); automatic feeding systems in order to achieve a continuous feeding; new types of feeding in order to improve mixing through; reproducibility of the tests

Continuous fermentation tests (here, fully mixed through stirred tank) are carried out in order to obtain statements regarding the fermentability of biogenous substrates under a continuous mode of operation. In this, data regarding gas yield, gas composition, the VS decomposition and any process disruptions and/or process limitation occurring can be determined under defined conditions. In the case of a sufficient test duration (at least 3 times the retention time), these tests can reflect the stationary state, i.e., the changes to the process parameters are zero.
Continuous tests are carried out in order to gain insights regarding:

- Specific gas yield in the case of
  - different organic loading rates and hydraulic retention times as well as multi-phase and/or multi-stage process flow
  - Different feeding regime, mixing through and fermentation temperature
  - Impact of additives (e.g. enzymes)
  - Application of digestion processes
- Accumulation and impact of inhibitors
- Material flows, mass balances and concentrations of substrates and inert substances in the stationary state
- Obtaining achievable degree of degradation of the organic components.

For continuous fermentation tests there are to date no standardised methods due to the large number of variants regarding the setup and execution. VDI Guideline 4630 provides action recommendations regarding select process flows and describes fundamental aspects. The following presentation describes the approach for continuous fermentation tests in the quasi-continuously operated stirred tank reactor at laboratory scale.

Substrate sampling and storage
The substrates must be obtained as a representative sample; this is the decisive prerequisite for the meaningfulness of the test. In addition to VDI Guideline 4630, rules and regulations from the waste industry or water analysis may also provide good action recommendations for the sampling, depending on the type of substrate. In order to keep the quality of the substrate constant, a preserving storage must take place. Dry substances can be stored dry at room temperature. Substances that spoil easily or contain highly volatile components should be subjected to a vacuum, sealed in transparent film, and put into cold or frozen storage. In the case of freezing, it must be taken into consideration that a disintegration of the cells may occur and as such a sort of pretreatment may take place. Otherwise, the input substance must, in principle, be placed in cold storage (+4 °C). A substrate pretreatment for easier handling in the test should be avoided, if possible, in order to ensure real-life conditions. Changes to the substrate, such as cutting or crushing, can result in a changed degree of degradation.

Devices and materials
A test setup for carrying out a continuous fermentation test includes the following components:

- PVC reactor (Figure 76)
  - Double-walled
  - Feeding nozzle with funnel (immersed)
  - Stirrer shaft immersion
  - Bleeder for retrieval of digestate
  - Gas extraction nozzle for gas discharge and pressure equalization
  - Inspection window
- Stirrer system/stirrer with gas-tight passage through cover
- Heating by means of circulation thermostat
- Gas meter
- Gas bag
- Gas analysis device
- Possibly measuring and control technology

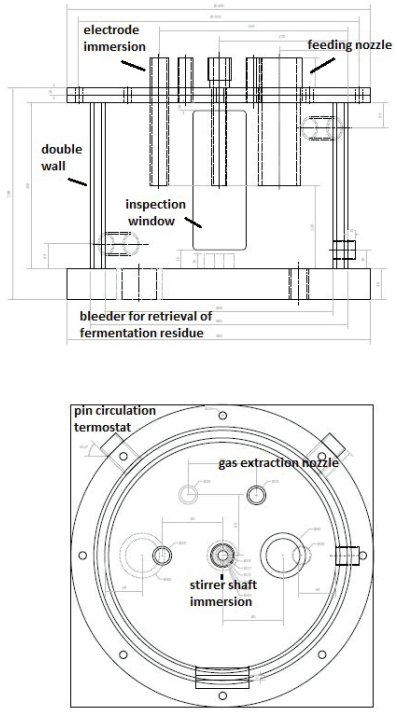


Figure 76: Technical drawing of a digester with a gross volume of 15 L

Figure 77: Laboratory for continuous fermentation tests at the DBFZ

The reactors are manufactured in double-walled construction. Located in the shell is water which circulates and is heated by means of a circulation thermostat. The stirrer system is installed as central stirrer system. Liquid fermentation mixtures are mixed through with paddle or pitched-blade stirrers. For highly viscous media, similar models of anchor stirrers have been developed. The test setup must be gas-tight. Prior to commissioning, a thorough leak test of the system should take place. Particularly susceptible to leaks are the cover gasket, the gas extraction nozzle and the hose connections. Figure 77 shows six biogas reactors in the complete test setup.

Execution method

The reactors are operated in the known temperature range for mesophilic and/or thermophilic mode of operation. A continuous mixing through is taking place in the rotational frequency range of 50–100 min^{-1}. In the case of paddle and pitched-blade stirrers, the stirring is carried out at a higher rotational frequency. A method that is gentler to the microbiology is the slower homogenisation with anchor stirrers.

At the start of the test, the digester is filled with digestate/fermentation mixture of a biogas plant which, if possible, is fed with substrates that are similar to those in the test plan. In the ideal case, the organic loading rate of the plant corresponds to that of the test plan. In accordance with the organic loading rate and retention time specified in the test plan, the substrate is weighed out fresh daily. Prior to feeding, digester content is retrieved via a spherical valve that is located at the bottom of the reactor. It is used to conduct process-accompanying analytics and/or disposed off as discharge. In this, it has to be taken into consideration that dead zones may occur in the discharge pipe and/or on the digester's floor in which solid components are deposited. To obtain a sample that is as representative as possible, it is therefore recommended to first return a correspondingly large amount of fermentation mixture and to retrieve the sample thereafter. The feeding is carried out at least once a day manually via the feeding funnel. To make dry or long-fibre substrates more flowable for the feeding, they can be diluted with fermentation mixture. In the case of very liquid input substances, the feeding is carried out by means of hose pump multiple times a day. It must be ensured that the fill level in the reactor remains constant in any case. This can be checked visually with the help of a scale at the inspection window or by means of a measuring rod via the feeding pipe. During the digestate retrieval and the feeding, the pressure equalization in the system must be ensured in order to avoid the entry of air or negative pressure at the gas meters. For this, a gas bag filled with biogas is attached to a bypass on the digester cover and the measurement of the gas amount is temporarily disconnected.

For process control, the pH value in the discharge is determined daily (or, even better, continuously in the process). Depending on the test plan and necessity, the parameters VOA, VOA/TIC, NH_4-N (TAN), TS/VS and/or COD and the individual acid spectrum are recorded 1–2 times per week. The biogas generated is fed through a gas meter in order to determine its volume. From there, it is passed into a gas bag and automatically checked for its composition by a process analysis system. In this, the concentration of the gases methane, carbon dioxide, oxygen and hydrogen is determined.

The adaptation to the desired process state (in the start-up phase or over the course of the test) – in the majority of tests, this is associated with an increase of the organic loading rate – is therefore conducted differently. In the case of well-researched input materials, the load can continuously be increased every day by 0.1 g_{VS}/L d. In this, an eye must be kept on the gas production and the concentration of organic acids in order to be able to intervene in case an overloading of the process occurs.

An optional approach is to increase the organic loading rate by 0.5 g_{VS}/L d and then wait until the process parameters remain unchanged over the course of approx. two weeks. Then, the next increase can take place. This variant is considerably more time-consuming.

If the digester is fed with a constant amount and composition of substrate and the fill level is maintained, the process approaches the stationary state. In the stationary state, the change of the process parameters is zero. Inert substances, in this, show the following temporal progression of the concentration in response to a step function.

Corresponding to Figure 78, the process is approaching the stationary state; depending on the test objective, the test can be terminated in the case of a sufficient approximation. However, it must be taken into consideration that the biological system may also have a delayed response to changes in the process. As such, the temporal forecast, for example, of process fluctuations due to deficiency symptoms (e.g. lack of trace elements) is very difficult.

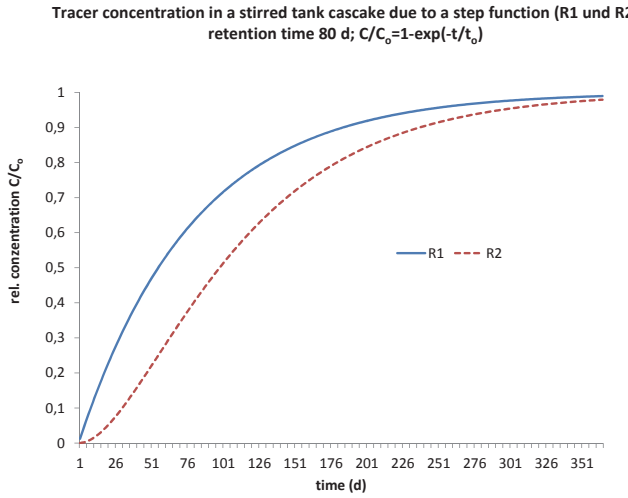


Figure 78: Tracer concentration in a stirred tank cascade due to a step function in the case of a retention time of 80 d (Source: DBFZ)

Weekend feeds

For a continuous process flow, a feeding should also take place on weekends and holidays. To shorten the work flow, on weekends the fermentation mixture is withdrawn only in exceptional cases. The correct fill level is then once again established the next workday.

Analysis

If it cannot be ensured that the gas production is measured every day at the exact same time, the gas meter reading and the corresponding time must be recorded twice a day for the calculation of the daily biogas volume produced. Between the two reading points, at least 30 min should have passed, the second reading must be carried out shortly before the daily feeding. Then, the biogas volume flow standardised to 24 h can be calculated with the following equations.

