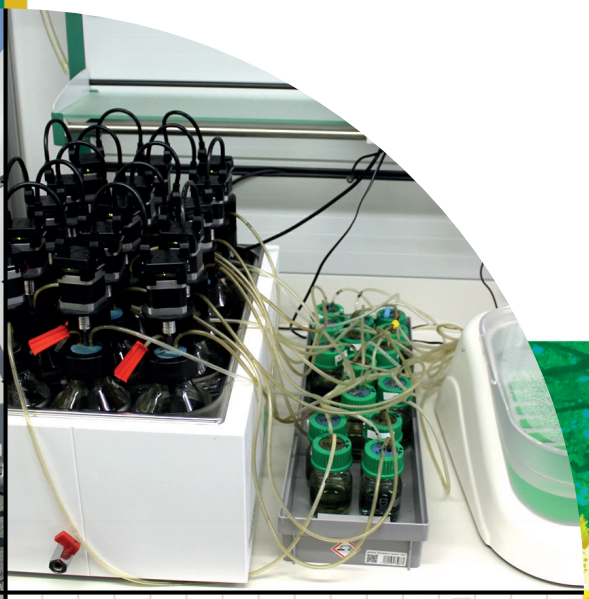
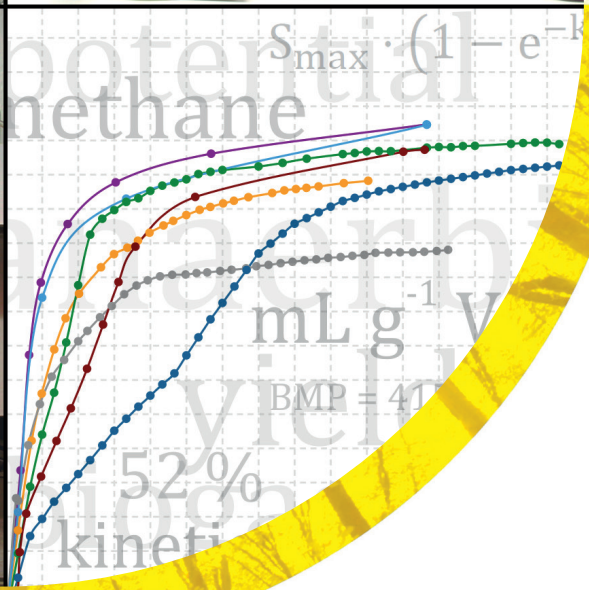
VALUE OF BATCH TESTS FOR BIOGAS POTENTIAL ANALYSIS

Method comparison and challenges of substrate and efficiency evaluation of biogas plants





IEA Bioenergy Task 37

IEA Bioenergy: Task 37: 2018:10

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IEA Bioenergy

Method comparison and challenges of substrate and efficiency evaluation of biogas plants

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CITATION
Weinrich, S., Schäfer, F., Bochmann, G., Liebetrau, J., (2018). Value of batch tests for biogas potential analysis; method comparison and challenges of substrate and efficiency evaluation of biogas plants. Murphy, J.D. (Ed.) IEA Bioenergy Task 37, 2018: 10
ISBN: 978-1-910154-49-6 (eBook electronic edition), **978-1-910154-48-9** (printed paper edition)

ACKNOWLEDGEMENTS:

Parts of the work presented was funded in the research project "Evaluating substrates for biogas production – from laboratory to industrial scale" (Grant 22034614) by the Federal Ministry of Food and Agriculture of Germany. This work was made possible with funding from IEA Bioenergy Task 37, and the support of: DBFZ, Germany; BOKU, Austria; ZHAW, Switzerland; the MaREI centre, University College Cork, Ireland.

ISBN: 978-1-910154-48-9 (printed paper edition)
ISBN: 978-1-910154-49-6 (eBook electronic edition)

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Published by IEA Bioenergy

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Executive summary

The key parameter for the evaluation of substrates to be used in anaerobic digestion plants is the biogas potential. It states the maximum amount of biogas that can be obtained from a given amount of substrate and therefore represents the benchmark for any technical application for biogas production. The biogas yield describes the amount of gas retrieved under technical conditions at a given biogas facility.

The biogas potential of a specific substrate defines the maximum amount of biogas that can be produced during anaerobic digestion, but it includes a certain amount of substrate utilised for microbial growth and maintenance, which consequently lowers the amount of degradable substrate available for biogas production. There are several methods of substrate characterisation available which are used to determine, or correlate to, biogas potential. Most common are total solids and volatile solids determination, chemical composition analysis, chemical oxygen demand, total organic carbon and nutrient composition. For a direct determination of the biogas potential via a chemical analysis, these methods all lack the precise direct determination of the degradable fraction of the substrate and the amount of substrate used for microbial growth. Therefore they use calculation methods based on empirical correlation or coefficients to estimate the biogas potential.

Discontinuous batch tests (or continuous tests) are biological test systems, which allow for the direct assessment of factors not considered by the chemical test analysis, but add the uncertainty of a biological test. Several standards and guidelines are available (e.g. VDI 4630, 2016, DIN EN ISO 11734, 1998 or Angelidaki et al., 2009) for performing anaerobic digestion by means of batch experiments. The experiments give a biogas yield which can be used for estimation of the biogas potential and provide additional information on degradation kinetics.

One of the major factors influencing the results of a batch test is the inoculum used. Source and sampling of the inoculum, pre-treatment and storage and in particular the adaption to the substrate of choice have a significant impact on results. However, there is no measure yet to judge the adaption of an inoculum to an available substrate other than a monitored adaption process. Substrate sampling and pre-treatment are also important factors, which influence the results. Sampling needs to deliver a representative sample. Pre-treatment of the sample should be minimised in order to compare with real world applications in the biogas facility.

Major factors, which influence the results of the test are: the test equipment; the reference system used; the blank test; and the inoculum to substrate ratio. The impact of the test equipment has not been analysed extensively. For the reference system, the blank and the inoculum to substrate ratio, standard conditions should be met. The criteria used to signify termination of the test is also an important factor. The evaluation must include for standardisation of the gas volume at standard temperature and pressure, include for subtraction of water vapour and allow for gas production from inoculum. Most analyses give the gas produced over a period of time assuming only a negligible amount of gas would have been produced in case of longer retention times. A more precise evaluation of the test results includes model-based estimations of the biogas potential assuming an infinite retention time. Inter-laboratory tests help to identify variability in results within several laboratories and reduce errors in test execution.

Recommendations

The purpose of the test whether to inform plant design or plant performance analysis or pretreatment technology evaluation must be known in advance of the test. Different purposes require different approaches and additional supporting measurements. For a successful test series it is essential that the aim of the study is defined; this leads to the development of a sampling procedure and test scenario and allows for an evaluation of uncertainties and interpretation of results. The representability of the sample analysed is crucial to the veracity of the output of the test. The adaptation of the inoculum to the substrate must be considered. The evaluation of the results – besides the validity of the test according to the standard protocols – should include a model-based estimation of the biogas potential at infinite retention time. When interpreting the results, the uncertainty and inherent variability should be considered and highlighted. In

order to ensure the quality of lab-scale experiments for determination of biogas potential a regular participation in inter-laboratory tests is recommended. For those who employ labs to undertake biogas potential tests it is recommended to check if the lab is participating in such tests and is deemed to be a reputable laboratory for such tests.

Conclusion and outlook

The batch test is an established test system for the determination of the biogas potential of organic materials. Inter-laboratory tests and investigations analysing the impact of inoculum have revealed a significant variability in the results of the test. Other methods for the determination of the biogas potential based on chemical analysis show a significant lower variability in the results, but limited correlation with batch tests. Which test result is more accurate and free of bias remains unknown since there is no absolute value or method to be compared with.

Summing up the advantages and disadvantages of the batch test can be described as in the table below.

A limited literature screening showed coefficients of variation of 1 – 20 % (in some cases >30 %) are possible when comparing different inocula. This translates into a similar variation possible in case non-adapted inocula are in use. The only measure for adaption available is the execution of an adaption process which increases the effort of the test considerably. Inter-laboratory reproducibility of batch tests is in the range of 8 – 26 % when looking at the results of three national inter-laboratory tests. Therefore any result should be interpreted carefully under consideration of available literature data, calculations based on chemical or physical substrate analysis and the known intra-laboratory variability of the lab analysing the sample.

A further reduction in the variability seems to be possible when examining the impact of the test procedures and the inoculum. A standard method for the transfer of batch test to continuous test or full-scale systems is as yet not available.

Revisions of the available protocols and identification and elimination of causes for the variability is needed. If the variability of the batch test can be reduced, the development of biochemical analysis combined with regression analysis might become more precise and result in a higher accuracy. A further series of inter-laboratory tests (including for continuous processes and chemical analysis such as nutrient assessment) and the publication of these results are necessary for further improvement of applied test procedures and more precise results.

Pros	Cons
Direct measurement of sum of biochemical parameters (microbial growth, degradability or water incorporation)	No distinctive, separate determination of biochemical parameters (microbial growth, degradability or water incorporation)
Standard protocols for test methodology available	Numerous influencing factors and still large variability compared to chemical analysis
Availability of many reference values and long-term experience	Details about test methodology often incomplete
Limited effort compared to continuous tests	Does not give sufficient data for continuous full-scale plants on factors such as: kinetic process behaviour, idealised retention time or operation at these retention times, effects of inhibitory substances, trace element deficiencies, impact on rheology or mixing properties.
Substrate independent methodology	Comparably high effort and costs (compared to single chemical analysis)

1. Introduction

The key parameter for the evaluation of substrates to be used in anaerobic digestion plants is the biogas potential. It states the maximum volume of biogas that can be obtained from a given amount of substrate. An accurate assessment of the biogas potential allows for accurate mass balances and analyses of process performance for full-scale facilities either existing or proposed. The biogas potential is also the basis for the economic performance of biogas facilities. However, the biogas yield, the amount of gas retrieved under technical conditions at a given biogas facility, is dependent on many factors or variables such as: the kinetics of the degradation process; the rheology and mixing properties of the digestate; the presence of inhibitory substances; and potential nutrient/trace element deficiency. Besides these factors, pre-treatment technologies and technical limitations resulting from disturbing materials or the formation of swimming layers can also have significant impacts on the specific process performance. The plant operator or developer must identify the crucial factors and undertake an accurate evaluation in order to minimise the risk of misjudgement of plant performance.

The batch test is one method which helps to assess biogas or methane potential of a given substrate. **The current report gives an overview on information to be gained from the test, the relation to other test methods and the limitations of the test.**

2. Analysis of the biogas potential

2.1. Definitions

In this report both the terms biogas potential/yield and methane potential/yield refer to a method of substrate characterisation associated with the production of a certain amount of an energy rich gas from a certain amount of organic material. For methane yield, the energy yield of the biogas can be deduced directly. For a full mass balance of the anaerobic digestion process the amount of produced gases other than methane must be known, in particular levels of CO_2 are required. Some of the determination methods described below allow exclusively for the determination of either methane or biogas potentials/yields. Biogas potential and biogas yield are often used as synonyms. However, in the context of this report a clear distinction between potential and yield has been made as follows.

The biogas potential of a specific substrate defines the maximum amount of biogas that can potentially be produced during anaerobic digestion. Due to diverse metabolic pathways during biochemical conversion a certain amount of substrate is also utilised for microbial growth or maintenance, which consequently lowers the amount of degradable substrate available for biogas production. Furthermore, the definition refers to the individual state of the sample as it is analysed or utilised in the respective digestion processes. This means substrate pretreatment or disintegration processes prior to the analysis can change the biogas potential. The report addresses the specific case of estimating the biogas potential based on batch tests.

The biogas yield describes the achieved fraction of the biogas potential under practical conditions during an experimental or technical digestion process. Thus, the yield can depend on numerous impact factors, such as retention time, organic loading rate, inhibitory effects or nutrient deficiency (Figure 1). By definition the specific biogas yield has to be lower or equal to the respective biogas potential of the utilised substrates.

2.2. General remarks

The characterisation of organic materials comes with several challenges. First of all, the biogas potential is only one criterion for successful implementation of a biogas facility. Secondly, the biogas potential can be described in different ways and based on different analytics. Individual determination procedures are based on different assumptions and require different efforts; they have different limitations and advantages. Before starting a testing campaign it is recommended to identify the critical knowledge gaps of the particular substrate and then select the proper measurement scenario.

The heterogeneous and seasonal characteristics of organic materials leading to variability in composition of the substrate must be highlighted. The question as to whether the analysed sample actually represents the significant characteristics of the material sampled in all its variability is crucial. All organic materials are variable in composition. In the case of energy crops the changes might be less significant than for example the variation in the organic fraction of municipal solid waste, which has a highly seasonal composition.

Therefore, the first question to be answered before analysing substrate characteristics (such as biogas potential) is which population mean represents the investigated substrate and if the available sample can describe the entire substrate population. In particular for estimation of the biogas production for full-scale facilities, the long-term analysis of relevant substrate characteristics can be crucial to determine the variation within the population. Since such a variation analysis requires usually a large number of samples to be analysed, it is recommended to include laborious tests (such as the biogas potential test) at a later stage when the variation can be described and related to a single sample taken for detailed analysis.

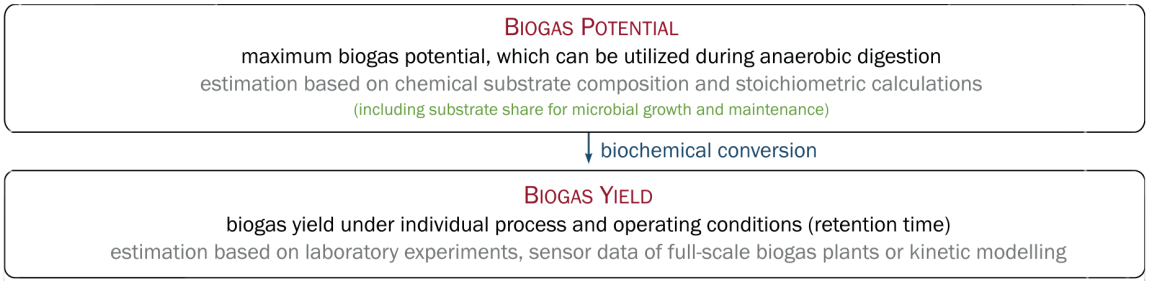


Figure 1: Distinction between biogas potential and biogas yield of substrates (modified from Liebetrau et al., 2017; adapted by permission from the copyright holders, Springer Nature)

One more important point to be considered is the necessity of pre-treatment for any analysis. It is well known that mechanical and chemical pre-treatment can change the characteristics and in particular the biogas potential. Some of the methods described here require pre-treatment of the sample such as for example drying and milling. It has to be considered that this will have an impact on the analysis results and hence it has to be taken into account for data interpretation.

2.3. Theoretical determination of biogas potential

In the following, methods using physical or chemical analyses combined with stoichiometric calculations or reference values from literature for biogas potential analysis are discussed in order to provide a brief comparison to the biological analysis via batch tests. In general, the degradation of an organic substrate can be described as shown in Figure 2. Several analytical methods allow the determination of different fractions of the substrate and provide consequently different information about the sample. It can be stated that there is no method available which allows the direct and precise determination of all relevant fractions by means of a chemical or physical analysis. The available approaches allow an approximation, address different fractions and have different limitations. Crucial fractions for the determination of the biogas potential which cannot be determined directly by means of a chemical analysis are:

- Degradable fraction of volatile solids (or any other basis such as chemical oxygen demand (COD));
- Fraction of substrates used for microbial growth.

