Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters

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Article Outline

Glossary

Anaerobic digestion/anaerobic fermentation - Microbial degradation of organic material under exclusion from oxygen. The product spectrum reaches from mainly methane over organic acids to hydrogen.

Biogas potential Maximum amount of biogas that can be generated from a specific substrate.

Biogas yield Amount of biogas that is produced from a specific substrate or a substrate mixture under individual process conditions.

Biogas Moisture-saturated gas mixture that mainly consists of CO_2 and CH_4 with traces of H_2S, NH_3, and H_2.

Chemical oxygen demand The amount of oxygen that is necessary to completely oxidize a substance. It thereby represents the chemical energy content of the substance.

Continuous stirred tank reactor Agitated tank reactor that represent an ideal reactor type assuming constant density of the reactor content and constant temperature (deviates from standard definition)

Kinetic modeling Quantitative description of a complex process, e.g., the anaerobic digestion process, over time on basis of a model. Das lässt sich an dieser Stelle schlecht verallgemeinern - würde ich rausnehmen. The estimation of specific kinetic parameters is one central aim of kinetic modeling.

Methanogenesis/methanation Last step of the anaerobic digestion process performed by methanogenic archaea. Mainly the microbial conversion of acetate to CH_4 and CO_2 (acetoclastic methanation) as well as H_2 and CO_2 to CH_4 and H_20 (hydrogenotrophic methanation).

Methanogens Microorganisms within the domain "Archaea" that perform methanogenesis under strict anaerobic conditions.

Definition of the Subject

Anaerobic digestion (AD), i.e., the microbial conversion of organic material to biogas, which consists of mainly methane and carbon dioxide, is used since centuries to produce heat and later also electricity. The technical application of this natural process is used to provide a renewable energy carrier (biogas or methane). Furthermore, it is used to reduce the content of organic matter of waste material prior to disposal or to provide organic acids with different chain lengths.

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Technical applications nowadays can be found within the sewage sludge treatment, the treatment of industrial and municipal solid wastes (including landfill gas utilization) and wastewaters, and the conversion of agricultural residues and energy crops.

The understanding of basic principles behind the anaerobic digestion (AD) process is a crucial requirement for the successful implementation and operation of the process. Therefore, the following entry addresses microbial basics of the anaerobic digestion (AD) process, important aspects of substrate characterization and degradation kinetics, as well as chemical and technical process parameters that are relevant for practical implementation.

Introduction

Already in the eleventh century, Assyrian baths are said to be heated with biogas. In the eighteenth century, Alessandro Volta realized that the amount of decaying organic matter and the amount of biogas are linked to each other [1]. In 1856, the first documented biogas plant was built in Bombay, India [2]. In the first half of the twentieth century, the microbiology of anaerobic digestion (AD) became object of intensive research [3] with the aim to identify process parameters and to promote methane production from organic matter. Later, the syntrophic interaction between acetate-producing and hydrogen-consuming microorganisms was described [4].

Once transferred to the industrial scale, it was possible to provide sufficient amounts of electricity and heat from biogas. The installed electrical capacity of biogas plants worldwide is listed with approx. 15 GW in 2015, whereas Europe contributed with an installed capacity of approx. 10 GW [5]. Usually, biogas plants are operated to produce a constant amount of biogas that is burned in combined heat and power (CHP) plants or cleaned, upgraded to methane, and injected into the natural gas grid. Besides a constant biogas production, it is also possible to use biogas plants to provide flexible power for residual load management [6]. Nevertheless, this requires special plant configurations such as additional combined heat and power plant (CHP) capacities, extra gas storages, or an adapted feeding and process management system. Furthermore, conclusive process monitoring and substrate characterization procedures as well as suitable process control strategies (e.g., model-based process control [7]) and sensors are needed to ensure a safe and stable but dynamic plant operation.

Microbial Basics

Anaerobic digestion (AD) is based on the activity of a microbial community. Under anoxic conditions, complex organic matter is degraded to methane and carbon dioxide. Depending on the composition of the substrate, also compounds like ammonia or hydrogen sulfide are produced as side products. As the organic matter is usually not completely degradable due to inert compounds, solid or liquid residues remain, called digestate, which also contain the microorganisms. A part of the substrate is used for microbial growth.

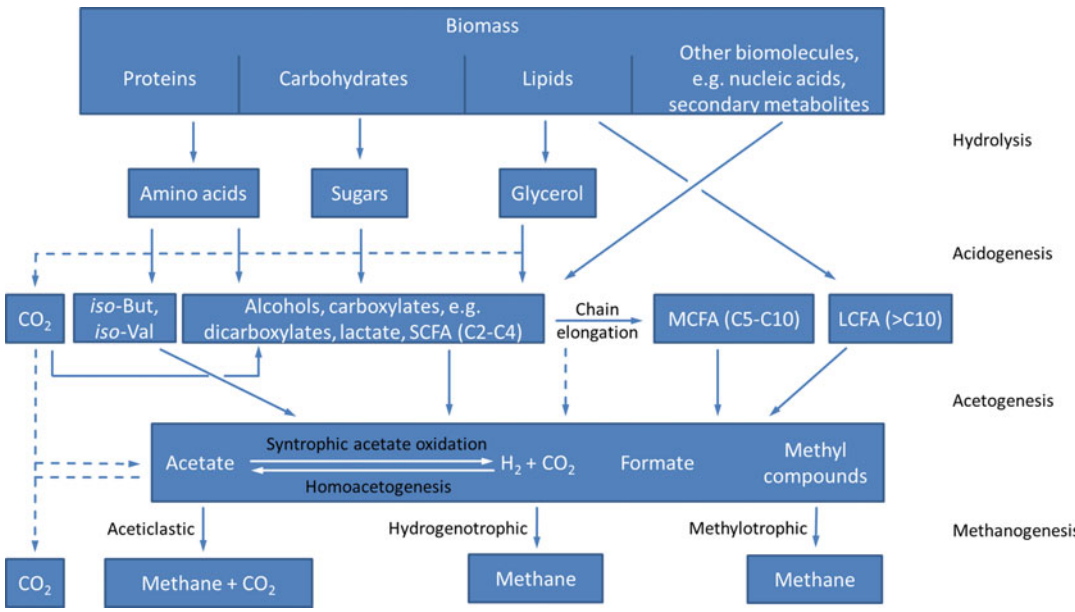
Anaerobic digestion (AD) occurs in natural habitats and anthropogenic systems. In nature, biogas is produced, for example, in swamps, marine and freshwater sediments, and soil and by ruminants, while anthropogenic sources are depositories or reservoirs of livestock excrement and bio-based waste as well as biogas plants and wastewater treatment plants. The conditions in natural and anthropogenic systems differ, and so do the microbial communities. What they all have in common are microbial functional groups catalyzing the four metabolic anaerobic digestion (AD) steps. In the complex metabolic network, microorganisms with their individual performances and whole functional groups work in tight interaction. They compete for nutrients, exchange chemical compounds, or mutually degrade substrate compounds or metabolites. In the end, a degradation process results that is remarkably stable considering the billions of different involved microorganisms. The process stability is supported by functional redundancy within the microbial groups. Thus, individual sensitivities of single species, e.g., process or environmental conditions, do not affect the anaerobic

digestion (AD) process. However, specific conditions can also influence the activity of whole microbial groups [8], e.g., high organic acid or ammonia concentration [9], as well as secondary plant metabolites [10, 11] which can inhibit methane formation. It has been shown that particularly microbial groups that are involved in later conversion steps (i.e., acetogens and methanogens) are more sensitive to inhibition than those of the earlier degradation steps. This is due to the lower phylogenetic diversity and the higher specialization within these groups limiting the potential for functional redundancy. These groups are comparatively well investigated, whereas less is known about microbial ecology features of hydrolytic/fermenting bacteria.

For biogas production, open reactor microbiomes are used, which means that the substrates are unsterile and the process tolerates a steady input of microorganisms not belonging to the anaerobic digestion (AD) process. As the external microorganisms are not adapted to the process conditions, they usually do not influence the process itself. Although it is theoretically possible to start an anaerobic digestion (AD) process without any inoculum by exploiting natural microbial communities in the substrates, their activity is often low and the process accordingly slow. To avoid long adaptation times, it is strongly recommended to use an inoculum from another anaerobic digestion (AD) plant or from a source with appropriate and highly active microorganisms (e.g., cow manure). Microorganisms enriched from natural habitats can support the anaerobic digestion (AD) in technical systems. They can be applied by bioaugmentation for increased biogas yields from recalcitrant substrates like lignocellulose or to overcome inhibition states [12, 13].

Below, the four metabolic anaerobic digestion (AD) steps are described in more detail and shown in Fig. 1.

- *Hydrolysis.* Large parts of complex organic matter are usually composed of polymers (e.g., carbohydrates and proteins) as well as fats, which cannot be taken up into microbial cells and have to be hydrolyzed first. Hydrolytic



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Fig. 1 Carbon flow during anaerobic digestion of biomass. *Dashed lines* indicate exclusive CO_2 evolution by decarboxylation and its transfer. Abbreviations: *iso-But* *iso*butyrate, *iso-Val* *iso*valerate, *SCFA* short-chain fatty acids, *MCFA* medium-chain fatty acids, *LCFA* long-chain fatty acids

bacteria excrete hydrolases (e.g., amylases, lipases, proteases, cellulases, hemicellulases) which cleave these substances into oligomeric as well as di- and monomeric compounds (e.g., sugars, amino and fatty acids, glycerol). Some bacteria produce large hydrolytic enzyme complexes, called cellulosomes, or proteins enabling adhesion of microbial cells to substrate particles. Bacteria do not gain energy by hydrolysis; hence, they take up soluble hydrolysis products into the cell for fermentation. When solid substrates are used, hydrolysis is often the rate-limiting step of anaerobic digestion (AD). Particularly, cellulose and hemicellulose can only slowly be hydrolyzed. Intermeshed with lignin in lignocellulose, the hydrolysis is even slower as anaerobic microorganisms cannot attack lignin. This affects the access of hydrolytic bacteria to degradable compounds thus lowering their bioavailability.

- *Acidogenesis*. In the second step, the anaerobic fermentation of water-soluble hydrolysis products occurs and a mixture of different organic acids (mainly acetate, propionate, and *n*-butyrate), alcohols, carbon dioxide (CO_2), and hydrogen (H_2) is produced. Not always the fermentation products are the direct result of a linear degradation way. Instead, the metabolic processes meander whereby carbon chain degradation and elongation alternate or run in parallel depending on the process conditions and the metabolic needs of single fermenting species. The phylogenetic diversity of the bacterial fermenters is high and comprises several thousand of different facultative and strict anaerobic *Clostridia, Bacteroidia, Gammaproteobacteria, Actinobacteria*, and *Bacilli*.

- *Acetogenesis*. In the third step, products of the acidogenesis are further converted to acetate. Additionally, alcohols and organic acids are oxidized to H_2, CO_2, and other C1 compounds. A low hydrogen partial pressure is essential for these reactions to be thermodynamically favorable [14]. Hence, acetogenic bacteria are in close spatial proximity to hydrogen-consuming microorganisms for interspecies hydrogen transfer. Instead of hydrogen, also formate can be used for transfer of electrons. This strong trophic interdependency of microbial species is known as syntrophy. In anaerobic digestion (AD), the main syntrophic partners of acetogens are hydrogen-consuming methanogens. Acetate can also be produced from H_2 and CO_2 in a homoacetogenic reaction. Principally, homoacetogens compete with hydrogenotrophic methanogens (see below) both utilizing the same compounds. However, in common anaerobic digestion (AD) processes, homoacetogens are outcompeted, as they need high hydrogen concentrations. The reverse reaction of homoacetogenesis (i.e., syntrophic acetate oxidation) plays a bigger role in anaerobic digestion (AD). It is part of an alternative route for methane production from acetate. The phylogenetic diversity of syntrophic acetogenic bacterial specialists is much lower than that of fermenting bacteria. Different phylotypes of *Synergistales*, *Syntrophobacterales*, *Clostridiales*, and *Thermoanaerobacteriales* were identified as abundant acetogens.

- *Methanogenesis*. Methane formation by strict anaerobic microorganisms belonging to *Euryarchaeota* is the final step in anaerobic digestion (AD). When mainly easily degradable organic matter is used as anaerobic digestion (AD) substrate (e.g., dairy wastewater, food waste, or specific crops), methane production is the rate-limiting step as methanogens and acetogens have comparably low growth rates. Depending on their preferred substrate, methanogens are affiliated to hydrogenotrophs, methylotrophs, or acetotrophs. For hydrogenotrophic methanogenesis, CO_2 is reduced with hydrogen. Methyl compounds like methanol are the substrate for the methylotrophs. Acetate can be either directly cleaved in methane and CO_2 (aceticlastic methanogenesis) or first converted to $H_2 + CO_2$ by syntrophic acetate oxidizers (see above) and then to methane by hydrogenotrophic methanogens. The shares of the different metabolic pathways depend on substrate and process conditions. Seven different orders of methanogenic archaea are known (i.e., *Methanobacteriales*, *Methanococcales*,

Methanocellales, *Methanomicrobiales*, *Methanosarcinales*, *Methanomassiliicoccales*, and *Methanopyrales*). Most of the known methanogens catalyze hydrogenotrophic and methylotrophic methanogenesis. Only *Methanosarcina* and *Methanosaeta*, both belonging to *Methanosarcinales*, can directly utilize acetate as substrate. *Methanosaetaceae* can solely use acetate for methane production.