First, the average gas production is determined.

$$V = \frac{GM_1 - GM_2}{\Delta t} \quad (80)$$

V	Average gas production	(mL/h)
GM_1	Gas meter reading at first reading	(mL)
GM_2	Gas meter reading at second reading	(mL)
Δt	Time difference between 1st and 2nd reading	(h)

Since the feeding does not take place every day at the same time, the time difference to 24 h is taken into consideration.

$$\Delta t_{24} = 24 - \Delta t_F \quad (81)$$

Δt_{24}	Time difference to 24 h	(h)
Δt_F	Time difference between the point in time of feeding on the current test day and the point in time of feeding on the preceding test day	(h)

The conversion calculation to standard pressure (101.325 kPa), standard temperature (273.15 K) and the standardisation to dry gas are carried out by means of the Antoine equation (Bierwerth 2011).

$$V_{STD} = (V \cdot \Delta t_{24} + GM_{2\text{-}2}) \cdot \frac{\left(\left(p_a - \overbrace{10^{7.19621 - \frac{1730.63}{233.426 + T_a}}}^{[kPa]}\right) + \Delta p_{GM}\right) \cdot 273.15K}{101.325kPa \cdot (273.15K + T_a)} \quad (82)$$

$GM_{2\text{-}2}$	Gas production since the last feeding according to the gas meter reading	(mL)
V_{STD}	Standardised gas volume	(mL (STP)/d)
p_a	Ambient pressure	(kPa)
Δp_{GM}	Pressure loss of the gas meter	(kPa)
T_a	Ambient temperature	(°C)

For the calculation of the TS decomposition, the masses of the input and the discharge as well as the TS contents are put in relation to one another. Since some substrates and digestates contain volatile substances that get lost in the determination of the total solids in accordance with DIN 12880, the total solids are corrected with the concentration of volatile organic acids.

$$TS_{decomp} = \frac{(m_{TS,\,inp} + c_{inp}) - (m_{TS,\,dis} + c_{dis})}{(m_{TS,\,inp} + c_{inp}) \cdot 100} \quad (83)$$

TS_{decomp}	Decomposition of the total solids	(%)
$m_{TS,dis}$	Mass of total solids of the discharge	(g)
$m_{TS,inp}$	Mass of [total solids] of the input	(g)
c_{inp}	Concentration of the volatile organic acids in the input (inlet)	(g/kg_{ww})
c_{dis}	Concentration of the volatile organic acids in the discharge	(g/kg_{ww})

The mass of the output can – at constant fill level – be determined through weighing. A calculation of the digestate with the following equation is optional.

$$m_{biogas} = \left(M_{CH_4} \cdot \frac{V_{STD} \cdot c_{CH_4}}{V_m}\right) + \left(M_{CO_2} \cdot \frac{V_{STD} \cdot c_{CO_2}}{V_m}\right) + \left(M_{H_2S} \cdot \frac{V_{STDd} \cdot c_{H_2S}}{V_m}\right) + m_{WV} \quad (84)$$

m_{biogas}	Mass of the biogas produced	(g)
$M_{(x)}$	Molar mass of the respective index	(g/mol)
V_m	Molar volume of the ideal gas	(L (STP)/mol)
$c_{(x)}$	Concentration of the respective index	(%)
m_{WV}	Mass of the water vapour in the biogas	(g)

The fed and withdrawn masses of volatile solids are utilised for the calculation of the VS decomposition.

$$VS_{decomp} = \frac{m_{VS,inp} - m_{VS,dis}}{m_{VS,inp} \cdot 100} \quad (85)$$

VS_{decomp}	VS decomposition	(%)
$m_{VS,inp}$	Mass of VS in the input (inlet)	(g)
$m_{VS,dis}$	Mass of VS in discharge	(g)

The correction of the TS content also has an impact here. Therefore, the volatile solids are corrected with the concentration of the volatile organic acids for the decomposition of the organic substance.

$$oS_{decomp} = \frac{(c_{inp} + m_{VS,\,inp}) - (c_{dis} + m_{VS,\,dis})}{(c_{inp} + m_{VS,\,inp}) \cdot 100} \quad (86)$$

oS_{decomp} Decomposition of the organic substance (%)

For the assessment of the deviations of parallel test, the data of the gas production of two digesters operated in parallel was utilised. In the test, the fermentation behaviour of renewable resources in the case of increasing organic loading rate was investigated. Figure 79 shows the gas production and the organic loading rate. In comparison to this, Figure 80 shows the cumulated gas production of the same test. The results of the gas production of the whole test period of 210 days (n = 210) were analysed. When comparing the average values of the production of gas of both reactors, a relative standard deviation of the daily gas production of 1.2 % results.
The relative standard deviation of the daily gas production of the individual digesters is 6.2 %, on average. For this, the values starting on the 126nd test day (n = 84) were utilised. This period is considered to be a stable state. If during a test significantly higher relative standard deviations occur here, a systematic error may be the reasons for that.
The quality of the continuous fermentation tests depends – in addition to the measuring errors of the measuring devices – essentially on a thorough and constant test support.

If the deviation of the average gas production of the single (or multiple) reference system(s) is to be assessed, statistical test methods must be utilised for this (e.g. Tukey Kramer).

Figure 79: Gas production and organic loading rate of two continuous fermentation tests in parallel test (Source: DBFZ)

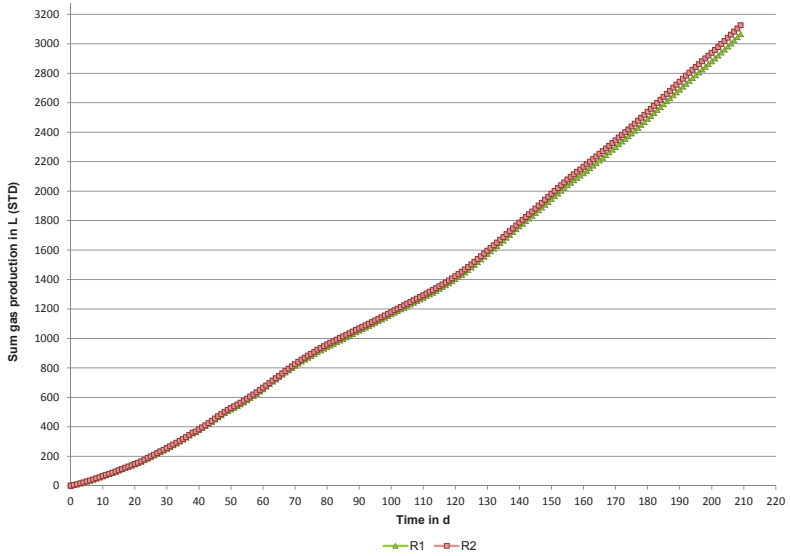


Figure 80: Cumulated gas production of two digesters in parallel operation (Source: DBFZ)

6.5 Microbiological tests

Tobias Lienen, Hilke Würdemann, GFZ

Status	The cultivation-independent molecular biology methods presented below are at the state of the art of science. Nevertheless, these methods are being optimised constantly and new high-throughput methods are being developed with which within an ever shorter period of time larger numbers of sample can be tested and characterised in even more detail.
Associated standards	The principle of the DGGE, qPCR and FISH is standardised. However, the protocols must be adjusted to the substrate mixtures to be tested. The methods are in application in a broad range.
Area of application	The methods can be applied to different substrate spectra. Limitations with respect to the meaningfulness of the test results exist insofar as a lot of the active microorganisms in biogas plants haven not yet been sufficiently characterised. This makes the interpretation of the test results and the recommendation of countermeasures more difficult.

Need for research: Comparability to results of other methods;
A short time ago, new high-throughput methods such as the Next Generation Sequencing (NGS) were established which allow for a qualitative and quantitative analysis of the sample in a single step and which make it possible to conduct comprehensive analyses at the same time. The affiliation of the microorganisms to specific functions, however, often is only insufficient since a lot of bacteria and archaea have not yet been cultivated and a physiological characterisation has not yet been performed. However, knowledge regarding the physiology and biochemistry of organisms is decisive in order to optimise biogas plants and to uncover the reasons for process disruptions. Therefore, in addition to cultivation-independent methods, cultivations of microorganisms occurring in plants as well as their characterisation are necessary. There still is a significant need for a correlation of chemical and biological process data for the enhancement of the understanding of the process with the objective of process optimisation, of improvement of the process stability, and of a performance increase.

The microbial composition and the number of cells of individual groups of microorganisms provide indications of the stability of the biogas production process and can provide indications of the causes of process disruptions. The four-stage biogas production process from hydrolysis via acidogenesis and acetogenesis to methanogenesis is carried out by different microorganisms that must encounter optimal conditions in order to ensure a stable biogas process. The cultivation of individual microorganisms from an environmental sample is time-consuming and costly. To obtain data regarding the composition and number of the microorganisms rather quickly, molecular biology techniques can be used. This includes so-called "fingerprint" analyses such as the PCR-DGGE (polymerase chain reaction – denaturing gradient gel electrophoresis) and methods for quantification such as the fluorescence *in situ* hybridisation (FISH) or the quantitative polymerase chain reaction (qPCR). In addition to these three techniques which will be covered in more detail below, there are numerous other molecular biology methods to better examine the microorganisms (Rittmann et al. 2008).

Polymerase chain reaction – denaturing gradient gel electrophoresis

The polymerase chain reaction – denaturing gradient gel electrophoresis (PCR-DGGE) serves for the molecular biology screening of an environmental sample such as the sample from a biogas reactor (Lerm et al. 2012). At first, the whole DNA (deoxyribonucleic acid), that consists of a specific sequence of bases, is isolated from the sample. In this, different techniques can be used in which the cell lysis is based on different methods. For example, the cell lysis can be performed with heat, mechanically via small ceramic spheres, or via

reagents. Often, commercially available DNA extraction kits, in which a defined protocol is followed, are used for this. Nevertheless, these protocols need to be optimised in most cases, depending on the type of sample and the microorganisms to be analysed (Weiss et al. 2007). In the polymerase chain reaction, a specific section of the overall DNA is amplified. This section is particularly well suited for the identification and the derivation of the capabilities of the organism found based thereupon. The amplified DNA fragments are subsequently applied to a denaturing acrylamide gel. This gel contains a urea gradient which must be adjusted to the microorganisms to be analysed. The gel is connected to an electric field and due to the negative charge of the DNA molecules, these migrate in the gel in the direction of the plus pole. Depending on the sequence of bases of the DNA fragment, the double strand is denaturing more or less, and the further or the short the DNA travels in the denaturing gel. A banding pattern shows on the gel in which, in an ideal case, each band corresponds to one microorganism. The bands are sequenced and the microorganisms and/or their closest relatives can be identified via a comparison to a database freely accessible on the Internet (e.g. Basic Local Alignment Search Tool, BLAST). The affiliation helps in deriving the role of the different organisms in the digester and their significance for process stability.

Quantitative PCR

The quantitative PCR relies on the same principle as the polymerase chain reaction mentioned. Here, too, a specific section of the isolated DNA is amplified. In contrast to conventional PCR, in which only a semi-quantitative analysis is possible, the amplified DNA can be quantified in the quantitative PCR so that the amount of DNA from specific microorganisms can be determined. For this, a fluorescent dye that binds to the DNA is added to the reaction. Alternatively, specific probes may also be used. The more DNA source material is available, the sooner the amplication can be detected so that a quantity comparison becomes possible. In this, the quantification can be carried out absolute, with the help of an internal standard, or relative. In the case of relative quantification, the DNA amount of the target microorganism is put in relation to the DNA amount of the total bacterial or archaeal DNA. Herein, differences in the DNA extraction of several samples can also be relativised. The quantitative PCR is very well suited to compare already characterized microorganisms of different samples in their quantity. In addition to the DNA, RNA (ribonucleic acid) may also be analysed by means of quantitative PCR. This can be meaningful if microorganisms can be detected in a sample, yet there is no certainty whether these are also metabolically active or whether only the DNA was introduced. The RNA is an evidence for the activity of organisms. Differences in the number and activity of microorganisms can be reasons for process disruptions and/or indicate them. A reduction of the activity of methanogenic organisms, for instance, is directly related to a lower biogas yield.

