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ORIGINAL PAPER

Online monitoring of concentration and dynamics of volatile fatty acids in anaerobic digestion processes with mid-infrared spectroscopy

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Abstract An ATR-MIR-FTIR spectrometer was integrated into a laboratory scale anaerobic digestion setup. Automatically, a sludge sample from the digester was transferred to a measurement cell; an IR spectrum was recorded and evaluated by chemometric models to estimate the concentration of the individual volatile fatty acids (VFA). The calibration set included semi-artificial samples spiked with known concentrations of the VFA as well as original samples from a continuous fermentation. High-performance liquid chromatography (HPLC) was used as a reference analysis of the samples. The models were optimized for a low root mean square error of prediction (RMSEP). R^2 for acetic acid, propionic acid, isobutyric acid, butyric acid, valeric acid, and isovaleric acid were 0.94, 0.88, 0.83, 0.75, 0.59, and 0.90, respectively. The accuracy of the models was validated in a second experiment. Considering the complex and heterogeneous sludge composition and the chemical similarity of VFA, absolute concentration and dynamic (increasing and decreasing concentration of VFA) was predicted well for acetic, propionic, isobutyric, and isovaleric acid (in their respective concentration range); Butyric acid could not be detected. The installed setup was able to gather and measure native samples from the digester (every 2 h) automatically over a period of 6 months without problems of clogging or biofouling. The instant and continuous analysis of the concentration of the VFA made it possible to evaluate the current bioprocess status and adjust the organic loading rate accordingly.

Keywords Anaerobic digestion (AD) · Biogas · Volatile fatty acids (VFA) · FTIR · Mid-infrared spectroscopy (MIR)

Abbreviations

ATR	Attenuated total reflectance
CSTR	Continuous stirred-tank reactor
FTIR	Fourier transform infrared
GC	Gas chromatography
HPLC	High-performance liquid chromatography
HRT	Hydraulic retention time
MIR	Mid-infrared
NIR	Near infrared
OLR	Organic loading rate
PLS	Partial least squares
RMSEP	Root mean square error of prediction
VFA	Volatile fatty acids
VS	Volatile solids

Introduction

Over the last decade, the anaerobic digestion of biomass to biogas received a strong impulse with the rise of the energy prices of fossil fuels or possible restrictions of natural gas supply. Especially in Germany, the introduction of a renewable energy law in 2000 led to the construction of a great number of biogas plants and thus a more independent energy market. Although the biological and chemical principles of the process are known, the complex

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interaction of the different microorganisms is not fully understood. The challenge for every biogas plant operator is to maintain a stable fermentation process and consistent biogas production with a high percentage of biomass utilization in a short hydraulic retention time. (HRT) Measuring and control technology can thereby provide operator support. Parameters like pH, biogas volumetric production rate and concentration of methane and carbon dioxide are easy to analyze, but are unfortunately not preferred indicators for the actual biological process status. Instead, composition and concentration of volatile acids (VFA) within the digester seem to provide a meaningful measure for the process status [1–3].

The degradation of biomass and the formation of methane is a complex biochemical process that can be separated into four main phases: hydrolysis, acidogenesis, acetogenesis, and methanation. During hydrolysis and acidogenesis, the biomass is degraded and different intermediates are formed, most prominently alcohol, lactate, hydrogen, carbon dioxide, and short chain volatile fatty acids (VFA), in particular, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid. Subsequently, these VFA get metabolized to acetic acid, which together with carbon dioxide and hydrogen form the direct substrates for the methanation process [4]. A kinetic uncoupling between the syntrophic bacteria of acid producers and consumers in these different fermentation steps can be observed by an accumulation of the different VFA [5]. Therefore, concentration and composition of the VFA are the best parameters to reflect the metabolic state of the biochemical process [1–3]. Off-line determinations of the VFA are usually performed with chromatographic methods, including gas chromatography (GC) [6], headspace GC [7] or high-performance liquid chromatography (HPLC) [8]. Online and off-line titrimetric methods can achieve a low cost and quick result of a VFA sum parameter, but ignore the individual composition of the specific VFA [9]. Pind et al. [10], however, concluded from their research into anaerobic fermentation that measurements of all individual VFA are important for control purposes. The dynamics should always be evaluated in close relationship to the conversion of other VFA and the history of the fermentation process.

Recently, few online methods for GC [10–12] and HPLC [13] have been applied to the measurement of VFA. Unfortunately, problems with sample preparation and biofouling are the downside of these methods. FTIR spectroscopy has been proven to be a real alternative without these problems. This method is widely used in pharmaceutical, food, medical, and bioprocess applications [14]. Near infrared spectroscopy (NIR 0.78–3 μm) was shown to be able to evaluate the VFA content in glycerol-boosted anaerobic digestion processes [15, 16] and in a H_2-producing bioreactor [17]. Mid-infrared spectroscopy (MIR 3–50 μm) was already shown to be suitable for monitoring volatile fatty acid content, chemical oxygen demand, alkalinity, sulfate, ammonia, and nitrate content in industrial wastewater treatment [18, 19]. In anaerobic batch composting studies, FTIR spectroscopy was used to evaluate the different decomposition states of biowaste [20, 21].

The aim of this study was to develop and validate chemometric partial least square (PLS) models to predict the concentration of VFA with ATR-MIR-FTIR spectroscopy in anaerobic digestion systems. Furthermore, the established models should be integrated in an automated and sturdy online sampling and sensor system. Native heterogeneous and viscous digester slurry (total solids >6 %) had to be sampled, transferred, analyzed, and disposed with little to none maintenance necessary. The complete history of VFA dynamics, in combination with additional parameters (volumetric biogas rate, pH, biogas composition), should make it possible to adapt the effective organic loading rate (OLR) and HRT for optimal process efficiency.

Materials and methods

Lab-scale biogas plant

An anaerobic continuous stirred-tank reactor (CSTR) with an active sludge volume of 10 l was operated at 40 °C and 75 RPM (Biostat MD, Braun, Melsungen). The inoculum sludge was taken from an operating biogas plant (Biogasanlage Leese, Lower Saxony, Germany). Electrochemical parameters (pH, redox potential) were monitored by electrodes (Mettler Toledo, Greifensee, Switzerland), the biogas flow was measured with a biogas flow meter based on the liquid displacement principle [22], operated with a 75 % saturated NaCl solution at pH 2 [23] and corrected to standard conditions (0 °C, 1,013 hPa). Sampling and feeding of the fermenter were done once a day. Two different feedstocks were used: substrate A was composed of a mixture of 10 % peptone with corn starch. Substrate B contained wheat flower. The powdered substrates were manually fed into the digester to increase or decrease the OLR. The HRT was kept constant because 20 ml of digestate were removed at every measurement and balanced by the addition of fresh water. The resulting HRT was 42 days with a varying OLR between 0 and 4 gVS $(l\ d)^{-1}$.