In biogas plants, all four metabolic steps run in parallel in the reactor. However, hydrolysis and acidogenesis can also be separated from acetogenesis and methanogenesis running the anaerobic digestion (AD) in two stages. No spatial separation of hydrolysis and acidogenesis is possible as right after hydrolysis, bacteria take up compounds into the cells for fermentation. Furthermore, acetogenic bacteria need close proximity to methanogens due to their syntrophic relationship requiring electron transfer as explained above. The spatial separation of the process in two stages allows the production of more than biogas from biomass. The first stage can be operated for optimal H_2 production (dark fermentation) or for optimal production of organic acids. Thus, biomass can be utilized for production of bioenergy only (H_2 + CH_4) or chemicals and bioenergy (organic acids + CH_4). In case of a pure energetic use, the separation of the two stages is often not advantageous as the costs for additional expenditure (two reactors including all control technology instead of one reactor) are not covered by a higher energetic yield.

The production of organic acids and methane in a two-stage system can be feasible if (i) the organic acid and methane production processes are optimized and (ii) the organic acids can be separated from the fermentation broth and sold to the chemical industry or are further converted. Not all organic acids from anaerobic fermentation are worth to be produced. Particularly acetate, propionate, and *n*-butyrate (short-chain fatty acids, SCFA), which are usually produced in highest concentrations, have a too low prize for their commercial exploitation. In contrast, branched chain fatty acids like *iso*butyrate and *iso*valerate as well as unbranched fatty acids with longer carbon chains (C5–C8 = medium-chain fatty acids, MCFA), i.e., *n*-valerate, *n*-caproate, and *n*-caprylate, have a higher prize, and their production from biomass by anaerobic fermentation might be feasible.

Medium-chain fatty acids (MCFA) can be produced from short-chain fatty acids (SCFA) by microbial chain elongation in the anaerobic fermentation stage. The reaction is catalyzed by fermenting bacteria, and this specific function seems to be quite distributed. Although much is known about the biochemical reactions for microbial chain elongation [15], there are still many open questions regarding the ecology of chain-elongating and other fermenting bacteria as well as their dependencies from process conditions. Extended knowledge regarding this metabolic function might help in developing viable fermentation processes. Besides short-chain fatty acids (SCFA), short-chain alcohols (ethanol, propanol) or lactate is necessary for chain elongation. Hence, substrates containing ethanol like residues from breweries or those with high lactate concentrations like crop silages or food waste are particularly good substrates for medium-chain fatty acid (MCFA) production. In contrast, lignocellulosic substrates or untreated plant biomass is less suitable as alcohol and lactate concentrations are low or missing. Alcohol or lactic acid fermentation within the acidogenesis is usually not sufficient for high medium-chain fatty acid (MCFA) yields. Hence, mainly short-chain fatty acids (SCFA) are produced from such substrates.

Another microbial reaction derived from the anaerobic digestion (AD) process is the methanation of CO_2 using H_2. It can be used to store H_2 in the form of CH_4 in the natural gas grid, whereas H_2 can be produced from excess electric energy via water electrolysis, e.g., from wind or solar power (power-to-gas technology). In the final anaerobic digestion (AD) step, H_2 and CO_2 are converted to methane by hydrogenotrophic methanogens. At high H_2 concentrations, it can also be used for acetate production by homoacetogens (see above), and acetate can be cleaved by aceticlastic methanogens releasing methane and CO_2. Although reactors in pilot scale for H_2 methanization are already operated, the knowledge about the ecology of microbial communities in such systems is still scarce.

Degradation of organic material results in production of biomass in the form of microorganism. For the portion of substrate which is converted into microbial biomass, quite different numbers are given in literature. For the specific groups involved in the process, the portion varies between 3 and 20%.

Substrate Characterization

For technical application of anaerobic digestion (AD) of organic substances (e.g., wastewater, energy crops, organic residues, and wastes from industry or agriculture), the following substrate and digestion medium characteristics are crucial for the selection of appropriate process technology:

- Biogas and methane potential
- Kinetics of the substrate degradation
- Total solid (TS) content and particle size
- Nutrients and trace elements
- Content of inhibitory substances
- Impurities or unwanted materials

The characteristics of the substrate determine the design of the technical process which is appropriate for the treatment as well as the physical and biochemical conditions within the digestion medium. The main parameters will be discussed in the following sections.

Biogas Potential and Yield

A key parameter for the evaluation of substrates with regard to the applicability within a biogas process is the biogas potential (Fig. 2). The biogas potential defines the maximum amount of biogas to be produced during anaerobic digestion (AD) of a specific substrate (including substrate share for microbial biomass formation). The potential is typically stated in relation to fresh matter (FM) or organic fractions of the substrate, such as volatile solids (VS)/organic dry matter, chemical oxygen demand (COD), or total organic carbon (TOC).

The biogas yield of a substrate (Fig. 2) is defined by the technically achievable fraction of the biogas potential (degradation efficiency) under individual process and operation conditions. Thus, the yield is a result of the specific retention time, the concentration of inhibitory substances, the availability of crucial nutrients, and the substrate degradation kinetics.

The potential can be estimated based on chemical substrate composition and stoichiometric calculations. Discontinuously (batch) or continuously operated laboratory digestion tests as well as process data from full-scale biogas plants enable the experimental determination of the biogas yield. Based on mass balances or kinetic modeling, the underlying biogas potential of the substrate mixture utilized in the respective experimental or large-scale process can be derived.

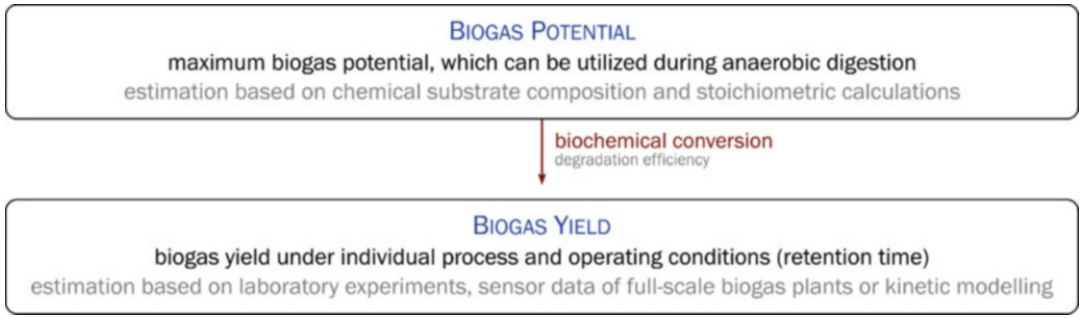
The main characteristics determining the biogas potential of a substrate are the water content, the portion of inert substances, and the chemical composition of the substrate. The chemical composition (or structure) in turn determines which portion of the substrate is anaerobically degradable and how much water is incorporated during the digestion process. Figure 3 shows the characteristic substrate fractions during the anaerobic digestion (AD) process as well as the conversion step from biogas potential to biogas yield.

In the following sections, several methods for the determination of the biogas potential and the deduction of the biogas yield are presented.

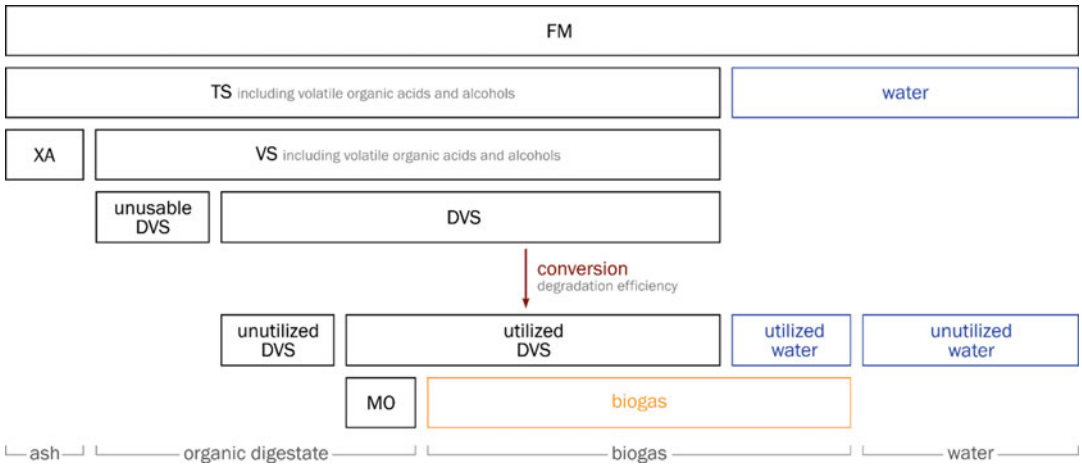
Biogas Potential Based on Total or Volatile Solids

The major analysis for substrate characterization of solid materials in practice is the determination of total solid (TS) and volatile solids (VS). Both parameters are fairly simple to determine. The total solid (TS) content is defined as the substrate fresh matter (FM) subtracted by the amount of water lost during drying [16]. Based on the mass of dried substrate, the amount of volatile solids (VS) is determined by subtracting the remaining inert fraction after incineration from the mass of total solids (TS) [16].

The determination cannot avoid a certain error when applied to substrates with high content of volatile substances (e.g., alcohols or organic acids), which are lost during the drying process. Therefore, the results have to be corrected for a



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Fig. 2 Distinction between biogas potential and biogas yield of substrates



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Fig. 3 Characteristics substrate fractions during anaerobic digestion of organic material. Abbreviations: *FM* fresh matter, *TS* total solids, *VS* volatile solids, *XA* ash, *DVS* degradable volatile solids, *MO* microorganisms

precise calculation of total solid (TS) and volatile solid (VS) content and subsequently the biogas potential [17–19].

Specific biogas or methane yields are usually related to the content of volatile solids (VS) in the substrate. Therefore, a variety of literature values are available for measured biogas yields from numerous substrates – either from laboratory experiments or full-scale application. Some collections of yields are referred to as "standard values" (e.g., KTBL [20]). However, usually the process conditions under which these values have been obtained are not presented; therefore, the transfer to other processes or comparison between different substrate types is not possible. For that reason, such compilations are rather an orientation than a source of precise information.

A direct and reliable determination of the biogas or methane potential of an unknown substrate based on volatile solid (VS) analysis is impossible, since the substrate fraction of nondegradable organic components as well the individual chemical composition is unknown. Thus, additional analysis is necessary for an accurate and detailed estimation of the biogas potential.

Correction of Total Solid Content for Precise Determination of Gas Yield

While drying an organic material, volatile substances, e.g., alcohols or organic acids might

evaporate too. Consequently, the mass of substrate fractions available in the substrate for gas production might be underestimated. If biomethane potential tests or continuous digestion tests are carried out with dried material, the gas potential of the original substrate is underestimated by the portion of the evaporated volatile substances. In case these tests are carried out with the original wet material, the resulting specific gas potential overestimates the gas production from the volatile solids (VS) since it relates only to the dried sample [21].

It should be clearly stated that if the analysis was carried out with a dried sample and in case of ratios (e.g., the specific gas yield in L/g VS), the resulting error should be calculated. Furthermore, it should be stated if the error can be neglected or has to be considered for further calculations. One option is the correction of total solids (TS) and volatile solids (VS) considering the volatile fractions (in [17–19], equations for the correction for defined substrates such as silages from maize, grass, and sugar beets have been developed).

Drying of, e.g., 182 samples [16] caused a loss of volatile substances of around 4%. Some samples showed maximum losses of 16% volatile substances. Equation 1 describes the correction of the loss on drying. TS_k describes the corrected total solids, TS_n the total solid content (two drying steps), *NFS* the content of short-chain fatty acids, *MS* the content of lactic acid, *PD* the content of 1,2-propandiol, *BD* the content of 2,3-butandiol, and *AA* the content of alcohol results:

$$TS_k = TS_n + (1.05 - 0.059\,pH)NFS + 0.08MS + 0.77\,PD + 0.87BD + AA \quad (1)$$

Biogas Potential Based on Chemical Composition (Elementary Analysis)

In 1952, Buswell and Mueller developed a chemical equation for the calculation of the stoichiometric biogas potential and composition based on the elementary constitution of a specific substrate (Eq. 2) [22]. If applied correctly, the sum formula is only valid for organic materials or substances which are completely degradable under anaerobic conditions. In 1977 Boyle added the elements sulfur and nitrogen to enable the calculation of sulfurous and nitrogenous substrate components such as proteins (Eq. 3) [23]. The number of carbon, hydrogen, oxygen, nitrogen, and sulfur atoms of the degradable substrate or individual molecule must be known for the determination of the biogas potential:

$$\mathrm{C}_a\mathrm{H}_b\mathrm{O}_c + \left(a - \frac{b}{4} - \frac{c}{2}\right)\mathrm{H_2O} \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4}\right)\mathrm{CH_4} + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4}\right)\mathrm{CO_2} \quad (2)$$

$$\mathrm{C}_a\mathrm{H}_b\mathrm{O}_c\mathrm{N}_d\mathrm{S}_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)\mathrm{H_2O} \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)\mathrm{CH_4} + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)\mathrm{CO_2} + d\mathrm{NH_3} + e\mathrm{H_2S} \quad (3)$$

It is obvious that the resulting amounts of methane and carbon dioxide are changing with the presence of nitrogen and sulfur. Consequently, Eq. 3 has to be used if those substances are present; otherwise, the estimation of the biogas potential and composition will be incorrect.

