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.

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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 thermolitho-trophicus* 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
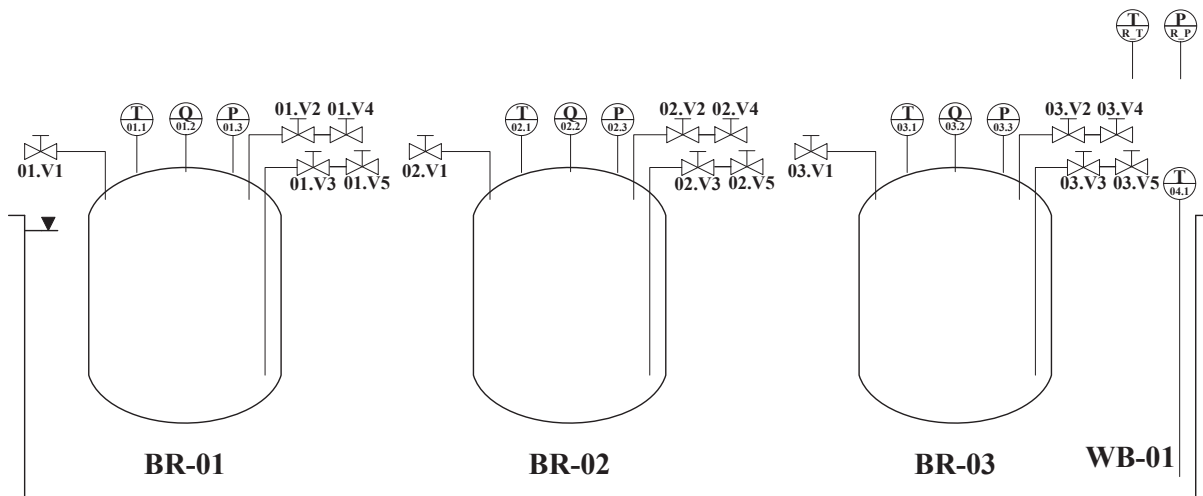

Fig. 1. Piping and instrumentation diagram of the high-pressure batch anaerobic digestion system.

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.

$$\sum_a \tilde{m}_a z_a = \sum_c \tilde{m}_c z_c \tag{1}$$

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].

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

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

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

$$HA \leftrightarrow H^+ + A^- \tag{4}$$

$$K_S = \frac{\tilde{A}^- \cdot \tilde{H}^+}{\widetilde{HA}} \cdot \frac{\gamma_{A^-} \cdot \gamma_{H^+}}{\gamma_{HA}} \tag{5}$$

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].

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

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

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.

$$Dissolved\ CO_2\ excluded: \tilde{m}_{HS^-} + \tilde{m}_{HAc} + \tilde{m}_{OH^-}$$
$$= \tilde{m}_{H^+} + \tilde{m}_{TN} + \tilde{m}_{Base^+} \tag{8}$$

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 γ_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 γ_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 λ as duration of the lag phase time (d). The constants P, R_m and λ 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
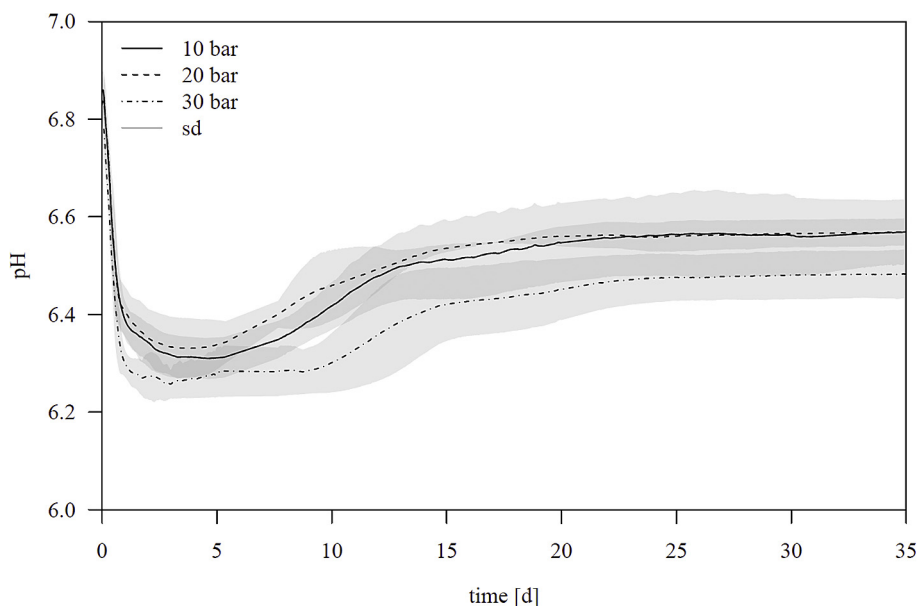

