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# Demand-Driven Biogas Production in Anaerobic Filters

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# Demand-Driven Biogas Production in Anaerobic Filters

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# 1 Introduction

## 1.1 Framework

Within the EU-28 the share of renewable energy (RE) in primary energy production has increased to 24.3 % in 2013, signifying a 84.4 % increase between the years 2003 and 2013 (EU, 2016). Guidelines for the transformation of the energy supply in Germany aim at increasing the share of RE to 35 % in 2020 and to more than 80 % in 2050 (BMWi, 2010). The European Union predicts the share of renewable energy in gross final energy consumption to achieve 55 % to 97 % in 2050 (EU, 2012).

Fluctuating energy sources, namely wind turbines and photovoltaic, will be the mayor contributors to this increase (Steinke et al., 2013). The intermittent energy supply by these sources poses challenges for the electricity grid and needs to be counter balanced. Balancing power, positive or negative, can be categorized by their response time into primary, secondary and tertiary balancing power with reaction times <30 s, <15 min and >15 min, respectively (Hirth and Ziegenhagen, 2015). A demand-driven energy supply by weather independent biomass conversion can offer these grid services (BMWi, 2010; Thrän et al., 2015). Flexible energy production from biogas has been identified as a vital approach to provide the grid with positive and negative balancing power. It can either be achieved by increasing gas storage capacity, a demand-driven biogas production or a combination of both (Hahn et al., 2014b). Recent research in demand-driven biogas production focuses on traditional continuously stirred tank reactor (CSTR) plant designs, demonstrating its feasibility, opportunities and limitations (Mauky et al., 2015, 2016; Mulat et al., 2016).

## 1.2 Flexible Biogas Production

In recent years the interest in flexible biogas production has increased. Bekkering et al. (2013) investigated a seasonal gas supply on a theoretical basis, since at the time no relevant literature was available concerning actively controlling the gas output quantity of a digester. Modeling three scenarios, the research group found that gas storage is by far the most expensive option, whereas a flexible gas production was revealed as the most cost effective.

Changing the focus towards flexible power generation, Hahn et al. (2014b) reviewed concepts to achieve flexibility by a demand-driven biogas supply. The concepts discussed were (1) biogas storing or (2) flexible biogas production. The latter accomplished either by variable substrate feeding using CSTRs or adapted biogas plant configurations, including

two-staged anaerobic digestion. It was concluded that on-site gas storage can provide the shortest reaction times in terms of power generation, albeit long term balancing power is limited, due to the size of gas storage and potential permissions under law. Furthermore it was concluded that a flexible biogas production can provide long term balancing power, but in order to provide tertiary and secondary balancing power a gas storage is needed. Primary balancing power was not considered specifically. Though, explicitly mentioned is the lower risk of process disturbance and enhanced flexibility of biogas production when adapted plant configurations such as two-staged anaerobic digestion are utilized.

The above findings are in line with Grim et al. (2015) who modeled flexible biogas production of a CSTR by using the anaerobic digestion model one (ADM1). Under the Swedish conditions presented herein, flexible biogas production could increase income by 6 % to 10 % for simple electricity production strategies. For more advanced flexibility concepts, the monetary framework needs to be adapted. Higher fluctuations in the electricity price as well as subsidies or system optimization would be necessary for flexible biogas production.

A first experimental study on flexible biogas production was executed by Mauky et al. (2015), using lab-scale CSTRs with organic loading rates on volatile solid base ($OLR_{VS}$) ranging between $1\,kg\,m^{-3}\,d^{-1}$ to $7\,kg\,m^{-3}\,d^{-1}$. The feeding regime was changed to less feedings per day. Easily degradable substrates like sugar beet were used in the mix to produce peaks in gas production. It was demonstrated that biogas can be produced highly flexible, minimizing the necessary gas storage system. No process disturbances could be detected, although the diurnal variation lead to a daily alternation of gas concentration, pH and acid concentrations.

Taking a deeper look into the dynamics of the process Mulat et al. (2016) compared the feeding regimes 'once per day' or 'once every second day' to 'once every 2 h'. It was found that less frequent feeding, while keeping the same overall OLR, leads to improved process stability and changes the bacterial community composition, whereas the methanogenic community remains stable. A sudden increase in volatile fatty acid (VFA) concentrations after feeding could be detected, but returned to normal levels in the periods when no substrate addition took place. The overall efficiency of the process could be improved, leading to a 14 % increase in methane yield. Feeding high amounts at certain points in time may prevent shortcut flows, ensuring recalcitrant components of the substrate to remain in the reactor. The higher diversity of the bacterial community in less frequently fed systems supports this suggestion.

In a trial by Mauky et al. (2016), not only the feasibility of a flexible biogas production, but also the prediction of gas production by means of simplified dynamic models was demonstrated in full scale. In addition a 42 % to 45 % saving in gas storage capacity could be achieved compared to constant gas production.

So far all of the published literature on demand-driven gas production focuses on

single-staged anaerobic digestion using CSTRs. There are possible advantages for flexible gas production in using a two-staged approach, which have not been examined yet.

## 1.3 Two-Staged Anaerobic Digestion

Anaerobic digestion (AD) of complex organic material is a sequence of processes with different microorganisms involved in each step. Based on substrate and produced intermediates, the overall process is divided into (1)hydrolysis, (2)acidogenesis, (3)acetogenesis and (4)methanogenesis (Bischofsberger, 2005). The initial breakdown of complex organics into soluble products is achieved by secretion of exo-enzymes by fermentative bacteria and is described as the rate limiting step in the anaerobic treatment of complex substrates (Pavlostathis and Giraldo-Gomez, 1991). Membrane permeable products of hydrolysis such as monosaccharides, amino acids and long chain fatty acids are further degraded to volatile fatty acids (VFA), hydrogen gas ($H_2$) and carbon dioxide ($CO_2$) during acidogenesis.

Acetogenesis is termed after its main product acetic acid (HAc) and is achieved either by oxidation of VFAs or by homoacetogenesis, the reduction of carbon dioxide with hydrogen gas. The latter does seem to play only a minor role in anaerobic treatment (Gehring et al., 2015).

For acetate production i.e. oxidation of VFAs, a low $H_2$ partial pressure needs to be maintained. In anaerobic environments this is mainly accomplished by the close interaction of fermentative bacteria and $H_2$ consuming methanogenic archea. This syntrophic cooperation enables otherwise thermodynamically unfavorable reactions to be performed. The overall reaction becomes exergonic through the work of hydrogenotrophic methanogens, reducing $CO_2$ with $H_2$ to methane ($CH_4$). Under the low $H_2$ partial pressure ($<$10 Pa) the energy yield of fatty acid oxidation is then sufficient to form ATP from the oxidizing reaction (Schink, 1997). About one third of total methane production in anaerobic treatment is attributed to hydrogenotrophic methanogenesis (John S. Jeris, 1965). However, this share changes with substrate type and was found to be the dominant pathway in the studies of Mulat et al. (2016) for flexible gas production. In an anaerobic filter of a two-staged system Gehring et al. (2015) determined a share of hydrogenotrophic methanogenesis in the range of 28 % to 44 % of total methane production and it was positively correlated to OLR. The second mayor pathway of methane formation is accomplished by acetate cleavage, which forms 1 mol of $CH_4$ from the methyl group and 1 mol of $CO_2$ from the carboxylic group of 1 mol HAc and thereby completing the anaerobic digestion.

In traditional one-staged AD all these steps take place in one reaction vessel, where a delicate balance between acid forming and methane forming microorganisms is maintained. A lack of stability in the anaerobic process is often due to imbalances between

these groups of microorganisms(Cohen et al., 1979; Demirel and Yenigün, 2002). Both groups differ widely in their physiology, nutritional demand, growth kinetics, sensitivity to environmental changes and optimum growth conditions (Pohland and Ghosh, 1971). To take account of these differences and to provide optimal conditions for both groups, Pohland and Ghosh (1971) proposed a system to physically divide the process into two reactors, increasing process stability and control.

Throughout the last years the two-staged AD has been applied to treat a wide variety of substrates, ranging from liquid model substances like glucose over highly complex industrial wastewaters and animal products to solid organic waste or purposefully grown energy crops (Ghosh et al., 1985; Verrier et al., 1987; Raynal et al., 1998; Ince, 1998; Demirel and Yenigün, 2002; Yu et al., 2002; Demirer and Chen, 2005; Cysneiros et al., 2012; Zielonka et al., 2010; Lindner et al., 2016). The reactor configuration employed is tailored to the primary substrates of the system. Most commonly it is put into practice as a combination of CSTR or leach-bed reactor (LBR) as acidification stage and an anaerobic filter (AF) or upflow anaerobic sludge blanket reactor (UASB) as the secondary methanation stage. The spatio-temporal separation of hydrolysis/acidogenesis and acetogenesis/methanogenesis yields a liquid rich in easily degradable intermediates in the acidification recator (AR) and is subsequently degraded to biogas in the methane reactor (MR).

Recent developments show that the composition of produced hydrolysate in the AR can be influenced towards certain main products by controlling pH and redox-potential (Eh). In studies by Ren et al. (1997, 2007) treating molasses from sugar beet refinery an ethanol-type fermentation occurred at pH$<$4.5. Higher butyric acid (HBu) concentrations were achieved at pH$>$6 and propionic acid (HPr) was the main product under conditions of pH 5.5 and Eh$>-278\,\mathrm{mV}$. A complex medium containing glucose and yeast extract was the substrate used by Horiuchi et al. (2002). A change in composition of produced hydrolysate from mainly butyric to propionic and acetic acid was observed, when increasing the pH of the AR from 5.0 to 8.0. Highest HBu concentration were measured at pH 6.0. In experiments using maize silage, the hydrolysate composition changed with the pH, reducing the butyric acid concentration by $>90\,\%$ while going from pH 5.5 to 7.5 Lindner et al. (2015). Here propionic acid concentrations increased accordingly until its highest values at pH 7.0, displacing acetate as the main VFA. Total acids measured in the hydrolysate were reduced, due to the onset of methanogenesis at pH 6.0.

Two-staged AD offers several advantages over single-staged AD and can be summarized as follows:

- provision of optimal conditions for the microorganism consortia taking part in the respective step (Pohland and Ghosh, 1971; Cohen et al., 1979),
- thereby increasing turnover rates and enabling a reduction in total reactor volume

(Cohen et al., 1979),

- disposal of slugde from the AR without loss of slowly growing methanogens (Cohen et al., 1979),
- a fractionation of produced biogas (Muha et al., 2013), enabling high methane concentrations in the MR and
- higher overall methane yields for specific substrates (Ghosh, 1987).

Further advantages, possibly improving demand-driven gas production and thereby enhancing the scale of flexibility compared to single- stage AD, are that

- the hydrolysis as rate limiting step (Pavlostathis and Giraldo-Gomez, 1991) is decoupled from methanogenesis, thereby enabling a shift of the gas production into times of higher demand,
- selective production of certain intermediates in the AR by controlling pH and redox-potential (Ren et al., 1997, 2007; Lindner et al., 2015),
- generally higher process stability and ruggedness against shock loads (Rajeshwari et al., 2000),
- operation of the MR at much higher OLRs than CSTRs (Rajeshwari et al., 2000) and
- the possibility to keep the MR in dormancy (Tauseef et al., 2013).

The above advantages come at the expense of a decreased overall degradation degree when treating lignocellulosic material. Lindner et al. (2016) determined a decrease in methane yield using a two-staged AD system in comparison to the methane potential determined by batch tests. Methane yields were reduced by 70.6 %, 31.3 % and 7.8 % for hay/straw, maize silage and sugar beet, respectively. It was concluded from this study that only easily degradable substrates with low lignocellulosic components should be used in two-staged AD. Supporting evidence of a decreased hydrolysis rate of cellulose under slightly acidic conditions were presented by Koeck et al. (2015), who found the highest degradation rates for cellulose filter paper by different clostridia strains at a starting pH between 7.19-7.51. At lower and higher starting pH the time needed for complete degradation was extended. Other disadvantages of two-staged AD are the need for well-trained plant operators and possibly higher investment and maintenance cost due to a more complex plant design.

### 1.3.1 Anaerobic Filters

In two-staged AD the majority of methane is produced in the MR, accounting for approximately 80 % of total methane production when treating agricultural crops like sugarbeet, hay/straw or maize silage (Lindner et al., 2016). Therefore the focus for demand-driven energy production is the MR, as it is the key for a flexible biogas production in two-staged AD. Anaerobic Filters marked the first step towards the so called "second generation anaerobic digesters" and their potentially high efficiency was first demonstrated by Young and McCarty (1969). Anaerobic Filters can be operated in upflow or downflow and they use the ability of the microorganisms to form a biofilm and attach to support media - the packing bed (Bischofsberger, 2005). The immobilization of the biomass prevents the loss of slowly growing methanogens and thereby enables high organic loading rates with a mean $OLR_{COD}$ of $9.6\,g\,L^{-1}\,d^{-1}$ for installed industrial anaerobic filters (Bischofsberger, 2005) and maximum $OLRs_{COD}$ up to $\approx 40\,g\,L^{-1}\,d^{-1}$ (Tauseef et al., 2013; Bischofsberger, 2005). These high OLRs are combined with short HRTs as low as $<1\,d$ (Tauseef et al., 2013) and maintaining high degradation degrees.

The media surface texture, media pore size and porosity of the packing bed have a significant influence on biomass retention of attached biomass as well as on suspended biomass trapped in the interstitial void spaces, both increasing the efficiency of the AF (Tay et al., 1997; Show and Tay, 1999). Specific surface area seems to be of importance only if the amount of attached biomass is large relative to the suspended biomass (Tay et al., 1997). In packing beds with a porosity of 90 % the majority (56 %) of methane production in the AF could be attributed to the suspended biomass. In contrast 56 % to 58 % of methane production was attributed to attached biomass in the packing bed with a porosity of 75 % (Show and Tay, 1999). During development of the biofilm the void volume is reduced markedly by 43 % to 57 % (Show and Tay, 1999; Jawed and Tare, 2000) and thus decreasing the effective HRT. Biofilm development can be partitioned into at least four distinct stages: (1)reversible attachment, (2)"irreversible" attachment, (2)maturation and (4)detachment. Each of the stages is accompanied by a profound change in phenotype of the microorganisms (Stoodley et al., 2002) and the average difference in protein production between phases is as high as 35 % for *Pseudomonas aeruginosa* (Sauer et al., 2002). Mature biofilms are complex structures with matrix-enclosed microcolonies interspersed between channels which deliver nutrients into the deeper levels of thick biofilms. The biofilm structure is largely determined by production of slime-like extracellular polymeric substances (EPS) which make up 10 % to 90 % of total organic matter in biofilms (Nielsen et al., 1997). But also the physical environment plays an important role to develop density and strength (Stoodley et al., 2002). Biofilms grown under high shear forces seem to exhibit a smoother and denser matrix than those grown under low shear forces (Liu and Tay, 2001).

# 2 Problem and Objective

Two-staged AD is a viable option for demand-driven biogas production (Hahn et al., 2014b). It is known that anaerobic filters are characterized by high process stability and enable the operation at very high OLRs, compared to single-staged AD (Ghosh, 1991; Tauseef et al., 2013). Yet, gas production profiles matching sudden increases and decreases in energy demand haven't been examined in literature.

Quick adaptions within a timeframe <15 min could open new marketing opportunities for plant operators and provide decentralized balancing services for grid integrity. The advantages of two-staged AD over traditional CSTRs could enhance the range of flexibility in a large scale. Therefore a major question is how fast the methane production can be adapted to sudden changes in demand and to what extent these adaptions are reproducible.

The ability to react might be influenced by substrate composition and controlled hydrolysis towards certain intermediates could improve the reaction times towards increased demand. It is therefore another focus of this research to examine intrinsic methane production kinetics of common intermediates of AD. A reliable prediction of methane provision is needed and the groundwork for future prediction models is laid out in this research. From the above the following research objectives are deduced:

- Determine the intrinsic kinetics of gas production in Anaerobic Filters for the most common intermediates found in hydrolysate,
- demonstrate the feasibility, reproducibility and the possible extent of demand-driven biogas production in Anaerobic Filters with respect to changing substrate composition and
- evaluate the process efficiency based on carbon fluxes to unfold effects resulting from changing operational conditions.

# 3 Kinetics of Biogasproduction in Anaerobic Filters

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## Abstract

This study investigates methane production kinetics from individual volatile fatty acids (VFA) in an Upflow Anaerobic Filter (AF). $1\,g_{COD}$ in the form of acetic (HAc), propionic (HPr) or butyric acid (HBu) was injected into the AF while operating at an organic loading rate ($OLR_{COD}$) of $3.5\,g\,L^{-1}\,d^{-1}$. A methodology is introduced to separate gas production of the baseload from the product formation of VFA degradation after the injection. The lag phase, fractional rate of gas production and half-life has been determined for the methane generation of the three VFAs. The half-lifes were in the order HAc<HBu<HPr showing a slower gas production from the C-3 acid than from the C-4 acid. The results can be used for prediction models for on-demand biogas production which has been identified as a vital approach to provide balancing power for a transforming energy market.

*Keywords:* on demand, two-staged, anaerobic digestion, volatile fatty acids, biogas

## 3.1 Introduction

The growing share of renewable energy worldwide demands for balancing power to compensate its fluctuating energy supply. On-demand biogas production and its subsequent electrification or its implementation in Power to Gas strategies has been identified by several researchers as a vital approach to provide short and long term as well as secondary and tertiary, positive and negative balancing power (Hahn et al., 2014; Mauky et al., 2015; Ahern et al., 2015; Linke et al., 2015). In micro gas grids the biogas production can avoid the necessity of large gas reservoirs by following demand curves and provide gas when needed. In particular two-staged anaerobic digestion has several advantages emphasizing its suitability to meet the requirements imposed by a transforming energy market and several special applications.

Two-staged AD systems are mostly realized by a continuously stirred tank reactor (CSTR) or leach-bed reactor (LBR) as acidification-stage in combination with a high rate methanation stage i.e. Upflow sludge blanket reactor (UASB) or anaerobic filter (AF) where the majority of biogas production takes place (Linke et al., 2015; Nizami and Murphy, 2010; Demirel and Yenigün, 2002). In the acidification stage a hydrolysate rich in organic acids and other intermediary products such as alcohols, sugars, and amino acids is produced. The methanation stage completes the AD and degrades the intermediary products to methane and carbon dioxide as its main products.

The advantages of two-staged AD are rooted in the spatial separation of the hydrolysis and acidogenesis from the acetogenesis and methanogenesis, thus facilitating an enhancement of AD by optimizing the environment for the microorganisms involved in the respective step (Fox and Pohland, 1994), enabling shorter retention times, higher loading rates and an overall more robust process (Nizami and Murphy, 2010; Demirel and Yenigün, 2002). Due to the selection towards faster growing microorganisms in the first stage, two-staged systems can accomplish higher substrate flexibility and withstand changes in operational control (Fox and Pohland, 1994; Lindner et al., 2015). Provided that the composition of the hydrolysate is known, the timed feeding to the AF could be used to produce gas when needed and cover peaks in demand.

From degradation kinetic studies it is known that individual organic substances show different rates of degradation (Wang et al., 1999; Aguilar et al., 1995; Öztürk, 1991; Rebac et al., 1999) resulting in different rates of product formation, in particular biogas production rates. However the rate of biogas production in this respect has not been a main focus of research yet. In anaerobic digestion for energy production (as opposed to waste treatment) the main product is methane and the time needed for its production from different substances is of special interest for demand driven biogas production.

Since the interactions of operational conditions are versatile and complex, this study is taking a step towards elucidating methane production kinetics from individual substances

in the AF. A detailed look into the gas production is provided after injecting VFAs into the AF while under normal operation. The aquired data can be used to develop prediction models of methane formation which is essential for demand driven biogas production using AFs. In the following a new methodology is described to determine the methane yield as well as its production kinetics from individual intermediates.

## 3.2 Methods

### 3.2.1 Experimental Setup

Three upflow Anaerobic Filters (AF) of identical construction with an internal free volume of $2801 \pm 16\,\mathrm{mL}$ (measured before inocculation) were used. The packed bed consists of Type HX-9 (Christian Stöhr GmbH & Co.KG) with a surface area of $940\,\mathrm{m^2 m^{-3}}$. Each of the three AF is equipped with two peristaltic pumps (Watson Marlow, 114FDC/DC) for feeding and recirculation, a combined pH/redox electrode (Endress & Hauser, CPS16D), temperature- (Endress & Hauser, Easytemp TMR31) and pressure sensor- (Endress & Hauser, Cerabar T PMC131), electrical heating (thermo GmbH) and gas cooling with integrated condensate reflux into the reactor. The produced biogas is measured volumetrically with a Milligascounter (Ritter, MGC10) and recalculated to STP conditions (0 °C and 1013.25 hPa). Gas samples are drawn via a multipositioning valve connecting through the three reactors to analyse the gas quality by gas chromatography (Inficon, 3000 $\mu$-GC) every 20 min for each reactor. An interpolated value of the gas quality is assigned to each count of the Milligascounter depending on the time difference between the count and the two gas quality measurements before and after the volume count.