Generally, the individual biogas potential and respective composition of typical organic substances such as simple sugars and individual amino or fatty acids can easily be calculated based on stoichiometric equations. However, for a reliable calculation of the biogas potential of complex substrates, all degradable components (characteristic molecules) have to be known. Thus, a correct application for a precise calculation of the biogas or methane potential of complex substrate is rarely possible, since not all substrate components as well as their degradability and representing sum formula are available.

Methane Potential Based on the Chemical Oxygen Demand

The theoretical methane potential can be calculated based on the chemical oxygen demand (COD). The chemical oxygen demand COD represents the amount of oxygen necessary for a complete oxidation of the substance and thereby represents the chemical energy content of the substance. The value allows only the estimation of the methane potential of the substrate. Generally, the oxidation of methane is given as shown in Eq. 4:

$$CH_4 + 2\,O_2 \rightarrow CO_2 + 2\,H_2O \qquad (4)$$

At standard conditions, 1 mol CH_4 equals a volume of 22.4 L CH_4. Two mol O_2 are needed for oxidation of 1 mol methane, which corresponds to a mass of 64 g O_2. Therefore, 1 g of oxygen is utilized to oxidize 350 mL methane (22,400 mL CH_4 divided by 64 g O_2). Consequently, 1 g chemical oxygen demand (COD) in an organic substance can be converted to a maximum of 350 mL of methane (without consideration of microbial growth) [21].

The chemical oxygen demand (COD) is analyzed by means of a reaction of the substrate with potassium dichromate or potassium permanganate and is typically applied to liquid substrates (e.g., sewage sludge). Since the analysis oxidizes all components within the matrix, high content of nitrogen or sulfur might lead to an error in regard to the methane potential. Furthermore, the calculation of the methane potential based on the chemical oxygen demand the chemical oxygen demand COD does not distinguish between anaerobically degradable and nondegradable substrate components. Also the substrate share utilized for microbial growth is neglected. Thus, the true methane potential of a specific substrate is lower than the respective methane potential calculated using chemical oxygen demand (COD) analysis.

Biogas Potential Based on Total Organic Carbon

The biogas potential can be calculated based on the total organic carbon (TOC) content of a substrate. By anaerobic degradation of 1 mol carbon (which equals 12 g of the C-12 Isotope), 1 mol biogas can be produced, which equals a volume of 22.4 L biogas. Thus, from 1 g organic carbon, a maximum amount of 1.868 L of biogas can be expected (without consideration of microbial growth) [24].

Total organic carbon (TOC) measurement is mostly used in wastewater treatment and is determined through combustion or wet oxidation. Total organic carbon (TOC) has a higher precision compared to chemical oxygen demand (COD) analysis (in particular on solid materials), requires less environmental relevant chemicals, and is easier to be automated. However, the sole knowledge of the amount of carbon does not allow the determination of the methane or carbon dioxide fraction of the produced biogas. Furthermore, the microbiologically available share of the total organic carbon (TOC) cannot be characterized, which also hinders a reliable approximation of the biogas potential, since anaerobically nondegradable carbon sources (such as lignin) cannot be identified separately.

Biogas Potential Based on Characteristic Nutrients or Feedstuff Analysis

Keymer and Schilcher developed an approach for the estimation of the biogas potential of substrates for agricultural biogas plants [25]. The assumption for the estimation is that the biogas process is comparable to the digestion in ruminants and that the gas potential from a substrate is defined by the content of proteins, fats, and carbohydrates and their respective digestibility.

The digestibility and the substrate composition can be deduced from various analyses and experiences available from the animal nutrition. Weissbach supported this approach with his analysis [26]. This approach is in comparison to Buswell, Boyle, chemical oxygen demand (COD), or total organic carbon (TOC), more realistic, since it considers a fraction of the organic material as nondegradable. Below the assumptions are explained.

Conversion coefficient. The conversion coefficient used in animal husbandry is the ratio of nutrients in the fodder to the ratio of nutrients in the feces [27]. The coefficient can be determined for a fraction of the substrate or the substrate itself

by Eq. 5. V_Q describes the conversion coefficient, F the uptake, and K the amount remaining within the feces:

$$V_{Q=\frac{F-K}{F}\cdot 100} \quad (5)$$

The feces contain besides nondegradable fraction of the substrate also intermediates of the degradation which leads to an underestimation of the conversion coefficient [26, 27]. Therefore, this coefficient is called apparent digestibility coefficient. In particular, for the estimation of the biogas potential, this difference is of relevance.

The conversion coefficient is either determined by means of laboratory experiments [28] or conventional animal feeding experiments [27]. Digestibility is dependent on the type of animal, substrate amount, and composition [27].

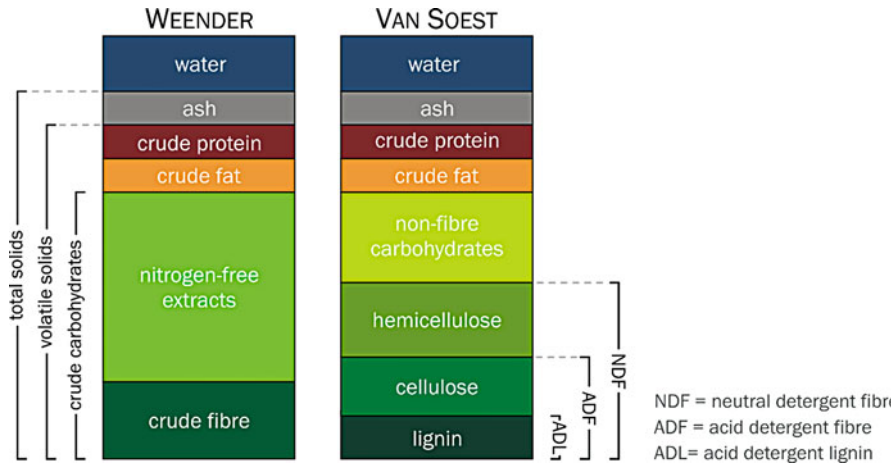
The database "Futtermittel" [29] is a collection of 1,300 substrates from conventional and organic farming with composition, unwanted components, digestibility, energy content, and additional quality characteristics [29]. Additional data such as the content of lipids, starch, proteins, sugars, cellulose, hemicellulose, and lignin from the animal feeding can be deduced, e.g., from [30, 31].

In general, it can be assumed that digestion within the animals is in correlation with the degradation during the biogas process, but not identical. The advantage of the method is the availability of a large database for specific agricultural substrates. However, new substrates or substrates which have not been or can not be tested in digestibility tests are difficult to investigate.

Characteristic nutrients. The volatile solids (VS) of a specific substrate can be divided into substrate fractions that basically consist of carbohydrates, proteins, and lipids. Depending on the used determination method, there exist several subcategories (e.g., structural carbohydrates). The nutrient composition of substrates can be obtained from the literature or has to be determined by laboratory tests. Two major methods are available: the Weender analysis and the van Soest analysis (extension of Weender analysis). The characteristic components of each analysis are depicted in Fig. 4. Based on the individual conversion coefficients, the anaerobically degradable masses of each nutrient or substrate fractions can be determined.

Biogas Potential of the Digestible Substrate Fractions

The challenge for the determination of the biogas potential and the biogas composition from the masses of the individual substrate fractions is the knowledge of their chemical composition. Since the determination of the individual chemical composition of all subcomponents is most often impossible, the overall chemical composition of each nutrient fraction needs to be characterized by means of



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Fig. 4 Characteristic nutrients and components of Weender and van Soest analysis

estimations or assumptions. Therefore, reference substances which represent the characteristic biogas potential of the specific nutrient need to be defined (e.g., glucose or cellulose for carbohydrates). Based on the stoichiometric sum formula of each reference substance, the overall biogas potential of the nutrient fraction can be determined according to Buswell and Mueller or Boyle (Eqs. 2 and 3), respectively [32]. Typical biogas or methane potentials for the degradable share of carbohydrates, proteins, and lipids are shown in Table 1.

Biogas Potential Based on the Fermentable Volatile Solid Content

Weissbach [35] introduced the parameter fermentable volatile solid content (FoTS). He defined it as the "amount of organic total solids, which can be potentially degraded by microorganism under anaerobic conditions and will be biologically converted under optimal process conditions and sufficient retention times [35]."

Weissbach [35] investigated the impact of the nutrient composition and the volatile solids (VS) of the substrate on the biogas potential based on typical chemical composition and biogas potentials of substrate components according to Baserga [33]. He based his investigations on digestion tests with sheep [36, 37] and developed models for defined substrates (grain, maize, grain plants, forage). According to Weissbach [35], the calculation of the fermentable volatile solid content (FoTS) is quick, cheap, and reproducible – if the model is available and applicable [26].

Experiments for the Determination of the Biogas Potential

Biogas or methane potential can be determined by means of a biological degradation test. They can be operated batchwise or continuously. The estimation of the biogas potential from such tests requires either dynamic modeling (and dynamic periods during operation) or stationary test periods with a variation of the retention time.

Batch tests. Several standards or guidelines are available to analyze the substrates (e.g., VDI 4630 [21]) with so-called batch tests. The result of such tests is a gas- yield which can be used to calculate the biogas potential.

The batch test underlies quite high variations – in particular if results of different laboratories are compared to each other. Heuwinkel et al. [38] state a realistic coefficient of variation of 10% (test within one laboratory), if VDI 4630 [21] is applied.

Continuous tests. Nonstandardized continuously operated tests are also an option for the determination of yields and, with some additional effort, also for the potential. The batch test is usually terminated when a certain criterion for the biogas production is reached. Otherwise the duration of the test would be far too long for an economically viable test. Consequently, the test shows a yield at a certain retention time which has to be extrapolated to the potential. This extrapolation is done by fitting kinetic calculations.

The continuously operated tests require more effort than the batch test. No standard detailed procedure is available. The results give more insight into possible process conditions (e.g., yields at certain retention times, organic loading rates, impact of nutrient availability, and inhibitory substances). VDI 4630 [21] contains some basic considerations regarding the operation of continuous tests.

Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Table 1 Biogas potential of characteristic nutrients [32]

	Carbohydrates		Proteins		Lipids	
	Biogas (mL/g)	Methane (%)	Biogas (mL/g)	Methane (%)	Biogas (mL/g)	Methane (%)
Baserga [33]	790	50	700	71	1,250	68
VDI [21]	750	50	800	60	1,390	72
Weiland [34]	700–800	50–55	600–700	70–75	1,000–1,250	68–73
Weissbach [32]	787–796	50.0–51.1	714–883	50.9–51.4	1,340–1,360	70.5–71.3

Conclusion

The analyses considering most of the unknown relevant parameters influencing gas and methane potential are the nutrient component-based analysis (including fermentable volatile solid content (FoTS)) and the batch test. All of them have restrictions with regard to the quality of the results and the effort. The nutrient component-based test also has a limitation to substrates which are used as animal fodder (Table 2). The continuous digestion test in the lab is an alternative to the batch test. However, the (additional) information obtained comes with a higher technical and time effort.

The described methods are all used to analyze a small sample of an inhomogeneous material. Therefore, it is important to consider that any analysis is pointless, if the sample is not representative for the material. In particular, waste material should be sampled very carefully in order to get representative information from the material used. It might be the case that a larger number of samples are needed to be analyzed in order to cover changes in the substrates over time. Therefore, the variability of the substrate quality has an impact on the numbers of samples necessary to get a reliable characterization of the substrate. The number of samples in turn translates into effort and costs for the overall analysis. The selection of one of the methods for biogas potential determination described here might also be dependent on the costs for sampling and the information needed.

Most complex substrates contain a substrate-specific, nondegradable fraction. A precise analytical determination of the biogas potential requires a determination of this fraction. However, a universal and substrate-independent method for this purpose is still not available.

The analysis of the parameters total organic carbon (TOC) and chemical oxygen demand (COD) or elementary analysis do not allow the determination of the degradable fraction of an unknown substrate. However, they can be used for mass balances of existing processes. Such an evaluation gives the fraction of substrate which has been converted. In comparison to literature values or other processes, this can be used for benchmarking purposes.