Additionally, the elementary composition of the degradable substrate fraction is rarely available for the total solids, and almost never for the degradable fraction. However, this is required to calculate the gas composition and the amount of water to be incorporated during degradation. Usually literature values or regression models are used to estimate missing parameters.

The common way to present a biogas potential or yield of an organic material is the specific biogas potential or yield. Thus, the amount of biogas is given in relation to a substrate fraction (e.g. volatile solids or chemical oxygen demand) in order to reduce uncertainty caused by water content and inorganic compounds within the material. The measurements described in the following are consequently used either for direct estimation of the biogas potential or as a basis for estimation of biogas potential or yield based on reference values.

2.3.1 Total solids and volatile solids

The basis for most substrate analysis includes the determination of total solid (TS) and volatile solid (VS) content. The TS content of a substrate is determined by drying the substrate and thereby removing the water (and volatile organic compounds) from the fresh matter of the substrate

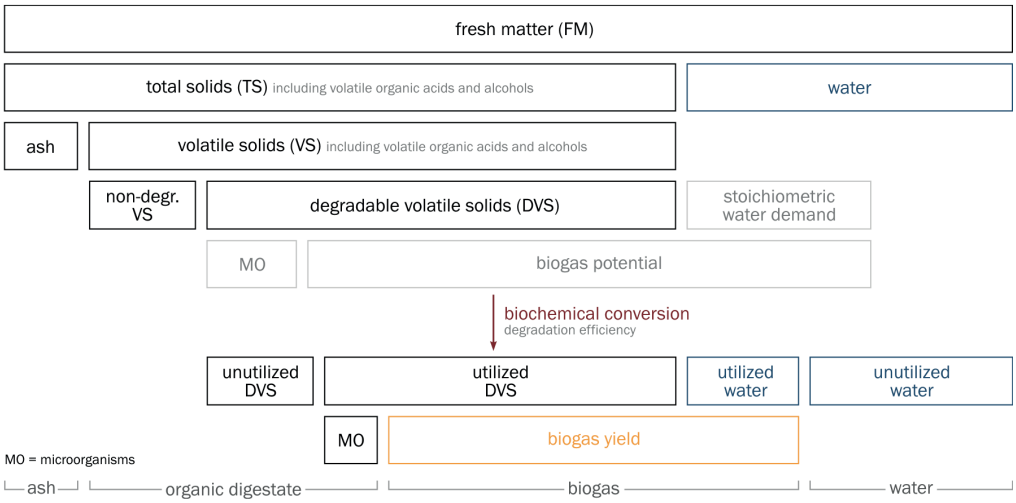


Figure 2: Utilization of individual substrate components during anaerobic digestion (modified from Liebetrau et al., 2017; adapted by permission from the copyright holders, Springer Nature)

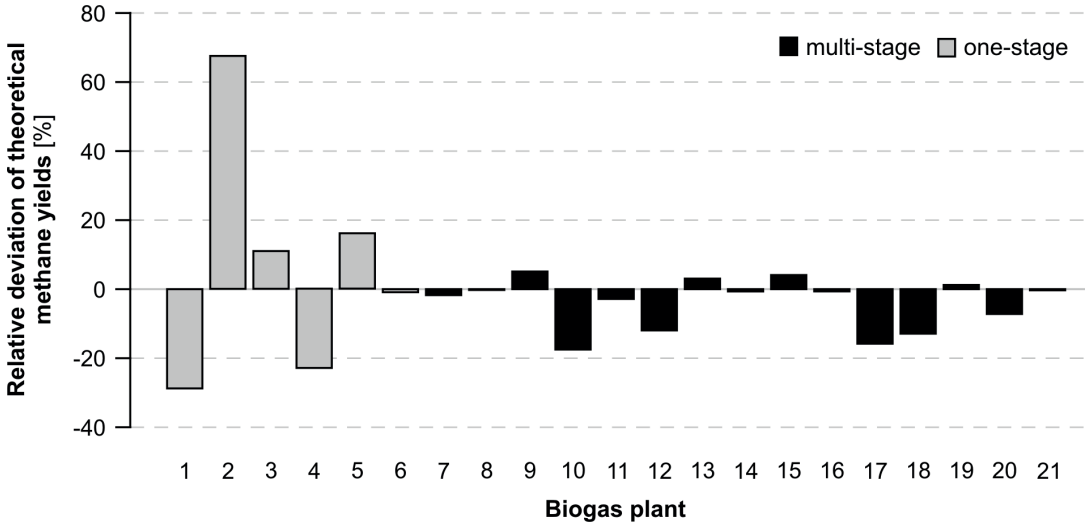


Figure 3: Full scale performance in comparison to VS-based standard literature values (KTBL 2013) for the calculation of the theoretical methane yield (Reprinted from Ruile et al., 2015, Copyright with permission from Elsevier)

(DIN EN 12880:2001-02, 2001). In case of substrates containing significant fractions of volatile compounds (such as silages) the overall procedure has to consider the losses of organic compounds during drying, otherwise the biogas production from the original material might be misjudged (Weissbach & Strubelt, 2008a-c).

By incinerating the dry mass of the sample in a muffle furnace the organic components are oxidised and the inert fraction remains. Subtracting the remaining inert fraction from the initial dry mass results in the content of volatile solids (VS) of the substrate (DIN EN 12879:2001-02, 2001; Figure 2). The VS content represents an approximation of the organic fraction of the substrate. Combined with reference/standard values for given substrates from literature (e.g. KTBL, 2015) the biogas potential of the sample can be estimated. Due to the variability of the quality of the organic materials and the often incomplete information about the generation of reference data (such as if yield or potential is given) and the presentation of ranges of potential gas amounts in literature, the precision of such an estimation is limited. Ruile et al. (2015) compared full scale performance with values calculated based on reference values from literature (KTBL, 2013) (Figure 3). To some extent, the deviation was disproportionally high.

In addition the method is not suitable for biogas potential determination of an unknown substrate. For precise determination of the biogas potential of a particular sample or the characterisation of unknown substrates additional analyses are necessary.

2.3.2. Chemical substrate composition and stoichiometric calculations

The chemical substrate composition derived by elementary analysis provides the information for stoichiometric calculation of the theoretical biogas potential. The estimation can be carried out based on the model of Buswell and Müller (1952) (Equation 1) or the extended model (addition of sulphur and nitrogen) by Boyle (1977) (Equation 2).

Equation 1:

$$C_aH_bO_c + \left(a - \frac{b}{4} - \frac{c}{2}\right) H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4}\right) CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4}\right) CO_2$$

Equation 2:

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right) H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} + \frac{e}{4}\right) CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right) CO_2 + dNH_3 + eH_2S$$

Since the degradable fraction of the organic material has to be known for a precise biogas potential analysis, the knowledge of the chemical composition alone is not sufficient for the characterisation of complex substrates with a significant fraction of non-degradable material. For substrates such as plain glucose or cellulose it represents an important method to validate experimental setups (e.g. VDI 4630, 2016). Furthermore, the necessary amount of energy and substrate for microbial metabolism to convert the substrate is not determined directly by means of the elementary analysis. If the substrate demand for microbial growth and maintenance is known extended balances developed by McCarty (1972) can be utilized to account for microbial growth during stoichiometric calculations.

2.3.3. Chemical oxygen demand

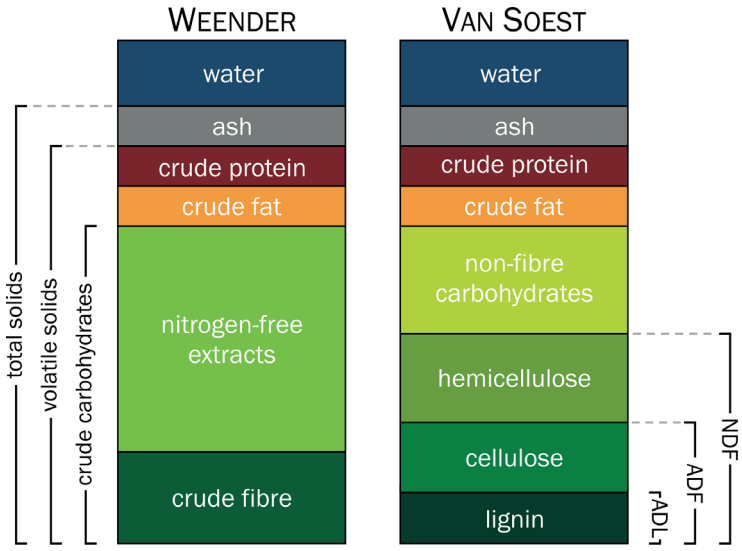
The chemical oxygen demand (COD) method is based on the fact that 1 g COD (assuming that only organic carbon compounds are oxidised) is equivalent to a potential of 350 mL methane (VDI 4630, 2016; DIN 38414-9:1986-09, 1986). Consequently, 1 g of converted COD can produce a maximum of 350 mL of methane. A deduction of the amount of produced carbon dioxide from the COD measurement is not possible. The estimation based on the COD lacks also the direct determination of the degradable fraction of organic material. The COD method is mainly used within the waste water treatment industry and still has limitations when it comes to the analysis of complex and particulate matter. Microbial growth is not considered within the method and a high content of other oxidisable compounds (nitrogen, sulphur) might lead to overestimation of methane potentials (VDI 4630, 2016).

2.3.4. Total organic carbon

Whereas the COD method is suitable only for methane potential determination, the total organic carbon (TOC) provides the biogas potential with no information about gas composition. Anaerobic degradation of 1 mol organic carbon results in 1 mol biogas (22.414 L), which is equivalent to 1.868 L biogas per g TOC (without consideration of microbial growth). TOC is determined via combustion or wet oxidation and is more precise as compared to the COD approach (Liebetrau et al., 2017) in assessment of solid materials. Again, the degradable TOC fraction as well as the amount utilised for microbial growth can not be characterised, which compromises the estimation.

2.3.5. Calculations based on nutrient composition

Several researchers developed individual approaches to estimate the biogas potential based on the characteristic nutrient composition of specific substrates. The analytical determination of various nutrients is typically based on either the Weender and/or van Soest method (Naumann & Bassler, 1976), which both originated from plant or feedstock characterisation for (livestock) husbandry. Thus, individual components of either method are commonly utilised to determine biogas potentials of agricultural substrates (such as energy crops) and lignocellulosic biomass, Figure 4.



NDF = neutral detergent fibre
ADF = acid detergent fibre
ADL= acid detergent lignin

Figure 4: Components of Weender and van Soest analysis of characteristic nutrients (Liebetrau et al., 2017; adapted by permission from the copyright holders, Springer Nature)

Table 1: Biogas potential of the degradable nutrient fractions (adapted according to Weissbach, 2009b)

	Weiland 2001		Baserga 1998		VDI 4630, 2016		Weissbach 2009b	
	Biogas [L kg^{-1}]	Methane [%]	Biogas [L kg^{-1}]	Methane [%]	Biogas [L kg^{-1}]	Methane [%]	Biogas [L kg^{-1}]	Methane [%]
Carbohydrates	700 – 800	50 – 55	790	50	750	50	787 – 796	50.0 – 51.1
Proteins	600 – 700	70 – 75	700	71	793	50	714 – 883	50.9 – 51.4
Lipids	1000 – 1250	68 – 73	1250	68	1390	72	1340 – 1360	70.5 – 71.3

Generally, available estimation procedures can be divided into two main categories. The first kind utilises the composition and degradability of macro nutrients such as crude carbohydrates, proteins and lipids determined by Weender analysis to calculate the biogas potential of the respective substrate. For this purpose the characteristic biogas potential of each nutrient is determined by suitable reference values (Weiland, 2001; Baserga, 1998; VDI 4630, 2016) or detailed stoichiometric calculations (Weissbach, 2009b), Table 1.

By utilising the results of digestibility experiments on ruminants the anaerobically degradable fraction of each nutrient (degradable carbohydrates, proteins and lipids) is approximated (such as DLG, 1997). Thus, by multiplying the degradable share of each nutrient with the respective biogas potential (Table 1) the overall biogas potential of the substrate is calculated (Keymer & Schilcher, 1999). Generally, this method can be applied for distinctive and detailed substrate characterisation. However, the validity depends very much on a realistic approximation of the specific biogas potential and degradability of the characteristic nutrients. Furthermore, reference data on the digestible fraction of individual nutrients is typically limited to agricultural substrates.

Weissbach (2008) developed a modified version of this procedure. Based on digestibility experiments with sheep the method determines the total share of degradable volatile solids (DVS) by an empirical regression model in correlation with crude fibres (Weender analysis in Figure 4). By multiplying the DVS with an average biogas and methane potential for agricultural energy crops based on a detailed one-time chemical analysis (Weissbach, 2009b) the overall biogas potential of individual substrates can be determined. Furthermore, Weissbach (2009a) utilises an empirical approximation between 2 and 5 % of the degradable biomass fraction to account for microbial growth and maintenance.

Table 2: Typical regression models to determine biogas yields based on anaerobic batch tests and nutrient analysis of agricultural substrates (such as energy crops) and lignocellulosic biomass

Source	Gas components	Required Parameters	Validated based on
Amon et al., 2006	CH_4	crude protein, crude fat, crude fibre and nitrogen-free-extracts	energy crops (maize, cereals and grass)
Amon et al., 2007	CH_4	crude protein, crude fat, cellulose and hemicellulose	maize
Stoffe & Köller 2012	CH_4	lignin and starch	maize
Gunaseelan, 2006	CH_4	crude carbohydrates, lignin, acid detergent fiber, nitrogen and ash	fruits and vegetable solid wastes, sorghum and napiergrass
Thomsen et al. 2014	CH_4	cellulose, hemicellulose and lignin	lignocellulosic biomass
Triolo et al. 2011	CH_4	cellulose, lignin, acid detergent fiber and neutral detergent fiber	energy crops and manure
Rath et al. 2013	Biogas	hemicellulose, crude fat, water-soluble carbohydrates and lignin	maize
Dandikas et al. 2014	Biogas and CH_4	hemicellulose and lignin	energy crops (barley, clover, cup plant, grass, maize, millet, potato, rye, sugar beet, sunflower, triticale)
Dandikas et al. 2015	Biogas and CH_4	crude proteins, hemicellulose and lignin	grass and legumes
Kaiser, 2007	Biogas and CH_4	crude protein, crude lipids, crude fibres and nitrogen-free-extracts (additionally also hemicellulose, cellulose and lignin)	maize, grass and energy crops (miscanthus, sugar beet, wheat, peas, hemp)

The second category utilises the results of Weender and van Soest analysis to develop substrate specific regression functions between various nutrient fractions and the biogas yield determined in experimental batch tests (Table 2). Since these methods only depend on the functional behaviour and typically do not respect fundamental biochemical dependencies they are only valid for a specific substrate type (and individual analytical or experimental procedures applied during model development/validation).

2.3.6. Conclusions on theoretical biogas potential analysis

The methods described allow a quick approximation of the biogas potential; these methods are compromised by the unknown variability of the degradable fraction and consumption by microbial growth, both of which are only included in results of biological test systems. Some methods are also not able to specify the biogas composition or the stoichiometric water demand incorporated during decomposition.

The shortcomings of the analytical methods can be reduced by using regression analysis, reference or literature values for estimation of the unknown variables. This might be more or less precise, depending on the analytical method, the substrate and the parameter to be considered. In particular the methods, which estimate the biogas potential based on the nutrient composition place considerable emphasis on the evaluation of the degradable fraction of the substrate and in some cases on the chemical substrate composition (Weissbach 2008, 2009b), which should result in a higher precision of the determination of the biogas potential.

