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Membrane-free dissolved hydrogen monitoring in anaerobic digestion

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ABSTRACT

Dissolved hydrogen (dH_2) is an important parameter in anaerobic digestion (AD) processes, in particular in a two-stage operation mode encompassing separate hydrolytic/acidogenic and methanogenic stages. Then, monitoring of dH_2 as a substrate of hydrogenotrophic methanogenesis is essential to avoid feast and famine conditions. Despite its significance, there is currently a lack of established monitoring systems which are capable of reliably measuring dH_2 concentrations in culture broth. To address this challenge, we propose a novel measurement system, which is based on the membrane-free extraction of hydrogen in an extraction chamber and the subsequent analysis by a metal-oxide (MOX) gas sensor. The response time of the MOX sensor lies in the range of seconds, while the entire measurement process completes within a total processing time of about 70 min). This study explores the measurement performance of the dH_2 sensor in the hydrolytic/acidogenic and methanogenic stage during lab-scale anaerobic digestion. The measurement principle was consistently applied for over three months. During this period, the methanogenic stage was partly sparged with gaseous hydrogen to monitor the dH_2 response afterwards. The dH_2 sensor responded reliably to these and other dynamic changes. Depending on the process conditions, concentrations between < 10 and $> 4{,}000$ Pa were detected, corresponding to dH_2 concentrations of < 0.074 to > 30 μmol L^{-1}. The findings demonstrated the importance of dH_2 monitoring and show that it facilitates the control of H_2 addition, thereby preventing both under- and oversupply during methanogenesis.

1. Introduction

The conversion of biogenic residues into biogas is part of any renewable energy provision scenario. Energy from biomass, especially in the form of biogas, has the potential to partially offset shortages and surpluses from wind and solar energy, if the methane production is controllable and can be altered to a certain extent and in an appropriate time frame [1–3]. By using waste streams as substrates for microbiological degradation and by preventing methanogenesis, anaerobic digestion (AD) can play a key role in solid waste valorization. Serving as a producer of value-added byproducts such as short-chain carboxylic acids (SCCA), a combined material and energy use of the biogenic feedstock becomes possible [4]. The pre-requisite for utilizing the full capacity of AD is a stable and robust microbial digestion system, which allows the alternating use of different feedstock and quantities [5]. One way to achieve a high level of flexibility is to separate the process phases and their dominant microorganisms into different fermenters. A good compromise between process efficiency and operating expenses is represented by two-stage digestion [2,6]. This way, comparably high rates of hydrolysis and acidogenesis can be achieved at low pH-values (4−5) by bacteria in the first stage. The effluent can subsequently be transformed into biogas by archaea in the second stage at neutral pH-values. However, it is necessary that the processes of AD can be specifically monitored and finally controlled to enable i) a flexible feedstock utilization at the typically rather robust hydrolysis stage, and ii) a flexible methane production in the second stage by feeding the products of the first stage in a controllable manner. In order to achieve this, continuous process monitoring is required for the early detection of disturbances, in

Abbreviations: AD, anaerobic digestion; CSTR, continuous stirred-tank reactor; dH_2, dissolved hydrogen; k_H, Henry constant; HSA, humidified synthetic air; MFC, mass flow controller; MOX, metal-oxide; PLC, programmable logic control; pH_2, partial pressure of hydrogen; pdH_2, partial pressure of dissolved hydrogen; ppbv, parts per billion by volume; ppmv, parts per million by volume; SCCA, short-chain carboxylic acids.

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particular in the second stage containing rather sensitive archaea, as an under- or oversupply of nutrients has to be avoided. This is generally difficult at dynamic operation conditions, which as such are a pre-requisite for a flexible feedstock use and dynamic product synthesis. The monitoring of dissolved hydrogen (dH_2) and other gases can provide important information to prevent an under- or oversupply of nutrients or the gas itself if sparged. Changes in concentrations in the liquid phase might appear faster than in the gas phase, which might lead to earlier detection of disturbances when a dissolved gas measurement is applied.

1.1. The role of hydrogen in anaerobic digestion

Hydrogen production as a part of AD happens during hydrolysis, acido- and acetogenesis. In full AD, hydrogen is consumed during methanogenesis. If these processes are not balanced in a one-stage AD application, process disturbances may appear. If dH_2 accumulates, e.g., due to a slow uptake by archaea, the metabolism of hydrolytic and acidogenic bacteria is inhibited with a rising hydrogen partial pressure. Most methane producing hydrogenotrophic archaea rely on fermentative bacteria, as these provide the hydrogen. The partial pressure should, however, stay between 3 and 10 Pa [7,8]. Higher partial pressures of hydrogen during methanogenesis may lead to a simultaneous production of acetate (*acetobacter/clostridia*) and methane (archaea), as homoacetogenesis and hydrogenotrophic methanogenesis, that are two ways of metabolic conversion of hydrogen and carbon dioxide, are increased. Furthermore, due to the inhibition of propionic acid oxidizing microorganisms, hydrogen concentrations above 100 Pa may lead to an accumulation of propionic acid and other SCCA [9–11]. A resulting drop of the pH-value can cause an inhibition of the methane production. In most AD processes, microbial hydrogen assimilation is, however, much higher than the interspecies hydrogen transfer, resulting in the transfer being the limiting step [12]. Results from Luo and Angelidaki [13] also showed no signs of an inhibition of the methanogenesis at partial pressures of dH_2 up to 300 Pa and more during hydrogen addition. Hence, it is believed that an undersupply of hydrogen and a subsequent microbial starvation is more crucial than any oversupply.

While methane, which is synthesized from carbon dioxide and hydrogen, is typically considered to be responsible for around 30% of the methane yield of a typical biogas plant [14,15], some studies highlight, that hydrogenotrophic methanogenesis has probably an even higher impact on the energy yield [16]. Luo and Angelidaki [13] showed that external addition of hydrogen and the resulting higher partial pressure of it leads to a higher hydrogenotrophic activity. Furthermore, it caused a shift in the archaeal community towards hydrogenotrophic archaea and methanogenesis mediating microorganisms like *M. thermoautotrophicus* for the co-digestion of manure and whey in an anaerobic reactor [13]. The concept of hydrogen addition suits especially well for two-stage AD applications, where hydrogen can be dosed directly into the methanogenic stage, without disturbing the hydrolysis. Several studies show that the methane yield can be increased by over 20–30% through the addition of hydrogen into the methanogenic stage [13,14,17].

1.2. Measurement of dissolved gases and other volatile components

Due to the low solubility of hydrogen and the technical challenges for measuring dissolved gases, it is commonly monitored in the off-gas of AD applications only. Miebach et al. [18] described the state-of-the-art gas-phase measurement for biohydrogen production in anaerobic monocultures. Castro-Carranza et al. describe different dissolved hydrogen monitoring methods in their recent review [19]. Methods for *in situ* determination of partial pressure or concentration of dissolved gases and other volatile components such as volatile SCCA in AD processes by means of various electrochemical measurement techniques have been known already for some time, but are often unprecise or rather expensive [20,21]. Chromatography, Raman or infrared spectroscopy (for CO_2, O_2, N_2, CH_4 and H_2) have been successfully used in more simple environments, such as sea or fresh water [20,22]. NIRS measurements were applied in transformer oils to detect dissolved gases (H_2, CH_4 etc.) [23] and in biogas plants to measure volatile SCCA [24,25]. Castellano and Ituarte [26] describe a system and several methods for removing dissolved gases from a liquid. The gas is removed by creating a pressure differential, applying heat, or sonicating dry solute and degassed solvent. Viitanen [27] describes a membrane-less extraction system for gases from transformer oils consisting of a bellows flask for liquid removal, gas extraction by low pressure, and subsequent gas compression. This form of extraction is typically avoided in biotechnological processes, since it would lead to the destruction of microorganisms, resulting in partial pressures that do not accurately represent the actual conditions. Another process for membrane-free gas recovery from transformer oils is described by Bräsel et al. [28]. In their process, the liquid medium, from which dissolved gases are to be extracted, flows in a thin film through the quiescent extraction gas at normal pressure. This extraction method is, however, not easily applicable in many biotechnological processes, due to the multiphase nature and risk of contamination. Alternatively, for many measurement methods, the sensor system is separated from the fermentation broth by a thin polymer membrane that is permeable to the analyzed gas [29]. This setup operates on the principle that gas permeates from the measurement medium through the membrane into a sensor medium driven by a concentration gradient. It is analyzed with a variety of different methods, mostly *offline*. As membrane-based gas extraction in mixed culture media carries the risk of membrane clogging, their long-term application is challenging.