This approach is also used when referring to standard gas yields which usually are given as specific gas production. These yields are an orientation since they cannot represent specific process

Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Table 2 Major characteristics of methodologies for determination of biogas potentials

	Biogas composition	Determination of degradable fraction	Effort for determination	Information on kinetics	Remarks
Buswell and Boyle	Yes	No	High	No	
COD	Only methane	No	Medium	No	Difficult to analyze for solids
TOC	No	No	Medium	No	
Nutrient components	Based on reference values	Apparent digestibility	Medium, new substrates, high	No	Requires data from fodder tests for animals
FoTS	Yes	Yes	Medium, new substrates, high	No	Requires data from fodder tests for animals
Batch test	Yes	Yes	High	Yes (transfer to full-scale questionable)	High variation
Continuous test	Yes	Yes	Very high	Yes (additional effort)	Also information on yield, inhibition, digestate composition

COD chemical oxygen demand, *TOC* total organic carbon, *FoTS* fermentable volatile solid content

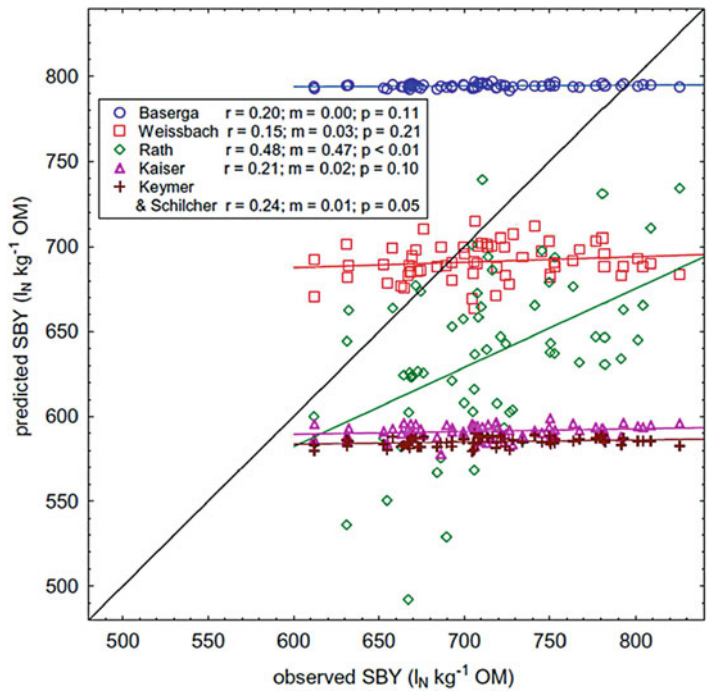
conditions or substrate variations (as seen with biowaste). Therefore, some sources from literature give a (wide) range of potential biogas yields from a specific substrate. Often the conditions how these yields have been obtained are not described.

The elementary composition approach, the fermentable volatile solid content (FoTS), and the nutrient component-based approach have been correlated with the batch tests (Fig. 5) [39]. Obviously the variation of the batch test does not represent the variation within all the chemical analyses. Baserga [33] obviously overestimates the gas potential (since he does not account for nondegradable fraction). Keymer and Schilcher [25] underestimate the gas potential due to the use of the apparent digestibility. For all it seems obvious that either the variation within the batch test is too high or the chemical analysis does not aim at right parameters to describe the biogas potential.

Biogas Production Kinetics and Yield

The degradation kinetics and the resulting biogas yield describe the individual conversion rate of the utilized substrates under respective process conditions. Thus, the kinetic properties depend on the substrate composition as well as the specific process characteristics (including microbial growth and process inhibitors). In connection with basic operational parameters such as the amount of degradable substrate components in the input or the effective reaction volume, the reaction kinetics are essential for the definition of an appropriate retention time to ensure efficient and stable process conditions.

For the application of kinetic dependencies with regard to the plant design or process evaluation, the respective kinetic approach has to be implemented into a general reaction model. For this purpose, the mass balance of the specific reactor or plant concept has to be defined. For a single continuous-stirred tank reactor (CSTR), the change of mass of the degradable substrate (V_{liq} . S in kg) over time depends on the specific substrate concentration in the input (S_{in} in kg/m^3), the input and output volume flow (q_{in} and q_{out} in m^3/d) as well as the substrate concentration in the digester (S in kg/m^3), the liquid reaction volume (V_{liq} in m^3), and reaction rate (r_S in kg/(m^3 d)) due to biochemical conversion (Eq. 6):



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters,
Fig. 5 Calculated biogas potential (maize silage) of different methodologies in relation to batch test results, specific biogas yield (SBY), organic matter (OM) [39]

$$\frac{d\left(V_{liq} \cdot S\right)}{dt} = q_{\text{in}} \cdot S_{\text{in}} - q_{\text{out}} \cdot S + V_{liq} \cdot r_{\text{S}} \quad (6)$$

Assuming a constant reaction volume (V_{liq} = constant) and therefore utilizing an identical input and output volume flow ($q_{in} = q_{out} = q_{liq}$), Eq. 6 can be simplified to Eq. 7:

$$\frac{dS}{dt} = \frac{q_{liq}}{V_{liq}} \cdot (S_{\text{in}} - S) + r_{\text{S}} \quad (7)$$

Depending on the kind of application, the basic reaction model can then be solved to describe discontinuous or continuous (steady-state or dynamic) processes. To describe the individual anaerobic digestion (AD) process, basic operational parameters, such as the hydraulic retention time (HRT) ($V_{liq}/q_{\text{in}} = HRT$ in d) or concentration of degradable substrate components in the input (biogas potential), have to be defined. Furthermore, a suitable kinetic function has to be implemented and parameterized based on measurements (parameter estimation) or typical literature values.

Numerous kinetic models have been developed in the past [40–42]. Generally, two kinds of kinetic functions have been established to depict the characteristic process phases in anaerobic digestion (AD). The respective degradation processes during enzymatic hydrolysis are typically described by simple first-order reactions [43]. Thus, the hydrolytic degradation rate of particulate substrates r_S can be described by a first-order reaction constant (k in 1/d) and the specific substrate concentration (S in kg/m^3) in the digester (independently from the specific biomass growth or enzyme concentration) (Eq. 8):

$$r_s = k \,.\, S \quad (8)$$

The biochemical conversion of soluble substrate components or intermediates is typically depicted by the growth rate (μ) of the involved microorganisms (Eq. 9) (X microbial biomass):

$$r_X = \mu\, X \quad (9)$$

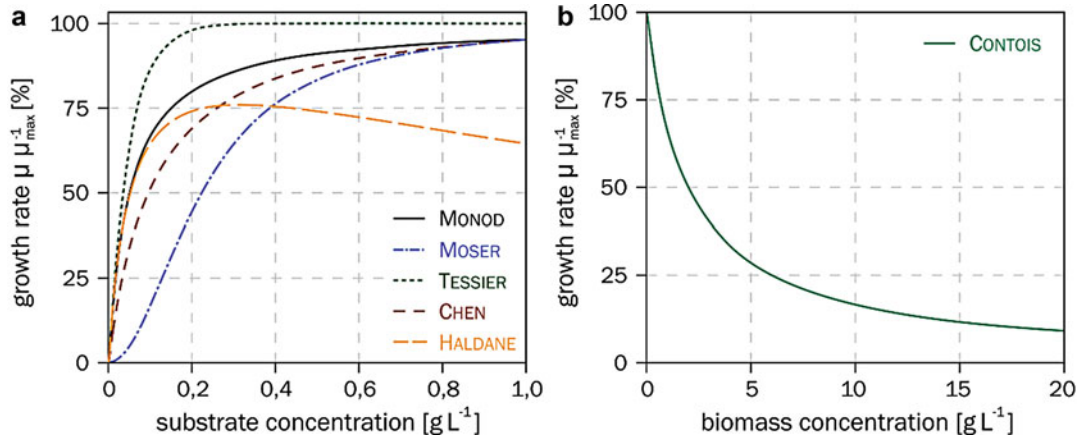
Based on the microbial growth, the substrate consumption (substrate degradation) and product formation can be calculated. Depending on the specific substrate, product or biomass concentration, as well as additional biochemical and physicochemical influencing factors (such as pH, temperature, or inhibitory substances), numerous mathematical functions have been developed for a realistic description of microbial growth kinetics [42–44]. Many kinetic modeling approaches are based on empirical or phenomenological dependencies to depict individual growth or process behavior. During practical and scientific implementation, a small group of kinetic functions have proven their general applicability (Table 3).

The established Monod equation describes microbial biomass growth based on the specific substrate concentration as well as the maximal growth rate and the half saturation constant of the involved species. Based on experimental measurements of cell divisions during glucose fermentation, Monod [45] utilized the Michaelis–Menten kinetics (originally created to describe enzyme-catalyzed reactions) for an empirical description of microbial growth [45]. As shown in Fig. 6a, additional mathematical expressions developed from Moser, Tessier, or Chen and Hashimoto [46, 47, 49] also enable the calculation of sigmoidal growth kinetics by individual parameter

Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Table 3 Typical kinetic functions to describe microbial growth during anaerobic digestion

Monod [45]	$\mu = \mu_m \cdot \frac{S}{K_S+S}$	Tessier [46]	$\mu = \mu_m \cdot \left(1 - e^{-\frac{S}{K_S}}\right)$
Moser [47]	$\mu = \mu_m \cdot \frac{S^n}{K_S+S^n}$	Chen and Hashimoto [48]	$\mu = \mu_m \cdot \frac{S}{K_S \cdot (S_{\text{in}}-S)+S}$
Contois [49]	$\mu = \mu_m \cdot \frac{S}{B \cdot X+S}$	Haldane [50]	$\mu = \mu_m \cdot \frac{S}{K_S+S+\frac{S^2}{K_I}}$

μ growth rate in 1/d, μ_m maximal growth rate in d, S substrate concentration in g/L, K_I inhibition constant in g/L, K_S half saturation constant in g/L, X microbial biomass in g/L, S_{in} input substrate concentration in g/L



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Fig. 6 Qualitative progression of typical kinetic functions to depict microbial growth. Relative growth rate depending on (a) the substrate concentration and (b) biomass concentration. Calculations based on Table 3 (K_S = 50 mg 1/L, S_{in} = 2 g 1/L, n = 2, K_I = 2 g 1/L, X = 300 mg 1/L, S = 1 g 1/L, B = 0.5)

adaptation [46, 47, 49]. The kinetic formula from Haldane [50] additionally includes inhibition of high substrate concentrations [50], whereas Contois [49] depicts growth rate reduction due to high concentrations of microorganisms [49] (Fig. 6b).

Furthermore, additional expressions can be included to depict inhibition by specific intermediates or process components. Thus, the established Monod kinetic is typically extended by growth-limiting or growth-inhibiting dependencies (section "Inhibitory Substances").

Depending on the model complexity, state of inhibition, and available measurements, these additional inhibition functions enable a clear distinction between maximal growth rate (under optimal and uninhibited process conditions) and inhibitory effects. However, if no information on the kind and strength of inhibition is available, no additional inhibition function can be implemented and the growth-limiting effect will directly result in slower growth or degradation kinetics.

Anaerobic digestion (AD) of complex substrates involves numerous microbial species and many degradation pathways. Generally, the overall degradation rate is governed by the slowest process phase. This characteristic degradation phase varies depending on the utilized substrate and the individual process conditions. During anaerobic digestion (AD) of particulate substrates (e.g., energy crops), hydrolysis defines the rate-limiting process phase, whereas acetoclastic methanogenesis becomes rate limiting during degradation of soluble or acidic substrates (e.g., sewage sludge).

For kinetic modeling, the growth conditions of individual species are typically summarized in functional groups (e.g., acetic acid degraders) to simulate the entire process in a reasonable degree of complexity and precision. Thus, dynamic models such as the *Anaerobic Digestion Model No. 1 (ADM1)* [43] have been developed as standardized tools for scientific application and model-based investigation of the anaerobic digestion (AD) process. The *Anaerobic Digestion Model No. 1 (ADM1)* includes all major process phases (Fig. 1) as well as typical process variables, inhibitors, or physicochemical dependencies (e.g., pH value or phase transition processes). Therefore, utilizing an extensive measuring scenario for parameter estimation, the *Anaerobic Digestion Model No. 1 (ADM1)* enables a detailed simulation and profound process understanding. However, simplified modeling approaches based on the rate-limiting process phase or overall degradation kinetics can be applied for a reasonable and sufficient plant design or efficiency evaluation in practice.

In the following paragraphs, the basic principles of kinetic modeling are applied for process design and evaluation of discontinuously (batch) and continuously operated laboratory or full-scale anaerobic digestion (AD) processes.

