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Regulation and optimization of the biogas process: Propionate as a key parameter

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Abstract

The use of volatile fatty acids (VFA) as process indicators in biogas reactors treating manure together with industrial waste was studied. At a full-scale biogas plant, an online VFA sensor was installed in order to study VFA dynamics during stable and unstable operation. During stable operation acetate increased significantly during the feeding periods from a level of 2–4 to 12–17 mM, but the concentration generally dropped to about the same level as before feeding. The fluctuations in the propionate were more moderate than for acetate but the average level rose during 1 week of operation from 0.6 to 2.9 mM. A process disturbance caused by overloading with industrial waste was reflected by a significant increase in all VFA concentrations. During the recovery of the process, the return of propionate back to the steady-state level was 2–3 days slower than any other VFA and propionate could best describe the normalizing of the process. In a lab-scale continuously stirred tank reactor experiment, with manure as main substrate, the prospective of using either propionate concentration or methane production as single process indicators was studied. Propionate was found to be the best indicator. Thus, a process breakdown caused by organic overloading with meat and bone meal and lipids was indicated by changes in propionate concentration 12–18 days before a decrease in methane production was observed. Furthermore, a more efficient and stable utilization of the substrate was observed when propionate was used as process indicator.
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Keywords: Biogas process; Regulation; Process imbalance; Propionate; Volatile fatty acids; Methane

1. Introduction

Anaerobic digestion of organic matter with a simultaneous production of biogas is an environmentally attractive way for treatment of organic waste. In Denmark, several joint large-scale biogas plants combine the treatment of manure together with waste from slaughterhouses and food processing industries (codigestion). The degradation process is complex and depends on a balanced action of several microbial groups [1]. During the process biopolymers are initially hydrolyzed and fermented to volatile fatty acids (VFA) H_2 and CO_2, by the hydrolytic/fermentative bacteria. VFA such as propionate, butyrate and isobutyrate are subsequently oxidized by acetogenic bacteria producing acetate, H_2 and CO_2, and finally these products are converted to CH_4, CO_2 and H_2O by methanogens [2]. The growth rates and the sensitivity toward environmental changes differ widely between the different groups. As a consequence of this, an unrestrained reactor operation can lead to disturbances in the balance between the different microbial groups, which might lead to reactor failure. Therefore, development of reliable tools for efficient process control and understanding are necessary. Normally, during a process imbalance intermediates such as H_2, VFA and alcohols will accumulate, accompanied by fluctuations in gas production. In theory, this provides several parameters that can be used as indicators of process instability. However, the complexity of the process has made it difficult to find a simple and suitable control parameter reflecting the metabolic state of the entire process. Steyer et al. [3] successfully used biogas production and pH as control parameters in a high-rate

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fluidized bed reactor treating a more or less uniform wine distillery waste. The control strategy was suitable for safe operation of the studied system, but can hardly be transferred to biogas plants treating manure in combination with complex organic waste because of the buffering capacity of the manure, the high HRT of the reactor and the unknown substrate composition. Nevertheless, at Danish centralized biogas plants, biogas production is normally the only continuously measured parameter.

The transfer of hydrogen plays an important role in the overall regulation of the anaerobic digestion process and especially in the oxidation of VFA. For that reason hydrogen could seem as an obvious control parameter. However, complicated dynamics of hydrogen in anaerobic ecosystems and variability for given reactors and substrates makes hydrogen inadequate as a single control parameter [4]. The use of hydrogen as a control parameter should always be during simultaneous measurement of other process parameters.

It is well accepted that VFA concentrations can serve as efficient indicators of process imbalances, but controversy exists about how and which VFAs that should be included in the control strategies [5–9]. Hill et al. [7] suggested that a propionate/acetate ratio higher than 1.4 indicated impending digester failure, but other results have clearly contradicted this statement [5,10]. Ahring et al. [5] suggested that a combined parameter reflecting the concentrations of both butyrate and isobutyrate could be a reliable tool for indication of process instability caused by changes in hydraulic loading, organic loading and temperature. Various associations between the actual concentration of individual VFA concentrations and process imbalance have also been suggested. Hill et al. [7] concluded that an acetate concentration higher than 13 mM would indicate process imbalance and Hill and Holmberg [8] suggested that concentrations of isobutyrate and isovalerate higher than 0.06 mM was an indication of process instability. However, several experiments in our laboratory show that stable reactor performance can occur at VFA concentrations well above these limits [11–13]. No useful control strategies that includes VFA have, therefore, yet been developed.

