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# Two-stage high pressure anaerobic digestion for biomethane production

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# TWO-STAGE HIGH PRESSURE ANAEROBIC DIGESTION FOR BIOMETHANE PRODUCTION

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# Acknowledgments

# Contents

# Abbreviations

| | |
|---|---|
| CHP | combined heat and power |
| $CH_4$ | methane |
| $CO_2$ | carbon dioxide |
| COD | chemical oxygen demand |
| g | gram |
| $H_2$ | hydrogen |
| $H_2O$ | water |
| $H_2S$ | hydrogen sulfide |
| HAc | acetic acid-equivalent |
| kg | kilogram |
| km | kilometer |
| kWh | kilowatt hour |
| L | standard liter (1,013 hPa; 0 °C) |
| MW | Megawatt |
| $m^3$ | cubic meter |
| $N_2$ | nitrogen |
| $NH_3$ | ammonia |
| $O_2$ | oxygen |
| P | pressure increase potential |
| PSA | pressure swing adsorption |
| $R_m$ | maximum daily pressure increase rate |
| SCOD | soluble chemical oxygen demand |
| SMY | specific methane yield |
| TIC | total inorganic carbon/ total alkalinity |
| $t_{max}$ | time of the maximum pressure increase per day |
| VFA | volatile fatty acids |
| λ | duration of the lag phase |

# 1 Introduction

## 1.1 Natural Gas

The demand of natural gas for power and heat generation in the EU grew rapidly since 1990. Due to increased energy efficiency and a significant increase in renewable energy generation, a reduction in natural gas consumption has been observed since 2010 [1]. In 2015, the gross inland consumption of natural gas in the EU-28 was about 16,733 thousand terajoules, which is equal to the consumption in 1995 [1]. 75% of the EU-28 gas demand is consumed by the six European countries Germany, UK, Italy, France, Netherlands and Spain. In 2015, 4,989 thousand terajoules of natural gas were produced in the EU-28 [1]. This corresponds to a natural gas import dependency of about 69.3% [1]. In 2014, Russia supplied 37.5% of the natural gas consumed, followed by Norway (31.6%), Algeria (12.3%) and Qatar (6.9%) [2].

Due to the dependence on gas imports and seasonal fluctuations in gas demand, the storage of gas has become important. In May 2015, in the EU-28 a total underground gas storage capacity of 108.3 billion m³ was available; 7.4 billion m³ more were under construction and 29.3 billion m³ planned [3]. The available capacity represents about 20% of the yearly demand of natural gas. The natural gas enters and leaves Europe at 26 cross-border interconnections [4]. Furthermore, 28 import terminals for feeding liquefied natural gas (LNG) into the transmission network are available since 2015 [5].

In 2012, the gas was transported via pipelines in a gas transmission network with a length of 2.15 million km across the EU to the consumers [6]. The operating pressures of long-distance pipelines vary between 60-150 bar, of local and regional pipelines between 8-40 bar and of distribution pipelines to customers between 0.05-0.1 bar [7, 8]. The gas qualities ranges between methane ($CH_4$) contents of 80% and 98%, depending on the origin of the natural gas, and can be characterized as L-gas (low heating value) and H-gas (high heating value) [7]. Natural gas can be extracted from fossil resources such as oil fields, coal beds, shale or natural gas fields. Alternatively, biomethane obtained from biogas by an upgrading process, offers an energy supply based on renewable resources.

## 1.2 Biogas as natural gas

Biogas is produced by microbial anaerobic conversion of biodegradable material and is regarded as a carbon dioxide ($CO_2$) neutral energy source. Biogas primarily consists of $CH_4$ (55 to 60%) [9] and $CO_2$ (40 to 45%), dependent on the fermentation process and on the

converted initial substrates. It is predominantly used for combined heat and power (CHP) generation [10] due to its low calorific value.

In 2014, 17,240 biogas plants were operating in Europe with a total installed capacity of 8,339 MW [11]. Germany had the largest share with 10,786 biogas plants [11] fostered by the introduction of the Renewable Energy Law in 2000 [12] and led to a steady increase in renewable energy generation.

Instead of using the biogas in CHP units, the purification of biogas to biomethane and its subsequent injection into the natural gas grids presents an alternative method of ensuring high energy utilization, especially for biogas stations located in rural areas without heat sinks [13]. By applying upgrading techniques, the gas production and its utilization can be decoupled in terms of time and space [14]. In 2014, 367 biomethane plants with a total upgrading capacity of 310,000 $m^3$ $h^{-1}$ raw biogas were operated in Europe [11]. 178 of these are located in Germany, injecting approximately 638 million $m^3$ biomethane into the gas grid yearly [15].

Prior to injection into the gas grid, raw biogas must be purified and its heating value adapted to the natural gas quality. In this step, the $CO_2$ content and the typical trace amounts of the raw biogas such as water ($H_2O$, 5-10%), oxygen ($O_2$, 0-1%), nitrogen ($N_2$, 0-2%), hydrogen sulfide ($H_2S$, 0.005-2%), ammonia ($NH_3$, <1%) and siloxane (0-0.02%) have to be reduced [16]. Furthermore, the purified gas is subsequently compressed to the current pressure level of the grid section.

The produced biomethane has to meet specific standards in order to ensure a safe and reliable operation of gas grids, gas infrastructures and facilities. Several countries have implemented different standards for its utilization as a vehicle fuel or for grid injection [17]. The requirements differ in terms of gas quality ($CH_4$-, $CO_2$-, $O_2$- and hydrogen ($H_2$)-content) and Wobbe Index. In Germany, the gas has to fulfil the specific injection requirements determined by various technical guidelines such as the G 260 and G 261 published by the Deutscher Verein des Gas- und Wasserfaches e. V. (DVGW) [18, 19].

Commonly used upgrading technologies for reducing the $CO_2$ content in the raw gas are water and amine scrubbing as well as gas separation membranes and pressure swing adsorption (PSA) [9]. In Germany and the Netherlands water and amine scrubbers, PSA units and membrane technology are commonly used whereas water scrubbing systems are often installed in Sweden [16, 20].

However, all of these methods have significant disadvantages when operated in large scale. PSA often requires an upstream desulfurization unit whereas for amine scrubbing high amounts of auxiliary chemicals and additives are necessary. Gas separation membranes are usually

operate only as partial flow filters [9]. Additionally, some of these technologies generate waste and waste water which has to be cost-intensively treated and removed. Furthermore the upgraded biomethane is usually generated at quite low pressures and has to be subsequently compressed for grid injection, thus leading to high operating costs [21]. Available alternative technologies, such as low-temperature cryogenic separation or the biological removal of $CO_2$ through the addition of hydrogen into the digester, are either expensive or only used in experimental scale [16]. In contrast to the established techniques, the innovative two-stage high pressure anaerobic digestion integrates biogas production, upgrading and pressure boosting within one process with the help of microorganisms.

## 1.3 Two-stage high pressure anaerobic digestion

The microbial degradation of organic material under anaerobic digestion to biogas can be separated into four main steps [22, 23]. In the first step called hydrolysis, the complex organic molecules are degraded to monomers by enzymes. Subsequently, acidogenic microorgansims convert the monomers into volatile fatty acids, alcohols, $CO_2$ and $H_2$. These volatile fatty acids are split into acetate, $H_2$, $CO_2$ and water in the acetogenic step, also named dehydrogenation. Finally, methanogetic microorganisms convert acetic acid into biogas by disproportionation or produce $CH_4$ and water by the anaerobic oxidation of hydrogen whereby simultaneously $CO_2$ is reduced [24].

In the two-stage anaerobic digestion process, the degradation steps of hydrolysis/acidification are spatially separated from acetogenesis/methanogenesis to provide optimum environmental conditions for the different groups of microorganisms [25-27]. The advantages over single stage anaerobic digestion demonstrated by lab scale experiments are providing a constant high organic loading rate (OLR) in the methane reactor, no loss of methanogens by the removal of digestate and an improved degree of degradation of the substrate [28].

In two-stage high pressure anaerobic digestion, the second process stage runs under increased pressure. The methanogenic bacteria in the methane reactor autogeneratively increase the pressure of the gas. This concept uses the higher solubility of $CO_2$ in liquid compared to $CH_4$ [29-31], providing an opportunity to discharge the $CO_2$ via the liquid stream from the methane reactor. Through that, it integrates biogas production, purification and pressure boosting within one process and results in a high-calorific biogenic gas gained from the methane reactor. This innovative concept is based on the following theoretical basis:

### 1.3.1 Gas solubility under pressure

The main components of biogas are $CH_4$, $CO_2$ and hydrogen sulfide ($H_2S$). In several recent publications the solubility of these gases in water have been studied and described [32-36]. The results with pure gases showed a high solubility of $CO_2$ and $H_2S$ in contrast to $CH_4$, which hardly dissolved in water. With decreasing temperature or raising pressure, the solubility of $CO_2$ and $H_2S$ in water was further increasing. The dissolved amount of the gases in water at different temperatures and pressures can be calculated using Henry's Law [37, 38].

### 1.3.2 pH-value under pressure

The pH-value in the anaerobic digestion process has a significant effect on the digestion process [39-41]. Capri et al. mentioned a maximum pH range between 6.0 and 7.5 for anaerobic digestion [42] and studies from Lie et al. found an optimal pH range in anaerobic digestion to be 6.5–7.5, depending on the substrate and digestion technique [43]. Within this pH range, the buffer capacity of the aqueous phase depends almost totally on the carbonic acid dissociation [42]. Between pH 6.0 and 7.5, the dissolved $CO_2$ is reduced to hydrogen carbonate ($HCO_3^-$) and the amount of carbonate ion ($CO_3^{2-}$) is negligible [42].

In pressurized anaerobic digestion, an increase in pressure increases the solubility of $CO_2$ in the aqueous phase and enables more $CO_2$ to be dissolved in the liquid. The dissolved $CO_2$ forms carbonic acid ($H_2CO_3$), which immediately dissociates to $HCO_3^-$, depressing the pH-value in the reactor due to the liberated protons in the solution. Previous studies by Chen et al. using continuously operated methane reactors showed a significant decrease in pH from 7.2 to 6.5 by raising the pressure from 1 to 9 bar, due to the increased partial pressures of $CO_2$ [44]. At even higher operating pressures, the partial pressures of $CO_2$ further increase and lower pH-values can be expected.

### 1.3.3 Microbiology under pressure

The operating pressure in a two-stage high pressure anaerobic digestion system is "autogeneratively" produced by the gas production of the methanogenic microorganisms. Former studies have shown that different microorganisms tolerate various pressure thresholds [45]. They can be divided into 3 categories:

Piezophilic microorganisms demonstrate optimal growth rates at pressures above atmospheric pressure; piezotolerant microorganisms are capable of growth at atmospheric pressure as well as at higher pressures, with their optimal growth rate being at atmospheric

pressure; piezosensitive microorganisms demonstrate optimal growth at atmospheric pressure and stop reproduction at around 500 bar [45-49].

Research by deep-sea microbiologists found piezophilic microorganisms (bacteria and archaea) living in the deep ocean waters at pressures of 100 bar to 1,030 bar [50] and studies from Bernhardt et al. mentioned an enhancement of the growth rate of *Methanococcus thermolithotrophicus* at high pressures of up to 500 bar [51]. The effect of pressure over 100 bar on pressure sensitive processes such as motility, growth, etc. have recently been described in literature for *Escherichia coli* [50].

It can be assumed that the microorganisms in anaerobic digesters are usually piezosensitive or piezotolerant as they are inoculated from animal excrements, wastewater treatment sludge or sewage slurry under atmospheric conditions.

## 1.4 Objectives of the study

Two-stage high pressure anaerobic digestion systems have been recently described in literature for operating pressures up to 10 bar [44, 52]. To inject gas into regional and transnational pipelines, even higher pressure loads from 60 to 150 bar are necessary. Consequently, the novel approach of this study was to develop and test a two-stage high pressure anaerobic digestion system at operating pressures up to 100 bar; thereby reducing the pressure boosting costs for injecting the biomethane into transnational gas grids. To gain a better understanding of this innovative technology, lab-scale batch- and continuously operated test rigs were developed and installed at the State Institute of Agricultural Engineering and Bioenergy in Hohenheim, Germany. The focus was set in this study on the methane reactor fed with hydrolysate from batch production. The aims of the conducted experiments were divided into three subtasks:

Introduction

*Process stability: Influence of $CO_2$ partial pressure on pH-value during anaerobic digestion*

For measuring the process stability in anaerobic digestion the pH-value is an important parameter. Due to the significant effect of pH-value on the digestion process and a limited optimal pH range between 6.0 and 7.5 for anaerobic digestion, an increased attention should be paid on the behavior of the pH-value under pressure. In contrast to common biogas stations operated under atmospheric pressure, the pH-value at high operating pressures is highly affected by the dissolved $CO_2$. For this reason, the effect of $CO_2$ partial pressure on the pH-value during anaerobic digestion at different initial pressure levels (10, 20, 30 bar) was examined. In addition, the effect of pressure during anaerobic digestion on organic degradation was analyzed, to investigate the effect of pressure during anaerobic digestion.

*Biogas production: Effect of pressure on production kinetics and specific methane yield*

After proving the process stability of anaerobic digestion, the research focused on the pressure effects on the production kinetics and specific methane yields (SMY). By comparing the production kinetics or SMYs, a potential negative impact of the high operating pressures on the microbial activity could be detected. In order to explore the effect of pressures required for gas injection into transnational pipelines, the performance of the pressurized methane reactors was studied at pressures up to 100 bar.

*Transferability: Performance of a continuously operated methane reactor*

Previous research studies showed that high pressure anaerobic digestion up to 100 bar is technical feasible under batch conditions. However, for future applications, research on the performance of a continuously operated high pressure methane reactor is essential. For this purpose, experiments with a continuously operated methane reactor with pressures up to 50 bar were conducted for the first time. The performance of this reactor and important process parameters for further improvement of the system were investigated.

## 1.5 Structure of the thesis

Within the framework of this thesis, first, the effect of $CO_2$ partial pressure on the pH-value during anaerobic digestion was investigated in batch experiments (10, 20 and 30 bar) to evaluate the process stability at high pressures [Publication 1: Title: "Effects of high-pressure anaerobic digestion up to 30 bar on pH-value, production kinetics and specific methane yield"]. In a second step, the study focused on the gaseous phase, estimating the influence of even higher pressures on the production kinetics and specific methane yields in batch rigs (1, 50, 100 bar) [Publication 2: Title: "High-pressure anaerobic digestion up to 100 bar: influence of initial pressure on production kinetics and specific methane yields"]. Finally, the performance of a continuously operated pressurized methane reactor was proven in 10, 25 and 50 bar test runs [Publication 3: Title: "Influence of pressures up to 50 bar on two-stage anaerobic digestion"].

## 2 Publication 1: Effects of high-pressure anaerobic digestion up to 30 bar on pH-value, production kinetics and specific methane yield

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## Abstract

The production of biogas for combined heat and power generation represents a common method in Germany. An alternative is the purification of biogas to biomethane and injection into the natural gas grid to decouple gas production from usage in terms of space and time. The concept of pressurized two-stage anaerobic digestion integrates biogas production, upgrading and pressure boosting within one process. The increasing solubility of $CO_2$ in process liquid at high pressures results in high methane contents in gaseous phase and in drop of pH. To investigate the effects of high initial pressures (10, 20, 30 bar) and dissolved $CO_2$ on pH-value, production kinetics and specific methane yields, pressurized batch methane reactors were built up. Additionally, a method for indirect measuring of pH-value was determined. The results of the experiment showed a decrease in pH from 7 to 6.31 (10 bar) and 6.25 (30 bar). Furthermore, neither a significant influence of initial pressures on the pressure increase nor on the degradation of organics and the specific methane yields was observed. However, the results show that anaerobic digestion at high pressures up to 30 bar might be a promising alternative to post purification and pressure boosting applications.

Publication 1: Lemmer et al., 2017

# Effects of high-pressure anaerobic digestion up to 30 bar on pH-value, production kinetics and specific methane yield

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## ARTICLE INFO

## ABSTRACT

The production of biogas for combined heat and power generation represents a common method in Germany. An alternative is the purification of biogas to biomethane and injection into the natural gas grid to decouple gas production from usage in terms of space and time. The concept of pressurized two-stage anaerobic digestion integrates biogas production, upgrading and pressure boosting within one process. The increasing solubility of $CO_2$ in process liquid at high pressures results in high methane contents in gaseous phase and in drop of pH. To investigate the effects of high initial pressures (10, 20, 30 bar) and dissolved $CO_2$ on pH-value, production kinetics and specific methane yields, pressurized batch methane reactors were built up. Additionally, a method for indirect measuring of pH-value was determined. The results of the experiment showed a decrease in pH from 7 to 6.31 (10 bar) and 6.25 (30 bar). Furthermore, neither a significant influence of initial pressures on the pressure increase nor on the degradation of organics and the specific methane yields was observed. However, the results show that anaerobic digestion at high pressures up to 30 bar might be a promising alternative to post purification and pressure boosting applications.

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## 1. Introduction

Biogas, as a carbon dioxide ($CO_2$) neutral energy source, is produced by anaerobic digestion of biodegradable substrates. In industrial biogas plants in Germany, the produced biogas has a methane ($CH_4$) content of 55%–60% depending on the initial substrates [1] and is commonly used for combined heat and power generation. Due to the high content of $CO_2$ in the raw gas, the usage possibilities of biogas are limited to the low calorific value. For further applications, the $CO_2$ content of biogas has to be reduced significantly. After upgrading the raw biogas by commonly used technologies such as amine and pressure water scrubbing, pressure swing adsorption (PSA) or gas separation membranes [1] followed by cost-intensive compression [2]. This gas can be fed into the natural gas grid if the relevant injection regulations are fulfilled: In Germany, these regulations are mainly determined by the technical guidelines G 260 and G 261 issued by the Deutscher Verein des Gas- und Wasserfaches e. V. (DVGW) [3,4]. Alternatively, the purified biogas can be used as fuel for vehicles [5]. The high energy demand

of the upgrading units [6] may prevent further expansion of these technologies due to economic reasons.

Biogas stations are normally run nearly under ambient pressure. Due to typically used low-pressure gas storage systems, the maximum operating pressure of biogas stations is 10 mbar above the local atmospheric pressure. Also the microorganisms in anaerobic digestion, which are introduced into the system by added liquid or solid manure, sewage sludge or wastewater treatment sludge, are normally adapted only to ambient pressure. These microorganisms involved in the anaerobic digestion (AD) process can be subdivided into three groups of which the first two are primary and secondary fermenting bacteria. In the last step of AD, methanogenic *Archaebacteria*, which are present on earth since approximately 3.8–4.1 billion years [7], are forming methane, carbon dioxide and water. These methanogenic microorganisms can also be found in a diversity of extreme habitats like marine sediments, digestive and intestinal tracts of animals, as well as in geothermal springs and both shallow and deep-sea hydrothermal vents [8]. In contrast to piezosensitive strains, most of them are piezotolerant or even piezophilic strains, which means that they are able to grow under high pressures or reach their optimal growth rates at pressures considerably above atmospheric pressure [9].

Examples of such piezophilic microorganisms (bacteria and archaea) were isolated in deep ocean waters at pressures of 100 bar to 1030 bar [10]. Other studies from Bernhardt et al., 1988 mentioned enhancing growth rates of *Methanococcus thermolithotrophicus* at high pressures up to 500 bar [11].