Beyond the determination of the biogas potential the methods described obviously cannot give any information about biological induced effects such as degradation kinetics or effects of inhibitory substances. Table 3 gives an overview of the major characteristics of the available methods for determination of biogas potential and yield. The consideration of different parameters has been distinguished into direct measurement of a parameter within the individual analysis of the respective substrate and the potential consideration of additional values based on regression models, reference values or literature data.

Table 3: Major characteristics of methodologies for direct determination of biogas potentials (based on Liebetrau et al., 2017)

	Direct determination/measurement of				Practical applicability	
	Biogas composition	Degradable fraction	Stoichiometric water demand	Microbial growth	Effort for determination	Available data for reference
TS/VS	No [a]	No [a]	No [a]	No [a]	Low	High
Chemical substrate composition	Yes	No	Yes	No	High	Low
COD	No (only methane)	No	Yes	No	Medium	Wastewater sector high, solid materials low
TOC	No	No	No	No	Medium	Low
Nutrient composition via statistical methods	No [a]	No [a]	No [a]	No [a]	Medium	In general plant materials for fodder high, specific for biogas medium and waste and wastewater none
Nutrient composition (incl. DVS) via digestibility analysis	No [b]	No [c]	No [b]	No [b]	Medium, new substrates high	In general plant materials for fodder high, specific for biogas low and waste and waste water none
Batch test	Yes	Yes	Yes	Yes	High	High
Continuous test	Yes	Yes	Yes	Yes	Very high	Medium

[a] reference values based on experimental batch tests available
[b] reference values based on stoichiometric calculations available
[c] reference values based on feeding experiments for agricultural materials

3. Experimental determination of biogas yield and potential via batch test

Laboratory-scale digestion experiments for the determination of biogas yield and the subsequent evaluation of the underlying biogas potential can be performed using a discontinuous (batch) or continuous stirred tank reactor (CSTR) approach. Several standards and guidelines are available (see Table 4) for performing anaerobic digestion by means of batch experiments. The experiments give a biogas yield which can be used for estimation of the biogas potential and facilitate the construction of degradation kinetic models.

Continuous experiments are more laborious, require a longer test duration but provide additional information on inhibitory effects, nutrient availability, organic loading rates, retention times, viscosity of digestate and operating parameters at these loading rates such as levels of total ammonical nitrogen (TAN), volatile fatty acids (VFA), gas composition and ratios of acidity to alkalinity. Due to their continuous operation they give a better representation of the conditions of continuous operated biogas plants. Up to now, there are no methodological standards on the performance of continuous laboratory digestion tests (besides some basic considerations in VDI 4630, 2016).

Biogas potential estimation from experimental data or process data from full-scale plants requires either dynamic modelling or several test periods in steady state. Typically, at full-scale, plant operators do not collect sufficient data for reliable detailed analyses. Therefore, an evaluation/verification process is necessary whereby laboratory tests are undertaken to mimic full-scale systems and to confirm full-scale performance analysis. Experimental determination of the biogas yield should generally result in lower values in comparison to biogas potential (Figure 2).

3.1. Test conditions and standards for batch tests

The report focuses on biogas potential analysis via batch tests. A variety of methods and guidelines for laboratory batch experiments are in use and numerous test results and publications are available. All available standards result rather in a biogas yield and not in a biogas potential in the way that they end the experiment after a certain retention time (typically around 30 days) under the assumption that the gas production following the termination criterion is negligible.

Since formulation of important test parameters and conditions is often vague or not applicable to other substrates in older standards (DIN 38414-8:1985-06, 1985; DIN EN ISO 11734, 1998), different interpretations and test protocols have been developed and applied. Assuming an effect of the protocol on the results it is very likely that the results of tests from different standards are hardly comparable. Over the last number of years several guidelines focussed on batch assay protocol development in order to improve the test result comparability. Table 4 gives an overview on individual characteristics of selected standards and guidelines for batch test approaches.

An overview of current methods for experimental determination of biogas yields via batch tests and methods for theoretical interpretation are given in the following section, followed by a discussion on the most influential factors and a general evaluation of test methods.

3.2. Experimental setup

Batch tests require gastight equipment (no oxygen inflow and fugitive biogas outflow) and typically consist of a reaction vessel and gas collection/measurement system. Glass is the preferred material for the reaction vessel and if possible for all biogas containing parts of the apparatus (VDI 4630, 2016). There are several types of testing devices, which measure the amount of produced biogas as a function of biomass activity in the presence of the test substrate (Guwy, 2004). For this purpose, manometric or volumetric (water replacement, flow meter, syringe extension) methods are used. The main principles of biogas quantification are shown in Figure 5.

Table 4: Comparison of standards and guidelines for batch tests

		DIN 38414-8:1985-06 (1985)	DIN EN ISO 11734 (1998)	VDI 4630 (2016)	Angelidaki et al., 2009	Holliger et al., 2016
Scope		Fermentation of wastewater treatment sludge	Fermentation of organic materials	Fermentation of organic materials	Fermentation of solid organic wastes and energy crops	Fermentation of organic materials
Experimental setup	Volume	Eudiometer, 0.5 L	0.1 to 1 L	0.5 to 2 L, 10 to 20 L in case of inhomogeneous substrate	~0.1 L to 2 L depending on the substrate homogeneity	~0.1 L to 2 L depending on the substrate homogeneity
	Temperature	Mesophilic	Mesophilic	Mesophilic or thermophilic	Mesophilic or thermophilic	Mesophilic or thermophilic
	Stirring	–	Manual mixing or stirring twice or 3 times a week	Continuous stirring or periodical manual mixing	Continuous stirring	Gentle continuous or manual mixing once a day
Inoculum	Source	Digested and active sludge	Municipal sewage sludge or digestate from laboratory-scale reactors	Active anaerobic digester (municipal sewage treatment or agricultural plants)	Active anaerobic digester (sludge, manure)	Active anaerobic digester (municipal sewage treatment or agricultural plants)
	Pre-treatment	–	Washing and resuspension in specified test medium	Removal of coarse material	Homogeneity required Removal of coarse material	Dilution if necessary Sieving, 1 to 5 mm mesh size
	Nutrients	pH and nutrition adjustment		Optional according to DIN EN ISO 11734 (1998)	Macro- and micro nutrient supplementation	Macro- and micro nutrient supplementation
	Degassing	Degassing at 35°C	Optional degassing of 5 to 7 days at 35 ± 2°C	Degassing at test temperature	Degassing of 2 to 5 days at test temperature	Degassing up to 7 days at test temperature
	Storage	Extended storage (1 month) at 35°C under anaerobic conditions	–	Optional according to DIN 38414-8 (1985)	Usage of fresh inoculum	Inocula should be used as fresh as possible
	Adaptation	–	Optional pre-incubation of slow to degradable substrates	Optional repetition of batch test with digestate from the preceding test	–	Not necessary
	Gas production/ Activity	–	–	endogenous gas production of the inoculum < 20 % of the sample (substrate and inoculum)	activity test on cellulose, glucose, casein and organic acids (propionic, n-butyric or acetic acid)	endogenous gas production of the inoculum < 20 % of the sample (substrate and inoculum)
Reference		Reference sludge municipal sewage plant	Sodium benzoate, phenol, polyethylene glycol (degradation > 60 % by weight)	Microcrystalline cellulose (745 L biogas kg^{-1} VS ± 10 %) acetate	Microcrystalline cellulose, gelatine	Microcrystalline cellulose (>85 % and <100 % of the stoichiometric gas potential >352 L CH_4 kg^{-1} VS and >414 L CH_4 kg^{-1} VS), tributyrin
Inoculum to substrate ratio		5 – 20 % substrate by mass	100 mg substrate per L organic carbon	> 2.5 (VS based substrate to inoculum ratio should be < 0.5)	Detailed substrate specific recommendation given	Between 2 and 4 (VS based)
Termination criterion		Daily biogas rate ≤ 1 % of total volume	60 d, degradation > 50 % (plateau phase of the degradation kinetics)	Daily biogas rate < 0.5 % of total volume	–	Daily biogas rate < 1 % of total volume
Repetition/ Validity		–	Triplicate	Deviation between two of the triplicates max. 15 % Microcrystalline cellulose (reference) yields 745 ± 10 % biogas or 373 ± 10 % methane (in L kg^{-1} VS)	Triplicate Inter-laboratory evaluation	Triplicate Inter-laboratory evaluation Outlier elimination: relative standard deviation >5 % Microcrystalline cellulose (reference) yields between 352 and 414 methane in L CH_4 kg^{-1} VS
Data analysis		Dry gas correction Volume correction under standard conditions (273.15 K, 101.33 kPa)	Dry gas correction Volume correction under standard conditions (273.15 K, 101.33 kPa)	Dry gas correction Volume correction under standard conditions (273.15 K, 101.33 kPa) TS correction if necessary	Volume correction under standard conditions (273.15 K, 101.33 kPa)	Dry gas correction Volume correction under standard conditions (273.15 K, 101.33 kPa)

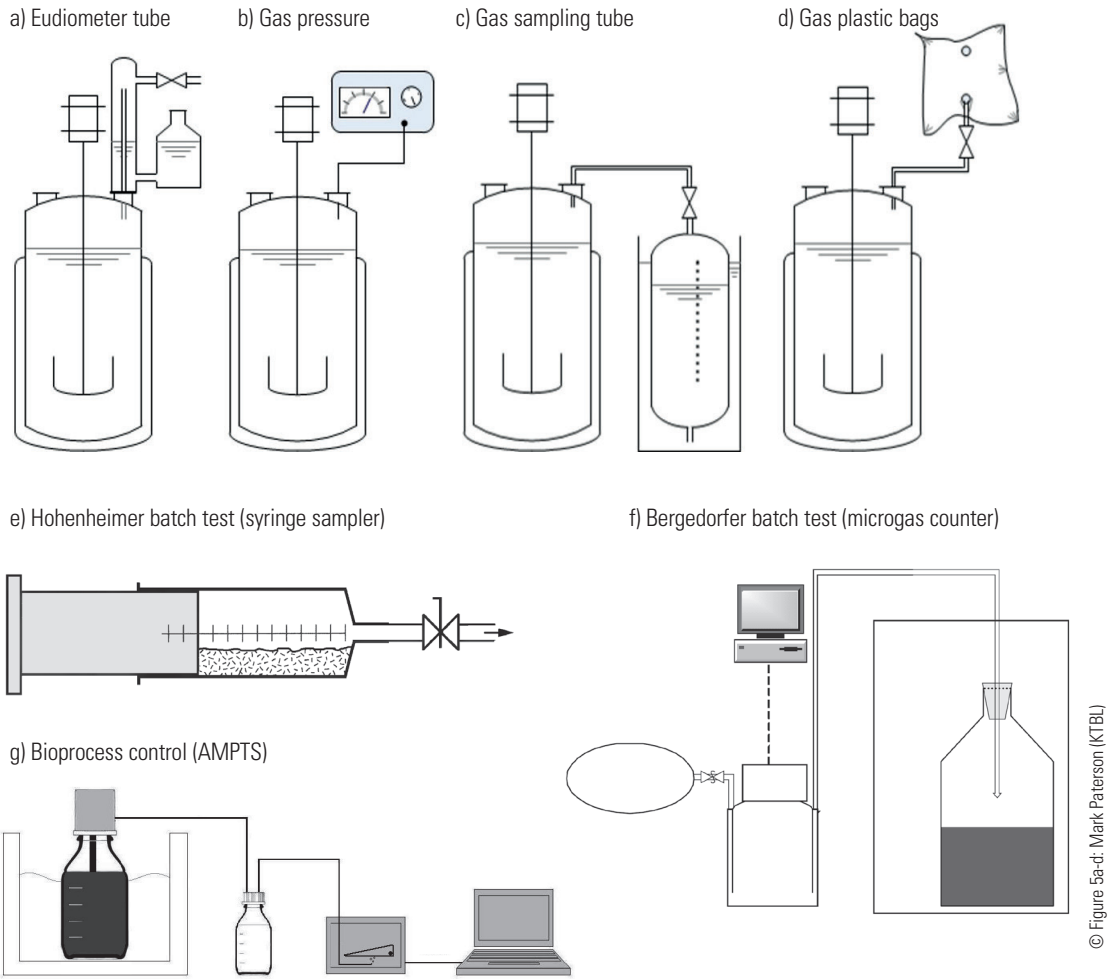


Figure 5: Main principles of biogas quantification used for batch tests a) Eudiometer tube (DIN 38414-8:1985-06, 1985) b) Gas pressure measurement (DIN EN ISO 11734, 1998) c) Gas sampling tube d) Gas plastic bags e) Hohenheimer batch test (Helffrich & Oechsner., 2003) f) Bergdorfer batch test (Scherer, 2001) g) AMPTS (Bioprocess Control, Sweden)

Batch test reactor volumes vary from 0.1 to 2 L flasks (Figure 5a – d, g and f). Inhomogeneous substrates may require larger fermentation volumes (10 to 20 L) to obtain representative sample amounts (VDI 4630, 2016). Reaction volumes at the lower end such as the Hohenheimer syringe sampler (100 mL, Figure 5e) require sample pretreatment (drying and milling to 1 mm particle size) to achieve representative samples (Helffrich & Oechsner, 2003) for adequate inoculation.

The analysis of gas composition (gas analyser) is usually implemented by collecting the gas in a plastic bag or the direct connection of the equipment to a measurement device. In contrast, the measurement device provided by Bioprocess Control allows automatic measurement of methane production (AMPTS, Figure 5g) by separating carbon dioxide from the gas flow by means of a base (NaOH) and measuring only methane in a flow meter (tipping counter).

Incubation of anaerobic digestion tests is usually conducted under mesophilic (37 °C ± 2 °C) or thermophilic (55 °C ± 1 °C) conditions in climatic chambers or water baths (VDI 4630, 2016). A mixing device is useful to prevent floating layer formation and to guarantee homogeneous degradation conditions. Different implementations of mixing from manual mixing once a day to continuous stirring is applied (Holliger et al., 2016). However, the effects of mixing on the general performance of anaerobic digestion are contradicted in the literature (Raposo et al., 2012). Whereas mixing increases the contact between substrate and microorganisms, it may also

destroy formed structures (such as flocs and granules) and thereby decrease interaction between microbial populations.

Test duration depends on the kinetics of the process. The batch test should be continued until the daily biogas production equals 0.5 % of the total biogas production on three consecutive days (VDI 4630, 2016). The performance of triplicates (VDI 4630, 2016; Holliger et al., 2016) or even more repetitions depending on the complexity of the substrate is recommended (Angelidaki et al., 2009).

3.3. Inoculum

Several standards and literature references highlight the importance of the quality of the inoculum and describe general quality criteria such as, origin and preparation/storage of batch test inocula (Angelidaki et al., 2009; VDI 4630, 2016; VDLUFA, 2011; Holliger et al., 2016). For inoculum evaluation, determination of basic values such as TS and VS are essential. In addition, determination of pH value, VFA, TAN and alkalinity might be necessary for quality evaluation of inocula (Holliger et al., 2016). Detailed activity assessment is described in (Angelidaki et al., 2009).