Chemicals

All reference compounds (acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid) were of analytical grade (Fluka, Roth, and Applichem).

Reference sample preparation and analysis by HPLC

Sludge samples were extracted with a syringe and centrifuged at 13,000 rpm for 10 min to pelletize the solids. The supernatant was further filtered with a 0.45 µm micro-filter (Minisart RC 25, Sartorius GmbH) into a HPLC sample vial and stored at −20 °C. These samples were analyzed with a HPLC setup consisting of a binary HPLC pump and an auto sampler (Waters, MA). To detect the VFA, two successively connected detectors were used (UV/Vis detector 2489 at 210 nm and Refractive Index Detector 2414). An Aminex HPX-87-H column (Bio-Rad Laboratories, Richmond, CA) combined with a suitable guard column (Bio-Rad Micro-Guard Cation-H) was used as stationary phase. The column temperature was 40 °C. 5 mM sulfuric acid with a flow rate of 0.6 ml min^{-1} served as mobile phase. The injection volume was 20 µl and the sample run time 60 min. For calibration, an external standard with varying concentrations for the different VFA was prepared (acetic acid 0–4 g l^{-1}, propionic acid 0–3 g l^{-1}, isobutyric acid, butyric acid, isovaleric acid, and valeric acid 0–1 g l^{-1}). Integration and quantification of the recorded chromatograms was done with the Breeze2 software (Waters, MA).

Sample preparation (FTIR)

Native, filtered samples were used for IR spectra recording. Suspended solids were removed with a raw mesh filter (manually or with a rotating filter unit mounted on the stirrer axis in the digester; modified according to Pind et al. [10]). Pore size was approximately 1 mm^2 and the rotation shearing force removed solids, which accumulated on the surface of the filter.

MIR-ATR-FTIR equipment and data analysis

TENSOR 27 (Bruker Optics, Ettlingen, Germany) FTIR spectrometer fitted with an ATR Cell at room temperature (25 °C) was used to record the IR spectra. Zinc Selenide (ZnSe) was used as the ATR crystal. The horizontal, open position allowed an easy manual sample application as well as automatic application of the digestate with a pump. Wavenumbers from 2,800 to 900 cm^{-1} were scanned at a resolution of 4 cm^{-1} for reference measurements with fresh water and samples.

Two hundred and fifty six spectra were recorded in absorbance mode and averaged to smooth and minimize temperature drifts (digester temperature 40 °C—spectrum measurement at room temperature).

Partial least square algorithm was used to create separate multivariate calibration models for acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid with the QUANT2 software package (Bruker Optics, Ettlingen, Germany). Principles of chemometrics and multivariate calibration are described by Martens and Næs [24].

Automatic sampling and sensor system

The process of sludge extraction, spectrum recording and processing during the fermentation experiments was automated. An acrylic glass chamber with two inlets (sample and flushing water) and one outlet (waste) was installed and sealed on top of the ATR Cell (c.f. Fig. 1).

A process cycle included the following steps (c.f. Fig. 1): (1) The acrylic glass chamber was flushed with fresh water by a peristaltic pump (2) (Millipore, USA), (2) A background spectrum of the water was recorded immediately afterward. (3) A multichannel peristaltic pump (1) (Ismatec, Wertheim, Germany) transferred approximately 20 ml of the digestate from the filter unit to the flow chamber. The filling level of the fermenter was leveled out with fresh water. (4) An MIR spectrum of the digestate was recorded and processed. (5) The flow chamber got flushed again to minimize biofouling and the cycle restarted after 2 h. Twelve spectra per day were recorded.

PLS model development and calibration set

For the development of the different PLS models to predict the concentration of the VFAs in the digester, a calibration set of samples of two sources was used. Figure 2a presents a summary of the strategy for calibration set part A and B.

Calibration set part A: spiked samples

Similar to Udén and Sjaunja [25], who estimated the VFA concentration in rumen samples by MIR-FTIR, a calibration set with spiked samples was used to developed the PLS methods for the different VFAs. Therefore, the feeding of the biogas plant was halted until the concentrations of the VFAs had decreased below the detection level of the HPLC. This VFA-free digestate was used as matrix to prepare spiked samples with known concentrations of VFA. The Calibration Design Tool of the QUANT2 software package was used to develop a scheme, where the variations of the concentrations of VFAs were independent of each other (R^2 of acetic acid vs. propionic acid low, same for all other combinations of VFA). This guaranteed that an observed variation in the recorded spectrum could be traced back to a variation in concentration of the individual VFA. Analytical grade fatty acids were added to 5 ml of native digestate and mixed. Afterward, MIR spectra were recorded and HPLC samples to verify the concentration of VFA were prepared.