This pressure tolerance of methanogenic microorganisms provides the theoretical background for high pressure anaerobic digestion. In pressurized AD, the operating pressure inside the methane reactor is autogeneratively raised by microbial biogas production. In this process, the reactors's gas outlet is opened only if the aimed operating pressure is achieved. By running an AD process under high operating pressures, the formed $CO_2$ is partially dissolved in the liquid digestate and can be removed by the effluent from the reactor. Due to the higher solubility of $CO_2$ compared to methane ($CH_4$) [12—14], the $CH_4$ content in the gaseous phase can be increased. Previous research in two-stage high-pressure anaerobic digestion show that $CH_4$ contents of up to 87% at 5.9 bar in continuous operation and 95% at pressures up to 90 bar in batch tests can be achieved [15,16]. By the integration of biogas production, purification and pressure boosting within this novel process, the cost for the post-production biogas upgrading and pressure adjustment for grid injection can be substantially reduced.

Nevertheless, pressurized AD is a challenging process. The increased $CO_2$ partial pressures in high-pressure digestion systems are leading to an augmented formation of hydrogen carbonate, thus dropping the pH-value in the reactor. Previous studies from Chen et al., 2014 on continuous pressurized two stage anaerobic digestion showed a significant drop of the pH-value from 7.2 to 6.5 by raising the pressure from 1 to 9 bar, without any additional pH-adjustment [17]. Lie et al., 2008 mentioned an optimal range of pH in anaerobic digestion of 6.5—7.5, depending on substrate and digestion technique [18]. At even higher operating pressures, higher partial pressures of $CO_2$ and lower pH-values are assumed. Therefore, the pH monitoring plays an essential role in high-pressure digestion systems.

For pH measuring, standard pH sensors with glass electrodes and gel-filled reference electrolytes are available for pressures up to 10—16 bar. At higher pressures, special adapted pH sensors [19—21] are needed which are more expensive. Although for further full-scale applications, the significance of pH measuring at one certain point is limited. For that reason, an indirect method for the prediction of pH-value under different working conditions is of great interest. This method, described in a study from Lemmer et al., 2015 considers the ion concentration in the liquid and estimates the

dissolved $CO_2$ by its solubility considering all relevant environmental conditions in the reactor [15].

In this study the effect of high operating pressures up to 30 bar in AD systems on the pH-value in the digestate and its subsequent influence on the produced biogas quantity and quality were examined. Therefore, lab-scale batch experiments were performed at the State Institute of Agricultural Engineering and Bioenergy (University of Hohenheim). Additionally, the influence of different operation pressures on the substrate's pH-value and the method for indirect measuring of pH-value was determined by the DVGW Research Centre at the Engler-Bunte-Institute of Karlsruhe Institute of technology (KIT).

## 2. Material and methods

### 2.1. Reactors

The used AD system consists of three high-pressure batch reactors (BR-500, Berghof, Eningen, Germany) with a volume 0.64 L each, shown in Fig. 1. For temperature control the reactors were heated in a water bath by a thermostat (ED, Julabo, Seelbach, Germany). Each reactor was equipped with one valve (V1) for gas inlet and a double-valve system (V2, V4) for the gas outlet. Furthermore, liquid samples were taken by a double-valve system with an immersion tube (V3, V5). Parameters such as pressure (DMU 01, Error ± 1% FSO, Afriso, Gueglingen, Germany) and temperature (PT100, Berghof, Eningen, Germany) were measured in each reactor. pH-values were measured by high pressure pH sensors (Polilyte Plus XP VP 120, Hamilton, Reno, USA), which had a pressure range between 0 and 50 bar at temperatures up to 60 °C. For measuring the temperature of the water bath a TMR31 was used (Endress + Hauser, Error ± 0.15 K, Weil am Rhein, Germany). The air pressure (ALD-I, Error ± 1.5% of final value, S + S Regeltechnik, Nuernberg, Germany) and air temperature (KFTF-35, Error ± 0.5 K, S + S Regeltechnik, Nuernberg, Germany) were logged during the measurements.

### 2.2. Experimental procedure

At the beginning, each reactor was filled with a mixture (0.55 L, pH 7.1 under ambient pressure) made of hydrolysate from leach-bed-reactors and effluent from an anaerobic filter, both run under atmospheric conditions. The hydrolysate of the mixture was produced in four acidogenic-leach-bed-reactors with a volume of 50 L
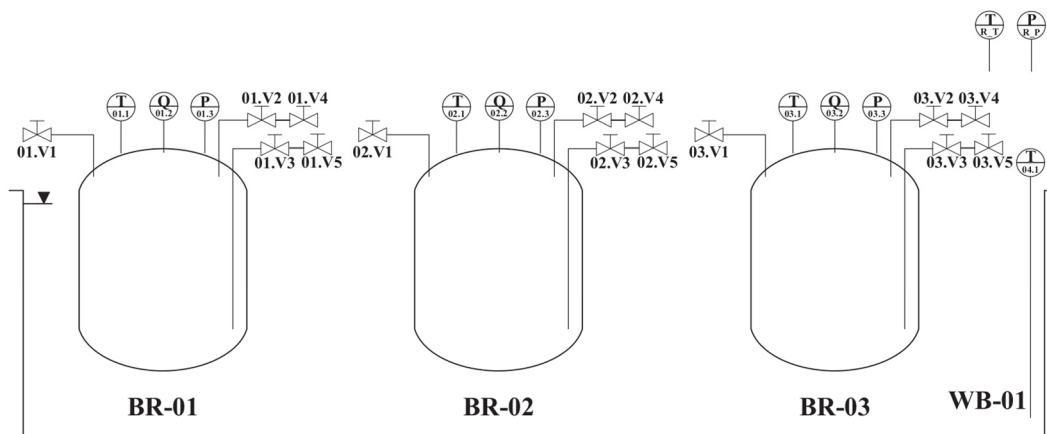


**Fig. 1.** Piping and instrumentation diagram of the high-pressure batch anaerobic digestion system.

each, operated at 55 °C as described by Chen et al., 2014 [17]. They were fed with a substrate mixture of 4.3 kg (fresh mass) grass and 4.8 kg (fresh mass) maize silage [22] from the Field-test station of the University Hohenheim (Unterer Lindenhof, Eningen, Germany). The organic dry matters (ODM) of grass and maize silage were 309.61 ± 3.54 g kg$^{-1}$ and 343.75 ± 34.86 g kg$^{-1}$, respectively. This hydrolysate was mixed up with the effluent from an anaerobic filter for inoculation and for the adjustment of starting pH-value.

The soluble chemical oxygen demand (SCOD) concentration of the mixture was 8.8 ± 0.8 g L$^{-1}$ and the temperature in the water bath was at 37.7 ± 0.1 °C. Three different initial pressures (10, 20, 30 bar) were tested with three repetitions each. For initial inertisation of the reactors and for applying initial pressure levels, nitrogen (N$_2$) was used. After 35 days, no more pressure increase was observed and gas and liquid samples were extracted from the reactors with 100 ml high pressure syringe (SYR H-CX, ILS, Stuetzerbach, Germany). To determine the gas volume, the gas in the reactor was released into the gas bags (TECOBAG, Tesseraux, Buerstadt, Germany) for 1 h, as the gases dissolved in the liquid could become undissolved in mean time.

### 2.3. Analytical methods and data acquisition

In this study, pressure, temperature and pH-value of each reactor was monitored and logged via LabView (National Instruments, Austin, USA). For data acquisition a CompactDAQ controller (National Instruments, Austin, USA) was used. The composition of the produced gas was measured under atmospheric pressure at the end of each run (MicroGC 3000, Inficon, Bad Ragaz, Swiss) and the quantity by a 100 ml high pressure syringe (SYR H-CX, ILS, Stuetzerbach, Germany). The reactor liquid was analyzed before and after the run for volatile fatty acids, content of sugar, alcohol, total carbon (TC), total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN) and SCOD. The concentration of acetate, propionate, n- and iso-butyrate, n- and iso-valerate and capronate was determined with a capillary column gas chromatography (GC 2010 Plus + Autoinjector AOC-20, Shimadzu, Kyoto, Japan). For measuring D/L-lactic acid, formic acid, sucrose, glucose, fructose, ethanol and propylene glycol a high pressure liquid chromatography (HPLC, Bischoff, Leonberg, Germany) was used. TC, TOC, IC and TN were analyzed with a TOC/TNb analyser (Analytik Jena AG Type multi N/C 2100). The volatile fatty acids (VFA) (FOS in the German technical literature), total inorganic carbon/alkalinity (TIC) and VFA/TIC ratio was measured with a titrator (Metrohm Type 785 DMP Titrino). For SCOD determination the samples were first filtered (0.2 μm) with a syringe filter holder followed by a cuvette test from Hach Lange (LCK 014) with a high temperature thermostat (HT 200 S) and a sensor array photometer (LASA 20).

### 2.4. Theory/Calculation

#### 2.4.1. pH-value
The pH-value was calculated using the charge balance between cations c and anions a.

$$\sum_a \tilde{m}_a z_a = \sum_c \tilde{m}_c z_c \qquad (1)$$

Mainly existing ions in the pressurized methane reactor are protons (H$^+$), carbonic acid (HCO$_3^-$), carbonate (CO$_3^{2-}$), volatile fatty acids, hydroxide ion (OH$^-$) and ammonia (NH$_4^+$). Other alkaline substances (all cations like calcium, magnesium, potassium, sodium, alcohols) in the methane reactor were measured once and estimated to be the same like Chen et al., 2014 mentioned approximately 0.115 mol L$^{-1}$ [17]. The value was validated from the data of a previous research study from Chen et al., 2014 [17].

$$\tilde{m}_{HCO_3^-} + 2 \cdot \tilde{m}_{CO_3^{2-}} + \tilde{m}_{vfa^-} + \tilde{m}_{OH^-} = \tilde{m}_{H^+} + \tilde{m}_{NH_4^+} + \tilde{m}_{Base^+} \qquad (2)$$

$$pH = -log(a_{H^+}) = -log\left(\frac{\tilde{m}_{H^+} \cdot \gamma_{H^+}}{\tilde{m}_i^0}\right) \qquad (3)$$

Every term is affected by the concentration of H$^+$-ions and can be described by the acid dissociation constant K$_s$ as follows:

$$HA \leftrightarrow H^+ + A^- \qquad (4)$$

$$K_s = \frac{\tilde{A}^- \cdot \tilde{H}^+}{\widetilde{HA}} \cdot \frac{\gamma_{A^-} \cdot \gamma_{H^+}}{\gamma_{HA}} \qquad (5)$$

In the definition of the acid dissociation constant K$_s$, HA is the acid, A$^-$ its conjugated base in water and γ the respective activity coefficient. All acids measured with a capillary column gas chromatography or HPLC were standardized to acetic acid and named with "HAc". Also self-ionization of water was taken into account to calculate $\tilde{m}_{OH^-}$. Equation (2) can be solved for $\tilde{m}_{H^+}$ and the pH-value can be calculated by Equation (3).

Another contrary approach was based on sum parameters like VFA (FOS in the German technical literature) by titration (Equation (6)), where H$_2$SO$_4$ consumption was measured up to pH-value of 5 (TIC) and from 5 to 4.4 (VFA). The TIC-value reflects the buffer capacity of the carbonate buffer system and was given in mg CaCO$_3$/L [23–25].

The dissolved amount of CO$_2$ can either be calculated or measured. Most methods and analyzers degas the liquid and measure the volume of the gas. However, fermentation liquid has a high buffer capacity and depending on the pH-value, most CO$_2$ remains dissolved in the liquid under atmospheric pressure. The TIC value is a parameter for the dissolved CO$_2$ and several forms of carbonate in the liquid. In a determination with titration, it appears to be appropriate to use TIC as "total alkalinity of carbonates" [24].

$$\text{Sum parameter}: \tilde{m}_{TIC} + \tilde{m}_{HS^-} + \tilde{m}_{VFA} + \tilde{m}_{OH^-}$$
$$= \tilde{m}_{H^+} + \tilde{m}_{TN} + \tilde{m}_{Base^+} \qquad (6)$$

$$\text{Acetic acid standardization}: \tilde{m}_{TIC} + \tilde{m}_{HS^-} + \tilde{m}_{HAc} + \tilde{m}_{OH^-}$$
$$= \tilde{m}_{H^+} + \tilde{m}_{TN} + \tilde{m}_{Base^+}$$
$$(7)$$

The dissolved CO$_2$ couldn't be easily detected with HPLC, so that Equation (8) represents the standard liquid analysis results without taking the dissolved CO$_2$ into account.

$$\text{Dissolved CO}_2 \text{ excluded}: \tilde{m}_{HS^-} + \tilde{m}_{HAc} + \tilde{m}_{OH^-}$$
$$= \tilde{m}_{H^+} + \tilde{m}_{TN} + \tilde{m}_{Base^+} \qquad (8)$$

pH-value can be calculated with Equation (3) and with dissociation constant of ammonia, hydroxide ion that was described by Chen et al., 2014 and Lemmer et al., 2015 [15,17], Equations (6)–(8) can be solved. As described above, dissolved amount of CO$_2$ can be detected via titration at atmospheric pressure. Under reaction conditions, highly pH sensitive CO$_2$ and its ions needs to be considered.

At the DVGW-EBI the effect of components in the fermentation liquid on the solubility of methane and carbon dioxide was investigated. The solubility can be described by Henry's law.

$$H_{i,lm} = \lim_{x_i \to 0} \frac{p_i}{x_{i,lm}} \qquad (9)$$

where, $x_i$ represents the mole fraction of gas i in the solvent lm and its partial pressure $p_i$. Henry's law only considers the physical dissolved gases but according to the pH-value carbonic acid reaction with water cannot be ignored [26]. This leads to an apparent Henry coefficient with a temperature dependent Henry correlation [27], pH-value, the activity coefficient $\gamma_0$ and the equilibrium coefficient $K_{CO_2}$ for the first and with the additional second dissociation reaction.

$$H^*_{CO_2,ely}(T,pH) = H_{CO_2,H_2O}(T) \cdot \frac{\gamma_0}{\gamma_{+/-}}$$

$$\cdot \left[ 1 + \frac{K_{S,CO_2,1}}{\left(10^{-pH}\frac{mol}{kg}\right)} \frac{1}{\gamma_{+/-}} + \frac{K_{S,CO_2,1} \cdot K_{S,CO_2,2}}{\left(10^{-pH}\frac{mol}{kg}\right)^2} \frac{1}{\left(\gamma_{+/-}\right)^2} \right]^{-1} \qquad (10)$$

Due to the fact that with increasing salt concentration, gas solubility often decreases, this "salting-out" effect was considered in Equation (10) with $\gamma_0$ and can be described with the Sechenov relation and model parameters measured by Weisenberger et al. [28]. Activity coefficient for ions $\gamma_{+/-}$ was calculated with Pitzer-Debye-Hückel [29]. The temperature dependency for $CO_2$ ($H_{CO_2,H_2O}(T)$) can be described with the Peng-Robinson equation [27] and for $CH_4$ with Jaeschke et al. [23]. Results of solubility measurements and comparison with solubility calculations as described in Equation (10) are published [15,17,22,26,30].

### 2.4.2. Production kinetics

For the experiments the ideal gas equation partial has to be adapted to the behavior of real gases. The compressibility of the gas cannot be disregarded as experiments run at pressures of up to 30 bar.

$$p_i \cdot V = Z_i \cdot n_i \cdot R \cdot T \qquad (11)$$

In Equation (11), the ideal gas law where $p_i$ is the partial pressure of gas i (bar), V as volume (L), $n_i$ the amount of substance i (mol), R as universal gas constant (J mol$^{-1}$ K$^{-1}$) and T as temperature (K), is extended by the compressibility factor $Z_i$. $Z_i$ is calculated with the equations of SGerg-88 [31] for $CH_4$ and Peng-Robinson [27] for $CO_2$. At standard pressure and temperature (STP: p = 1.013 bar, T = 273.15 K), Z = 1 for any gas and decreases for both applied gases with rising pressure.

The pressure increase was calculated by the measured pressures in the reactors which are temperature corrected by using the ideal gas equation with a constant volume.

$$p_n = \frac{\left(\frac{p_m(t)}{Z(t) \cdot T_m} - \frac{p_1}{Z_1 \cdot T_1}\right) \times T_n}{SCOD_{added}} \qquad (12)$$

where $p_n$ is the temperature corrected pressure increase (bar g$^{-1}$ SCOD), calculated by the difference between the quotient of the measured pressure $p_m$ (bar) and temperature $T_m$ (°C) and at the minimum pressure $p_1$ and temperature $T_1$ of the experiment divided by the SCOD added (g). The temperature variations of 37.7 ± 0.1 °C during the experiments are compensated by multiplying with the desired temperature ($T_n$) of 37 °C. The compressibility factor Z could be neglected due to the fact that the initial pressure was adjusted with a high amount of $N_2$ in comparison to $CH_4$ and $CO_2$.

The degradation kinetics are estimated by the cumulative pressure increase which was fitted to the modified Gompertz equation [32] by assuming that pressure increase is a function of bacterial growth.

$$M = P \times exp\left\{ -exp\left[\frac{R_m \times e}{P}(\lambda - t) + 1\right]\right\} \qquad (13)$$

M is the cumulative pressure increase (bar g$^{-1}$ SCOD), P as pressure increase potential (bar g$^{-1}$ SCOD), $R_m$ is maximum daily pressure increase rate (bar d$^{-1}$ g$^{-1}$ SCOD), t as duration of run (d) and $\lambda$ as duration of the lag phase time (d). The constants P, $R_m$ and $\lambda$ are calculated by a non-linear regression. With the first derivative of Equation (3) the daily pressure increase rate over time was determined to find the point in time of maximum daily pressure increase rate (t = $t_{max}$). The Kruskal-Wallis test was used for statistical analyses performed by the statistical software R [33].

### 2.4.3. Specific methane yield

To calculate the amount of $CH_4$ produced Equation (14) was used, incorporating the experimental pressure increase, temperature and reactor gas volume. The accumulated amount of mole methane $n_{gas,CH_4}$ was calculated as follows, regarding the measured parameters pressure $p_m$ (bar), gas quality $y_{CH_4}$, temperature $T_m$ (K), and the known gas volume above the liquid phase $V_{gas}$ (L).

$$n_{gas,CH_4} = \frac{p_m \cdot y_{CH_4} \cdot V_{gas}}{T_m \cdot R \cdot Z_{CH_4}} \qquad (14)$$

The specific methane yield (SMY) (L kg$^{-1}$) was calculated by Equation (15) with the accumulated amount of mole methane in gas $n_{gas,CH_4}$ (mol) after decompression at standard temperature $T_{STP}$ (K) and pressure $p_{STP}$ (bar) related to the input of SCOD (g L$^{-1}$).

$$SMY = \frac{n_{gas,CH_4} \cdot R \cdot T_{STP}}{p_{STP} \cdot SCOD_{added}} \qquad (15)$$

## 3. Results and discussion

### 3.1. Process stability

The compositions of initially added liquid feed mixture and the effluent at the end of the experiment after 35 days trial time are summarized in Table 1. All originally added alcohols and sugars as well as n-butyric acid and lactic acid are completely degraded and could not be detected in the effluent anymore. In contrast, a slight accumulation of iso- and n-valeric acid and low concentrations of acetic acid could be observed in the effluent. It must be noted that propionic acid concentration increase during the experiment, irrespective of the applied initial pressures. The TOC in the liquid decreased from 3.153 g L$^{-1}$ till 1.473 g L$^{-1}$ after the experiment. The IC increased slightly from 0.749 g L$^{-1}$ to 1.107 g L$^{-1}$ (20 bar) or to 1.287 g L$^{-1}$ (30 bar), respectively. The total carbon and nitrogen content (TC, TN) as well as SCOD content decreased during the process. The VFA/TIC ratio declined from 0.521 to 0.366. The SCOD degradation grade varies between 52.5± 1.1% at 10 bar and 49.9± 6.9% at 30 bar. All measured variations of the organic acids, TOC, IC, TC, TN, VFA/TIC ratio and SCOD in the effluent of the three different initial pressures were not significant (p > 0.05).