Fluorescence *in-situ* hybridisation

The fluorescence *in-situ* hybridisation (FISH), too, is a method for the determination of the number of metabolically active microorganisms. In this, a specific probe coupled with a fluorescent dye binds to the ribosomal RNA (rRNA) of the microorganisms. Subsequently, the number of cells is determined with a fluorescence microscope. With this method, primarily metabolically active cells are detected since the rRNA content correlate to the metabolic activity. With the help of specific probes for different physiology groups, the

microbial biocenoses in dependence on different process conditions can be analysed. The detection of the activity is an important parameter for the assessment of biological processes since under disadvantageous conditions the activity changes much quicker than the number of cells. A lower cell division rate results from the reduction of the metabolic activity, which in continuously operated reactors, such as in many biogas plants, quickly leads to a reduction in the number of cells due to eluviation processes.

6.6 Determination of the residual gas/residual methane potential

Hans Oechsner, State Institute of Agricultural Engineering and Bioenergy, University of Hohenheim

Status:	The method was tested, and a standard is currently under development as a part of the VDI Guideline 4630 (Grünndruck 2014). An interlaboratory comparison is currently taking place for the validation of the method.
Associated standard	VDI 4630
Substrates	The method is applicable for solid and liquid digestate.
Limitations of the method	The setting in relation to an amount of methane generated presupposes the estimation of digestate mass flows and biogas yields at the biogas plant.
Advantages:	Easy to implement
Need for research	Relation between the residual gas potential and emissions in the case of open storage of digestate (i.e., fermentation residue); precise mass balance of substrates.

The biogas plants, in which renewable bioresources are used as feedstock (i.e. substrate for fermentation), seek to explore ways in achieving economic viability and maximizing the utilisation of the energy potential of the feedstocks. Biogas plants select the fermentation process (i.e., plant design and operation mode) depending on the characteristics of the feedstocks used. The feedstock utilization rate varies significantly with the design (e.g., single stage and double stage) and operational parameters (e.g., pH, temperature, organic loading rate, and retention time among others).
The following questions can be answered by determining the residual gas potential of the digestate from the biogas plant:

- How much biogas/methane potential of the substrate is not utilized following the digestate leaving the gas-tight section of the biogas plant?
- How much economic benefit (if any) does the cover of the digestate storage unit have?
- How large are the maximum emissions potential and the corresponding environmental impact? For additional information, refer to VDI Guideline 3475, part 4.

Depending on the objective of the analysis, the digestate sample could be incubated at different temperature:

- To determine an available energy potential (i.e., economic aspects), the fermentation of the digestate at a mesophilic temperature (37 ± 2 °C) or at a thermophilic temperature (50 ± 1 °C) is recommended. If possible, a temperature range should be selected based on the operating temperature of the fermenter from which the digestate sample was collected. Operating at similar temperature allows a rapid gas production, which is comparable to the gas production from the substrate in the fermenter from where the digestate sample was taken.
- In accordance with VDI 3475 part 4, the residual gas potential of the digestate can also be estimated at 20 ± 2 °C. Additionally, this can be used to determine the emissions potential, given that the digestate are stored in an open and cold storage tank. Therefore, it is assumed that lower temperature prevail in digestate storage units than in fermenter systems. However, currently there is no any scientific evidence that establishes a close relation between residual gas potential measurement and the actual emissions from the digestate of the evaluated plant.

Sampling from the biogas plant and data collection
The digestate samples must be collected from the biogas plant in order to determine the residual gas potential. This method requires diligence and compliance with the following procedures in order to obtain representative digestate samples for the selected biogas plant.

The digestate that leaves the last fermentation stage may, depending on the mode of operation and the feedstock used, be inhomogeneous and may also vary over the course of time. Therefore, the sampling should not be conducted only at a certain point of time, but rather for multiple times per day (e.g., three times). Additionally, it should preferably be for various days over the week. If it is not possible, for technical reasons, to withdraw samples at multiple times over a day or week, the fermenter content must be thoroughly homogenised prior to the retrieval of the sample. It is also possible to analyse the digestate from solids fermentation plants and the freshly separated solids from liquid fermenters. In the case of the latter, however, the substrate flow must be thoroughly recorded in order to make a statement about the digestate generation potential of the biogas plant. Immediately after its retrieval, the digestate sample must be cooled to a temperature of approximately 4 °C. Such cooling will quickly stop any further decomposition of the digestate. Later, the digestate samples which are collected multiple times over a day or week are mixed together to create a cumulative sample. If sample contains fibre components, a coarse cutting/crushing can ensure that a homogeneous and representative sample is available for the fermentation test.
The residual gas potential is usually based on the amount of biogas and the methane yield of the biogas plant in a stationary mode of operation. Therefore, in addition to taking digestate samples for analysis, the amount of biogas and methane generation at the plant

must also be recorded. Besides, the results of the digestate analysis must be put in relation to the volume of the digestate flow. Thus, it is necessary to determine the volume of digestate that leaves the plant section under review (i.e., overflowing fermentation mixture/fermentation residue). The sample of the fermentation mixture (i.e., digestate) represents a mixture of a long period with respect to the feeding. In the case of stirred tank reactors, an approximation of the stationary state is achieved after approximately three retention times. It is therefore recommended that the data from three retention times be used to calculate an average amount of gas and digestate produced per day.

The volume of fermentation mixture/digestate can be determined through calculation if the measuring equipment does not quantify it. For example, the mass of input substrate (i.e., mass flow of substrate into the biogas plant) and the mass of the biogas produced are recorded. Later, the mass of the biogas generated is subtracted from the mass of the input substrate to derive the amount of the fermenter content/digestate (see in text below). The loss of mass caused by the biogas production should not be neglected since it can, for example, constitute up to 30 % of the input mass flow in the case of maize silage.

Fermentation approach

The representative digestate sample should be analysed for the contents of total solids (TS), volatile solids (VS), volatile fatty acids (VFAs) and pH value. The required amount of digestate is added into the fermentation test apparatuses without adding inoculum. The fermentation test should be replicated no less than three times.

If digestate from solid-state digestion plants and solids from liquid-state digestion plants are analysed after separation, tap water should be added in order to reduce the total solids content of the mixture below 10 %. The addition of water is necessary in order to obtain an optimally fermentable and stirrable mixture in the fermentation test apparatus. The fermentation test apparatus should be heated to the desired temperature. Additionally, a constant temperature and homogenisation conditions should be maintained throughout the fermentation test period as described in the gas yield test (batch) (see ch. 6.2). The produced biogas should be quantified for its volume and methane content, which later provides the information on the temporal gas production. The fermentation test for the determination of the residual gas potential should be conducted over a defined period of 60 days, as stated in VDI Guideline 3475.

Analysis

The data can be analysed analogous to the batch fermentation test. It is simplified by the fact that no inoculum is used. A gas production graph should be created to display the development of the specific biogas/methane production throughout the test period.

The methane yield (L (STP) methane/kg VS) from the digestate is less than the methane yield from the feedstock/raw substrate. Moreover, the daily average amount of methane generated at the biogas plant from where the digestate sample was collected completes the analysis. Currently, there are no reliable functioning system for the determination of the amount and quality of produced biogas in all biogas plants. However, the average amount

of electricity generated per day can be used to estimate the amount of methane produced at the biogas plant. The latter must be combined with the efficiency of the CHP unit in order to obtain the amount of methane utilised daily. In this estimation, the amount of gas that passes through the gas flare is not taken into consideration, and may lead to a corresponding overestimation of the residual gas potential.

The following data should be collected from the biogas plant under study:

- Biogas/methane production [m³ (STP) methane/d] at the biogas plant over a period of three retention times. This is done through recording data (e.g., standard biogas amount and methane content) at the biogas plant. Alternatively, the daily electricity production [kWh/d] can be recorded for estimating the amount of methane production per day. The electricity generation data in combination with an electrical efficiency of the CHP unit provides an estimation of the daily methane production [m³ (STP) methane/d]
- Daily discharge of digestate and its VS content from the respective last gas-tight covered fermenter/digestate storage unit [t fermentation residue * VS [%] / 100]. The mass flow can be estimated via the mass of the substrate input (i.e., liquid and solid substrates) per day minus the mass of the biogas produced daily.

$$m_D = m_S - m_{BG} \quad [m^3] \tag{87}$$

m_D	Average mass flow of digestate per day	(kg/d)
m_S	Average mass flow of substrate input per day	(kg/d)
m_{BG}	Average mass flow of the biogas generated per day ($m_{BG} = V_{BG} * \rho_{BG}$)	(kg/d)
V_{BG}	Biogas rate, standardised volume of the biogas generated per day	(m³ (STP)/d)
ρ_{BG}	Density of the biogas (= 1.25 kg/m³ at 55 % methane content, dry gas, standard conditions)	(kg/m³)

These data are put in relation to the following values determined in the fermentation test: Specific methane yield from the residual gas analysis [m³ (STP) methane/kg VS].

$$V_{RG} = V_{SRG} \cdot VS_{RG} \quad [m^3] \tag{88}$$

V_{RG}	Average methane potential of the digestate from the biogas plant per day	(m³ (STP)/d)
V_{SRG}	Specific methane yield from the residual gas analysis	(m³ (STP)/kg VS)
VS_{RG}	Average amount of volatile solids that exit the last gas-tight tank of the biogas plant per day	(kg/d)

From this the residual gas potential of the biogas plant in [%] is determined using the following formula:

$$RGP = \frac{V_{RG}}{V_{BGP}} \cdot 100 \quad [\%] \qquad (89)$$

RGP	Residual gas potential	(%)
V_{BGP}	Average methane production of the biogas plant per day	(m³ (STP)/d)

6.7 Determination of the maximum possible biogas output of substrates by disintegration with sodium hydroxide

Björn Schwarz, Fraunhofer IKTS

Status	The method described is based on a regulation from the sewage sludge sector (among others, published through Müller et al. 2000), in which the maximum disintegration potential of sewage sludge is determined via the analysis of the dissolved COD. In this, the samples are diluted 1:1 with 1-molar NaOH and treated for 10 min at 90 °C. This method was transferred to vegetable substrates at IKTS and currently represents an interim processing state.
Area of application	The method can be utilised for the determination of the maximum disintegration potential (release of dissolved COD) as well as for the determination of the maximum possible biogas production from biogas substrates such as renewable resources, residues (e.g. straw) or digestates.
Advantage of the method	The maximum decomposition and/or energy potential possible by means of anaerobic conversion is determined without including fractions that are not biologically available (such as lignin). Any barriers within the substrate structure are removed and all biologically available substrates are made available to be decomposed. The method can easily be carried out as pretreatment method for comparative fermentation tests (low technical expenditure).
Need for research	In the test of model substrates, it was determined that for the framework conditions selected to date for some groups of substances losses in the energy content were caused by the method of disintegration. A possible cause is the generation of biologically non-usable interim products. For real substances, these effects are difficult to estimate due to the heterogeneous composition. To eliminate these uncertainties, systematic investigations in combination with analyses regarding the material composition and structure would be necessary.