Gas chromatographs, when combined with gas extraction units and appropriate detection equipment, offer a solid approach for quantifying dissolved gases. However, these methods require relatively high initial investment costs and need thorough maintenance [29,30]. A new concept for the extraction of dH_2 from anaerobic fermentation broth was presented by Zosel and co-authors based on the membrane-free extraction of dissolved volatiles from the liquid phase by an extraction gas stream of a high purity inert gas [31,32]. In their approach the hydrogen in the sample medium is extracted without the interposition of a gas permeation membrane via an open interface between the sample medium and a carrier gas stream. The gas transfer is conducted in a special extraction vessel at a defined interface between the two phases. The contact time of the extraction gas at the surface of the liquid phase is so short that the concentrations of the extracted gases in the gas phase are far below their equilibrium concentrations. After the extraction, the carrier gas is fed into a gas chromatography system for the selective measurement of the components' concentrations.

1.3. Dissolved hydrogen measurement with metal-oxide sensors

An alternative to chromatographic detection is the use of highly selective metal-oxide (MOX) gas sensors. These sensors are cost-effective (approximately 10 to 50 € per sensor) and ensure stable long-term measurement of hydrogen within a suitable carrier gas. Yet, their effective use requires coupling with a membrane-free extraction unit for the dissolved components.

In this study, a new measurement system for dH_2 is introduced. It combines the advantages of membrane-free extraction with the cheap and effective detection with MOX gas sensors. For the sake of simplicity, the system will be referred to as dH_2 sensor hereinafter. The performance of the sensor is investigated under dynamic AD in a two-stage lab-scale bioreactor cascade. The primary objectives of the study are to determine the reliability of the dH_2 sensor in AD culture broth over a period of several months, and to assess the potential benefits of this method over traditional off-gas measurements, such as providing insights into microbial uptake rates. Furthermore, its long-term stability resulting from the membrane-free extraction and the short exposure of the MOX to the biogas components shall be examined. As feedstock, a

combination of established substrate (maize silage) and residual recalcitrant substrate (bedding straw) was chosen as it is suggested to be relevant already now and in future.

2. Material and methods

2.1. The two-stage lab-scale anaerobic digester

The experiments for testing the dH_2 sensor in anaerobic fermentation broth were conducted as a continuous process in a tailor-made lab-scale bioreactor system consisting of two stirred-tank reactors (CSTR) in a cascade, shown in Fig. 1. A photo of the setup is additionally provided in fig. S1 in the supplementary material. The basis for the bioreactor system are two similar cuboid-shaped fermenter tanks made from polycarbonate (Makrolon®, Bayer, Leverkusen, DE), which are open at the top. The outer dimensions are 112 × 112 × 250 mm with a wall thickness of 6 mm. The total inner volume of each tank is 2.44 L, with 1.5 L of liquid volume (working volume). Each fermenter has two boreholes on the sides with 0.5″ pipe threads for tube connections and sampling / harvesting spots. The expanded upper part of each tank possesses 8 boreholes (ø 7 mm) for the bolted connection with the top cover. Both identical top covers are 3D-printed and made of polyamide (PA12). To reduce the surface roughness in favor of a waterproofed design, the top covers were slide grinded. The dimensions of each top cover are 142 × 142 × 25 mm with an additional 32.5 mm on top for a GL14 external screw thread for the stirrer connection. Furthermore, the top covers are equipped with 8 boreholes for secure attachment of both the top cover and the tank vessel with M6 screws (6 mm threads) and 3 boreholes with Pg13.5 screw threads for the integration of *online* sensors for the monitoring of the liquid phase. Another borehole (ø 8.8 mm) is designed for the off-gas tube connection, and a larger 45 mm borehole for the attachment of the dH_2 sensor. Two 25 mm openings are added into the top cover for feeding and as sample ports. The contact area between the tank and the top cover is sealed with a rubber ring sealing (EPDM rubber) to reduce the likelihood of gas leakage. The sensor and gas tube connections are tightened with a combination of O-rings (EPDM rubber) and seal tape (PTFE). Each fermenter is equipped with an overhead stirrer (Eurostar 40 digital, IKA® Labortechnik, Staufen, DE) with tailor made (3D-printed, PA12) inclined 3-wing paddles (each 32 ×40 mm), ensuring a good mixing and a downwards directed flow in order to reduce or prevent swimming layers. The stirrer shaft (stainless steel) is connected with the top cover through a double-sided Teflon-silicone seal. Additional sealing grease helps to further reduce gas leakage. The fermenter tanks are equipped with sealed hose nozzles, which are interconnected via Tygon® tubes (ø 20 mm, Saint-Gobain Plastics, La Défense, France). The same material is used for the off-gas tubes (ø 6 mm). For the connection between the fermenters and the dH_2 sensor, a metal ring (ø 45 mm, height 10 mm, stainless steel variant 1.4571) was glued to the top cover borehole using epoxy resin. An O-ring (EPDM rubber) provides the sealing between the dH_2 sensor and the fermenter, allowing easy attachment and detachment. The fermenters were temperature-controlled via heating pads and insulated with a rubber foam jacket.

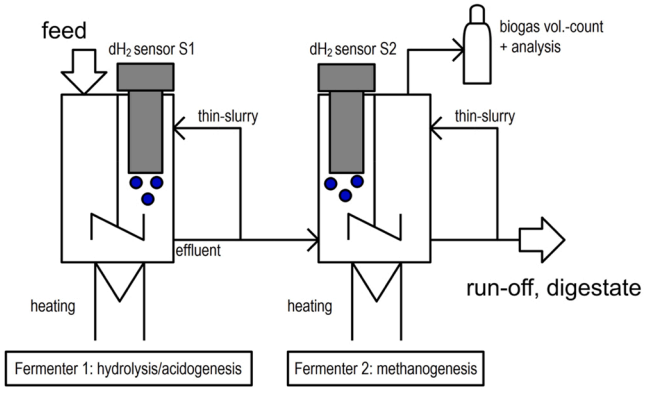

Fig. 1. Schematic drawing of the CSTR setup with dual thin-slurry recirculation and integrated dH_2 sensors.

Each fermenter is equipped with a temperature and pH-value sensor (EasyFerm Plus, Hamilton Bonaduz, Switzerland), which is connected to a power source (Voltcraft HPS 13015, Conrad, Hirschau, DE) and a Modbus USB-RS485 converter (Hamilton) via VP8 data cables (Hamilton). Data recording is done with the EloFerm bioreactor control software (EloSystems, Berlin, DE). The off-gas volume of the methanogenic stage is monitored with a BlueVcount gas counter (BlueSens, Herten, DE) and the data are collected with the BlueVIS software (v2.2, BlueSens). A steam trap is connected upstream to prevent residual moisture from entering into the sensor chambers. The off-gas composition measurement is conducted via *in situ* gas analysers, which are connected to the second fermenter downstream of the volume counter. Methane (BCP-CH_4; max. range 100 vol% CH_4) and carbon dioxide (BCP-CO_2; max. range 50 vol% CO_2) are detected and quantified with dual-wavelength infrared sensors. Hydrogen concentration is determined with a thermal conductivity sensor (BCP-H_2; max. range 50 vol% H_2; all off-gas sensors from BlueSens). The data are recorded with LabView (version 2018 – National Instruments, Newbury, UK). Overpressure is avoided by the open off-gas tube directed towards the gas measurement.

2.1.1. Offline analyses

The SCCA composition in the suspension phase was analyzed using previously frozen 2 mL samples from both fermenters as described by Longis et al. [33]. The samples were thawed at room temperature, and stored at 4 °C for 24 h afterwards, facilitating salt precipitation. Next, samples were centrifuged for 20 min at 13,300g. The supernatant was subsequently filtered through 0.2 μm Nylon filters (Carl Roth, Karlsruhe, DE). A clarification kit (Carrez clarification, Merck, Darmstadt, DE) was used to remove the remaining protein. The quantification of the SCCA was conducted with a 1200-series HPLC system (Agilent Technologies, Waldbronn, DE), equipped with a refractive index detector and an HyperRez XP Carbo-hydrate H^+ column (Fisher Scientific, Waltham, MA), as described by Gómez-Camacho et al. [34]. Data evaluation was performed with OpenLab ChemStation (v. B.04.03; Agilent Technologies, Santa Clara, CA). To determine the soluble chemical oxygen demand (sCOD) of the fermentation broth, frozen liquid samples were filtered through 0.45 μm cellulose filters (Merck) and diluted 1:100 in deionized water. 2 mL of the filtered samples were analyzed with a chemical oxygen demand mercury-free TNTplus vial test kit (Hach Lange, Düsseldorf, DE) according to the manufacturer's instructions.