Start up was completed using seed sludge from similar reactors in our laboratory and the reactors have been in operation for eight month. Prior to this experiment the packed beds have been removed, mixed and were then redistributed to each reactor to provide same conditions and to level effects of previous experiments. After mixing the packed beds, the reactors were operated for three weeks while increasing the $\mathrm{OLR_{COD}}$ to $15\,\mathrm{g\,L^{-1}\,d^{-1}}$ with COD degradation degrees of $94.2 \pm 1.5\,\%$ which was considered as stable operation.

The reactors were then operated at $38.8 \pm 0.5\,°\mathrm{C}$ and the $\mathrm{OLR_{COD}}$ was set to $3.5\,\mathrm{g\,L^{-1}\,d^{-1}}$ with hydraulic retention times (HRT) of 9.33 d over the whole course of the experiment. The applied OLR is below the actual capacity of the reactors, as mentioned above. For the base-feed a hydrolysate, produced from 1:1 maize and grass silage was used to provide essential macro- and micronutrients as well as simulating a base load for the reactors.LBRs were used to produce the hydrolysate similar to Chen et al. (2013). The hydrolysate has a total COD of $33\,574\,\mathrm{mg\,L^{-1}}$ and is composed by $3546\,\mathrm{mg\,L^{-1}}$ Acetic Acid, $381\,\mathrm{mg\,L^{-1}}$ Propionic Acid, $2213\,\mathrm{mg\,L^{-1}}$ n-Butyric Acid, $8053\,\mathrm{mg\,L^{-1}}$ Lactic Acid,

1878 mg $L^{-1}$ Ethanol, 1516 mg $L^{-1}$ Propanediol, 693 mg $L^{-1}$ Fructose and 513 mg $L^{-1}$ particulate matter. Concentrations are given on a COD-basis. The remaining share is not detected by the applied methods for single component analysis.

Twice a week (Tuesdays and Thursdays) a 10 mL dilution was injected into the lower part of the reactor via a septum, containing a COD of 1 g in the form of acetic acid (HAc), propionic acid (HPr) or butyric acid (HBu). Dilutions were prepared by calculating the theoretical COD using formula 3.1, weighing 10 $g_{COD}$ into a 100 mL flask and filling to the mark with distilled water.

$$COD_t = \frac{8(4x + y - 2z)}{12x + y + 16z}[g_{COD} g^{-1} C_x H_y O_z] \tag{3.1}$$

The chronological order of the injected VFA was randomised for each reactor. Each reactor received three replicates of each VFA. Data recording started three hours prior to injection and lasted for eight hours in total. The effluent was collected in plastic sample vials which were attached to the free overflow of the reactor and were exchanged hourly. The effluent samples were analyzed for soluble chemical oxygen demand (SCOD) to check for VFAs which were not degraded.

### 3.2.2 Analytical

SCOD is measured using Hach Lange cuvette test (LCK014) after filtering (0.2 µm) the samples with a syringe filter holder.

Volatile fatty acids are analyzed by adding 1 ml of 17 % ortho-phosphoric-acid and 1 ml of n-methyl-valeric-acid to 1 ml of sample. By adding distilled water a 1:10 dilution is prepared which is then transferred into vials for the autosampler of the Varian CP-3800 gas chromatograph. It operates with FID-detector and a WCOT fused silica column (50 m, 0.32 mm) at 60 °C for two minutes, then increasing the temperature to 150 °C with 30 °C $min^{-1}$ and finally increasing to 240 °C with 8 °C $min^{-1}$.The carrier gas is Helium.

Alcohols, sugars and lactic acid are analyzed with a Bischoff HPLC with RI-detector and a BioRad Aminex HPX-87H column (7.8 x 300 mm, part size 5 µm) operated at a flow rate of 0.6 ml $min^{-1}$,35 °C,6.0 MPa and 0.02 N $H_2SO_4$ eluent. The samples are prepared by adding 1 ml of 0.2 N $H_2SO_4$ to 5 ml of sample and filling up with bi-distilled water to 10 ml in order to yield a 1:2 dilution. It is then transferred into vials for the autosampler of the HPLC-system.

Gas quality is analyzed by the Inficon3000 $\mu$-GC with two columns. $H_2$, $N_2$, $O_2$ and $CH_4$ is analyzed by channel A with backflush-injector, fix and variable sample loop and a 5 Å Molsieve column (30 µm film, 320 µm diameter, 14 m length) and a Poraplot U pre column (30 µm film, 320 µm diameter, 2 m length). Backflush is activated after 10 s, Injection-time of the variable sample loop is set to 0 ms. Column A is operated at 2.0 bar and 80 °C. The carrier gas is Argon. Channel B, for the analysis of $CO_2$ and

$H_2S$, is equipped with a variable sample loop and a Pora Plot Q column (20 µm film, 320 µm diameter, 8 m length). Injection-time is set to 30 ms. It is operated at 1.4 bar and 50 °C. The carriergas is Helium. Both channels are connected to an individual TCD-sensor. Injector temperature as well as sample inlet for both channels are set to 60 °C. To purge the line from the sample point to the $\mu$-GC the internal pump operates for 45 s at approximately 15 ml min$^{-1}$ to 30 ml min$^{-1}$.

### 3.2.3 Analysis

To separate the gas production of the continuous base-feed from the gas production of VFA injection a baseline is determined for each injection. Therefore a linear regression using the method of least squares has been fitted to the cumulative gas production of methane and carbon dioxide starting from three hours prior to injection. Let $i \in \{CH_4, CO_2\}$, then the base gas production $B_i$ is given by

$$B_i(t) = \alpha_i + r_i \cdot t \tag{3.2}$$

where $t$ is the time[h], $t \in [-3, 0]$, $t_0 = 0$, $\alpha_i$ is the approximated intercept at $t_0$ and $r_i$ is the approximated rate of the respective gas production.

To determine the gas production resulting from the actual injection, the baseline determined by equation (3.2) is subtracted from the cumulative sum of the respective gas component, giving the gas yield curve of VFA degradation

$$\tilde{X}_i(t) = X_i(t) - B_i(t) \tag{3.3}$$

where $X_i(t)$ is the measured cumulative sum of gas produced at $t$, $t \in [-3, 5]$ and $\tilde{X}_i(t)$ is the resulting gas production from VFA degradation. To given $\tilde{X}_i(t)$ within the interval $t \in [0.05, 5]$, a modified Gompertz-function (equation (3.4)) is approximated via non-linear regression using the Gauss-Newton Algorithm. The classical Gompertz function was modified by adding a parameter $z_i$. This restriction and modification is done to take account of the initial gas ejection after VFA addition.

$$V_i(t) = a_i \cdot exp(-exp(1 - k_i \cdot (t - \lambda_i))) + z_i \tag{3.4}$$

with $V_i(t)$ for the approximated sum of produced gas from the injection at time $t$. $a_i + z_i$ [mL] is the final asymptotic gas volume, $z_i$ [mL] is the intercept at $t = 0.05$, $\lambda_i$ indicates the lag-phase [h] and $k_i$ [h$^{-1}$] is the fractional rate of gas production.

To find the point in time of maximum methane-production-rate equation (3.4) is rearranged to equation (3.5) using the parameters $a_{CH_4}$, $\lambda_{CH_4}$, $k_{CH_4}$ and $z_{CH_4}$. This is done to correct for the displacement caused by the initial gas ejection.

$$V_{CH_4}(t) = (a_{CH_4} + z_{CH_4}) \cdot exp(-exp(1 - k_{CH_4} \cdot (t - \lambda_{CH_4}))) \tag{3.5}$$

The first derivative of equation (3.5) will give the methane production rate over time and enables to find the point in time of maximum methane production rate ($t = t_{max_{CH_4}}$). The half-life of $CH_4$ production $t_{0.5_{CH_4}}$ is found in analogy to Wang et al. (2011) by

$$t_{0.5_{CH_4}} = \lambda_{CH_4} + \frac{1 - ln(ln(2))}{k_{CH_4}} \tag{3.6}$$

For a statistical analysis the fitted parameters $\lambda_{CH_4}$, $k_{CH_4}$ and the points in time for $t_{0.5_{CH_4}}$ and $t_{max_{CH_4}}$ of individual runs have been analyzed. These variables have been tested by an ANOVA and a consequent Tukey HSD test to determine differences between means.

## 3.3 Results and Discussion

The injection of VFAs into the AF showed a complex interaction of several mechanisms. Due to the injection a pH drop could be observed immediately after injection, causing an ejection of large quantities of gas. This rapid ejection is completed in the first three minutes after injection. For this reason the fitting of the Gompertz function is restricted to the data from 3 min after injection and parameter $z_i$ in formula (3.4) is introduced, which is reflecting the amount of gas ejected in the first three minutes. Due to the very low solubility of $CH_4$ compared to $CO_2$, assuming $z_i$ to be $CO_2$ only, is reasonable. Thus, the overall $CO_2$ production caused by the injection can be divided into two separate fractions: $CO_2$ ejected by the pH drop and $CO_2$ produced by the degradation of the injected VFA.

The fraction of $CO_2$ determined as parameter $z_i$ are $99.30 \pm 23.23$ mL, $77.02 \pm 13.46$ mL and $62.35 \pm 5.10$ mL for acetic, propionic and butyric acid respectively. The decrease in $z_i$ can be explained by the higher Mol-concentration added, the shorter the carbon chain of the VFA. Thus providing more $H^+$ for lowering the pH in the vicinity of the septum and causing a transition from $HCO_3^-$ to $CO_2$. The immediate $CO_2$ release leads to a lower $CH_4$ concentration of the gas composition (data not shown) directly after injection. The initial concentration levels are reached after 5 hours when the gas production from VFA degradation is finished and the head space of the reactors is purged by the produced gas. This is in contrast to the findings of Pind et al. (2003) who observed an increase in $CH_4$ concentration as well as in pH after injecting VFAs. The reason for the converse reactions may be attributed to the neutralization with NaOH of the VFAs by Pind et al. (2003). This way excess $OH^-$ after the degradation of the VFA is present to increase the pH and in turn providing a higher solubility for $CO_2$ and a transformation to $HCO_3^-$ in

the liquids.

In Figure 3.1 the gas production ($CO_2$ + $CH_4$) over time for the individual VFA is presented graphically. The data of six individual runs for each VFA are combined and shaded in gray. Solid lines depict the mean of the fitted Gompertz functions, while the dashed lines represent the fitted ± MAE (mean absolute error) for both gas components. For the fitted function, the corresponding gas composition after 5 h is depicted in the bar plot with indication of the two separate $CO_2$-fractions as discussed earlier.

Under the conditions presented acetic acid yields $298.94 \pm 19.72\,\mathrm{mL/g_{COD}}$ $CH_4$ and $348.22 \pm 16.93\,\mathrm{mL/g_{COD}}$ $CO_2$. Propionic acid yields $330.73 \pm 5.42\,\mathrm{mL/g_{COD}}$ $CH_4$ and $295.07 \pm 6.84\,\mathrm{mL/g_{COD}}$ $CO_2$ and butyric acid yields $313.80 \pm 8.62\,\mathrm{mL/g_{COD}}$ $CH_4$ and $247.42 \pm 10.24\,\mathrm{mL/g_{COD}}$ $CO_2$. Taking a look back and subtracting parameter $z_i$ from the total $CO_2$ produced, the methane content in the gas produced from the degradation of the VFA can be estimated, which is $54.7 \pm 3.0\,\%$, $60.3 \pm 1.9\,\%$ and $62.9 \pm 1.1\,\%$ for acetic, propionic and butyric acid respectively.

The total methane yield of acetic acid is off the theoretical value and showing a high standard deviation. To clarify the origin of the underestimation in methane yield for acetic acid a second set of injections has been done with half the original concentration yielding $351.26 \pm 8.76\,\mathrm{mL/g_{COD}}$ of methane, which fits to the expected value. Thus it was concluded that the initial $CO_2$ ejection results in a gas flow exceeding the capabilities of the used gas meter. However for the subsequent analysis on methane production rates this has only minor effects since the underestimation takes place in the short time frame of the first three minutes and does not affect the gas production rate thereafter.

The presented values are in good agreement with the stoichiometric considerations by Buswell and Mueller (1952) for complete conversion to the gas products or by Pavlostathis and Giraldo-Gomez (1991) using equations developed by McCarty (1972) who are taking cell synthesis with sufficient $NH_4^+$ supply into account. The slightly higher methane concentrations are possibly due to the free capacity to dissolve $CO_2$ back into the liquids, which has been emitted earlier by the pH drop. The total methane yield for propionic- and butyric acid are very close to the theoretical values and resemble the slightly lower methane yield for butyric acid compared to propionic acid as calculated by Pavlostathis and Giraldo-Gomez (1991). The second set of injections for HAc suggests a complete conversion to $CH_4$ and $CO_2$ according to Buswell and Mueller (1952), while the injections for HPr and HBu are closer to the calculations by Pavlostathis and Giraldo-Gomez (1991). This may be due to the low amount of HAc which is quickly used for cell maintenance by abundant HAc degrading organisms, while the HPr and HBu injections trigger cell synthesis and replication due to the increased supply for the respective microorganisms.

Although gas production kinetics are commonly described by the Gompertz function and showing a superior fit to the data compared to first order kinetics, it has mostly been used to assess complex substrates(Wang et al., 2011; Lo et al., 2010; Krishania et al.,

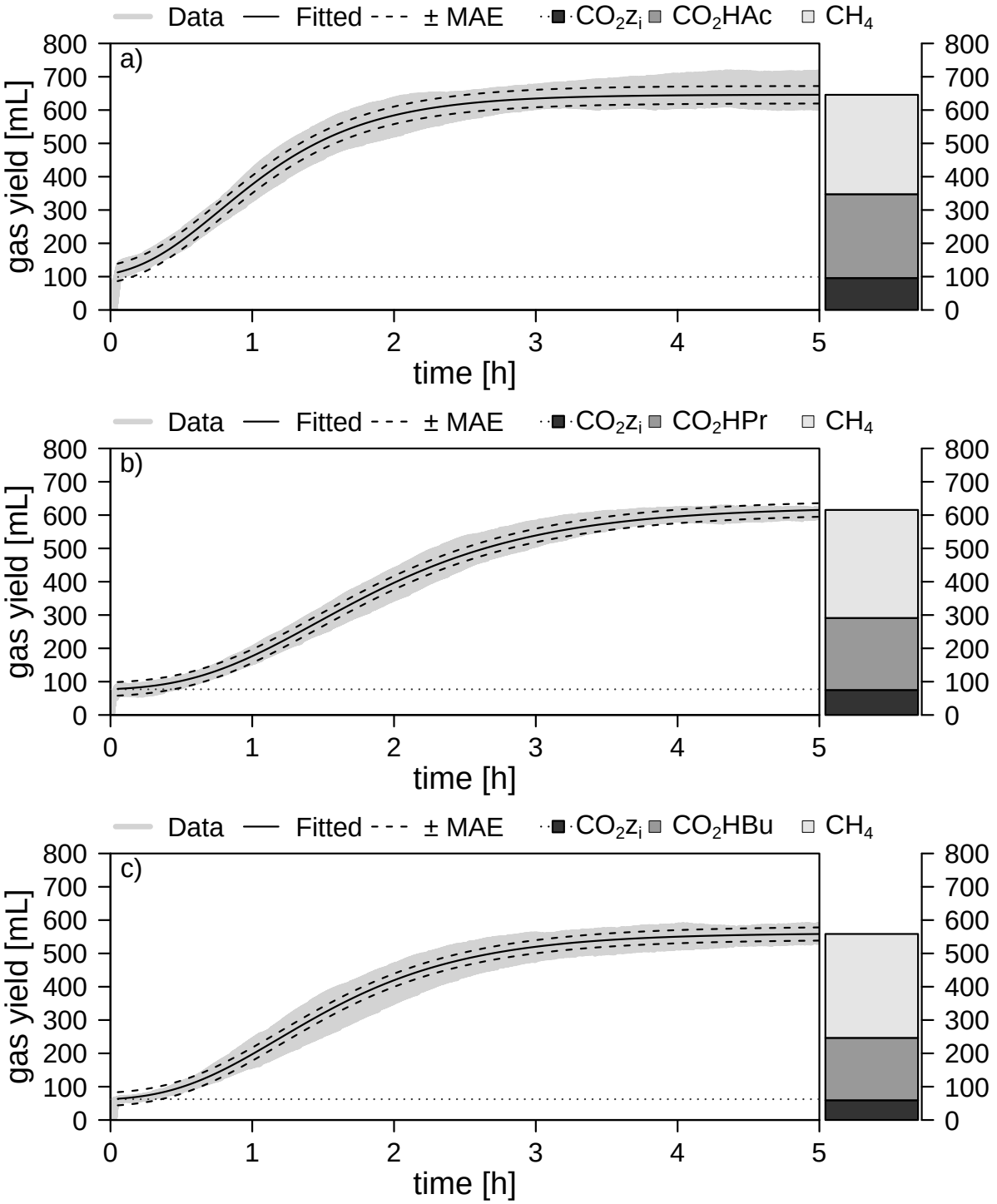


**Figure 3.1:** Cumulative gas production from the injection of 1 $g_{COD}$ as VFA: a)HAc , b)HPr and c)HBu. The barplot indicates the gas composition after 5 hours. $CO_2z_i$ is the share of $CO_2$ emitted from the liquids by pH drop (see text). $CO_2$HAc, $CO_2$HPr and $CO_2$HBu indicates the $CO_2$ production from degradation of the respective VFA, MAE = mean absolute error

**Table 3.1:** Overview of theoretical and experimental methane yields from the injection of 1 $g_{COD}$ as VFA and fitted parameters of the Gompertz function of equation (3.4) for methane. Significant differences in the mean are marked by different letters

| VFA | theoretical methane yield | | | $\lambda_{CH_4}$ | $k_{CH_4}$ | $t_{max_{CH_4}}$ | $t_{0.5_{CH_4}}$ |
|---|---|---|---|---|---|---|---|
| | Ref. A | Ref. B | this study | | | | |
| | [mL/$g_{COD}$] | [mL/$g_{COD}$] | [mL/$g_{COD}$] | [h] | [$h^{-1}$] | [h] | [h] |
| HAc | 350 | 334 | $351 \pm 9$* | $0.26 \pm 0.10^a$ | $1.77 \pm 0.26^a$ | $0.82 \pm 0.09^a$ | $1.05 \pm 0.12^a$ |
| HPr | 350 | 323 | $330 \pm 5$ | $0.70 \pm 0.12^b$ | $1.14 \pm 0.09^b$ | $1.54 \pm 0.11^b$ | $1.90 \pm 0.19^b$ |
| HBu | 350 | 309 | $313 \pm 9$ | $0.50 \pm 0.09^c$ | $1.35 \pm 0.12^c$ | $1.25 \pm 0.14^c$ | $1.42 \pm 0.14^c$ |

Ref. A: Buswell and Mueller (1952) ; Ref. B: Pavlostathis and Giraldo-Gomez (1991); * value taken from the second set of injections with 0.5 $g_{COD}$ per injection (see text); $\lambda_{CH_4}$ = lagphase; $k_{CH_4}$ = fractional rate of methane production; $t_{max_{CH_4}}$ = point in time of maximum $CH_4$ production; $t_{0.5_{CH_4}}$ half life of $CH_4$ production

2013; Kafle and Kim, 2013; Budiyono et al., 2014). To our knowledge the present study is evaluating gas production kinetics from single VFAs with the Gompertz function for the first time. By using equation (3.5) a good estimate of the actual $CH_4$ production over time is given. In this respect the parameters determined for the lag phase $\lambda_{CH_4}$ and fractional rate of methane production $k_{CH_4}$ are significantly different between all tested VFAs. Consequently the resulting half-life $t_{0.5_{CH_4}}$ and the point in time of maximum methane production rate $t_{max_{CH_4}}$ are also significantly different between HAc, HPr and HBu. A summary of estimated parameters and methane yields for the present study is given in Table 3.1.

In Figure 3.2 the change in the methane production rate over time is depicted with indicated $t_{max_{CH_4}}$. More specifically the determined $t_{max_{CH_4}}$ are $49 \pm 5$ min, $92 \pm 7$ min and $75 \pm 8$ min after the injection, for acetic, propionic and butyric acid respectively. The time necessary for complete degradation of the VFA and its intermediary products to $CO_2$ and $CH_4$ is in the order HAc<HBu<HPr. The slow conversion of HPr is most likely due to the thermodynamically unfavorable degradation pathway in anaerobic digestion which involves a number of unusual enzymes (Wang et al., 1999; Öztürk, 1991).

Another approach was used by Refai et al. (2014), using serum flasks in batch experiments to determine $CH_4$ production rates. The order of reported $CH_4$ production rates is in accordance with our results showing the same trend, although a significant difference between HPr and HBu could not be determined by Refai et al. (2014).