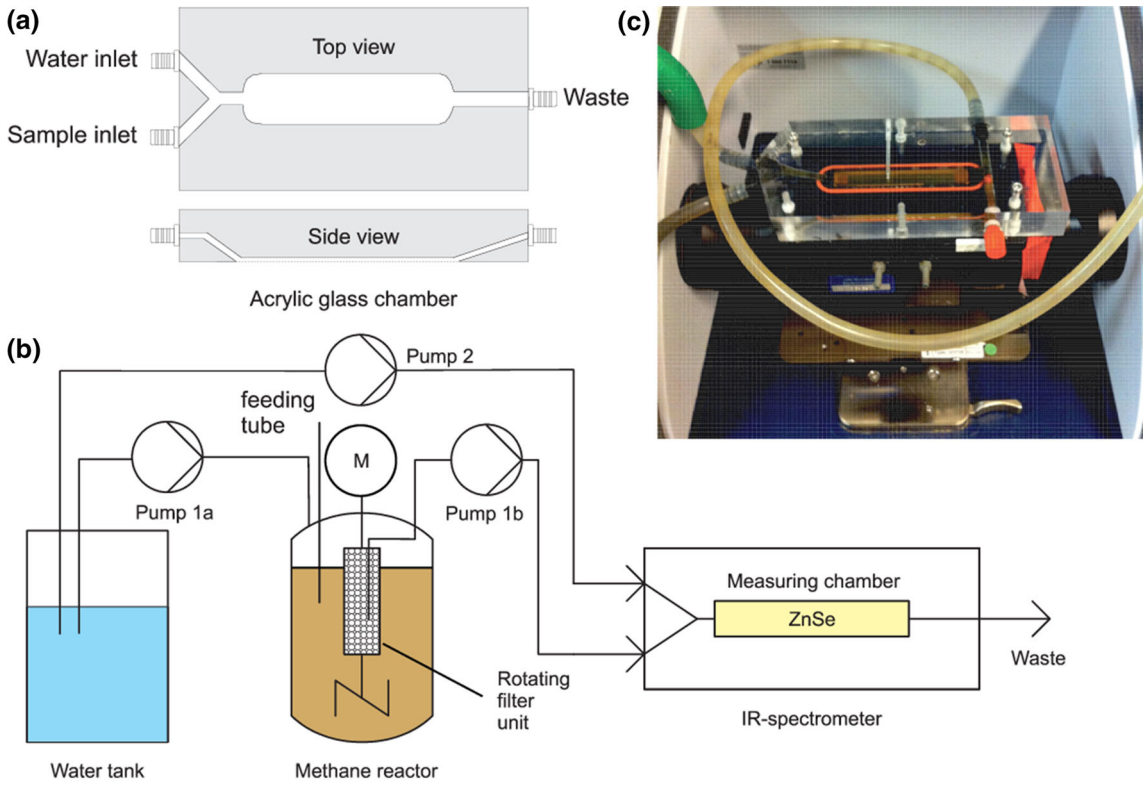

Fig. 1 **a** Schematic of the acrylic glass chamber mounted on top of the ZnSe crystal, *side view* and *top view*. **b** Piping and instrumentation scheme of the anaerobic digester and the IR spectrometer. A filtered sample is generated by a rotating filter unit mounted on the stirrer axis and transferred to the measurement chamber by peristaltic pump 1b. Simultaneously, fresh water is transferred to the fermenter to balance the filling level (peristaltic pump 1a). After spectrum recording, both crystal and chamber are cleaned with fresh water via peristaltic pump 2. **c** Picture of the acrylic glass chamber. Water and sample inlets on the left side, waste outlet on the long distal side

Calibration set part B: samples from a continuous fermentation

In addition to the spiked samples, a continuous fermentation experiment was performed. As the goal was to detect varying concentrations of specific VFA in a real bioprocess, a mixture of cornstarch and peptone powder was used as a single substrate. It was known from previous anaerobic digestion tests, that due to the fast and high degradability, these substrates are fast acid formers, even at low OLR. Figure 6a presents an overview of the digestion experiment: the dashed line represents the cumulative biogas volume and the solid line reflects changes in pH. Figure 6b–f present the concentration of the individual VFA, from acetic acid to isovaleric acid.

Prior to day 0, the sludge was adapting to the laboratory conditions for 10 days. The feeding started on day 0 with an OLR of 1.0 gVS $(l\ d)^{-1}$. After 1 week, it was increased to 2.0 gVS $(l\ d)^{-1}$ and stopped at day 20, because of the drop in pH. The feeding was restarted at day 43, after the pH had stabilized again with an OLR of 1.0 gVS $(l\ d)^{-1}$. Due to the second rapid pH drop, the feeding was stopped again at day 48 and resumed at day 58–72 with a reduced OLR of 0.5 gVS $(l\ d)^{-1}$.

This complex approach for the model development was chosen to generate both independent concentrations of VFA over a set concentration range (Calibration set—part A) and native samples from a fermentation, to include other varying factors (changing bacterial biomass, pH, OLR). Digestate was sampled daily; IR spectrum was recorded and VFA concentrations determined with HPLC.

Model validation and sensor system performance (c.f. Fig. 2b)

After a successful calibration (here PLS model development), a robust sensor system should be able to measure the desired values in a specified measuring and deviation range. Furthermore, the additionally gained data should help to make informed decisions about the biological process status and possible corrections in the method of operation (e.g., high VFA concentration: process overloaded → adaptation of the OLR to the current degree of capacity utilization). A continuous fermentation experiment was conducted to test

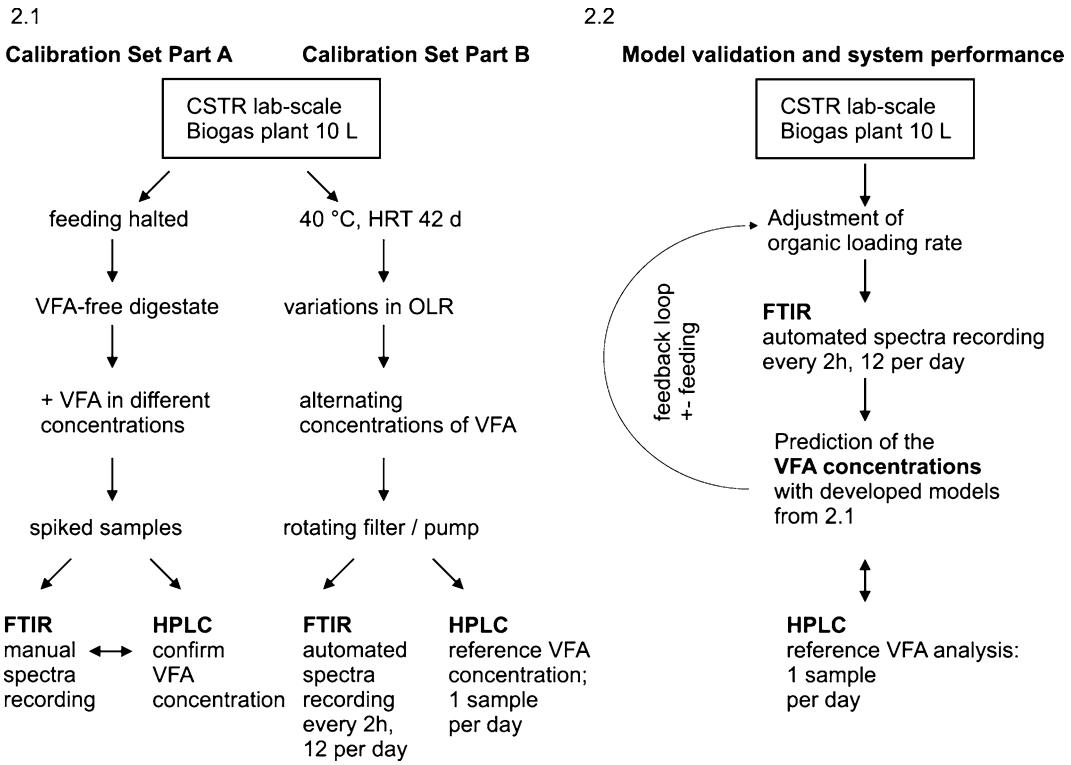

Fig. 2 Flow chart of PLS model development (**a**) and validation of the sensor system (**b**): *Calibration set part A* was created by manually spiking VFA-free digestate samples with analytical grade VFA in known concentrations. *Calibration set part B* consisted of samples from a fermentation experiment; one spectra and HPLC reference analysis was done daily

the abilities of the whole setup: the accuracy of the prediction of the VFA dynamics and concentration in the process by the PLS models, an overall mechanical stress test for the spectrometer and remaining components and an optimization of the feeding strategy, shortening the startup time to reach full throttle of the biogas plant.