Compared to other studies, certain concentrations of acids were still detectable in the effluent after a run of 35 days. Merkle et al., 2016 mentioned no significant concentrations of propionic acid, butyric acid and valeric acid after decompressing of pressurized batch experiments up to 100 bar [34]. In contrast, studies by

**Table 1**
Results of the analyses of volatile fatty acids, carbons, nitrogen, VFA/TIC and SCOD to describe the substrate properties of the feed mixture and the degradation.

| Parameters | Feed mixture | Effluent composition at operating pressure | | |
|---|---|---|---|---|
| | | 10 bar | 20 bar | 30 bar |
| acetic acid (g kg$^{-1}$) | 0.733 ± 0.034 | 0.068 ± 0.033 | 0.056 ± 0.039 | 0.159 ± 0.185 |
| propionic acid (g kg$^{-1}$) | 0.019 ± 0.001 | 1.483 ± 0.132 | 1.193 ± 0.803 | 1.058 ± 0.930 |
| iso-butyric acid (g kg$^{-1}$) | 0 | 0 | 0 | 0.006 ± 0.005 |
| n-butyric acid (g kg$^{-1}$) | 0.022 ± 0.002 | 0 | 0 | 0 |
| iso-valeric acid (g kg$^{-1}$) | 0 | 0.018 ± 0.006 | 0.020 ± 0.013 | 0.032 ± 0.017 |
| n-valeric acid (g kg$^{-1}$) | 0 | 0.007 ± 0.006 | 0.006 ± 0.007 | 0.004 ± 0.006 |
| lactic acid (g kg$^{-1}$) | 2.483 ± 0.161 | 0 | 0 | 0 |
| glucose (g kg$^{-1}$) | 0.083 ± 0.076 | 0 | 0 | 0 |
| ethanol (g kg$^{-1}$) | 0.283 ± 0.029 | 0 | 0 | 0 |
| TOC (g L$^{-1}$) | 3.153 ± 0.328 | 1.473 ± 0.244 | 1.600 ± 0.197 | 1.580 ± 0.333 |
| IC (g L$^{-1}$) | 0.749 ± 0.073 | 1.123 ± 0.103 | 1.107 ± 0.083 | 1.287 ± 0.186 |
| TC (g L$^{-1}$) | 3.900 ± 0.397 | 2.597 ± 0.267 | 2.703 ± 0.265 | 2.680 ± 0.306 |
| TN (g L$^{-1}$) | 0.951 ± 0.040 | 0.801 ± 0.082 | 0.811 ± 0.075 | 0.801 ± 0.084 |
| VFA/TIC | 0.521 ± 0.045 | 0.366 ± 0.038 | 0.368 ± 0.022 | 0.394 ± 0.054 |
| SCOD (mg L$^{-1}$) | 8825 ± 788 | 4191 ± 433 | 4428 ± 507 | 4419 ± 760 |

Lindeboom et al., 2011 also described significant concentrations of propionate, butyrate and valerate after decompressing 3 bar and 31 bar pressure experiments [16].

The accumulation of high amounts of propionic acid can be the reason of high amounts of lactic acid in the leachate. Zellner et al., 1994 described a shift in the degradation pathway of lactic acid from acetic acid to propionic acid at high concentrations of lactic acid of about 40 mM ≙ 3.632 g L$^{-1}$ [35]. The measured valeric acids in the effluent might be formed by the degradation of amino acids like Arginine, Leucine, Iso-leucine and Proline [36], which could not be measured in the mixture added.

### 3.2. pH-value

The pH-value curves of three different initial pressures (10, 20, 30 bar) and the standard deviations (sd) of the repetitions over 35 days are shown in Fig. 2. A drop of pH-value to 6.31 ± 0.04 after 4.16 ± 0.82 days (10 bar) and to 6.25 ± 0.03 after 3.16 ± 1.47 days (30 bar) was observed. After these initial drops, the pH-values increased again and reached a constant value of 6.57 ± 0.07

(10 bar) and 6.48 ± 0.05 (30 bar) after 35 days. These measured differences of pH-value by different initial pressures were not statistically significant ($p > 0.05$).

The measured and calculated pH-values after 35 days of three different initial pressures (10, 20, 30 bar) are shown in Fig. 3. The pH-values were calculated by sum parameter VFA (FOS in the German technical literature) and total alkalinity (TIC), by sum parameter acetic acid-equivalent (HAc) and total alkalinity (TIC) or by liquid analysis (LIQ), excluded the dissolved $CO_2$. The pH-values calculated by VFA, TIC were between 6.49 ± 0.1 (10 bar) and 6.34 ± 0.09 (30 bar) and by HAc, TIC between 6.55 ± 0.09 (10 bar) and 6.39 ± 0.1 (30 bar). The estimated pH-values by LIQ were between 9.48 ± 0.06 (10 bar) and 9.2 ± 0.12 (30 bar). No significant differences ($p > 0.05$) of the measured and the calculated pH-value by VFA, TIC and by HAc, TIC could be determined. The measured differences between measured or calculated pH-value by VFA, TIC and by HAc, TIC and the pH-value calculated by LIQ were statistically significant ($p < 0.05$).

The results of pH-value measurement showed no significant influence of initial pressure on the pH-value. As a result of the
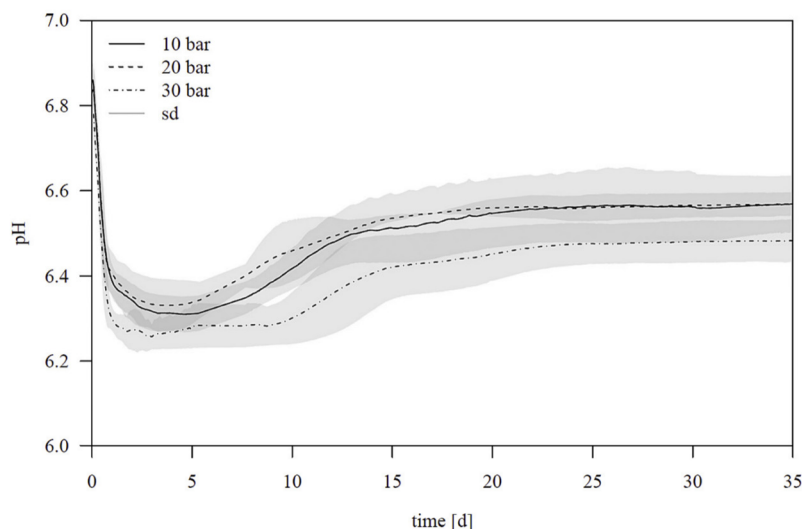


**Fig. 2.** pH-value curves for three different initial pressures (10, 20, 30 bar), standard deviation (sd) of three repetitions in grey, overlaps of sd in darkgrey.
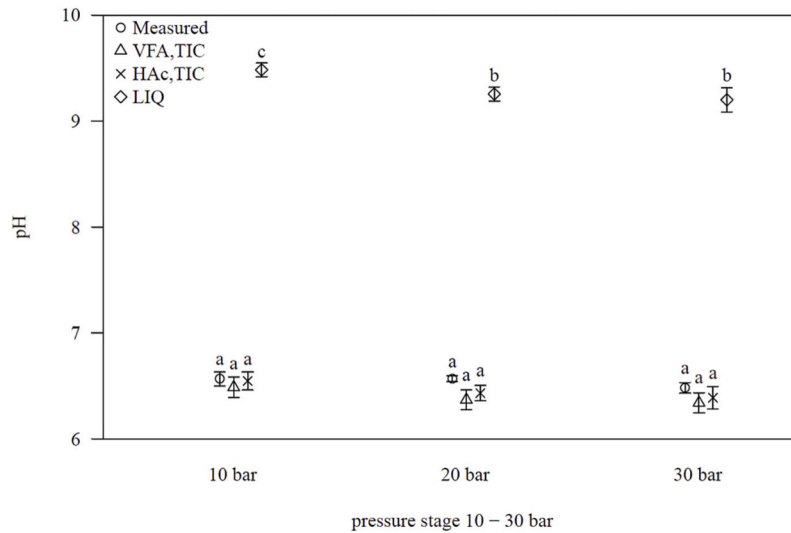
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**Fig. 3.** Measured and calculated pH-value for three different initial pressures (10, 20, 30 bar). The pH-value calculated either by volatile fatty acids (VFA) and total alkalinity (TIC), by acetic acid equivalent (HAc) and total alkalinity (TIC) or by liquid analysis (LIQ). The significant differences among the pH-values are marked with different letters ($p < 0.05$, LSD test).

initial pressure adjustment by $N_2$, only comparatively low $CO_2$ partial pressures were reached, indicated by low $CO_2$ content of the produced gas. Contrary to this, previous studies showed a clear effect of pressure on the pH-value under continuous conditions [17,22,30]. pH-values calculated by sum parameters like VFA, HAc and taken dissolved amount of $CO_2$ in the liquid into account, represents an alternative to the direct measuring of pH under pressure, thus offering an interesting alternative to forecast the process stability as well as the gas quality under high operating pressures. The estimated pH by liquid analysis, without considering the dissolved $CO_2$ in the liquid, is relatively imprecise when all substances are not known.

### 3.3. Production kinetics

The effects of three different initial pressures on mean pressure increase and pressure increase rate are shown in Fig. 4. The mean measured values of three repetitions, the adapted curves calculated with the modified Gompertz equation, their first derivatives and the standard deviations (sd) of the repetitions are included. For the modified Gompertz equations, the coefficients of determination were between 99.7% and 99.8%. With an initial pressure of 10 bar, the highest pressure increase with $2.19 \pm 0.12$ bar $g^{-1}$ $SCOD_{added}$ was observed after 35 days. At higher initial pressures of 20 bar and 30 bar, the pressure increased by $1.97 \pm 0.07$ bar $g^{-1}$ $SCOD_{added}$ and $1.84 \pm 0.33$ bar $g^{-1}$ $SCOD_{added}$. The time of maximum daily pressure increase rate ($t_{max}$) was reached between $10.63 \pm 1.52$ days (10 bar) and $13.54 \pm 2.85$ days (30 bar). The measured differences of the pressure increases and times of the maximum pressure increase rate by different initial pressures were not statistically significant ($p > 0.05$), due to rising of standard deviation by increasing the operating pressure.(See Fig. 5).

The results of the degradation kinetics are shown in Table 2. There was only minor deviation in the pressure increase at different initial pressures. The pressure increase potential (P) ranged from $2.22 \pm 0.13$ bar $g^{-1}$ $SCOD_{added}$ (10 bar) to $1.84 \pm 0.25$ bar $g^{-1}$ $SCOD_{added}$ (30 bar). Significant differences between $3.98 \pm 1.15$ days (10 bar) and $7.59 \pm 1.86$ days (30 bar) could be observed in the

duration of the lag phase ($\lambda$). By increasing the initial pressures, the maximum daily pressure increase rate ($R_m$) has slightly diminished from $0.13 \pm 0.03$ bar $d^{-1}$ $g^{-1}$ $SCOD_{added}$ (10 bar) to $0.12 \pm 0.04$ bar $d^{-1}$ $g^{-1}$ $SCOD_{added}$ (30 bar). The measured differences of pressure increase potential and maximum pressure increase rate were not significant ($p > 0.05$).

The results of this analysis showed a visible but not a significant influence of initial pressure in the batch reactor on pressure increase and pressure increase rates. A slightly lower pressure increase at 30 bar might be a result of the fact, that the solubility of $N_2$ in the used percolate is unknown, which led to an underestimation of the pressure increase at high initial pressures, mentioned by Merkle et al., 2016 [34]. In pure water and at a temperature of 40 °C the solubility of nitrogen increased from 0.0054 mol $kg^{-1}$ at 10 bar to 0.0133 mol $kg^{-1}$ at 25 bar [37]. Due to the fact, that the batch reactors have not been stirred, it cannot be excluded that the liquid was not completely saturated with nitrogen at the beginning of the experiment and still dissolving in the liquid, counteracting the pressure increase by biogas production. Nonetheless the experiments showed similar results to studies from the University of Hohenheim and the University of Wageningen, where no detrimental harm on methanogens at even higher pressure was observed [16,34].

### 3.4. Gas quality and specific methane yield

The measured gas components and the gas volume of the gas samples taken after 35 days are shown in Table 3. By increasing the initial pressure, the $N_2$ content raised from $38.3 \pm 2.1\%$ at 10 bar to $61.6 \pm 2.2\%$ at 30 bar. This led to a decrease in $CH_4$ content by $39.3 \pm 1.1\%$ to $21.7 \pm 1.3\%$ and in the $CO_2$ content from $14.5 \pm 0.4\%$ to $8.7 \pm 0.7\%$. Other gases like hydrogen sulphide ($H_2S$) and $H_2$ have not been detected. Higher initial pressures resulted in an increase of the total gas volume from $2.18 \pm 0.02$ L to $4.14 \pm 0.21$ L, due to the higher initial $N_2$ volumes added at the beginning.

The decreasing $CH_4$ and $CO_2$ contents at high pressures were not related to a lower microbiological activity, but were a result of initial $N_2$, also mentioned by Merkle et al., 2016 [34].

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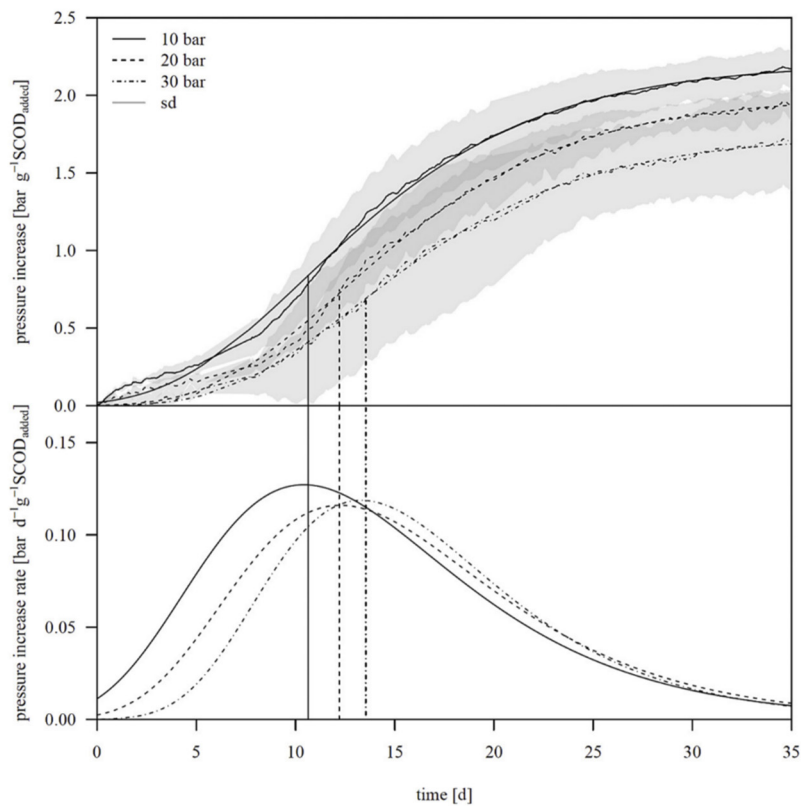
**Fig. 4.** Measured pressure increase curves and pressure increase rates per SCOD added over time and the Gompertz adaptions of three different initial pressures (10, 20, 30 bar) at 37 °C, standard deviation (sd) of three repetitions in grey, overlaps of sd in darkgrey.
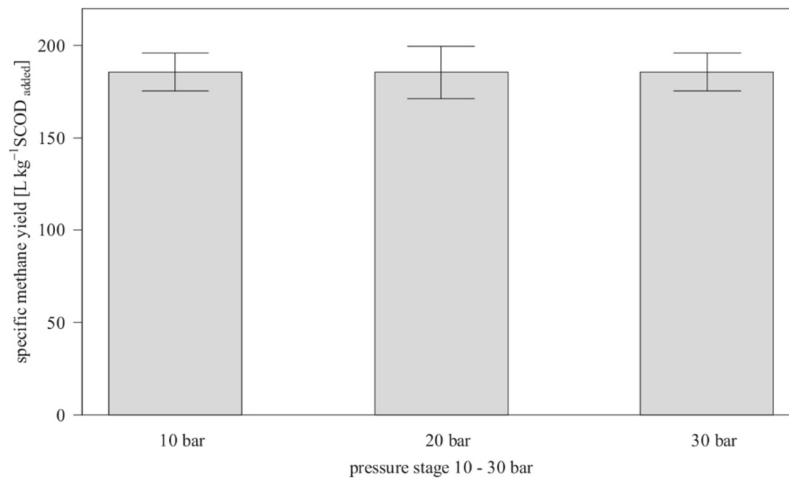


**Fig. 5.** Specific methane yield per kg SCOD added at T = 37 °C calculated from the gas analysis for three different initial pressures (10, 20, 30 bar), $p > 0.05$.

The specific methane yields (SMY) related to the $SCOD_{added}$ were calculated for three different initial pressures (10, 20, 30 bar) based on the gas analysis (Table 3) and are shown in Fig. 5. These SMY varied between $180 \pm 16$ L $kg^{-1}$ $SCOD_{added}$ at 10 bar and $185 \pm 14$ L $kg^{-1}$ $SCOD_{added}$ at 30 bar. The differences in SMY did not

meet the statistical significance ($p > 0.05$).

The results of these experiments showed no influence of initial pressure on the SMY. Studies from Merkle et al., 2016 till 100 bar also determined no influence of initial pressure on the SMY [34]. In contrast to these findings, Chen et al., 2014 reported a little impact

**Table 2**
Overview of calculated parameters of the Gompertz equation and the first derivative for three different initial pressure levels (10, 20, 30 bar). P = pressure increase potential per g SCOD added, $\lambda$ = duration of the lag phase time, $R_m$ = maximum daily pressure increase rate and $t_{max}$ = time of the maximum pressure increase per day. Significant differences in the mean are marked by different letters.

| Pressure levels | P (bar $g^{-1}$ $SCOD_{added}$) | $\lambda$ (d) | $R_m$ (bar $d^{-1}$ $g^{-1}$ $SCOD_{added}$) | $t_{max}$ (d) |
|---|---|---|---|---|
| 10 bar | $2.22 \pm 0.13^a$ | $3.98 \pm 1.15^a$ | $0.13 \pm 0.03^a$ | $10.63 \pm 1.52^a$ |
| 20 bar | $2.02 \pm 0.11^a$ | $5.86 \pm 0.64^{ab}$ | $0.12 \pm 0.02^a$ | $12.21 \pm 1.21^a$ |
| 30 bar | $1.84 \pm 0.25^a$ | $7.59 \pm 1.86^b$ | $0.12 \pm 0.04^a$ | $13.54 \pm 2.85^a$ |

**Table 3**
Results of the analyses of mean $N_2$ content, mean $CH_4$ content, mean $CO_2$ content and mean measured gas volume for three different initial pressures (10, 20, 30 bar).

| Initial pressure (bar) | Mean $N_2$ content (%) | Mean $CH_4$ content (%) | Mean $CO_2$ content (%) | mean measured gas volume (l) |
|---|---|---|---|---|
| 10 | $38.3 \pm 2.1$ | $39.3 \pm 1.1$ | $14.5 \pm 0.4$ | $2.18 \pm 0.02$ |
| 20 | $54.1 \pm 2.5$ | $28.2 \pm 0.1$ | $10.2 \pm 1.0$ | $3.18 \pm 0.14$ |
| 30 | $61.6 \pm 2.2$ | $21.7 \pm 1.3$ | $8.7 \pm 0.7$ | $4.14 \pm 0.21$ |

of pressure on the SMY under continuous conditions without adding $N_2$, working at pressures up to 9 bar [17,30].