Batstone et al. (2003) determined parameters for the degradation of several VFAs in thermophilic AD, using substrate based Monod-type kinetics.The determined maximum uptake rates ($k_m$) and half-saturation constants ($K_s$) indicate a faster degradation for HBu than for HAc up to a certain substrate concentration and here HPr shows the slowest degradation as well. This parameter estimation is in agreement with other sources in the literature with different operational conditions (Pind et al., 2003; Wang et al., 1999;

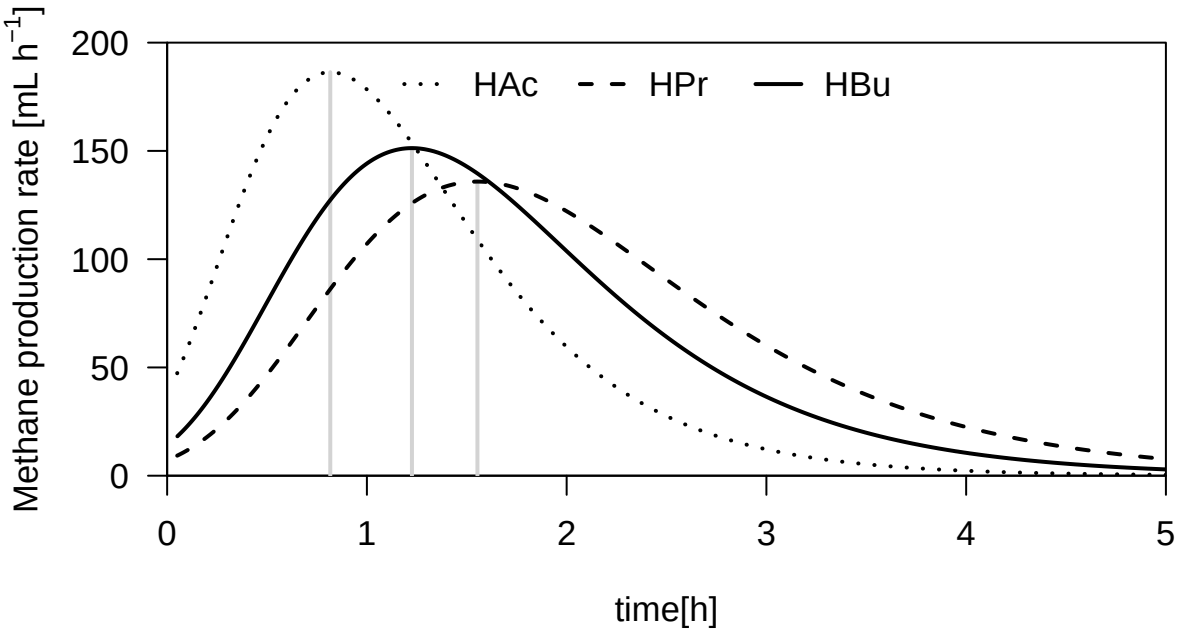


**Figure 3.2:** Methane production-rate of HAc,HPr and HBu with indicated time of maximum methane production rate $t_{max_{CH_4}}$

Rebac et al., 1999), giving the rate of degradation in the order HBu>HAc>HPr. As pointed out by Wang et al. (1999) HBu is degraded not only by beta-oxidation but also by isomerization producing iso-HBu. Additionally HBu degradation by beta-oxidation accumulates HAc which also needs to be oxidized to produce biogas. Although the degradation rate is in the order HBu>HAc>HPr, the rate of gas production will be HAc>HBu>HPr taking full degradation to the final products $CH_4$ and $CO_2$ into account. The apparent order is consistent throughout the reviewed literature, however the value of determined parameters can be very different for a single VFA as outlined by Nielsen et al. (2008), who emphasizes that the parameters are depending on biomass composition, reactor type, substrate, temperature and HRT.

## 3.4 Conclusions

In the presented study a method could be developed to measure gas production from single VFA injection into an Anaerobic Filter with high precision while under normal operation. Determined gas yields and concentrations are matching theoretical values of the tested VFAs. Acetic (HAc), Propionic (HPr) and Butyric Acid (HBu) show significantly different kinetic parameters between all VFAs. Furthermore it could be approved that the time needed for full degradation to $CO_2$ and $CH_4$ is faster for the C-4 Butyric Acid than for the C-3 Propionic Acid in Anaerobic Filters, more specifically the order is HAc<HBu<HPr.

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# 4 Intrinsic Gas Production Kinetics of Selected Intermediates in Anaerobic Filters for Demand Orientated Energy Supply

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## Abstract

In consequence of a growing share of solar and wind power, recent research on biogas production highlighted a need in demand-orientated, flexible gas production to provide grid services and enable a decentralized stabilization of the electricity infrastructure. Two-staged anaerobic digestion is particularly suitable for shifting the methane production into times of higher demand due to the spatio-temporal separation of hydrolysis and methanogenesis. To provide a basis for predicting gas production in an Anaerobic Filter, kinetic parameters of gas production have been determined experimentally in this study. A new methodology is used, enabling their determination during continuous operation. An order in methane production rate could be established by comparing the half lives of methane production. The order was, beginning with the fastest: acetic acid>ethanol>butyric acid>iso-butyric acid>valeric acid>propionic acid>1,2propanediol>lactic acid. However the mixture of a natural hydrolysate from the acidification tank appeared to produce methane faster than all single components tested.

*Keywords:* anaerobic digestion, VFA degradation, biogas, two stage, two phase

## 4.1 Introduction

In consequence of a growing share of solar and wind power, recent research on biogas production highlighted a need in demand-orientated, flexible gas production to provide grid services and enable a decentralized stabilization of the electricity infrastructure (Thrän et al., 2015; Hahn et al., 2014; Mauky et al., 2015). In the traditional CSTR biogas plant, treating energy crops or agricultural wastes, all four steps of anaerobic digestion (hydrolysis, acidogenesis, acetogenesis, methanogenesis) take place in one reaction chamber. The initial breakdown of lignocellulosic substrates to soluble products is described as the rate-limiting step in anaerobic digestion (AD) (Pavlostathis and Giraldo-Gomez, 1991), defining the boundaries in organic loading rate (OLR) and therefore the adaption to possible demand-curves. In a two-staged plant setup with acidification reactor and a subsequent high rate methanation reactor, the hydrolysis and methanogenesis are separated into two reaction vessels, as described extensively in the literature (Fox and Pohland, 1994; Demirel and Yenigün, 2002; Yu et al., 2002; Lehtomäki and Björnsson, 2006; Parawira et al., 2007; Zhang and He, 2014; Lindner et al., 2015, 2016). With this spatio-temporal separation, the two staged AD system is particularly suitable for shifting the methane production into times of higher demand and could open new marketing opportunities for plant operators.

To produce biogas on point in such a system a supporting model is desirable to provide future plant operators with reliable predictions for their gas production. Yet, current modeling approaches in the literature focus on degradation kinetics (Yang et al., 2015), rather than gas production kinetics. To provide a basis to predict gas production from hydrolysate in a high rate methanation reactor, kinetic parameters of gas production for individual intermediates have been determined experimentally in this study.

## 4.2 Material and Methods

### 4.2.1 Experimental Setup

The experiments were conducted in three Anaerobic Filters (AF) of identical construction. The mean free volume of the reactors was 2.8 L. A schematic representation of the reactors is given in Figure 4.1. The reactors were in operation for approximately two years after inoculation with separated liquid digestate from the research biogas plant "Unterer Lindenhof, University of Hohenheim". Previous experiments included the one described in Krümpel et al. (2016) as well as feeding similar substrates as the base feed used in this experiment at various organic loading rates (OLR) up to $OLR_{COD}$ of $20\,g\,L^{-1}\,d^{-1}$.

The preparation of the reactors for the experiment at hand included removal, mixing and redistribution of the packed beds to level effects of previous experiments. The react-

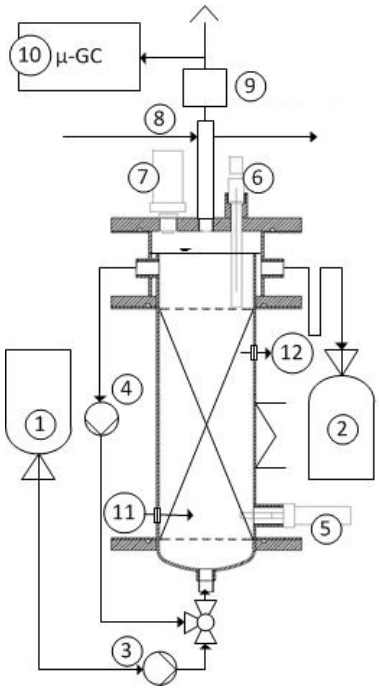


**Figure 4.1:** experimental setup of the anaerobic filter: (1)&(2) Hydrolysate & Effluent storage bags, (3)&(4) peristaltic pumps for feeding and recirculation, (5) Temperature Sensor, (6) pH-Redox-Temperature Sensor, (7) Pressure Sensor, (8) Gas Cooling System (Liebig-Cooler), (9)Milligascounter, (10) $\mu$-GC, (11) Injection-port (Septum),(12) Sample-port (Septum)

ors were operated approximately four hydraulic retention times (HRT), until stable gas production at an $OLR_{COD}$ of $10\,g\,L^{-1}\,d^{-1}$ was achieved before starting this experiment. The reactors were fed continuously via a peristaltic pump and were completely mixed by a recycle pump of the same type. Effluent was disposed via a free overflow and collected in plastic bags, which were exchanged daily.

The base feed was a hydrolysate produced from a 1:1 mixture of maize and grass-silage by leach-bed acidification reactors and contained $2.08\,g\,L^{-1}$ acetic acid, $0.47\,g\,L^{-1}$ propionic acid, $1.44\,g\,L^{-1}$ n-butyric acid, $0.01\,g\,L^{-1}$ iso-butyric acid, $0.03\,g\,L^{-1}$ n-valeric acid, $0.02\,g\,L^{-1}$ iso-valeric acid, $0.60\,g\,L^{-1}$ caproic acid, $3.90\,g\,L^{-1}$ lactic acid and $0.90\,g\,L^{-1}$ ethanol. The total chemical oxygen demand (COD) was $20.89\,g\,L^{-1}$. It was used to maintain a continuous operation and likely provided essential macro and micro-nutrients which have not been analyzed individually.

The feeding regime was constant throughout the experiment with a resulting $OLR_{COD}$ of $7.2\,g\,L^{-1}\,d^{-1}$ and a hydraulic retention time (HRT) of 2.9 d with mesophilic conditions at 38 °C.

In addition to the base feed, the reactors received a 10 mL injection, three times per week over a course of five weeks. Each injection was given through a septum in the lower part of the reactor (see (11) in Figure 4.1) and contained $1\,g_{COD}$ of a specific intermediate of AD. In total eight injections per substance were given. The order was randomized and

contained one of the following substances: acetic acid (HAc), propionic acid (HPr), lactic acid (HLac), butyric acid (HBu), ethanol (Eth) or 1,2propanediol (Prd).

After completing this set of injections a second set was prepared. This time it was not randomized. Each reactor received two injections of valeric acid (HVal), followed by three injections of the base-hydrolysate (Hyd) and finally receiving two injections of iso-butyric acid (i-HBu) over a course of three weeks. The acquired data of this set is reported, but not taken into account for the statistical analysis.

### 4.2.2 Analytical

Liquid samples from hydrolysate and samples taken from the reactor were analyzed by a combination of gas chromatography (GC) and high performance liquid chromatography (HPLC) to detect volatile fatty acids (VFAs), lactic acid, alcohols and sugars. The GC was equipped with a WCOT fused silica column and FID-detector with the carrier gas helium. The HPLC-system was operated with 0.02 N H2SO4 eluent, a Biorad Aminex HPX87-H column and a refractory index detector. COD was measured using Hach Lange cuvette tests (LCK 014).

Produced biogas volume was measured online by Ritter Milligascounters and was recalculated to STP conditions (0 °C, 1013.25 hPa). Gas quality was analyzed by gas chromatography every 20 minutes with a two-channel Inficon 3000 $\mu$-GC. Channel A was equipped with a Molsieve 5 Å column and channel B with a Poraplot Q column. Carrier gases were Argon and Helium respectively. Both channels were equipped with a thermal conductivity detector (TCD). To each count of the Milligascounter an interpolated value of the gas quality was assigned, depending on the time difference between the quality measurements and the volume-count.

The exact operating conditions of GC, HPLC and $\mu$-GC are described elsewhere (Krümpel et al., 2016).

### 4.2.3 Analysis

To determine wether tested intermediates show differences in methane production kinetics the following procedure was applied: A baseline of gas production was recorded during the three hours prior to injection. The baseline was a simple linear fit to the data of cumulative gas production. The baseline was then subtracted from the cumulative gas production over the time period from three hours prior injection to six hours after injection. The resulting curve represented the cumulative gas production resulting from the injection. In order to describe the observed gas production from the injected intermediate, a nonlinear fit using a modified Gompertz equation (after Wang et al. (2011)) was fitted to the resulting curve. The fit was accomplished by a method of least squares using the built in 'NL2SOL'-Algorithm of the free R-statistical software (R Development Core Team, 2008).

The Gompertz equation (4.1) given below includes three parameters, giving the final gas yield $a_i$ [mL], the lag phase $\lambda_i$ [h] and the fractional rate of gas production $k_i$ [$h^{-1}$] with $i \in \{CH_4, CO_2\}$.

$$V_i(t) = a_i \cdot exp(-exp(1 - k_i \cdot (t - \lambda_i))) \tag{4.1}$$

In case of acidic intermediates the above equation is extended by a fourth parameter $z_i$ [mL]. It is introduced to correct for initial $CO_2$ ejections due to lowered pH as described in Krümpel et al. (2016):

$$V_i(t) = a_i \cdot exp(-exp(1 - k_i \cdot (t - \lambda_i))) + z_i \tag{4.2}$$

The final yield in this case changes to $a_i + z_i$ and the corrected methane production kinetic is therefore (Krümpel et al., 2016):

$$\begin{aligned} V_{CH_4}(t) =& (a_{CH_4} + z_{CH_4}) \\ & \cdot exp(-exp(1 - k_{CH_4} \cdot (t - \lambda_{CH_4}))) \end{aligned} \tag{4.3}$$

A detailed description of this method is given in Krümpel et al. (2016). The parameters have been estimated for every injection. To establish an order of methane production rates the half lives for each injection were calculated with the following formula, according to Wang et al. (2011):

$$t_{0.5,CH_4} = \lambda_{CH_4} + \frac{1 - ln(ln(2))}{k_{CH_4}} \tag{4.4}$$

Where $t_{0.5,CH_4}$ is the point in time where half of the final methane yield is reached.

## 4.3 Results and Discussion

Due to technical difficulties one of the three reactors had to be taken out of analysis. Nevertheless the remaining injections were analyzed as described. After subtracting the baseline for each individual injection, all gas production curves showed a sigmoid shape which is shown in Figure 4.2 and 4.3.

The Gompertz function shown is constructed from the mean of parameter estimations for each substance and aggregates the resulting $CO_2$ and $CH_4$ production curves. After the injection of acidic intermediates, the pH dropped as expected. In consequence $CO_2$ was ejected as dissolved carbon in the form of $HCO_3^-$ transformed to gaseous $CO_2$. The amount of immediate gas ejection was highest for acetic acid with $40 \pm 11\,\%$ of total $CO_2$ release after injection. This share decreased roughly with lower molar concentration of the injected intermediate. The specific shares of immediate ejection in total $CO_2$

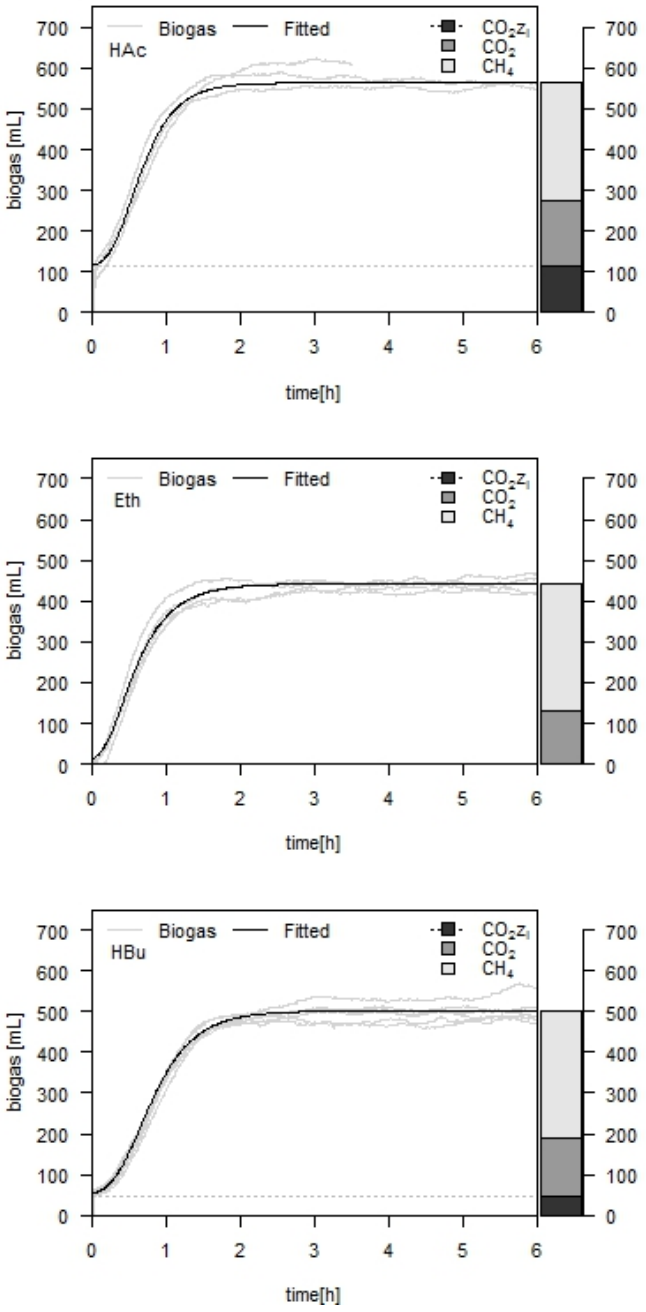


**Figure 4.2:** Cumulative gas production after subtraction of baseline with indicated gas composition and fitted Gompertz function. Each gray line represents the time course of gas production resulting from one single injection. The black line represents the mean of fitted Gompertz functions. Each injection contained 1 $g_{COD}$ (HAc = acetic acid, Eth = ethanol, HBu = butyric acid). $CO_{2,zi}$ indicates the share of $CO_2$ released by lowering pH in succession to injecting acidic intermediates (see text)

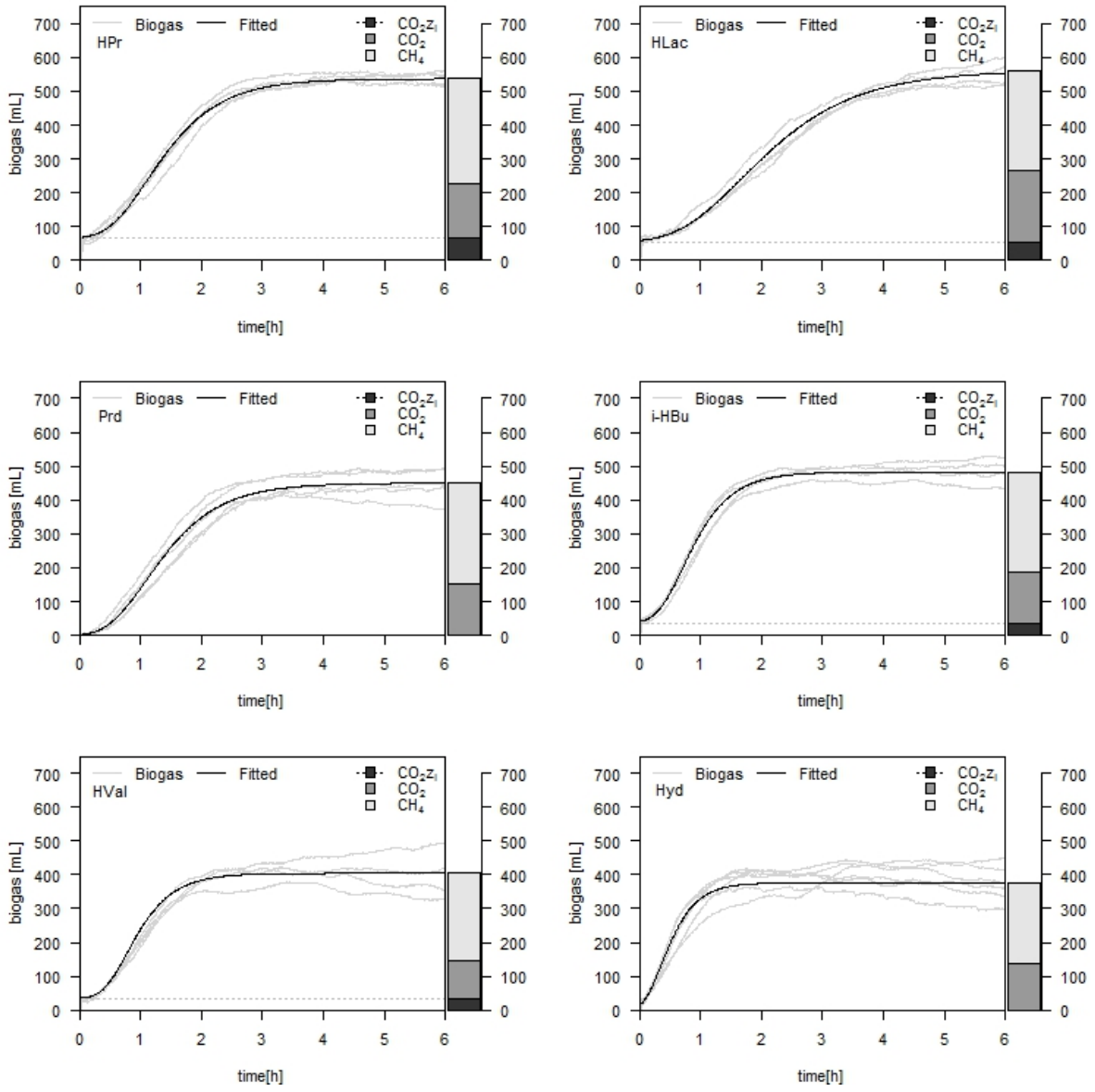


**Figure 4.3:** Cumulative gas production after subtraction of baseline with indicated gas composition and fitted Gompertz function. Each gray line represents the time course of gas production resulting from one single injection. The black line represents the mean of fitted Gompertz functions. Each injection contained 1 $g_{COD}$ (HPr = propionic acid, HLac = lactic acid, Prd = 1,2propanediol, iHBu = iso butyric acid, HVal = valeric acid, Hyd = hydrolysate). $CO_{2,zi}$ indicates the share of $CO_2$ released by lowering pH in succession to injecting acidic intermediates (see text)

release were $29 \pm 12\,\%$, $25 \pm 10\,\%$, $25 \pm 14\,\%$, $20 \pm 8\,\%$ and $20 \pm 9\,\%$ for HPr, HBu, HVal, HLac and i-HBu respectively. For the non-acidic intermediates, namely ethanol and 1,2-propanediol, this immediate gas ejection did not take place (see Figure 4.2 and 4.3). The gas composition indicated in the accompanying barplot was calculated as the mean of individual parameter estimations from each repetition. The split $CO_2$ bars illustrate the aforementioned gas ejection as $CO_{2,zi}$ and the remainder which was released from degradation of the injected intermediate. After the $CO_2$ ejection and the proceeding degradation of the intermediate, the pH recovers and free capacity to solubilize $CO_2$ emerges. These processes lead to a gas composition, which is off the stoichiometrical values predicted by Buswell and Mueller (1952). The methane yield however is not biased by this, since its solubility is very low compared to that of $CO_2$.