Based on the recorded dynamics of the VFA, a decision was made daily to adapt the OLR to the bioprocess status, e.g., increase of OLR if the concentration of VFA stayed low, decrease of OLR if concentration of VFA increase or leave it unaltered (as anaerobic digestion systems tend to react slowly to changes).

Results and discussion

Spectral analysis of VFA

The region between 1,750 and 950 cm^{-1} was used for calibration as it represents the area of highest absorption of VFAs at a given pH. Figure 3a shows the absorbance of acetic acid at different pH. The spectra changed clearly with the state of protonation of acetic acid. At a pH of 2.5, two major peaks could be observed at 1,712 and 1,281 cm^{-1}. With increasing pH, these peaks shifted to 1,551 and 1,415 cm^{-1}. The pK_s was the barrier for this change. At pH of 4.75, peaks at all mentioned wavenumbers could be seen. The pH in a typical stable anaerobic digestion was between pH 7–8. The developed methods, therefore, were optimized to detect the ionic forms of the different organic acids. Figure 3b presents the spectra of the different VFA at pH 5.5. The spectra were very similar due to the almost identical chemical structure of the compounds, but not congruent, which made an individual detection of fatty acids possible. The major peak could be identified around the wavenumber of 1,551 cm^{-1}, but also other peaks could be identified in the given wavenumber range from 1,850 cm^{-1} down to 900 cm^{-1}.

Spectra of digestate

IR- spectra taken at various time points (day 6, 16, 26, 37, 47, and 57) during a fermentation (c.f. Fig. 6) experiment are presented in Fig. 4 to give a compact overview of the spectra change with high and low VFA concentration in the digestate. The main absorbance region was found between 1,800 and 900 cm^{-1}, which represent the absorption region for early decomposition products like aldehydes, ketones, esters, and short chain carboxylic acids. A typical IR band is present at 1,640 cm^{-1}, which reflects the aromatic C=C

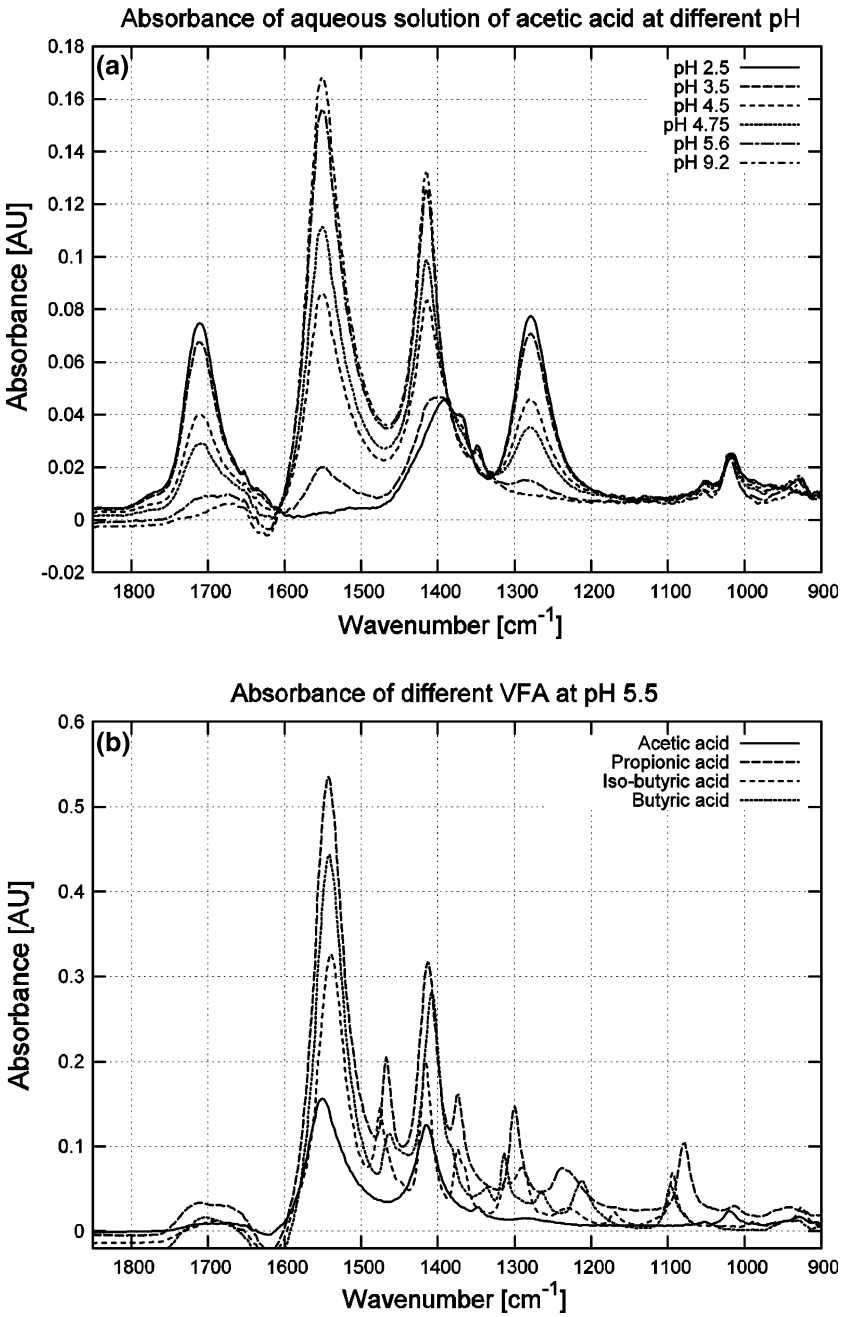

Fig. 3 **a** Absorbance spectrum of an aqueous solution of acetic acid (0.5 g l^{-1}) at different pH values. **b** Absorbance spectrum of acetic acid, propionic acid, isobutyric acid, and butyric acid (concentrations of all acids 0.5 g l^{-1}) at a pH of 5.5

bond and C=O group absorption of amides (Amid I band) or carboxylates. A second band of protein origin, an indicator for protein rich components, the Amid II band is located around 1,570 and 1,540 cm^{-1}, due to the N–H in-plane bend vibration and can be assigned to secondary amides [20, 21, 26]. The shoulder at 1,425 cm^{-1} is due to the COO^{-} stretch of carboxylates and the C–O stretch of carbonyls. Another characteristic region for the VFA is between 1,265 and 1,240 cm^{-1}, where the C–O vibration can be observed.