## 4. Conclusions

This study examined the pressure effects on the anaerobic digestion, in terms of pH-values, production kinetics and specific methane yields by batch experiments. While operating the lab-scale batch reactors at pressures 10, 20 and 30 bar for 35 days, the experimental investigation showed no significant differences in the degradation of sugars, alcohols and organic acids were determined between the three different pressure levels. An accumulation of propionic acid, iso- and n-valeric acid could be observed after 35 days, not depending on the initial pressure.

The production and solution of $CO_2$ in the liquid caused a drop of the pH-value from 7 to $6.31 \pm 0.04$ after $4.16 \pm 0.82$ days (10 bar) and to $6.25 \pm 0.03$ after $3.16 \pm 1.47$ days (30 bar). During the following days of the experimental runs, the pH-values increased to a constant value of $6.57 \pm 0.07$ (10 bar) and $6.48 \pm 0.05$ (30 bar) after 35 days. It could be shown, that the indirect post calculation of pH-value by sum parameters like VFA, TIC gained by simple titration and considering the dissolved $CO_2$ in the liquid, represents an interesting alternative to the direct measuring of pH under pressure.

No significant influence of initial pressure on the pressure increase was observed. The initial pressure increased by $2.19 \pm 0.12$ bar $g^{-1}$ $SCOD_{added}$ (10 bar) and $1.84 \pm 0.33$ bar $g^{-1}$ $SCOD_{added}$ (30 bar), respectively. The maximum pressure increase rates ranged from $0.13 \pm 0.03$ bar $d^{-1}$ $g^{-1}$ $SCOD_{added}$ (10 bar) to $0.12 \pm 0.04$ bar $d^{-1}$ $g^{-1}$ $SCOD_{added}$ (30 bar) and varied only slightly. Higher initial pressures slowed down the digestion process slightly, shown by the days when the maximum pressure increase was achieved ($10.63 \pm 1.52$ days (10 bar) and $13.54 \pm 2.85$ days (30 bar)). The resulting specific methane yields varied between $180 \pm 16$ L $kg^{-1}$ $SCOD_{added}$ (10 bar) and $185 \pm 14$ L $kg^{-1}$ $SCOD_{added}$ (30 bar) and were not significantly different.

Additional research would be worthwhile to determine the influence of high pressures and decreasing pH-value on the microorganisms in the methane reactor by the dissolution of $CO_2$ in the liquid.

## Acknowledgements

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# 3   Publication 2: High-pressure anaerobic digestion up to 100 bar: influence of initial pressure on production kinetics and specific methane yields

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## Abstract

To ensure an efficient use of biogas produced by anaerobic digestion, in some cases it would be advisable to upgrade the biogenic gases and inject them into the transnational gas grids. To investigate biogas production under high-pressure conditions up to 100 bar, new pressure batch methane reactors were developed for preliminary lab-scale experiments with a mixture of grass and maize silage hydrolysate. During this investigation, the effects of different initial pressures (1, 50 and 100 bar) on pressure increase, gas production and the specific methane yield using nitrogen as inert gas were determined. Based on the experimental findings increasing initial pressures alter neither significantly, further pressure increases nor pressure increase rates. All supplied organic acids were degraded and no measurable inhibition of the microorganisms was observed. The results show that methane reactors can be operated at operating pressures up to 100 bar without any negative effects on methane production.

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# High-pressure anaerobic digestion up to 100 bar: influence of initial pressure on production kinetics and specific methane yields

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**ABSTRACT**

To ensure an efficient use of biogas produced by anaerobic digestion, in some cases it would be advisable to upgrade the biogenic gases and inject them into the transnational gas grids. To investigate biogas production under high-pressure conditions up to 100 bar, new pressure batch methane reactors were developed for preliminary lab-scale experiments with a mixture of grass and maize silage hydrolysate. During this investigation, the effects of different initial pressures (1, 50 and 100 bar) on pressure increase, gas production and the specific methane yield using nitrogen as inert gas were determined. Based on the experimental findings increasing initial pressures alter neither significantly, further pressure increases nor pressure increase rates. All supplied organic acids were degraded and no measurable inhibition of the microorganisms was observed. The results show that methane reactors can be operated at operating pressures up to 100 bar without any negative effects on methane production.

## Introduction

Following the introduction of the Renewable Energy Law in 2000,[1] Germany has recorded a steady increase in renewable energy generation. Today, in Germany 8928 biogas plants are in operation with an installed electric capacity of 4177 MW.[2] The biogas is predominantly used for combined heat and power generation. The available heat is mainly used for the heat demand of the plant as well as for hot water supply and heating of buildings.[3] In case of missing heat sinks in rural areas, the purification of biogas to biomethane and the injection into gas grids can be an alternative as it timely and spatially decouples the gas production and its utilization resulting in a higher overall energy utilization efficiency.[4,5]

Biomethane production as a carbon dioxide ($CO_2$) neutral energy source and its injection into the natural gas grid has high potential as it can take advantage of an already existing large, energy storage system. Biogas with a methane ($CH_4$) content of 55–60% [6] must be purified before it can be fed into the grid, in order to fulfil the necessary injection requirements determined by the technical guidelines G 260 and G 261 of the DVGW.[7,8] Furthermore, the gas has to be compressed to the required pressure level of the grid section.[8]

At present, the most commonly used upgrading technologies are amine scrubbing, water scrubbing, pressure swing adsorption (PSA) and gas separation membranes.[6] However, all of these methods have significant disadvantages. Amine scrubbing requires a large amount of additives and auxiliary chemicals, PSA often needs a preliminary desulphurization before and gas separation membranes are usually used only as partial flow filters.[6] Furthermore, some of these technologies generate waste and waste water that has to be treated and removed. After the purification process, the gas is generally at a low pressure and has to be compressed for grid injection which incurs high-energy costs.[9]

An alternative approach for the production of biomethane is pressurized two-stage anaerobic digestion.[10–14] This takes advantage of the technical separation of hydrolysis/acidification and acetogenesis/methanogenesis to guarantee optimum environmental conditions for each group of microorganisms.[13] In contrast to commonly applied non-pressurized two-stage anaerobic digestion processes in the literature,[15–20] the second process stage is run under elevated pressure up to 90 bar. In the process, methanogenic bacteria auto-generatively increase the pressure of the gas. It is possible to apply an internal scrubbing cycle, which allows $CO_2$ to discharge from the reactor. As $CO_2$ is more

soluble than methane,[21–23] this results in a higher $CO_2$ content in the liquid phase at increased pressures. Previous research in two-stage high-pressure fermentation has indicated that $CH_4$ contents of up to 87% are possible at operating pressures up to 5.9 bar in continuous operation using maize silage, and of up to 95% at pressures up to 90 bar in batch tests using wastewater.[13,24]

For future applications and longer transport routes, transnational gas grids with operating pressures of 60–150 bar [25] represent a possible alternative to international biomethane trading. The aim is to integrate biogas production, purification and pressure boosting in one process at operating pressures of 100 bar, so that the produced methane-rich biogas inside the methane reactor can be fed directly into the transnational gas grids. In comparison to the purification technologies that are frequently used, no further compression of the gas is required.

Research in the field of deep-sea microbiology has revealed pressure adapted bacteria and archaea. Piezophilic microorganisms (bacteria and archaea) are living in the deep ocean waters at pressures of 100–1030 bar.[26] The effects of pressure on different pressure sensitive processes such as motility and growth are described in the literature for pressures over 100 bar, using *Escherichia coli* as an example.[26]

The aim of this study was to investigate the effects of high-operating pressures up to 100 bar on biogas quantity and quality produced at the second stage methane reactor, using a mixture of grass and maize silage hydrolysate as substrate. The ability of the anaerobic microorganisms to adapt within 5 min to different pressure increases and possible microbiological inhibitions were investigated, too. Lab-scale pressure batch experiments

were performed at the University of Hohenheim and accompanying modelling work was carried out at the DVGW Research Centre at the Engler-Bunte-Institute of Karlsruhe Institute of technology (KIT) to evaluate the amount of $CH_4$ produced.

## Materials and methods

### Reactors

Figure 1 shows a piping and instrumentation diagram of the experimental set-up of the high-pressure batch anaerobic digestion system. The batch system consists of three high-pressure batch reactors (BR-500, Berghof, Eningen, Germany) with a volume of 0.64 L each. The reactors were heated in a water bath by a thermostat (ED, Julabo, Seelbach, Germany). Each reactor has a gas inlet (V1) and a double-valve system (V2, V4) for the gas outlet. Additionally, liquid samples can be extracted by a double-valve system with an immersion tube (V3, V5). In each reactor, the temperature (PT100, Berghof, Eningen, Germany) and pressure (DMU 01, Error ± 1% FSO, Afriso, Gueglingen, Germany) were measured. The temperature in the water bath was measured by a TMR31 (Endress +Hauser, Error ± 0.15 K, Weil am Rhein, Germany). The air temperature (KFTF-35, Error ± 0.5 K, S+S Regeltechnik, Nuernberg, Germany) and air pressure (ALD-I, Error ± 1.5% of final value, S+S Regeltechnik, Nuernberg, Germany) were logged during the measurements.

### Experimental procedure

Each reactor was initially filled with 0.5 L of hydrolysate mixture (pH 7.4) from an acidogenesis-leach-bed-
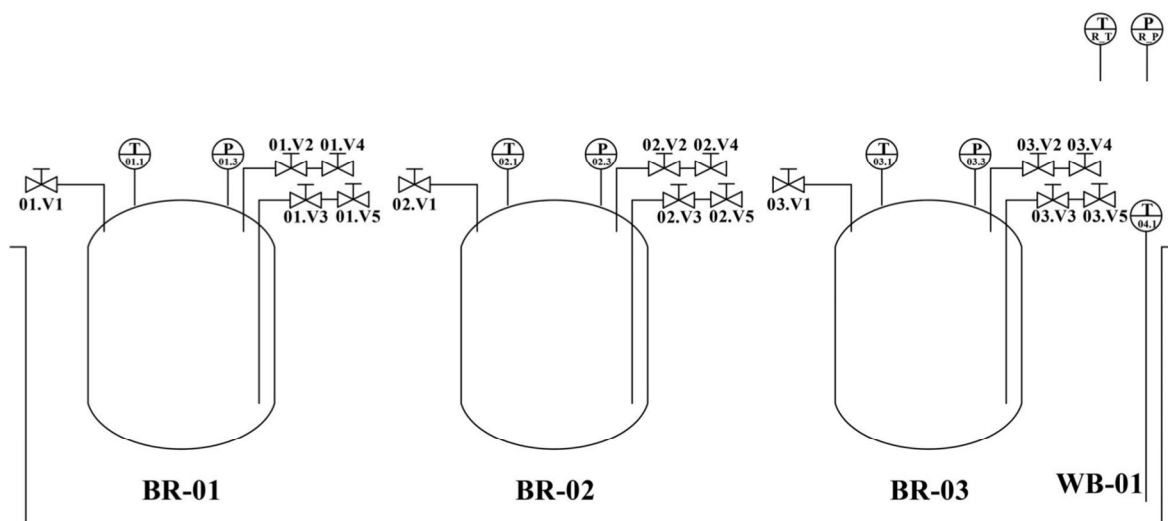


**Figure 1.** Piping and instrumentation diagram of the experimental setup of the high-pressure batch anaerobic digestion system.

reactor operated at 55°C described by Chen et al. [10] which was fed with a mixture of grass and maize silage,[14] and with effluent from an anaerobic filter both operated under atmospheric conditions.

The acidogenesis-leach-bed-reactor had a volume of 50 L, and was fed once with a mixture of 4.3 kg (fresh mass) grass and 4.8 kg (fresh mass) maize silage [14] from the Field-test station of the University Hohenheim (Unterer Lindenhof, Eningen, Germany). The organic dry matters of maize and grass silage were 343.75 ± 34.86 and 309.61 ± 3.54 g kg$^{-1}$, respectively. The effluent from an anaerobic filter was used as inoculum to simulate conditions similar to anaerobic filter with packings as well as to have a starting pH value above 7. Pre-studies in the lab with packings from the anaerobic filter in the batch reactors have shown no difference to the use of effluent instead.

The hydrolysate mixture had a chemical oxygen demand (COD) concentration of 3.9 ± 0.6 g L$^{-1}$ and a temperature in the water bath of 36.9 ± 0.3°C. Three different initial pressures (1, 50, 100 bar) were tested for three repetitions each. Nitrogen (N$_2$) was used for initial inertisation of the reactors and for applying initial pressure levels because of the fact that microorganisms do not use N$_2$ for their degradation kinetics. The initial pressures were applied within 5 min without any adapting phases. After 21 days, pressure increase was no longer measured and gas and liquid samples were taken. The samples were extracted from the reactors with a 100-mL high-pressure syringe (SYR H-CX, ILS, Stuetzerbach, Germany). For gas volume measurements, the reactors gassed once into a gas bag (TECOBAG, Tesseraux, Buerstadt, Germany) for one hour.

### Analytical methods and data acquisition

In this study, pressure and temperature of each reactor were monitored and data were logged via LabView (National Instruments, Austin, USA). A CompactDAQ controller (National Instruments, Austin, USA) was used for data acquisition. The gas composition was measured at atmospheric pressure with a micro GC (MicroGC 3000, Inficon, Bad Ragaz, Switzerland). The total amount of gas produced was determined with a gas meter (TG 20/5, Error ± 0.5%, Ritter, Bochum, Germany; temperature sensor: GTF, Error ± 0.03°C, Greisinger, Regenstauf, Germany; pressure sensor: Ceraphant T PTC31, Error ± 0.5%, Endress+Hauser, Weil am Rhein, Germany) under standard temperature and pressure (STP: $T$ = 273.15 K, $p$ = 1.013 bar). The liquid phase was analysed in advance and after the runs. The concentration of acetate, propionate, $n$- and iso-butyrate, $n$- and iso-valerate and capronate were determined by capillary column

gas chromatography (CP-3800, Varian, Walnut Creek, USA). d/l-Lactic acid, formic acid, sucrose, glucose, fructose, ethanol and propylene glycol were measured by high-pressure liquid chromatography (HPLC, Bischoff, Leonberg, Germany). Total carbon (TC), total organic carbon (TOC) and inorganic carbon (IC) were analysed with a titrator (785 DMP Titrino, Metrohm, Filderstadt, Germany). Total nitrogen (TN) was determined by a versatile infrared rapid Kjeldahl digestion system (Turbotherm, Gerhardt, Koenigswinter, Germany) and afterwards distilled (Vapodest 50, Gerhardt, Koenigswinter, Germany) and back titrated. For COD determination, a cuvette test from Hach Lange was used (LCK 014, Hach Lange, Düsseldorf, Germany) with a high-temperature thermostat (HT 200 S, Hach Lange, Düsseldorf, Germany) and a sensor array photometer (LASA 20, Hach Lange, Düsseldorf, Germany).

### Evaluation of the experiment

To describe the behaviour of real gases, the ideal gas equation partial has to be adapted. As experiments run at pressures of up to 100 bar, compressibility of the gas cannot be disregarded

$$p_i V = Z_i n_i RT. \tag{1}$$

In Equation (1), the compressibility factor $Z_i$ is added to ideal the gas law where $p_i$ is the partial pressure of gas $i$ (bar), $V$ is the volume (L), $n_i$ is the amount of substance $i$ (mol), $R$ is the universal gas constant (J mol$^{-1}$ K$^{-1}$) and $T$ is the temperature (K). $Z_i$ is calculated with Peng–Robinson [27] for CO$_2$ and SGerg-88 [28] for CH$_4$. At standard temperature and pressure (STP: $T$ = 273.15 K, $p$ = 1.013 bar), $Z$ = 1 for any gas and decreases for both applied gases with increasing pressure.

To calculate the pressure increase, the measured pressures in the reactors were temperature corrected by using the ideal gas equation with a constant volume

$$p_n = \frac{(p_m(t)/Z(t)T_m - p_1/Z_1 T_1) \times T_n}{COD_{input}}. \tag{2}$$

$p_n$ is the temperature corrected pressure increase (bar g$^{-1}$ COD), which is the difference between the quotient of the measured pressure $p_m$ (bar) and temperature $T_m$ (°C) and at the minimum pressure $p_1$ and temperature $T_1$ of the experiment divided by the COD input (g). To compensate temperature variations of 36.9 ± 0.3°C during the experiments, the term is multiplied by the desired temperature ($T_n$) of 37°C. By considering that the initial pressure was adjusted with a high amount of N$_2$ in comparison to CH$_4$ and CO$_2$, alterations in the compressibility factor $Z$ could be neglected.

To estimate the degradation kinetics, the cumulative pressure increase was fitted to the modified Gompertz equation [29] by assuming that pressure increase is a function of bacterial growth:

$$M = P \times \exp\left\{-\exp\left[\frac{R_m \times e}{P}(\lambda - t) + 1\right]\right\}, \quad (3)$$

where $M$ is the cumulative pressure increase (bar g$^{-1}$ COD), $P$ is the pressure increase potential (bar g$^{-1}$ COD), $R_m$ is the maximum daily pressure increase rate (bar d$^{-1}$ g$^{-1}$ COD), $\lambda$ is the duration of the lag phase time (d) and $t$ is the duration of the run (d). $P$, $R_m$ and $\lambda$ are constants and calculated by a non-linear regression. The first derivative of (3) was used to determine a time for $R_m$. For statistical analyses, the Kruskal–Wallis test was used with the statistical software R.[30]

The amount of $CH_4$ produced was calculated using Equation (4), which incorporates the experimental pressure increase, temperature and reactor gas volume. Regarding the measured pressure $p_m$ (bar), the gas quality $y_{CH_4}$, temperature $T_m$ (K), and the known gas volume above the liquid phase $V_{gas}$ (l), the accumulated amount of mole methane $n_{gas,CH_4}$ was calculated as follows:

$$n_{gas,CH_4} = \frac{p_m y_{CH_4} V_{gas}}{T_m R Z_{CH_4}}. \quad (4)$$

With Equation (5), the specific methane yield (SMY$_{gas}$) (L kg$^{-1}$) was calculated with the sum of the accumulated amount of mole methane in gas $n_{gas,CH_4}$ (mol) and liquid $n_{liq,CH_4}$ (mol) at standard temperature $T_{STP}$ (K) and pressure $p_{STP}$ (bar) related to the input of COD (g L$^{-1}$).

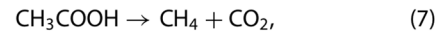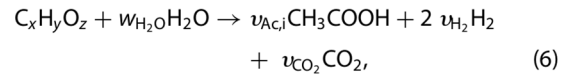$$SMY_{gas} = \frac{(n_{gas,CH_4} + n_{liq,CH_4})RT_{STP}}{p_{STP}COD_{input}}. \quad (5)$$

Previous studies have shown that the dissolved amount of methane in fermentation liquid can be calculated with Henry's law and the value for methane in water at 37°C with $H_{CH_4,H_2O} = 4775.5$ MPa.[28]

**Table 1.** Overview of calculated parameters of the Gompertz equation and the first derivative for three different initial pressure levels (1, 50, 100 bar). $P$ = pressure increase potential per g COD input, $\lambda$ = duration of the lag phase time, $R_m$ = maximum daily pressure increase rate and $t_{max}$ = time of the maximum pressure increase per day.

| Pressure levels (bar) | $P$ (bar g$^{-1}$ COD$_{input}$) | $\lambda$ (d) | $R_m$ (bar d$^{-1}$ COD$_{input}$) | $t_{max}$ (d) |
|---|---|---|---|---|
| 1 | $2.09 \pm 0.07^a$ | $2.73 \pm 0.46^a$ | $0.26 \pm 0.06^a$ | $5.74 \pm 0.34^a$ |
| 50 | $1.89 \pm 0.04^a$ | $2.45 \pm 0.73^a$ | $0.21 \pm 0.05^a$ | $5.85 \pm 0.44^a$ |
| 100 | $1.81 \pm 0.18^a$ | $2.64 \pm 0.9^a$ | $0.18 \pm 0.05^a$ | $6.39 \pm 0.28^a$ |

Note: Different superscript letters indicate significant differences ($p \leq .05$).