None of the injected intermediates reached the methane yield of 350 mL which is predicted by stoichiometry assuming complete degradation Khanal (2008). Under the conditions presented, the methane yields for the tested substances in the first set of injections were $287 \pm 13$ mL, $310 \pm 19$ mL, $311 \pm 33$ mL, $296 \pm 39$ mL, $312 \pm 16$ mL and $300 \pm 38$ mL for acetic acid, propionic acid, butyric acid, lactic acid, ethanol and 1,2-propanediol, respectively. In the second set of injections the yields were $298 \pm 30$ mL, $257 \pm 54$ mL and $239 \pm 37$ mL for iso-butyric acid, valeric acid and the hydrolysate, respectively.

The actual methane yield is affected by microbial growth during degradation as well as possible side routes with inorganic electron acceptors in the reactor liquid, resulting in other products, predominantly $H_2S$. The microbial growth is particularly pronounced in the studies of Yang et al. (2015), who studied methane formation kinetics of selected C1 to C5 organic acids in batch serum bottles. Here the methane yields were between $0.17\,g_{COD}/g_{COD}$ for lactic acid and $0.58\,g_{COD}/g_{COD}$ for acetic acid. This stresses the distinctive differences between batch and continuous operation or in other words between a growing micro organism (MO) population and a steady state population.

In Table 4.1

the estimated parameters of the Gompertz functions are summarized for $CH_4$. Herein significant differences are only indicated between substances of the first set of injections. By calculating the half lives of methane production according to formula (4.4) an order of methane production rates could be established and is depicted in Figure 4.4.

The order determined, beginning with the fastest is HAc >Eth >HBu >HiBu >HVal >HPr >Prd >HLac. At this point it should be noted that intermediates with an odd-numbered C chain are more likely to be found on the lower rates end. This is most commonly explained by the thermodynamically unfavorable conversion of propionic acid to acetate, $CO_2$ and hydrogen (Öztürk, 1991). Straight chain VFAs with odd-numbered C-atoms will mostly be degraded by beta oxidation and eventually yield propionic acid as an intermediate. Odd-numbered straight chain VFAs will therefore include this unfavorable pathway and are thus more likely to be found at the lower end of methane production

**Table 4.1:** Fitted parameters of the Gompertz function for methane (equation (4.1)&(4.3)) after injection of 1 $g_{COD}$ as well as calculated half lives (equation (4.4)). Significant differences in the mean are marked by different letters (only first set of injections))

| inter-mediate | methane yield [mL/$g_{COD}$] | $\lambda_{CH_4}$ [h] | $k_{CH_4}$ [$h^{-1}$] | $t_{0.5,CH_4}$ [h] |
|---|---|---|---|---|
| HAc | $287 \pm 13$ | $0.24 \pm 0.04$ | $3.41 \pm 0.60$ | $0.65 \pm 0.11^{c}$ |
| HPr | $310 \pm 19$ | $0.45 \pm 0.19$ | $1.39 \pm 0.26$ | $1.45 \pm 0.18^{b}$ |
| HBu | $311 \pm 33$ | $0.18 \pm 0.18$ | $2.26 \pm 0.75$ | $0.84 \pm 0.07^{c}$ |
| HLac | $296 \pm 39$ | $0.74 \pm 0.31$ | $1.01 \pm 0.27$ | $2.17 \pm 0.30^{a}$ |
| Eth | $312 \pm 16$ | $0.08 \pm 0.07$ | $2.53 \pm 0.69$ | $0.66 \pm 0.10^{c}$ |
| Prd | $300 \pm 38$ | $0.43 \pm 0.10$ | $1.43 \pm 0.31$ | $1.42 \pm 0.17^{b}$ |
| i-HBu | $298 \pm 30$ | $0.21 \pm 0.16$ | $2.05 \pm 0.53$ | $0.92 \pm 0.09$ |
| HVal | $257 \pm 54$ | $0.34 \pm 0.23$ | $2.20 \pm 1.02$ | $1.12 \pm 0.27$ |
| Hyd | $239 \pm 37$ | $0.03 \pm 0.03$ | $2.99 \pm 1.10$ | $0.55 \pm 0.16$ |

rates when compared to even numbered VFAs in their vicinity. Interestingly, degrading the C5 valeric acid produced methane faster than the C3 compounds. With the first degradation step of valeric acid, acetic acid and hydrogen is produced. Both can enter methanogenesis directly, thus accelerating $CH_4$ production compared to C3 compounds.

Within the block of C3 compounds the lactic acid is standing out as the slowest methane yielding substance tested. This is in accordance with Yang et al. (2015), who also found lactic acid to have the lowest microbial growth rate ($\mu_{max}$) of the tested C1 - C5 acids and showing a distinctive lag phase in the beginning of the experiment. Lactic acid was the only alpha-hydroxy acid and must therefore undergo different degradation pathways than straight chain VFAs. In Zellner et al. (1994) several degradation pathways of lactic acid are discussed. One pathway forming acetate only, another forming acetate, $CO_2$ and hydrogen and a third forming propionate, acetate and $CO_2$. Here defined mixed cultures were used and fed only with low sulfate substrates. It was concluded that at high lactate concentrations lactate degradation takes place via propionate, while at low lactate concentrations the degradation takes place via acetate. For clarification of the pathways taken in our system, two injections of lactic acid were accompanied by sampling the reactor liquid every 30 min through septum (12) in Figure 4.1. While propionate accumulation and degradation could be confirmed, no acetate but two other intermediates appeared in the HPLC-chromatogram which hadn't been identified. The absence of acetate accumulation is probably due to its rapid degradation and the low amounts formed. One of the unidentified intermediates was detectable in high concentrations 30 min after injection and degraded slowly until no lactic acid was detectable anymore. Their presence in the reactor lasted longer than that of propionic acid. Thus it is concluded that there must be an unreported degradation pathway for lactic acid in anaerobic digestion, which

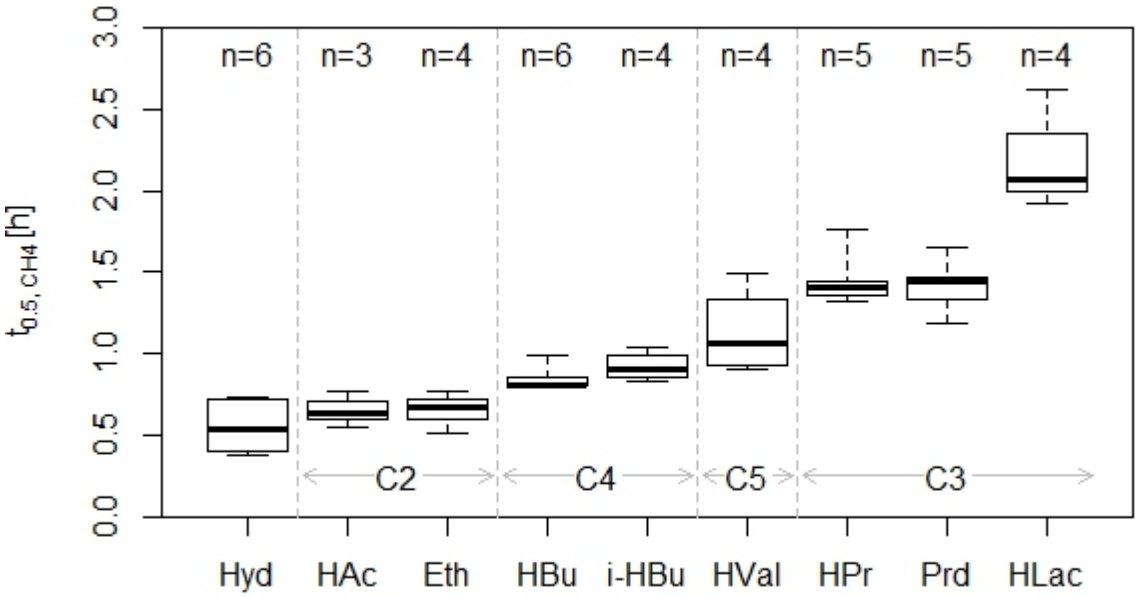


**Figure 4.4:** Half lives $t_{0.5,CH_4}$ (equation (4.4)) per injected substance; Hyd = hydrolysate, HAc = acetic acid, Eth = ethanol, HBu = butyric acid, i-HBu = iso-butyric acid, HVal = valeric acid, HPr = propionic acid, Prd = 1,2propanediol, HLac = lactic acid

was in this case more dominant. However since the mixed culture of the reactors at hand was not characterized, and the occurring intermediates could not be identified, no further statements can be made at this point.

Differences in the degradation kinetics between straight chain HBu and branched chain i-HBu have been reported by other researchers (Wang et al., 1999), with generally higher degradation rates for HBu than for i-HBu (Angelidaki and Ahring, 1995). The degradation in methanogenic cultures involves a reciprocal isomerization of i-HBu and HBu which has been studied extensively (Tholozan et al., 1988; Matthies and Schink, 1992; Angelidaki and Ahring, 1995; Wang et al., 1999). Both forms are eventually degraded via $\beta$-oxidation yielding HAc as an intermediate, which will then enter methanogenesis. In our study only slight tendencies pointing in the direction of a faster methane production rate for HBu could be observed when compared to i-HBu.

Comparing the results of this study with previous parameter determination,(Krümpel et al., 2016) it can be clearly stated that the order of methane production kinetics is the same for the three VFAs tested earlier: HAc>HBu>HPr. However, the absolute values of determined parameters are markedly different in the experiment at hand and calculated half lives were shortened by 38 %, 24 % and 41 % for acetic acid, propionic acid

and butyric acid, respectively, although the same digesters and methods were used. The main difference between the two experiments was the base-$OLR_{COD}$, with $3.5\,g\,L^{-1}\,d^{-1}$ in (Krümpel et al., 2016) and $7.2\,g\,L^{-1}\,d^{-1}$ in this study. Therefore the OLR applied has most likely a major impact on the results of this method. That is actually not surprising as the Monod Kinetic, the most widely used kinetic model for AD, expressed in terms of specific utilization rate $U$ is (Pavlostathis and Giraldo-Gomez, 1991):

$$U = \frac{kS}{K_S + S} \tag{4.5}$$

where: $k$ is the maximum specific utilization rate, $S$ is the concentration of the growth limiting substrate and $K_S$ is the half velocity coefficient (i.e. substrate concentration at one half maximum specific growth rate). This implies that the specific utilization rate is dependent on substrate concentration. It stands to reason that at a higher OLR the substrate concentrations are higher and therefore a faster methane production was observed in this experiment. Other factors influencing the level of estimated parameters in kinetic studies outlined by Nielsen et al. (2008) are a more adapted microorganism (MO) population, reactor type, substrate, temperature and HRT.

As a surprising result, hydrolysate produced methane faster than all other tested substances, even faster than acetic acid, which can be readily converted to $CH_4$ and $CO_2$ by acetoclastic methanogens. That is particularly unexpected because a major portion of the COD in the hydrolysate is comprised by lactic acid, the slowest methane forming intermediate in the trial. The hydrolysate has the lowest lag-phase ($\lambda_{CH_4}$) determined in the trials, but only the second highest fractional rate of gas production ($k_{CH_4}$) after HAc. The higher lag-phase of HAc could be governed by the higher acidity of the injected solution. When HAc was injected, the pH in the vicinity of the injection port may have been decreased to inhibitory levels for a short period of time and thus delaying the methane production. The half lives as the frame of reference could therefore be slightly biased by this effect.

However when providing several intermediates at once, they are degraded simultaneously by different specialized MOs releasing $H_2$, $CO_2$ and acetate. While acetate is evidently producing methane very quickly, hydrogenotrophic methanogenesis as a subsequent reaction to interspecies hydrogen transfer is possibly a main driving force for faster methane production in the case of hydrolysate. This is in agreement with Wiegant et al. (1986) who found the highest microbial specific growth rates of $0.330\,h^{-1}$ when $H_2/CO_2$ was the substrate compared to $0.040\,h^{-1}$, $0.030\,h^{-1}$ and $0.109\,h^{-1}$ for acetate, propionate and butyrate respectively. Following the argument that propionate degradation is a slow process, one could argue that due to the thermodynamically favorable conversion of $H_2$ and $CO_2$ to $CH_4$ hydrogenotrophic methanogenesis is a very fast conversion step. This argumentation has its flaws since it is known that thermodynamic constants such as

$\Delta G'^{\circ}$ do not state how fast equilibrium is reached in a certain reaction.Nelson and Cox (2013) This is rather the field of enzyme kinetics. But the higher growth rates found in the literature for thermodynamically favorable reactions lead to a denser population of MOs specifically performing the reaction of interest. This is supported by the fact that MO populations can adapt to substrates and increase the degradation rates as shown by several studies (Öztürk, 1991; Wang et al., 1999; Aguilar et al., 1995) for various VFAs. Thus the higher microbial biomass in the fixed bed performing the specific reaction are likely to be the underpinning reason for faster reactions.

## 4.4 Conclusion

Kinetic parameters of gas production can be determined during continuous operation of a high rate methanation reactor. The gas production rates of tested single intermediates can be ordered by determining their half lives of gas production. The order determined after injection of $1\,g_{COD}$, beginning with the fastest is HAc >Eth >HBu >HiBu >HVal >HPr >Prd >HLac. Odd-numbered C-chains of the specific intermediate lead to a slow gas production due to the thermodynamically unfavorable degradation of propionate in the process of complete breakdown to $CH_4$ and $CO_2$. However, the mixture used as a base feed (hydrolysate) has shown to have the fastest methane production rate, even though it was comprised of lactic acid to a large extent. This is due to multiple factors such as the parallel degradation of the compounds by different microorganisms, the inter-species hydrogen transfer and following hydrogenotrophic methanogenesis, the possibly perfectly adapted MO population to the hydrolysate or the low acidity compared to other acidic injections. The findings can be used for modeling and point towards the possibility to lump gas production kinetics of several intermediates into one simple parameter describing the hydrolysate. Further research on this is recommended. Especially with an eye on more extreme situations and fluctuating gas production over the course of the day to meet the requirements of flexible gas production.

## Acknowledgments

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# 5 Demand-Driven Biogas Production in Anaerobic Filters

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## Abstract

The growth in electricity generated from renewable energy sources is posing challenges for grid integrity and the need to counter balance the intermittent power supply by these sources. Biogas technology can offer such grid services by adapting the biogas production to the balancing demand and subsequent electricity production of the combined heat and power unit. Innovative plant designs such as two-staged anaerobic digestion could possibly adapt to imbalances in the electricity grid in shorter time frames than traditional completely mixed tank reactors (CSTR). Scope of this research paper is to demonstrate the feasibility of operating an anaerobic filter for highly flexible gas production. The repeatability of this type of operation is examined to demonstrate its predictability. Based on gas production profiles a measure of responsiveness is introduced to determine how quick adaptions in gas production can be made. Furthermore the influence of substrate composition is tested and finally a carbon balance is derived to evaluate the performance of this approach of operation. The results indicate that anaerobic filters are well suited for flexible gas production and show a good reproducibility under the conditions presented. From the comparison of rate of increases in methane production it was found that the composition of the two substrates tested, does not have an influence on the rate of increase in methane production. The pH affects the solubility of $CO_2$ and $HCO_3^-$ and therefore marks an important parameter determining the gas composition, especially under varying OLRs. The carbon balance showed that the largest output fraction is $CH_4$, followed by $CO_2$, inorganic carbon, dissolved organic carbon and particulate carbon with varying shares depending on the experimental phase.

*Keywords:* balancing power, anaerobic digestion, two-staged, flexibility, carbon balance

## 5.1 Introduction

As a result of greenhouse gas(GHG) emission reduction policies, the share of renewable energy (RE) in energy production has seen a considerable increase in the last decade. Within the EU-28, the share had increased by 84.4 % between 2003 and 2013. Electricity generated from renewable energy sources contributed to more than one quarter (25.4 %) of the EU-28's gross electricity consumption. The growth in electricity generated from renewable energy sources during the period 2003 to 2013 largely reflects an expansion in three renewable energy sources, namely, wind turbines, solar power and biomass (EU, 2016).

Scenarios developed by the EU predict a significant share of 55 % to 97 % renewable energy supply in gross energy production by th year 2050 (EU, 2012). As outlined by Steinke et al. (2013), the increase will mainly be provided by solar and wind, thereby posing challenges for grid stability and the need to counter balance the intermittent power supply by these sources.

Biogas technology not only reduces GHG emissions when compared to fossil fuel electricity production (Bacenetti et al., 2016), but can offer grid services by making electricity production of the combined heat and power unit (CHP) more flexible. With enhanced flexibility in energy production the biogas technology could play a significant role in smart grid applications such as the models presented in (Brouwer et al., 2015; Calvillo et al., 2016; Zamani et al., 2016), and help increase the revenues for future aggregated distributed energy resources or virtual power plants.

Shifting the electricity output into times of higher demand or alternatively lowering the power production to amend excess supply by other sources can be achieved by several adjustments to biogas technology. One frequently discussed option is to adapt the biogas production to the electricity demand, thereby opening new marketing options for plant operators (Thrän et al., 2015; Hahn et al., 2014b; Grim et al., 2015; Mulat et al., 2016).

Existing biogas plants in Germany are currently increasing gas storage volumes and expanding CHP capacity to achieve higher flexibility (Hahn et al., 2014a). Current reaction times are approximately one day and weekend shutdowns are not the usual practice at the moment. Improvements in flexibility could be achieved by altering the feeding regime to adapt gas production within several hours. Mauky et al. (2015) not only demonstrated the general feasibility but also determined savings in biogas storage capacity of 42 % to 45 % and found a more stable overall performance for continuously stirred tank reactors (CSTR) when operated in a flexible feeding regime.

Innovative plant designs such as two-staged anaerobic digestion (AD) with separ-

ated hydrolysis/acidogenesis and acetogenesis/methanogenesis could possibly adapt to imbalances in the electricity grid in even shorter time frames. Two-staged AD systems commonly consist of a CSTR or leach-bed reactor to produce a liquid that is rich in volatile fatty acids and alcohols. The acidification reactor (AR) is coupled to a methanation reactor (MR) where the soluble organics can be quickly degraded to yield biogas. The second stage is often put into practice as an anaerobic filter (AF), upflow sludge blanket reactor (UASB) or more sophisticated versions thereof (Lindner et al., 2015; Zielonka et al., 2010; Nizami and Murphy, 2010; Cysneiros et al., 2012; Tauseef et al., 2013; Chen et al., 2013). The advantages of two-staged setups found in literature are:

- Providing optimal conditions for the microorganism consortia taking part in the respective step of AD(Pohland and Ghosh, 1971; Cohen et al., 1979),
- thereby increasing turnover rates and enabling a reduction in total reactor volume (Cohen et al., 1979),
- disposal of slugde from the AR without loss of slowly growing methanogens (Cohen et al., 1979),
- fractionating the produced biogas (Muha et al., 2013) and enabling high methane concentrations in the MR and
- higher overall methane yields for specific substrates (Ghosh, 1987).