2.2. MOX sensor for dissolved hydrogen measurement

The newly developed dH_2 sensor consists of a sensor head, connected with a programmable logic control (PLC) unit for data logging and processing as shown in Fig. 2a and b. The diameter of the complete cylindrical sensor head is 40 mm while its length can be adapted to different headspace heights depending on the size of the respective digester. In this study, the length of the sensor head was 20 cm. Beside the PLC, the sensor head is connected to a gas supply system, providing humidified synthetic air (HSA) and one or more calibration gas mixtures containing hydrogen diluted in synthetic air. The flow rate of this gas supply was set to 10 mL min^{-1}.

A chamber with a volume of about 1 mL is situated in the center of the sensor head, which contains the MOX. The planar substrate of this sensor is based on the design of the hydrogen gas sensor GGS 6530 T (UST Umweltsensortechnik, Geschwenda, DE), which is equipped with a sensitive layer made by screen printing of a SnO_2-paste with Pd particles. This layer is subsequently coated with a thin glassy film to improve its selectivity to hydrogen. For the measurement, the MOX gas sensor is heated to 400 °C and the resistance of the sensitive layer is measured

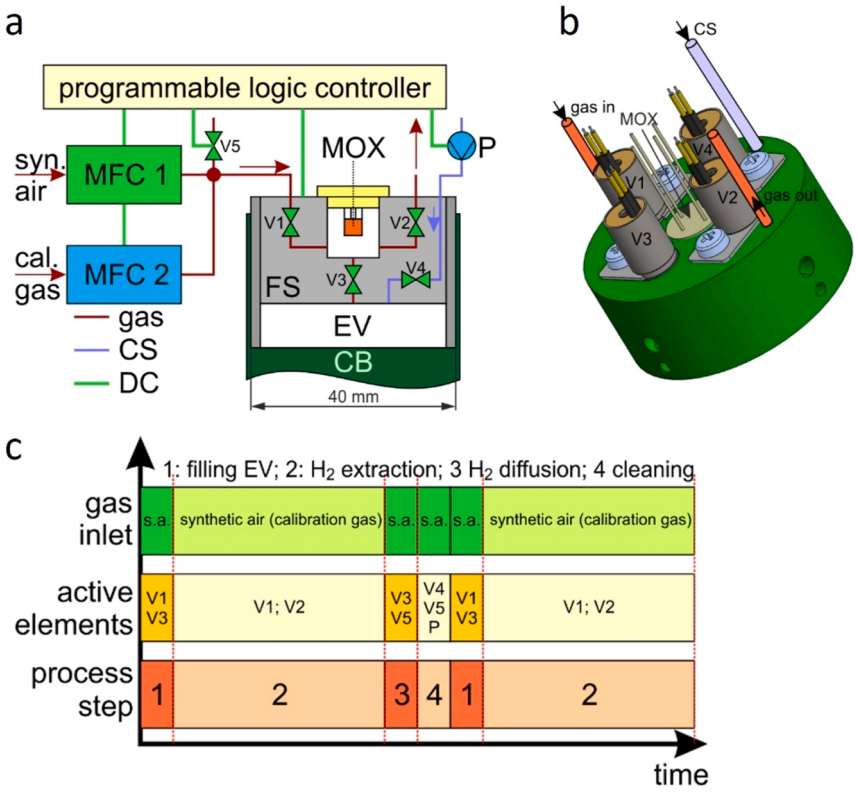

Fig. 2. a: schematic drawing of the measurement system for dH_2; b: 3D illustration of the whole sensor head; c: scheme of the measurement cycle with step 1 = filling of extraction volume, 2 = extraction of dH_2 and equilibration between culture broth and extraction gas, 3 = diffusion of extracted H_2 into the MOX chamber, and 4 = cleaning of extraction chamber (optional). Abbreviations: cal. gas = calibration gas, CB = culture broth, CS = cleaning water stream, DC = data/control line, EV = extraction volume (21 mL), FS = fluidic system, MFC = mass flow controller, MOX = metal oxide gas sensor, s. a. = synthetic air, P = pump, V_i = micro-solenoid valves.

with direct current. If this MOX was to be used in direct contact with biogas for longer periods, its sensitive layer would degrade rapidly and thus a stable measurement during long-term operation would not be possible. Therefore, the sensor head contains a fluidic arrangement around the MOX chamber, providing a defined short diffusion time of measuring gas to the MOX, its conditioning with the synthetic air mentioned above and its periodic recalibration with the miniaturized solenoid valves V_1 and V_2. The bottom of the sensor head contains a cylindrical extraction chamber of about 21 mL, which is open at its lower side to the culture broth and can be connected to the MOX chamber by a gas channel (controlled by valve V_3). The membrane-free operation leads to a direct contact of the boundary layers of the gas and liquid phase. This enables a rapid reliable establishment of a steady-state equilibrium with respect to the partial pressures of the components of interest. A fourth valve and a pump are used to transport cleaning solution to the extraction chamber.

The measurement cycle was optimized to increase hydrogen transfer to the MOX compared to the transfer of other extracted components. The optimized sequence, as shown in Fig. 2c, starts with opening valves V_1 and V_3 to introduce fresh HSA into the extraction chamber for 3 min. The broth, which is displaced in the extraction chamber is pressed into the core of the liquid phase during this filling step. In the next step of the dH_2 extraction, valves V_1 and V_2 are opened while V_3 and V_4 are closed. The MOX is purged with HSA, and the equilibrium is established between the partial pressures of the dissolved components in the culture broth on the one hand and the gas in the extraction volume on the other. This dH_2 extraction step (step 2) lasts 65 min and is followed by step 3 for diffusion of the extracted gas phase into the MOX chamber. To enable this diffusion, valves V_1 and V_2 are closed while only V_3 is opened. To prevent an unwanted pressure increase in the gas supply line during this diffusion step, vent valve V_5 is also opened. To prevent MOX-harmful biogas components (like H_2S) from entering the MOX chamber in significant quantities, the duration of this step is limited to 1 min. Since hydrogen has a much higher diffusion coefficient than the other gases or volatile components present in the extraction volume ($D(H_2) = 0.71$, $D(CH_4) = 0.196$, $D(CO_2) = 0.148$; $D(H_2S) = 0.15$; all values in $cm^2\ s^{-1}$, diffusion in air at 101.325 kPa and 9 °C [35]), its concentration increase in the MOX chamber during diffusion step 3 exceeds that of the other components more than three times. The corresponding resistance drop of the MOX sensor during this step is used as a measure of the hydrogen concentration (partial pressure) in the extraction volume.

A measuring cycle is terminated with the cleaning of the extraction chamber by rinsing with water. Valves V_4 and V_5, or V_4, V_1 and V_2, alternatively, are opened during this cleaning step and the pump is activated. This regular cleaning prevents the growth of biofilms on the walls of the extraction chamber, which would disturb the setting of the equilibrium. Duration and frequency of this cleaning phase are adapted to the measurement conditions. For measurements in very small fermenters, like in this study, the automatic *inline* cleaning is replaced by a manual external cleaning of the extraction chamber in order to avoid considerable dilution of the fermentation broth. This is executed once a week by taking the dH_2 sensor out of the fermenter system and cleaning the extraction chamber with a paper towel and distilled water. During cleaning, the top cover of the fermenter is sealed with a rubber plug.

At the applied setup, the sensor provides one value for partial pressure of dH_2 at one measuring cycle of a duration of 70 min. The value, at the end of the time frame, represents the equilibrium H_2 partial pressure in the extraction volume just before diffusion step 3. With about 22 measurements per day, a biogas fermenter is monitored quasi continuously.