To determine the specific methane yield potential (SMY$_{liq}$) by the degradation of the acids, the assumption of total degradation of acids with acetic acid as intermediate product, and the amount of $CH_4$ produced were calculated with the following equations:

$$C_xH_yO_z + w_{H_2O}H_2O \rightarrow v_{Ac,i}CH_3COOH + 2\,v_{H_2}H_2$$
$$+ \ v_{CO_2}CO_2, \quad (6)$$

$$CH_3COOH \rightarrow CH_4 + CO_2, \quad (7)$$

where $C_xH_yO_z$ is the carbohydrate, $w_{H_2O}$ is the amount of water ($H_2O$), $v$ is the stoichiometric coefficient (mol) and $v_{Ac,i}$ is the stoichiometric coefficient of the intermediate product acetic acid (mol). The degradation of detected acids such as butyric acid, lactic acid, propionic acid, valerianic acid and caproic acid can be found in the literature.[31,32] Taking into account that no hydrogen ($H_2$) was measured in the product gas, the methanation reaction (Equation (8)) is assumed as an additional methane source. In Equation (8), the letters u and s stand for the stoichiometric coefficient of the substances:

$$uCO_2 + 2(2v_{H_2})H_2 \rightarrow (v_{Ac,i} + 1/4v_{H_2})CH_4 + sH_2O. \quad (8)$$
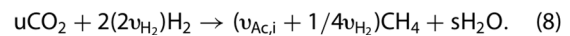
Following the previous equations, the produced amount of methane $n_{CH_4, Potential}$ (mol) was calculated:

$$\sum_{i=acids}\left(c_i v_{Ac,i} + c_i\,v_{H_2,i}\frac{1}{4}\right)\frac{M_{liq}}{\tilde{M}_i} = n_{CH_4,Potential}. \quad (9)$$

where $c_i$ is the concentration of the substance (g kg$^{-1}$), $M_{liq}$ is the mass of the liquid (kg) and $\tilde{M}_i$ is the molar mass (g mol$^{-1}$).

With Equation (10), the specific methane yield potential (SMY$_{liq}$) was calculated with $\rho_{liq}$ being the density of liquid at standard temperature $T_{STP}$ and pressure $p_{STP}$ related to the input of COD:

$$SMY_{liq} = \frac{n_{CH_4, Potential}\rho_{liq}R\,T_{STP}}{M_{liq}p_{STP}COD_{input}} \quad (10)$$

### Results and discussion

#### Pressure increase

The effects of different initial pressures on the mean pressure increase are shown in Figure 2. The graphic includes the mean measured values of three repetitions, the fitted curves calculated with the modified Gompertz equation and the standard deviations (sd) of the repetitions. The coefficients of determination of the modified Gompertz equations are between 99.1% and 99.7%. When 1 bar of initial pressure is applied, the highest pressure increase with $2.16 \pm 0.08$ bar g$^{-1}$ COD$_{input}$ is observed after 21 days. With an initial pressure of
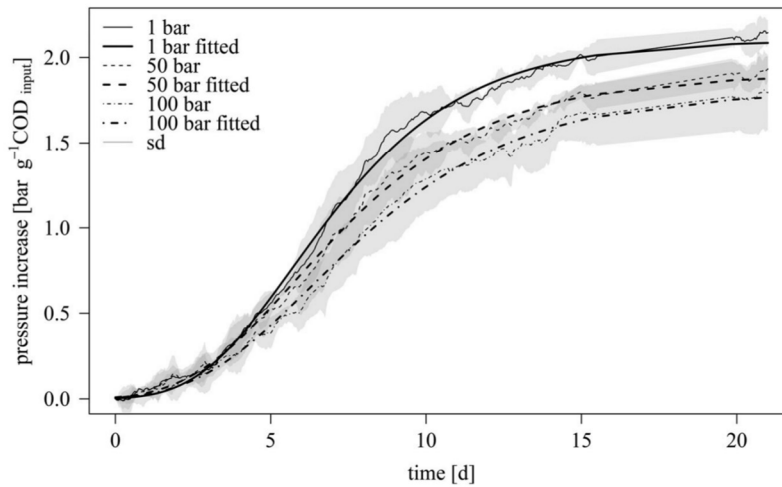
**Figure 2.** Measured pressure increase curves per COD input over time and the fitted Gompertz functions of three different initial pressures (1, 50, 100 bar) at 37°C, standard deviation (sd) of three repetitions in grey, overlaps of sd in darkgrey.

50 bar, the pressure increases by $1.95 \pm 0.08$ bar $g^{-1}$ $COD_{input}$ and with 100 bar pressure by $1.82 \pm 0.23$ bar $g^{-1}$ $COD_{input}$. The statistical analyses ($p > .05$) show that the changes of pressure are not subject to different initial pressures.

The results of degradation kinetics calculated by the modified Gompertz equations fitted to the cumulative pressure increases are shown in Table 1. Only minor deviation in the pressure increase could be observed: the pressure increase ($P$) ranges from $2.09 \pm 0.07$ bar $g^{-1}$ $COD_{input}$ (1 bar) to $1.81 \pm 0.18$ bar $g^{-1}$ $COD_{input}$ (100 bar) and the duration of the lag phase ($\lambda$) is between $2.45 \pm 0.73$ days (50 bar) and $2.73 \pm 0.46$ days (1 bar). By increasing the initial pressures, the maximum daily pressure increase ($R_m$) has diminished from $0.26 \pm 0.06$ bar $d^{-1}$ $g^{-1}$ $COD_{input}$ (1 bar) to $0.18 \pm 0.05$ bar $d^{-1}$ $g^{-1}$ $COD_{input}$ (100 bar) and the time of the maximum daily pressure increase ($t_{max}$) postpones from $5.74 \pm 0.34$ days (1 bar) to $6.39 \pm 0.28$ days (100 bar). The detected differences of the pressure increase potential, the duration of the lag phase, the maximum pressure increase rate and time of the maximum pressure increase are not significant ($p > .05$).

The results of this analysis show no significant influence of the initial pressure in the batch reactor on the pressure increase and pressure increase rates. A slightly lower pressure increase at 100 bar may be a result of the fact that the solubility of $N_2$, which was used for initial inertisation, is according to Henry's law mainly depending on the partial pressure. Due to the fact that the Henry index for $N_2$ and the used percolate is unknown, the potential initial pressure drop could not be considered completely, thus leading to an underestimation of the pressure increase at high-initial pressures.

## Degradation kinetics

Table 2 summarizes the composition of the liquid phase of the leachate and the effluent after the degradation within 21 days. No sugars and alcohols are detected in the leachate and after degradation. All acids are degraded after 21 days within all experiments, independent of the initial pressures. The TOC in the liquid decreases from 1.204 to 0.37 till 0.392 g $L^{-1}$ after the experiment. The IC increases slightly from 0.887 to 1.013 up until 1.045 g $L^{-1}$. TC, TN and COD decreases after degradation. All variations of TOC, IC, TC, TN and COD of the three different initial pressures are not significant ($p > .05$).

In comparison to other literature, no acids are detected in the liquid after 21 days at the end of the experiment. Lindeboom et al. [24] mention significant concentrations of propionate, butyrate and valerate after decompressing the 3 and 31 bar pressure experiments with wastewater. Other experiments under continuous operation show the same degree of degradation of nearly all acids in the methane reactor at pressures of up to 9 bar.[10,14] This indicates a stable biological process and stands in line with the experimental finding that high operating pressures do not cause any inhibitions.

After 21 days, gas samples are taken for measuring gas quality and quantity. The measured gas components and the gas volume after decompression are shown in Table 3. By raising the initial pressure, the $N_2$ content increases from $19.9 \pm 2.0\%$ at 1 bar and to $94.9 \pm 1.1\%$ at 100 bar. This results in a decrease $CH_4$ content by $68.7 \pm 1.5\%$ to $3.4 \pm 0.9\%$ and in the $CO_2$ content from $9.5 \pm 1.5\%$ to $0.5 \pm 0.2\%$. Hydrogen sulphide ($H_2S$) and $H_2$ cannot be detected. Measured gas volume has

**Table 2.** Results of the analyses of volatile fatty acids, carbons, nitrogen and COD to describe the substrate properties of leachate and the degradation.

| Parameters | Leachate = feed for reactors | Effluent composition at operating pressure | | |
|---|---|---|---|---|
| | | 1 bar | 50 bar | 100 bar |
| Acetic acid (g kg$^{-1}$) | $0.342 \pm 0.162$ | 0 | 0 | 0 |
| Propionic acid (g kg$^{-1}$) | $0.138 \pm 0.03$ | 0 | 0 | 0 |
| $n$-Butyric acid (g kg$^{-1}$) | $0.843 \pm 0.195$ | 0 | 0 | 0 |
| Iso-valeric acid (g kg$^{-1}$) | $0.035 \pm 0.006$ | 0 | 0 | 0 |
| $n$-Valeric acid (g kg$^{-1}$) | $0.062 \pm 0.014$ | 0 | 0 | 0 |
| Caproic acid (g kg$^{-1}$) | $0.058 \pm 0.014$ | 0 | 0 | 0 |
| TOC (g L$^{-1}$) | $1.204 \pm 0.645$ | $0.379 \pm 0.048$ | $0.37 \pm 0.023$ | $0.392 \pm 0.049$ |
| IC (g L$^{-1}$) | $0.887 \pm 0.311$ | $1.013 \pm 0.044$ | $1.016 \pm 0.044$ | $1.045 \pm 0.018$ |
| TC (g L$^{-1}$) | $2.090 \pm 0.363$ | $1.388 \pm 0.083$ | $1.385 \pm 0.052$ | $1.437 \pm 0.049$ |
| TN (g L$^{-1}$) | $0.685 \pm 0.045$ | $0.628 \pm 0.027$ | $0.613 \pm 0.012$ | $0.633 \pm 0.027$ |
| COD (mg L$^{-1}$) | $3881 \pm 580$ | $1008 \pm 44$ | $986 \pm 42$ | $1011 \pm 52$ |

**Table 3.** Results of the analyses of mean $N_2$ content, mean $CH_4$ content, mean $CO_2$ content and mean measured gas volume for three different initial pressures (1, 50, 100 bar).

| Initial pressure (bar) | Mean $N_2$ content (%) | Mean $CH_4$ content (%) | Mean $CO_2$ content (%) | Mean measured gas volume (L) |
|---|---|---|---|---|
| 1 | $19.9 \pm 2.0$ | $68.7 \pm 1.5$ | $9.5 \pm 1.5$ | $0.76 \pm 0.01$ |
| 50 | $90.8 \pm 0.1$ | $5.7 \pm 2.6$ | $0.8 \pm 0.5$ | $7.84 \pm 0.15$ |
| 100 | $94.9 \pm 1.1$ | $3.4 \pm 0.9$ | $0.5 \pm 0.2$ | $15.10 \pm 1.42$ |

increased with higher initial pressures from $0.76 \pm 0.01$ to $15.1 \pm 1.42$ L because of higher initial $N_2$ contents.

Decreasing $CH_4$ and $CO_2$ contents at high pressures is a result of the initial $N_2$ and not related to a lower microbiological activity. The use of $N_2$ was necessary to calculate the $CH_4$ and $CO_2$ produced by the microorganisms. Under batch conditions, there was no possibility to reach 100 bar autogeneratively by the degradation of the acids. In contrast to that, continuous two-stage systems are producing the pressure autogeneratively without using $N_2$, which makes it feasible to produce methane-rich gas.

In Figure 3, the specific methane yield related to the COD added to the reactors is expressed as SMY calculated for three different initial pressures (1, 50, 100 bar) based on the liquid and gas analysis (Table 3). The SMY potential calculated by liquid potential sums up to $210 \pm 25$ L kg$^{-1}$ $COD_{input}$ at each pressure. The SMYs derived from the measured gas analysis are between $230 \pm 15$ L kg$^{-1}$ $COD_{input}$ at 1 bar and $244 \pm 36$ L kg$^{-1}$ $COD_{input}$ at 100 bar. In all, 9.6% of methane measured by gas analysis was dissolved in the liquid phase. The differences in SMY between the three pressures calculated from liquid potential and gas analysis are not significant ($p > .05$).

In these experiments, the pressure has no influence on the calculated SMY. This is in contrast to other two-stage pressurized anaerobic digestion experiments till 9 bar. Without adding $N_2$, Chen et al. [10,11] measured a little impact of pressure on SMY under continuous conditions without using any chemicals for pH control.
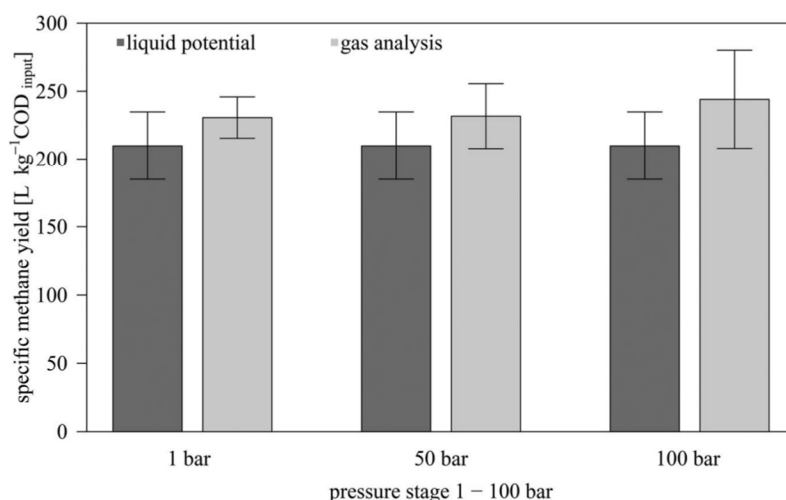


**Figure 3.** Specific methane yield per kg COD input at T = 37 °C calculated from the liquid and gas analysis for three different initial pressures (1, 50, 100 bar), $p > .05$.

## Conclusions and outlook

This paper reports an experimental investigation and mathematical modelling of the initial pressure on the methane production kinetics during anaerobic digestion. Based on the experimental findings, there was no observable effect exhibited by increasing pressure. Furthermore, no significant differences were determined between the three different pressure levels, working with initial pressures of 1, 50 and 100 bar. The pressure increase was between $2.09 \pm 0.07$ bar $g^{-1}$ $COD_{input}$ (1 bar) and $1.81 \pm 0.18$ bar $g^{-1}$ $COD_{input}$ (100 bar) after 21 days. The maximum pressure increase rates showed as well no significant difference. They were between $0.26 \pm 0.06$ bar $d^{-1}$ $g^{-1}$ $COD_{input}$ (1 bar) and $0.18 \pm 0.05$ bar $d^{-1}$ $g^{-1}$ $COD_{input}$ (100 bar). The time of the maximum daily pressure increase was between $5.85 \pm 0.44$ days (50 bar) and $6.39 \pm 0.28$ days (100 bar).

All acids were degraded by the end of the experiment at each pressure level. As specific methane yields derived from the gas analyses did not differ at any pressure levels from the calculated potentials based on the added organic acids, this study assumes that microorganisms were not inhibited. The specific methane yield varied between $230 \pm 15$ L $kg^{-1}$ $COD_{input}$ (1 bar) and $244 \pm 36$ L $kg^{-1}$ $COD_{input}$ (100 bar).

Further scientific research would be worthwhile in order to determine the solubility of the produced gases under pressure in the leachate and to further describe the pressure increase. With this in mind, pH sensors are needed for calculating the pH-dependent dissolved amount of $CO_2$ in the liquid phase.[12,27] With regard to gas composition, experiments under continuous autogenerative two-stage conditions are recommended to confirm that SMY is not pressure dependent and that high methane contents can be reached without using nitrogen, providing a valuable alternative to commonly used purification methods.

## Funding information

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## 4 Publication 3: Influence of pressures up to 50 bar on two-stage anaerobic digestion

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## Abstract

The concept of pressurized two-stage anaerobic digestion integrates biogas production, purification and pressure boosting within one process. The produced methane-rich biogas can be fed into gas grids with considerably less purification effort. To investigate biogas production under high pressures up to 50 bar, a lab scale two-stage anaerobic digestion system was constructed including one continuously operated pressurized methane reactor. This investigation examined the effects of different operating pressures in methane reactor (10, 25, 50 bar) on biogas quantity and quality, pH value and process stability. By increasing operating pressures in methane reactor, the pH value decreased from 6.65 at 10 bar to 6.55 at 50 bar. Simultaneously, methane content increased from 79.08% at 10 bar to 90.45% at 50 bar. The results show that methane reactors can be operated up to 50 bar pressure continuously representing a viable alternative to commonly used gas upgrading methods because of reduced purification effort.

# Influence of pressures up to 50 bar on two-stage anaerobic digestion

CrossMark

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## HIGHLIGHTS

- The influence of pressure on anaerobic digestion up to 50 bar was examined experimentally.
- Increasing pressure decreases pH value in the methane reactor until 6.53.
- Increasing pressure increases methane content over 90%.
- Increasing pressure decreases specific methane yield until 0.26 L g$^{-1}$ COD$_{added}$.
- The pressurized methane reactor operates very stable.

## ARTICLE INFO

## ABSTRACT

The concept of pressurized two-stage anaerobic digestion integrates biogas production, purification and pressure boosting within one process. The produced methane-rich biogas can be fed into gas grids with considerably less purification effort. To investigate biogas production under high pressures up to 50 bar, a lab scale two-stage anaerobic digestion system was constructed including one continuously operated pressurized methane reactor. This investigation examined the effects of different operating pressures in methane reactor (10, 25, 50 bar) on biogas quantity and quality, pH value and process stability. By increasing operating pressures in methane reactor, the pH value decreased from 6.65 at 10 bar to 6.55 at 50 bar. Simultaneously, methane content increased from 79.08% at 10 bar to 90.45% at 50 bar. The results show that methane reactors can be operated up to 50 bar pressure continuously representing a viable alternative to commonly used gas upgrading methods because of reduced purification effort.

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## 1. Introduction

Considering Germany's energy supply, there is a need to supplement the increasing expansion of volatile energy sources. In 2015, wind and solar energy had a share of 86.6% on the installed capacity for renewables-based electricity generation in Germany (AGEE-Stat, 2016). This challenge can be mastered by expanding demand power generation and developing new energy storage concepts. The production of biomethane as a renewable energy source, in this case, has the potential to play a major role. As biomethane gas can be fed directly into the public natural gas grid, therefore utilizes a large existing energy storage system and furthermore decouples the gas production and its utilisation in terms of time and space (Niesner et al., 2013; Barchmann et al., 2016). In 2014, the total upgrading capacity of 310,000 m$^3$ h$^{-1}$ raw biogas was

contributed by 178 biomethane plants in Germany and 189 plants from rest of Europe (EBA, 2016).