Further advantages of two-staged AD over single-staged AD, potentially improving demand-driven gas production, are that the hydrolysis as rate limiting step (Pavlostathis and Giraldo-Gomez, 1991) is decoupled from methanogenesis and thereby enables a shift of the gas production into times of higher demand. It could furthermore be shown that selectively producing certain intermediates in the AR is possible by controlling pH and redox-potential (Ren et al., 1997, 2007; Lindner et al., 2015), which may be used for a targeted production of a hydrolysate with a high methane production rate. It is also known that attached biomass reactors have a generally higher process stability and ruggedness towards shock loads and can operate at much higher organic loading rates (OLR) than CSTRs (Rajeshwari et al., 2000). Moreover, maintaining dormancy in the MR is also possible (Tauseef et al., 2013).

The scope of this research paper was to demonstrate the feasibility of operating an anaerobic filter for highly flexible gas production. The replicability of this type of operation was examined to demonstrate its predictability. Based on gas production profiles, a measure of responsiveness was introduced to determine whether and how rapidly adaptions to the production process are possible. Furthermore, the influence of substrate composition was tested and finally a carbon balance was derived to evaluate operation performance.

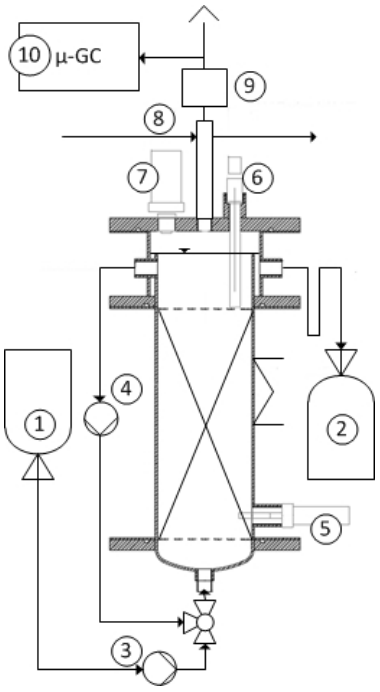


**Figure 5.1:** Experimental setup of the anaerobic filter: (1)&(2) hydrolysate & effluent storage bags, (3)&(4) peristaltic pumps for feeding and recirculation, (5) temperature sensor, (6) pH-temperature sensor, (7) pressure sensor, (8) gas cooling system (Liebig-cooler), (9)gas meter, (10) $\mu$-GC

## 5.2 Material and Methods

### 5.2.1 Experimental Setup

The experiments were executed in three anaerobic filters of identical construction, each comprising three compartments: the inlet, the packed bed and the headspace. The packed bed consisted of type HX-9 (Christian Stöhr GmbH & Co.KG) with a surface area of $940\,m^2m^{-3}$ and a porosity of 85 %, left the packed bed with a net void volume of $2070 \pm 15\,mL$. The total internal free volume was $2801 \pm 16\,mL$, measured before inoculation. A schematic representation is depicted in Figure 5.1.

The reactors were operated under mesophillic conditions with $37.5 \pm 1.3\,°C$. Substrate and effluent were either stored in inflatable plastic bags, connected to the inlet at the bottom, or to the free overflow at the top of the reactor. Each reactor was equipped with two peristaltic pumps (Watson Marlow, 114FDC/DC) for feeding and recycling.

Process parameters such as temperature, pH as well as produced gas volume were recorded online. The gas meters (Ritter, MGC10) gave a signal for every $\approx 3\,mL$ produced. Gas volume was corrected to STP conditions ($0\,°C$ and $1013.25\,hPa$). Gas quality was measured by gas chromatography (Inficon, 3000 $\mu$-GC) every 20 minutes for each reactor. To every signal of the gas meter an interpolated gas composition was assigned and was based on the time difference to the quality measurements before and after the signal. All

**Table 5.1:** Mean composition of Substrate A and B

| Component | Hydrolysate A | | Hydrolysate B | |
|---|---|---|---|---|
| | concentration [g L$^{-1}$] | share of TC [%] | concentration [g L$^{-1}$] | share of TC [%] |
| acetic acid | 6.214 | 22.67 | 3.089 | 10.00 |
| propionic acid | 2.943 | 13.05 | 0.451 | 1.78 |
| lactic acid | 1.833 | 6.69 | 9.612 | 31.13 |
| n-butyric acid | 4.656 | 23.15 | 0.537 | 2.37 |
| iso-butyric acid | 0.396 | 1.97 | 0.012 | 0.05 |
| n-valeric acid | 0.230 | 1.23 | 0.012 | 0.06 |
| iso-valeric acid | 0.085 | 0.46 | 0.015 | 0.07 |
| ethanol | 0.667 | 3.17 | 0.887 | 3.75 |
| 1,2propanediol | 0.316 | 1.37 | 0.587 | 2.25 |
| sucrose | 5.000 | 19.20 | | |
| glucose | 0.150 | 0.55 | | |
| fructose | 0.067 | 0.24 | | |
| particulate C | | 0.82 | | 0.73 |
| unidentified | | 5.44 | | 47.81 |
| total carbon (TC) | 10.967 | 100.00 | 12.353 | 100.00 |
| total nitrogen (TN) | 0.457 | | 1.214 | |
| $NH_4$-N | 0.384 | | 0.416 | |
| COD | 37.405 | | 36.462 | |

data was recorded in a mySQL database for further analysis.

Two different substrates as a feedstock were prepared. Substrate 'A' was an effluent collected from the same anaerobic filters, supplemented with a mix of volatile fatty acids and low amounts of alcohols to mimic a typical hydrolysate produced in leach-bed reactors. Sucrose was added to account for soluble substances which are usually not identified. Lactate has been found to exhibit slow methane production kinetics (Yang et al., 2015) and therefore lactate concentrations were deliberately reduced in this case and substituted by acetic and n-butyric acid. This was done in order to substantially differentiate substrate 'A' from substrate 'B'. Substrate 'B' was a hydrolysate produced from a 1:1 mixture of maize- and grass-silage in leach bed reactors at 60 °C. Table 5.1 gives an overview of the two substrates and their final chemical composition.

The components which could not be identified with the applied methodology are summarized as "unidentified". The share of $NH_4$-N in total nitrogen is ≈84 % and ≈34 % for 'A' and 'B', respectively. The remainder are most likely nitrogen-containing compounds such as amino-acids, proteins and alike and contribute to the unidentified carbon fraction. Additionally some sugars and sugar-alcohols could not be identified with the applied methodology. These unidentified components originate from the base-effluent for substrate 'A' or are naturally produced during hydrolysis of the maize and grass silage in

the case of substrate 'B'.

In addition to the two substrates, two OLR-modes of operation were tested. The first OLR-mode featured characteristics of a diurnal "double peak"-pattern of electricity-price development (Li and Flynn, 2004). These characteristics are defined by starting low and shooting up quickly in the early morning hours, continuing with a mid-day depression and a second increase in the later evening hours, to finally come back to a low level during the night. The second mode of operation featured two extreme increases in OLR per day for three hours to simulate peak-production. In the following these two OLR-modes of operation will be referred to as 'demand' and 'peak', respectively.

The AFs were operated in parallel, processing the substrate and OLR-mode combinations in the sequence: 'demand.A' → 'peak.A' → 'demand.B' → 'peak.B'. Each combination was planned to last four days. The corresponding mean hydraulic retention times (HRT), based on the measured free volume of the packing bed were 2.4 d and 3.6 d for 'demand' and 'peak', respectively. The true HRT is lower due to the establishment of the biofilm. A change of HRT due to biomass accumulation during the experiment is thought to be negligible since a fully grown biofilm was established beforehand.

Changes to the OLR during the day were realized by increasing or decreasing the pause-intervals between feeding events. Each feeding event lasted 15 seconds throughout the experiment and equaled the amount of ≈3.8 mL.

Hydrolysate samples were drawn once a week during bag filling. The prepared bags were stored at 4 °C for a maximum of one week. No gas production could be observed in the feed bags while storing. The low pH of the hydrolysate (between 3-4) hindered any significant gas development during the time when the bags were attached to the plants. The substrate and effluent bags connected to the AFs were exchanged daily and weight losses and gains were recorded with a weighing scale. Effluent samples were drawn each day after disconnecting and weighing the bags.

### 5.2.2 Analytical

Liquid samples were analyzed by gas chromatography (GC) to determine volatile fatty acids (VFA). The Shimadzu GC-2010plus was equipped with a SGE 25 m × 0.32 mm column with a Bonded Polyethylene Glycol BP21 0.25 µm film, a flame ionization detector and helium as a carrier gas. The temperature program started at 60 °C for two minutes, then the temperature increased to 150 °C at 30 °C $min^{-1}$ and finally to 240 °C at 8 °C $min^{-1}$. The injector temperature was set to 80 °C.

A supplementary analysis was performed to determine sugars, alcohols, formic acid and lactic acid with a Bischoff-HPLC system, equipped with a BioRad Aminex HPX-87H column (7.8 × 300 mm, part size 5 µm), refractory index detector and 0.02 N $H_2SO_4$ eluent. The system was operated at a flow rate of 0.6 mL $min^{-1}$, 35 °C and 6.0 MPa.

Total carbon (TC), inorganic carbon (IC) and total nitrogen (TN) were analyzed by the Analytik Jena multi N/C 2100 S (ChD) BU. Herein the sample is catalytically oxidized within an oxygen atmosphere at 850 °C. The gas stream is carried towards an nondispersive infrared adsorption (NDIR) detector to detect $CO_2$. An inline electrochemical detector was used to detect nitric oxides. The resulting area under the curve is correlated to the TC or TN content, respectively. A second injection into another reaction vessel was performed to analyze IC by acidifying the sample with $H_3PO_4$ and purging the $CO_2$ towards the detector. The total organic carbon (TOC) was calculated by the difference between TC and IC. By filtering with a 0.25 µm syringe filter holder prior to analysis, the dissolved carbon (DC) and dissolved organic carbon (DOC) were determined with the same procedure. Chemical oxygen demand (COD) was measured using cuvette tests (Hach Lange, LCK014).

$NH_4$-N was analyzed by using the automatic distillation system Gerhardt Vapodest50s. Herein the ammonium was distilled as ammonia by adding 32 %-NaOH to a 5 mL sample and was collected in a 2 % boric acid ($H_3BO_3$) condenser. After completed distillation the pH of the condenser liquid is titrated back to its initial pH with 0.1 molar HCl. The HCl consumption was correlated to the ammonium content of the sample. The Vapodest50s was set to 90 % steam power and 5 min distillation time. Added reactants were 74 mL water, 86 mL NaOH and 86 mL $H_3BO_3$.

Gas quality was analyzed by the Inficon3000 $\mu$-GC with two separate analytical channels. Both channels were equipped with a thermal conductivity detector (TCD). Channel A was characterized by having a variable and fixed sample loop, 5 Å molsieve column (30 µm film, 320 µm diameter, 14 m length), a Poraplot U pre column (30 µm film, 320 µm diameter, 2 m length) and argon as a carrier gas to analyze concentrations of $H_2$, $N_2$, $O_2$ and $CH_4$. Column A was operated at 2.0 bar and 80 °C. Backflush was activated after 10 s and the injection time of the variable sample loop was set to 0 s. Channel B, for the analysis of $CO_2$ and $H_2S$, was equipped with a Pora Plot Q column (20 µm film, 320 µm diameter, 8 m length) operating at 1.4 bar and 50 °C. The injection-time was set to 30 ms and the carrier gas was helium.

### 5.2.3 Analysis

The recorded gas production data were compiled into 20 min intervals for each day. The mean gas flow and the standard deviation (SD) per interval was calculated over all three reactors and four days of operation, equaling n=12 days per substrate×OLR combination.

As a measure of responsiveness to changes in OLR, the rate of increase in methane production ($r_{CH_4}$) was calculated for each 20 minute interval. This was done by using the mean of the produced methane in each interval and determining the slope by linear regression in a moving window of three consecutive intervals.

Intra-daily variations were calculated as the span of measured values during one day according to equation 5.1:

$$\Delta(i)_d = max(i)_d - min(i)_d, \tag{5.1}$$

Where i is the value measured, i.e. pH on day d.

The carbon balance was calculated on a daily basis with the start and end point marked by the exchange of feed and effluent bags. The produced gas was assigned to the same period and was recalculated to the respective carbon mass. The analyzed concentrations of individual components in the process-liquids by GC and HPLC were also recalculated to their respective carbon mass. In combination with the data from the carbon analyzer, the following carbon pools could be established: TC, IC, particulate carbon (pC), unidentified dissolved organic carbon (uDOC) and carbon by component i ($Cc_i$). Wherein the sum of $Cc_i$ and uDOC gave DOC, DOC + pC gave TOC and TOC + IC gave TC. The degradation degree was calculated based on TOC by equation 5.2 to evaluate the process performance:

$$\eta_{TOC} = \frac{TOC_{in}[\mathrm{g/d}] - TOC_{out}[\mathrm{g/d}]}{TOC_{in}[\mathrm{g/d}]} \times 100\%, \tag{5.2}$$

where $\eta_{TOC}$ is the degradation degree, $TOC_{in}$ is the TOC fed into the AF and $TOC_{out}$ is the TOC leaving the system via the effluent during the same time period.

All carbon fractions have been directly measured with the exception of dissolved methane. The solubility of methane at atmospheric pressure is extremely low and therefore thought to be negligible.

## 5.3 Results

### 5.3.1 Flexible Gas Production

Figure 5.2 depicts the phases 'demand.A' and 'demand.B', individual methane as well as total biogas production. The mean SD per 20 min interval for biogas production were as low as ±12.6 mL for 'A', and ±13.1 mL for 'B'. The according mean SD for methane production were ±7.5 mL and ±8.6 mL, respectively. The highest deviations were reached within the hour after the steep increase in OLR during the morning hours, with a maximum SD of ±27.4 mL and ±28.9 mL. This demonstrated an excellent replicability, considering that three independent reactors were involved in the analysis.

Notably, the gas production followed the changes in OLR immediately and every increase or decrease in OLR found its expression in the gas production profile. The two instances per day, when the $OLR_{COD}$ had increased to its highest value of $\approx 20\,\mathrm{g\,L^{-1}\,d^{-1}}$, are worth taking a closer look. Although the applied OLR was the same for both instances,

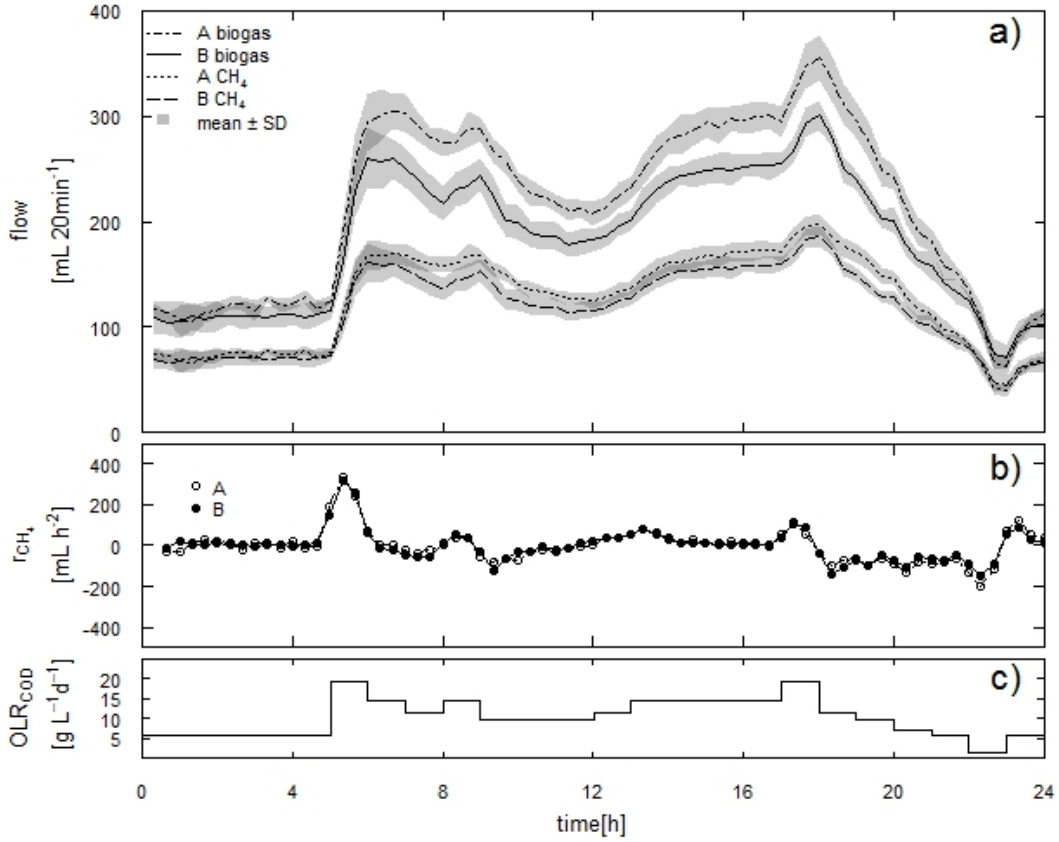


**Figure 5.2:** Compiled data of OLR-mode 'demand' for both substrates A and B with daily gas and methane production: a), rate of increase in methane production $r_{CH_4}$: b), applied OLR: c)

the peak flow of methane was 16.5% and 14.8% higher in the latter instance, for 'A' and 'B' respectively. This is due to the higher OLR applied in the hours before and therefore higher concentrations of intermediates were still present in the system.

The rate of increase in methane production ($r_{CH_4}$) was derived in order to determine how rapidly the gas production can be adapted. The maximum $r_{CH_4}$ achieved for 'demand.A' and 'demand.B' was 336.56 mL h$^{-2}$ and 319.78 mL h$^{-2}$, respectively. Figure 5.2 a) and b) outline that the shut down of gas production was able to be controlled and the slope was held stable by a stepwise decrease in the OLR, as demonstrated in the later evening hours.

The second OLR-mode of operation, namely phases 'peak.A' and 'peak.B' are depicted in Figure 5.3. These phases represent extreme increases to OLR to comply with short term balancing power. During peak production, the OLR was increased by a factor of 5.33 over three hours, twice a day. Here, the mean SD per 20 min interval for biogas production was recorded at ±12.2 mL for 'A', and ±8.5 mL for 'B'. The according mean SD for methane production were ±7.6 mL and ±5.5 mL, respectively. Again, these results demonstrate excellent replicability.

The rates of increase of methane production in phases 'peak.A' and 'peak.B' reached higher maximum values with 475.92 mL h$^{-2}$ and 456.69 mL h$^{-2}$. In this case, $r_{CH_4}$ had most likely neared the maximum possible increase in gas production, corresponding to the maximum rate of acetoclastic methanogenesis, which is generally considered to be the rate-limiting step, when treating liquid substrates (Pavlostathis and Giraldo-Gomez, 1991). Comparably, the minimum slope was determined as −358.70 mL h$^{-2}$ and −407.02 mL h$^{-2}$, enabling quick shut down of gas production. These values do not represent the full potential of a shut down as feeding had continued at a low level during that time.

Comparing the methane production kinetics, no significant differences were observed between the two substrates. By plotting $r_{CH_4}$ for 'A' and 'B' against each other, linear slopes of 1.02 and 0.93 were obtained for 'demand' and 'peak', respectively. These indicated that the compositions of substrate 'A' and 'B' did not largely influence methane production kinetics. These results support the rate-limiting step approach, which is often highlighted in literature and marks methanogenesis as the rate-limiting step when no lignocellulosic substrates are involved. In a recent, yet unpublished work, Krümpel et.al determined intrinsic methane production kinetics of specific intermediates as well as for a complete hydrolysate, indicating that the mixture produces gas faster than every intermediate as a single injection. This was attributed to the kinetically saturated degradation pathways for single substances vs multiple degradation pathways for the whole bandwidth of substances, thus providing high concentrations of precursors to methanogenesis and again marking it as the rate limiting step. These findings, together with this study, suggest that a controlled hydrolysis towards specific intermediates is not necessary to achieve faster gas production.