To establish a relationship between the dH_2 concentration and the dynamic MOX sensor signal, the sensors were calibrated in distilled water (25 °C) with the arrangement shown in Fig. 3. It enables the precise adjustment of hydrogen partial pressures in model liquids (distilled water) by sparging a stirred and temperature-controlled medium with a defined test gas (H_2 in N_2). H_2 dissolves in accordance with the partial pressure via the gas bubbles in the liquid phase. The dH_2 diffuses into the extraction chamber in the sensor head until the two partial pressures are equal in both phases. The pre-calibration shown in Fig. 3 can be extended to a broader parameter field with respect to temperature, pressure and oxygen concentration if this will be required for other sensor applications.

In order to assess and maintain the reliability of the MOX sensor during measurements with culture broth, periodic *inline* calibrations are performed as one-point calibrations. Therefore, the flow rate of the HSA for rinsing the sensor during extraction step 2 was reduced from 10 to 5 mL min^{-1} and a flow of synthetic air (containing 100 ppmv H_2) was added (5 mL min^{-1}). The resulting calibration gas mixture (flow rate 10 mL min^{-1}) contained 50 ppmv H_2 in HSA. The signal of the dH_2 sensor to the gas mix can be used to detect shifts in the sensor signals due to a degeneration of the active layer.

To transfer the molar dH_2 partial pressures into molar dH_2 concentrations, the Henry constant for hydrogen was considered. 30 °C and a 1 bar hydrogen atmosphere lead to an aqueous solubility of 1.5 mg L^{-1} or 0.75 mmol L^{-1} [36]. This results in a Henry constant of $k_H = 7.5 * 10^{-4}$ mol (L bar)$^{-1}$. The exact Henry constants for complex anaerobic fermentation broths are difficult to determine, but should be around a value of $7.4 * 10^{-4}$ mol (L bar)$^{-1}$ at 35 °C and normal pressure, as the solubility of hydrogen does not seem to be much affected by the typical components of anaerobic fermentation broth [12,37]. The molar concentration of dH_2 was calculated by multiplying the Henry constant with the partial pressure of dH_2.

2.3. Process operation of the fermenter system

Both digesters were operated under mesophilic conditions (T ~ 37 °C) due to the purpose of the study, namely the investigation how already installed biogas plants with two stirred tank digesters, fed with agricultural feedstock, can be operated with a dedicated separation of the hydrolysis/acidogenesis in one and the methanogenesis in the other stirred tank. Such plants have been mostly operated at mesophilic conditions. The pH-value was controlled separately for each fermenter. To favor hydrolytic and acidogenic bacteria, the pH-values were kept in a range between ~~3~~ 4.5 and 5.5 by addition of appropriate amounts of 1 molar HCl in the first stage. The second stage was operated at pH-values between 6.5 and 7.5 (addition of 1 molar NaOH) to maintain methanogenesis [6]. A combination of shredded and sieved (mash width of 2.5 mm) maize silage and bedding straw (50/50 w/w) was fed as substrate to the first fermenter. The effluent from the first fermenter was then added to the second stage, without any further treatment. The feeding and harvesting were conducted twice a week with a liquid volume of 400 mL, which resulted in a hydraulic retention time of 10 d in each fermenter. Thus, the total retention time in the fermenter system adds up to 20 days. The same retention time was chosen for each stage as this is among realistic scenarios for biogas plants of two stirred tank digesters and a subsequent post-digester so that the full plant capacity can be used. The hydrolytic fermenter was operated with a dry matter content of 5 to 7% (w/w). Higher concentrations were not suitable due to the stirrer setup. Agitation was set to 40 to 60 rpm. The resulting power inputs into the liquid phases of the fermenters were calculated according to Platas Barradas et al. [38]. The dry matter content in the second fermenter was measured to be in between 3% and 5% (w/w). The organic loading rate was in between 1.2 and 1.5 g (L d)$^{-1}$ in the hydrolytic fermenter. To stabilize the respective microbial consortia, both fermenters were equipped with a thin-slurry recirculation, that equaled the feed flux in volume. The thin-slurry was led back into the fermenter, while the thick sludge was transferred to the second reactor and discarded. As the broth for the recirculation was harvested additionally to the normal feed-harvest cycle, no change of retention times occurred during recirculation. The standard process conditions for the anaerobic digesters are summarized in Table 1.

Different cleaning, feeding and harvesting cycles as well as stirrer speeds were applied during the measurement of dH_2 in order to investigate, if these external process changes influence the sensor signals. Due to the regular automatic rinsing of the sensors with a defined hydrogen-containing gas mix, statements can be made regarding a possible time-induced mitigation or drift of the sensor signals and the reproducibility of measurements. Furthermore, the dH_2 sensor was exposed to hydrogen addition into the methanogenic fermenter as hydrogen addition can increase the share of methane in the off-gas. Therefore, pure hydrogen gas (99.99% (v/v), Air Liquide, Paris, France) was inserted through a 1/16″ high-grade steel tube to the bottom of the fermenter at a rate between 0.5 and 1.0 mL min^{-1} and for between one and four hours during a two-week experimental phase. The constant flux was controlled by a mass flow controller (MFC, Smart Mass Flow Meter, Brooks Instrument, Hatfield, PA).

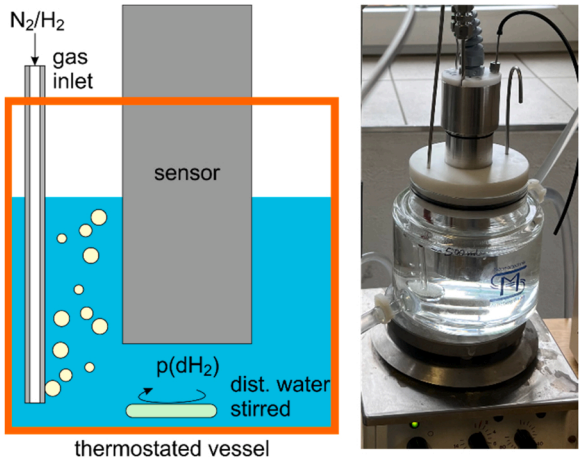

Fig. 3. Scheme and photo of the setup for laboratory calibration of the dH_2 sensor in distilled water and H_2 sparging.

3. Results and discussion

The hypothesis of this paper was that monitoring the partial pressure of dH_2 in the liquid phase of AD would allow to detect changes in process dynamics earlier than by common headspace measurements.

3.1. AD process performance

The two-stage AD digester system (2 CSTRs, 1.5 L working volume, each) was operated with maize silage and bedding straw at mesophilic

Table 1
Process conditions in the two-stage fermenter system for biogas production.

Fermenter	substrate	pH-value	HRT [d]	OLR [g (L d)$^{-1}$]	DM [% (w/w)]	Temperature [°C]
1	Shredded maize silage + bedding straw (50/50 w/w)	4.5-5.5	10	1.2-1.5	5-7	37
2	Harvest from 1st fermenter	6.5-7.5	10		3-5	37

conditions for four months. SSCA and H_2 were produced in the first fermenter (hydrolysis and acidogenesis) at a pH-value between 4.5 and 5.5. In the second fermenter (methanogenesis), the Effluent from the first was metabolized into methane at pH-values between 6.5 and 7.5. Biogas yield and production of SCCA during experiments are shown in Table 2. The biogas production process was proceeding well in the two-stage reactor system: 200 to 300 mL of methane were produced per gram of COD from maize silage and bedding straw added to the first fermenter (50/50 w/w, organic loading rate: 1.2 - 1.5 g (L d)$^{-1}$). The methane content in the biogas reached up to 60% (v/v). Concentrations of more than 10 g L^{-1} of SCCA were reached in the first fermenter, mainly acetic and butyric acid. Gas and acid production were in the same range compared to AD applications of a similar size like from Colussi et al. [39].

3.2. Suitability of the dH_2 sensor for AD

The response curves of two MOX sensors to different H_2 (gas) concentrations in test procedures (Fig. 4) indicate the expected logarithmic sensitivity, a lower limit of detection below 50 ppbv (~ 0.005 Pa or 3.7 *10^{-5} µmol L^{-1}) and a short response time in the range of a few seconds (shown by the rapid change in sensor signal after the adjustment of the dH_2 concentration, Fig. 4, left). The signal depends also on humidity and temperature in a relatively complex scenario. The relative sensitivity S/S0.3 (Fig. 4, right) was calculated by dividing the actual resistance by its value at c(H_2) = 0.3 ppmv. The response time of the sensor itself could not be measured, because a setup for rapid gas exchange at the sensor surface was not available for a time range below t_{90} < 5 s. This calibration result, where external hydrogen gas is applied to the sensors during a certain amount of time, cannot be used to calculate dH_2 concentration in media with the sensor head described above, because the diffusion step 3 is too fast to achieve a concentration equilibrium between the extraction volume and the MOX chamber.