Fig. 2. pH-value curves for three different initial pressures (10, 20, 30 bar), standard deviation (sd) of three repetitions in grey, overlaps of sd in darkgrey.
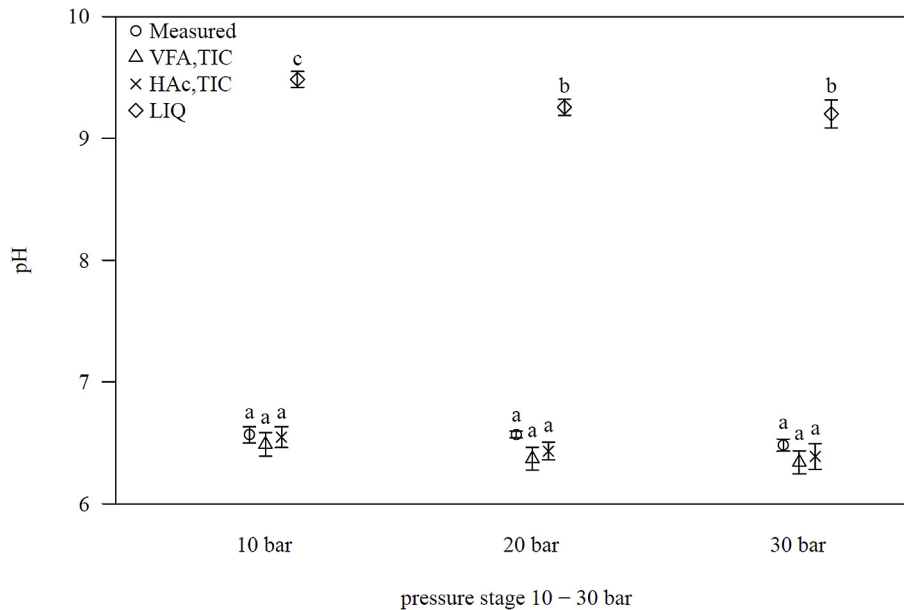
Fig. 3. Measured and calculated pH-value for three different initial pressures (10, 20, 30 bar). The pH-value calculated either by volatile fatty acids (VFA) and total alkalinity (TIC), by acetic acid equivalent (HAc) and total alkalinity (TIC) or by liquid analysis (LIQ). The significant differences 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 ± 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 ± 0.07 bar g^{-1} $SCOD_{added}$ and 1.84 ± 0.33 bar g^{-1} $SCOD_{added}$. The time of maximum daily pressure increase rate (t_{max}) was reached between 10.63 ± 1.52 days (10 bar) and 13.54 ± 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 ± 0.13 bar g^{-1} $SCOD_{added}$ (10 bar) to 1.84 ± 0.25 bar g^{-1} $SCOD_{added}$ (30 bar). Significant differences between 3.98 ± 1.15 days (10 bar) and 7.59 ± 1.86 days (30 bar) could be observed in the