3.3.1. Source and sampling

The most frequently used inoculum sources are biogas plants treating agricultural residues, sewage sludge or biowaste (Raposo et al., 2011; Holliger et al., 2016; VDI 4630, 2016). Digestate from municipal sewage treatment plants is recommended due to its diverse biocenosis, resulting from contact with a large variety of substances (VDI 4630, 2016) and the consistent composition at different plant sites. According to Koch et al. the highest diversity is found within this digestate (mix of carbohydrates, proteins, lipids, cellulose from toilet paper) (Koch et al., 2017). In order to increase the microbial community diversity, Holliger et al. (2016) suggest mixing of inocula from different sources. Inoculum adaptation to the utilised substrates prior to the batch test might shorten the test duration, but is not mandatory (Koch et al., 2017; Holliger et al., 2016). Besides the microbial diversity the activity conditions of the digestate are important (Holliger et al., 2016). Inoculum quality can be tested by performing activity tests using standard substrates (e.g. minimum activity of 0.1 g CH_4-COD g^{-1} acetate) (Angelidaki et al., 2009). Additional quality criteria indicated by Holliger et al. (2016) include for a pH range between 7.0 and 8.5, an amount of VFA < 1.0 g L^{-1}, a TAN value < 2.5 g L^{-1} and alkalinity > 3 g $CaCO_3$ L^{-1} (Holliger et al., 2016). VS should be above 50 % TS (VDI 4630, 2016).

3.3.2. Pretreatment and storage

Consistent information is found in the scientific literature in relation to degassing of inocula via a pre-incubation process at test temperatures. Pre-incubation of 2 to 7 days is recommended to reduce endogenous gas production (VDI 4630, 2016; ISO 11734, 1998; Angelidaki et al., 2009; Holliger et al., 2016). According to ISO 11734 (1998) additional adaptation prior to the batch test by upstream fermentation using the test substrate is beneficial, whereas other literature sources did not detect a need for adaptation (Koch et al., 2017; Holliger et al., 2016). Dilution might be necessary in case of high VS contents (> 100 g L^{-1}) (VDI 4630, 2016; Holliger et al., 2016).

Whereas DIN EN ISO 11734 (1998) recommends a washing step and resuspension in a specified test medium to achieve adequate nutrient supply and buffering capacity, avoidance of washing and reduction of pre-treatment to a minimum is proposed by others (Angelidaki et al., 2009; Holliger et al., 2016). In case of micro-nutrient or trace element depletion, supplementation to the origin medium is suggested to be applied combined with pH adjustment (Angelidaki et al., 2009; Raposo et al., 2012). Some inocula may require sieving (1 – 5 mm mesh size) to remove coarse materials (stones, wood) for optimisation of test conditions (Angelidaki et al., 2009; Holliger et al., 2016).

Long term storage should be avoided; there is a stated preference for fresh inocula. If storage is unavoidable, temperatures should be adjusted to the test temperature (Holliger et al., 2016). Some larger laboratories have their specific inoculum digestion system and feed the inoculum according to a given protocol.

3.3.3. Adaptation of the inoculum

It can be stated that application of a universal inoculum bears the risk of limited adaptation to specific substrates which might compromise the results. There have been several studies showing that the inoculum can have a significant impact on the result (see section 3.3.4 below). A labori-

ous solution might be the adaptation of the inoculum to the substrate tested in order to rule out the lack of adaptation (DIN EN ISO 11734, 1998; VDI 4630, 2016). In particular while testing pre-treatment technologies via batch tests the adaptation of the microbial fauna to the substrates in continuous tests might make differences found in batch tests disappear as compared to continuous tests. Moeller et al.(2018) studied the effect of the disintegration grade of triticale grain (coarse triticale in two grain sizes and grain kernels) on biogas production in batch and continuous approaches (see Table 8). Batch tests showed an 11 % lower biogas yield with the kernels, whereas semi-continuous fermentation showed similar biogas yields for all pre-treatments (Moeller et al., 2018). The modified substrate composition and lack of adaptation potential during the batch assay may have caused this difference. Tabassum et al. (2016) compared anaerobic digestion of seaweed species in batch tests using initial and acclimatised inoculum (after continuous trials with a similar substrate mixture) and achieved 2 to 13 % higher methane yields in case of the adapted inoculum. In contrast to that, some studies found no significant effect of inocula adaptation (Koch et al., 2017; Li et al., 2013).

3.3.4. Influence of the inoculum on batch test results

Compliance with general quality parameters for the inoculum as described in several norms and scientific literature on batch test, standardisation (Angelidaki et al., 2009; VDI 4630, 2016; VDLUFA, 2011; Holliger et al., 2016; Raposo et al., 2012) does not prevent varying test results based on different inocula sources. Table 5 gives an overview on the impact of the inoculum origin on the methane yield detected via batch tests, by showing results of substrates tested with different inocula. Some studies detected no effect, whereas other studies proved a significant impact on the resulting methane yield. Different levels of microbial population (abundance of methanogens) are discussed as possible reasons (Raposo et al., 2012; De Vrieze et al., 2015). Furthermore, inhibiting effects of the inoculum itself (De Vrieze et al., 2015) or stimulation of carbon lacking inocula by addition of carbon-rich substrates (Koch et al., 2017) could contribute to these differences. Only a larger data set and repetition of experiments (e.g. inter-laboratory comparison) can potentially compensate natural fluctuations.

Based on the results presented in Table 5 it can be stated that an effect of the inoculum on the results with the given

Table 5: Influence of the inoculum on batch test results

Substrate	Standard/ reference method	Number of tested inocula	Specific methane yield[a] [L kg^{-1} VS]	Coefficient of variation [%]	Range [%]	Reference
Sewage sludge	VDI 4630, 2016 Holliger et al., 2016	3	300 ± 8[b]	3	5	Koch et al., 2017
Maize		3	345 ± 5[b]	1	3	
Food waste		3	452 ± 16[b]	4	7	
Cellulose		3	356 ± 19[b]	5	11	
Rice straw	–	6	91 ± 74[b]	81	191	Gu et al., 2014
Chicken manure	–	2	325 ± 37	12	16	Li et al., 2013
Corn stover		2	259 ± 58	22	32	
Para grass : pig manure (75 : 25)	Hansen et al., 2004	2	448 ± 7	2	2	Dechrugsa et al., 2013
Para grass		2	470 ± 74	16	22	
Pig manure		2	135 ± 173	128	181	
Food waste	–	2	865 ± 106	12	17	Elbeshbishy et al., 2012
Molasses	Angelidaki et al., 2009	4	360 ± 12[b]	3	8	De Vrieze et al., 2015
Bio-refinery waste		4	212 ± 38[b]	18	40	
Liquid pig manure		4	239 ± 80[b]	33	81	
High-rate activated sludge		4	378 ± 66[b]	18	40	

[a] mean of tested inocula for the respective substrate
[b] estimated based on a diagram/graphical representation of the experimental results (no data table available)

conditioning procedures in the standards cannot be eliminated for sure. When interpreting results of a test, it has to be assumed that the inoculum has an impact on the biogas potential and contributes to a certain variability in the results. This is also of importance when comparing tests conducted with the same inoculum. The inoculum might be adapted differently to individual substrates (and substrate components) and this might be particularly of interest in case of the evaluation of pre-treatment methods. The inoculum might have at this point an unwanted and unkown impact on the results which compromises the evaluation of the pre-treatment technology.

3.4. Residual biogas yield

A particular test is the batch test of an active digestate without the addition of substrate. The test usually aims at the quantification of the amount of degradable substrate available in the digestate after the digestion process. It is used for efficiency analysis of the digestion process (remaining gas potential) as well as the estimation of potential methane emissions from the digestate if stored openly (VDI 4630, 2016). For the latter purpose, tests at lower temperatures (20°C) have been presented (VDI 3475, 2010).

3.5. Substrate

3.5.1. Sampling

Whereas sampling of a representative substrate fraction is quite simple in case of liquid and homogeneous substrates, solid materials with increasing heterogeneity require a high level of technical knowledge to achieve representativeness. Variability of substrate quality requires special effort on the sampling strategy to obtain representative samples. A detailed description of appropriate techniques is available in the German guideline VDI 4630 (2016). Subsequent substrate analyses necessary for substrate characterisation include TS, VS and pH as basic parameters and VFA, total Kjeldahl nitrogen (TKN), TAN and alkalinity for estimation of potential inhibition problems (Holliger et al., 2016). Further data on the composition of substrates can be used for calculation of theoretical biogas potential by several approaches (see section 2.3.2). Sampling of the substrate as well as sample preparation are essential components for the evaluation of anaerobic digestion properties of organic material and have a high impact on reliable batch test results.

3.5.2. Pretreatment and storage

Substrate preparation should be minimized in order to avoid alteration of properties and degradability. Removal of coarse inert material (gravel, sand, plastics) as well as shredding or grinding (particle size of 10 mm) should be applied only if necessary (Holliger et al., 2016). Since particle size is an important parameter, especially for degradation kinetics, standardisation is recommended to increase reproducibility of testing results (Angelidaki et al., 2009). Consistent to this conclusion Raposo et al. (2012) proposed a particle size ≤ 10 mm to achieve comparable results. Surface area and particle size are supposed to be important determinants of the initial degradation rate (Raposo et al., 2012). As described for the inoculum, the substrate should be used as fresh as possible and storage should be avoided. A storage of 2 to 5 days at 4 °C is acceptable. Freezing and drying may significantly alter the substrate conditions and should be avoided (Holliger et al., 2016).

Several studies investigated the influence of substrate particle size on specific biogas or methane yield obtained by batch tests. Besides the effect on specific methane yield an influence on the initial degradation rate has been detected (Mshandete et al., 2006). Most of the studies achieved higher specific biogas or methane yields by reducing the substrate particle size (Moeller et al., 2018; Weiss & Brückner, 2008; Herrmann & Rath, 2012; Moorhead & Nordsted, 1993; Sharma et al., 1988; Mshandete et al., 2006). Only a few studies reported no specific or only small effects (Llabres-Luengo & Mata-Alvarez, 1988). Drying of substrate may alter the specific biogas yield if the volatile fraction has a significantly different chemical composition than the remaining substrate and if it is not compensated for in the analysis of the test. Batch assays using dried substrate may result in an underestimation of the gas production from the original wet material in proportion to the evaporated volatile compounds. Using wet substrates may lead to overestimation of specific biogas potential if compensation for losses during drying is not done properly (VDI 4630, 2016).

3.6. Test procedure

3.6.1. Reference and blank

For estimation of background methane production resulting from the inoculum, performance of a blank assay without substrate is necessary (Angelidaki et al., 2009; Holliger et al., 2016; VDI 4630, 2016). The substrate specific gas yield should be more than 80 % of the total gas yield (VDI 4630, 2016), which requires low inoculum generated gas production (degassing, dilution; see section 3.3.2 above) and an appropriate substrate amount (see section 3.6.2 below).

For evaluation of the inoculum activity and ability to degrade organic material, a reference substrate is used as a positive control. Suitable substrates should have a known gas potential so completely degradable materials with a known composition are commonly used. Microcrystalline cellulose or acetate are most abundant. The threshold for the validity of the test according to VDI 4630 (2016) is 745 mL g^{-1} VS ± 10 % for microcrystalline cellulose. This value is deduced based on the stoichiometric biogas potential of 829 mL g^{-1} VS for cellulose ($C_6H_{10}O_5$) minus 10 % of the degradable substrate components for microbial growth and maintenance. Angelidaki et al. (2009) suggested application of standard substrates dependent on tested substrate composition; cellulose in case of agro-waste or municipal waste and gelatine in case of meat and fish. Furthermore, some laboratories use their own internal standard such as dried concentrated fodder or synthetic mixtures (adjusted to their specific field of application) for internal validation of individual trails.

3.6.2. Inoculum to substrate ratio

The concentration of organic matter from substrate and inoculum is supposed to be between 20 and 60 g VS L^{-1} in sum (Holliger et al., 2016) and should not exceed a TS content of 10 % (VDI 4630, 2016). VS-based inoculum to substrate ratio (ISR) should be between 2 and 4 (Holliger et al., 2016; VDI 4630, 2016). In order to avoid overload or inhibition, comparison of different ISR is recommended for unknown substrates (≥ 4 in case of easy degradable substrates, ≤ 1 for less degradable substrates like lignocellulosic organic matter) (Holliger et al., 2016). The ISR is an additional parameter discussed as a determinant of batch test results. Whereas Raposo et al. (2006) showed only slightly variations of specific methane yield for maize at ISR of 1, 1.5, 2 and 3 (211 ± 6 mL CH_4 g^{-1} VS), Neves et al. (2004) described a major influence of ISR on anaerobic batch digestion of kitchen waste. The study showed drastically reduced specific methane yields at substrate to inoculum ratios > 0.5 based on VS (equivalent to an ISR of 2), which had a greater influence than the inoculum activity (granular inoculum in comparison to suspended sludge; Neves et al., 2004). Since ISR suitable for certain test conditions varies with substrate composition, different threshold values are documented. Hashimoto reported significantly reduced specific methane yields using wheat straw at ISR < 0.25 (Hashimoto, 1989).

3.7. Methods for test evaluation

Generally, there is no standardised or mandatory procedure for evaluating and interpreting experimental batch test results beyond the termination criterion and the standardisation of the gas produced. The German VDI Guideline 4630 (2016) includes a comprehensive list of necessary/useful calculations and corrections. Thus, biogas production is typically normalised to standard temperature and pressure (T = 273.15 K and p = 101.33 kPa). Further calculations include corrections for water vapour (Magnus formula) or individual head space volume to indicate dry biogas and valid biogas composition. The resulting biogas potential production is usually expressed in a ratio or volume of biogas in L per kg VS (or COD) of substrate added.

Since volatile components – such as short chain organic acids or alcohols – are lost during TS and VS determination (drying and incineration) a correction of TS and/or VS is necessary to provide a precise and realistic determination of the respective biogas potential (Schumacher, 2016). Weissbach & Strubelt (2008a) showed a TS loss of around 4 % by analysing 182 grass silages; some samples showed maximum losses up to 16 %.

Furthermore, additional information on the

- Origin, sampling, storage, pre-treatment and physico-chemical parameters of the tested substrate and utilised inoculum;
- Test conditions, experimental setup and procedures (including measuring techniques);
- Individual results of gross biogas production of substrate, blanks and control;
- Statistic evaluation of replicates (relative average and standard deviation)

should be included in the final report to increase transparency and enable comparability of different trails or methods (Angelidaki et al., 2009). Besides thorough data processing, statistic evaluation and error checking using model-based evaluations can significantly increase validity of batch test.

3.7.1. Model-based evaluation

Based on simplified reaction kinetics or regression functions mathematical models can provide additional information for substrate characterisation. Generally, two main cornerstones during batch test simulation and interpretation can be defined as follows:

Estimation of biogas potential

Most often the measured cumulative biogas yield (final value) during batch operation is considered equivalent to the total biogas potential of the respective substrate. However, due to comparably short test duration (typically < 30 days) and numerous influencing factors (Holliger et al., 2016) slowly degradable, particulate or lignocellulosic substrates are not entirely degraded after test termination. Diverse opinions about individual termination criteria (see Table 4) as well as the general validity of batch tests to determine the maximum biogas potential are still being discussed today. Suitable models can be used to increase meaningfulness of batch tests by extrapolating biogas production during batch operation and estimating the total biogas potential (at infinite retention time).