A significant peak of the spectra shown in Fig. 4 can be seen between 1,070 and 1,000 cm^{-1}. This region is assigned to the C–O stretching of polysaccharides and polysaccharide-like substances, Si–O of silicate impurities and clay minerals possibly in a complex with humic acids [20]. The heterogeneous and complex matrix of the sludge led to an elevated background noise. For the PLS model development, all spectra were smoothed and preprocessed with the first or second derivative to remove this background signal [27].

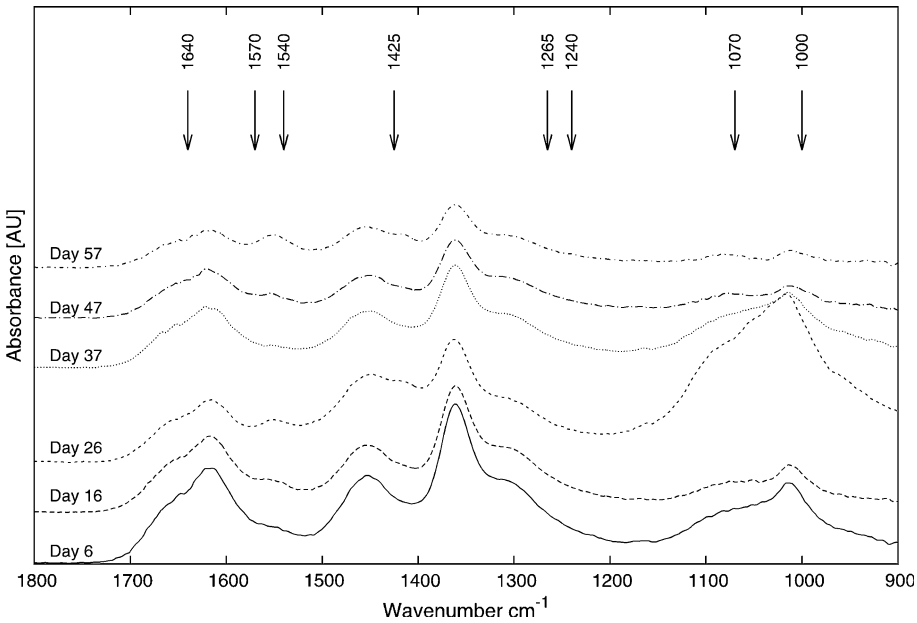

Fig. 4 Spectra recorded at different time points (low and high concentration of VFA in digestate) during the validation fermentation experiment (c.f. Figs. 2b, 7). Spectra are stacked on the *y*-axis for better clarity

Calibration set part B

The focus was to generate changing concentrations of VFA to generate test samples with a variability and VFA composition. Therefore, substrates with a high degradability were chosen for the feeding of the laboratory digester.

With the beginning of the feeding, almost instantly the concentration of all VFA started to increase rapidly (day 10). After the feeding stopped on day 20, the VFA were degraded fast, only propionic acid persisted for nearly 30 days. After the concentration of propionic acid decreased under 0.5 g l^{-1}, the feeding was restarted at day 45, resulting in a second increase of the VFA, especially acetic acid, isobutyric acid, butyric acid, and isovaleric acid. The experiment was successful, as HPLC samples and spectra with different VFA composition could be collected. Different important phases can be distinguished: no VFA in process: day 50–60; high concentration of all VFA: day 10–20; only propionic acid: day 30–40; high concentration of all VFA with low propionic acid, day 45–55 as well as changing levels of pH over the whole experiment.

PLS models

Based on the Calibration set, PLS models have been developed for acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid. Other organic intermediates such as formic and lactic acids were not relevant to assess the process status in anaerobic digestion. For every volatile fatty acid, a different concentration range was chosen (acetic acid 0–3 g l^{-1}, propionic acid 0–3 g l^{-1}, other VFA 0–0.3 g l^{-1}), close to their expected concentration in a stable anaerobic digestion process.

The quality and robustness of the developed PLS models can be estimated with two important factors: the root mean square error of prediction (RMSEP) and the number of internal latent variables. In both cases, lower values describe more robust models. The whole dataset was divided into two parts: calibration and test points. PLS models were created based solely on the calibration points. These models are further predicting the concentration of the test points. The advantage was that calibration and prediction data set are truly independent of each other. This external test-set validation was used and the RMSEP was calculated: It describes the error of the reference (standard deviation SD) of the residuals calculated from the difference between the predicted values and HPLC analysis data within the calibration set. Table 1 summarizes the properties (number of calibration spectra, number of test spectra, internal latent variables, RMSEP) for each model and Table 2 presents the frequency ranges used in the models, respectively. Furthermore, Fig. 5 gives a graphical representation of these results, as it shows the predicted values plotted against the reference analysis with HPLC.

Acetic acid is one of the direct precursors for methane production during methanogenesis. The collected samples cover smoothly a wide concentration range from 0 to 3 g l^{-1}. The predictability was good, with a R^2 of 0.94 and a RMSEP of 0.16 g l^{-1}. Inhibition of anaerobic fermentation coincides usually with an increase of propionic acid concentration [28–31], therefore making it a suitable indicator for reactor imbalances. The developed method

Table 1 Summary of the properties of the developed PLS models for the different components

Component/PLS model	No. calibration spectra	No. test spectra	Data pretreatment	Concentration range (g l^{-1})	Internal latent variables	R^2	RMSEP (g l^{-1})
Acetic acid	71	69	1st derivative	0–2.75	7	0.94	0.156
Propionic acid	67	66	2nd derivative	0–3.33	7	0.88	0.235
Iso-butyric acid	42	41	1st derivative	0–0.26	2	0.83	0.0318
Butyric acid	44	44	1st derivative	0–0.33	6	0.75	0.0472
Iso-valeric acid	40	39	1st derivative	0–0.25	3	0.59	0.0496
Valeric acid	44	44	1st derivative	0–0.26	6	0.90	0.0191

The data include spectra from the artificial calibration and the results from the continuous fermentation experiments