duration of the lag phase (λ). By increasing the initial pressures, the maximum daily pressure increase rate (R_m) has slightly diminished from 0.13 ± 0.03 bar d^{-1} g^{-1} $SCOD_{added}$ (10 bar) to 0.12 ± 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 ± 0.02 L to 4.14 ± 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].
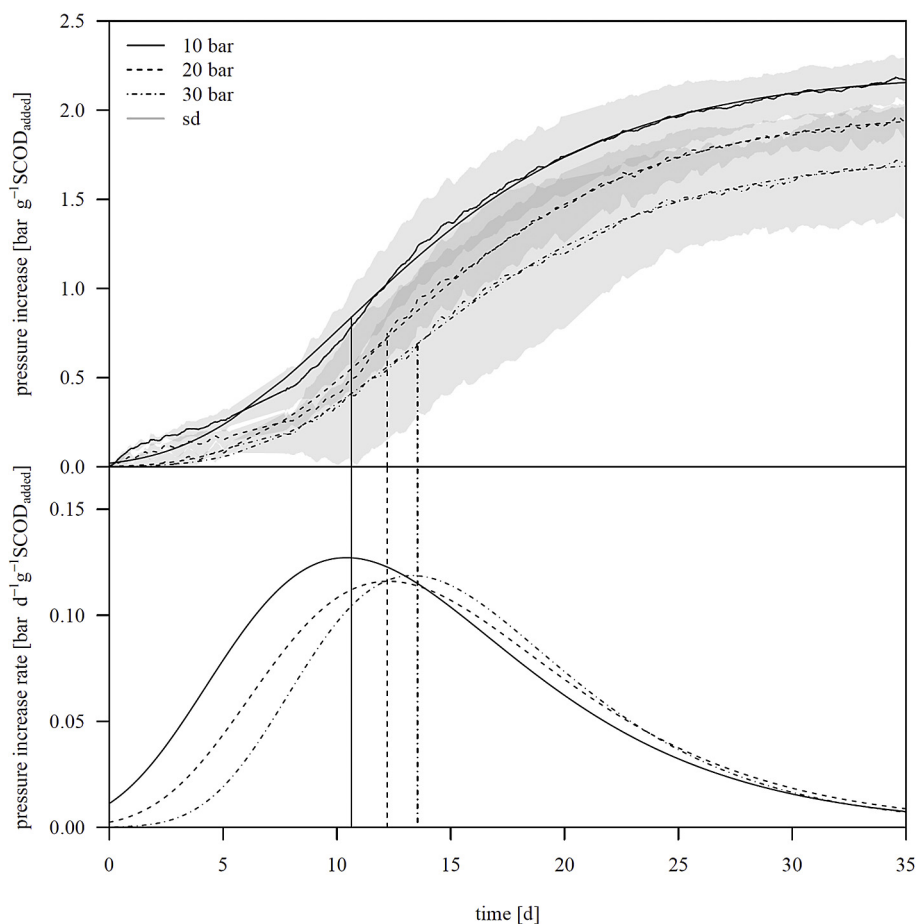
Fig. 4. Measured pressure increase curves and pressure increase rates per SCOD added over time and the Gompertz adaptions of three different initial pressures (10, 20, 30 bar) at 37 °C, standard deviation (sd) of three repetitions in grey, overlaps of sd in darkgrey.
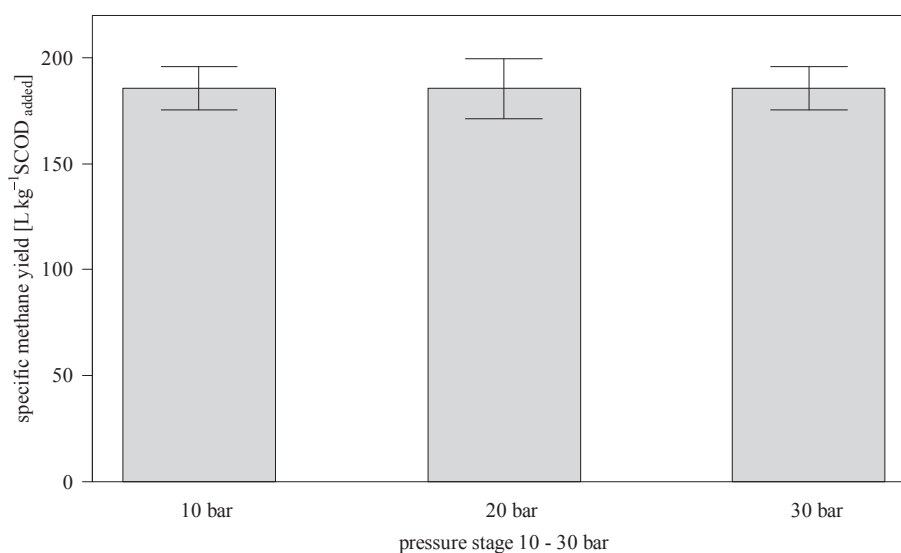

Fig. 5. Specific methane yield per kg SCOD added at T = 37 °C calculated from the gas analysis for three different initial pressures (10, 20, 30 bar), p > 0.05.

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 ± 16 L kg^{-1} $SCOD_{added}$ at 10 bar and 185 ± 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, λ = 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} $SCOD_{added}$)	λ (d)	R_m (bar d^{-1} g^{-1} $SCOD_{added}$)	t_{max} (d)
10 bar	2.22 ± 0.13[a]	3.98 ± 1.15[a]	0.13 ± 0.03[a]	10.63 ± 1.52[a]
20 bar	2.02 ± 0.11[a]	5.86 ± 0.64[ab]	0.12 ± 0.02[a]	12.21 ± 1.21[a]
30 bar	1.84 ± 0.25[a]	7.59 ± 1.86[b]	0.12 ± 0.04[a]	13.54 ± 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 ± 2.1	39.3 ± 1.1	14.5 ± 0.4	2.18 ± 0.02
20	54.1 ± 2.5	28.2 ± 0.1	10.2 ± 1.0	3.18 ± 0.14
30	61.6 ± 2.2	21.7 ± 1.3	8.7 ± 0.7	4.14 ± 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 ± 0.04 after 4.16 ± 0.82 days (10 bar) and to 6.25 ± 0.03 after 3.16 ± 1.47 days (30 bar). During the following days of the experimental runs, the pH-values increased to a constant value of 6.57 ± 0.07 (10 bar) and 6.48 ± 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 ± 0.12 bar g^{-1} $SCOD_{added}$ (10 bar) and 1.84 ± 0.33 bar g^{-1} $SCOD_{added}$ (30 bar), respectively. The maximum pressure increase rates ranged from 0.13 ± 0.03 bar d^{-1} g^{-1} $SCOD_{added}$ (10 bar) to 0.12 ± 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 ± 1.52 days (10 bar) and 13.54 ± 2.85 days (30 bar)). The resulting specific methane yields varied between 180 ± 16 L kg^{-1} $SCOD_{added}$ (10 bar) and 185 ± 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.

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