Devices and chemicals

- Analytical scale
- Heatable magnetic stirrer plate incl. magnetic stir bar
- pH-meter
- Beaker
- Laboratory fume hood
- 1-molar NaOH
- 1-molar HCl (for neutralisation – other chemicals may also be possible)
- Distilled water

Execution method

For the determination of the dissolved COD, it is necessary to exactly record – in addition to the empty weight of the beaker – all weighed-in materials as well as the final weight prior to the COD determination so that evaporation and dilution effects are known.

For the determination of the gas potential of the fully disintegrated sample, the amount of sample should fit the size of the batch reactor (gas yield test) in order to avoid unnecessary splitting of samples and losses. In this case, only the exact weight of the sample must be recorded since the added chemicals as well as any evaporation losses do not have any impact on the result of the gas yield test.

The sample and the 1-molar NaOH solution are mixed in the beaker at a ratio of 14 g NaOH solution to 1 g of VS of the substrate (for instance, approx. 63.2 g NaOH [1-molar] to 16.8 g

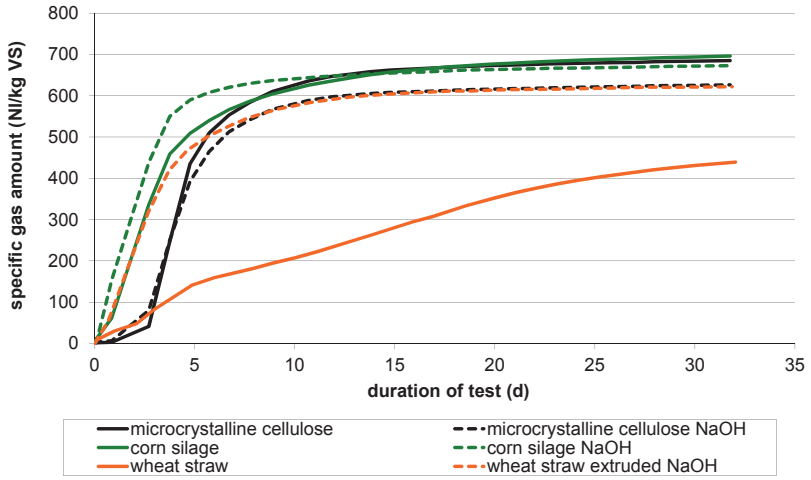


Figure 81: Sample gas yield graphs before and after a disintegration by means of NaOH (Source: IKTS)

leaves [31.7 % TS, 84.4 % VS]). For an improvement of the stirrability of the preparation that may be necessary, distilled water can be added (e.g. 20 g). Under constant stirring, the beaker is heated on a heatable magnetic stirrer plate within approx. 15 min to at least 90 °C. A temperature of 90–95 °C is maintained for 20 min. During this disintegration, evaporation losses can be minimised through heat-stable and humidity-stable covers. The vapours generated are potentially noxious and should be exhausted (working under a hood is recommended). Subsequently, the preparation is cooled down to room temperature and neutralised to pH-values of 6–8 by means of 1-molar hydrochloric acid.

The analysis of the dissolved COD is performed through pressure filtration of the sample via 0.45 µm as well as by means of cuvette tests.

For the determination of the gas yield, the complete preparation is transferred timely into a batch reactor. To minimise losses, a flushing with a small amount of distilled water can be performed.

Sample results and open questions

Via an analysis of the development of the gas yield graphs, it can be determined whether it is worthwhile to carry out a substrate pretreatment for the substrate being tested. By means of a disintegration via NaOH, for example, only an acceleration can be observed in Figure 81 for maize silage after a 30 d retention time, but no increase in yield. This roughly corresponds to the results of numerous tests regarding the pretreatment of maize silage that did not achieve significant rates of increase under the ideal conditions of the laboratory fermentation tests. For the disintegration of straw, on the other hand, it was possible to achieve a significant improvement of the gas production velocity and yield. This is a clear indication of the aptness of disintegration methods in the area of residues containing lignocellulose.

But generally there are still uncertainties in the interpretation of the effects of the disintegration by means of NaOH since obviously lower gas yields are obtained for select groups of substances than without the disintegration. One example is the micro-crystalline cellulose, which is also depicted in Figure 81. Subsequent to the disintegration by means of NaOH, about 10 % lower gas yields are obtained which also were evident in case of shorter treatment times and in case of lower utilisation of NaOH. A return of condensed vapours produced during the disintegration led to the same result. Possibly, individual groups of substances or components of them are converted into no longer usable interim products during the disintegration. Tests with peptone (protein model substrate) and glucose have also resulted in reductions of approx. 15 % and 50 % regarding the gas yield by the disintegration by means of NaOH. To which extent these reductions also occur in real substrates has not been investigated yet systematically.

7 Calculation and assessment methods (parameters that describe processes/ meta-parameters)

7.1 SWOT analysis

Christof Heußner, Oliver Kugelstadt, Tobias Bahr, Klaus Fricke, TU Braunschweig

The SWOT analysis (S-Strength, W-Weaknesses, O-Opportunities, T-Threats) is a tool that originates from the area of strategic management but is also used for formative evaluations and quality developments. This analysis method serves for deriving suitable strategic solution alternatives for the achievement of previously defined objectives from the strengths or weaknesses of an organisation or enterprise (internal view) and the opportunities and risks (external view), and presenting them clearly. The internal analysis is intended to make it possible to recognise strengths and weaknesses of a company – or, in this case, of a process. These result from the competencies, skills (and/or technical capabilities) and resources of the unit under review.

In the course of the external analysis, the closer and more distant environment of this unit is investigated. Only through the alignment with the environment (and/or the framework conditions) can strengths and weaknesses be weighted subsequent to a stocktaking and transitioned into options for action (Lombriser & Abplanalp 1998).

The objective of the analysis consists of working out to what extent the strengths of the currently applied technologies and methods for the utilisation of the available biomass are suitable and – given the background of the current circumstances and those expected in the future – relevant. As a result, it becomes possible to react to the current and future needs based on the energetic and material utilisation of existing biomass. This objective is achieved by reworking the aforementioned sub-areas of the SWOT analysis.

The SW portion (strengths and weaknesses) deals with the internal factors. These are the technical options and development potentials of the method under review. Here, there are numerous forms of development that essentially result from the specifics of the individual case which, however, due to the viewpoint from which it is viewed, also feature a lot of commonalities. Therefore, a prior identification of decisive factors (so-called critical/ key success factors), (Panagiotou 2003), is helpful for the SW analysis. In relation to these factors, strengths and weaknesses are then checked. These factors or objectives must be defined before working on the actual analysis. In doing so, concrete and non-abstract objectives must be selected. Abstract objectives such as an increase of the energy efficiency must be put into concrete terms based on test results and must be evaluated by means of existing literature references and own assessments (Lombriser & Abplanalp 1998). Furthermore, it must be ensured that all identified strengths and weaknesses are relative. They only gain meaningfulness through a benchmarking against other methods or utilisation options. In the case of the energy efficiency, the method is – differing from traditional SWOT analyses – applied to a multitude of technical solutions and processes. The more important it is therefore to find a suitable benchmark based on which the state of develop-

ment (in particular the energy efficiency) of the individual processes can be categorised. For this, an objectivisation of the rather subjective factors of the individual results of the SWOT analyses is necessary.

1. Comparison of the respective processes to the best assessed plant on the market (real comparison);
2. Comparison of the respective processes to a hypothetical best practice plant by combining the best assessed sub-processes of multiple plants available on the market (hypothetical comparison – state of technology);
3. Comparison of the respective processes to a hypothetical best practice plant through idealised combination of optimised sub-processes taking into consideration the latest state of research (hypothetical comparison – state of research);
4. Comparison of the respective processes with a hypothetical theoretical approach. Reference here is the energy content as target parameter (100 % efficiency) – exploratory comparison of fundamental utilisation variants (thermal utilisation, material utilisation, fermentation, ethanolisation)

The OT portion (opportunities and threats) includes trends and changes that result from the general and specific circumstances for the respective process. To be considered to be external factors they must be those on which a company (in the case at hand, a plant operator or technology provider) does not have any direct influence. This could, for instance, be statutory regulations, limit values, or funding programmes (Lombriser & Abplanalp 1998):
Criteria are defined for both the internal as well as the external analysis that ensure a standardised approach and a comparable method response even in the case of the analysis of different processes for biomass utilisation (e.g. composting, fermentation, thermal utilisation). A compilation of superordinated criteria can be found in Table 22. These criteria are in turn refined by subordinate criteria (c.f. R&D potential and ecological framework conditions). During the collection of the criteria, often the problem arises to what extent individual criteria are being categorised based on their importance and/or how important the criterion is with respect to the objective of the analysis. Furthermore, a multitude of criteria renders the subsequent analysis more difficult. In the categorisation of the criteria, SWOT analysis of the University of Warwick may serve as an exemplary model to follow (Dyson 2004). Here, the strengths, weaknesses, opportunities and threats are collected and subsequently assessed with a corresponding point scale. If a criterion does, for example, not reach a certain number of points, it is removed from the catalogue of criteria.
From the actual SWOT analysis itself, no concrete measures are decided or implemented but rather first only states are described in relation to best case scenarios and in a subsequent step strategies are developed. To transition these strategic approaches into concrete measures, typically a concrete action plan is required (Dyson 2004). The plants to be reviewed are technically complex and feature different stages of treatment and levels of utilisation. For the identification of optimisation measure that are as effective as possible, the processor has permission to compare individual process steps (where applicable: individual aggregates) to the state of technology and derive optimisation potentials. The data necessary for this are captured at the plants in parallel to the SWOT analyses, specific values are bundled into groups and summarised in evaluation indexes.