Discontinuous Operation

During discontinuous operation, no substrate or digestate is fed or removed over the system boundary of the reactor. Assuming a single first-order reaction, the basic reaction model (Eq. 6) can be simplified to Eq. 10. By integration of the differential equation, the explicit solution can be derived directly (Eq. 11):

$$\frac{dS}{dt} = -k \cdot S \tag{10}$$

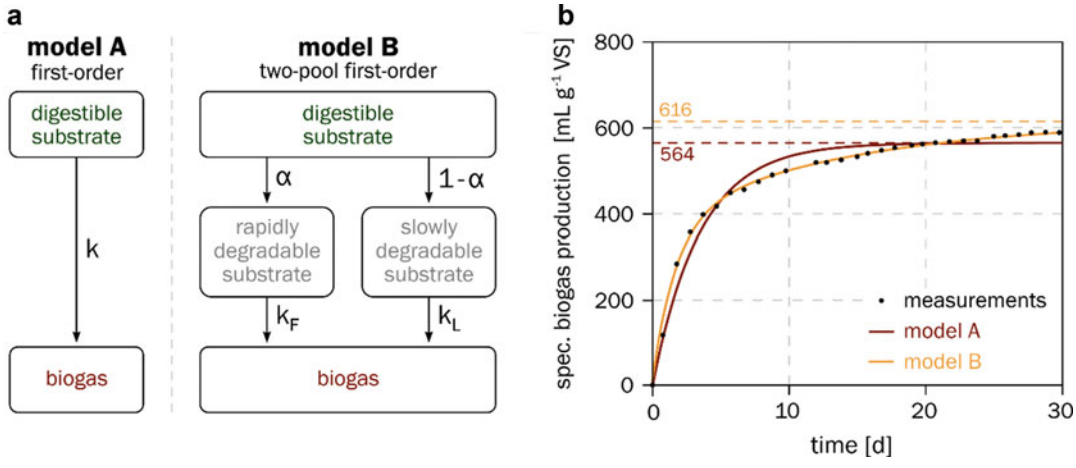
$$S(t) = S_0 \cdot e^{-k \cdot t} \tag{11}$$

Therefore, the progression of substrate degradation (and product formation) only depends on the initial substrate concentration (S_0 in kg/m^3) and the respective degradation kinetics (k in 1/d). By estimating both parameters (S_0 and k) based on experimental batch tests, the respective biogas potential as well as the degradation kinetics during batch operation can be derived. However, single first-order kinetics are not suitable to describe individual degradation characteristics such as inhibitory acid accumulation or temperature effects. Thus, additional dependencies can be implemented into the specific reaction equations to depict the individual process behavior.

Starting with the basic first-order reaction kinetics, different kinetic approaches or mathematical functions have been developed to describe batch operation [51].

Model A describes the degradation of the entire substrate by a single first-order reaction, whereas model B divides between rapidly and slowly degradable substrate components. According to Fig. 7, models that are more complex can depict the individual progression in more detail. Furthermore, due to the extended model structure, additional information about the utilized substrate can be gained (e.g., amount of rapidly or slowly degradable substrate).

However, parameter estimation during model adaptation does not guarantee reliable and realistic model parameters on principal. Therefore, the identified parameters – such as the maximum biogas potential (start concentration of degradable substrate) or the individual kinetic constants – should thoroughly be reviewed to provide a



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Fig. 7 Kinetic modeling of discontinuous AD (batch). (**a**) Basic model structure of two first-order reaction models [51]. (**b**) Simulation results during discontinuous digestion of maize silage (model A, $Y = 564$ mL 1/d VS, $k = 0.29$ 1/d, and $R^2 = 0.97$; model B, $Y = 616$ mL 1/d VS, $\alpha = 0.62$, $k_F = 0.57$ 1/d, $k_L = 0.07$ 1/d, and $R^2 = 1.00$)

meaningful set of parameters inside a reasonable value range.

Providing a good fit of the respective simulated and experimental results, the estimated parameters can then be utilized for substrate classification. Furthermore, the identified start concentration (or biogas potential) S_0 occasionally shows higher values than the respective measuring results, which indicates that the underlying methane potential is slightly higher than the final value of the batch experiments (Fig. 7). In this case, the estimated start concentration (or biogas potential) S is a reliable approximation of the maximum biogas potential (at infinite retention time).

Continuous Operation

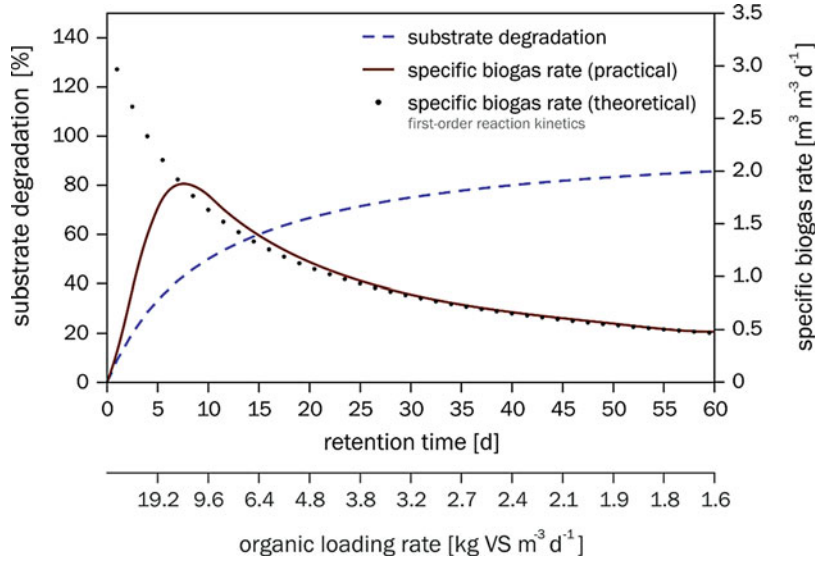
Commonly, industrial biogas plants are operated in continuous (or semicontinuous) mode. For plant design and efficiency evaluation, the general mass balance, and resulting reaction, equations for a continuous stirred tank reactor (CSTR) are typically solved for steady-state conditions. The steady state is characterized by optimal degradation and microbial growth conditions with regard to the specific retention time and organic loading rate. To depict steady state in the general reactor model, the change of the substrate concentration over time equals zero (Eq. 6). Based on simplified first-order reaction, the explicit solution of the substrate concentration for steady state condtions (S_ss) therefore complies to Eq. 12:

$$S_{ss} = \frac{S_0}{1 + \frac{k}{D}} = \frac{S_0}{1 + k\,HRT} \tag{12}$$

The anaerobic digestion (AD) process in continuous stirred tank reactors (CSTR) operated at retention times below approx. 8 days runs the risk of washing out the involved microorganisms. Below this retention time, many microorganisms cannot reproduce themselves fast enough. The operating point that defines the lowest possible retention time (and therefore the highest possible organic loading rate) under stable and uninhibited process conditions defines the maximum capacity utilization and highest volumetric gas production rate. However, at this operation point, the substrate-specific biogas yield (substrate degradation) is lowest and increases with longer retention times as shown in Fig. 8.

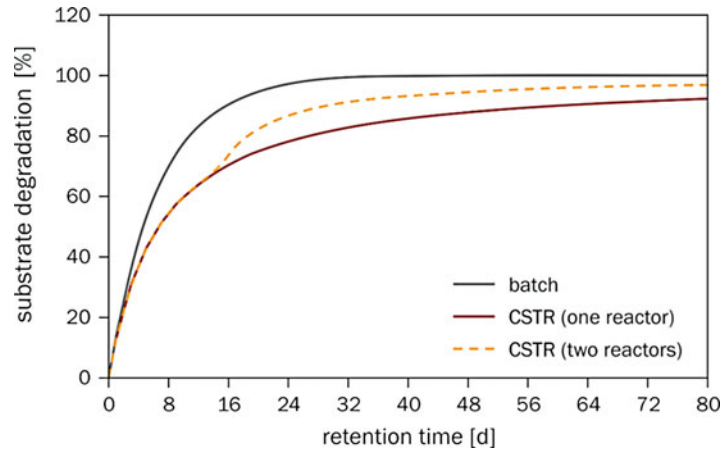
The comparison between discontinuously (batch) and continuously operated reactors indicates the characteristic degradation performance as shown in Fig. 9. Thus, discontinuously operated reactors achieve the same substrate degradation (biogas yield) in shorter retention times.

By connecting several continuous stirred tank reactors (CSTR) in series, more substrate can be



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters,
Fig. 8 Retention time, organic loading rate, substrate degradation, and specific biogas yield based on first-order kinetics (CSTR, input substrate – 12% TS, 80% VS, $k = 0.1$ 1/d, biogas potential = 0.34 L 1/g VS)

Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters,
Fig. 9 Substrate degradation in different operating modes (plant types)



degraded compared to a single reactor with the same reaction volume. If numerus digesters are connected, the degradation characteristics will eventually yield batch performance. The ideal plug flow reactor shows the same kinetic behavior as discontinuous (batch) reactor. However, a true plug flow without any back-mixing is difficult to establish in full-scale application of industrial biogas plants.

Kinetic modeling enables the prediction of important intermediates or products and therefore can be utilized for profound process design or process development. Additionally, kinetic modeling can be used to examine degradation efficiencies or inhibitory effects and enables the direct comparability of continuously operated laboratory, pilot, or industrial scale processes (scale-up).

Process Parameters

Total Solids/Dry Matter Content

The anaerobic digestion (AD) process is carried out in an aqueous environment under anaerobic conditions. Water is needed as transportation and distribution medium and as reaction component.

Therefore, substrates with high water content are particularly suitable for the process. Additionally, the content of nondegradable fractions (organic and inorganic, e.g., lignin and sand) should be as low as possible.

The biogas plant technology is adjusted to the substrate characteristics and the total solid (TS) content of the In general, substrates can be subdivided into liquid substrates (e.g., wastewater total solid (TS) <6%), sludge (e.g., liquid manure 6% < total solid (TS) < 15%), and solid substrates (e.g., organic waste total solid (TS) >15%).

The structure of the substrate and the viscosity are also critical factors for the selection of the anaerobic digestion (AD) technology. The degradation process reduces the total solid (TS) content of the substrate and changes rheological characteristics and structure of the substrate.

Therefore, the digestate has significant different rheological characteristics than the substrate. The use of solid/liquid separation technology within the process has additional impact on these digestate characteristics.

The total solid (TS) concentration has not only a direct impact on the available substrate concentration; it also defines the technologies that are necessary for material handling, e.g., substrate feeding, mixing, transportation, and post-treatment of digestate. An important aspect of the rheological characteristics is the sedimentation behavior of inert material within the digestate. Inert components tend to settle on the digester bottom depending on the retention time, mixing conditions, size of the particle, and the rheological conditions of the substrate.

A measure to control the total solid (TS) concentration in the process (besides simple water addition) is the use of solid/liquid separation as bypass and the subsequent lockout of the solids. By doing so, the total solid

(TS) content is reduced and the solid retention time is uncoupled from the liquid retention time. Sometimes the liquid fraction is also used for substrate conditioning to meet the requirements of the feeding technology and to adjust the substrate concentration in the first digester. The accumulation of unwanted components in the liquid fraction needs to be considered due to the recirculation and the consequent impact on the process.

Total solid (TS) content of more than 15% in the process is usually combined with the so-called plug flow digesters (continuous operation) or garage-style digester (batch operation). Below 15% of total solid (TS) content continuous stirred tank reactors (CSTR) are most often.

For substrates like wastewater with low total solid (TS) and particulate content, digestion systems with biomass retention as, e.g., fixed-bed or upflow anaerobic sludge blanket, are used.

Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Table 4 Comparison of mesophilic and thermophilic digestion systems

Mesophilic anaerobic digestions	Thermophilic anaerobic digestions
Lower water content within the biogas Higher CO_2 solubility in digestate Lower heat demand for heating substrate Higher diversity of microorganisms, higher biological stability Option of low-temperature heating systems Lower ammonia concentration in ammonium-rich digestates, lower inhibitory effect	Higher degradation rates Better sanitation compared to mesophilic conditions Lower solubility of oxygen

Temperature

For technical use of anaerobic processes, three temperature ranges are used to describe the temperature regime:

- Psychrophilic: up to 25 °C
- Mesophilic: 35–42 °C
- Thermophilic: 55–60 °C

These temperature ranges are rather an orientation since overstepping the given limits does not necessarily result in drastic process changes. In particular, the range between mesophilic and thermophilic conditions is used in different applications. Nevertheless, both temperature ranges account for specific biological, energetic, and kinetic process properties (Table 4). Temperature has a direct impact on conversion kinetics. The higher the temperature, the higher the degradation rates – as long as the biological system is not compromised by limitations. Microorganisms usually have a preferred temperature optimum. Therefore, the different temperature ranges favor different microbial consortia. The choice of the optimal temperature needs also to consider:

- The water content of the substrate (resulting concentrations of relevant substances (e.g., ammonia) and heat demand for temperature control)
- Formation of inhibitory substances (in particular if temperature dependent as, e.g., ammonia)
- Necessity of sanitation

If the substrate needs to be heated and has a high water content (e.g., liquid manure), a high temperature is not recommended since a large fraction of the energy available from the gas utilization is needed for temperature control.

The psychrophilic temperature range is most often used in uncontrolled applications (e.g., lagoons, domestic wastewater treatment, and digestate storage). The temperature in such systems will change with seasonal temperature changes.