Characterisation of degradation kinetics

Additionally, kinetic models can be utilised to access temporal progression of biogas production (substrate degradation). Thus, the fundamental model structure and respective kinetic function has to be defined. Generally, scientific literature offers numerous kinetic approaches and empirical models for modelling anaerobic digestion processes (Bastin & Dochain, 1990; Kythreotou et al., 2014). However, a small share of simplified model approaches have proven their applicability for model-based evaluation of batch test in practise (see Table 6).

The individual model structures clearly distinguish between the biogas potential and degradation kinetics. Furthermore, the kinetic behaviour is described by characteristic model parameters to enable comparability of individual substrates and trials. Thus, a change of the biogas potential (e.g. S in L kg^{-1} VS) will increase or lower cumulative biogas production whereas a change of degradation kinetics (e.g. k in d^{-1}) will affect the rate of degradation (see Figure 6).

For evaluation of experimental batch tests and substrate characterisation, individual model parameters have to be adjusted to guarantee realistic simulation results in comparison to the respective experimental measurements. For model adaption unknown parameters can either be fitted manually or identified based on numerical optimisation procedures (Isermann & Münchhof, 2010). Thus, the choice of a respective optimisation algorithm, objective function and additional constraints will affect the outcome of parameter estimation and model accuracy.

Table 6: Established model approaches for dynamic simulation of cumulative biogas production during anaerobic batch tests

First-order Angelidaki et al. 2009	$S \cdot (1 - e^{-k \cdot t})$	S = biogas potential in L kg^{-1} VS k = first-order reaction constant in d^{-1}
Two-fractions first-order Brulé et al. 2014	$S \cdot (1-\alpha \cdot e^{-k_{fast} \cdot t} - (1-\alpha) \cdot e^{-k_{slow} \cdot t})$	S = biogas potential in L kg^{-1} VS α = share of rapidly degradable substrate components k_{fast} = first-order reaction constant of rapidly degradable fraction in d^{-1} k_{slow} = first-order reaction constant of slowly degradable fraction in d^{-1}
Monod-type Koch et al. 2017	$S \cdot \left(\frac{k \cdot t}{1+k \cdot t}\right)$	S = biogas potential in L kg^{-1} VS k = rate constant in d^{-1}
Modified Gompertz Donoso-Bravo et al. 2010	$S \cdot e^{-e\left(\frac{Rm \cdot e}{S} \cdot (\lambda - t)+1\right)}$	S = biogas potential in L g^{-1} VS Rm = maximum biogas production rate mL g^{-1} VS d^{-1} λ = lag time in d

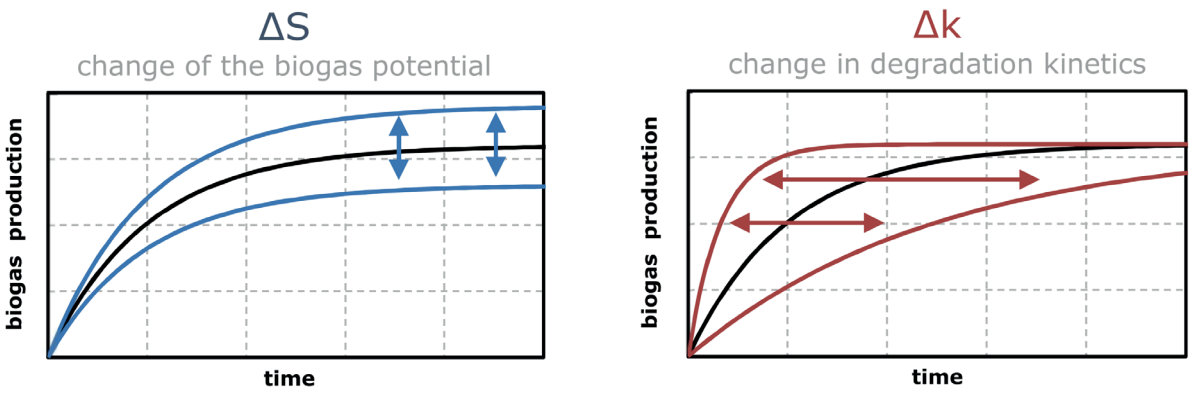


Figure 6: Characteristic dependencies of the biogas potential and degradation kinetics on the resulting biogas production during batch test modelling

As shown in Figure 7 single first-order models describe the degradation of the entire digestible substrate by a single first-order reaction, whereas a two-fraction first-order approach distinguishes between rapidly and slowly degradable substrate components. Generally, more complex models can depict the individual progression in more detail. Furthermore, due to the extended model structure additional information about the utilised substrate can be gained (e.g. amount of rapidly or slowly degradable substrate).

Providing a good fit of the respective simulated and experimental results the estimated parameters can then be utilised for substrate classification. Furthermore, the identified biogas potential occasionally shows higher values than the respective measuring results, which indicates that the underlying biogas potential is slightly higher than the final value of the batch experiment (Figure 7). In this case the estimated biogas potential S (or start concentration of degradable solids) is a reliable approximation of the maximum biogas potential (at infinite retention time). However, even for precise simulation results the estimated parameters do not guarantee reliable and realistic parameter values on principal. Therefore, the identified parameters – such as the maximum biogas potential or the individual kinetic constants – should thoroughly be reviewed to provide a meaningful set of parameters inside a reasonable value range for substrate characterisation.

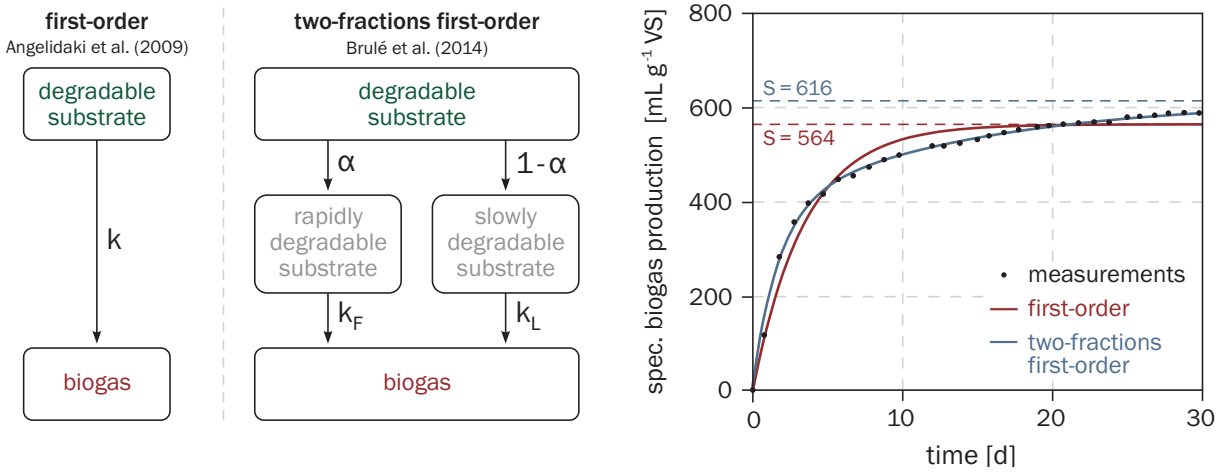


Figure 7: Simulation example of two characteristic model structures (adapted from Liebetrau et al., 2017)

4. Inter-laboratory tests and variability of batch test results

Inter-laboratory tests play a decisive role in quality evaluation of biogas laboratories, especially when considering results of biogas potential analyses. Although, detailed guidelines for batch tests exist (section 3.1), national and international inter-laboratory tests have shown a distinct variability in test results (Raposo et al., 2011). The reasons for varying batch test results within inter-laboratory studies corresponds to influencing factors already discussed for intra-laboratory deviation (section 3) in addition to variations caused by different technical equipment. A report on data of an international inter-laboratory study identified a significant influence of ISR on the methane yield, whereas inoculum source and experimental factors (stirring) had no significant influence on methane yield but on production rate (Raposo et al., 2011).

Since 2006 an inter-laboratory study on determination of biogas potential, substrate characterisation (TS, VS, etc.) and residual biogas potential has been performed by KTBL/VDLUFA on a regular basis (KTBL/VDLUFA, 2017). The methodical approach of the study refers to the VDLUFA method protocol and VDI 4630 (VDLUFA, 2011; VDI 4630, 2016). Results of the inter-laboratory study in 2016 comparing 21 laboratories analysing 4 samples are shown in Table 7.

Whereas the inter-laboratory variation of TS and VS is quite low for all tested substrates, the variation regarding the methane yield determined by batch tests is much higher. The inter-laboratory reproducibility (accuracy across different laboratories) is given in the different tests in a range between 8 – 26 %. In comparison to that, the impact on methane yield from different inocula, which resulted in variations from 1 – 20 % (in exceptional cases > 30 %) is quite high (Table 5), hinting at a major impact of the inoculum on the results. The common substrates cellulose and maize silage seem to have at least in the KTBL/VDLUFA (2017) test a lower inter-laboratory reproducibility than uncommon substrates. It should be highlighted that the range or spread of the values is higher.

An inter-laboratory study by ADEME (France) showed quite good intra-laboratory repeatability (accuracy within specific lab within one test set up) (4 %), while inter-laboratory reproducibility was much lower (13 to 21 %) (Table 7, Cresson et al., 2014). The BFE financed international inter-laboratory study achieved similar results (7 – 9 % intra-laboratory repeatability and 15 – 17 % inter-laboratory reproducibility, Fruteau de Laclos & Holliger, 2018). Intra-laboratory reproducibility (accuracy within specific lab,

Table 7: Comparison of batch test results within inter-laboratory studies

Substrate	TS [% FM]	Range [%]	VS [% TS]	Range [%]	Mean specific methane yield [L kg^{-1}VS]	Min-Max [L kg^{-1}VS]	Range [%]	Intra-laboratory repeatability [%]	Inter-laboratory reproducibility [%]	Reference
Cellulose	96 ± 1	5	96 ± 1	4	371	324 – 440	31	3	8	KTBL/VDLUFA, 2017
Maize silage	33 ± 1	5	32 ± 1	9	369	292 – 421	35	5	11	
Oat bran	91 ± 0.4	2	86 ± 1	7	208	174 – 436	126	8	26	
Animal feed	92 ± 0.3	1	81 ± 2	13	452	202 – 567	81	4	19	
Cellulose	95 ± 1		95 ± 1		363	237 – 669	119	9	17	Fruteau de Laclos & Holliger, 2018
Pig feed	89 ± 1		82 ± 4		376	273 – 718	118	7	16	
Fodder flour	89 ± 1		86 ± 4		383	305 – 692	101	7	15	
Fodder	92 ± 1		80 ± 3		490	302 – 683	78	9	15	
Crushed mixture					405	260 – 525	65	4	19	Cresson et al., 2014
Straw					277	195 – 370	63	4	21	
Mayonnaise					848	660 – 1026	43	4	13	

time intervals between test conduction) was tested by ADEME and highlighted slightly higher variability (5 to 7 %) than the intra-laboratory repeatability (4 %) (Cresson et al., 2014).

The text box outlines the details and results of the KTBL/VDLUFA-Proficiency Test Biogas authored by Mark Paterson (German Association for Technology and Structures in Agriculture, KTBL), Hans Oechsner, (University of Hohenheim) and Peter Tillmann (VDLUFA Quality Assurance NIRS GmbH, VDLUFA).

It shows that frequent participation at inter-laboratory tests led to stable, but still quite variable results. It should be highlighted that it is not always the same laboratories taking part each year. There seems to be a limit in using the existing standards to reduce variation further. Participation at an inter-laboratory test is not mandatory and the results are not published yet. The "true" variability of results from batch tests in the overall biogas sector might therefore be different to the picture given by the inter-laboratory tests.

KTBL/VDLUFA-Proficiency Test Biogas

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Parts of the presented summary on the KTBL/VDLUA Proficiency Test Biogas were taken from the submitted manuscript "KTBL/VDLUFA-Proficiency Test Biogas" to be published in the Series of the funding programme "Biomass energy use" on Collection of Methods for Biogas (Methods to determine parameters for analysis purposes and parameters that describe processes in the biogas sector, Liebetrau et al., 2015), supported by the Ministry of Economic Affairs and Energy, Germany

The aim of the KTBL/VDLUFA-Proficiency Test Biogas (also referred to as inter-laboratory test) is essentially the comprehensive quality-assurance of biogas laboratories in the determination of gas yield and residual methane potential by means of discontinuous laboratory tests (batch tests). To this end, possible influencing factors and causes of deviations in the measurement results are analysed in order to increase the measurement accuracy of the biogas laboratories. This improves the comparability of the results of fermentation tests. Since 2006, the German Association for Technology and Structures in Agriculture (KTBL) together with VDLUFA Quality Assurance NIRS GmbH (VDLUFA) has carried out the Proficiency Test Biogas for biogas laboratories. The number of participating laboratories is between 20 and 30 per year, which come from Germany and abroad and participants utilise different experimental setups. In examining the selected analysis processes of the last three years, the focus in determining the gas yield (mean 24 laboratories per test) and determining the residual gas potential (mean 17 laboratories per test) is clear (Weinrich & Paterson, 2017). In order to obtain a uniform procedure and a good basis for the comparison of the test results from the biogas inter-laboratory test, the VDI Guideline 4630 (2016) or the VDLUFA Association Method (2011) procedures for carrying out the test were specified.

Implementation of the Proficiency Test Biogas

The scope of the annual KTBL/VDLUFA inter-laboratory test biogas is determined individually by the participating biogas laboratories. The participants choose from the following analysis scopes:

- Determination of the biogas and methane yield for at least 3 sample materials, including determination of dry matter, organic dry matter, crude ash and fermentation acids;
- Determination of raw nutrients, crude protein, crude fibre, crude fat, crude starch, sugar and other characteristics of feed evaluation and/or;
- Determination of the residual methane potential (at 20 °C and 37 °C), including the determination of C2-C5 fatty acids.

The basic requirement for the proficiency test participation is compliance with the VDLUFA methodology "Determination of biogas and methane yield in fermentation tests" (2011) or the VDI Guideline "Fermentation of organic substances; substrate characterisation, sampling, collection of material data, fermentation tests" VDI 4630 (2016) for the analysis scope biogas and methane yield and residual methane potential determination. At least three different samples are sent for the fermentation test in the inter-laboratory test. For this purpose, identical sample material is sent to all laboratories in the quantity required for the respective test setup. The sample material sent should be handled and analysed as usual in the laboratory. The sample material contains one sample of microcrystalline cellulose as a reference substrate and maize silage as a typical test material. The other fermentation substrates shipped should cover the usual range of substrate variations in practice. Other throughput substrates included wheat grain, grass silage, cup plant, oat bran, forage and brewer's grains. The raw nutrients are usually determined in maize silage samples. The analysis of the residual methane potential is based on fermentation residue (digestate) samples from an agricultural biogas plant. When sending fresh silages, the influence of sample storage and sample homogenization on the result must be considered. Normally, the samples are therefore sent in insulated boxes in a cool state in an express parcel. All samples, including the inoculum, must be analysed by the laboratory with at least three repetitions. The analysis period for the laboratories from when the analysis results are handed over to the organisers is about 4 months. The results and measured values are submitted in particular data sheets (MS Excel-based), if necessary with the corresponding curves of biogas and methane formation. Since the quality assurance of the biogas laboratories is the goal of the proficiency test, no plausibility check is carried out on the submitted laboratory data by the organisation. The inter-laboratory test is carried out and evaluated anonymously; each participating laboratory receives an individual identification number. At the end of the proficiency test, the laboratories receive a comprehensive written report of the proficiency test evaluation including the laboratory assessments, all relevant comments, method descriptions and individual results of the complete test. Usually, the organisers of the proficiency test arrange a final meeting after the evaluation has been completed, at which the laboratory representatives can discuss the results of the evaluation together with the KTBL working group and discuss possible sources of errors or problems that have arisen. This results in some interesting indications for the improvement of the laboratory work.