One concept for biomethane production combining biogas production, purification and pressure boosting in one process is the pressurized two-stage anaerobic digestion system (Wonneberger et al., 2011; Chen et al., 2014a, 2014b; Lemmer et al., 2015a). Through this, energetic efforts for grid injection could be significantly reduced. In commonly applied non-pressurized two-stage anaerobic digestion processes found in literature (Lehtomäki and Björnsson, 2006; Parawira et al., 2007; Muha et al., 2013; Lindner et al., 2015), the degradation steps of hydrolysis/acidification and acetogenesis/methanogenesis are spatially separated to ensure optimum environmental conditions for each group of microorganisms (Muha et al., 2013; Lemmer et al., 2015a; Lindner et al., 2015). In contrast, in the pressurized two-stage anaerobic digestion process, methanogenic bacteria autogeneratively increase the pressure of gas in the second stage. Besides, this applied process makes use of the fact that $CO_2$ is more soluble in liquid than

methane (Clever and Young, 1987; Crovetto, 1991; Gevantman, 1992), allowing $CO_2$ discharge from the methane reactor with liquid stream. Therefore this system represents an alternative to commonly used post upgrading technologies pressure swing adsorption (PSA), gas separation membranes, amine and water scrubbing (Niesner et al., 2013). Previous studies in continuous two-stage high pressure fermentation indicated that high $CH_4$ contents of over 80% are possible at operating pressures of up to 9 bar (Chen et al., 2014a,b; Lemmer et al., 2015a,b). Other studies with batch systems up to 100 bar showed no inhibition of methanogenic bacteria, still producing biogas under high pressures (Lindeboom et al., 2011; Merkle et al., 2016).

For future applications, even higher operating pressures of 60 to 125 bar (Chandra, 2006) are possible, for e.g. biomethane transportation via transnational gas grids. These pressure levels were not yet achieved in former studies under continuous pressurized two-stage anaerobic digestion process. Therefore, a continuously operating pressurized methane reactor was build. The aim of this study was to investigate the effect of high operating pressures up to 50 bar on pressurized two-stage anaerobic digestion by testing three different operating pressure levels (10 bar, 25 bar and 50 bar) in the methane reactor. The effects on biogas quantity and quality, pH value and process stability were investigated using a lab-scale pressurized two-stage anaerobic digestion system at the University of Hohenheim. The accompanying modelling work was conducted by the Deutscher Verein des Gas- und Wasserfaches e. V. (DVGW) Research Centre at the Engler-Bunte-Institute of Karlsruhe Institute of Technology (KIT).

## 2. Materials and methods

### 2.1. Reactors

The design of the two-stage high pressure anaerobic digestion system without first stage is shown in Fig. 1. The first stage hydrolysis-acidification was performed in four parallel-operated acidogenesis-leach-bed-reactors with a volume of 50 L each (Chen et al., 2014a), in which the supplied biomass was degraded to organic acids and alcohols. An internal circulation of liquid was used to leach out organic acids and alcohols produced.

The pressurized anaerobic filter system consisted storage tanks HP-T1 for the percolate, HP-T4 for the effluent, high pressure methane reactor as an upflow anaerobic filter with a total volume of 21 L and two flash tanks (Flash 1 + 2) with a total volume of 10 L each. The percolate was pumped with P-1, a piston diaphragm pump, (PROMINENT, Type HP3A) from HP-T1 into high pressure methane reactor in order to convert the organic fraction into biogas. The liquid in methane reactor was circulated by a separate circulation gear pump (GATHER Industrie, Type 1MA-A/12-11/X-SS/Q/K100/) to guarantee a homogeneous distribution of organic acids and provide uniform temperatures in the reactor. The fixed bed consisted of randomly packed polyethylene fillers (STÖHR, Type HX09: surface area 861 $m^2$ $m^{-3}$, porosity 83%) as a carrier material to aid in immobilizing the microorganisms and biofilm development. The fillers had an effective packing surface of 24.38 $m^2$.

The aim of high pressure fermentation system was to have an autogenerative increase in reactor pressure by microorganisms
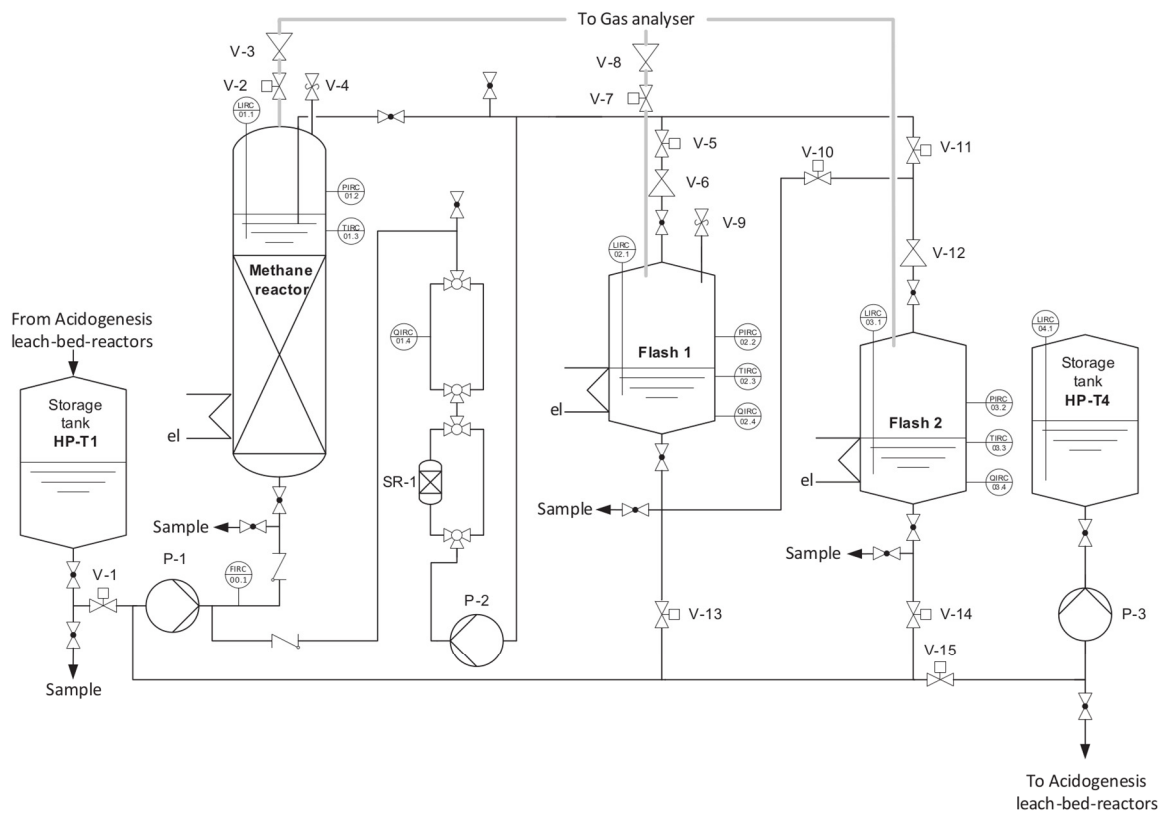


**Fig. 1.** Schematic diagram of the pressurized anaerobic filter system without the first stage

induced biogas production. As a measure to control this pressure, the produced biogas was left to accumulate in methane reactor until the desired reactor pressure was achieved. Liquid level inside methane reactor was measured by a level sensor (Endress + Hauser, Liquicap M FMI51). Depending on the liquid level, either the gas outlet (V-2) was automatically opened, and the produced biogas was released to a gasbag or the liquid outlet (V-5) was opened to release and depressurize the liquid to lower pressure in Flash 1. Afterwards this liquid was released to atmospheric pressure in Flash 2, finally this liquid was further removed from the system and stored in another storage tank HP-T4. Due to the release of gas or liquid, there is a slight drop of pressure in the methane reactor. Once the release valve was closed again, the microorganisms autogeneratively re-increased the biogas pressure to the desired value.

### 2.2. Experimental procedure

At the beginning methane reactor was inoculated with the effluent from another fixed-bed anaerobic filter, which had been fed with leachate of grass and maize silage and operated at atmospheric pressure. A steady state that was needed to begin the experiments on the effect of pressure on two-stage anaerobic digestion was reached after a start-up period and preliminary testing phase of approximately eight months.

The four parallely-operated acidogenesis-leach-bed-reactors had an operating temperature of 55 C and were run at ambient pressure. Every two weeks they were filled with 4.8 kg (fresh mass) maize silage and 4.3 kg grass silage from the Field-test station of University of Hohenheim (Unterer Lindenhof, Eningen, Germany). The grass and maize silage were not pre-treated and stored as feedstock for a full scale biogas plant. Mean organic dry matter (ODM) of the grass and maize silage was $343.75 \pm 34.86$ g kg$^{-1}$ and $309.61 \pm 3.54$ g kg$^{-1}$, respectively. Also 20 L of tap water was added and micro nutrients following the recommendation of Vintiloiu et al. (2012) in order to avoid biological inhibitions that would further result in reduced microbial growth and disturbance in biological process. An internal circulation was used to leach out

the produced organic acids and alcohols. With this process 400 L of percolate was produced in advance, pumped through a 10 μm filter to avoid blockage of the used feeding pump P-1 and stored at 4 °C, to ensure an equal and stable quality for the whole experiment.

Approximately 12 L of the stored leachate was mixed once a week with 12 L of effluent from tank HP-T4 and introduced into tank HP-T1 for storage. In Table 1 the properties of these mixed percolates are presented in detail.

Every 12 h, a certain amount of leachate was pumped into the high pressure methane reactor. Although feeding amount was dependent on the percolate's chemical oxygen demand (COD) concentration, so the organic loading rate (OLR) of methane reactor remained unchanged. To guarantee a stable operating volume, the same amount of liquid was eluted from the methane reactor to Flash 1 operating at 4 bar and then to Flash 2 at atmospheric pressure.

In the experiment, three different operating pressures in the methane reactor were tested at 37 °C (Table 2). Here, the OLR related to COD was $4.42 \pm 0.44$ kg m$^3$ d$^{-1}$ at 10 bar, $4.25 \pm 0.31$ kg m$^{-3}$d$^{-1}$ at 25 bar and $4.19 \pm 0.09$ kg m$^{-3}$d$^{-1}$ at 50 bar. The resulting hydraulic retention times (HRT) amounted to $4.07 \pm 0.09$ d at 10 bar, $4.16 \pm 0.10$ d at 25 bar and to $4.19 \pm 0.06$ d at 50 bar. The entire amount of liquid within the methane reactor was circulated in several intervals once hourly. No additional caustic chemicals were added for pH adjustment throughout this experiment.

### 2.3. Analytical methods and data acquisition

In this study, pH, pressure and temperature values of the pressurized methane reactor, Flash 1 and Flash 2 were monitored in real-time (pH-sensor in methane reactor: Corr Instruments Type pH-G-14"-Tube375-316SS; pH-sensors in Flash: Endress + Hauser Type Orbisint CPS11D; pressure sensors: JUMO Type dTrans p30; temperature sensors: Hitec Zang Type IS-T-PT100-1/3-VA-d-l). The data was logged in a mysql database by Labview 14.0.1 (National Instruments). The quality and quantity of the produced gas was measured under ambient conditions by a gas meter (Ritter Type TG20/5) and a gas analyzer (Sick Maihak Type S710), which was calibrated before every pressure experiment. The measured gas volume was corrected to dry gas at a standard temperature and pressure (273.15 K and 1.013 bar). The working volumes of the methane reactor, tanks Flash 1 and Flash 2, were controlled by liquid level sensors (Endress + Hauser Type Liquicap M FMI51). The leachate from HP-T1 and the effluent from the methane reactor were sampled every second day and analyzed to determine COD, volatile fatty acids (VFAs) as well as alcohol and sugar contents, total carbon (TC), total organic carbon (TOC), inorganic carbon (IC) and ammonium nitrogen (NH$_4^+$-N). COD was determined using a cuvette test from Hach Lange (Hach Lange Type LCK 014) with a high temperature thermostat (Hach Lange Type HT 200 S) and a sensor array photometer (Hach Lange Type LASA 20). The concentration of the VFAs acetic acid, propionic acid, n- and iso-butyric acid, n- and iso-valeric acid and caproic acid were determined by capillary column gas chromatography (Shimadzu

**Table 1**
Chemical characteristics of the percolate used for pressurized two-stage anaerobic digestion experiments operated at different pressure levels.

|  | Percolate = feed for methane reactor | | |
|---|---|---|---|
|  | 10 bar | 25 bar | 50 bar |
| Acetic acid (g kg$^{-1}$) | $3.36 \pm 0.55$ | $2.94 \pm 0.24$ | $3.13 \pm 0.37$ |
| Propionic acid (g kg$^{-1}$) | $1.53 \pm 0.38$ | $1.14 \pm 0.26$ | $1.22 \pm 0.28$ |
| iso-Butyric acid (g kg$^{-1}$) | $0.10 \pm 0.04$ | $0.09 \pm 0.04$ | $0.13 \pm 0.06$ |
| n-Butyric acid (g kg$^{-1}$) | $1.31 \pm 0.45$ | $1.25 \pm 0.49$ | $1.20 \pm 0.42$ |
| iso-Valeric acid (g kg$^{-1}$) | $0.21 \pm 0.07$ | $0.18 \pm 0.07$ | $0.19 \pm 0.07$ |
| n-Valeric acid (g kg$^{-1}$) | $0.44 \pm 0.44$ | $0.75 \pm 0.30$ | $0.68 \pm 0.27$ |
| Caproic acid (g kg$^{-1}$) | $0.33 \pm 0.28$ | $0.47 \pm 0.35$ | $0.51 \pm 0.34$ |
| DL-lactic acid (g kg$^{-1}$) | $0.83 \pm 1.23$ | $0.97 \pm 1.35$ | $0.67 \pm 1.35$ |
| Ethanol (g kg$^{-1}$) | $0.30 \pm 0.18$ | $0.31 \pm 0.12$ | $0.36 \pm 0.21$ |
| TOC (g L$^{-1}$) | $6.69 \pm 0.30$ | $6.47 \pm 0.19$ | $5.97 \pm 0.29$ |
| IC (g L$^{-1}$) | $0.26 \pm 0.06$ | $0.26 \pm 0.05$ | $0.29 \pm 0.07$ |
| TC (g L$^{-1}$) | $6.95 \pm 0.31$ | $6.73 \pm 0.17$ | $6.26 \pm 0.25$ |
| NH$_4^+$-N (g L$^{-1}$) | $1.24 \pm 0.03$ | $1.10 \pm 0.10$ | $1.04 \pm 0.05$ |
| COD (mg L$^{-1}$) | $17,888 \pm 419$ | $17,484 \pm 647$ | $17,115 \pm 581$ |

**Table 2**
Operating parameters for each run of the experiment.

| Run | Duration (days) | Absolute pressure in the methane reactor (bar) | | | |
|---|---|---|---|---|---|
|  |  | Target value | Hourly mean | Min. | Max. |
| 1 | 25 | 10 | $9.83 \pm 0.06$ | 9.58 | 9.92 |
| 2 | 16 | 25 | $24.66 \pm 0.17$ | 23.68 | 24.86 |
| 3 | 19 | 50 | $49.09 \pm 0.44$ | 46.01 | 49.71 |

Type GC 2010Plus, Autoinjector AOC-20). Other VFAs like D/L-lactic acid and formic acid, as well as sucrose, glucose, ethanol and propylene glycol were measured by high pressure liquid chromatography (Bischoff Type HPLC). TC, TOC and IC were analyzed with a TOC/TNb analyzer (Analytik Jena AG Type multi N/C® 2100). The ratio of volatile fatty acids and total alkalinity (VFA/TIC) was determined using an automatic titrator (Metrohm Type 785 DMP Titrino). $NH_4^+$-N was determined via distillation (Gerhardt Type Vapodest 50) and back titration. The acquired data was statistically analyzed using the nonparametric Kruskal Wallis test and subsequently Tukey's Test (p < 0.05) in R Studio (v 0.99.902).

## 3. Results and discussion

### 3.1. Effect of pressure on process stability

The characteristics of the effluent from methane reactor are summarized in Table 3. Hardly any volatile fatty acids and alcohols were detected. The concentration of propionic, acetic, *iso*-butyric and *iso*-valeric acid had slightly increased for the experimental pressure level of 50 bar. At 50 bar the acetic and propionic acid concentrations rose to $0.08 \pm 0.1$ g $kg^{-1}$ and $0.43 \pm 0.14$ g $kg^{-1}$, respectively. The TOC and TC in the effluent had slightly increased with pressure, however this was not observed for IC. Decreasing concentrations of ammonium nitrogen in the methane reactor was due to decreasing ammonium contents in the leachate. The VFA/TIC ratios remained between 0.13 and 0.21. The COD concentration in the effluent of methane reactor increased from $2.2 \pm 0.2$ g $L^{-1}$ at 10 bar to $3.5 \pm 0.5$ g $L^{-1}$ at 50 bar. The degradation rate in the methane reactor remained more than 87% until 25 and decreased to $80.4 \pm 1.7\%$ at 50 bar (Fig. 2).

The results of the characteristics of the effluent showed a slight acid accumulation in the methane reactor. One reason can be due to the increasing partial pressure of $CO_2$ from 2.06 bar to 4.69 bar by increasing the working pressures from 10 bar to 50 bar. Previous studies from Hansson and Molin (1981) and Lindeboom et al. (2016) showed a significant influence of $CO_2$ partial pressure on the degradation rate of propionic acid. Thus, the results from Lindeboom et al. (2016) demonstrated an inhibition of propionic acid degradation rate of 90% by increasing $CO_2$ partial pressures up to 5 bar.

The accumulation of propionic acid in methane reactor could be prevented by expanding the volume of methane reactor and the

resulting increase of HRT. Furthermore by using a continuous acidification reactor, instead of acidogenesis-leach-bed-reactor, the pH value in the acidification reactor can be controlled, leading to produce percolate with little amounts of propionic acid. Thus, Lindner et al. (2015) showed a shift from propionic acid production to acetic and n-butyric acid by changing the operation pH value from 7 to 5.5.

However, the acetic and propionic acid concentration remained below 1 g $kg^{-1}$ and 0.7 g $kg^{-1}$ (Deublein and Steinhauser, 2011) at all times. This is much lower than the critical value and therefore, indicates a very stable biological process. As acid concentrations increased with pressure, this might have led to a slight reduction in process stability. The low VFA/TIC ratios indicated a continuously stable pressurized methane reactor. The degree of degradation in the methane reactor remained more than 87% until reaching the operating pressure of 50 bar at an OLR of 4.2 kg $COD \cdot m^{-3} \cdot d^{-1}$. Higher OLRs should be possible, at least at operating pressures up to 25 bar.

Fig. 3 shows the pH value of methane reactor at different operating pressures. The pH value varied between $6.65 \pm 0.05$ at 10 bar, $6.53 \pm 0.04$ at 25 bar and $6.55 \pm 0.02$ at 50 bar. The pH value at 10 bar was significantly higher in comparison to 25 bar and 50 bar experiments

The drop in pH value is caused by dissolution of $CO_2$ in the liquid. $CO_2$ solubility is proportional to the partial pressure of $CO_2$ in the gaseous phase and can be modelled by using Henry's Law (Carroll and Mather, 1992). The process remained stable despite low pH-values until 6.53 was measured, which was at the lower end of the optimal range of pH value 6.5–7.5 for anaerobic digestion under atmospheric conditions (Liu et al., 2008). Nevertheless, other studies had also measured pH values as low as 6.4 at even lower pressures up to 10 bar using hydrolysate from maize and grass silage (Chen et al., 2014a; Lemmer et al., 2015b). Due to the high concentration of ammonium ($NH_4^+$) of 1.04 till 1.24 g $kg^{-1}$ in the leachate mixture of hydrolysate and effluent, representing the total ammonia nitrogen (TAN), it can be assumed that a higher buffering capacity was present than in the studies from Chen et al. (2014a) and Lemmer et al. (2015b).