There is a transition between the temperature ranges. The process can be transferred to another temperature, if the gradient is not too large and the process is not operated at the capacity limit. Large changes and uncontrolled temperature changes should be avoided in any case, since this might lead to instable conditions. Since

different microorganisms might become predominant with changing temperature conditions, the response of the process to other parameters, e.g., lack of trace elements, inhibitory substances, and process conditions (e.g., retention time, pH), might be different and result in different process behaviors.

Impurities

For the technical implementation of anaerobic digestion (AD) processes, the content of impurities like sand, stones, glass, metals, or plastics is a crucial factor. Such substances lead to deterioration at pumps and mixing devices; they also might sediment in the pipes or digesters. Particle size distribution, density, and substrate rheology define potential methods for separation of such materials prior to the digestion process. Alternatively, digesters can also be equipped with scrapers or other sand removal systems. For example, selected substrates, e.g., chicken manure, separately collected organic fraction of municipal waste (biowaste), or solid manure from open barns (in particular without concrete flooring), usually contain significant amounts of inert materials.

A particle size distribution of the inert particles and a rheological analysis of the digester medium can be used to forecast the occurrence of sedimentation. Since the rheology of the substrate changes with ongoing degradation and separation of solids, the sedimentation of inert materials can vary with the processing in the digestion process.

Particle Size Distribution

Since the organic material needs to be broken down to soluble monomers, the particle size distribution is a crucial factor for the microbial degradation. The degradation occurs from the surfaces of the substrate particles, and consequently, the larger the surface area, the faster is the degradation, and the smaller the particles, the better is the degradation.

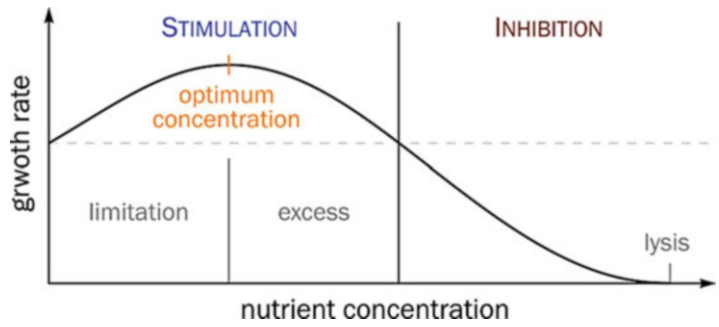
Since the mechanical pretreatment of solid organic materials requires technical effort, the optimum between adequate retention time within the biological process and pretreatment technologies to speed up the process has to be identified. Pumps and mixing technologies need to be selected according to the resulting substrate characteristics.

Dry fermentation is a specific process that is carried out in so-called garage-style or box digesters. These systems percolate a liquid phase over a heap of substrate (or a mixture of substrate and inoculum). The percolate trickles through the substrate and ensures the transport of solubilized substrate, microorganisms, nutrients, and heat. The particle size distribution in such a system needs to be stable and sufficient for the percolation process.

In some cases, the digestate is posttreated by solid-liquid separation and/or composting of the separated solids or the digestate. In such a case, structure and particle size of the material need to be sufficient for oxygen supply within the heap. The addition of bulk material is most common in such cases.

Mineral Nutrients

Besides the supply of organic material, microorganisms need access to a variety of essential mineral nutrients. The maximum growth rate of microorganisms can only be achieved if the conditions are optimal (Fig. 10).



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Fig. 10 Effects of nutrient concentration on microbial growth rates

Some processes quickly show a deficiency of mineral nutrients (e.g., maize mono -fermentation), and the addition of such nutrients becomes necessary. Due to the variety of process conditions (e.g., total solids (TS) and volatile solids (VS), temperature and retention time) and the impact of substrate characteristics, technical conditions, inhibitory substances, and the mutual interferences of mineral nutrients on the growth rate, the identification of optimal amounts of a specific nutrient is difficult if not impossible. Additionally, the chemical speciation of micronutrients (e.g., electrical charge, degree of dissociation, degree of complexation) influences their bioavailability and, therefore, the micronutrient uptake by microorganisms. Consequently, a wide range of sufficient amounts of micronutrients are available in the literature, whereas a definitive number on necessary concentrations are difficult to find.

A very basic value is the ratio of carbon to nitrogen and phosphorus. This ratio is given in different literature sources with a certain variation. According to Ottow and Bidlingmaier [52], a C:N:P:S ratio of 2,000:15:5:3 is optimal, whereas Weiland [53] states a ratio of 600:15:5:1. These concentrations stand for a minimum supply; an oversupply does not result necessarily in a negative effect. However, the ratio does not tell anything about the concentration of the nutrients in the digester medium.

Other elements like sodium, potassium, and calcium and trace elements like iron, zinc, molybdenum, cobalt, nickel, and selenium are essential for microorganisms. On the contrary, high concentrations can inhibit the biogas process and have a negative environmental impact. In particular, in processes with high growth rates, the impact of insufficient nutrient supply becomes apparent through instabilities and underperformance. If a substrate change leads to critical shortage of mineral nutrients, the effect will become obvious depending on the retention time, since the washout of nutrients will proceed accordingly.

The use of the recycled liquid phase from a solid/liquid separation might lead to accumulation of substances in the liquid phase of the digestate. This effect can be used to keep crucial substances in the process but can also lead to accumulation of unwanted substances. In such a case, the effect to the digester medium needs to be predicted with mass balances or closely monitored to avoid critical process conditions.

Several publications highlight the importance of trace elements in anaerobic digestion (AD) (e.g., Graf and Bajohr [54] provide an orientation for optimal trace element concentrations). In comparison to aerobic conditions, the nutrient demand under anaerobic conditions is lower since the biomass yield is much lower.

Inhibitory Substances

The content of inhibitory substances – either occurring in the substrate or created because of degradation processes – needs to be considered when designing the process, since it can have a severe impact on the degradation performance.

Inhibitory substances can be metabolites like ammonia, hydrogen sulfide, or organic acids as well as substances like disinfectants, antibiotics, heavy metals, salts, or surfactants. Some of the mentioned substances can also have toxic properties. Microorganisms can adapt to a certain degree to toxic compounds depending on their type and concentration.

The impact of inhibitory substances on the process is of complex nature and depends on their concentration, the process conditions (e.g., retention time, temperature, pH value), interaction with other substances, and the adaptability of microorganisms. Additionally, only little information is available about the time microorganisms need to adapt to inhibitory substances. Fast concentration changes might lead to inhibition of microorganisms, although the process would be able to adapt with time for transition. Further, the manner of addition influences the process. A one-time addition of an inhibitory substance to the process has a different impact compared to a continuous addition.

Some processes with inhibitory substances even allow a stable operation under obvious inhibition. An example is the ammonia inhibition, which sometimes leads to stable but increased organic acid concentrations. The buffering capacity of ammonia compensates the high acid concentration. However, the process is more sensitive

to significant changes of, e.g., the feeding regime or the temperature.

The definition and the description of the impact of toxic substances are far from simple. First, toxic effects are specific to certain metabolic processes, and consequently toxic effects can be limited to certain groups of microorganisms with specific functions. Additionally, some toxic substances can be degraded under specific conditions, so anaerobic processes might even be used to degrade toxic components [55]. In Table 5, selected inhibitory substances and remarks are listed.

Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Table 5 Selected inhibitory substances

Sodium	Potential negative effect between 5 and 15 g/L; at 15 g/L degradation performance reduced to 50%; interaction with potassium [56]
Potassium	Regulation of osmosis of methanogens; average inhibitory concentration (IC 50) at 4,800 mg/L [56]
Calcium	IC 50 at 4,800 mg/L [56]
Magnesium	IC 50 at 1,900 mg/L [56]
Ammonia	Solution equilibrium of ammonia/ammonium depends on pH value and temperature; inhibition between 2.7 and 10 g/L
Sulfide	Inhibitory >30 mg/L H_2S (undissociated) [57], precipitation with metal ions
Heavy metals	Inhibitory as free ions: Ni >10 mg/L, Cu >40 mg/L, Cr >100 mg/L, Pb >340 mg/L, Zn >150 mg/L [57] Sulfide precipitation and formation of polyphosphates (Cu, Cd, Pb, Zn) can neutralize the inhibitory effect
Short-chain fatty acids	Undissociated fraction is inhibitory (pH dependent) [57]
Petrochemical products	IC 50 of phenol 1,500 mg/L [56]
Hydrogen	Interrelation with accumulation and/or degradation of short-chain fatty acids, low concentrations for degradation of, e.g., propionic acid necessary
Disinfectants and antibiotics	Specific effect; not all anaerobic microorganisms are influenced by antibiotics (e.g., penicillin affects only aerobic organisms)

The interaction of several inhibitory substances is an important aspect. For instance, the inhibition induced by heavy metals depends on the occurrence of other ions or complexing agents in the liquid phase, since metals will bind to some ions, e.g., sulfide, or complexing agents. This strongly affects their bioavailability and toxicity (see section "Mineral Nutrients"). Sulfide ions can also be inhibitory, as precipitation can lead to sludge formation or incrustation. Consequently, the addition of antagonistic substances might become problematic due to resulting interactions.

Concentration of Organic Acids

Organic acids and in particular short-chain fatty acids are intermediates in the anaerobic digestion (AD) process. Since only acetic acid can be directly converted to methane, all other acids need to be converted to acetic acid and/or hydrogen, carbon dioxide, and formate to become further converted to methane.

In a well-balanced system, the formation rate of mainly acetic, propionic, butyric, and valeric acid equals the conversion to other intermediates or methane. Accordingly, the concentrations of these intermediates are low and constant. Disturbances as, e.g., addition of large amounts of substrate, inhibitory substances, or a deficiency of nutrients, lead to an imbalanced degradation. Since the acid formation is usually more robust than the acid degradation, the consequence of disturbances is in most cases an increasing acid concentration. High concentrations of organic acids inhibit the acid conversion process. However, an increased acid concentration alone is not a definitive sign for process imbalances. In case of the presence of inhibitory substances (e.g., high ammonia concentration), increased but stable acid concentrations can appear. Furthermore, the evaluation of the process state should include in any case the concentration change and the degree of dissociation of the organic acids as undissociated acids and therefore uncharged molecules can pass the membranes of the microbial cells and damage them much easier as dissociated (charged) molecules.

On a short term, higher acid concentrations (e.g., from substrate feed) can result in short-

term increased growth rates and can have a stimulating function. Such fast concentration increases (e.g., after each feeding event) are quickly decreased to the stationary level.

Besides the classical analysis of organic acids via gas chromatography and high-performance liquid chromatography, for first information, the titration has become an often-used method. In Germany, the simple and quick-to-determine FOS/TAC value [16] is used to estimate the ratio of acid concentration and buffer capacity. The change of the parameter rather than the absolute value shows disturbances of the process. A direct comparison of different processes is not possible because of the described interactions of organic acids with other inhibitory substances and process parameters, e.g., the pH value.

Concentration of Ammonia

Ammonia is released when nitrogen containing substrates are degraded at anoxic conditions. In particular, protein-rich substrates lead to a high concentration of ammonia. In water, ammonia is in corresponding solution equilibrium with the ammonium ion. Nitrogen is a crucial nutrient for the microorganisms. Similar to the organic acids, if undissociated, it becomes inhibitory at high concentrations. The dissociation equilibrium depends on the temperature and the pH (Fig. 11), and consequently at higher temperatures, the process is more susceptible to high ammonia concentrations. Again, the variety of process conditions and interacting substrate components makes it impossible to give limiting concentrations.

The increase of the ammonia concentration should be handled with care and, if possible, be performed very slowly. Since the growth rate is limited by ammonia, the increase of the retention time in continuous stirred tank reactors (CSTR) is a strategy to reduce inhibitory effects of ammonia. In a continuous stirred tank reactor (CSTR), high ammonia concentrations can go along with higher acid concentrations [58]. Since the ammonia provides buffer capacity, elevated acid concentrations do not lead necessarily to pH changes and a stable operation is possible even under such difficult conditions. However, such a process is more sensitive to process disturbances (see also section "Inhibitory Substances").

pH Value

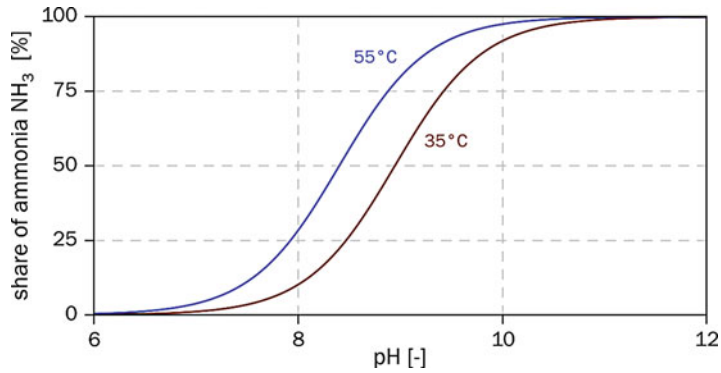
The optimal pH value in the anaerobic digestion (AD) is given in a range of 7–7.5. The pH in the process is usually affected by many process-relevant substances, e.g., carbon dioxide, organic acids, ammonia, hydrogen sulfide, and salts as well as inorganic acids and bases. In processes where all metabolic steps are conducted in one digester, the buffering substances and low acid concentration lead usually to a pH in the desired range. The process can also be operated outside of the optimal pH range, as long as the limitations resulting from the suboptimal conditions do not conflict with the requirements resulting from the process conditions (e.g., retention time).