Table 2 Frequency ranges (cm^{-1}) used in the different PLS models

Component/PLS model	Frequency ranges (cm^{-1})			
Acetic acid	1,801–1,641	1,589–1,535	1,429–1,375	1,323–1,269
Propionic acid	1,801–1,747	1,535–1,269		
Iso-butyric acid	1,811–1,622	1,496–1,433	1,371–1,182	
Butyric acid	1,492–1,178			
Iso-valeric acid	1,716–1,550	1,389–1,346		
Valeric acid	1,717–1,661	1,390.6–1,335	1,282.3–1,173	

spans a concentration range of 0–3 g l^{-1} with a RMSEP of 0.26 g l^{-1} and R^2 of 0.88, with equally distributed values across the concentration range. These standard errors of predictions are consistent with the findings of other studies with NIR (acetic acid 0.28–0.57 g l^{-1}, propionic acid 0.53 g l^{-1}) or MIR (acetic acid 0.1–0.9 g l^{-1}) applications [17, 27]. Similar to propionic acid, the existence and especially increasing concentrations of butyric acid and valeric acid are a sign of process disturbances [1, 30]. Their ability to inhibit the process is inverse to their concentration (inhibition at concentrations of 50 mg l^{-1} undissociated fatty acids without prior adaption for isobutyric and isovaleric acid [4]). It was therefore decided to use a smaller concentration range from 0 to a maximum of 300 mg l^{-1} to account for this factor. All models exhibit a clustering of values around 0 g/l, because of their usual lack in the anaerobic digestion process. They could only be detected in samples, that were either manually spiked and part of calibration set part A or in phases of higher disturbance while collecting samples for calibration set part B (c.f. Fig. 6, between days 10–20, 45–55, after day 65). This shows the challenge of creating valid models for these higher chain fatty acids with samples form a fermentation process alone. The R^2 of these methods are 0.83, 0.75, 0.59, and 0.90 for isobutyric acid, butyric acid, isovaleric acid, and valeric acid, respectively.

Continuous fermentation—validation of the developed methods, Fig. 7

Feeding strategy

The final step was the validation of the performance of the developed PLS models in an independent anaerobic digestion experiment. HPLC analysis served as a reference only to evaluate the accuracy of the predicted concentration values for the individual VFA. Figure 7 shows the responses of pH, biogas generation (Fig. 7a) and the volatile fatty acid concentration (Fig. 7b–f). In addition to the concentrations estimated with HPLC (points), the result of the PLS models are depicted as dots (single measurement) and a line (smoothed average).

The feeding started with an OLR of 0.5 gVS (l d)$^{-1}$ at day 4 with cornstarch and peptone. Due to the rapid drop of pH and the increase of the VFA concentration, the feeding was stopped at day 11 until the concentrations of the VFA recovered. Feeding was restarted at day 17 with grinded wheat (slower degradability, to put less stress on the anaerobic digestion) at the same OLR of 0.5 gVS (l d)$^{-1}$. On day 24, the OLR was increased to 1 gVS (l d)$^{-1}$ and on day 37 further to 2 gVS (l d)$^{-1}$. On day 42 was an increase to 3 gVS (l d)$^{-1}$ and a final step to 4 gVS (l d)$^{-1}$ on day 48.

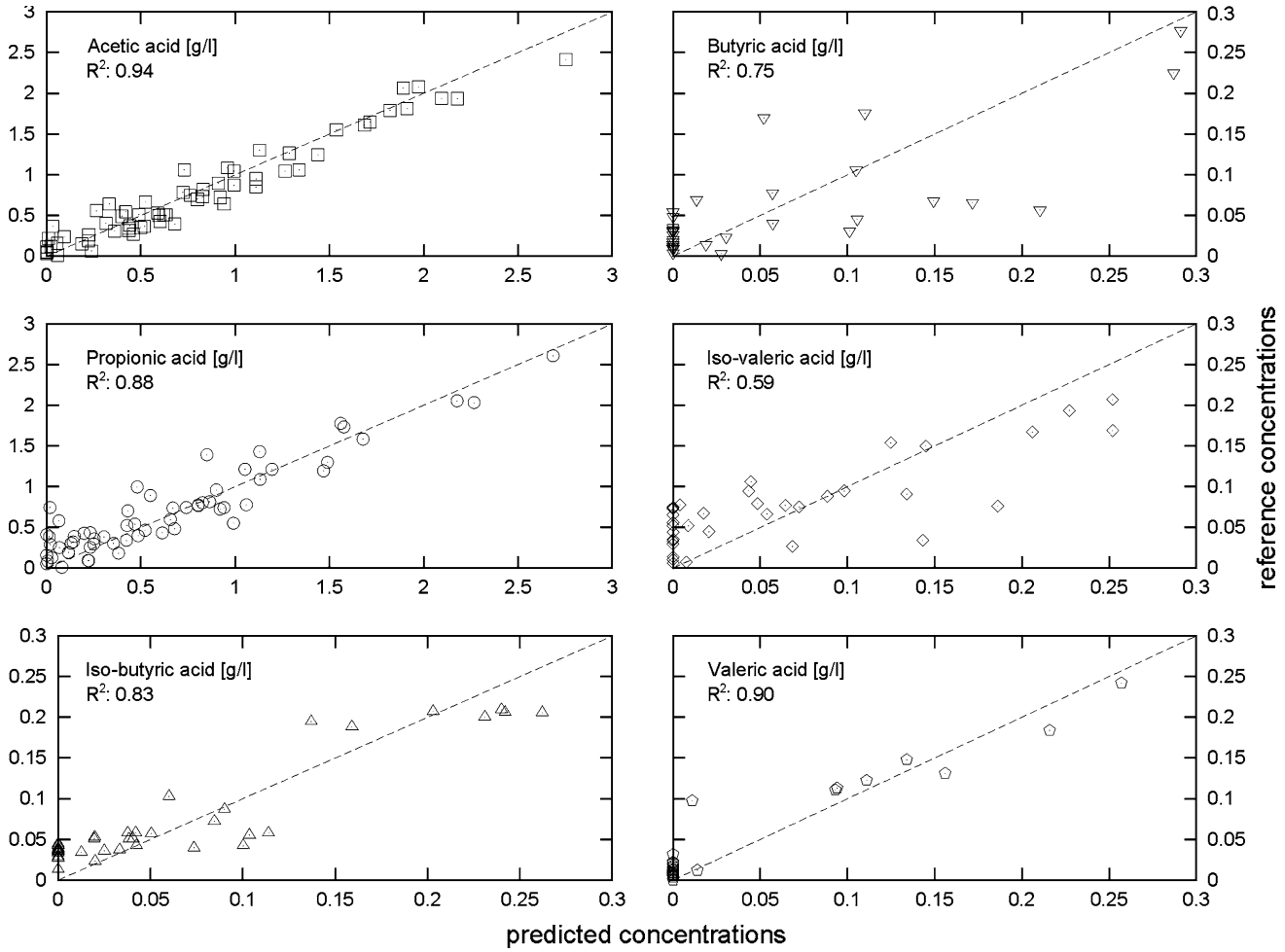

Fig. 5 Regression plots from the PLS- models for the individual VFA. Concentrations predicted by the developed PLS models plotted against the HPLC reference analyses

Predictability of PLS models

The absolute reference concentrations of the HPLC results were not matched by the PLS models. A maximum deviation of about 0.5 g l^{-1} for acetic acid (c.f. Fig. 7b), for predicted and reference values was observed. Nevertheless, especially the dynamic of acetic acid was described very well by the PLS models. In a concentration range between 0 and 2.5 g l^{-1}, the increase phase (day 7–12, day 20–32), degradation phase (day 33–40) and phase with elevated level (day 55–70) could be monitored by IR spectroscopy.