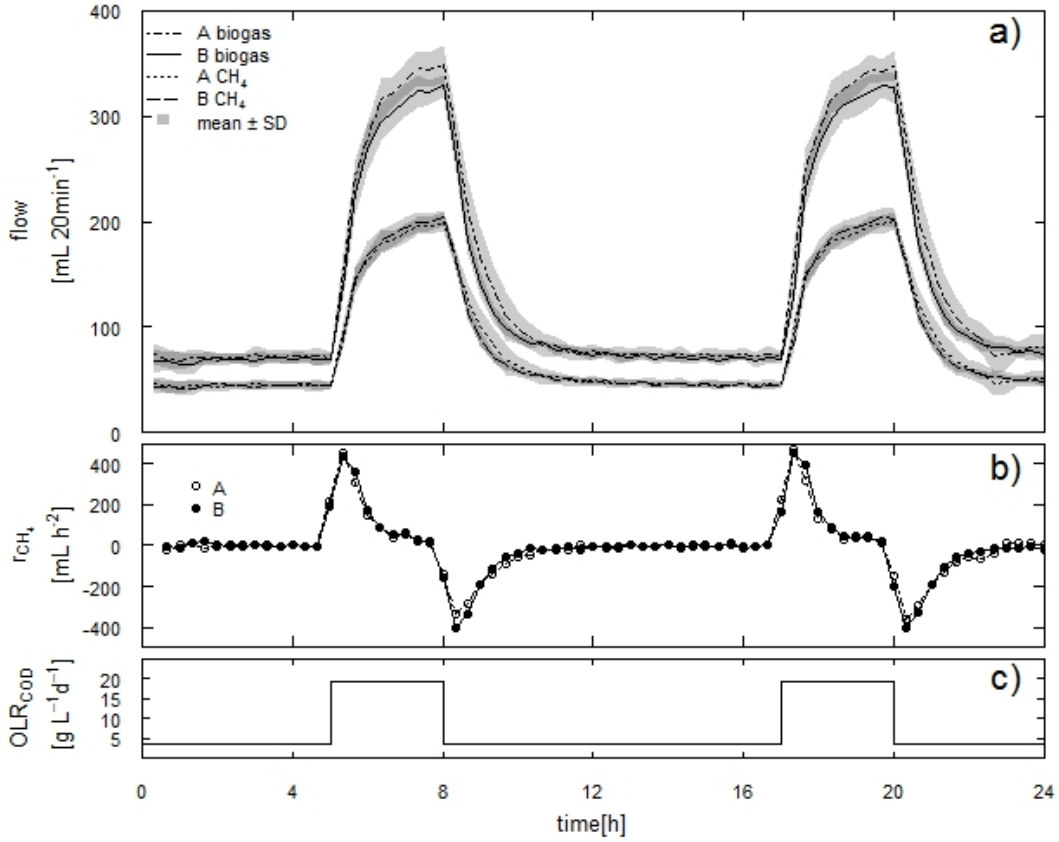


**Figure 5.3:** Compiled data of OLR-mode 'peak' for both substrates A and B with daily biogas and methane production: a), rate of increase in methane production $r_{CH_4}$: b), applied OLR: c)

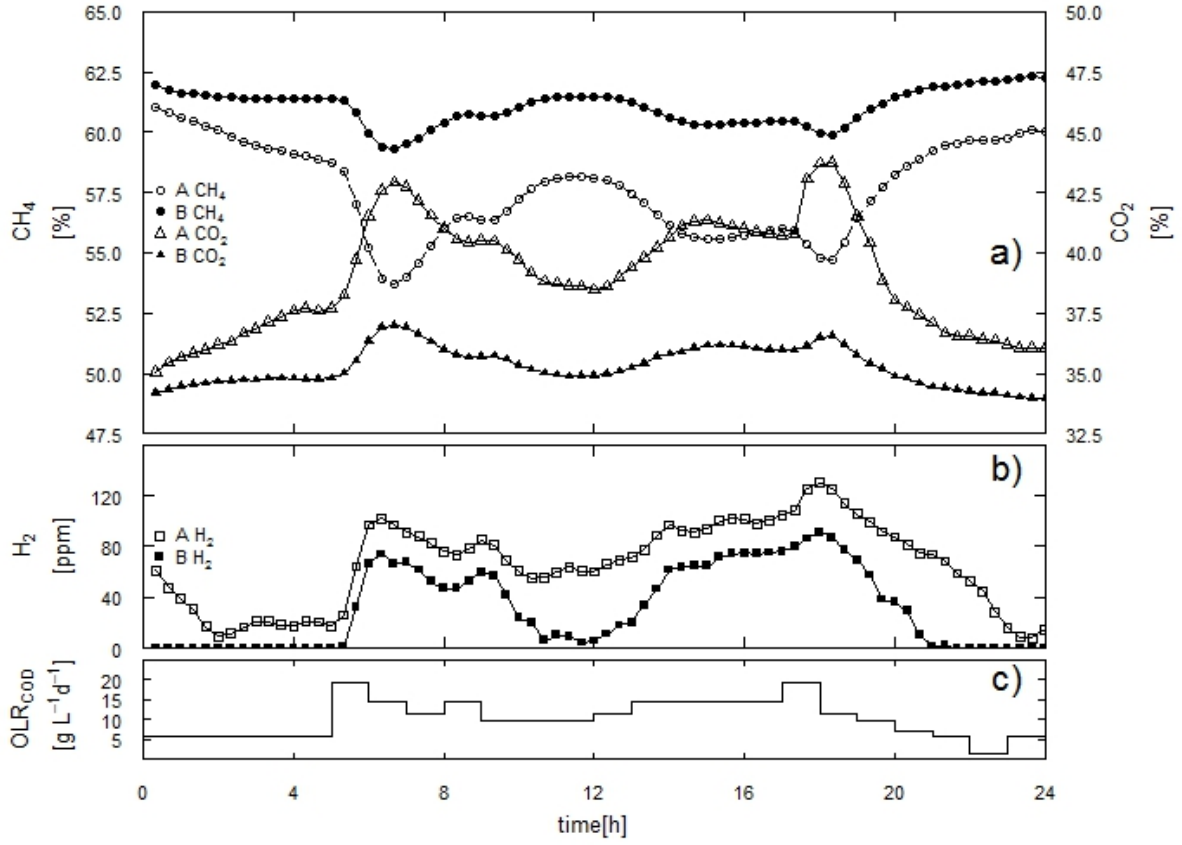


**Figure 5.4:** OLR-mode 'demand' for both substrates 'A' and 'B' with mean $CH_4$ and $CO_2$ concentrations: a), $H_2$ concentrations: b) and applied OLR: c)

### 5.3.2 Variability of Gas Concentrations

Not only the produced gas volume but also the gas composition had varied with changing OLR. During times of increased OLR, elevated $CO_2$ concentrations could be measured while $CH_4$ concentrations reacted conversely. A mean $CH_4$ concentration was recorded at $57.56 \pm 2.03$ % for 'demand.A'.

Intra-daily variations were in the order of $6.16 \pm 0.99$ %-points, excluding day one of 'demand.A', where the intra-daily variation was highest with 10.29 %-points. In phase 'demand.B', the $CH_4$ concentrations were generally higher with a recorded $61.05 \pm 0.85$ % and showed smaller intra-daily variations to 'demand.A' of $3.00 \pm 0.77$ %-points. The course of mean gas concentrations during the phases 'demand.A' and 'demand.B' is shown in Figure 5.4. The gas concentrations during 'peak.A' and 'peak.B' responded similarly to increasing OLR. The mean $CH_4$ concentrations were $59.02 \pm 2.39$ % and $61.74 \pm 1.06$ % for 'A' and 'B', respectively. The according intra-daily variations were slightly higher than those in the OLR-mode demand, with $7.26 \pm 2.05$ %-points and $3.58 \pm 0.37$ %-points. The course of mean gas concentrations during the phases 'peak.A' and 'peak.B' is shown in

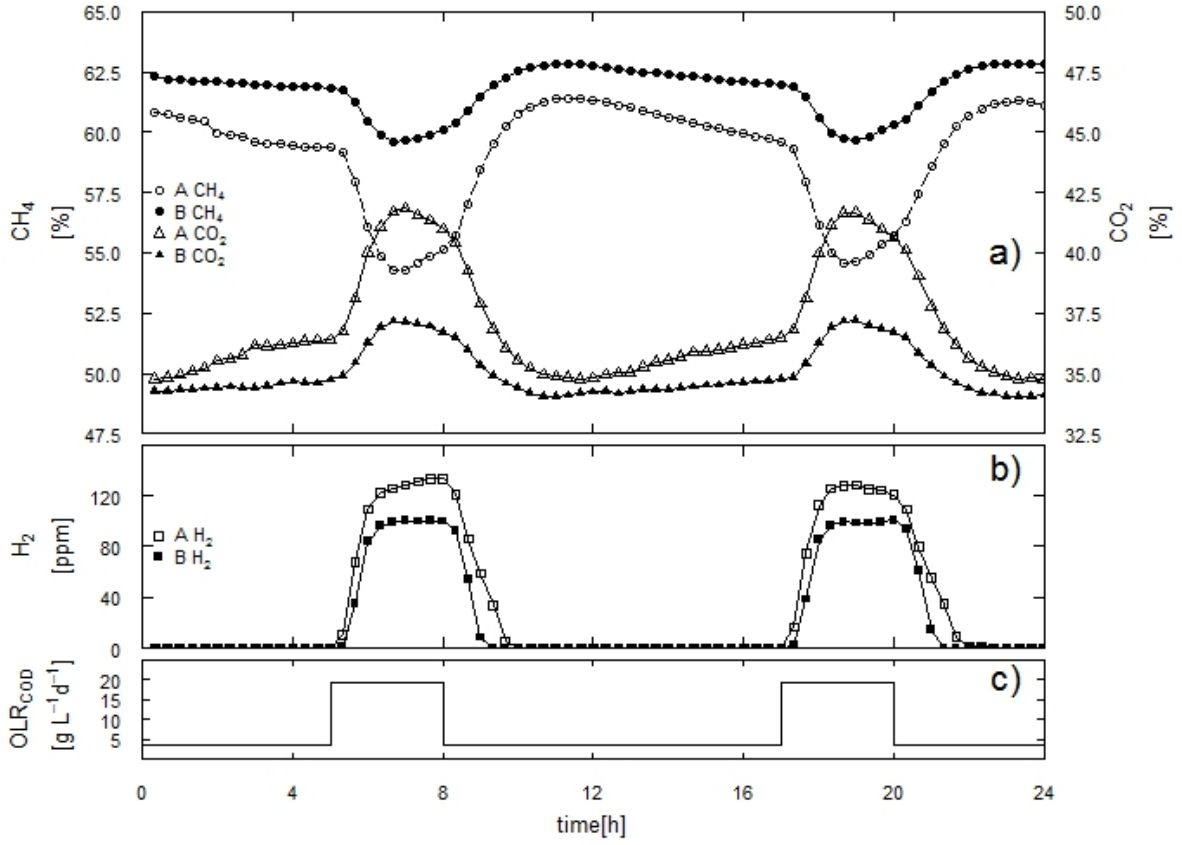


**Figure 5.5:** OLR-mode 'peak' for both substrates 'A' and 'B' with mean $CH_4$ and $CO_2$ concentrations: a), $H_2$ concentrations: b) and applied OLR: c)

Figure 5.5.

A large influence on gas concentrations can be attributed to the pH. The pH at the beginning of the experiment was recorded at $7.46 \pm 0.05$ for the three reactors and decreased to $7.22 \pm 0.05$ at the end of phase 'demand.A'. During that phase, the pH had varied in response to OLR, decreasing during times of high OLR and increasing again during lower OLRs. The intra-daily variation was $0.13 \pm 0.02$ units in pH. Continuing to feed 'A', the pH further decreased to $7.05 \pm 0.02$ and stabilizing at this value in phase 'peak.A' for all three reactors. The intra-daily variation here was similar to 'demand.A' with $0.13 \pm 0.04$ units in pH. By changing the feed to 'B', the pH increased from $7.05 \pm 0.01$ to $7.35 \pm 0.05$ during 'demand.B' and further increased to $7.43 \pm 0.07$ during 'peak.B'. Intra-daily variations in pH for phase 'demand.B' and 'peak.B' were $0.13 \pm 0.04$ and $0.08 \pm 0.01$ units in pH, respectively. Overall, the pH never reached critically low values, which therefore verified biological stability, even under such challenging conditions. For substrate 'A', the intra-daily variation of pH was a reliable predictor for intra-daily variation of $CH_4$ concentration with a $\Delta CH_4 = 0.429 + 49.93 \times \Delta pH$ and $R^2 = 0.842$. This was not the

case for substrate 'B' with $\Delta CH_4 = 3.13 + 3.04 \times \Delta pH$ and $R^2 = 0.036$.

The reasoning behind the different responses to a changing pH was found in the buffer system acting in anaerobic digestion, which is mainly the carbonate buffer. This system could be labeled as open buffer system since a decreasing pH causes $CO_2$ to leave the system via the gas stream. Thus it is not available anymore for buffering.

In contrast, the $NH_3$ / $NH_4^+$ buffer could be termed as closed buffer, since both species will remain in the liquid phase of the system. Taking a look at the substrate composition, substrate 'B' featured higher nitrogen contents, whether it is in the form of proteins or ammonia. In the pH range where the experiments were conducted, all nitrogen contributing to the $NH_3$ / $NH_4^+$ buffer would be in the state of $NH_4^+$. By introducing nitrogenous compounds through the feed stream, accumulated VFAs were immediately neutralized and reduced the extent of action by the carbonate buffer and thus smaller variations in gas composition were observed. Therefore the $NH_3$ / $NH_4^+$ buffer could be valued higher as it prevented extreme variations in gas quality. While nitrogenous compounds help to stabilize the gas quality under these conditions, it must be assured that nitrogen levels will not reach inhibitory levels.

Similarly to $CO_2$ and $CH_4$, the measured mean $H_2$ concentration varied with changing OLR. A maximum in $H_2$ concentration was reached during the second increase in OLR during the evening hours with 130 ppm and 90 ppm for 'demand.A' and 'demand.B', respectively. During 'peak.A' and 'peak.B', $H_2$ could only be measured at increased OLR and reached 130 ppm and 100 ppm.

About one third of total methane production in anaerobic treatment is attributed to hydrogenotrophic methanogenesis (John S. Jeris, 1965). However, this share changes with substrate type and was found to be the dominant pathway for flexible gas production (Mulat et al., 2016). In an anaerobic filter of a two-staged AD system a share of hydrogenotrophic methanogenesis in the range of 28 % to 44 % of total methane production was found and it was positively correlated to OLR (Gehring et al., 2015). Due to the sensitive response of $H_2$ concentrations to changing OLR shown here (see Figure 5.4 and 5.5) and the apparent increased share of hydrogenotrophic methanogenesis in flexibly fed AD systems, it may serve as a controlling measure to prevent overloading.

### 5.3.3 Carbon Balance

All reactors performed in a stable manner throughout the experiment. The TOC degradation degree was high and ranged between 92.35 % and 95.42 %. During the OLR-mode 'demand', the degradation degree was the lowest for both substrates, as it reflects the higher mean OLR. Herein the degradation degree for 'A' was $92.84 \pm 1.11$ %, whereas 'B' was $93.63 \pm 0.59$ %. Applying the OLR-mode 'peak', the degradation degree for 'A' and 'B' were $94.39 \pm 0.68$ % and $94.17 \pm 0.39$ %, respectively. A graphical representation of

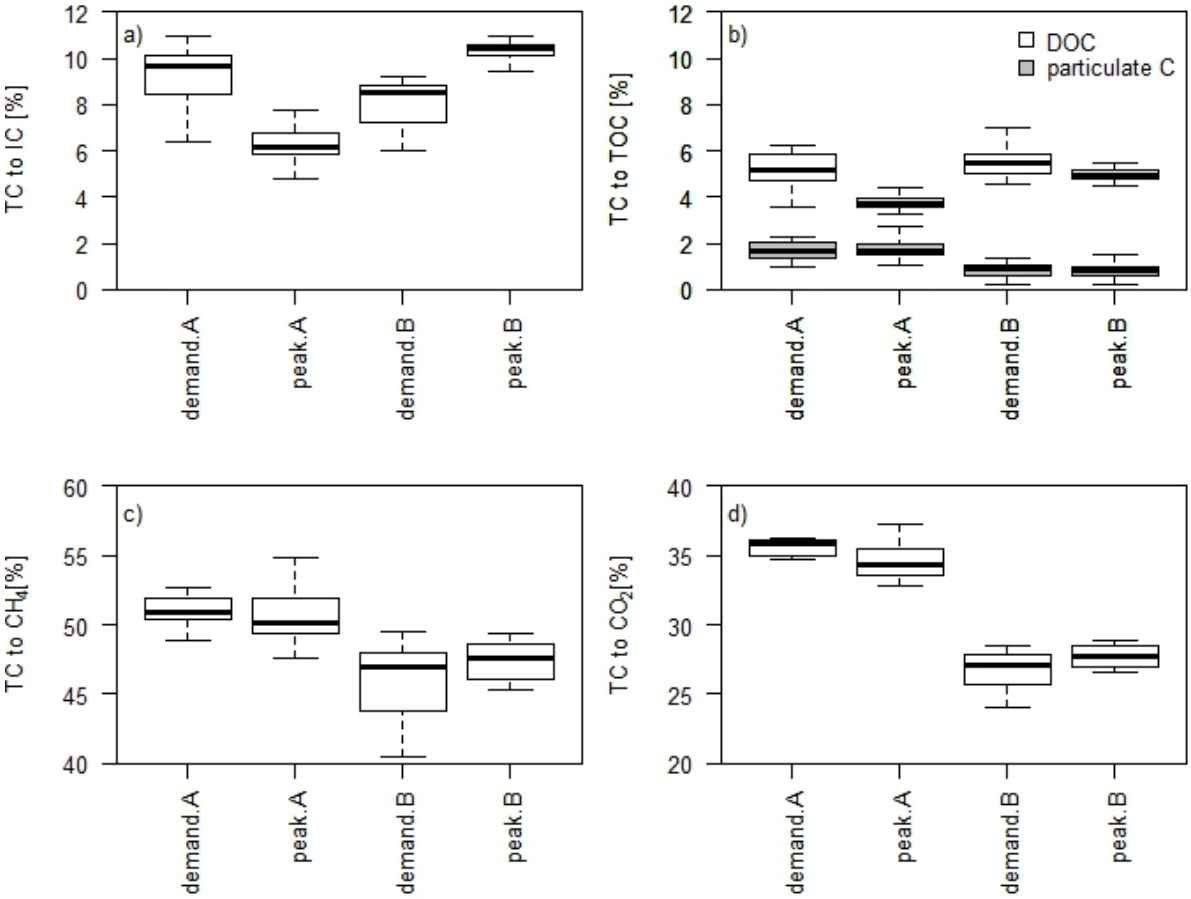


**Figure 5.6:** Carbon balance based on total carbon (TC) input per phase of the experiment. Conversion of TC to inorganic carbon (IC): a), conversion of TC to total organic carbon (TOC) separated into dissolved organic carbon (DOC) and particulate carbon (pC): b), conversion of TC to methane: c) and conversion of TC to carbon dioxide: d)

the carbon balance based on TC-input is given in Figure 5.6, showing the four major C-output fractions in a) to d), with TOC divided into pC and DOC. Each diagram shows a boxplot for each phase of the experiment representing the calculated share based on TC-input. The majority of TC-input was converted to $CH_4$, the second largest output fraction was $CO_2$, followed by IC, DOC and particulate C.

The carbon which was converted to microbial biomass could not be measured directly, but was estimated based on the nitrogen input and output. Figure 5.7 shows the mean difference of TN-input and TN-output per day over the three reactors. With both substrates, the difference is approaching $0.1\,\mathrm{g\,d^{-1}}$ (dashed line in Figure 5.7). Assuming the difference to be converted to microbial biomass and considering the chemical composition of microbial biomass to be $C_5H_7O_2N$ Pavlostathis and Giraldo-Gomez (1991), the corresponding carbon mass would be $0.43\,\mathrm{g\,d^{-1}}$. Thus the conversion of TC-input to microbial biomass approximates 4 % in case of substrate 'A' and 3.5 % for 'B'. Since these values are only estimates they are not included in the following balance calculations.