As described in the Materials and Methods section, the sensors were calibrated in distilled water with a pH_2 adjusted by MFC with a maximum uncertainty of 2% (Fig. 3). An example of the resulting signal curves is shown in Fig. 5a: a stable baseline of the MOX resistance and its steep decrease during diffusion step 3. The minimum of the resistance at the end of step 3 is the measurement of the pH_2 in the extraction volume as present at the end of step 2. After a change of pH_2 in the sparging gas of the setup (Fig. 3), it takes up to four measurement cycles until the new equilibrium is established. The mean values of six consecutive minima (shown with higher resolution in Fig. 5b) were used to calculate a third order logarithmic calibration equation (Fig. 5c):

$$\log\left(p(H_{2,diss})\right) = A \bullet (\log(S))^3 + B \bullet (\log(S))^2 + C \bullet \log(S) + D \quad (1)$$

The values for A-D are the result of a logarithmic regression. This equation is valid for the special set of parameters of measuring cycle and sensor head (equilibration time, diffusion time, channel dimensions, chamber volumes).

Correspondingly, the temperature measured in the sensor head also drops about 1 K during the diffusion step, caused by the lower heat production of the solenoid valve V_3, which is active during this step. The temperature level also indicates that the transfer of thermal energy from the hot MOX substrate into the sensor head is neglectable.

Fig. 5d shows the time course of the whole calibration process, calculated from the signal minima with the logarithmic Eq. (1). It proves that the actual partial pressure in the model liquid is measured with a relatively low noise in a broad range. A maximum noise of 20% was found for sensor 2 after increasing p(dH_2) to 100 Pa again. This value indicates also the maximum expectable uncertainty for one measured value, while the typical noise is substantially lower, leading to an estimation of maximum values of approx. ± 10% (as noted also in Table 3). The response time of the apparatus increases with decreasing p(dH_2) as expected for the discharge of gas traces from the 250 mL of distilled water. The sensitivity (lower detection limit <50 ppbv), selectivity (>0.8 for hydrogen), stability (no significant changes in sensor signals after >3 months of continuous measurement) and accuracy (better than 20% of the measured value) of the dH_2 sensor, indicated by these results, are sufficient for the *online* measurement of dH_2 in a AD culture broth. The measurement range of the whole dH_2 sensor (‡ detection range of the MOX) is in between < 0.1 and 1,000 Pa (between <0.00074 and 7.4 µmol L^{-1}), but can easily be extended to > 10,000 Pa by decreasing the duration of the diffusion step 2 within a laboratory calibration.

The harsh conditions of the suspension phase of AD with abrasive material, low pH-values, multiple unknown components that are partly corrosive, and finally the low concentrations of dH_2 makes any design of sensor probes a challenge. Nevertheless, any sensor still needs to fulfill high metrological standards, especially with respect to reliability and long-term stable detection [40]. Long-term measurements need to be feasible with the dH_2 sensor, without an immoderate time-induced shift in the sensor signal, e.g., due to biofilm formation or abrasion of the MOX sensors' active layer. The regular one-point calibrations during the experiments cause a decline of the signal to a plateau value as shown in Fig. 6a. This value was applied to monitor the sensor accuracy and to adjust the value D of the calibration Eq. (1) regularly. D is responsible to shift the values of the dH_2 calibration in accordance with the change of the response behavior of the MOX. The time courses of the plateau minima of both systems are provided in Fig. 6b. They indicate a slight drift and acceptable noise during a measurement period of over three months. Since the humidification of the calibration gas was not temperature-controlled, the humidity of the gas varies between different calibrations, which could be one of the reasons for drift and noise. The influence of the humidity on the sensor signal can explain, why there is a fluctuation of about 0.04 kΩ in both sensors during the first 50 day of measurement (Fig. 6b). The results of the one-point calibrations prove that optimal *inline* calibration is necessary and suited to assure the required accuracy of long-term measurement of dH_2. The active layer of the MOX gas sensor maintained its functionality and reactiveness during the measurement period despite the constant exposure to the gas phase of the AD fermenter system. It can contain aggressive gas components, such as hydrogen sulfide. The great advantage of the hydrogen measurement is, however, the higher diffusion rate of hydrogen compared to all other relevant gases or volatile molecules. Thus, the exposure of the MOX sensor to the rest of the gas phase in the extraction chamber is minimal.

As described before, the dH_2 sensor also features an automatic water-jet cleaning for the extraction chamber, which is in contact with the fermentation broth. Due to the risk of dilution, this feature was not used for the lab-scale fermenters. Without cleaning, biofilm formation was detected around the extraction chamber after one week, which was growing bigger over time, as seen in Fig. S2a in the supplementary material. This formation was probably enhanced by the availability of oxygen at the interfacial area between the gas and the liquid phase. The response time of the sensor was apparently not immediately affected by the biofilm. However, it led to a degradation in the general functionality of the system, as valve V_3 was partly clogged by the film. The growth of hydrogen-releasing microorganisms inside the extraction chamber can lead to false positive results, while a clogging of the valve between the extraction chamber and the sensor results in a hindered gas diffusion

Table 2
Biogas and acid production performance.

Gas production (fermenter 2)					Acid production (fermenter 1)		
CH_4 [%]	CO_2 [%]	H_2 [%]	Biogas yield [mL (L d)$^{-1}$]	Spec. biogas yield [mL $g_{COD,added}^{-1}$]	Acetic ac. [g L^{-1}]	Butyric ac. [g L^{-1}]	Other [g L^{-1}]
50-60	30-45	1-4	500-750	200-300	2	5	3

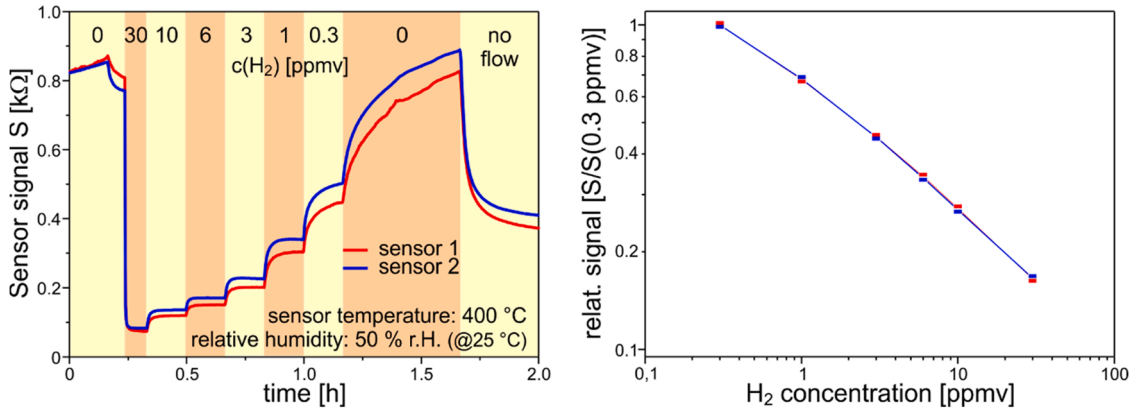

Fig. 4. MOX response to different H_2 concentrations in HSA at 400 °C sensor temperature; left = temporal course, right = calculated relative signal dependency; the relative sensitivity S/S(0.3 ppmv) was calculated by dividing the actual resistance by that at $c(H_2) = 0.3$ ppmv.