Table 22: Working draft of a catalogue of criteria for the internal and external part of the SWOT analysis (incomplete)

Criteria catalogue strengths/weaknesses analysis	Criteria catalogue opportunity/risk analysis
■ General process characteristics: • Temperatures • Pressures • Corrosivity • Residues • Heat integration potential ■ Offer potential (products, services) ■ Distribution (collection and distribution) ■ Market communication ■ Prices and conditions ■ Procurement (educts, aids and operating resources) ■ Finances ■ Personnel ■ Management and organisation ■ R&D potential: • Amount of R&D investments • R&D know-how • Technical equipment • Patents and licenses • Access to external R&D sources/cooperations	■ Market structures ■ Competition ■ Statutory/state framework conditions ■ Societal framework conditions ■ Procurement of raw material (prices and supply) ■ Energy prices ■ Other economic conditions ■ Technological/technical development ■ Ecological framework conditions: • Air quality management • Water pollution control • Soil conservation • Waste disposal and avoidance • Energy utilisation • Utilisation of raw materials

7.2 Calculation of plant/process indexes (optimisation potential of individual process steps)

Christof Heußner, Oliver Kugelstadt, Tobias Bahr, Klaus Fricke, TU Braunschweig

Plant and process indexes serve for the assessment and comparison of individual process areas, plant components or treatment concepts and thus support the working out of optimisation potentials with respect to the treatment objective. For this, specific energy consumptions of fermentation and composting plants are recorded and weighted.
Based on the recorded data, the different plants are assessed individually (part A). For this, it is necessary to subdivide the process steps within the plant into main process stages in order to allow for a delineation from other process steps (part B). It is, for example, defined where the area of the feed ends and where the sorting starts.
The data of the separated process steps are standardised to a functional unit (e.g. 1 Mg total solids) in order to ensure comparability, and specific indicators are derived (e.g. specific thermal and/or electrical energy demand). Since it is to be expected that the specific energy consumption of large plants is lower than that of small plants, initially a categorisation based on the plant throughput is performed to establish comparability and to avoid distortions of the results. A final classification and categorisation is conducted after the recording of the underlying data.
Parts of the plant that do not correlate to the mass throughput are reviewed separately. This includes rooms and any buildings that cannot solely and directly be allocated to the

plant operation such as the location's fire brigade, the administration, the central weighing station, the canteen, staff buildings, etc.
For the mass balance, the mass flows generated based on the type of plant are taken into consideration. This includes, for example, process water and exhaust air as well as the input and output of the processed substrate (part C). Furthermore, particularly process-relevant individual substances (special valuable materials or harmful substances) as well as valuable materials produced (for instance compost and other fertiliser substituents) are recorded for later analysis since they constitute an impact on the life-cycle assessment efficiency of the respective plant or process due to energy/raw material savings and/or consumption (in the case of substitution or utilisation of industry products that are using a lot of energy or of fossil resources). For the energy balance, the consumption data of thermal, electrical and chemical energy are recorded separately for individual aggregates or process steps.
The data determined in parts A–C is compiled into a data matrix. From this matrix, the minimum and maximum values for each mass and energy balance are determined. As such, the lowest individual value of the gas yield of all plants recorded is utilised as pessimum of the gas production potential and the highest one as optimum. With the help of these minimum and maximum values, an energy and/or mass indicator is calculated for each process step. Via this indicator it is possible to categorise the results of the energy and/or mass balance of the process steps under review and to compare it to results of energy and/or mass balances of the same process of other plants (part D). Even for the same plant and the same process, different energy efficiencies may be achieved, for instance dependent on the current load or quality of the input materials. The processes may feature a different "minimal load capability" and may, for instance, become very inefficient at a reduced load. This is, for example, relevant in the case of a pronounced seasonality in quantity and/or quality of the input material. These differences are taken into consideration in the assessment, too.
Energy and mass indicators are aggregated into a process indicator in a next step. This indicator allows for both the comparison of the energy efficiency of the same process in other plants as well as the relative comparison to other processes in the same plant (part E). With an analysis, plant-internal optimisation opportunities can be determined on the one hand, while, on the other, allowing for comparisons to the same process in different plants. In a last step, a plant index is determined from the process indicators calculated (part F). The plant index allows for the classification of all systems relative to one another, wherein the model makes it possible to compare different types of plants (e.g. biowaste fermentation, composting) with one another. The model is designed such that through the utilisation of the specific minimum and maximum values of each energy and mass balance for each process step and standardisation is achieved that is independent of the type of plant analysed.
Systems with little or a lot of technical equipment remain in detail comparable to one another since each individual process step is compared to the same process step in other plants. In the calculation of the evaluation index, the lower number of individual plant-specific indicators for smaller or more simple plants is taken into consideration. Depending on the scope of the analysis, energy and mass balances of individual parts of the plant may be recorded down to individual aggregates and thus may feature a previously unachieved acuity and depth of analysis.

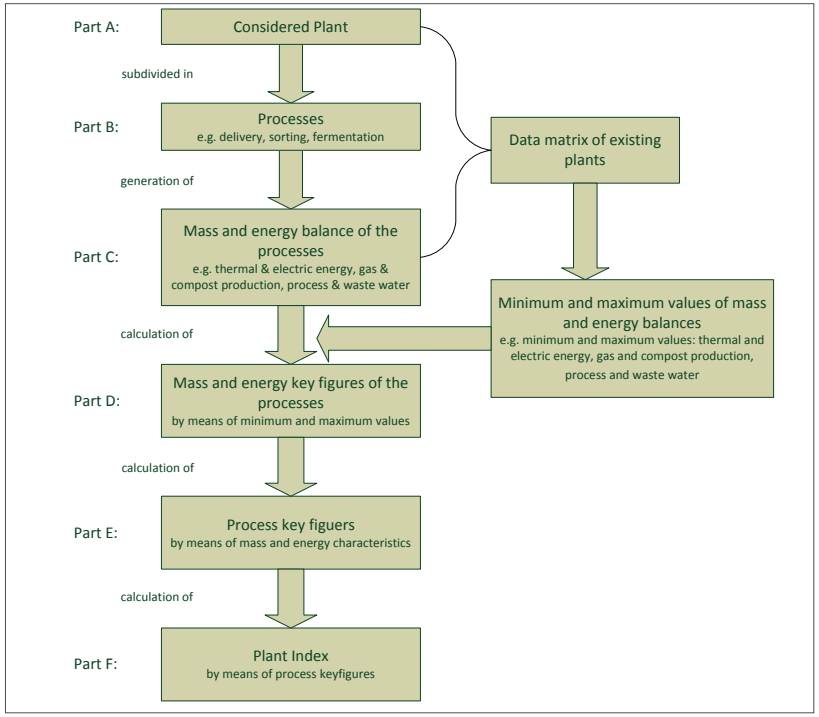


Figure 82: Schematic for the determination the plant index (Source: TU Braunschweig)

The maximum value utilised for the standardisation is a real best practice value that is taken from the data matrix, c.f. Figure 82. In the sense of an ideal borderline case consideration, the real maximum value for the determination of the energy and mass indicators can be replaced by a hypothetical maximum value – determined from theoretical considerations and the latest research results. While the latter has not yet been implemented in practice, it does allow for an estimation of the best possible progress in the implementation of the current state of research.
This method of simulation can also be used for developing a plant with the latest state of technology (best practice approach). For this, for each energy and mass indicator the maximum value (from the data matrix, c.f. Figure 83) is assumed, from which a maximum process indicator and/or plant indicator is calculated. In comparison to this indicator, the optimisation potential can be estimated for each plant since the maximum values in the case of this simulation consist of real, existing values. However, in the near future the frame of consideration can be expanded via the inclusion of currently hypothetical, yet realistically achievable energy efficiency of relevant plant components (state of research).
Another important option for the utilisation of this model is the optimisation of individual plants. With the help of this model it can be determined which plant component features

the greatest optimisation potential. This is possible because different processes become comparable to one another through standardisation. The higher the indicator, the more costly and expensive an optimisation of this process will be.

Database	e.g. plant X, plant Y
Breakdown of data according the process stage and allocation of variables	e.g. delivery = A; fermenter = B; gas purification = C
Mass and energy balance of the process stages and normalization to 1 Mg_{input}; allocation of variables	e.g. water consumption/Mg = α; KW_{el}/Mg = β; kW_{therm}/Mg = γ; gas production/Mg = δ
Determination of the min/max-values for the mass and energy balances on the basis of the overall database	e.g. $\alpha_{min}/\alpha_{max}$; β_{min}/β_{max}
Calculation of the corresponding mass and energy indicator of the reviewed plant	e.g. $\alpha_n = (\alpha_{act} - \alpha_{min})/(\alpha_{max} - \alpha_{min})$ n = variable of the process stage α_{act} = specific actual value of the corresponding plant act = actual
Calculation of the process key figure of the corresponding process stage of the reviewed plant	e.g. $A_{tot} = (\alpha_a + \beta_a + \gamma_a)/n_{balance\ steps}$ tot = total
Calculation of the evaluation index (EI) of the reviewed plant	e.g. $EI_x = (A_{tot} + B_{tot} + C_{tot})/n_{process\ stages}$

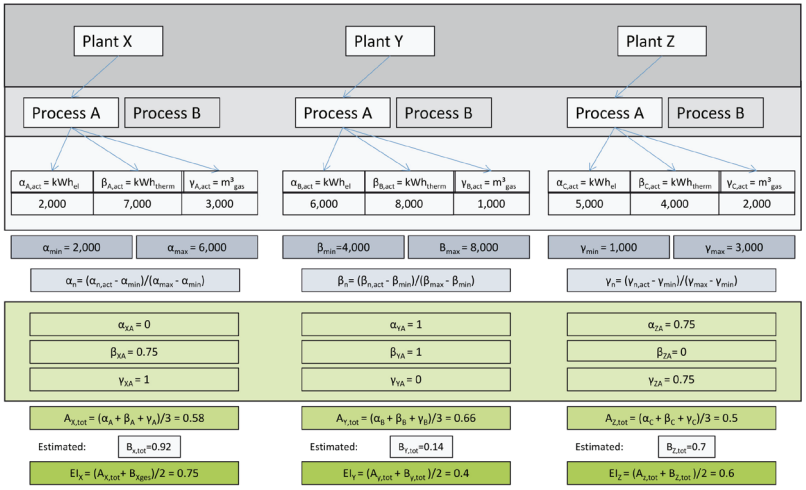


Figure 83: Mathematical depiction regarding the calculation of the plant index (both figures) (Source: TU Braunschweig)

7.3 Determination of the effect of organic fertilisers on the humus supply of soils

Doreen Zirkler and Peter Dominik, TU Berlin

Status	The method introduced for the determination of the impact of organic fertiliser on the humus supply of soils (laboratory experiment) is modelled on ISO standard 16072: Soil quality – Laboratory methods for determination of microbial soil respiration (2001).
Associated standards	ISO 10381-6:1993, Soil Quality – Sampling – Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory ISO 11274:1998, Soil Quality – Determination of the water retention characteristics ISO 11465, Soil quality – Determination of dry matter and water content on mass basis – Gravimetric method ISO 4796, Laboratory glassware
Area of application of the method	Applicable to all organic fertilisers and terrestrial soils. A soil with a $\delta^{13}C$ signal that differs from that of the fertiliser should be selected.
Limitations of the method	The plant roots that exist under natural conditions change soil chemistry and soil biology, which have an essential influence on the decomposition of organic matter. This aspect is not taken into consideration in the respiration experiment. The time span of incubation that is needed until the humus contribution of the fertiliser remains in the soil for longer periods is controversial.
Advantages	Compared to the VDLUFA method, the respiration method has the advantage that the effect of the organic fertiliser on the humus supply of soils does not only have to be gained from long-term field experiments in which a lot of other factors play an important role, too, but can be determined specifically for the given fertiliser and the corresponding soil. Furthermore, novel organic fertilisers that have not been listed in the VDLUFA position paper so far can also be tested. In comparison to field experiments, the respiration method is time and cost saving.
Need for research	The method can be improved by the simulation of plant roots. Currently, experiments are conducted to test the integration of ion exchangers as plant root substitute. Moreover the simulation of root exudates in respiration experiments should be developed and investigated.