The PLS prediction capability was similar for longer chain VFA. Comparing the smooth average of the prediction values with the reference, propionic acid shows a deviation of about 0.175 g l^{-1}. The first increase of propionic acid from day 4 to 13 was detected well and matched the reference values from the HPLC. The subsequent degradation of propionic acid was not matched precisely. In contrast to the reference analysis, which shows a level of propionic acid near 0 g l^{-1} between day 25 and 50, the PLS models predict a low, but persistent concentration of about 0.15 g l^{-1}. The further increase of propionic acid after day 50 due to the fast increase of the OLR, however, is recognized by the sensor system.

The dynamics of the isoforms of butyric and valeric acid were also detected. As soon as they appeared in fermentation process, from day 30 to 35 and again beginning with day 50, they could be detected with by the PLS models. Butyric acid seems not to be detectable in this experiment. All the spectra from day 0 to 52 for butyric acid failed the internal validity tests and are therefore not shown in Fig. 7e. But also the evaluation of the collected chromatograms showed problems for butyric acid as mostly no defined peak could be integrated. Therefore, none of the two methods provided valid concentration values over a long period during the experiment.

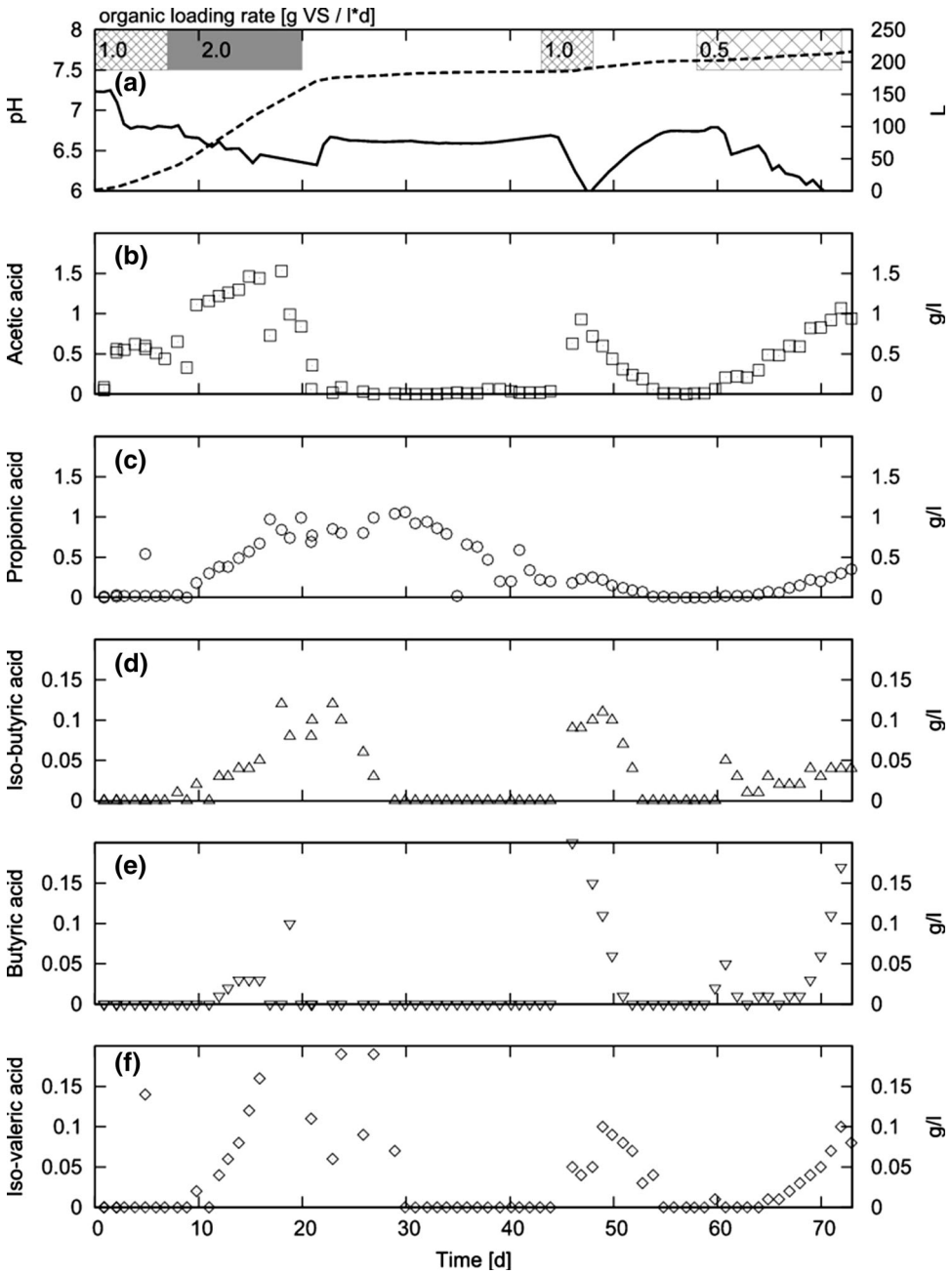

Fig. 6 Calibration set part B; continuous fermentation experiment to collect data for the PLS model development, **a** *solid line* pH, *dashed line* cumulative biogas volume, organic loading rate indicated in *top row*, **b–f** points: concentration of VFA, determined with HPLC

VFA concentration as control parameter for feeding

In this experiment, the predicted concentration of the VFA was already used for modulating the OLR. After the increase of OLR from 0.5 to 1 gVS (l d)$^{-1}$ on day 24, the responses of the VFA were closely monitored. The next increase to 2.0 gVS (l d)$^{-1}$ happened only after the VFA were almost completely degraded again. This strategy also explains the following short time interval between the next increasing steps. After the increase of OLR to 3.0 gVS (l d)$^{-1}$ the VFA measured with the sensor system showed no significant response and therefore the final step to and 4.0 gVS (l d)$^{-1}$ was only a few days later. The sensor system and the strategy to adapt the substrate feed to the actual VFA concentrations made it possible to increase the OLR from 0.5 to 4 gVS (l d)$^{-1}$ in only 1 month (day 17–48), without any overloading.