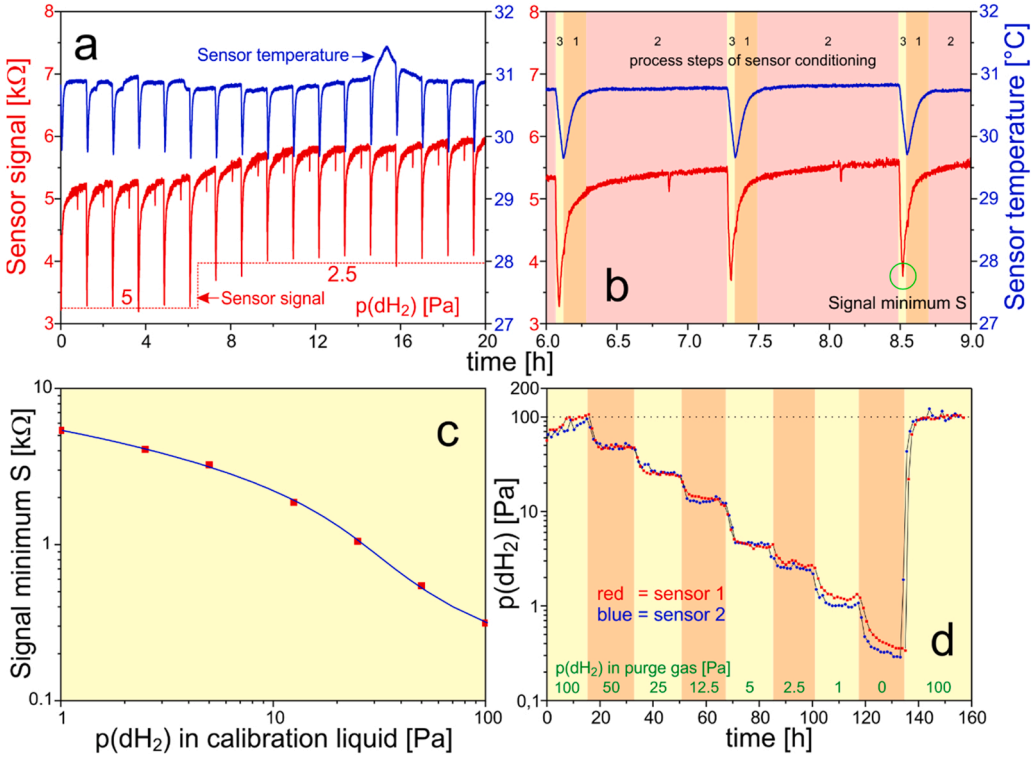

Fig. 5. a and b: signal time courses of MOX resistance and sensor head temperature during calibration in the setup shown in Fig. 3; c: extracted calibration function of the MOX; d: time course of the calculated partial pressure of dissolved hydrogen $p(dH_2)$ during the long-term lab calibration in the setup shown in Fig. 3.

Table 3
Measurement properties of the dH_2 sensor.

Parameter	Range of values
Measurement range	< 0.1 – 1,000 Pa (> 10,000 Pa after calibration) < 7.4•10^{-4} – 7.4 µmol L^{-1} (> 74 µmol L^{-1})
Lower detection limit of MOX	50 ppbv (~0.37•10^{-4} µmol L^{-1})
Accuracy (permissible error)	~10% of measured value
Measurement cycle time	≤ 70 min
Response time of the MOX	t_{90} < 5 s
Selectivity for H_2	> 0.8
Maintenance-free operation	> 3 months

towards the sensor. Any visible biofilm could be removed by manual cleaning with rinsing water, which was performed once per week, as seen in Fig. S2b in the supplementary material.

The data from the regular one-point calibration (Fig. 6) prove the effectivity of the cleaning process, because there is hardly any shift in the sensitivity of the sensor due to biofilm formation or similar microbiological or physicochemical factors over a time period of more than three months of permanent operation in anaerobic fermentation broth.

Table 3 summarizes the measurement properties of the dH_2 sensor. The measurement range is compared to the saturation coefficients (concentration at half-maximal reaction speed) from literature for dH_2 for different methanogens (Table 4). Although these literature data differ considerably, the measurement range of the sensor covers the whole relevant dH_2 concentration range for AD. Accuracy, response time, and the other properties of the sensor are well-suited for the application in AD.

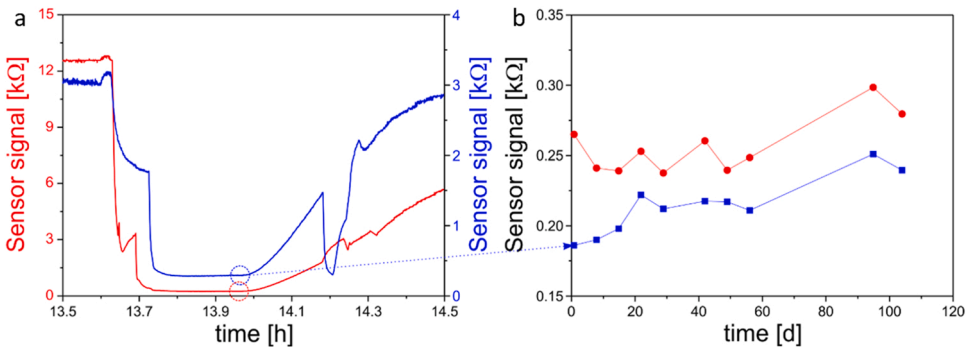

Fig. 6. a: Time course of sensor signals during an *inline* calibration with 50 ppmv H_2 for 15 min b: development of the signal minima during the long-term application in the lab fermenter system. Red line: dH_2 sensor 1; blue line: dH_2 sensor 2.

Table 4
K_s values for dH_2 of different methanogens.

K_S-values [µmol L^{-1}]	Microorganism	Cultivation conditions and H_2 detection	Reference
0.14	Hydrogenotrophic methanogens	4 L batch reactors, reduction gas analyzer	[41]
6	*Methanobrevibacter arboriphilus*	500 mL glass fermenter, hydrogen electrode	[42]
5.81 – 7.3	*Methanospirillium hungatei*	2 L flasks, gas chromatograph	[43]
6.6	*Methanogens*	2 L flasks, gas chromatograph	[43]
1 – 10	Rumen fluid and *Methanobrevibacter ruminantium*	Rumen fluid in wash bottle, gas chromatograph	[44]
22.36	Granular bio-sludge	4 L batch UASB, pressure transducer, dH_2 concentrations calculated from partial pressures	[45]
0.06 – 1050	Methanogens	Summary of Literature data	[46]

3.3. Monitoring of dissolved hydrogen in a two-stage AD process

Continuous dH_2 and off-gas measurements (in the methanogenic stage) under dynamic process conditions were conducted for more than 500 h. On the basis of the calibration data and the Henry constant k_H, the resistance measurements of the dH_2 sensor were directly transformed into molar concentrations with the help of a Microsoft® Excel script. The sensor in the hydrolytic stage showed higher average dH_2 concentrations than the sensor of the methanogenic stage. This was to be expected. While the hydrolytic stage usually contains a surplus of hydrogen releasing microorganisms under the given conditions, the archaea in the methanogenic stage are mostly hydrogen consumers. The spikes in dH_2 concentrations in the methanogenic stage were a direct result of feeding the fermenter with effluent from fermenter 1. The reason for this effect is the influx of hydrogen-rich substrate from the first fermenter, which is subsequently utilized by the methanogenic archaea within the following hours. One measurement cycle of the dH_2 sensor has a duration of ≤ 70 min, sufficiently low for every feeding process to be detected. The influence of the regular internal one-point calibration of the sensors with calibration gas (as explained in the previous section) was also detected with the dH_2 sensor. The hydrogen peaks, which were directed downwards (Fig. 7a, dotted line M), are resulting from an exposure of the sensor to hydrogen-free ambient air during the manual cleaning of the measurement system. After the cleaning, the dH_2 sensor was inserted back into the fermenters and hydrogen from the liquid phase could enter the extraction chamber once again. Depending on when and how long the cleaning takes place, process step 2 of the measurement (Fig. 2c) can be shortened. The dH_2 equilibration is influenced by this procedure. The sensor detects lower dH_2 concentrations during this operation, which do not reflect the real values. this, however, represents an issue only in small-scale fermenters, as there is no manual cleaning required in industrial scale AD plants.

After approximately 430 h, the dH_2 sensor detected a huge steady increase of the hydrogen concentration in the hydrolytic stage. During this time, a forced shift of the pH-value was induced to keep the ~~pH~~ pH-value in the hydrolytic fermenter between 4.5 and 5.5 The reason for the instantaneous rise in dH_2 is probably a growth of hydrogen-producing bacteria like *Clostridia* spp. [47–49]. In order to investigate the reliability of the sensors, both sensors were taken out of the fermenters after 530 h for an inspection and thorough manual cleaning. Afterwards the measurement was continued under dynamic process conditions for another 450 h. The results are shown in Fig. 7.