Evaluation of the Proficiency Test Biogas

The evaluation report of the proficiency test biogas of KTBL and VDLUFA includes all analysis areas and the corresponding parameters as well as all relevant notes, method descriptions and individual results for the respective year. The results of the laboratory evaluation are mainly presented using the systematic deviations/comparability of the laboratory results.

The evaluation is carried out according to DIN ISO standard No. 5725-1 (1997) "Accuracy (correctness and precision) of measuring methods and results" (DIN ISO 5725-1, 1997 and DIN ISO 5725-2, 2002), in order to describe the performance of the analysis method, and DIN standard No. 38402-45 (2014) "Standard methods for water, wastewater and sludge analysis – Part 45: inter-laboratory tests for suitability testing of laboratories".

The evaluation of the proficiency test by means of DIN ISO standard No. 5725-1 (1997) and 5725-2 (2002) serves to describe the possibilities of the method and in particular the comparability of the results across the laboratories. The precision of results is calculated, among other things, as inter-laboratory reproducibility (sR) and intra-laboratory repeatability standard deviation (sr). According to DIN ISO standard 5725-2 (2002), individual values are determined as outliers, if they do not match the other values of this laboratory. Furthermore, all values of a laboratory are marked, if the laboratory mean value of this laboratory deviates statistically significantly from the mean value of all laboratories or if the laboratory internal dispersion is increased. For all

three types of outliers, a distinction is made as to whether these outliers are significant at the 1 % level (then these measurement results are removed from the evaluation) or whether the significance is only given at the 5 % level (then these values are marked and considered in further calculations). Outliers are also removed from the evaluation by hand, without statistical calculations, if there are justified doubts about the data. The proficiency test organisers document these decisions.

Because of the evaluation according to DIN ISO standard 5725-1 (1997) and 5725-2 (2002), the following characteristic data of the method are obtained:

- Intra-laboratory repeatability variation coefficient (CV_r) – Relative accuracy of values within a single laboratory;
- Intra-laboratory repeatability standard deviation (s_r) – Precision of individual values within a single laboratory
- Inter-laboratory reproducibility variation coefficient (CV_R) – Relative accuracy between different laboratories;
- Inter-laboratory reproducibility standard deviation (s_R) – Precision of the mean values between different laboratories.

The following Figure 8 from the evaluation report of the inter-laboratory test 2016 (KTBL/VDLUFA, 2017) shows the resulting spread of the submitted analysis values for comparative laboratory evaluation, exemplified by the parameter methane yield for the sample microcrystalline cellulose.

The solid horizontal line indicates the mean value of the analyses from this inter-laboratory test. The dashed lines – if any – mark the "true value". The green dashed lines mark the tolerance limits calculated with the standard deviation of the method according to the standard.

In addition, DIN standard 38402-45 (2014) is used in the evaluation to enable the laboratory assessment. For this purpose, an existing method description – see paragraph above on DIN ISO 5725-1 (1997) and 5725-2 (2002) – is presupposed. The z scores (standardised normal distribution) are calculated and displayed. The tolerance limits are determined with m ± 2 standard deviation (s_R) or x_a ± 2 s_R if a "true value" has been assigned to the samples, where m is the mean value of the analyses from the proficiency test and x_a is the target value of the samples. Furthermore, the reproducibility standard deviation of s_R = 33 L per kg VS as defined in the VDLUFA Association Method (2011)

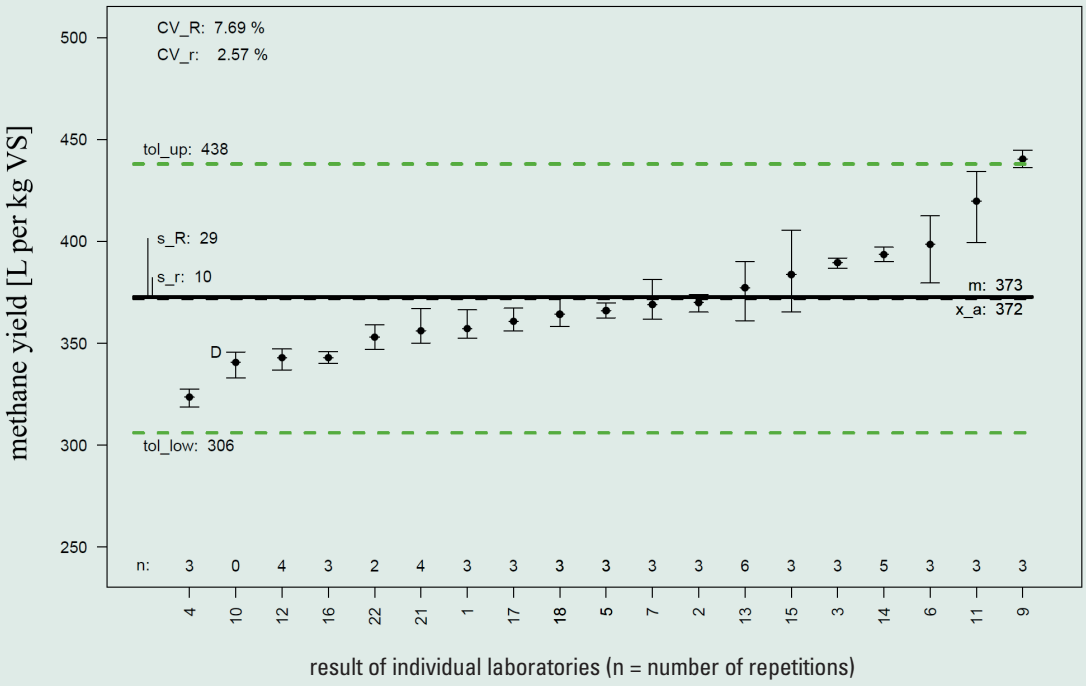

Figure 8: Scattering of the submitted analysis values for comparative laboratory assessment of the methane yield for the sample microcrystalline cellulose as example (KTBL/VDLUFA, 2017)

(based on an average over several inter-laboratory tests) is utilised to calculate the respective lower and upper tolerance boundaries.

Evaluation over the years

The first findings from the study of the inter-laboratory test data show that the results of the analysis have improved significantly over the past years of the inter-laboratory tests in determining the biogas and methane yield. Despite the increasing demands on the measurements, changing laboratories among the participants and varying numbers of participants over the years (Weinrich & Paterson, 2017). Since the composition of the laboratories participating in the proficiency test changes annually, a comparison of the evaluation over the duration of the test is only possible to a limited extent.

The inter-laboratory precision is represented by the variation coefficient of repeatability (CV_r) and variation coefficient of reproducibility (CV_R) for microcrystalline cellulose (reference standard) and maize silage samples (see Figure 9).

It is striking, that at the first run in 2006 the results for cellulose showed a relatively wide spread, although a standardized and very homogeneous test substrate was used. The inter-laboratory reproducibility coefficient of variation of methane yield between laboratories was 19.5 %. When comparing the test setups and the results, it became clear that the deviations were not related to the type and size of the respective test facilities. Rather, the procedure of data collection, the accuracy of methane measuring instruments, their regular calibration, the mathematical evaluation considering the reference values for standard conditions and the consideration of water vapour correction in the event of deviations played a clearly more recognizable role. In the meantime, the CV_R values for the methane yield for cellulose are around 8 %. The intra-laboratory repeatability coefficient (CV_r), which describes the accuracy of the values within a single laboratory, was reduced to less than 3 % for the methane yield in the years of the test runs.

A slightly different picture emerges by looking at the results of determining the methane yield of maize silage. The intra-laboratory repeatability variation coefficient (CV_r) of the laboratories could be improved from initially more than 6 % to about 4 % in 2017. The inter-laboratory reproducibility

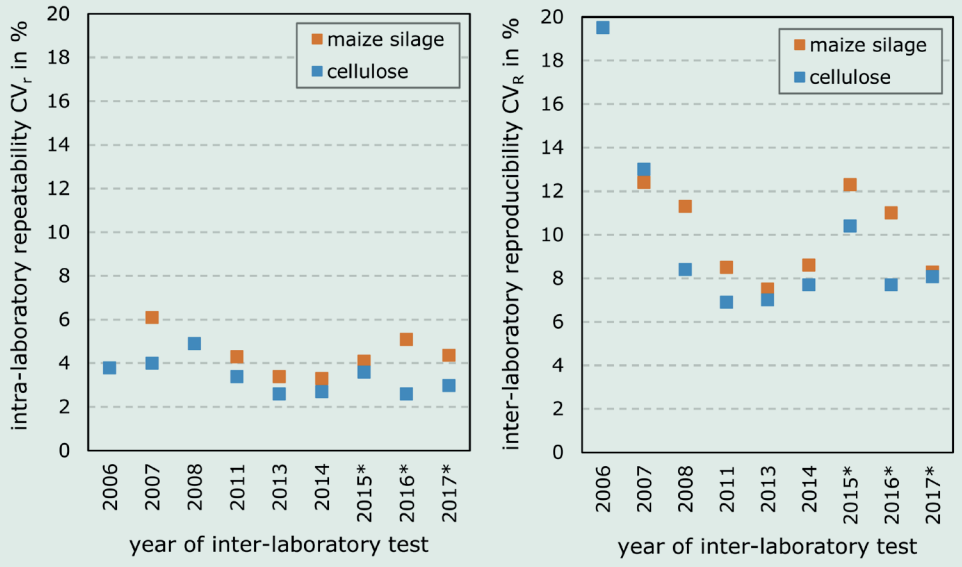

Figure 9: Development of the intra-laboratory repeatability coefficient (relative precision in a single laboratory) and inter-laboratory reproducibility (relative precision between laboratories) in the KTBL/VDLUFA (2017) proficiency test biogas (years 2006 to 2017) for the determination of biogas and methane yield of microcrystalline cellulose (reference standard) and maize silage. *The change in the objective of the inter-laboratory test from testing the implementation of the VDLUFA method (with plausibility control of incoming laboratory data) to quality assessment of laboratories (without plausibility control of incoming laboratory data) explains the changes in the repeatability coefficients

began with high CV_R values (of over 12 %) and over the years of the inter-laboratory test the scattering for this sample material has reduced to around 8 %. In the meantime, however, these values rose again slightly in some cases. For such a substrate, possible natural quality differences between the cultivation years, the influence of comminution technology and the influence of silage play a role in the development of the results. It has to be mentioned, that correction for volatile fatty acids was not mandatory and therefore not included in all test results. This can also lead to certain distortions of the results. Furthermore, in 2015, the objective of the inter-laboratory test was changed towards the quality assessment of the laboratories. Thus, the plausibility check of the incoming laboratory data applied up to then was omitted, which partly explains the deterioration in the evaluation in the final years of the comparison.

Also the homogeneity of the sample has had an influence on the comparison of the test evaluations over the years; maize silage is sent to the laboratories without pre-comminution and the sample is prepared as is customary in the respective laboratory. For this reason, higher and more fluctuating CV_R values are generally plausible for maize silage compared to cellulose.

Due to the increasing relevance of the determination of the residual methane potential for the efficiency assessment of existing biogas plants, the KTBL working group decided to include the residual methane determination of digestate in the analysis spectrum of the proficiency test biogas. Against the background of quality assurance, this is viewed as a good decision. During the first evaluations of the proficiency test with regard to the determination of residual methane, numerous biogas laboratories showed considerable potential for increased optimisation when determining this parameter. This is similar to the results at the beginning of the inter-laboratory test for biogas yield measurements. This knowledge and the fact that special requirements must also be observed for practical test results led to the VDLUFA method “Determination of the residual gas potential from digestate in the laboratory test” (VDLUFA, 2018).

Summary

Despite the established methodological regulations, it is essential for laboratories to carry out laboratory-wide quality improvement measures in order to test their performance. The analytical performance of a laboratory can best be tested in an inter-laboratory test in comparison with other laboratories.

The quality of the results of the KTBL/VDLUFA Proficiency Test Biogas has been continuously improving over the years of the inter-laboratory tests. However, it also emerged that it is essential for the basic conditions of the directives to be compiled in order to obtain comparable results. These include for example: the selection and quality of the inoculum; the appropriate mixing ratios of the test substrate and the inoculum: gas-tight test equipment; regular calibration of measuring instruments; and an optimised evaluation of the gas yields taking into account the temperature and pressure conditions.

Efforts to identify and eliminate sources of error in the participating laboratories are currently underway in order to achieve further improvement of the internal and cross-laboratory standard deviations.

Further information on the Proficiency Test Biogas of KTBL and VDLUFA can be found at:
www.ringversuch-biogas.de.

5. Comparison of methods for biogas potential and yield estimation at different scales

5.1. Interpretation of results from chemical analysis as compared to batch test

The biogas potential calculated by a chemical substrate analysis gives a rough estimation of the methane potential but has obvious limitations when it comes to the representation of biological processes. Several studies show the relationship between chemical analyses and batch tests in particular for known, homogenous substrates with limited variability within the degradation characteristics. However, the variability of the batch test itself raises the question as to whether the chemical analysis can match the batch test for accuracy.

Rath et al. (2015) compared different calculation methods for determination of the biogas potential based on nutrient specific reference values, degradable volatile solids and regression models (see chapter 3.2) to the results of laboratory batch tests during discontinuous digestion of different maize silage samples analysed in a single laboratory (Figure 10). It is obvious that the variation found in the batch tests is different from the variation of the chemical analysis. Whether the high variance of the batch test is caused by different substrate qualities or the fundamental uncertainty in the experimental setup and test procedure still remains unclear.

In Figure 10 it becomes obvious that the variation obtained from the chemical analysis differ from the variation of the batch test. Calculation of biogas potential based on chemical substrate analysis as applied here does not correlate to biological availability as given from the batch test.

The approach from Baserga (1998) overestimates the biogas potential due to the lack of information on the degradable fraction of the substrate and neglection of microbial biomass synthesis. Methods based on the composition of degradable nutrients (see chapter 2.3.5) named as Kaiser (2007) or Keymer and Schilcher (1999) underestimate the degradable fraction of the substrate and show a similar lack of variation regarding the composition.

The method of Weissbach (2008) seems at least to show a higher variation and is scattered around the target area. However the variation of the batch test does not correlate clearly with the calculation referring to nutrient (here mainly crude fibre) composition.

Additionally, it should be highlighted that most regression models published use data from one specific laboratory. Sources of variation of the prediction models might be based on methodical reasons when collecting specific biogas production data for model calibration (different sample pre-treatment and storage, chemical composition analysis) and the implementation of batch test procedures (Rath et al., 2015). Inter-laboratory tests evaluating batch tests show that the variation of results within a lab is considerably smaller than the inter-laboratory variation. This might also hold true for the chemical analysis which is used to correlate batch tests.