### 3.2. Effect of pressure on biogas production

Fig. 4 illustrates the specific quantity of produced gas under different operating pressures of methane reactor. The specific methane yield (SMY) of the gas produced in the methane reactor and measured in gaseous phase, is related to the COD fed into the digester. Increasing pressure resulted in a significant decrease of the SMY. The SMY decreased from $0.33 \pm 0.02$ L $g^{-1}$ $COD_{added}$ at 10 bar to $0.26 \pm 0.04$ L $g^{-1}$ $COD_{added}$ at 50 bar. Taking the dissolved $CH_4$ in liquid into account, which was released to the Flash 1, the SMY of the methane reactor at 10 bar represents 97.9% and at 50 bar only 76% of the total produced $CH_4$ of the whole anaerobic filter system. By increasing the pressure, more $CH_4$ was dissolved in the process liquid and transferred to the Flash 1, where this dissolved $CH_4$ was released due to the reduced partial pressure. Summarizing the $CH_4$ production of the whole anaerobic filter system, the SMY decreased from 0.34 L $g^{-1}$ $COD_{added}$ at 10 bar to 0.3 L $g^{-1}$ $COD_{added}$ at 50 bar.

Throughout the experiment, the SMY till 25 bar corresponds with other studies (Nallathambi Gunaseelan, 1997; Chen et al., 2014a; Lemmer et al., 2015b). Acid accumulation in the methane reactor may explain the decrease in SMY at pressures up to 50 bar. At this pressure level, acid conversion to methane has been incomplete.

Significant differences were observed in the biogas composition under different operating pressures (Fig. 5). The methane content increased from $79.08 \pm 1.01\%$ at 10 bar to $90.45 \pm 0.73\%$ at 50 bar,

**Table 3**
Chemical characteristics of the effluent from methane reactor operated at different pressure levels.

| | Effluent composition at different operating pressures (bar) | | |
| --- | --- | --- | --- |
| | 10 | 25 | 50 |
| Acetic acid (g $kg^{-1}$) | 0 ± 0 | 0 ± 0 | 0.08 ± 0.10 |
| Propionic acid (g $kg^{-1}$) | 0 ± 0 | 0 ± 0 | 0.43 ± 0.14 |
| *iso*-Butyric acid (g $kg^{-1}$) | 0 ± 0 | 0 ± 0 | 0.04 ± 0.02 |
| n-Butyric acid (g $kg^{-1}$) | 0 ± 0 | 0 ± 0 | 0.00 ± 0.01 |
| *iso*-Valeric acid (g $kg^{-1}$) | 0 ± 0 | 0 ± 0 | 0.04 ± 0.01 |
| n-Valeric acid (g $kg^{-1}$) | 0 ± 0 | 0 ± 0 | 0.01 ± 0.04 |
| Caproic acid (g $kg^{-1}$) | 0 ± 0 | 0 ± 0 | 0.01 ± 0.02 |
| DL-Lactic acid (g $kg^{-1}$) | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| Ethanol (g $kg^{-1}$) | 0 ± 0 | 0 ± 0 | 0.02 ± 0.04 |
| TOC (g $L^{-1}$) | 0.80 ± 0.09 | 0.78 ± 0.10 | 1.14 ± 0.20 |
| IC (g $L^{-1}$) | 1.69 ± 0.09 | 1.64 ± 0.04 | 1.54 ± 0.06 |
| TC (g $L^{-1}$) | 2.49 ± 0.10 | 2.42 ± 0.10 | 2.68 ± 0.14 |
| $NH_4^+$-N (g $kg^{-1}$) | 1.40 ± 0.01 | 1.35 ± 0.02 | 1.24 ± 0.05 |
| VFA/TIC | 0.13 ± 0.01 | 0.14 ± 0.01 | 0.21 ± 0.04 |
| COD (mg $L^{-1}$) | 2215 ± 225 | 2249 ± 158 | 3538 ± 493 |

**Fig. 2.** COD degradation degree at different pressure levels. The significant differences between the pressure levels are marked with different alphabets ($p < 0.05$, Tukey's test).



**Fig. 3.** pH value measured in the methane reactor at different pressure levels. The significant differences among the pressure levels are marked with different alphabets ($p < 0.05$, Tukey's test).



**Fig. 4.** Specific methane yield in methane reactor at different pressure levels. The significant differences among the pressure levels are marked with different alphabets ($p < 0.05$, Tukey's test).

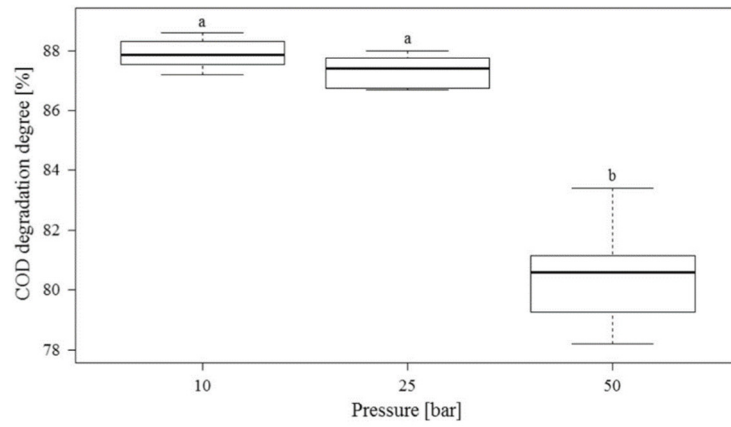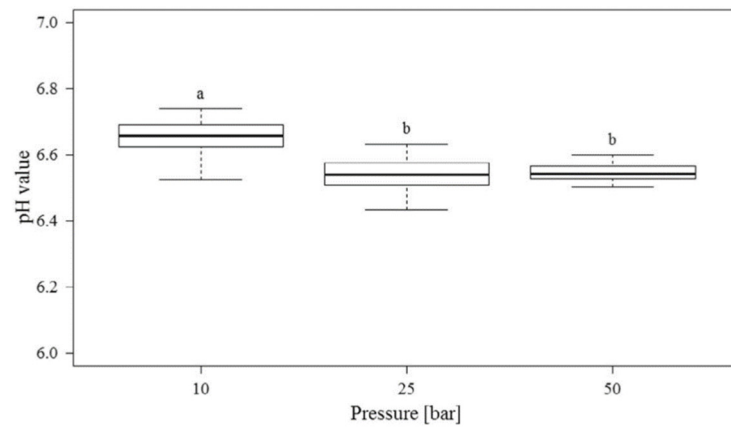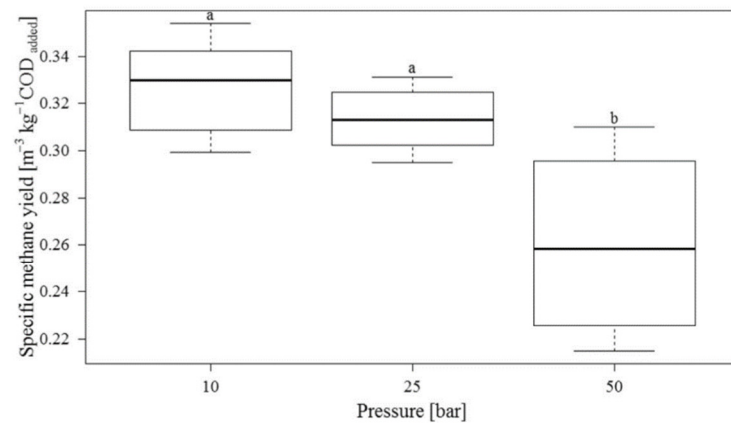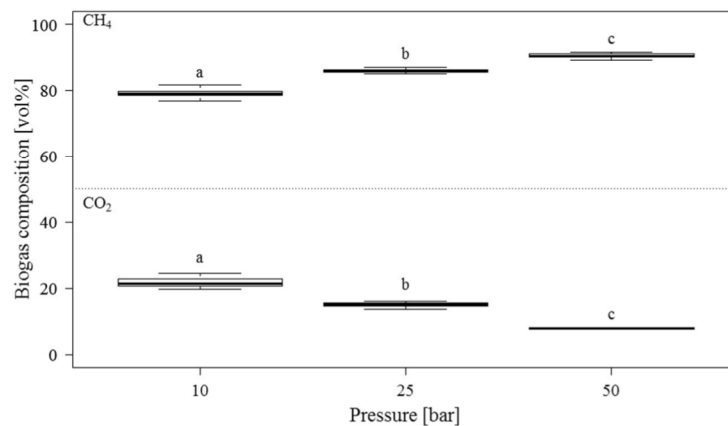**Fig. 5.** Methane and carbon dioxide content in the methane reactor measured at different pressure levels. The significant differences among the pressure levels are marked with different alphabets (p < 0.05, Tukey's test).

while the carbon dioxide content decreased from $21.62 \pm 1.28\%$ at 10 bar to $7.86 \pm 0.2\%$ at 50 bar. At higher operating pressures the methane content in the gas increased.

Biogas quality was also affected due to the significant differences in solubility of $CH_4$ and $CO_2$ under pressure. Therefore the optimal operating pressure is depending on the desired methane content in the produced gas and the utilization of low calorific value flash gas. At operating pressure of 50 bar, the $CH_4$ tends to be dominant in the biogas, resulting in a significant increase of $CH_4$ content more than 90%. Higher $CH_4$ contents might be achieved by increasing the pH value, as the pH value influences the amount of dissolved $CO_2$ in an equal way than pressure does. Chen et al. (2014a) mentioned that at the same pressure, the solubility of $CO_2$ at a pH value of 7.5 is about seven times higher than at a pH value of 6.5.

## 4. Conclusion

This investigation of two-stage high pressure anaerobic digestion integrating biogas production, purification and pressure boosting within one process, methane contents above 90% was obtained. The operation of the high pressure anaerobic digestion system at operating pressures up to 50 bar was technically feasible without any problems. The produced biomethane can be injected into the transnational gas grids without additional pressurization or can be used in transportation sector. Although being a promising process, an effective and economical method for maintaining pH level in the pressurized methane reactor should be further investigated.

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# 5 General discussion

The concept of two-stage high pressure anaerobic digestion integrates biogas production, purification and pressure boosting within one system, aiming to reduce the efforts and costs of subsequent gas purification. Within this innovative research project, lab-scale batch- and continuously operated test rigs were developed. Three different experimental set-ups, demonstrated in the publications 1-3, were chosen to examine the fundamentals of biomethane production in pressurized two-stage anaerobic digestion systems and to explore the potential of the concept. In the following chapter, the overall performance of the pressurized methane reactor in terms of process stability and biogas production will be discussed. The application prospect of this new concept for a full scale application will also be presented.

## 5.1 Process stability

### 5.1.1 pH-value

The fermentation process in anaerobic digestion is significantly influenced by the pH-value [39-41] and a neutral pH-value about 7 is recommended for a stable process [43]. At pH levels lower than 6 the methane producing of bacteria will be inhibited [42]. The results of the study (Pulication 1) showed that different operating pressures (10, 20 and 30 bar) in the batch reactor have a significant influence on the pH-value of the liquid phase. During the starting phase of the experiments, it could be observed that the pH-value drops beyond the optimal range for anaerobic digestion from 7 to $6.31 \pm 0.04$ at 10 bar and to $6.25 \pm 0.03$ at 30 bar. From the beginning to the end of the experiment, the pH-value steadily increased to a constant value of $6.57 \pm 0.07$ at 10 bar and $6.48 \pm 0.05$ at 30 bar. This can be explained as follows: At the beginning of the experiment, the partial pressure of $CO_2$ is almost zero, due to the use of $N_2$ to provide the initial pressure. After closing the batch reactors, the microorganisms immediately started to degrade sugars, alcohols and organic acids. Due to the related production of $CO_2$, the $CO_2$ partial pressure slightly increased. According to Henry's Law, the solubility of $CO_2$ is proportional to partial pressure, indicating that $CO_2$ dissolution rises in the aqueous phase. Furthermore, within the pH range of 6.0 to 7.5, the buffer capacity of the aqueous phase is almost totally dependent on the carbonic acid dissociation [42]. The dissolved $CO_2$ forms carbonic acid ($H_2CO_3$) which dissociates immediately to hydrogen carbonate ($HCO_3^-$), depressing the pH-value in the batch reactor due to the liberated protons in the solution. For this reason, the pH-value in the batch reactors at the end of the experiment cannot reach the same level as at the beginning, despite the further degradation of the organics.

In contrast to the batch experiments (Publication 1), the pH-value could be kept very stable in a continuously operated methane reactor up to pressures of 50 bar (Publication 3). Due to the continuous feeding and $CO_2$ release by the effluent from the methane reactor to the flash tank, there were only minimal changes in $CO_2$ partial pressure within one run. The pH-value varied between $6.65 \pm 0.05$ at 10 bar and $6.55 \pm 0.02$ at 50 bar, which was still within the range of the optimal pH-value for anaerobic digestion under atmospheric conditions [43]. However, in studies on continuously operated pressurized methane reactors from Chen et al. and Lemmer et al., pH-values lower than 6.4 at pressures up to 10 bar are reported [44, 53]. The difference in pH-drop is due to the higher concentration of ammonium ($NH_4^+$) of 1.04 to 1.24 g kg$^{-1}$ in the leachate (Publication 1) in these studies, leading to a high buffering capacity of the digestate. At pH-values below 7, the ammonium concentration represents the total ammonia nitrogen because all ammonia nitrogen turns into ammonium nitrogen [14].

Furthermore, in this study an innovative method was applied for indirect pH measuring in the methane reactor by combined liquid and gas quality analysis. The pH-values in Publication 1 were also calculated based on sum parameters such as VFA (Volatile Fatty Acids, FOS in the German technical literature), acetic acid-equivalent (HAc), total alkalinity (TIC) and taking also the dissolved amount of $CO_2$ in the liquid into account. The results were quite similar to the measured values, representing an alternative to the cost intensive direct measuring of pH under pressure. The pH-values calculated by VFA and TIC ranged between $6.49 \pm 0.1$ at 10 bar and $6.34 \pm 0.09$ at 30 bar in contrast to the measured pH-value, ranging between $6.57 \pm 0.07$ at 10 bar and $6.48 \pm 0.05$ at 30 bar. Without considering the dissolved amount of $CO_2$ in the liquid, the estimated pH-values by liquid analysis (LIQ) were between $9.48 \pm 0.06$ at 10 bar and $9.2 \pm 0.12$ at 30 bar.

An increasing $CO_2$ partial pressure is lowering the pH-value at all tested pressure stages. Especially in batch experiments, pH-value drops beyond the optimal range for anaerobic digestion to $6.25 \pm 0.03$ at 30 bar. The studies showed that a proper mathematical estimation of the pH-value in the process liquid is possible, if additional to the composition of the liquid the dissolved amount of $CO_2$ is taken into account.

## 5.1.2 Organic degradation

In the batch experiments in Publication 2 at pressures up to 100 bar, all organic acids were degraded after 21 days in all runs, independent of the initial pressures, thus indicating no inhibition of microorganisms.

General discussion

In contrast, in the batch experiments up to 30 bar in Publication 1, no complete degradation of the acids by the end of 35 days run was achieved. However, the incomplete degradation of organic acids was not depending on the initial pressure and could be observed at every pressure level. One reason for the accumulation of high amounts of propionic acid in the batch rigs in Publication 1 might be the high amount of lactic acid in the leachate. Zellner et al. described a shift from the degradation pathway of lactic acid from acetic acid to propionic acid at higher concentrations of lactic acid [54]. The present valeric acids in the effluent after 35 days might be a result of degradation of amino acids such as Arginine, Leucine, Iso-leucine and Proline [55]. Those acids could not be measured by the analyzing equipment available and should be subject to further studies. Another reason for the lower degradation could be the higher OLR of $8.8 \pm 0.8$ kg soluble chemical oxygen demand (SCOD) per m³ applied in the first experiments in contrast to the batch experiments up to 100 bar running only at an OLR of $3.9 \pm 0.6$ kg total COD per m³ (without taking the insoluble part of total chemical oxygen demand (COD) into account).

In the experiments in Publication 3, all detected acids of the initial leachate were degraded under operating pressures up to 25 bar. Only at an operating pressure of 50 bar a slight acid accumulation in the methane reactor was observed and the acetic and propionic acid concentration rose to $0.08 \pm 0.1$ g kg$^{-1}$ and $0.43 \pm 0.14$ g kg$^{-1}$. These values had remained below the critical value of 1 g kg$^{-1}$ and 0.7 g kg$^{-1}$ [56] at all times, indicating a still very stable biological process. The degradation degree in the methane reactor was higher than 87% at pressures up to 25 bar. It decreased to $80.4 \pm 1.7\%$ at 50 bar at an OLR of 4.2 kg COD·m$^{-3}$·d$^{-1}$ and an hydraulic retention time (HRT) of $4.19 \pm 0.06$ d. Chen et al. mentioned similar overall COD degradation grades of more than 90% at 9 bar at even higher OLR up to 12.5 kg COD m$^{-3}$ d$^{-1}$ and a HRT of 1.8 d [52]. In comparison to the batch experiments in Publication 1 and 2, the degradation degree in the continuous experiments (Publication 3) was always higher at a lower HRT. Based on the results it can be assumed, that at operating pressures of 25 bar, even higher OLRs with shorter HRTs are possible, making this process more suitable for full-scale applications.

## 5.2 Biogas production

The promising results of the process stability research in two-stage high pressure anaerobic digestion showed that a further look on the gaseous phase is absolutely necessary to explore the effect of pressure on biogas production and quality.

### 5.2.1  Production kinetics

Although the initial operating pressures in the batch experiments were applied within 5 minutes without any adaption phase for the microorganisms, there was no observable effect on the gas production kinetics detected. The batch experiments in Publication 1 showed no significant influence of the initial pressure on the measured pressure increase or on almost any of the calculated production parameter by the modified Gompertz equation [57]. The measured pressure increase per g added SCOD ($SCOD_{added}$) ranged between $2.19 \pm 0.12$ bar $g^{-1}$ at 10 bar and $1.84 \pm 0.33$ bar $g^{-1}$ at 30 bar. The calculated pressure increase potential (P) per $SCOD_{added}$ ranged between $2.22 \pm 0.13$ bar $g^{-1}$ at 10 bar and $1.84 \pm 0.25$ bar $g^{-1}$ at 30 bar. The maximum daily pressure increase rate ($R_m$) per $SCOD_{added}$ slightly diminished from $0.13 \pm 0.03$ bar $d^{-1}$ $g^{-1}$ at 10 bar to $0.12 \pm 0.04$ bar $d^{-1}$ $g^{-1}$ at 30 bar and the time of the maximum pressure increase per day ($t_{max}$) was postponed from day $10.63 \pm 1.52$ at 10 bar to day $13.54 \pm 2.85$ at 30 bar. Only the duration of the lag phase ($\lambda$) showed significant differences, varying between $3.98 \pm 1.15$ d at 10 bar and $7.59 \pm 1.86$ d at 30 bar. In contrast to the batch experiments in Publication 1, significant differences in $t_{max}$ at even higher operating pressures could be observed in the batch experiments at 1, 50 and 100 bar in Publication 2. One reason for this could be the difference in the composition of the leachate and in OLR.

Although the calculated parameters of the production kinetics showed no much difference in these experiments, this method offers an interesting opportunity to compare batch experiments under pressure. A new approach could be the use of single organic acids, sugars or alcohols as leachate for the pressurized batch reactors to evaluate the specific degradation times at different pressures. These findings could be used to simulate the production kinetics knowing the composition of the leachate at the beginning of the experiment. For atmospheric pressure, Krümpel et al. already evaluated the degradation times for different organic substances [58], also using the modified Gompertz equation to calculate degradation kinetic parameters.

### 5.2.2  Gas composition

Due to the use of $N_2$ for applying the initial pressure in the batch experiments in Publication 1 and 2, only low contents of $CH_4$ and $CO_2$ could be measured in the produced gas. The gas analysis at the end of each run in the batch experiments in Publication 1 showed a decrease in $CH_4$ content by $39.3 \pm 1.1\%$ at 10 bar to $21.7 \pm 1.3\%$ at 30 bar and in the $CO_2$ content from $14.5 \pm 0.4\%$ at 10 bar to $8.7 \pm 0.7\%$ at 30 bar. The calculated ratio only considering the contents

of $CH_4$ and $CO_2$ remained almost unchanged. At 10 bar, $CH_4$ had a calculated share of $73.4 \pm 1.1\%$ and at 30 bar $71.3 \pm 2.5\%$, respectively.