Mechanical evaluation

For the installation in an industrial scale biogas plant, the mechanical implementation has to be error prone and low maintenance. The sensor system, as it was evaluated in the laboratory fulfilled these requirements. The rotating filter

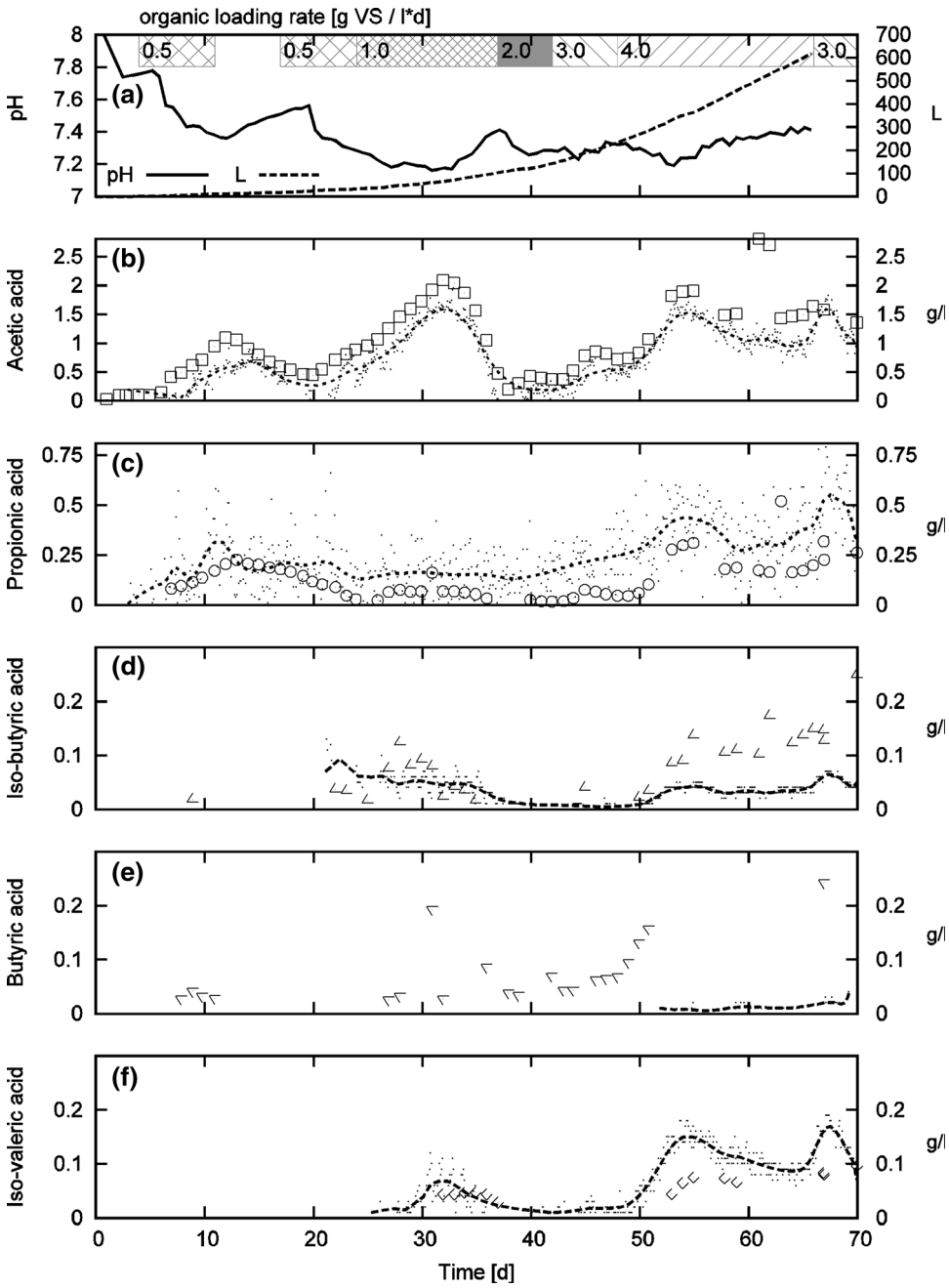

Fig. 7 Validation fermentation experiment of the developed sensor system (c.f. Fig. 2b), PLS models do not include the corresponding HPLC results; the plotted HPLC values serve as a reference only to estimate the accuracy of the predicted concentration values by the PLS models. **a** *Solid line* pH, *dashed line* cumulative biogas volume, organic loading rate indicated in *top row*, **b–f** dots: concentration values from PLS models. *Line* smoothed average of these values, *points* concentration reference by HPLC. All PLS predicted values shown in the figure passed the internal QUANT2 validity test

unit was working trouble free. Solids that accumulated over time on the surface were simply removed by increasing the RPM of the stirrer for a short period of time. With the solids removed, the filtered sample could be transferred easily to the acrylic glass chamber, a spectra recorded and flushed again with water. The only manual necessary maintenance was the periodically cleaning of the ZnSe crystal to prohibit the formation of a biofilm. The biggest installation problem to be solved is probably the environmental condition for the IR spectrometer. It has to be installed in a vibration-free and dry environment, but with short connections to the digester to be able to get fresh digestate samples. Only a vibration-cushioned, isolated, and sealed housing can fulfill these requirements.

Conclusions

This worked confirmed, that ATR-MIR-FTIR spectroscopy in combination with suitable PLS models could provide a satisfactory method to monitor the process of anaerobic digestion. These PLS models were developed based on a

calibration set with samples spiked with VFA and samples from an anaerobic digestion. The absolute concentration of VFA in the digestate could well be predicted for acetic and propionic acid. As the higher chain fatty acids only occur in a smaller concentration, the prediction of their absolute concentration value was difficult. The dynamics of increasing and decreasing concentration of VFA, however, could be monitored easily by the sensor system. As the anaerobic sensor system was required to record a sample every 2 h, an automatic sampling, sample transfer, and measurement unit was implemented with a rotating filter on the stirrer axis and an acrylic glass chamber with in- and outlets. Pumps were used to transfer the sample from the digester and to clean the ZnSe crystal. The monitoring of the VFA made it possible to implement a feeding strategy based on the current bioprocess status and hence shorten the startup time for a biogas plant. Also, the system might be used to detect problems of the bioprocess at an early stage, where time for possible countermeasures is still left. Future studies have to investigate how to install the sensor system on an industrial anaerobic fermentation system, the long time stability and the predictability of the PLS models with changing substrates and higher values of total solids in the process.

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