The accumulation of pH active intermediates or products (e.g., ammonia or organic acids) lead to a shift of the pH, which results in an intensification of the inhibitory effect of the substances since the share of undissociated fractions will increase. Therefore, it becomes difficult to differentiate between the effect of the pH and the resulting changes in the dissociation state of inhibitory substances. However, in the case of changing process parameters, the process needs to be monitored to evaluate if the process shifts to a different stable condition or if it collapses. In cases of acid accumulation and subsequent pH shift, a feeding stop might help to reduce the acids and increase the pH which would stabilize the process by itself. The pH is said to be a late warning indicator, since the buffer systems prevent a quick response to acid/base concentration changes. However, the online measurement of the pH value can provide valuable information.

Buffer Capacity

Many substrate components and intermediates (e.g., acids, ammonia, carbon dioxide, and phosphate) have an impact on the pH value. A sufficient buffer capacity helps to stabilize the pH.

Since most intermediates create buffer systems, most processes can stabilize themselves. The addition of pH-regulating chemicals can help to reduce the negative impact of low pH



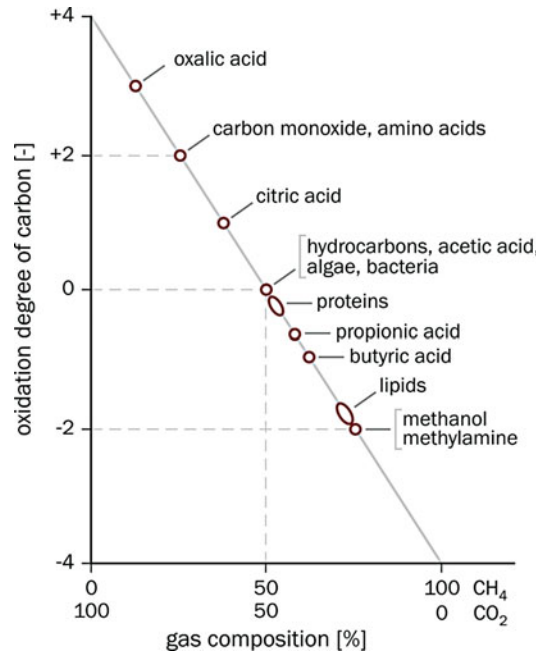
Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Fig. 11 Ammonia–ammonium equilibrium in relation to temperature and pH

and accumulated acids. In case of extensive disturbances and accumulation of acids, the digestion of specific substrates (e.g., wastewater) can require the addition of buffer substances.

Gas Composition

The gas composition (primarily the methane/carbon dioxide ratio) depends mainly on the chemical composition of the substrate (see section "Substrate Characterization" and Fig. 12) and the amount of carbon dioxide in the digestate. Concerning the chemical composition, not only oxygen but also nitrogen and sulfur have a significant impact on the methane concentration in the biogas.

The amount of carbon dioxide bound to the digestate depends on the water content of the digestate, the ratio of biogas production rate and hydraulic retention time, the temperature, the pressure (e.g., height of digesters), and the pH value. Acidogenesis usually leads to carbon dioxide formation. If the metabolic processes can be separated (hydrolysis/acidogenesis and acetogenesis/methanogenesis) and the first step does not lead to losses of hydrogen and/or methane, the methane concentration in the digester can be increased accordingly.



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Fig. 12 Relation of gas composition and oxidation degree of carbon [59]

Technological Process Parameters

A biogas plant is designed according to economic limitations, which means a compromise between technical and financial effort on the one hand and maximum substrate conversion on the other hand. The maximum substrate utilization corresponds to high retention times and consequently to great digester volumes, as the substrate-specific gas yield directly depends on the retention time. The process design aims

at sufficient operational conditions within economic given limits and a maximum degradation rate.

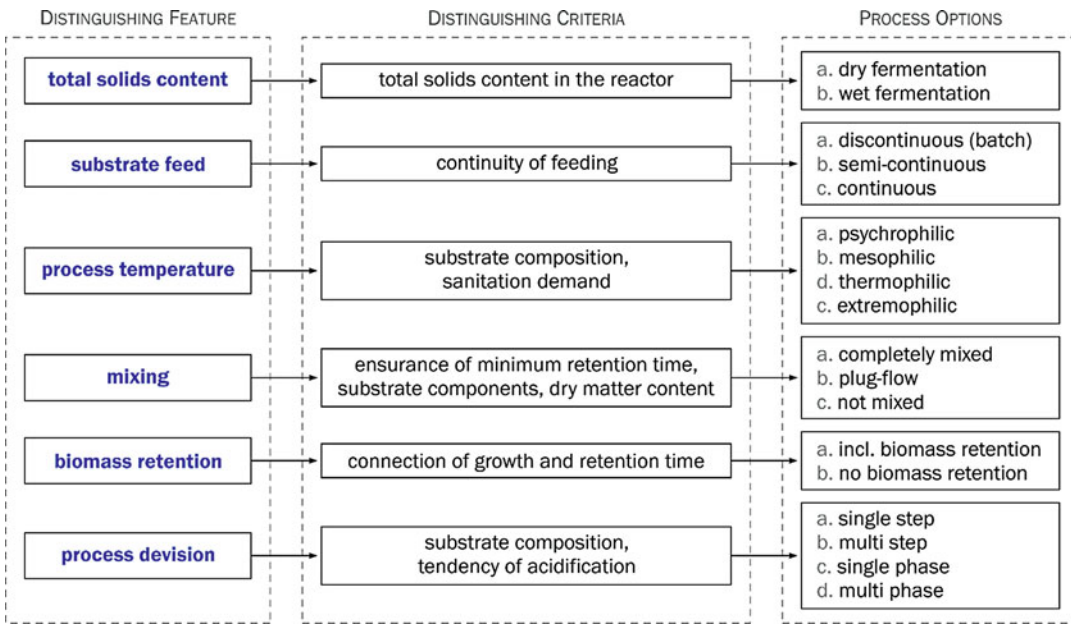
There are numerous variations in anaerobic digestion (AD) process design (Fig. 13). Most options can be combined with each other. A detailed analysis of all those options is not the purpose of this entry; it rather introduces the most important technical process parameters for anaerobic digestion process control (for further information, see, e.g., Speece [56] or Bischofsberger et al. [57]).

Hydraulic Retention Time

Hydraulic retention time (HRT) is a crucial parameter for process design. In case of solid-liquid separation in the process, the retention time of the respective fraction needs to be calculated separately.

The hydraulic retention time (HRT) in a continuous stirred tank reactor (CSTR) system needs to allow a growth rate sufficient to avoid washout of the crucial microorganisms. The growth needs to compensate the losses caused by the continuous digestate removal from the process. If the process is operated close to the maximum growth rate, the system is more sensitive toward disturbances and changes in load and substrate characteristics, since such changes cannot easily be compensated by increasing the growth rate. In case of biomass retention (here the term biomass refers to microorganisms, e.g., fixed-bed digesters or upflow anaerobic sludge blanket (UASB) reactors), the retention time can be lower than the growth rate of the microorganisms, since the growth and throughput do not depend on each other.

The hydraulic retention time (HRT) is defined as the ratio of reaction volume V (in m^3) and the input rate Q (e.g., in m^3/d) (Eq. 13). This definition applies only for a constant reactor volume. The assumption of a constant volume needs to be questioned in the case of high solids containing substrates. With 30% TS and a high degradability of these solids, a significant portion of the mass is degraded and the digestate mass and volume are reduced. In such cases, a precise calculation of the hydraulic retention time (HRT) needs to consider the volume reduction.



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Fig. 13 Technological characteristics of the anaerobic digestion processes

$$HRT = \frac{V}{Q} \tag{13}$$

The retention time of particulate material can be increased through specific measures to provide more time for degradation and achieve higher degradation rates. Anaerobic baffled reactors, for instance, have separation blades acting as flow breakers. Particulate material is kept inside, while the liquid phase is passing.

The relation of retention time and degree of degradation in plants using agricultural substrates like manure and energy crops is shown in Fig. 14. Since the substrates have quite different degradation kinetics, the data points are scattered, but the tendency is obvious. The throughput is the reciprocal of the hydraulic retention time (HRT) and often used as alternative process parameter to the retention time.

Organic Loading Rate

The organic loading rate (OLR) is defined as the quotient of the input rate of organic matter (F in kg VS/d) and the reactor volume (V in m^3) (Eq. 14).

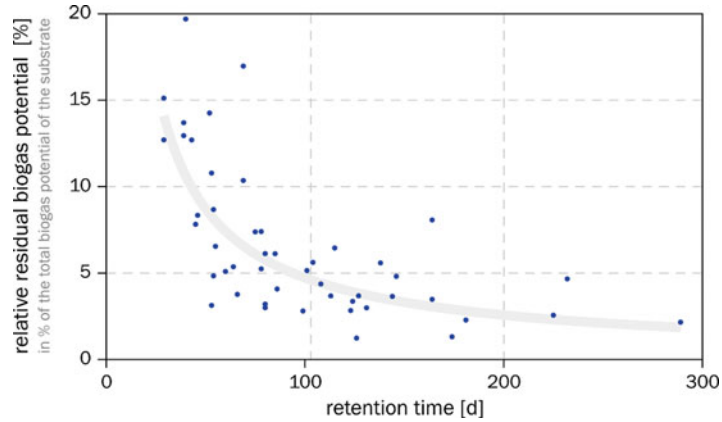
$$OLR = \frac{F}{V} \tag{14}$$

The organic loading rate (OLR) depends on the substrate concentration in the input and the retention time of the process. Assuming Monod kinetics for substrate degradation in combination with a continuous stirred tank reactor (CSTR), the growth rate depends only on the retention time. The amount of volatile solids (VS) in the input rather defines the necessary microorganism density. Critical process conditions usually occur when the growth rate reaches the limit. High organic loading rate (OLR) can be achieved in systems with biomass retention by means of high throughput or by a high share of volatile solids (VS) in the input, e.g., in dry fermentation systems. High organic loading rate (OLR) also stands for high specific gas production rates (specific to the digestion volume), which means that the digester volume is effectively used. High concentrations of substrate in the digester might lead to mixing issues, as well as problems to release the gas properly (mass transfer limitations).

In a plant screening program, the average organic loading rate (OLR) of 61 agricultural plants was determined with 3.0 kg VS/(m^3 /d) (the maximum was 9.9 kg VS/(m^3 /d) and the minimum 1.1 kg VS/(m^3 /d)) [60]. Table 6 summarizes typical retention times and organic loading rate (OLR) for anaerobic digestion processes.

Biogas or Methane Yield

The biogas yield represents the gas production achieved under typical process conditions (see also section "Biogas Potential and Yield"). Usually it is given as biogas or methane yield in relation to the organic substance added (most common based on volatile solids (VS) or chemical



Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters,
Fig. 14 Retention time and residual gas potential at agricultural biogas plants [60]

Anaerobic Fermentation of Organic Material: Biological Processes and Their Control Parameters, Table 6 Typical retention times and organic loading rate (OLR) for anaerobic digestion processes (possible extreme values in brackets)

Process	Organic loading rate (kg/(m^3 d))	Hydraulic retention time (d)
CSTR	2–4 (10)	(5) 10–40 (>50)
Plug flow reactors	5–15 (20)	10–40
UASB reactor	5–15	0.2–1 (5)
Fixed-bed reactors	5–15 (20)	0.5–8

oxygen demand (COD)) and as dry gas under standard conditions. In large-scale applications, besides the biological factors as described above, additional parameters influence the difference between biogas yield and biogas potential. Lack of mixing (mass transfer limitations), losses (e.g., leakages), or downtimes might lead to reduced gas amounts.

With the knowledge of yield and potential (section "Biogas Potential and Yield"), the efficiency of the process can be determined. An objective comparison with regard to the substrate conversion of different biogas plants is only possible if the kinetics and potential inhibition of the process are considered. Since the evaluation of all these parameters is an extensive work, it is most often not done. Therefore, reliable data for process and plant comparison are only rarely available. Consequently, standard values from literature are used to benchmark the plants. Since these numbers have been usually obtained under various conditions, they are valid only as orientation. Equally important is the careful sampling. The analysis will give erroneous interpretation, if the samples are not representative. Precise data from large-scale processes are difficult to get. The reasons are the lack of precise measurement devices (e.g., input masses, gas production) and, in particular at waste treating facilities, the representative sampling of substrates.

The specific gas production rate is defined by the ratio of gas production rate and digestion volume (m^3 gas/(m^3 $_{\text{working volume}}$ /d)). It is a measure for the utilization of the working volume (according to VDI 4631 [61]).