Humus supply plays an important role for the fertility of soils. Humus increases the nutrient supply for plants as well as the water storage capacity and aggregate stability of the soil (VDLUFA 2004). In crop production, humus decomposition differs depending on agricultural measures such as tilling or liming, but also on specifics of the type of crop such as soil cover duration. In contrast to natural vegetation, biomass is removed from the soil by harvesting. This deficit can be compensated for by returning organic residues such as harvest residues, slurry, manure, compost, but also biogas residues to the field.

In case an organic fertiliser provides insufficient or too easily degradable organic matter, the soil remains undersupplied with humus and the soil fertility decreases. In case it provides too much easily degradable and nutrient-rich organic matter, undesirable effects such as nutrient leaching or volatilisation (e.g. nitrogen) may occur (ibid). The extent of contribution of an organic fertiliser to the humus supply of soils can be tested in a variety of ways.

The VDLUFA method

The objective of the VDLUFA method (2004) is to estimate the organic matter supply of a field and to compensate deficits with organic fertilisers if necessary. The humus balance method regards humus dynamics as a balance, i.e., with inputs and outputs. For this, the humus demand of different crops and cultivation types is specified in kg humus-C $ha^{-1}a^{-1}$. This value can be positive (humus increasing) or negative (humus depleting). The derivation of these values was based on long-term tests and expert knowledge (Dominik et al. 2009). A negative value must be compensated for by the humus reproduction of organic fertilisers. The corresponding humus reproduction values of various organic fertilisers can be found in the VDLUFA position paper as well. They are specified in kg humus-C (t substrate wet weight)$^{-1}$. These humus reproduction values can be used for estimating the impact of organic fertilisers on the humus supply of soils. Although suggested by the unit (kg C_{org}*ha^{-1}*a^{-1}), actual changes of the humus reserves cannot be concluded from humus balances since the method neither takes local conditions such as type of soil and climate into account nor the former land use. Furthermore, changes of humus reserves are not linear but tend asymptotically to a new equilibrium. Thus it is possible that a specific location exhibits decreasing humus reserves, despite a positive humus balance. The VDLUFA method cannot be applied to ecological farming. For ecological farming, Leithold & Hülsbergen (1998) have developed the humus unit (HU) method. They indicate a higher humus depletion for crops than VDLUFA since no mineral N mineral fertilisers are used in ecological farming and the N supply for plants must be ensured by the mineralisation of soil organic matter (humus). Therefore, the demand for compensating reproduction-effective organic fertilisers is higher (Federal Environmental Agency 2008).

Determination in field experiment

TThe most exact method to determine changes of the humus content due to organic fertilisers are long-term field experiments. They take all influences of agricultural practice (such as crop rotation, tilling, sowing, harvest and weather conditions) into consideration. In field experiments, different (amounts of) fertilisers are applied to different field plots under otherwise identical test conditions, and the changes of humus content are measured. Since the humus contents of arable soils also vary locally and over time – the annual supply and decomposition of harvest residues can account for up to 10 % of the humus reserves – the parameter must be measured over many years in order to give reliable results about changes of humus content and their direction. In each land use system with constant cultivation and climate, an equilibrium between humus supply and decomposition will establish for a longer period, i.e. a stable humus content. A quantitative statement requires measurements for at least 20–30 years. As a result, this method becomes very expensive and is ill-suited for short-term predictions of the effect of novel organic fertilisers (such as biogas residues from renewable resources).

Determination in laboratory experiment

In respiration experiments at laboratory scale, the mineralisation time can be shortened by setting a temperature that is optimal for microorganisms. The number of days that are needed to get the same mineralisation efficiency as within one year in the field is called biological active time (BAT). It can be calculated with the help of the fine portion of the soil, the annual mean air temperature, and the annual amount of precipitation (Franko & Oelschlägel 1995).

In order to detect a priming effect, the soil used for the incubation should have a $\delta^{13}C$ signal different from that of the organic fertiliser. Soil and fertiliser are mixed with one another, adjusted to a water content of 40–60 % of the maximum water holding capacity and filled into sealable vessels. A smaller open vessel containing a base (potassium hydroxide, KOH) (Figure 84) is integrated in these vessels. The CO_2 emitted by the soil/fertiliser mixture is converted into carbonate ions in the base. The resulting increase in electrical conductivity is measured via electrodes installed in the base and converted into emitted CO_2. During the incubation, the vessels are placed in a water bath at constant temperature and under exclusion of light.

If no respirometer is available, the amount of CO_2 released can also be determined via titration of the base. For this, the CO_3^{2-} is firstly precipitated with barium chloride ($BaCl_2$) as $BaCO_3$. The rest of the base is titrated to pH 7 with a titration device or manually with hydrochloric acid (HCl) after addition of phenolphtalein. The amount of hydroxide ions neutralised during the titration in the base of the control (incubated soil without fertiliser) is deducted from the amount of hydroxide ions in the base of the soil/fertiliser sample. Subsequently, a conversion into CO_2 is carried out.

The shares of soil and fertiliser in the CO_2 emission can be gained by the determination of the $\delta^{13}C$ signal of the emitted CO_2 in the base and subsequent mixture calculation. The difference between the C amount of the fertiliser prior to incubation (measured by elemental analysis) and the amount of C emitted by the fertiliser corresponds to the amount of C added to the soil by the fertiliser. The corresponding humus content is given via multiplication with the factor 1.72 (Scheffer & Schachtschabel 2002). The $\delta^{13}C$ analysis can furthermore reveal whether the fertiliser is triggering a so-called priming effect, i.e., whether the application of the fertiliser leads to a higher amount of mineralised soil C than in the control. If a soil with a $\delta^{13}C$ signal that differs from that of the fertiliser is not available, the sources of the emitted CO_2 (soil and biogas residue) cannot be determined quantitatively. In this case, the difference between the amount of C emitted by the soil/fertiliser sample and the amount of C emitted by the control is calculated. The result is

finally deducted from the C amount of the fertiliser prior to incubation and multiplied with the factor 1.72 (see above). Any priming effects occurring cannot be identified this way.
A problematic aspect of the determination of the effect of organic fertilisers on the humus supply is that the mineralisation time required until a fertiliser is no longer decomposed quickly but is rather considered as long-term "humus effective" is controversial. It is recommended to include one or two well researched fertilisers (e.g. manure) as reference in the experiment for comparability and an approximate classification of the results (Dominik et al. 2009).

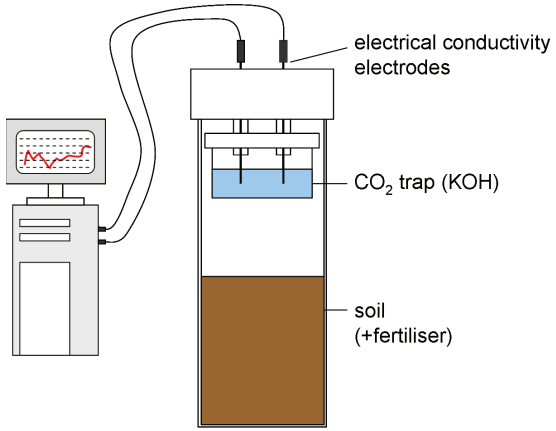


Figure 84: Example of a measuring cell of a respirometer

7.4 Mass balancing of biogas plants

Jürgen Pröter, Sören Weinrich, Josephine Hofman, DBFZ; Jürgen Kube, Agraferm Technologies AG

With increasing importance of energy production from biogas plants, the requirements posed to the description of the biogas process are increasing. In this, also moving into the foreground is the estimation of the potential biogas yield that is or can be produced as one of the most important criteria for the assessment of a biogas plant.
Different approaches exist in this for the presentation and assessment of the process engineering processes. Mass balancing (material balance), for example, provides the opportunity to assess the plant operation in dependence on: the respective amount and composition of the substrate used, the process engineering process parameters as well as the metabolic activity of the microorganisms involved. In addition to the prediction of the biogas rates or the residual gas potentials, the mass balancing therewith also allows for an optimisation of the process control and constitutes the foundation for a realistic plant sizing or economics calculation of biogas plants.

A direct, exact and comprehensive mass balancing is practical but often not implementable since the measurement of the material flows entering and exiting as well as the kinetic reactions cannot be realised with justifiable effort or the technical foundations are at times missing. Nevertheless, a variety of methods already exist today to estimate the maximum biogas potential or the actual biogas yield in real plant operation.
Based on basic considerations, the different methods, options and uncertainties of the mass balancing of biogas plants are detailed below.

Fundamentals of mass balancing

In order to be able to create a complete mass balance, a defined balance space must be specified in the beginning. Within the context of this short introduction, the balance or system boundary will encompass only a single reactor, Figure 85. However, in the respective application case it is possible to apply the methods described here to a whole plant design including the substrate storage or multiple digesters.

The masses fed to the system generally include the substrates and co-substrates utilised as well as additional additives (trace elements, enzymes or preparation for binding H_2S) and water. The biogas generated and the digestate exit the process as discharged masses. If a recirculation of digestate takes place, it may be sensible to leave the return within the system's boundaries in order to not have to additionally balance the recirculate. In addition to the direct transport of substances across the system boundary, the different biochemical and physical-chemical conversion reactions of the individual groups of substances have a decisive impact on the mass balancing of a biogas plant.

Based on these fundamental considerations, the general mass balance in the stationary operation of a continuous reactor can therefore be formulated to:

$$\frac{dm}{dt} = 0 = \underbrace{\text{masses fed} - \text{masses discharged}}_{\text{transport via system boundary}} \pm \underbrace{\text{material conversion}}_{\text{biochemichal reaction}} \qquad (90)$$

As such, no statements can be made under these conditions about the start-up and shut-down of a reactor or the dynamic operation at different load levels.