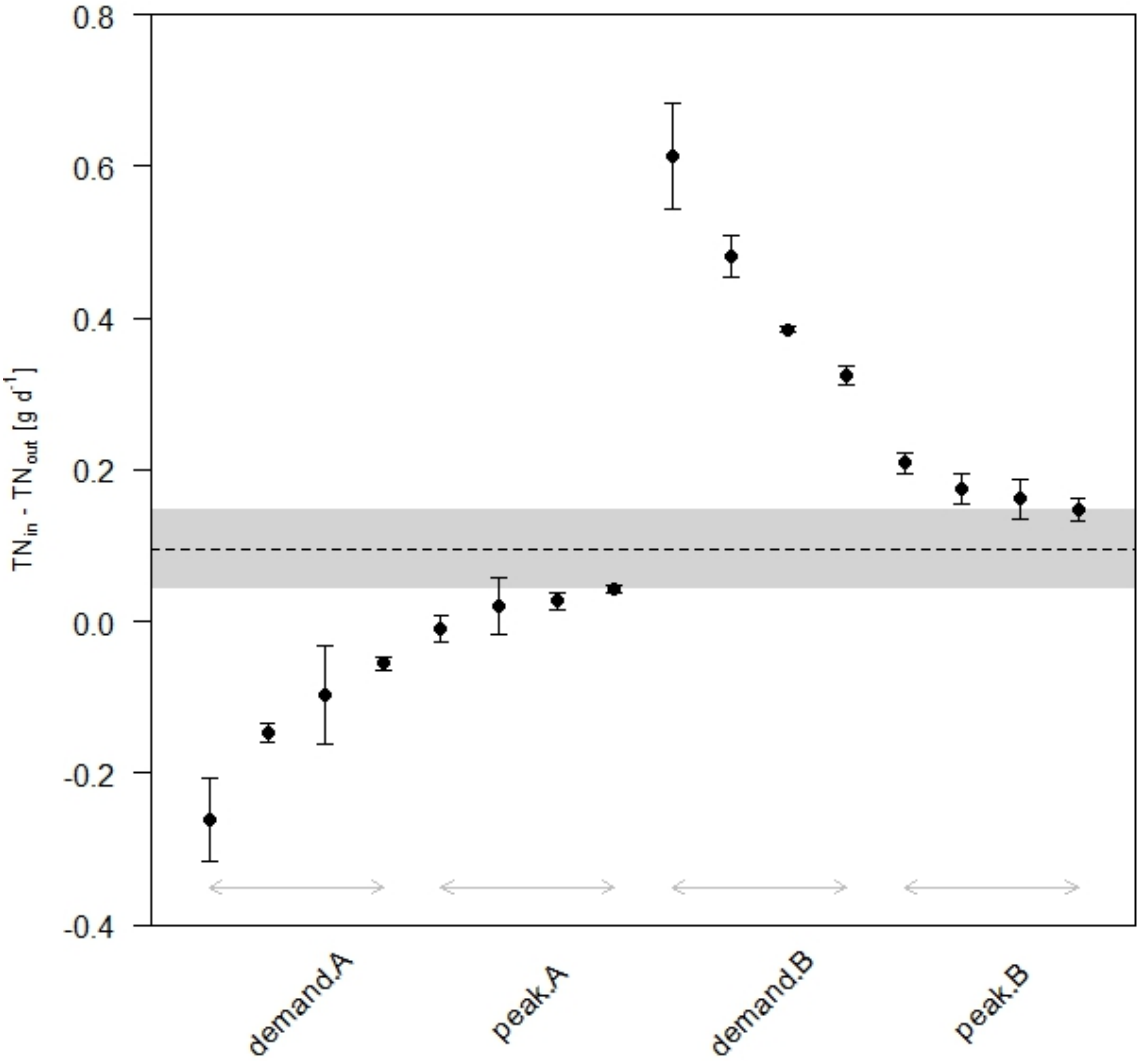


**Figure 5.7:** Mean difference of total nitrogen-input and total nitrogen-output per day over the course of the experiment

As a whole, the daily carbon balances based on TC-input were closed by $93.48 \pm 6.91\,\%$. The individual balance closures for each OLR-mode×substrate combination ordered chronologically were $103.16 \pm 2.82\,\%$ for 'demand.A', $95.39 \pm 7.00\,\%$ for 'peak.A', $86.89 \pm 5.50\,\%$ for 'demand.B' and $91.31 \pm 2.45\,\%$ for 'peak.B'. The differences in balance closure are mainly due to changes in pH over the course of the experiment which influenced the solubility for inorganic carbon. Initially, the carbon-saturated reactor liquid responded to the newly introduced substrate 'A' by a decrease in pH. $HCO_3^-$ was therefore transformed to $CO_2$ which could then leave the reactor via the gas stream. During 'peak.A', the pH remained at the lower level and inorganic carbon remained stable. With the introduction of substrate 'B', pH had began to rise, therefore providing a higher solubility for inorganic carbon. Due to the enrichment of the liquid during that phase, the carbon balance closure was reduced until saturation was reached again in phase 'peak.B'. This dynamic is supported by the measured IC concentrations, which follow the pH progression described earlier. Starting at $1.173 \pm 0.057\,\mathrm{g\,L^{-1}}$ for the three reactors, the IC concentration decreased over the course of 'demand.A' down to $0.850 \pm 0.113\,\mathrm{g\,L^{-1}}$. During 'peak.A' it further decreased to $0.680 \pm 0.004\,\mathrm{g\,L^{-1}}$. In 'demand.B' and 'peak.B', it increased to $1.153 \pm 0.020\,\mathrm{g\,L^{-1}}$and $1.402 \pm 0.061\,\mathrm{g\,L^{-1}}$, respectively.

A closer look into the effluent composition reveals that only a very small share of substances was identified. Substances in the effluent which were analyzed by GC and HPLC only accounted for $4.8 \pm 5.5\,\%$ of organic carbon in the effluent. These are most prominently acetic acid followed by propionic acid and n-butyric acid. No accumulation of VFAs could be detected which would instead have meant an imminent process failure. The maximum concentration of acetic acid in the effluent was $<0.3\,\mathrm{g\,L^{-1}}$. The unidentified remainder was a mixture of a wide variety of soluble and particulate substances including proteins, carbohydrates, polysaccharides, lipids, DNA, humic acid substances and other substances secreted by cells, dead cells and their fragments, as well as active biomass detaching from the biofilm. While these components are classified as soluble microbial products (SMP), extracellular polymeric substances (EPS) or subdivisions thereof, their exact definition and properties are a controversially discussed field (Laspidou and Rittmann, 2002; Ramesh et al., 2006). However, SMP are known to form the majority of effluent COD from biological treatment processes (Laspidou and Rittmann, 2002). For clarity SMP and EPS are referred to as uDOC and pC in the following, since sharp boundaries are defined by the methodologies to determine these fractions. The uDOC makes a share in relation to TC-input of $4.70 \pm 1.03\,\%$ over the whole experiment. Within one substrate-type the share of conversion from TC-input to uDOC-output decreases with lower mean OLR. This trend could not be determined as significant in this case, but is described in the literature as a possible response to shock loading, i.e. increased OLR and shortened HRT (Ketheesan and Stuckey, 2015). The share of pC in TOC of the effluent was distinctively different between phases of differing substrate. During the feeding

of substrate 'A', it increased from $24.21 \pm 8.11\,\%$ to $31.27 \pm 6.67\,\%$. Whereas in phase 'demand.B' and 'peak.B', it remained stable at $13.33 \pm 5.01\,\%$ and $14.02 \pm 5.09\,\%$. Although it was a major constituent of organic carbon in the effluent, it only accounted for $1.76 \pm 0.44\,\%$ and $0.88 \pm 0.32\,\%$ in relation to TC-input. Concerning the relation of TC-input to pC output, significant differences in the mean were observed between the substrates. Within one substrate-type no differences were observed between operation modes.

The interrelations here are complicated. During low OLR, biomass associated products (BAP) increase as a share of SMP due to endogenous decay. The share of utilization associated products (UAP), which are formed in proportion to the substrate used, increase at high OLRs (Schiener et al., 1998). Both are subdivisions of SMP. UAP are biodegradable (Laspidou and Rittmann, 2002), but due to shortened HRT with higher OLR these products may not be able to cycle back to become an electron-donor substrate for the cells and are instead washed out prematurely. On the other hand, part of extracellular polymeric substances (EPS), which are formed to stabilize the biofilm and also in proportion to substrate utilization, become inert biomass which is non biodegradable (Laspidou and Rittmann, 2002). In a two-staged AD system with liquid recycle, this may lead to an accumulation of inert biomass, thus increasing COD over time, which is not available for gas production. In this case, a special treatment might be required to combat increasing COD. This experiment has shown that EPS measured as pC are significantly different between substrates, while uDOC are more influenced by the mode of operation. Further research towards the effects of demand-driven biogas production on SMP and EPS production could be beneficial.

## 5.4 Conclusion

In this experiment, the suitability of anaerobic filters for demand-driven gas production was examined. The results indicate that anaerobic filters are well suited for highly flexible gas production. All three reactors showed degradation degrees over 90 % with no significant accumulation of intermediates. The replicability and therefore its predictability were evaluated which led to the finding that an excellent degree of replication can be achieved under the presented conditions. Even by introducing abrupt changes in $\mathrm{OLR_{COD}}$ by a factor >5 to $20\,\mathrm{g\,L^{-1}\,d^{-1}}$, the stability of the process was always guaranteed and high degradation degrees were ensured. As a possible measure for responsiveness to increased OLR, this study had introduced the rate of increase in methane production $r_{CH_4}$. By comparing $r_{CH_4}$ it was found that the substrate composition does not have an influence on the rate of increase. The pH is vital for the solubility of $CO_2$ and $HCO_3^-$ and therefore is an important parameter, especially under varying OLRs. When operating the AF for demand-driven gas production, nitrogen supplementation helps avoiding large fluctuations

in gas composition, which could ultimately lead to the shut down of the CHP when the methane content drops below 40 %. Preventing the action of the carbonate buffer is also important in order to maintain biofilm stability, since large quantities of $CO_2$ released from the liquid would introduce high shear forces and lead to biomass washout. The carbon balance shown here, together with pH observation supports the carbonate buffer dynamics and the importance of nitrogen supplementation. Further conclusions drawn from the carbon balance are that ≈6 % to 8 % of the carbon input is leaving the reactor in the form of DOC and particulate C. In a two-staged system with effluent recycle into an acidification reactor, this may lead to an accumulation of recalcitrant or inert carbon fractions, which result in increasing COD of the liquids over time. This is not limited to demand-driven operation, but may be exaggerated due to changing HRT and OLRs.

The proposed system, as a stand-alone AF, is suitable for substrates in which the hydrolysis rate does not play a significant role. High strength waste waters with considerable requirements towards hydrolysis are not suited. It may be used for high solid waste streams or energy crops in combination with a separated acidification reactor. This will ensure short reaction times concerning gas production.

In a full scale application several biogas plants would ideally be aggregated to a virtual power plant as part of a micro-grid or smart grid. By scattering the plants geographically, a decentralized tool for stabilizing the grid is established. Thereby local fluctuations in demand could be met locally, without the need of transition lines from a central power plant. It also spares large gas storage capacities which are restricted by law. Whenever positive or negative balancing power is requested, the CHPs of the according biogas plants are increasing or decreasing their power output and subsequently changing the biogas volume used by the CHP. The biogas plant operating system would then adjust the gas production accordingly by taking control over the feeding regime and produce the gas just in time. Implementation of the proposed system would not be restricted to the electricity sector. Gas production could also be guided by heat demand or a combination of both to support Smart Energy Systems on a national level (Mathiesen et al., 2015) or in a smaller scale like the Energy Hub approach (Orehounig et al., 2015).

Upcoming technologies, which aim at injecting into the natural gas grid, might also pose new requirements to stabilize low pressure gas-grids locally. This has been investigated by Abeysekera et al. (2016) and could be a future application for demand-driven biogas production as well.

As a further step into this research, it is recommended to investigate the boundaries of safe operation in a demand-driven feeding regime and its effects on the process to develop adequate control strategies.

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# 6 General Discussion

## 6.1 Methane Production Kinetics

In the literature several factors influencing the kinetics of methane production can be found. These include adaption of microorganisms to the substrate and nutrient supply. Since different microorganisms grow on specific substrates, the abundance of the respective organisms play an important role and determine the maximum rates of degradation, as well as the actual degradation pathway. These effects have been studied by Aguilar et al. (1995) using two differently enriched methanogenic cultures. The glucose pre-grown culture displayed an almost nine-fold higher utilization rate of propionic acid than the culture pre-grown with acetate as the sole carbon source. Similarly, n-butyric and iso-butyric acid were degraded faster by the glucose pre-grown culture as opposed to acetic acid degradation which was the same for both cultures. While this experiment was a long term selection over three years, changing the consortium of microorganisms to a large extent, Öztürk (1991) observed similar effects in a short time frame, using batch cultures which were fed twice. As a result of adaption of the sludge and its propionate-degrading bacteria, the second feeding showed a 2.5 times faster degradation of propionic acid and 1.45 to 1.75 times higher methanogenic activities.

Besides adaption to certain substrates, there are numerous other factors influencing the rate of degradation and ultimately the rate of methane production. In a study by Moestedt et al. (2015), the addition of Nickel increased the methanogenesis rate constants $k_m$ by 98 % to 220 % compared to the test runs where Nickel was omitted. Nickel is an important metal in key-enzymes of methanogenesis, such as methyl-coenzyme-M cofactor $F_{430}$, and its sufficient supply is essential to maintain high rates of methane production.

Alongside nickel the anaerobic process is reliant on appropriate supply of other nutrients to perform efficiently. These nutrients include nitrogen, phosphorous and several minor or micro nutrients such as cobalt, selenium, iron, manganese, molybdenum, potassium, calcium, and magnesium. Lack of certain nutrients deteriorates the performance of AD and may change the degradation pathways leading to a build up of intermediary products such as propionate. Results by Vintiloiu et al. (2012) showed strong statistically significant effects of macro- and micro-nutrients on the stability of the anaerobic process of agricultural biogas plants in Germany. Nickel, molybdenum, and sulfur showed the strongest effects, followed by selenium, whereas iron, cobalt and sodium only showed effects in combination with other elements. These findings emphasize the importance of a balanced macro and micro-nutrient supply to ensure a stable process.

In order to exclude effects of nutrient deficiencies, the produced hydrolysates used in

all experiments presented in the work at hand, were supplemented with sufficient amounts of trace elements. The aforementioned effects regarding the adaption of microorganisms to certain substrates was minimized by providing a base-OLR containing all kinds of intermediates. This procedure should enable the microorganisms to adapt to all substrates found in practice, specifically the ones tested in the experiments conducted in the publications "Kinetics of Biogas Production in Anaerobic Filters" and "Intrinsic Gas Production Kinetics of Selected Intermediates in Anaerobic Filters for Demand Orientated Energy Supply".

In these two publications the methane production kinetics for various components of hydrolysate have been determined. With the method presented, all injections were given as $1\,g_{COD}$, which stoichiometrically should yield the same amount of methane gas, assuming complete conversion to methane. From the determined kinetic parameters the half lives of methane production were calculated to order the tested substances according to their intrinsic gas production velocities. Significant differences in the methane production kinetics could be determined for individual substances with acetic acid representing the fastest intermediate and lactic acid as the slowest. The underpinning mechanisms which lead to these differences are complex. Concerning single substance injections, acetic acid represents the only direct precursor to methanogenesis, and therefore exhibits the fastest methane production in both experimental runs. However, the absolute values of determined parameters are markedly different in the second experiment and calculated half lives were shortened by 38 %, 24 % and 41 % for acetic acid, propionic acid and butyric acid, respectively, although the same digesters and methods were used. The relative order of the three substances remained the same. The main difference between the two experiments was the base-$OLR_{COD}$, with $3.5\,g\,L^{-1}\,d^{-1}$ in the first experiment and $7.2\,g\,L^{-1}\,d^{-1}$ in the latter. Therefore the OLR applied has most likely a major impact on the results of this method. That is actually not surprising as the Monod Kinetic, the most widely used kinetic model for AD, expressed in terms of specific utilization rate $U$ is (Pavlostathis and Giraldo-Gomez, 1991):

$$U = \frac{kS}{K_S + S} \tag{6.1}$$

where: $k$ is the maximum specific utilization rate, $S$ is the concentration of the growth limiting substrate and $K_S$ is the half velocity coefficient (i.e. substrate concentration at one half maximum specific growth rate). This implies that the specific utilization rate is dependent on substrate concentration. It stands to reason that at a higher OLR the substrate concentrations are higher and therefore a faster methane production was observed in the second experiment.

The complexity is underpinned by experiments by Wang et al. (1999), who found that the rate of propionic acid degradation was markedly decreased when acetic acid

concentrations were greater than 1400 mg $L^{-1}$. Conversely the rate of degradation of acetic acid was not altered by increased propionic acid concentrations. This relationship is confirmed by Kus and Wiesmann (1995), going even further and establishing the relation between unionized HAc and the HPr removal rate. That means that the dissociation state of the inhibitor is the actual player in that game and thus the 'true' substrate concentration is governed by pH. The stronger the acid i.e. the lower the $pK_A$ value of the respective acid, the lower the 'true' substrate concentration at elevated pH. This is considered in an extension of the Monod Kinetic and referred to as Haldane Kinetics.

Not only acetic acid has an influence on propionic acid degradation, but additionally the concentration of unionized propionic acid itself acts as inhibitor for its removal (Kus and Wiesmann, 1995). Furthermore unionized HAc inhibits the degradation of HAc and thus presenting a two sided optimization problem. For lower pH values the proportion of unionized HAc is high, thus substrate inhibition is induced, whereas at higher pH the concentration of unionized HAc is low and substrate limitation leads to lower degradation rates. Thus if VFA concentrations are sufficiently high they are self inhibitory to their degradation. Mawson et al. (1991) determined 50 % inhibition of acetic and propionate degradation at 14 mg $L^{-1}$ of undissociated VFA.

Apart from the unexplained degradation pathway discussed in chapter 4, this could be another reason for lactic acid to show such a low methane production rate. The $pK_A$ of lactic acid is considerably lower than that for the other substances tested, thus rendering unionized lactate as a scarce substrate, diminishing its degradation rate.

Other factors determining the kinetics of anaerobic digestion are discussed in the review by Pavlostathis and Giraldo-Gomez (1991) devoting separate chapters to the influences of bioenergetics, temperature, inhibition and mass transfer.

Nevertheless, the order of methane production rates for individual substances determined in chapter 3 and 4 is in agreement with most studies concerning VFA degradation (Wang et al., 1999; Aguilar et al., 1995; Öztürk, 1991), although with the exception of Yang et al. (2015) only the three major VFAs HAc, HPr and HBu were in the focus of interest. Lin et al. (1986) confirms the findings that a mixture of VFAs (2:1:1 mixture of HAc, HPr and HBu) increases the overall degradation rates compared to single VFA supplementation, without explicitly giving an explanation. As already suggested in chapter 4, the parallel degradation by many different organisms of the various substrates in the hydrolysate-mix and the concomitant production of precursors to methanogenesis are possibly the main factor of the positioning in the determined order of methane production rates.

The two hydrolysates tested in chapter 5 were relatively different in composition, substrate 'B' containing more than 30 % of TC as lactic acid, which was determined as the slowest methane producing intermediate tested in chapter 4. Yet, both substrates exhibited almost exactly the same methane production profiles for both OLR-modes of

operation. On the one hand this reinforces the findings that the mixture of intermediates inherits the highest methane production rates and on the other hand that acetoclastic methanogenesis is the rate limiting step in the Anaerobic Filters. This is most likely the reason that for both substrates the same methane production rates were observed.

It is obvious that all the above factors cannot be determined at once in an experimental run, and are thus not included in a number of kinetic studies. This also leads to high variations in reported kinetic constants for VFA degradation in the order of 1 - 30 fold (Yang et al., 2015) and make comparisons of those difficult, even when the same method is used.

## 6.2 Flexible Biogas Production

In order to operate an anaerobic digester with the aim of demand-driven or flexible biogas production, a highly stable process is required, which is characterized by

- low VFA concentrations,
- stable gas concentrations and
- high degradation degrees.

The attention towards demand-driven biogas production is relatively new and the literature on this quite limited. However there is another term that shares some key features, although the actual aims of these studies are different. These studies were published under the term hydraulic/organic shock/transient loads. Instantaneous changes in OLR can be defined as organic shock loading, while a gradual or stepwise variation in OLR over a specific time interval can be regarded as organic transient loading (Ketheesan and Stuckey, 2015). Hydraulic shock or hydraulic transient loading is termed similarly. During demand orientated biogas production, shock and transient loading occur on purpose and the findings on this matter are therefore relevant for this kind of operation.

It must be noted though that in the experiment conducted in chapter 5, the maximum $OLR_{COD}$ applied was $20\,g\,L^{-1}\,d^{-1}$. Although this is more than the doubled mean OLR for industrial anaerobic filters, it was only applied for a maximum of three hours in the OLR-mode 'peak'. The mean $OLR_{COD}$ over the day in the experiments conducted here was $10.14\,g\,L^{-1}\,d^{-1}$ and $7.38\,g\,L^{-1}\,d^{-1}$ for the OLR modes 'demand' and 'peak', respectively. Thus no extreme scenario compared to studies concerning shock loads was investigated here. The reason was the aim to achieve a gas production profile featuring characteristics of typical demand curves and not overloading the reactors. Additionally, the maximum OLR was limited to some extent by the used gas meter, which could only measure gas flows up to $1000\,mL\,h^{-1}$.

### 6.2.1 Cause and Effect of VFA Accumulation

The most reported response to shock loading is the decrease of degradation degree due to accumulation of VFAs and concomitant decrease in specific methane yield (Cohen et al., 1982; Grobicki and Stuckey, 1991; Borja and Banks, 1995; Chua et al., 1997; Angenent et al., 2002; Masse and Masse, 2005; Ketheesan and Stuckey, 2015). The accumulation is the consequence of kinetically saturated acetoclastic methanogenesis, the rate limiting step when liquid substrates are subjected to AD (Pavlostathis and Giraldo-Gomez, 1991) and thus controlling the maximum OLR that can be safely applied (Ketheesan and Stuckey, 2015). Acidogens on the other end seem to exhibit the most rapid conversion step and thus precursors to acetogenesis like propionate and butyrate accumulate. Acetogenetic organisms degrade the VFAs at a rate somewhere in between, while obligatory forming $H_2$. In order for these microorganisms to gain energy from $H_2$ forming reactions, a low $H_2$ partial pressure is mandatory and therefore live in syntrophic relation with $H_2$-consuming organisms like methanogens. Ultimately, kinetic differences between acidogenic, acetogenic and methanogenic microorganisms result in the accumulation of intermediates such as VFAs, $H_2$ and $CO_2$ (Ketheesan and Stuckey, 2015). Further accumulation leads to a drop in pH, depending on alkalinity of the system. The proportion of undissociated VFAs increases at lower pH and the uptake of undissociated VFAs is accelerated (Fukuzaki et al., 1990). In order to maintain intracellular pH and a functional gradient the excess protons need to be channeled out of the cell, amplifying the decrease in pH and the toxicity of undissociated VFAs (Fukuzaki et al., 1990). The accumulation of intermediary products such as formate, acetic acid and $H_2$ finally lead to the acetogenic pathway becoming thermodynamically unfavorable, completely blocking the degradation of precursors to acetogenesis with system failure as the result.