The dH_2 concentration in the methanogenic stage remained at a concentration of around 15 to 20 µmol L^{-1} after the sensor revision. This finding leads to the assumption that the increased hydrogen concentration derives from enhanced microbial hydrogen production in the hydrolysis stage and not from technical issues. The increased feeding frequency of effluent from the hydrolytic to the methanogenic stage in between 660 and 770 h led to an increase in the dH_2 concentration (~ 1 µmol L^{-1}). In general, under the constantly laminar conditions, no influences on the dH_2 concentration were expected and measured when the stirrer speed was changed.

Dynamic process conditions, e.g. from the feeding process (influx of ambient air together with the substrate leading to decreased methane and carbon dioxide concentrations) can be monitored by the off-gas measurement alone. The increased dH_2 concentration in the hydrolytic fermenter after 430 h could not be detected in the off-gas measurements at all, probably due to two factors. On the one hand, the changes in the dH_2 concentrations have very little effect on the composition of the gas phase with dominant methane and carbon dioxide fractions, even if more hydrogen is migrating from the liquid to the gas phase. These changes in the off-gas composition are hardly detectable and even probably not covered by the accuracy of the H_2 sensor (BCP-H_2, accuracy: <0.2% FS ± 3% of measured values [50]). On the other hand, hydrogen consumers are always present in each reactor to a certain extent. Thus, the dH_2 is detected by the sensor in the suspension, but is metabolized, before it is released and can be detected in the off-gas. Due to the slow transition of hydrogen from the liquid to the gas phase and vice-versa, under dynamic process conditions no equilibrium is formed between the two phases. As assumed in the introduction of this study, the dH_2 concentrations calculated from off-gas data might not reflect conditions in the suspension phase. The average off-gas hydrogen concentration was measured as ~2 vol% for some periods of time. The ambient pressure was ~1 atm during the measurements, as recorded by the off-gas sensors. Thus, the hydrogen concentration in the off-gas comprised around 20,000 ppmv and 2000 Pa partial pressure on average. With the Henry constant of $7.4 * 10^{-4}$ mol (L bar)$^{-1}$ at 35 °C

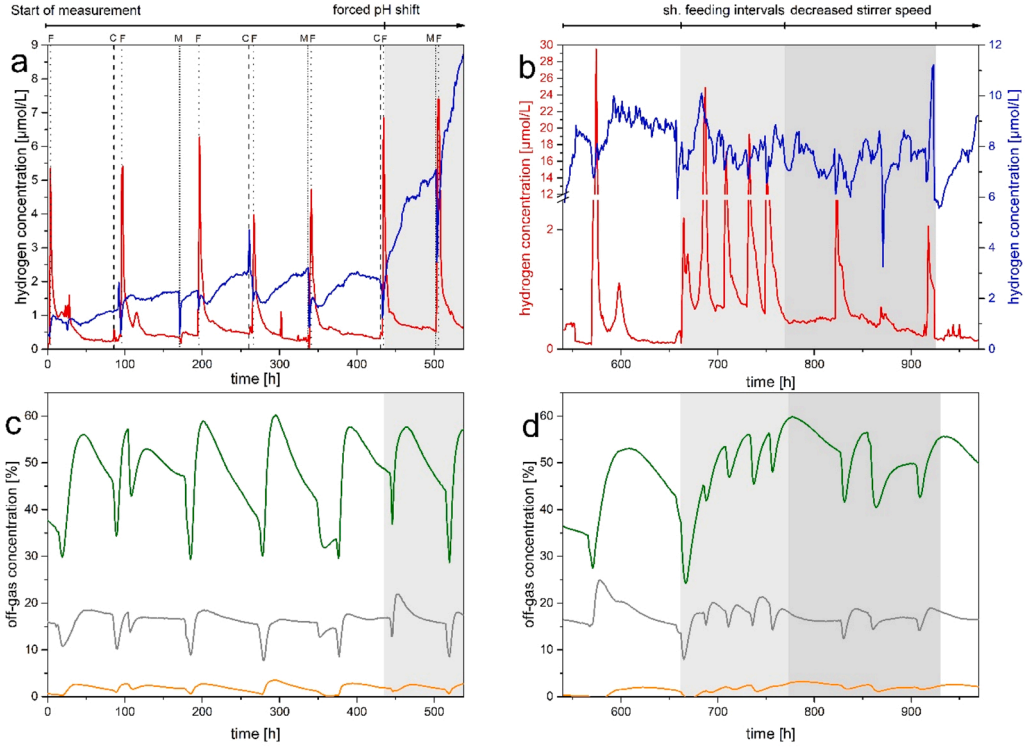

Fig. 7. dH_2 and off-gas concentrations under different dynamic process conditions (substrate: maize silage/bedding straw (50/50 w/w), OLR: 1.2 - 1.5 g (L d)$^{-1}$; a: dH_2 concentration in the hydrolytic (blue line) and the methanogenic (red line) stage before the revision of the dH_2 sensor with force shift of the pH-value after 430 h and growth of hydrogen releasing microorganisms (light grey) (symbols F: feeding of fermenters, C: internal calibration of the sensors, M: manual cleaning of the measurement system); b: dH_2 hydrogen concentration in the hydrolytic (blue line) and the methanogenic (red line) stage after the revision of the sensor with shortened feeding intervals (light grey), stirring speed in the methanogenic stage was decreased from 60 to 40 rpm after 770 h (dark grey); c and d: off-gas concentration in the methanogenic stage (green line: methane, gray line: carbon dioxide, orange line: hydrogen) before (c) and after (d) the dH_2 sensor revision.

and normal pressure, the theoretical dH_2 concentrations would be about 15 µmol L^{-1} at equilibrium conditions. Hence, off-gas data can overestimate the dH_2 concentrations measured by the dH_2 sensor to a great amount if re-assimilation of dH_2 is high.

The dH_2 concentration measured in this study are mostly comparable to the few published data. Dang et al. [51], Boulart et al. [29] and Bakar et al. [30] describe methods and research trends for measuring dissolved gases, such as hydrogen and methane in their extensive reviews. Pauss et al. [52] and Pauss and Guiot [12] measured 2 to 3.5 µmol L^{-1} dH_2 with a miniature fuel cell detector (dissolved hydrogen detector cell, Syprotec, Pointe-Claire, Québec, Canada) under AD conditions and up to 20 µmol L^{-1} in upflow sludge bed reactors, respectively. Platošová et al. [53] measured dH_2 concentrations between 0.039 and 0.425 mg L^{-1} (that is between 19.5 and 212.5 µmol L^{-1}) with an amperometric microsensor in a mesophilic single-stage rotatory drum reactor (substrate: food waste, working volume 15 L). The dH_2 concentrations over 200 µmol L^{-1}, which are much higher, than the values detected in this study were measured during an extreme overload of volatile fatty acids. Strong and Cord-Ruwisch [54] monitored dH_2 amperometrically in an anaerobic digester at glucose overload conditions. This would be probably a benchmark of what is achievable if there is a large surplus of easily available carbohydrates, and no separation of hydrogen producers and consumers is conducted at all. Partial pressures of 30 to 400 Pa were detected in the liquid phase, which corresponds to concentrations of 0.2 to 3 µmol L^{-1}. Thus, the values at which the dH_2 sensor was applied and showed reliable measurements in this study seem to be representative.

3.4. Measurement during hydrogen gas addition

To further test the dH_2 sensor under dynamic conditions, hydrogen was added to the methanogenic stage, as this can boost methane formation. The volumetric flow rates of hydrogen gas addition were selected based on a compromise between achieving a dH_2 concentration near the K_s values of the archaea, and the capabilities of the MFC, which had a minimum applicable flux of 0.5 mL min^{-1}. Different durations of the addition were chosen in order to make sure that the additional hydrogen was also detectable in the gas headspace of the fermenter and not assimilated completely in the liquid phase. The procedure of the addition is described in the material and methods section.

Results from experiments with different amounts of added hydrogen are shown in Fig. 8. The approximate K_S-values of the hydrogen uptake are calculated as the dH_2 concentrations, at which the hydrogen uptake rate is half of the maximum uptake rate after each hydrogen sparging.