Another option for process analysis is the energy-based process evaluation. The gas potential of the substrate can be converted into an energy potential (also possible is a direct determination of the heating value), and this energy potential can be directly put into relation with the electricity and/or heat produced. Such a balance includes all conversion efficiencies.

Future Directions

Even if the technology for biogas production can be seen as well established, the process monitoring and control, especially of dynamic anaerobic digestion (AD) processes, are still challenging. The described interactions of process parameters, such as the pH value and the degree of dissociation of process metabolites (e.g., acetate) and process inhibitors (e.g., NH_3), prevent the formulation of universal recommendations for process evaluation and control. The development and use of models and new sensors to predict and monitor process conditions deriving from different substrates and/or different modes of operation or process designs provide an opportunity for enhanced process control and automation.

The directed production of anaerobic digestion (AD) intermediates, e.g., medium-chain fatty acids, can increase the field of application of anaerobic digestion (AD). Medium-chain fatty acids can either be directly used or be processed to, e.g., alkanes and alkenes, via an electrochemical refining step [62], leading to either fuels or educts for the chemical industry. Furthermore, the hydrogenotrophic methanation pathway can be utilized to convert CO_2 to CH_4 using H_2. This process is called biological methanation and is one opportunity of methanation in the power to gas concept. It can contribute to power-grid stabilization as the H_2 can be provided by water electrolysis using surplus energy from fluctuating renewable energy sources such as wind and solar power [63]. Up to now, a few demonstration facilities for biological methanation exist in Europe. The further development and economic implementation of the biological methanation

mainly depend on the costs for hydrogen and carbon dioxide. A special form of the biological methanation is the microbial electrochemical methanation or bioelectrochemical methanation. The difference to the biological methanation is that instead of hydrogen, electrons are provided to the microorganisms, either direct or by the use of mediators [64].

Bibliography

1. Lusk P (1998) Methane recovery from animal manures – the current opportunities casebook. http://www.nrel.gov/docs/fy99osti/25145.pdf. Accessed 19 Oct 2016
2. Meynell PJ (1976) Methane: planning a digester. Schocken Books, New York
3. Buswell AM (1939) Anaerobic fermentations. State of Illinois, Department of Registration and Education, Division of the State Water Survey, Urbana, Illinois
4. Bryant MP, Wolin EA, Wolin MJ, Wolfe RS (1967) *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. Arch Mikrobiol 59:20–31
5. The International Renewable Energy Agency (2017) RESource database. http://resourceirena.irena.org/gateway/dashboard/?topic=4&subTopic=17. Accessed 19 Jul 2017
6. Hahn H, Krautkremer B, Hartmann K, Wachendorf M (2014) Review of concepts for a demand-driven biogas supply for flexible power generation. Renew Sust Energ Rev 29:383–393
7. Mauky E, Weinrich S, Nägele HJ, Jacobi HF, Liebetrau J, Nelles M (2016) Model predictive control for demand-driven biogas production in full scale. Chem Eng Technol 39:652–664
8. Chen Y, Cheng JJ, Creamer KS (2008) Inhibition of anaerobic digestion process: a review. Bioresour Technol 99:4044–4064
9. Schnürer A, Nordberg A (2008) Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. Water Sci Technol 57:735–740
10. Popp D, Plugge CM, Kleinsteuber S, Harms H, Sträuber H (2017) Inhibitory effect of coumarin on syntrophic fatty acid-oxidizing and methanogenic cultures and biogas reactor microbiomes. Appl Environ Microbiol 83(13):pii: e00438-17
11. Ngoma PM, Hiligsmann S, Zola ES, Ongena M, Thonart P (2015) Impact of different plant secondary metabolites addition: saponin, tannic acid, salicin and aloin on glucose anaerobic co-digestion. Ferment Technol 4:1
12. Sträuber H, Bühligen F, Kleinsteuber S, Nikolausz M, Porsch K (2015) Improved anaerobic fermentation of wheat straw by alkaline pre-treatment and addition of alkali-tolerant microorganisms. Bioengineering 2:66–93
13. Bagi Z, Acs N, Balint B, Horvath L, Dobo K, Perei KR, Rakhely G, Kovacs KL (2007) Biotechnological intensification of biogas production. Appl Microbiol Biotechnol 76:473–482
14. Conrad R, Schink B, Phelps TJ (1986) Thermodynamics of H2-consuming and H2-producing metabolic reactions in diverse methanogenic environments under *in situ* conditions. FEMS Microbiol Ecol 38:353–360
15. Angenent LT, Richter H, Buckel W, Spirito CM, Steinbusch KJ, Plugge C, Strik DP, Grootscholten TI, Buisman CJ, Hamelers HV (2016) Chain elongation with reactor microbiomes: open-culture biotechnology to produce biochemical. Environ Sci Technol 50:2796–2810
16. Liebetrau J, Pfeiffer D, Thrän D (eds) (2015) Collection of measurement methods for biogas – methods to determine parameters for analysis purposes and parameters that describe processes in the biogas sector. Series of the funding programme "Biomass energy use". DBFZ, Leipzig
17. Weissbach F, Strubelt C (2008) The correction of the total solids content of maize silages as substrate for biogas plants. Landtechnik 63:82–83
18. Weissbach F, Strubelt C (2008) The correction of the total solids content of grass silages as substrate for biogas plants. Landtechnik 63:210–211
19. Weissbach F, Strubelt C (2008) The correction of the total solids content of sugar-beet silages as substrate for biogas plants. Landtechnik 63:354–355
20. Kuratorium für Technik und Bauwesen in der Landwirtschaft (KTBL) (2015) Gasausbeute in landwirtschaftlichen Biogasanlagen. KTBL eV, Darmstadt
21. VDI Guideline 4630 (2016) Fermentation of organic materials – characterisation of the substrate, sampling, collection of material data, fermentation tests. Beuth Verlag GmbH, Berlin
22. Buswell AM, Mueller HF (1952) Mechanism of methane fermentation. Ind Eng Chem 44:550–552
23. Boyle WC (1976) Energy recovery from sanitary landfill: a review. In: Microbial energy conversion. Pergamon Press, New York, pp 119–138
24. DIN EN 15936 (2012) Sludge, treated biowaste, soil and waste – determination of total organic carbon (TOC) by dry combustion. Beuth Verlag GmbH, Berlin
25. Keymer U, Schilcher A (1999) Überlegungen zur Errechnung theoretischer Gasausbeuten vergärbarer substrate in Biogasanlagen. Landtechnik-Bericht No. 32, Freising
26. Weissbach F (2008) On assessing the gas production potential of renewable primary products. Landtechnik 63:356–358
27. Kirchgessner M (2014) Tierernährung: Leitfaden für Praxis, Beratung und Studium, 14th edn. DLG-Verlag, Frankfurt
28. Tilley JM, Terry RA (1963) A two stage technique for the *in vitro* digestion of forage crops. J Br Grassland Soc 18:104–111

29. Deutsche Landwirtschafts-Gesellschaft eV (DLG eV) Datenbank Futtermittel. http://www.dlg.org/futtermitteldatenbank.html
30. Givens DI, Owen O, Axford RFE (2000) Forage evaluation in ruminant nutrition. CABI Pub, Wallingford/Oxon/New York
31. Jeroch H, Flachowsky G, Weissbach F (1993) Futtermittelkunde mit 238 Tabellen. Fischer, Jena
32. Weissbach F (2009) Evaluation of the renewable primary products for biogas production. Part I: gas production potential of the fermentable nutrients. Pflanzenbauwissenschaften 13:72–85
33. Baserga U (1998) Landwirtschaftliche Co-Vergärungs-Bio-gasanlagen – Biogas aus organischen Reststoffen und Energiegras. FAT-Berichte 512:1–11
34. Weiland P (2001) Grundlagen der Methangärung – Biologie und Substrate. In: VDI-Berichte Nr. 1620: Biogas als regenerative Energie – Stand und Perspektiven. VDI Verlag, Düsseldorf, pp 19–32
35. Weissbach F (2009) Die Bewertung des Gasbildungspotenzials von nachwachsenden Rohstoffen. Biogas Sci 10:517–526
36. Weissbach F, Kuhla S, Schmidt L, Henkels A (1999) Schätzung der Verdaulichkeit und der Umsetzbaren Energie von Gras und Grasprodukten. Proc Soc Nutr Physiol 8:72
37. Weissbach F, Kuhla S, Schmidt L (1996) Schätzung der umsetzbaren Energie von Grundfutter mittels einer Cellulase-Methode. Proc Soc Nutr Physiol 5:115
38. Heuwinkel H, Aschmann A, Gerlach R, Gronauer A (2009) Optimierung der anaeroben Fermentierung durch mineralische Zusatzstoffe. Biogas Sci 1:85–95
39. Rath J, Heuwinkel H, Taube F, Herrmann A (2015) Predicting specific biogas yield of maize – validation of different model approaches. Bioenerg Res 8:832–842
40. Lyberatos G, Skiadas IV (1999) Modeling of anaerobic digestion – a review. Global NEST J 2:63–76
41. Pavlostathis S, Giraldo-Gomez E (1991) Kinetics of anaerobic treatment: a critical review. Crit Rev Environ Control 21:441–490
42. Gavala H, Angelidaki I, Ahring B (2003) Kinetics and modeling of anaerobic digestion process. In: Scheper T, Ahring B (eds) Biomethanation I. Springer, Berlin, pp 57–93
43. Batstone D, Keller J, Angelidaki I, Kalyuzhnyi S, Pavlostathis S, Rozzi A, Sanders W, Siegrist H, Vavilin V (2002) Anaerobic digestion model no. 1. IWA Publishing, London
44. Dochain D, Vanrolleghem P (2001) Dynamical modelling and estimation in wastewater treatment processes. IWA Publishing, London
45. Monod J (1949) The growth of bacterial cultures. Annu Rev Microbiol 3:371–394
46. Tessier G (1942) Croissance des populations bacteriennes et quantite d'aliment disponible. Rev Sci 80:209–216
47. Moser H (1958) The dynamics of bacterial populations maintained in the chemostat. no. 614. Carnegie Institution, Washington, DC
48. Chen YR, Hashimoto AG (1978) Kinetics of methane fermentation. Biotechnol Bioeng Symp 8:269–282
49. Contois DE (1959) Kinetics of bacterial growth: relationship between population density and specific growth. Rate of continuous cultures. J Gen Microbiol 21:40–50
50. Haldane JBS (1930) Enzymes. Longmans. Green and Company, London
51. Brulé M, Oechsner H, Jungbluth T (2014) Exponential model describing methane production kinetics in batch anaerobic digestion: a tool for evaluation of biochemical methane potential assays. Bioprocess Biosyst Eng 37:1759–1770
52. Ottow CGJ, Bidlingmaier W (1997) Umweltbiotechnologie. Gustav Fischer Verlag, Jena
53. Weiland P (2010) Biogas production current state and perspectives. Appl Microbiol Biotechnol 85:849–860
54. Graf F, Bajohr S (2013) Biogas, Erzeugung, Aufbereitung, Einspeisung. Deutscher Industrieverlag, München
55. Lorenz H (2006) Phytohygiene der biologischen Abfallbehandlung bei Kompostierung und Vergärung. Verlag Mensch und Buch, Berlin
56. Speece RE (1996) Anaerobic Biotechnology for industrial wastewater. Archae Press, Nashville
57. Bischofsberger W, Dichtl N, Rosenwinkel KH, Seyfried CF, Böhnke B (eds) (2005) Anaerobtechnik. Springer-Verlag, Heidelberg
58. Kroeker EJ, Schulte DD (1979) Anaerobic treatment process stability. J Water Pollut Control 51:719–772
59. Scherer P (2001) Mikrobiologie der Vergärung von festen Abfallstoffen. In: Kämpfer P, Weißenfels W (eds) Biologische Behandlung organischer Abfälle. Springer Verlag, Heidelberg, pp 45–80
60. Johann Heinrich von Thünen-Institut (vTI)/Institut für Agrartechnologie und Biosystemtechnik (2009) Biogas Messprogramm II, 61 Biogasanlagen im Vergleich. FNR, Gülzow
61. VDI Guideline 4631 (2008) Quality criteria for biogas plants. Beuth Verlag GmbH, Berlin
62. Harnisch F, Rosa LFM, Sträuber H, Kleinsteuber S, Dittrich-Zechendorf M, dos Santos TR, Schröder U (2016) Verfahren zur Herstellung organsicher Verbindung. German Patent Application DE 10 2014 214 582 A1; International application WO 2016012279A1, PCT/EP2015/065877
63. Götz M, Lefebvre J, Mörs F, McDaniel Koch A, Graf F, Bajohr S, Reimert R, Kolb T (2016) Renewable power-to-gas: a technological and economic review. Renew Energy 85:1371–1390
64. Geppert F, Liu D, van Eerten-Jansen M, Weidner E, Buisman C, ter Heijne A (2016) Bioelectrochemical power-to-gas. Trends Biotechnol 34:879–894