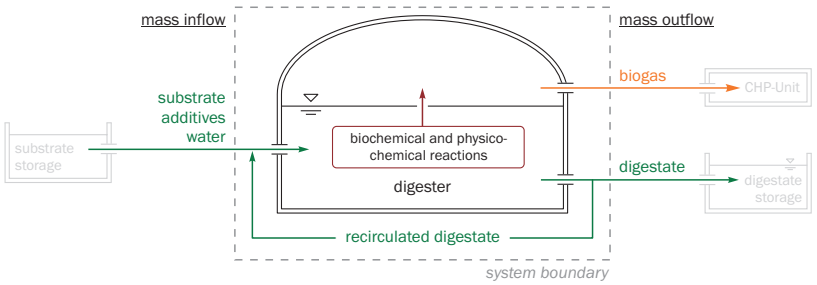


Figure 85: Mass balancing of a biogas digester (Source: DBFZ)

Supplied masses

Substrate

The material characterisation of the substrates fed is a core element of the mass balancing of a biogas plant. The different substrates do not solely consist of organic components but also contain water as well as inorganic and non-fermentable components. Gasses dissolved in the substrate can generally be neglected for a rough mass balance.
Errors in the characterisation of the different substrates therefore primarily occur already at the sampling as well as in the determination and interpretation of the individual measurement methods. In this, fundamentally different indicators exist in order to be able to asses and balance the different substrates:

Wet weight (WW)
Based on the wet weights (WW) fed and discharged as well as the respective biogas production, a meaningful mass balance can already be prepared. Since a lot of the problems for substrate characterisation and material conversion listed below are avoided here, the wet weight is as such already a robust and sensible indicator for a practise-oriented balancing of an industrial-scale biogas plant.

Volatile solids (VS)
The determination of the volatile solids is carried out based on standardised methods (Chapter 3.1). In the case of a high share of highly volatile substrate components, a total solids correction according to Weissbach must be performed (Chapter 3.2). In addition, the volatile solids do contain, for instance, non-fermentable components so that a futher estimation of the non-fermentable components is necessary for the calculation of the substrate-specific biogas potential.

Fermentable volatile solids (FVS)
The fermentable volatile solids (FVS) describe the total solids actually degradable under anaerobic conditions and can be calculated for selected substrates in first approximation according to Weissbach (2008, 2009). Since for the material conversion (biogas production) of the substrates also only the actually fermentable substrate components, the estimation of the non-fermentable substrate components is a prerequisite for a sensible mass balancing. Fundamentally, it is therefore important to further intensify the calculation, interpretation and utilisation of this indicator in practice. To date, standardized methods for analytical determination of the feremantable substrate components under practical conditions are still missing.

Feed analysis (Weender and van Soest analysis)
The feed analysis (c.f. Chapter 2 "Definitions" as well as Chapter 4.7–4.12) describes the various substrates based on the different composition of carbohydrates (structural substances and cell contents), proteins as well as fats and thereby constitutes often the basis for a differentiated description of the stoichiometric decomposition pathways of the different classes of nutrients. In addition to a reduced comparability of the measuring results due to different sample preparation and analytical methods, the assessment of the actually fermentable shares of the individual components is of decisive importance here, as well.

Chemical oxygen demand (COD)
The chemical oxygen demand (COD) indicates the amount of oxygen that is needed for the complete oxidation of the existing organic compounds of the respective sample. Similar to the volatile solids, the COD is a measure for the organic substrate components, but is typically utilised for the assessment of highly diluted samples in the area of wastewater analysis. During the anaerobic digestion of highly diluted substrates with a high share of volatile substances (for instance percolate) it may also be sensible to rely on the chemical oxygen demand since obviously no meaningfulness is to be expected here that is based on a determination of total solids.

Total carbon (TC)
The total carbon (TC) content describes the sum of the carbon from all inorganic and organic compounds of the substrate. In particular, the total organic carbon (TOC) is often utilised, similar to the chemical oxygen demand (COD), for the characterisation of the organic substrate shares in the area of wastewater analysis. Depending on the substrates and methods utilised, it is important to select the analytical parametersto be utilised based on the respective advantages and disadvantages with respect to the main objective of the mass balancing.

Additives

In practice, additives in the form of trace element mixtures, iron preparations or enzymes are utilised for stabilisation, desulphurisation or optimisation of the biogas process. Since the amount of such preparations utilised are often very small (< 0.1 % of the total substrate feed), the proportion of weight within the masses fed can generally be neglected. Rather, these additives change the activity and degradation velocity of the microorganisms involved and thereby may have a decisive impact on the growth-limiting and/or inhibiting processes in the kinetic description of the material conversion processes.

Water

If additional water is provided to the process in order to ensure the flow capacity of the substrates fed or of the digester content, it must be included in the balance. In this, the mass of water contained in the substrate is already included in the wet weight of the substrate fed and can be removed calculatorily based on the respective total solids content for the creation of a water balance.

Discharged masses

Biogas

In the balancing of the amount of biogas produced, a clear definition of the measuring point as well as of the measuring conditions (temperature and pressure) of the biogas should exist. Since moist biogas exits from the reactor, it must be ensured that the gas is dried upstream of the measuring site and that the temperature of the gas is measured. Typically, the gas temperature will approach the ambient temperature at the measuring site and will thereby deviate considerably from the digester temperature. In each case, a correction of the measured biogas to standard conditions and – where applicable – to dry biogas (steam correction) should be performed (STP).

From a reaction engineering point of view, a difference may exist between the biogas actually generated and the gas volume flow measured. This different can be traced back to the fact that part of the biogas generated exists dissolved in the substrate suspension. As such, the concentration of a gas in a liquid phase is directly dependent on the partial pressure of the gas and the substance-specific Henry constant. The temperature and the content of dissolved substances in the liquid also determine the solubility of a gas.

Digestate (fermentation resdiue)

In the characterisation of the digestate it must be taken into consideration that it does not consist solely of inorganic substance and water but rather additionally contains non-utilisable and non-convertable volatile solids as well as newly formed bacteria biomass. Furthermore, the digestate also contains portions of the generated biogas in dissolved form. The mass of the salts contained in the digestate (inorganic VS or ash) corresponds approximately to the mass of the salts fed. This applies exactly to all heavy metals. In an analysis of these substances, the mass of the digestate can be derived in the case of knowledge of the mass of substrate fed. Fundamentally, the analytical methods of substrate assessment already discussed are available for the characterisation of the different components of a digestate.

Material conversion

In addition to the characterisation of the masses fed and discharged, the description of the material conversion of the different substrate components is a central element of the mass balance.

In this, the amount of biogas produced is influenced both by the biogas (formation) potential of the substrate utilised as well as the kinetic growth conditions of the microorganisms involved so that the determination of both elements is decisive for a realistic mapping of the balanced process. But since a comprehensive description of the individual degradation pathways and intermediate products is possible only based on elaborate measurement methods and kinetic modelling approaches, these scientific balancing methods are rarely utilised in practice and are furthermore – due to the multi-layered dependencies – not usable for generally applicable and practice-oriented process evaluation as part of this short introduction.

Nevertheless, in uninhibited and continuous (stationary) reactor operation, simplified calculation methods and balancing approaches can also be utilised for a practice-oriented process description. Therefore, the differentiated decomposition of individual intermediate products is neglected below and only the overall reaction (brutto stoichiometry and kinetics) of the organic substrate into biogas is considered. However, in the respective application case it must be taken into consideration that individual growth-limiting intermediate products or inhibitors may severely inhibit the anaerobic digestion process and thereby may severely influence or relativize the meaningfulness of the balance.

Stoichiometry (biogas [formation] potential)

The biogas (formation) potential defines the biogas yield that maximally can be generated in the anaerobic digestion from the substrates utilised and must therefore not be mistaken for the actual biogas yield that can be achieved in real plant operation, taking into consideration the respective process conditions (retention time and reaction kinetics).

In principle, the biogas (formation) potential can be determined from experimental batch tests (c.f. Chapter 6.2) or otherwise base on stoichiometric calculations. But since the different test conditions (inocula/activity of the inoculum) and theoretical considerations (model substrates) differ considerably a comparability and/or transparency of the practical and analytical method does not exist to date. Fundamentally, different stoichiometric approaches exist for estimating the biogas potential based on the individual fermentable nutrient fractions.

Buswell & Müller

The anaerobic degradation of organic model substances can be described "by the simplified" reaction equation (oxidation reaction) of (Buswell & Mueller 1952). Based on the individual coefficients from the stoichiometric formula of the respective substrate components and pure substances, the biogas yield (methane and carbon dioxide) as well as the needed water share can be calculated. The bacteria biomass generated is not taken into consideration in this approach.

$$C_aH_bO_c + \left(a - \frac{b}{4} - \frac{c}{2}\right) H_2O \rightarrow \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4}\right) CO_2 + \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4}\right) CH_4 \quad (91)$$

Boyle

Starting from the reaction equation of Buswell & Mueller (1952), Boyle (1976) expanded the stoichiometric equation by the components nitrogen and sulphur. This way, in addition to carbon dioxide and methane, the share of ammonia and hydrogen sulphide in the biogas (and in solution) can be calculated. Furthermore, the calculation can now also be applied to substrate components that contain nitrogen and sulphur such as proteins and amino acids. However, the bacteria biomass generated continues to not be taken into consideration in this approach.

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right) H_2O \rightarrow \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right) CO_2 + \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right) CH_4 + d\ NH_3 + e\ H_2S \quad (92)$$

McCarty

To additionally map the share of the bacteria biomass generated, the reaction equation of Boyle (1976) can be expanded, modelled after McCarty (1972) in Pavlostathis & Giraldo-Gomez (1991), by the empirical formula $C_5H_7O_2N$ of biomass. In this, the stoichiometric yield coefficient α specifies how much substrate is utilised for the creation of bacteria biomass (Equation 93).

In addition to the introductory examples, comprehensive stoichiometric balances exist which describe the differentiated decomposition of the different intermediate products of

anaerobic digestion (Angelidaki et al. 1999; Batstone et al. 2002). In this, all methods depend on classifying the substrate mixture fed such that the individual substrate components can be characterised as close to reality as possible based on stoichiometric formulas, and can furthermore also be determined analytically.

$$C_aH_bO_cN_d + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} - 3\alpha\right) H_2O \rightarrow \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} - \frac{5\alpha}{2}\right) CO_2$$

$$+ \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{5\alpha}{2}\right) CH_4 + (d - \alpha)NH_3 + \alpha\, C_5H_7O_2N \qquad (93)$$

Mit $\alpha = Y \cdot \frac{M_S}{M_X}$ $\left[\frac{\text{mol biomass}}{\text{mol substrate}}\right]$

Y = yield coefficient [kg biomass/kg substrate]
M_S = molar mass of the substrate [kg/mol]
M_x = molar mass of the biomass [kg/mol]

Due to different model substrates and test methods, a variety of biogas potentials exist in the literature for the typical nutrient fractions, Table 23.