In the batch experiments up to 100 bar in Publication 2, even lower $CH_4$ contents of $0.5 \pm 0.2\%$ at 100 bar and $CO_2$ contents of $0.5 \pm 0.2\%$ at 100 bar were observed. The calculated share of $CH_4$ remained nearly unchanged between $88 \pm 1.5\%$ at 1 bar and $86.3 \pm 0.7\%$ at 100 bar.

In continuous experiments of Publication 3, the increased operating pressures of the reactor was achieved only by the gas production of the microorganisms without applying initial any pressures, thus leading to high contents of $CH_4$ in the product gas. The methane content increased from $79.08 \pm 1.01\%$ at 10 bar to $90.45 \pm 0.73\%$ at 50 bar. These results are similar to previous research in two-stage high pressure fermentation reaching $CH_4$ contents of up to 87% at 5.9 bar in continuous operation and recirculation of the effluent from the flash tank [26].

In batch experiments from Lindeboom et al. $CH_4$ contents up to 95% at pressures up to 90 bar have been even achieved [59]. As the pH-value influences the amount of dissolved $CO_2$ in an equal way to pressure, even higher methane contents can be reached by increasing the pH-value in the methane reactor. Chen et al. mentioned that the solubility of $CO_2$ at pH 7.5 is about seven times higher than at pH-value 6.5, at constant pressure level [44].

### 5.2.3 Methane production

In order to gain a clearer picture of the results, the methane production is related to the added COD or SCOD as SMY. As shown in Publication 1, the calculated SMY by gas analysis at the end of each run related to SCOD varied between $180 \pm 16$ L kg$^{-1}$ at 10 bar and $185 \pm 14$ L kg$^{-1}$ at 30 bar, without being affected by initial pressures.

The results of the batch experiments up to 100 bar in Publication 2 showed similar results. The SMY related to COD was slightly higher ranging between $208 \pm 14$ L kg$^{-1}$ at 1 bar and $220 \pm 33$ L kg$^{-1}$ at 100 bar, due to a complete degradation of the organic acids. Furthermore, a calculation of the SMY using only liquid analysis to estimate the production of the gases by the degradation pathways of the organic acids was possible because of a total degradation of the acids. This SMY by liquid potential related to COD summed up to $210 \pm 25$ L kg$^{-1}$ at each pressure level (1, 50, 100 bar). At a total degradation of the organic acids, this method represents a promising way to calculate the SMY.

In the continuous experiments in Publication 3, the SMY was calculated by the gas produced in the methane reactor and measured in the gas phase, and was related to the added COD into the methane reactor. The results showed a significant decrease of the SMY by increasing the

operating pressure. The SMY decreased from $330 \pm 20$ L kg$^{-1}$ at 10 bar and to $260 \pm 40$ L kg$^{-1}$ at 50 bar. Due to Henry's Law, the physically dissolved amount of the gases is proportional to the partial pressure [37, 38]. However, not only the solubility of $CO_2$ increased, but also the solubility of $CH_4$. At 10 bar, 2.1% of the $CH_4$ was dissolved in the liquid and transferred to the flash tank, where it was released due to the reduced partial pressure. By increasing the operating pressure to 50 bar, the share rose to 24%. Summarizing the $CH_4$ production of the total anaerobic filter system including methane reactor and flash tank, the SMY was slightly higher at 10 bar and 25 bar (340 L kg$^{-1}$) compared to 50 bar (300 L kg$^{-1}$). The results of the total anaerobic filter system up to 25 bar correspond with those other studies [44, 53, 60]. The lower SMY at 50 bar can be explained by the acid accumulation in the methane reactor.

In summary, high operating pressures have not led to an inhibition of microorganisms in the batch-rigs. Although the microorganisms were not adapted to these environmental conditions, no significant differences in the pressure increase curves could be detected. Furthermore, the SMYs in the batch experiments were only affected by the degradation degree of the organic substrates, independent of the operating pressure. In contrast, the operating pressure influenced the results of the experiments under continuous conditions. An inhibition of microorganisms could be observed which led to a decrease in SMY by increasing the pressure, despite an increase in $CH_4$ content in gaseous phase. Thus leading to the conclusion, that the $CO_2$ partial pressure has a major influence on the biological process stability compared to the absolute operating pressure.

## 5.3   Transferability

The results in Publication 1-3 showed that two-stage high pressure anaerobic digestion offers a promising technical solutions for biogas production and upgrading compared to commonly used post purification methods. It could be shown, that continuously operated pressurized methane reactor is technically entirely feasible and high contents of $CH_4$ above 90% in the product gas at pressures up to 50 bar are achievable. In order to provide high conversion efficiencies and a low methane slip, operating pressures below 50 bar should be aimed according to the current state of the present study. To address the overall applicability of this method, an economic evaluation is required, to prove the costs for biogas upgrading and pressure boosting in contrast to the commonly used methods. The specific costs for biogas upgrading systems nowadays range between 0.66 Cent per kWh and 2.32 Cent per kWh, depending on the used technique and size of the unit [61].

General discussion

In a previous project called "B2G - Innovative production of gaseous fuels from biomass" the performed simulations on different operating pressures in the methane reactor came to the conclusion that the costs for biogas upgrading are predominantly depending on the operating pressure of the methane reactor. The study further exhibited that at operating pressure of 40 bar the energy demand for pumping the liquid predominates the energetic savings by the autogenerative pressure increase [62]. In these calculations, it was economically preferable for a full scale application to operate the methane reactor beyond 40 bar.

To set up a full scale pressurized methane reactor, to refuel 25 cars a day by compressed natural gas (CNG), a reactor with a dimension of approximately 5 m in diameter and a height of 10 m is needed, which corresponds to a gross volume of 200 m³. Dependent on the available feed streams, the methane reactor could be operated as stand-alone system or be coupled with a preceding acidification reactor. Due to the use of a fixed bed in the methane reactor, the feed streams should contain a high amount of dissolved organic acids, sugars and alcohols such as wastewater from sugar production. In the case of using organic waste or energy crops as substrate an acidification reactor is required. Studies on the acidification reactor from Linder et al., testing different substrates in a two-stage systems, concluded, that the acidification reactor seems to be only recommendable for digesting sugar rich substrates [63].

Before up scaling the two-stage high pressure anaerobic digestion system, further research on the performance of the continuously operating two-stage system could be beneficial. Although being a promising process, an effective and economical method for maintaining pH level and reducing the methane slip of the pressurized methane reactor should be further investigated. For maintaining pH level, a recirculation of the effluent from flash tank into the methane reactor could be possible. By this, the amount of $CO_2$ dissolved in the liquid and released to the flash tank could be increased and also affect the pH-value. Additionally, an appropriate use of the gas from the flash tank with a $CH_4$ content of about 60%, can improve the overall efficiency. Furthermore, the OLR of the methane reactor has to be increased to make this process economically feasible. By increasing the OLR, the investment cost can be reduced due a smaller size of the methane reactor. For this reason further research is needed to prove the maximum possible OLR for the methane reactor at operating pressures below 50 bar.

## 5.4   Conclusions and outlook

In this study, the effect of pressure on several processing parameters during two-stage high pressure anaerobic digestion could be examined using lab-scale batch- and continuous systems. First the effect of $CO_2$ partial pressure on process stability during anaerobic digestion was

proven, then the influence of high pressures on biogas production was investigated and finally the performance of a continuously operated methane reactor was validated. The results can be summarized in the following conclusions:

- There is an influence of increased $CO_2$ partial pressure on the digestate's pH-value inside the methane reactor. It could be shown by batch experiments that the pH-value dropped beyond the optimal pH range of 6.5-7.5 for anaerobic digestion. At higher pressures even lower pH-values can be expected, without using pH buffering substances. Furthermore, high amounts of lactic acid in the leachate inhibit the whole degradation of the acids and might reduce the process stability.

- Even a rapid increase of the pressure up to 100 bar using nitrogen, has no significant influence on the production kinetics and the specific methane yield. No significant differences in the course of the increase in pressure could be observed, which indicates that no inhibition of microorganisms was caused by the pressure increase.

- Running a continuously operated pressurized methane reactor is technically entirely feasible, producing high contents of methane above 90% in the product gas at pressures up to 50 bar. It can be expected that, by increasing the operating pressure, the acid concentration in the effluent will increase slightly, resulting in a decrease in conversion efficiency as well as an increase in the methane slip to the flash tank caused by physical effects.

The results show that two-stage high pressure anaerobic digestion, integrating biogas production, purification and pressure boosting within one process, offers a promising alternative to commonly used post purification methods. Using this method, the costs usually associated with a subsequent gas purification unit can be significantly reduced, due to the high methane content in the product gas. The produced biogas is suitable for injection into the transnational gas grids without post pressurization or for use in the transportation sector as a fuel for cars or trucks.

Prior to an installation of a full scale application, further research on effective and economical methods of maintaining the pH level and reducing the methane slip of the pressurized methane reactor is needed. Furthermore, studies using leachate not containing lactic acid should be carried out under continuous conditions to prove the effect of lactic acid on conversion efficiencies.

## 6  Summary

The use of natural gas for power and heat generation in the EU has become particularly prominent since the 1990s. As a result, the whole natural gas infrastructure has been continuously expanded and today has a total length of 2.15 million km and a storage capacity of about 108.3 billion m³. Due to the fact, that the production of natural gas in the EU corresponds only one third (30.7%) of the annual natural gas demand, there is a strong dependency on imported fossil fuels. The production of biomethane in the EU and its distribution by natural gas network offers an interesting alternative for the reconfiguration of EU's energy supply system.

Up to now, biomethane is obtained by purifying and upgrading raw biogas in a complex process. In this process, primarily the carbon dioxide ($CO_2$) content of the educt gas is reduced from 40-45% to approximately 4% in the product gas. A number of different technologies are available for this upgrading, e.g. pressure water-scrubbing, pressure swing adsorption (PSA) or the non-pressurized amine purification. These systems are suitable only for large-scale applications.

In this study, a novel two-stage high pressure anaerobic digestion system was developed. This innovative concept aims to integrate biogas production, purification and pressure boosting within one system. The process is based on the enhanced water solubility of $CO_2$ compared to methane. By operating the methane reactor for biogas production at increased pressures, high amounts of dissolved $CO_2$ can be removed with the liquid effluent from the reactor, resulting in a high-calorific biogas. These two-stage high pressure anaerobic digestion systems have been described in literature for operating pressures up to 10 bar. The novel approach of this study was to develop and test a two-stage high pressure anaerobic digestion system aiming operating pressures up to 100 bar thus, reducing the pressure boosting costs for injecting the biomethane into transnational gas grids. To gain a better understanding of this innovative technology, lab-scale batch- and continuously operated test rigs were developed. The aims of the conducted experiments were divided into three subtasks:

(1) to prove the effect of increased $CO_2$ partial pressures on the pH-value during anaerobic digestion; (2) to estimate the influence of high operating pressures on production kinetics and specific methane yields; (3) to investigate the performance of a continuously operated methane reactor in a two-stage high pressure anaerobic digestion system.

During the anaerobic microbial conversion of organic substrates to methane, $CO_2$ is released, thus lowering the pH at higher operating pressures, due to the augmented formation of carbon hydroxide. At the beginning of the experiments, the initial pressures in the batch reactors were

raised by pressurized nitrogen to the aimed values within a few minutes. The study showed that different operating pressures in the batch reactor have a significant influence on the pH-value of the digestate during the experiments run at 10, 20 and 30 bar. During the starting phase of experiments pH-values dropped beyond the optimal range for anaerobic digestion to $6.25 \pm 0.03$ at 30 bar. However, the initial pressures had no significant influence, neither on the pH-value curve during the process nor on the process stability. The study on the effect of a rapid pressure increase up to 100 bar showed no inhibition of the microorganisms in the batch-rigs too, although the microorganisms were not adapted to these environmental conditions. There were also no significant differences in the pressure increase curves between the variants 1 bar, 50 bar and 100 bar. At the end of the experiment all acids were degraded and the specific methane yields related to the added chemical oxygen demand ($COD_{added}$) varied between $208 \pm 14$ L $kg^{-1}$ (1 bar) and $220 \pm 33$ L $kg^{-1}$ (100 bar). In the third part of the study, a continuously operated methane reactor was run at pressures up to 50 bar for the first time. The experiments showed that a stable anaerobic digestion process could be run at these pressures nearly without any problems. Methane contents above 90% could be achieved in the product gas at 50 bar operating pressure. Furthermore, the conversion efficiency related to COD removal decreased from 87% at 10 bar to 80% at 50 bar, due to a slight accumulation of acids in the effluent.

The present studies revealed the effects of high operating pressures in the methane reactor in a two-stage anaerobic digestion system. The promising results showed that this technology has great potential in producing on-site high calorific gas also in smaller units. In addition, the costs of post-production gas purification can be significantly reduced, due to the fact that the size of a subsequent gas purification unit can be decreased. Furthermore, the produced gas can be injected into the transnational gas grids without post pressurization or can be used in the transportation sector as fuel for cars or trucks. To summarize, the two-stage high pressure anaerobic digestion offers an interesting process for a sustainable and independent energy supply system in both economic and ecological perspectives.

# 7  Zusammenfassung

In der EU hat die Nutzung von Erdgas zur Erzeugung von Strom und Wärme seit den 1990er Jahren zunehmend an Bedeutung gewonnen. Infolge dessen wurde die Erdgasinfrastruktur kontinuierlich ausgebaut und umfasst heute eine Leitungslänge von 2,15 Mio. km und ein Speichervolumen von 108,3 Mrd. m³. Da die jährliche Erdgasförderung in der EU nur rund einem Drittel (30,7%) des Verbrauchs entspricht, besteht eine sehr große Abhängigkeit gegenüber importierten fossilen Brennstoffen. Eine interessante Alternative zur Neugestaltung des Energieversorgungssystems in der EU ist die Erzeugung von Biomethan und die anschließende Einspeisung in das bestehende Erdgasnetz.

Biomethan wird bisher in einem aufwendigen Reinigungs- und Aufbereitungsverfahren aus Rohbiogas gewonnen. Dieser Prozess basiert auf einer Reduktion des Kohlendioxidgehaltes von 40-45% im Eduktgas auf etwa 5% im Produktgas. Für die Gasaufbereitung werden eine Vielzahl von Technologien wie die Druckwasserwäsche, die Druckwechseladsorption oder die druckfreie Aminwäsche verwendet. Diese Verfahren werden bisher nur bei Großanlagen eingesetzt.

In der vorliegenden Studie wurde ein neuartiges zweistufiges Hochdruckfermentations-verfahren entwickelt. Ziel dieses innovativen Konzepts ist die Integration von Biogaserzeugung, -aufbereitung und -verdichtung in ein einziges Verfahren. Dieses Verfahren basiert auf der erhöhten Wasserlöslichkeit von Kohlenstoffdioxid im Vergleich zum Methan. Durch den Betrieb des Methanreaktors unter erhöhtem Druck können große Mengen an gelöstem Kohlenstoffdioxid mit dem Effluent aus dem Reaktor ausgetragen werden, was die Erzeugung eines hochkalorischen Biogases ermöglicht. Dieses zweistufige Hochdruckfermentationsverfahren wurde in der Literatur bereits bis zu Betriebsdrücken von 10 bar beschrieben. Der neuartige Ansatz dieser Studie bestand darin, ein zweistufiges Hochdruckfermentationsverfahren zu entwickeln und bei einem Druck von bis zu 100 bar zu betreiben, um die Kosten für die Gasverdichtung bei der Einspeisung des Biomethans in transnationale Gasnetze zu reduzieren.

Um ein besseres Verständnis dieser innovativen Technologie zu erhalten, wurden Untersuchungen im Labormaßstab mit Batch- sowie kontinuierlichen Systemen durchgeführt. In der vorliegenden Arbeit wurden folgende drei Teilziele bearbeitet:

(1) hat ein ansteigender $CO_2$ Partialdruck einen Einfluss auf den pH-Wert im Reaktor; (2) beeinflussen hohe Betriebsdrücke die Produktionskinetik und den spezifischen Methanertrag; (3) ist ein kontinuierlicher Betrieb des Methanreaktors in einer zweistufigen Hochdruckfermentationsanlage möglich.

Während der anaeroben mikrobiellen Umwandlung von organischen Substraten zu Methan wird $CO_2$ freigesetzt. Dies führt aufgrund der vermehrten Bildung von Hydrogencarbonat bei höheren Betriebsdrücken zu einem Absinken des pH-Wertes in der Reaktorflüssigkeit. Der Anfangsdruck in den Batch-Reaktoren wurde zu Beginn der Experimente schlagartig mit Stickstoff eingestellt. Bei Betriebsdrücken von 10, 20 und 30 bar, konnte ein signifikanter Einfluss des Drucks auf den pH-Wert im Reaktor ermittelt werden. Zu Beginn der Versuche fiel der pH-Wert bei allen Drücken (z.B. Betriebsdruck 30 bar; Absinken des pH-Wertes von 7,0 auf $6.25 \pm 0.03$) unter die Grenze des optimalen Bereichs für die methanogenen Mikroorganismen ab. Es konnte jedoch weder ein signifikanter Einfluss des Anfangsdrucks auf den Verlauf des pH-Wertes, noch auf die Prozessstabilität festgestellt werden. Die schnelle Druckerhöhung in den Batch-Reaktoren auf bis zu 100 bar zeigte keinerlei Hemmung der Mikroorganismen, obwohl diese nicht an die neuen Umgebungsbedingungen angepasst waren. Zwischen den Varianten 1, 50 und 100 bar konnten keine signifikanten Unterschiede im Druckanstieg durch die Gasproduktion der Mikroorganismen ermittelt werden. Darüber hinaus wurden in diesem Versuch alle Säuren vollständig abgebaut. Der spezifische Methanertrag betrug $208 \pm 14$ L $kg^{-1}$ bei 1 bar und $220 \pm 33$ L $kg^{-1}$ bei 100 bar bezogen auf den zugeführten Chemischen Sauerstoffbedarf ($CSB_{input}$). Im dritten Teil der Studie wurde erstmalig ein kontinuierlicher Methanreaktor bei Drücken bis 50 bar betrieben. Die Untersuchungen zeigten, dass ein kontinuierlicher Betrieb des Methanreaktors bei Drücken bis 50 bar nahezu problemlos möglich ist. Es konnten Methangehalte von über 90% realisiert werden. Jedoch konnte aufgrund einer leichten Anreicherung von Säuren im Methanreaktor ein Rückgang des CSB-Abbaugrades von 87% bei 10 bar auf $80,4 \pm 1,7\%$ bei 50 bar festgestellt werden.

In der vorliegenden Untersuchung wurden die Effekte hoher Betriebsdrücke im Methanreaktor in einer zweistufigen Fermentationsanlage auf die Prozessstabilität ermittelt. Diese vielversprechenden Ergebnisse zeigen, dass diese Technologie ein großes Potential für die Herstellung von hochkalorischem Gas auch in kleineren Maßstäben vor Ort bietet. Außerdem können die Kosten für eine nachgeschaltete Aufbereitungseinheit signifikant reduziert werden, da diese deutlich kleiner ausfallen könnte. Darüber hinaus kann das erzeugte Gas ohne weitere Verdichtung in das Erdgasnetz eingespeist oder im Transportsektor als Treibstoff für Autos oder LKWs eingesetzt werden. Die Studie zeigt, dass die zweistufige Hochdruckfermentation sowohl unter ökonomischen, wie auch unter ökologischen Gesichtspunkten ein zukunftsträchtiges Verfahren für eine nachhaltige und unabhängige Energieversorgung darstellt.

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