VFA accumulation likely occurred in the trials of chapter 5 after applying high OLRs. The effluent composition was analyzed only once per day and VFA dynamics could therefore not be pictured in detail. Nevertheless the lowest degradation degree accompanied by slightly higher VFA concentrations was reached in the OLR-mode 'demand', which represented the higher mean OLR of the two modes of operation. However, the measured VFA concentrations in the effluent never came close to any problematic values and renders evidence of a highly stable process, even under the challenging operational conditions applied in the trials.

When operating anaerobic filters for flexible biogas production they need to operate within the boundaries of the discussed self inhibitory VFA accumulation. The results presented in the study at hand suggest that anaerobic filters are well suited to handle variations in OLR throughout the day with quick responses in gas production. As demonstrated the gas production follows the applied OLR, with a distinctive expression of each change in the OLR. That marks the process as highly predictable and defined boundaries

within safe operation of AD in terms of VFA accumulation can possibly be satisfied by process control. The inclusion of three reactors in the analysis emphasize the repeatability and therefore predictability of such an approach of operation.

### 6.2.2 Variability of Gas Concentrations

The methane concentrations of the produced biogas changed in response to the variations of the OLR in the study presented here and has been reported throughout the literature for shock loading (Kennedy and van den Berg, 1982; Chua et al., 1997; Masse and Masse, 2005; Senturk et al., 2013). In all cases the $CO_2$ concentration increased in response to increased OLR, with the $CH_4$ concentration reacting conversely. This effect can be attributed to different mechanisms. First the accumulation of VFAs prevents the formation of their respective product gases. Secondly, depending on alkalinity, the accumulation of VFAs may cause a drop in pH and subsequently releasing $CO_2$ from the process liquid. Ultimately, high VFA concentrations or low pH would inhibit the methanogenic process.

In the experiment at hand substrate 'A' caused the gas quality to change by a maximum of >10 % during day one of the experiment and >7 % thereafter, presumably due to the release of large quantities of $CO_2$ after increasing the OLR to its maximum value. For substrate 'B' however, the gas concentrations only changed by $\approx 3\,\%$, although the intra-daily pH variations were similar for both substrates.

The main buffer acting in anaerobic digestion is the carbonate buffer. This system could be labeled as open buffer system since a decreasing pH causes $CO_2$ to leave the system via the gas stream, as could be shown in all the experiments conducted in this work. Thus it is not available anymore for buffering.

In contrast the $NH_3$ / $NH_4^+$ buffer could be termed as closed buffer, since both species will remain in the liquid phase of the system. Taking a look at the substrate composition, Substrate 'B' featured higher nitrogen contents, whether it is in the form of proteins or ammonia. In the pH range where the experiments were conducted all nitrogen contributing to the $NH_3$ / $NH_4^+$ buffer would be in the state of $NH_4^+$. By introducing nitrogenous compounds through the feed stream, the accumulated VFAs were immediately neutralized, reducing the extent of action by the carbonate buffer, and thus smaller variations in gas composition were observed. Therefore the $NH_3$ / $NH_4^+$ buffer could be valued higher as it prevents extreme variations in gas quality.

The converse reactions, i.e. higher methane and lower carbon dioxide concentrations can be observed during recovery from organic shock loading. Excess VFAs will be degraded and pH reaches pre-shock levels. The free capacity to dissolve $CO_2$ leads to an increased methane content since newly produced carbon dioxide partly remains in the liquid.

Recalling that the applied increases in OLR were somewhat limited by the gas meter

in this study, more extreme cases are imaginable in practice. If the gas quality dropped below a methane content of 40 %, the shutdown of the CHP would be initiated, destroying the revenue of demand orientated power supply. Hence the observation and control of alkalinity presents itself as crucial when demand orientated biogas/power production is targeted. The minimum observed $CH_4$ concentration during the whole experiment was 51.76 %, thus remaining in an area of safe operation. Again the AF proved itself to be well suited to handle these challenging operational conditions and its suitability for flexible biogas production.

### 6.2.3 Degradation Degree

The degradation degrees, based on TOC (see 5.2) during the trials remained high at >90 % under all substrate ×OLR-mode combinations tested. Thus it can be concluded that the anaerobic process in the AF was very stable under these flexible operational conditions. Yet, slight differences in degradation degree between the experimental phases could be observed and since VFA concentrations in the effluent were negligible, the cause of altered degradation degrees mus be rooted elsewhere.

Apart from VFA accumulation, other products summarized under the term 'soluble microbial products' (SMP) are affected by organic/hydraulic shock loads (Ketheesan and Stuckey, 2015). SMP can be further subdivided into utilization associated products (UAP) and biomass associated products (BAP). UAP are a direct result of substrate utilization, while BAP are formed from biomass, presumably as part of decay (Laspidou and Rittmann, 2002). A second large category of products is termed extracellular polymeric substances (EPS) and are largely associated with the solid phase and are therefore insoluble. EPS are bio-polymers in which biofilm microorganisms are embedded (Flemming et al., 2007). Laspidou and Rittmann (2002) presented a unification of the two schools of SMP and EPS, yet the exact definition of SMP, EPS and their subdivisions is still controversially debated (Ramesh et al., 2006). However three important remarks with respect to demand orientated biogas production can be made here. Firstly the biofilm structure is affected by shear stress induced for example by decreases in HRT, thus altering EPS composition and biofilm architecture Liu and Tay (2001). Secondly, partly degradation of EPS leads to BAP which can recycle to become electron donor substrate (Laspidou and Rittmann, 2002), but may be washed out prematurely when the HRT is low. Thirdly, true dead cell residuals as part of EPS are non-biodegradable (Laspidou and Rittmann, 2002) and thus could accumulate in a two-staged AD system with liquid recycling. This may lead to an increase in COD concentration of reactor liquids over time, with error of judgement regarding expected methane yields as the consequence. In chapter 5 the DOC measured in the effluent in deed decreased with lower mean OLR, thus pointing towards UAP. EPS measured as particulate C were not affected by the

OLR-mode but was altered slightly by the substrate type. Further research on this topic and its implication for two-staged AD, in particular demand-driven biogas production is desirable.

## 6.3 Reactor-Design for Demand-Driven Biogas Production

The extent to withstand organic/hydraulic shock loadings and therefore the feasibility of demand-orientated biogas production is governed by several factors. First and foremost the efficiency of acetoclastic methanogenesis determines the maximum OLR which can be applied without accumulation of VFAs. The buffer-capacity prevents the system from inhibitory low pH after shock loading. Other factors influencing the capability and efficiency of withstanding shock loads are mass transfer limitations, biomass washout, nutrient limitations and microbial diversity.

The appearance of the methanogenic aggregates, usually found in the form of flocs or biofilms with certain solid-/gas-/liquid-interfaces, influences the rate at which substrates can be degraded. The available surface area to volume ratio of such aggregates promotes or demotes the in and outflux of substrates and products. Thus a key factor for withstanding shock loads is the reactor design. Considering attached biomass reactors opposed to suspended biomass reactors, the suspended biomass may be more susceptible to washout of microorganisms than attached biomass.

Purposefully introduced organic shock loads for flexible biogas production could be achieved through higher substrate concentrations in combination with maintained HRT, as well as shortened HRT in combination with maintained substrate concentration. Yet, their effects can be very different. Shortened HRT may increase liquid velocities and shortening contact time for substrate metabolism, thus decreasing mass transfer into the aggregate. Differently, higher substrate concentrations lead to larger gradients in substrate concentration between the bulk liquid and the inward of the aggregate, increasing the mass transfer driving force. For flexible biogas production the shortened HRT with maintained substrate concentration are far more likely.

Higher shear forces during shock load may cause a loss of biomass due to increased liquid velocities, but even more due to increased gas production. A rapid drop in pH and concomitant $CO_2$evolution can cause extremely high shear forces and entrain large amounts of biofilm from the carrier material in anaerobic filters as observed during HAc injection in the experiments of chapter 3 and 4. Loss of active biomass from the reactor could cause serious problems and ultimately induce a chain reaction leading to system failure.

Kennedy and van den Berg (1982) examined anaerobic filters operating at 60 % to 70 %

of the maximum steady state OLR. These were overloaded for 24 h with loading rates 2-9 times higher than the control rate and a maximum $OLR_{COD}$ of 94.2 g $L^{-1}$ $d^{-1}$. Their conclusions were that the reactors were durable and regained stable operation quickly after overloading within 12 h to 48 h and that anaerobic filters were not prone to some of the operating instabilities associated with conventional anaerobic reactors. Other reactor types may be similarly suitable to variations in OLR, such as anaerobic migrating blanket reactors. In the studies of Angenent et al. (2002) these were subjected to a doubled $OLR_{COD}$ from 25 g $L^{-1}$ $d^{-1}$ to 50 g $L^{-1}$ $d^{-1}$ within six HRTs (42 h). Pre-shock conditions were reached "immediately" after the OLR was restored (Angenent et al., 2002). A fluidized bed reactor operated by Borja and Banks (1995) recovered within 11 h to 14 h after doubling the $OLR_{COD}$ from 15.6 g $L^{-1}$ $d^{-1}$ to 31.2 g $L^{-1}$ $d^{-1}$ for 6 h or 12 h. Anaerobic Baffled Reactors in the study of Grobicki and Stuckey (1991) were subjected to shock loads, increasing from an $OLR_{COD}$ 4.8 g $L^{-1}$ $d^{-1}$ to 96 g $L^{-1}$ $d^{-1}$ for 3 h, while decreasing the HRT from 20 h to 1 h. Here the biomass washout has been quantified and was less than 15 %. The recovery here was rapid and pre-shock degradation degrees were reached within 24 h. Masse and Masse (2005) also observed increased biomass loss during shock loads using anaerobic sequencing batch reactors, but without effect on performance, probably because initial biomass concentrations were high. Regarding biomass washout, fixed film, expanded and fluidized bed reactors withstand higher loading rates and have shown better stability compared to CSTR systems (Rajeshwari et al., 2000). For demand-driven biogas production this feature enhances stability and therefore marks attached biomass reactor designs as especially suitable.

## 6.4 Concluding Remarks

In the studies conducted in this work, differences in gas production kinetics for several important intermediates found in hydrolysate could be determined. In conclusion this demonstrated that:

- different intermediates formed in the AR have different methane production rates,
- extrapolating from individual methane production rates to the methane production rate of a mixture is questionable,
- the mixture of many different intermediates exhibits the fastest methane production rates and thus
- the control of the hydrolysis towards specific intermediates is not necessary.

Operation of anaerobic filters with the aim of flexible biogas production has proven its feasibility and reproducibility. Methane production immediately followed changes in OLR

when liquid substrates were used. A key factor in order to prevent large fluctuation in gas composition is the alkalinity of the process liquid, specifically the provision of nitrogenous compounds is vital to maintain stable gas concentrations. Anaerobic Filters or attached biomass reactors in general seem to exhibit superior performance towards shock loading and are therefore especially suited for demand orientated gas production as they recover quickly from overloading. Formation of soluble and particular C may be influenced or exaggerated by changing HRT and OLR. Further research in order to evaluate the limits of safe operation is recommended as more extreme scenarios are imaginable in practice.

In order to predict methane production or adjust operational conditions for demand orientated gas production mechanistic models picturing each degradation step and chemo-physical reactions seem too complex. A possible solution may be Autoregressive-Moving Average (ARMA) models which can be validated based on empiric data, such as the ones gathered in this work and are especially used in order to make short term forecasts.

# 7 Summary

Fluctuating energy sources, namely wind turbines and photovoltaic, will be the mayor contributors to the increase in share of renewable energies. The intermittent energy supply by these sources poses challenges for the power grid and need to be counter balanced. A demand-driven energy supply by weather independent biomass conversion can offer these grid services. Flexible energy production from biogas has been identified as a vital approach to provide the grid with positive and negative balancing power. The two-staged anaerobic digestion may be especially suitable for demand orientated gas production due to the advantages of the anaerobic filters to withstand high organic loading rates and shock loading. Two staged anaerobic digestion is characterized by a spatio-temporal separation of acidification and methane production. A liquid rich in soluble products, such as volatile fatty acids, alcohols and sugars is produced in the first stage and and is subsequently converted to biogas in the second stage. The methanation stage as the main gas producing unit in such a system is in the focus of this research.

The ability to react to sudden changes in demand might be influenced by substrate composition and controlled hydrolysis towards certain intermediates could improve the reaction times towards increased demand. It is therefore one focus of this research work to examine intrinsic methane production kinetics of common intermediates of anaerobic digestion. Other major questions are how fast the methane production can be adapted to sudden changes in demand and to what extent these adaptions are reproducible. It was therefore of interest to demonstrate the feasibility, reproducibility and the possible extent of demand-driven biogas production in anaerobic filters, with respect to changing substrate composition. Furthermore the evaluation of the process efficiency based on carbon fluxes should be examined to unfold effects resulting from changing operational conditions.

With a newly developed methodology, introduced in the publication "Kinetics of Biogas Production in Anaerobic Filters" kinetic parameters of methane production for individual volatile fatty acids (VFA) could be determined. The bandwidth of tested intermediates was broadened in the second research paper "Intrinsic Gas Production Kinetics of Selected Intermediates in Anaerobic Filters for Demand Orientated Energy Supply". It has been found that intermediates could be ordered according to their half-lives of methane production. The apparent order, beginning with the fastest was acetic acid >ethanol >butyric acid >iso-butyric acid> valeric acid> propionic acid> propanediol> lactic acid. However the mixture of these individual components administered as a naturally produced hydrolysate revealed the fastest methane production kinetics.

Differences in the absolute values of determined kinetic parameters between the two

experiments can be attributed to variations in organic loading rate (OLR), since degradation rates of a specific substrate are determined by substrate concentration. But also other parameters influence the absolute rate at which methane is produced, such as the concentration of products or unionized substrate itself, pH, nutrient availability, bioenergetics, temperature, inhibition, mass transfer and microbial population.

In the third research paper "Demand-Driven Biogas Production in Anaerobic Filters" the previous findings have been put to the test by applying changes in OLR throughout the day and examining different substrate compositions with respect to the methane production rates. As demonstrated, the gas production followed the applied OLR with a distinctive expression of each change in the OLR. That marks the process as highly predictable and defined boundaries within safe operation of AD, in terms of VFA accumulation, can possibly be satisfied by process control. The inclusion of three reactors in the analysis emphasizes the repeatability and therefore the predictability of such an approach of operation. Feasibility and reproducibility of demand-driven biogas production by anaerobic filters could thus be demonstrated. It has been found that the hydrolysate composition has no significant influence on methane production kinetics for demand orientated gas production, since the maximum rate is limited by acetoclastic methanogenesis. The control of the hydrolysis should focus on high overall degradation, rather than towards the production of specific intermediates.

A key factor in order to prevent large fluctuation in gas composition is alkalinity, specifically the provision of nitrogenous compounds is vital to maintain stable conditions. Anaerobic filters or attached biomass reactors in general seem to exhibit superior performance towards shock loading and are therefore especially suited for demand orientated gas production as they recover quickly from overloading.

Formation of soluble microbial products (SMP) and extracellular polymeric substances (EPS) may be influenced or exaggerated by constantly changing HRT and OLR. Further research in order to evaluate the limits of safe operation is recommended as more extreme scenarios than the ones examined in this work are imaginable in practice.

# 8 Zusammenfassung

Fluktuierende Energiequellen, vornehmlich in Form von Windturbinen und Photovoltaik-Anlagen werden den Großteil des Wachstums an Erneuerbaren Energien ausmachen. Die unstetige Energieversorgung dieser Quellen stellt eine Herausforderung für die Elektrizitätsnetze dar und muss mit entsprechender Regelenergie ausgeglichen werden. Eine bedarfsgerechte Energieversorgung durch wetterunabhängige Biomasse-Konversion kann diese Netzdienstleistungen erbringen. Flexible Energiebereitstellung durch Biogas wurde als wichtiger Ansatz erkannt, um Netze mit positiver und negativer Regelenergie zu stabilisieren. Die zwei-stufige anaerobe Vergärung, die aufgrund der hohen biologischen Stabilität des Anaerobfilters gegenüber Lastwechseln besonders für bedarfsgerechte Biogasproduktion geeignet ist, wird charakterisiert durch die räumlich-, zeitliche Trennung der Versäuerung von der Methanproduktion. Aus der zugeführten Biomasse wird in der ersten Stufe eine Prozessflüssigkeit, reich an organischen Säuren, Zuckern und Alkoholen produziert, die anschließend in der zweiten Stufe zu Biogas umgewandelt wird. Die Methan-Stufe, als maßgeblich gasproduzierende Einheit steht dabei im Fokus dieser Arbeit.

Im Rahmen dieser Arbeit sollte die Machbarkeit, Reproduzierbarkeit und das Ausmaß der bedarfsgerechten Biogasproduktion im Anaerobfilter begutachtet werden, auch im Hinblick auf den Einfluss der Substratzusammensetzung. Die Beurteilung der Prozesseffizienz soll anhand einer Kohlenstoffbilanz erfolgen um eventuelle Effekte der sich ändernden Betriebsparameter aufzudecken.

Mit einer neuen Methode, eingeführt in der Publikation „Kinetics of Biogas Production in Anaerobic Filters“, konnten kinetische Parameter der Methanbildung einzeler Fettsäuren bestimmt werden. Die Bandbreite der getesteten Intermediate wurde mit der zweiten Publikation „Intrinsic Gas Production Kinetics of Selected Intermediates in Anaerobic Filters for Demand Orientated Energy Supply“ erweitert. Anhand der „Halbwertszeiten der Methan Produktion“ konnte eine Reihenfolge der Gasbildungsgeschwindigkeiten etabliert werden. Die Reihenfolge der getesteten Intermediate, beginnend mit dem schnellsten, wurde wie folgt bestimmt: Essigsäure> Ethanol> Buttersäure> iso-Buttersäure> Valeriansäure> Propioinsäure> Milchsäure. Am schnellsten wurde Methan jedoch nach der Zugabe eines natürlich produzierten Hydrolysates, also eine Mischung aller einzelnen Intermediate, erzielt.

Unterschiede in den absoluten Werten der Kinetik-Parameter zwischen den beiden Experimenten können dem Einfluss der angelegten Raumbelastung zugeordnet werden, da Abbauraten spezifischer Substrate im Allgemeinen von der Substratkonzentration abhängig sind. Andere Faktoren, die für die absoluten Raten der Methanproduktion verantwort-

lich stehen, sind Produktkonzentrationen, unionisierte Substratkonzentration, pH-Wert, Nährstoffverfügbarkeit, Temperatur, Massetransfer und die mikrobielle Population.

In der dritten Publikation „Demand-Driven Biogas Production in Anaerobic Filters“ wurden die vorherigen Erkenntnisse überprüft, indem im laufe eines Tages wechselnde Raumbelastungen angelegt wurden und Methanproduktionsraten im Hinblick auf Substratzusammensetzung untersucht wurden. Die Gasproduktion folgte jeder Änderung der Raumbelastung mit einer sehr kurzen zeitlichen Verzögerung. Der Prozess zeichnet sich durch eine gute Voraussagbarkeit innerhalb der Grenzen des stabilen Betriebs der anaeroben Vergärung aus. Das Einbeziehen dreier Reaktoren in die Analyse unterstreicht die gute Reproduzierbarkeit und die damit einhergehenden Vorhersagemöglichkeiten eines solchen Ansatzes des Anlagenbetriebs. Die Machbarkeit und Reproduzierarkeit konnten demnach demonstriert werden. Die Substratzusammensetzung scheint in diesem Zusammenhang keinen signifikanten Einfluss auf die Gasbildungskinetik zu haben, da der Geschwindigkeitslimitierende Faktor die acetoklastische Methanogenese ist. Die Prozesskontrolle der Hydrolyse sollte daher in Richtung des Gesamtabbaugrades optimiert werden, anstatt gezielte Intermediate zu produzieren.

Die Produktion von gelösten mikrobiellen Produkten (SMP) und extrazellulären polymeren Substanzen (EPS) ist möglicherweise beeinflusst oder sogar verstärkt durch sich ständig ändernde Raumbelastung und Verweilzeit. Weitergehende Untersuchungen sind nötig um die Grenzen der sicheren Betriebsweise festzulegen, da in der Praxis durchaus extremere Szenarios denkbar sind.

Ein Schlüsselelement um starke Schwankungen in der Gaszusammensetzung zu vermeiden ist die Pufferkapazität der Flüssigkeiten im Fermenter. Insgesamt eignen sich Anaerobfilter oder Reaktoren mit entsprechendem Biomasserückhalt besser als klassische volldurchmischte Systeme, da sie eine deutlich bessere Wiederstandsfähigkeit gegenüber Stoßbelastungen aufzeigen und sich schnell von Überlastungen erholen. Sie sind daher besonders für die bedarfsgerechte Biogasproduktion geeignet.

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