Table 23: Biogas (formation) potential of the fermentable nutrient fractions (Weissbach 2009)

	(Weissbach 2009)[a]		(Weiland 2001)		(VDI Guideline 4630 2006)[b]		(Baserga 1998)	
	Biogas [L(STP)/kg]	CH_4 [%]	Biogas [L(STP)/kg]	CH4 [%]	Biogas [L(STP)/kg]	CH_4 [%]	Biogas [L(STP)/kg]	CH_4 [%]
Carbo-hydrates	787–796	50.0–51.1	700–800	50–55	750	50	790	50
Fats	1,340–1,360	70.5–71.3	1,000–1,250	68–73	1,390	72	1,250	68
Proteins	714–883	50.9–51.4	600–700	70–75	800	60	700	71

[a] Calculated for grains and cereals

[b] ... "without taking into consideration the bacterial biomass production from the fermented substrate" (VDI Guideline 4630 2006)

Fundamentally, it is of decisive importance that the whole biogas (formation) potential is calculated solely based on the actual amount of fermentable substrate components. In this, the calculation utilising the digestion factors from the classic feed(ing) value tables often provides very inaccurate results, so that the fermentable substrate components must be determined based on the non-fermentable shares according to Weissbach (2008, 2009). Depending on the stoichiometry utilised, an additional a correction of the biogas potential due to additionally produced bacteria biomass must also be performed.

Reaction kinetics

For a comprehensive and differentiated consideration of the microbial growth kinetics in the different decomposition stages of the anaerobic digestion, complex model considerations are necessary (Angelidaki et al. 1999; Batstone et al. 2002). Instead, in the case of a disruption-free operation, the biogas yield can also be calculated based on simple first-order reaction kinetics.

As such, the concentration of any substrate component is obtained in dependence on the reaction constant and the respective retention time in continuous and stationary reactor operation, in

$$c = \frac{c_{in}}{1 + k \cdot HRT} \left[\frac{kg}{m^3}\right] \qquad (94)$$

c_{in}	Substrate concentration in the input	(kg/m³)
k	First-order reaction constant	(1/d)
HRT	Average hydraulic retention time	(d)

Depending on the substrates, test conditions and models utilised, a multitude of different and at times contradictory kinetic constants exists in literature, so it is important in the future to develop a systematic and practice-oriented foundation for the selection and calculation (identification) of representative kinetic parameters of different substrates and/or substrate components.

Summary

The mass balancing provides a variety of options allowing for the assessment or forecasting of the substrate decomposition in a biogas plants. Even if comprehensive model approaches are necessary for a complete description of the anaerobic digestion process, the methods described above can already be utilised for a meaningful and – more importantly – practice-oriented balancing. Using the research regarding substrate assessment by Weissbach as a starting point, a considerable need for research continues to exist regarding the following questions:

- Development of standardised, experimental or analytical methods for the calculation of fermentable volatile solids (FVS) and individual substrate components
- Development of suitable methods for the characterisation and/or stoichiometric description (chemical formula) of practice-relevant and complex substrates
- Comparability of the experimental (batch test) and theoretical (stoichiometry) methods of testing the biogas (formation) potential of different substrates
- Impact of different disintegration methods on the biogas potential and degradation kinetics
- Application of simplified kinetic modelling approaches for the description of fundamental reaction engineering and process-engineering processes at laboratory scale and transfer (upscaling) to the practical operation of biogas plants
- Experimental test series regarding the identification of kinetic parameters of simple decomposition reactions (first-order) of practice-relevant substrates
- Impact and quantification of the activity of the microorganisms involved (inoculum) on the meaningfulness and comparability of standardised batch tests based on reaction engineering and micro-biological tests

- Amount and impact of the bacteria mass generated and/or died on the mass balancing of a biogas plant
- Impact of the solubility of gas components produced (carbon dioxide)

As such it is important in the future to include these essential approaches through further theoretical and practical research in a standardised and practice-oriented mass balancing approach in order to allow for an improved and close to reality assessment, optimisation and sizing of biogas plants with the help of reaction engineering balancing methods.

Example of a mass balance (Weinrich 2014)
In conclusion, the principle of a straightforward mass balancing based on a practice-focused example for the mono fermentation of maize silage in a single-stage biogas plant (500 kW_{el}) is presented (Weinrich 2014). The specific substrate characteristics of the maize silage utilised correspond to the average analysis values of various analyses at the German Biomass Research Centre (DBFZ Deutsches Biomasseforschungszentrum gemeinnützige GmbH), Table 24.
In accordance with common practice, the total solids content of the maize silage is being diluted via process water in the form of separated recirculate or fresh water in order to ensure the pumpability and stirrability in the digester, Figure 86.

Table 24: Substrate characteristics of the maize silage utilised for the calculation example (Weissbach 2009)

Parameters	Symbol	Value	Unit of measure
Total solids	TS	33.5	% WW
Organic dry matter (volatile solids)	VS	95.6	% TS
Fermentable organic dry matter/volatile solids[a]	FVS	78.5	% TS
Nitrogen-free extracts	NFE	626.1	g/kgTS
Crude fibre	CFI	221	g/kgTS
Crude protein	CP	78.8	g/kgTS
Crude fat	CF	29.6	g/kgTS
Crude ash	CA	44.5	g/kgTS

[a] Calculated with CA and CFI according to Weissbach (2008)

Under the assumption that 5 % of the FVS are incorporated into bacteria biomass, the stoichiometric calculations according to Buswell & Mueller for grains and cereals on average result in a gas production potential of 809 m³ biogas and 420 m³ methane per t of converted FVS (Weissbach 2009). The corresponding stoichiometric integration of water into the biogas amounts to 11.25 %. Based on the fermentable components of the substrate fed, the specific conversion of the FVS as well as the resulting amounts and characteristic properties of the generated fermentation products (biogas and digestate) in the case of stationary plant operation can be calculated unambiguously, Table 25.

As such, straightforward mass balancing provides a variety of options allowing for the assessment or forecasting of the substrate decomposition in a biogas plant. Even if comprehensive model approaches and measuring scenarios are possible for a detailed description of the decomposition processes and intermediate products, the calculation methods described here above can already be utilised for a meaningful, robust, and – more importantly – practice-oriented balancing.

Table 25: Calculation equations for the mass balancing of biogas plants (Weinrich 2014)

Options for the calculation of the conversion η of FVS in the biogas process	
Biogas yield:	$\eta = \frac{\dot{V}_B}{\dot{m}_S \cdot TS_S \cdot FVS_S \cdot Y_{FVS}}$
Residual gas potential:	$\eta = \frac{TS_S \cdot FVS_S \cdot Y_{FVS} - Y_D}{TS_S \cdot FVS_S \cdot Y_{FVS} \cdot (1 - Y_D \cdot \rho_B)}$
Reaction kinetics:	$\eta = \frac{k \cdot HRT}{1 + k \cdot HRT}$
VS in digestate:	$\eta = \frac{1}{FVS_S \cdot (1 - f_X)} \cdot \left(1 - \frac{1 - VS_S}{1 - VS_D}\right)$
TS in digestate:	$\eta = \frac{1}{FVS_S \cdot [1 - f_X - TS_D \cdot (1 - f_X + f_W)]} \cdot \left(1 - \frac{TS_S}{TS_D}\right)$
Calculation of the resulting amounts and properties of the fermentation products	
$\dot{V}_B = \frac{\dot{m}_S \cdot TS_S \cdot FVS_S \cdot \eta \cdot (1 - f_X + f_W)}{\rho_B} = \dot{m}_S \cdot TS_S \cdot FVS_S \cdot \eta \cdot Y_{FVS}$	
$Y_D = \frac{TS_S \cdot FVS_S \cdot (1 - \eta) \cdot (1 - f_X + f_W)}{[1 - TS_S \cdot FVS_S \cdot \eta \cdot (1 - f_X + f_W)] \cdot \rho_B} = \frac{TS_S \cdot FVS_S \cdot (1 - \eta) \cdot Y_{FVS}}{1 - TS_S \cdot FVS_S \cdot \eta \cdot Y_{FVS} \cdot \rho_B}$	
$\dot{m}_D = \dot{m}_S \cdot [1 - TS_S \cdot FVS_S \cdot \eta \cdot (1 - f_X + f_W)] = \dot{m}_S \cdot (1 - TS_S \cdot FVS_S \cdot \eta \cdot Y_{FVS} \cdot \rho_B)$	
$VS_D = \frac{VS_S - FVS_S \cdot \eta \cdot (1 - f_X)}{1 - FVS_S \cdot \eta \cdot (1 - f_X)}$	
$TS_D = \frac{TS_S - TS_S \cdot FVS_S \cdot \eta \cdot (1 - f_X)}{1 - TS_S \cdot FVS_S \cdot \eta \cdot (1 - f_X + f_W)}$	
$FVS_D = \frac{FVS_S \cdot (1 - \eta)}{1 - FVS_S \cdot \eta \cdot (1 - f_X)}$	

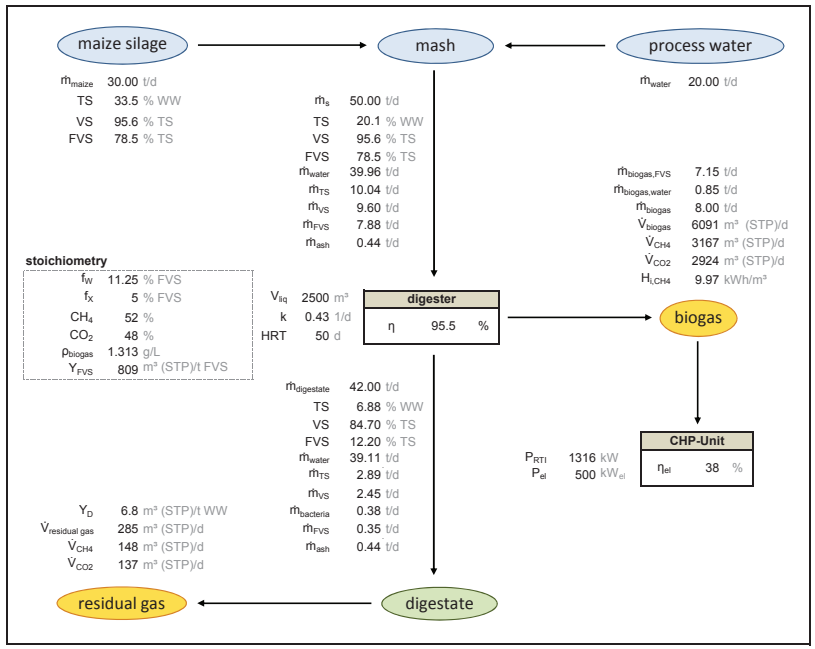


Figure 86: Calculation scheme of a straightforward mass balancing for the mono fermentation of maize silage (Weinrich 2014)

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