In general, both hydrogen monitoring methods, the dissolved measurement and the off-gas analysis, were able to detect the hydrogen infused into the methanogenic fermenter reliably. The hydrogen addition can be detected in form of spikes in the measurement signals (Fig. 8). The molar hydrogen concentrations and the hydrogen share in the off-gas are dependent on the flow rate and the duration of the hydrogen gas infusion. Naturally, higher rates and longer exposition times led to higher measurement signals. The base concentration (in between the hydrogen addition phases) of dH_2 in the methanogenic fermenter was decreasing over time, whereas the off-gas hydrogen share in the biogas increased at constant biogas production rates. The raise in the off-gas measurement was probably resulting simply from hydrogen gas bubbles, which passed the fermenter without dissolving into the solution. The power input was not able to distribute hydrogen back from gaseous headspace into the core of the liquid phase. The steeper decline of the dH_2 signal in later phases of the experiment may follow an adaptation of the microbial consortia towards the condition of regular hydrogen infusion.

Two main advantages of the dH_2 measurement become clear by these

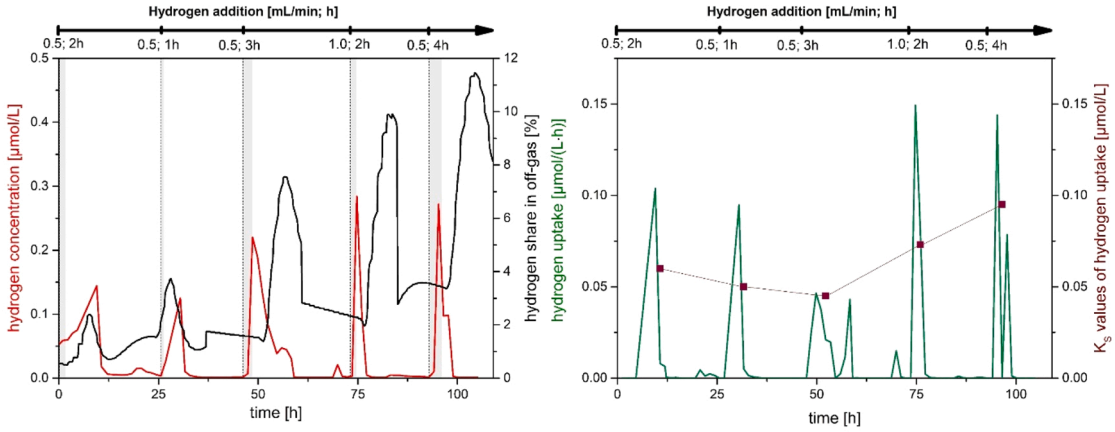

Fig. 8. Measurement of dH_2 during hydrogen gas pulse addition to the methanogenic stage with different gas flow rates and periods of addition (highlighted in grey, the dotted lines depict the start of the hydrogen gas addition); left: dH_2 concentration (red line) and off-gas hydrogen (black line); right: dissolved hydrogen uptake (green line) after the addition and approximate K_S values of the uptake (dark red squares and line).

results: i) The measurement cycle time of the dH_2 detection with a maximum of 70 min is sufficient to detect the dynamics of the process in case of hydrogen infusion and ii) the off-gas measurement responds slower as a dilution of the hydrogen gas in the headspace of the fermenter occurs. The interference of gas cleaning agents or other gas analyzing devices such as volume counters in front of the sensor or required tube connections increase the time until concentration rises are sufficiently detected at the sensor spot. Results from another experiment, where the off-gas hydrogen sensor was attached directly to the top of the methanogenic fermenter during hydrogen gas addition, can be found in the supplementary material (Fig. S3). Due to absence of any interference with other devices like mass flow controllers or long tube connections, the off-gas sensor reacted fast. Hence, a suitable location for installations in industrial scale plants would be, most likely, directly above the sparging location in the digester's headspace, which is not often easily applicable due to several reasons like moisture and biofilm formation as well as maintenance requirements.

Based on the dH_2 monitoring, hydrogen uptake rates of the microbial culture were determined (Fig. 8, right). During the experiments, the microbial culture exposed uptake rates of up to $r_{H2} = 0.15$ µmol $(L\,h)^{-1}$. The resulting half-velocity constants (saturation concentration) of dH_2 (K_S) were between 0.05 and 0.1 µmol. These values are comparable to the lower end of what is reported for methanogens elsewhere (Table 4). The information on (maximum) microbial uptake rates is necessary to set the ideal external hydrogen supply for the hydrogenotrophic archaea, without wasting it as off-gas or create starvation, thus, making the dH_2 monitoring especially interesting for future power-to-methane applications. There are, however, several uncertainties regarding the determined uptake rates and K_S-values, e.g., emission (penetration and leakage) of dH_2 to the surrounding.

In the headspace, due to typically large volumes and consequently dilution of trace gases, any change is visible only if the conditions last long enough to change the gas composition remarkably. The poor mixing at the gas-liquid interfacial area leads to layer formation, in which the dH_2 concentration can differ a lot from that one in the liquid bulk, with consequences for the equilibrium between the gas and liquid phases' partial pressures: Differences in the partial pressure between the bulk of the liquid and the gas phase in the headspace up to a factor of more than 10 have been reported [12]. Due to the fast utilization of the dH_2 by microorganisms, an equilibrium between the liquid and the gas phase in the head space can hardly be formed [42], if conditions change rapidly. If the hydrogen production exceeds assimilation by hydrogenotrophic microorganisms, it accumulates in raising bubbles to the gas phase. In case the ratio of microbial production and assimilation changes to the favor of assimilation, the dH_2 concentration in the bulk of the liquid phase becomes lower than that in the gas phase. Due to the typically low power input and the low solubility of hydrogen in the liquid phase, hydrogen from the head space is usually consumed by microbes already in the boundary layer. Hence, any calculated dH_2 concentrations from off-gas data might not necessarily reflect the dH_2 concentration in the liquid phase. No homogeneous liquid phase exists under the typically applied laminar flow conditions and common fluid viscosities in biogas production [55,56]. Thus, a measurement of dH_2 needs to be located at a suitable spot in the liquid phase. Then it is adding information about the process, as also shown under several process conditions in this study, e.g. in Fig. 8.

4. Conclusions and outlook

The newly developed dH_2 sensor can reliably detect dH_2 in anaerobic fermentation broth, proving its functionality. The results confirm the deep-seated detection limit of the system. Furthermore, the developed extraction unit is able to determine the hydrogen promptly with the least possible apparatus-related expenditure and an effective calibration. The sensor showed long-term stability during a period of more than three months, any shortcomings due to a very limited lifespan are not expected based on our results. Praxis-relevant alterations of the dH_2 concentration were monitored reliably.

The measurement of dH_2 provides additional advantages beside the conventional off-gas measurement. Since the extraction, detection, and analysis are performed in one constructive unit containing an extraction chamber and an analysis chamber, it is possible to integrate the dH_2 sensor in biogas plant monitoring and control concepts.

The evaluation of further experiments on biogas supply flexibilization with and without hydrogen addition will be supported by the additional information from the measurement of dH_2. Future power-to-gas and methanation applications, relying on the production of green hydrogen via electrolysis will especially benefit from the determination of ideal hydrogen supply to avoid over- and undersupply of microbes. This will be of great help if hydrogen is infused to increase the share of methane in the off-gas. With $\leq$ 70 min, the measurement cycle time of the dH_2 monitoring can be considered as suitable. Changes in process conditions in anaerobic fermentation broths occur typically within several hours up to days.

Beside hydrogen, the measurement device can be adapted to extract and analyze other dissolved gases and volatile components in a wide range of concentrations like carbon dioxide, oxygen, ammonia, methane, alcohols, volatile fatty acids or other low-molecular organic components, from any liquid fermentation media in future applications.

Author contributions

E.J. and R.R.M. performed experiments. E.J. wrote the manuscript with contributions from all other authors. All authors contributed to the data analysis and interpretation of results. S.J., J.Z. and P.N. edited the manuscript. A.L., S.J. and P.N. conceptualized wet-lab experiments and acquired funding together with J.Z.

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Janesch Eike: Data curation, Investigation, Visualization, Writing – original draft. **Retamal Marín Rodrigo:** Data curation, Investigation, Visualization, Writing – original draft. **Lemoine Anja:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Oelßner Wolfram:** Methodology, Resources, Software, Writing – review & editing. **Zosel Jens:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Junne Stefan:** Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **Mertig Michael:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Neubauer Peter:** Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Peter Neubauer reports financial support was provided by German Federal Ministry for Economic Affairs and Climate Action. Michael Mertig reports financial support was provided by German Federal Ministry for Economic Affairs and Climate Action. Jens Zosel has patent pending to Deutsches Patent- und Markenamt.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2024.112103.

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