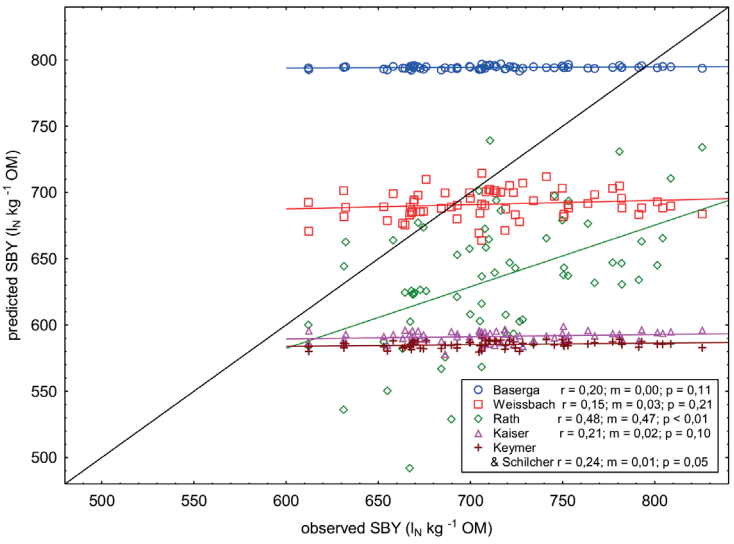

Figure 10: Calculated biogas potential of maize silage (predicted specific biogas yield, SBY) in relation to batch test results (observed SBY) with r = coefficient of correlation, m = slope and p = level of significance (by t-test) (Rath et al., 2015; adapted by permission from the copyright holders, Springer Nature)

In the national inter-laboratory test in Germany (KTBL/VDLUFA, 2017) experimental results from batch tests as well as from individual nutrient assessment from Weender or van Soest analysis (see Figure 4) were measured in participating laboratories. Figure 11 shows the comparison of inter-laboratory variation of experimental batch tests and available calculation procedures based on nutrient analysis (regression models or degradability analysis) to determine the methane potential of maize silage based on the results from the participating laboratories. Not all laboratories took part in all analysis categories. Figure 11 presents analytical results of the very same material from different laboratories, whereas Figure 10 presents results from different maize silages investigated in one laboratory. Furthermore, the KTBL reference value – based on 195 independent laboratory experiments of various types of maize silages with 27 to 36 % TS – is also depicted for comparison (KTBL, 2015).

Generally, the variance during the experimental determination by batch tests is significantly higher in comparison to individual calculation procedures based on degradability analysis or regression models as described in section 2.3.5. In accordance with the investigation of Rath et al. (2015) the individual calculation procedures for the methane potential tend to under estimate the methane yield measured in batch tests. Especially, the methods of Keymer and Schilcher (1999) and Kaiser (2007) evince a lower methane potential in comparison to the mean value of experimental batch results.

These results reveal a rather contradictory finding. Based on the fundamental definition in chapter 2.1 the methane or biogas potential based on stoichiometric calculations should always be higher than experimental results (with finite retention time and numerous influencing factors). In Figure 11 the characteristic methane potential determined by stoichiometric calculations (420 L CH_4 kg^{-1} DVS) and the fraction of degradable volatile solids based on the method of Weissbach (2008) as well as the regression model by Amon et al. (2006) lay close to the mean value of the inter-laboratory experimental results. Furthermore, the maximum share of degradable solids can be approximated by subtracting the content of volatile solids by the share of lignin (DVS = VS – lignin); multiplied with the stoichiometric methane potential it should define the maximum methane potential of the respective substrate. In Figure 11 this theoretical maximum based on lignin as well as the upper limit of methane production measured during the inter-laboratory batch tests correspond to each other.

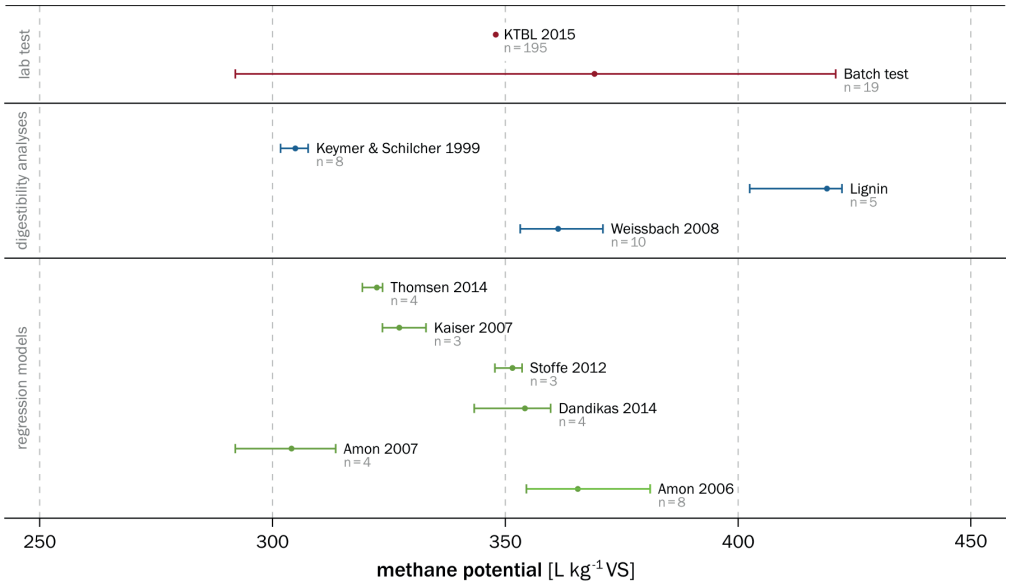

Figure 11: Comparison of inter-laboratory variation of experimental results and calculation procedures (regression models or degradability analysis) to describe methane potential of maize silage during the German inter-laboratory test (neglecting the influence of volatile organic compounds, which are lost during drying and incineration of the investigated maize silage) (Lignin method: Calculation method based on the maximum share of degradable volatile solids (DVS = VS – lignin); multiplied with the stoichiometric methane potential of 420 L CH_4 kg^{-1} DVS (Weissbach 2009b) (KTBL/VDLUFA, 2017)

Depending on the submitted analysis results of each laboratory the respective number (n) of samples available to calculate the methane potential based on individual methodologies (regression models or degradability analysis) varies significantly. Whereas, for example the calculation procedure of Weissbach (2009b) only requires ash and crude fibres as input parameters other calculation procedures such as Amon et al. (2007) or Kaiser (2007) utilise numerous components of nutrient analysis. Thereby, the number of required analytical parameters also reflects practical applicability and required effort during full-scale plant operation.

It can be concluded that the chemical analyses are an important way to obtain substrate characteristics. Usually they come with less effort than the biological test and are consequently an easier way to analyse the inhomogeneity and the variability of the substrate characteristics over time. Any chemical analysis needs information on the substrate type and the availability of reference values to compensate for the impossibility of determination of the degradable fraction of the substrate and the portion of biomass consumption for microbial growth.

A universal validity of the relationship of a chemical analysis to batch tests has not been published yet. Since the chemical analysis cannot mimic biological degradation and the biological process has an unknown variability, the veracity of both methods in terms of the biogas potential cannot be given. Consequently it is impossible to state which method gives the true value. The batch test remains the most profound experimental method since it includes most unknown variables but its results are compromised by the apparent variability of the results. There is still potential to improve the quality of the batch test results which might also lead to a better correlation of chemical analysis and biogas potential based on batch test results.

The obvious impact of the adaption of the inoculum questions in particular the comparison of pre-treatment methods by means of batch tests. Since the change of the substrate composition by the pre-treatment will have an impact on the "matching" of substrate and inoculum the method of such comparison by means of batch tests have to be questioned.

5.2. Interpretation of results from Batch versus continuous fermentation

Some studies compared biogas or methane potentials obtained by batch tests with yields based on continuous fermentation approaches (lab-, pilot- or full-scale) of the same substrate. Table 8 gives a selection of results from literature where batch and continuous results have been presented. A detailed comparison of the results is not possible without application of the kinetic characteristics of the substrates in the form of a model and knowledge of the process data.

Substrate conversion is time- and process-dependent. Figure 12 shows the yield as a percentage of the biogas potential in a first-order kinetic system for batch operation and a continuous stirred tank reactor (CSTR). The graphi-

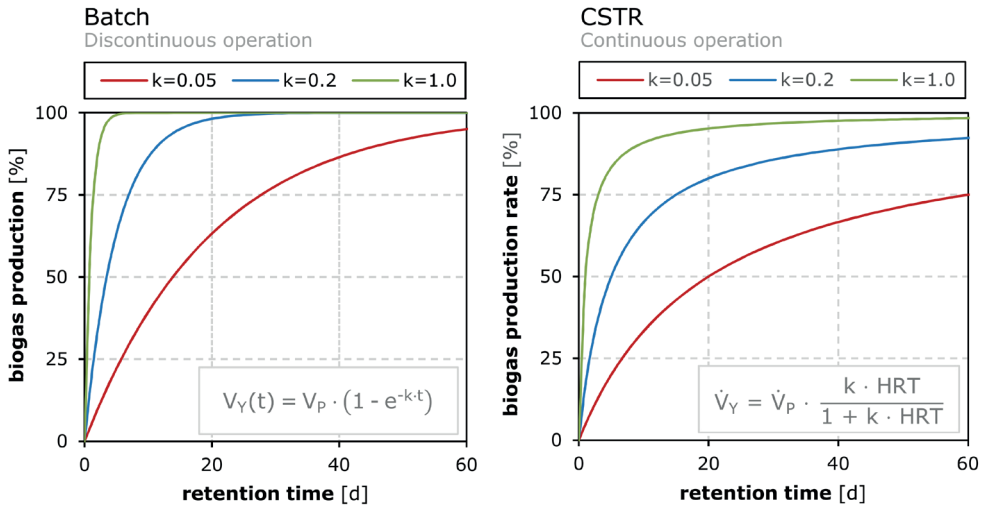

Figure 12: Qualitative relation of biogas yield and time in Batch or CSTR operation (k = first-order reaction constant, t = time, HRT = hydraulic retention time, V_P = biogas potential and V_Y = biogas yield)

cal representation assumes that the degradation rate characteristics (given as rate constant k) is transferable from batch to the continuous system. In theory this should be the case; for biogas processes this has not been proven yet. However, the figure is supposed to present the general relation of batch and continuous systems with different substrates.

A CSTR with 30 d retention time achieves less than 86 % of the biogas potential with a k value of 0.2 d^{-1}; slower degradable materials (k = 0.05 d^{-1}) achieve accordingly even lower gas yields at the same retention time. In theory the batch test degrades much faster than the continuous system.

As mentioned above, the terminated batch test delivers, according to the definition in this report a yield. However, in most of the standards the gas amount obtained after termination of the test is interpreted as the biogas potential. A theoretical correct determination of the biogas potential requires a model-based extrapolation as discussed in section 3.7.

The continuous test systems such as the CSTR in laboratory-, pilot- or full-scale will have (according to the substrate degradation kinetics and potentially additional limitations) a lower yield than the biogas potential and in theory also a lower yield than the batch test system at the same retention time.

An overview of data from selected available literature is presented in Table 8 in order to give an impression on the variation of the results from batch and continuous tests. Data given in Table 8 show similar results for batch and continuous digestion in some cases (Barbot et al., 2015). The majority of the values are, as expected, larger in case of the batch tests (Holliger et al., 2017; Ruffino et al., 2015; Zhang et al., 2013). In some cases, lower values in the batch tests than in the continuous tests have been reported. Browne et al. (2014) compared specific methane yields from batch and continuous fed systems and found a higher yield from continuous systems in comparison to batch fermentation of food waste. This was attributed to acclimatisation of the microbial fauna to the substrate in long term digestion in the CSTR over time.

The analysis from Figure 12 would suggest that the biogas yield of a complex substrate in a CSTR with moderate degradation characteristics and similar retention time should be significantly below the biogas potential taken from the batch test after termination or value from the calculation of the biogas potential. Otherwise it can be assumed that either the batch test or the continuous test delivered data which is not in agreement with kinetic theory. Since it is unlikely that the continuous test delivers values that are too high, it is more likely that the batch test delivers results too low for various reasons as discussed above such as lack of acclimatisation of microbial fauna.

There are some inter-laboratory studies available which describe the variation of biogas potential results for the batch test. For continuous tests such comparisons are not readily available in the literature. It can be deduced that the comparison and evaluation of combined batch and continuous tests has not been standardised yet and only a few studies address such comparisons in detail. The combination of unknown inter-laboratory variability of continuous tests with the uncertainty from the batch test alone makes it difficult to draw a clear conclusion on the comparability of biogas potentials from batch and yields from continuous test. A direct comparison of the results of biogas potential and yield in continuous tests would require the knowledge of the kinetics of the substrate degradation; there are few publications which discuss this comparison.

Table 8: Comparison of batch and continuous testing methods

Substrate	Batch test		Continuous test				Deviation to batch test [%]	Reference
	SMY [L kg^{-1} VS]	V [L]	SMY [L kg^{-1} VS]	OLR [kg VS m^{-3} d^{-1}]	HRT [d]	V [L]		
Triticale (coarse grain 0.5 – 1 mm)	416 ± 4	0.5	307	1.9	45	31	-26	Moeller et al., 2018
Triticale (coarse grain 4 mm)	394 ± 26	0.5	309	1.9	45	31	-22	
Triticale (grain kernels)	361 ± 6	0.5	301	1.9	45	31	-17	
Food waste (canteen, summer)	535	0.5	560	2	30	5	+5	Browne et al., 2014
Raw food waste	581	0.8	405	7	25	0.8	-30	Zhang et al., 2013
Food waste (vegetable mix)	294	6	223	1	30	300	-24	Ruffino et al., 2015
Wastes from macroalgae	172 – 214	2	173	2.5	40	10	+1 – -19	Barbot et al., 2015
Wastes from macroalgae	172 – 214	2	189	2	32	1700	+10 – -12	

Batstone et al. (2009) discussed the combination of degree of degradation and hydrolysis rate coefficient as the crucial parameters to describe the degradability characteristics of substrates. They found batch tests represented a conservative estimation of the substrate degradability at full scale; the hydrolysis rate coefficient was an order of magnitude lower. This has a strong impact on the transferability of individual model parameters from batch to continuous mode (Batstone et al., 2009). In a later study the biomethane potential (BMP) was found to be still conservative compared to the specific methane yield during continuous digestion, however, the values from both tests were closer (Figure 13). The used inoculum was derived from a continuous test using the same substrate origin as the batch test and the potential impact of the adaption of the inoculum was particularly mentioned in the respective publication (Jensen et al., 2011).

Besides the conformity of biogas potential and respective yield, the test systems deliver different answers on other crucial issues. Whereas batch tests provide results on the specific biogas potential, continuous lab-scale experiments are furthermore suitable for studying the degradation kinetics, the impact of inhibitory substances, trace element supply (Table 9), levels of VFAs, TAN, ratio of acidity to alkalinity, synergistic effects of co-substrates or substrate pretreatment (such as balance of bioavailability and characteristics of nutrients). Additional questions as to the evaluation of mixing properties and technical limitations (such as disturbing material, swimming layers) is reasonably achieved only at full-scale treatment.

5.3. Transferability to full-scale fermentation

The differences between batch and continuous systems for substrate biogas potential analyses represents the baseline when attempting to compare results of batch tests and full-scale processes. While the continuous test in the lab is conducted under controlled conditions, detailed analytics and monitoring, full-scale operation comes with some differences caused by scale up and a number of additional variables such as (adapted from Oechsner & Paterson, 2013):

- Fermentation volume (0.1 to 15 L versus > 2000 m³);
- Process conditions (organic loading rate and hydraulic retention time);
- Unknown mixing conditions (dead volume);
- Recirculation of digestate or separated liquids;
- Substrate conditioning and mixtures;

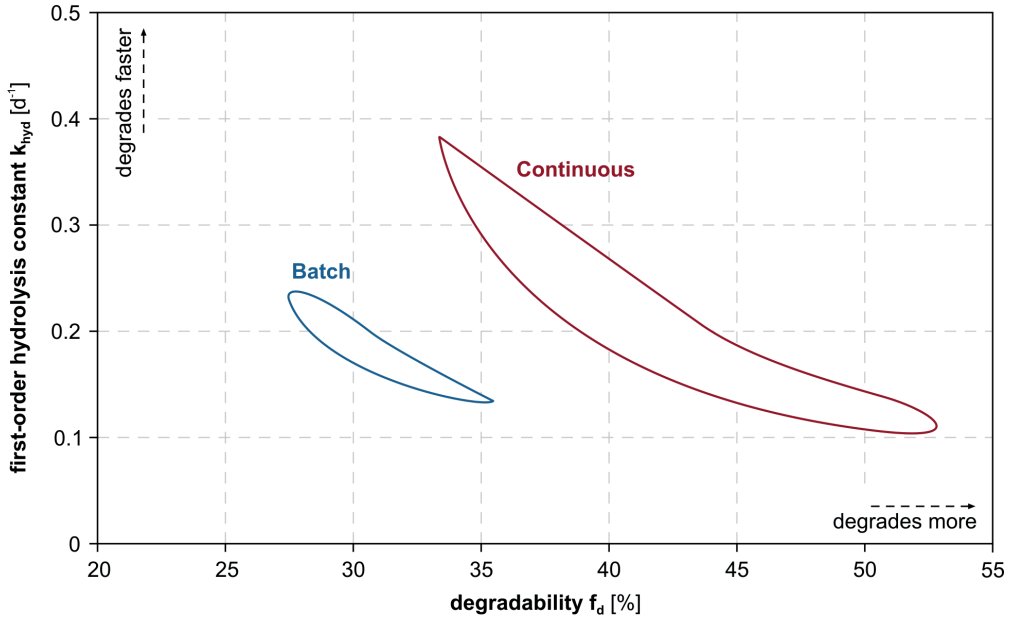

Figure 13: Comparison of characteristic value ranges for degradability and degradation kinetics (first-order hydrolysis constant) during batch and continuous operation (adapted from Jensen et al., 2011; with permission from the copyright holders, IWA Publishing).

- Measurement methods in particular weighing and gas quantity (exact in lab versus often imprecise in full-scale);
- Plant operator (undocumented changes in operation);
- Imprecise mass balances due to lack of monitoring (gas flaring or leakages).

Holliger et al. (2017) compared methane production from lab-scale batch tests of individual substrates and methane produced at full-scale co-digestion of these substrates. The results indicated a 10 % higher methane potential determined based on batch tests compared to the full-scale yield (Holliger et al., 2017).

The performance analysis of full-scale processes can be evaluated by means of a mass balance, however, additionally full-scale analysis often includes for analysis of the residual gas potential in the digestate. In combination with a gas potential analysis of the substrate and the gas yield at the plant the efficiency of the overall process can be checked for plausibility. Usually the uncertainties and the availability of sound data are different from plant to plant. As a result the reliability of the data on which the performance is interpreted may have different levels of accuracy or plausibility. A closed mass balance with negligible deviation is extremely unlikely due to the limited data quality.

Lehner et al. (2009) assessed 15 agricultural biogas plants and found a variation in residual biogas yields from 1.3 to 6.1 % related to the actual biogas yield determined for the biogas plant. Within a study of Ruile et al. (2015) 21 full-scale biogas plants were tested and found:

- Residual methane potentials of 4.1 ± 2.6 % in relation to the daily produced methane volume at the biogas plant;
- Mean degradation rate of 78 ± 7 %;
- Almost complete substrate degradation was reported at hydraulic retention times above 100 d (Ruile et al., 2015).

In a large plant evaluation program carried out in Germany the residual gas potential of the plants and the gas potential of the substrates were investigated (Johann Heinrich von Thünen-Institut (vTI) (2009)). Figure 14 shows results of the survey. The relationship between retention time and yield can be clearly seen. The variability in the results comes from the different substrates used, quality of data from full-scale operation and of course batch tests.

The study of Ward et al. (2018) showed a correlation between estimated methane yields by batch tests (kinetic and mass flow modelled) and measured yields at pilot-scale CSTR. In that case the inoculum to substrate ratio had a

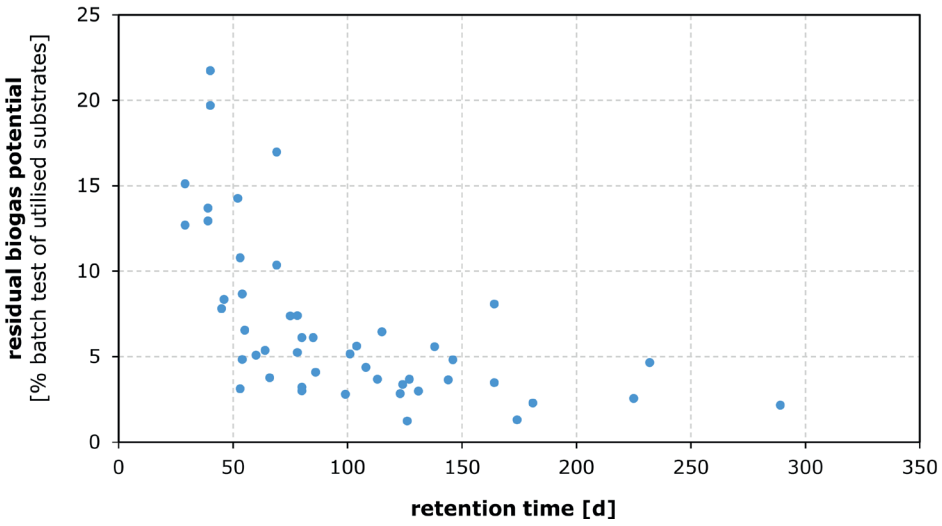

Figure 14: Remaining gas potential of plants evaluated in plant survey "Biogasmessprogramm II" (Johann Heinrich von Thünen-Institut (vTI) (2009)).

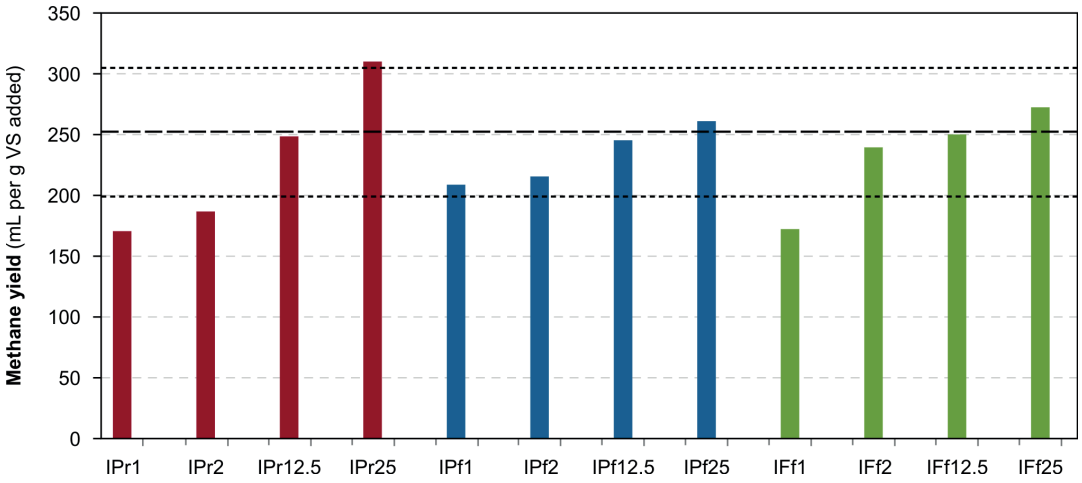

Figure 15: Estimated methane yields using three different inocula (IPr, IPf, IFf) at inoculum to substrate ratios as indicated (1, 2, 12.5 and 25:1) The line with longer dashes shows the CSTR mean measured methane yield and the lines with shorter dashes show the standard deviation during the measurement period. (Ward et al., 2018; © Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission)

stronger impact on the specific methane yield than the inoculum type (Figure 15).

In Table 9 several criteria which are crucial for efficient and predictable operation of full-scale plants are presented. The criteria are evaluated with regard to their precision, effort and availability to describe process behaviour in full-scale plant operation. Some of the criteria cannot be analysed within the test systems discussed in this report. Lab tests allow detailed approximation, a variety of operational modes and precise measurements. However, rheology and mixing properties cannot be depicted in small-scale reactors with rather ideal mixing conditions. Although full-scale operation is the measure of all things, since it represents the state of operation which is to be simulated by the other methods, it is limited in precision of measurements and is not available for new designs.

Table 9: Availability and precision of results from data obtained from different methodologies and scales to describe process behavior in full-scale plant operation

Criteria	Chemical/physical analysis	Batch test	Continuous lab tests	Full-scale
Gas yield/potential	+	++	+++	++
Kinetics	-	-	+++	++
Rheology/Mixing properties	-	-	-	+++
Impact of inhibitory substances	+	-	++++	+++
Nutrient/trace element deficiency	+	-	++++	+++
Effort to conduct test	low	medium	high	very high
Evaluation of pre-treatment measures	+	+	++++	+++
Technical limitations as disturbing material, swimming layer	+	-	+	++++

- not available, cannot be used to describe full-scale processes
+ available, very high uncertainty and/or high effort for full-scale process characterisation
++ available, medium uncertainty and/or high effort for full-scale process characterisation
+++ available, low uncertainty and/or high effort for full-scale process characterisation
++++ always available, direct measurement, very precise method to describe full-scale processes behaviour

6. Recommendations

First of all, the aim of the study should be defined in detail. Is the determination of the biogas potential required for plant design, plant performance analysis or pre-treatment technology evaluation? These aims require different approaches and have different options for additional supporting measurements. Other factors than the biogas potential might be as significant for the outcome of the study and should be considered as well. Accordingly, a detailed analysis scheme should be developed in order to cover all crucial aspects, including:

1. Definition of aim of study;
2. Development of a sampling procedure and test scenario;
3. Conduction of tests;
4. Statistical evaluation of uncertainties and interpretation of results.

The value of the results of batch tests for biogas potential analysis contain uncertainty and in particular, on an inter-laboratory comparison, a distinct variability. With all the limitations of the test, there are no sufficient alternatives since other methods have limitations or require much more effort. Accordingly, the selection of a proper way to determine the biogas potential should be done on the basis of acceptable variation for the purpose of the evaluation.

The importance of the representability of the sample analysed is crucial to the relevance and applicability of the result. The inoculum must be adapted to the substrate through acclimatisation in a way that the impact of the inoculum on the result can be considered negligible. Otherwise an adaptation/acclimatisation procedure is recommended. The assessment of the validity of the experimental setup by means of a test substrate (cellulose, acetate) does not give a definite indication on the capability of the inoculum to degrade the specific substrate of interest. The inoculum may be suited to degrade the test substrate but not the target substrate, in particular when it comes to specific cases such as seaweed.

The evaluation of the results – besides the validity of the test according to the standard protocols – should include a model-based estimation of the biogas potential (at infinite retention time). Reasonable simulation results of the experimental measurements are obtained using a model structure with at least two substrate fractions (such as fast and slowly degradable substrate components). Due to the uncertainty caused by the potential variability of the results the interpretation of results should be rather used as an orientation/approximation. Experimental results should be interpreted in context and cross checked for plausibility with:

- Literature values;
- Suitable calculations based results on available analytical measurements (e.g. nutrient analysis, lignin content or degradable VS);
- Known uncertainty within the lab.

Since intra-laboratory variability seems to be lower than inter-laboratory variability the results should be assessed in relation to the available data base within the same laboratory. For precise determination of biogas potential or degradation kinetics a higher effort, either in applying an adaptation procedure of inoculum or continuous operated digestion tests is recommended.

Furthermore, batch test setups can be used to determine the residual gas potential of individual digestates to determine degradation efficiency of anaerobic digestion.

In order to ensure the quality of lab-scale experiments for determination of biogas potential a regular participation at inter-laboratory tests is recommended. From the client perspective it is recommended to check if the lab of your choice is successfully participating in such tests.

7. Conclusion and outlook

The batch test is an established test system for the determination of the biogas potential of organic materials. Inter-laboratory tests and investigations, which have analysed the impact of inoculum revealed a significant variability of the results of the test. It will be a task for future research to minimise this variability as much as possible.

Other methods for the determination of the biogas potential based on chemical analysis show a significant lower variability in the results, but limited correlation with batch tests. The batch test is based on a biological system and includes more influencing factors than the majority of the chemical analysis. Which test result is more accurate and independent of bias remains unknown since there is no absolute value or method to be compared with.

Summing up the advantages and disadvantages of the test system can be described as in Table 10:

A limited literature screening showed that intra-laboratory variation of 1 – 20 % (with some cases up to 30 %) are possible when comparing different inocula. This translates into a similar variation possible in the case of non-adapted/non-acclimatised inocula use. Improving adaption or acclimatisation involves effort and time and typically requires repetition of batch processes or sourcing of the inoculum from a continuous digestion of the specific substrate. The obvious impact of the inoculum questions the method of comparing pre-treatment methods based on batch test with a single, non-adapted inoculum.

Inter-laboratory reproducibility was found in the range of 8 – 26 %. The cited inter-laboratory tests used standard protocols and over time the development of these tests has resulted in a reduction of variability between laboratories. A further reduction of the variability seems to be possible when examining the impact of the test setups, experimental procedures and the inoculum.

A standard method for the transfer of batch test to depict process behaviour of continuous tests or full-scale systems is as yet undeveloped. The few available publications highlight some matching results, but also cases with significant deviation. These results are difficult to interpret since an inter-laboratory comparison of continuous tests is as yet undocumented and as such not available; therefore, the accuracy of these tests remains unknown.

A further revision of the available protocols and the identification and elimination of causes for variability is needed. If the variability of the batch test can be reduced, the development of biochemical analysis combined with regression analysis may become more precise and result in a higher accuracy.

A further conduction of inter-laboratory tests (including continuous experiments and chemical analysis) and the publication of these results is necessary for a further improvement of the test execution and more precise results. A standard protocol for the transfer of batch test results to continuous test with a description of the inherent uncertainties needs to be developed.

Table 10: Summary of Pros and Cons of the batch test

Pros	Cons
Direct measurement of sum of biochemical parameters (microbial growth, degradability or water incorporation)	No distinctive, separate determination of biochemical parameters (microbial growth, degradability or water incorporation)
Standard protocols for test methodology available	Numerous influencing factors and still large variability compared to chemical analysis
Availability of many reference values and long-term experience	Details about test methodology often incomplete
Limited effort compared to continuous tests	Does not give sufficient data for continuous full-scale plants on factors such as: kinetic process behaviour, idealised retention time or operation at these retention times, effects of inhibitory substances, trace element deficiencies, impact on rheology or mixing properties.
Substrate independent methodology	Comparably high effort and costs (compared to single chemical analysis)

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Task 37 – Energy from Biogas

IEA Bioenergy aims to accelerate the use of environmentally sustainable and cost competitive bioenergy that will contribute to future low-carbon energy demands. This report is the result of the work of IEA Bioenergy Task 37: Energy from Biogas.

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IEA Bioenergy

PUBLISHED BY IEA BIOENERGY, October 2018

IMPRESSUM: Graphic Design by Susanne AUER/www.friedlundpartner.at

ISBN: 978-1-910154-49-6

http://www.iea-biogas.net

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