Traditionally measurement of VFA concentrations in anaerobic bioreactors treating livestock waste has been done by time-consuming manual sampling methods performed on a daily basis. The slow procedure is the major reason why VFA concentration is not measured at Danish centralized biogas plants. However, an *in-situ* microfiltration system, developed in our laboratory, has made online VFA monitoring of biogas reactors treating livestock waste possible [14]. The system provides the opportunity of gaining a more profound understanding of the complex VFA dynamics of the anaerobic digestion process and can help develop more efficient control strategies. In this context one purpose of the present study was to install an online VFA monitor at a less efficient full-scale Danish biogas plants and study the VFA dynamics during stable and unstable reactor performance.

With the application of the online VFA technique, we have previously studied VFA dynamics in thermophilic lab-scale reactors during periods of organic overloading (addition of protein), ammonia inhibition and long chain fatty acid (LCFA) inhibition [15,16]. In both studies all VFAs, except valerate, increased rapidly following each perturbation, but propionate showed the slowest recovery back to the original concentration and gave the best indication of when the biogas process had reestablished. This is in good agreement with kinetic studies showing that propionate degraders are the slowest growing and most sensitive VFA-degrading microorganisms in the anaerobic digestion process [12,17]. On the basis of these observations, the primary purpose of the present study was to compare the prospective of using either methane production or propionate concentration as single parameters for regulation of the anaerobic digestion process. This was done in lab-scale digesters treating pig and cattle manure together with complex organic industrial waste.

2. Materials and methods

2.1. Characterization of process parameters at different full-scale biogas plants

Reactor samples were randomly taken from 8 different centralized Danish biogas plants in order to select a less efficient biogas plant for installation of the VFA sensor. The samples were analyzed with regard to VFA levels, specific methanogenic activity (SMA) and biogas potential of the reactor effluent. All samples were taken within the same week during a tour visit in February 2002 and kept on ice for maximum 5 days before they were analysed. Measurement of SMA was based on the method by Sørensen and Ahring [18]. Nine millilitres of anaerobic basal medium was added into 30-ml serum bottles [19], which had been flushed with 100% N_2 to achieve the same pH as the various samples. The media were autoclaved at 141 °C for 40 min and 0.5 g l^{-1} Na_2S and 10 ml l^{-1} vitamin solution were added (Deutsche Sammlung von Mikroorganismen und Zellkulturen, medium no. 141, DSMZ 1989). Test vials were either supplemented with sterile-filtrated sodium acetate (0.2-µm minisart filter, Sartorius AG, Goettingen, Germany) in a final concentration of 50 mM or added H_2/CO_2 by pressurizing the vials with H_2/CO_2 (80%/20%) to 101 kPa overpressure. Bottles that served as controls were only added basal medium, Na_2S and vitamin solution. No substrate was added the control bottles. Finally, all bottles were inoculated with 1 ml reactor content and incubated at the same temperature as the corresponding reactor. All reactor samples had been preincubated for approximately 16 h before inoculation to ensure an active biomass. After inoculation the dilution rate of the biomass was 1:10.2 in vials containing acetate and 1:9.2 in control vials and vials with H_2/CO_2. Methane production was measured every second hour for 14 h. All experiments were conducted in triplicate.

The biogas potential of the reactor effluents were determined by distributing portions of 50 ml reactor effluents into 116-ml vials. The vials were flushed with N_2/CO_2 (80%/20%), closed with butyl rubber stoppers and aluminum crimps and incubated at the same temperature as the corresponding reactor. Methane production was measured every 4–5 days for a period of 60 days.

2.2. Online VFA measurement at a full-scale biogas plant

Based on the results from the random samples taken at the various biogas plants, which is discussed later, it was decided to install the online VFA sensor at Lintrup biogas plant (Linkogas Amba, Tornumvej 15, 6660 Lintrup, Denmark) in order to observe the VFA dynamics of the plant during continuous operation. The sensor was installed at the plant from December 2002 to October 2003. A detailed description of the sensor is given by Pind et al. [14].

2.3. Regulation and optimization of the biogas process in lab-scale reactors

Substrates: The main feedstock of the reactors was raw cattle manure and pig manure, which had been mixed in a ratio of 1:1 and blended for 1–2 min. The manure was obtained from the full-scale biogas plant in Hashøj, Denmark, and kept at $2\,^\circ C$ until used. Meat and bone meal (MBM) and fat were obtained from Green Farm Energy, Randers, Denmark. The fat, which was solid at room temperature, originated from a food processing industry producing margarine from palm oil. Palm oil has a high content of saturated fatty acids ($\approx 50\%$). The fat was mixed with rapeseed oil in a 1:1 ratio resulting in a thick fluid. The fat was mixed with the oil in order to get a constant distribution and concentration of the product in the manure. Daka Bio-industries, Ringsted, Denmark, produced the MBM. The dry and finely ground product was produced from discarded animal parts from slaughter-houses and fallen stock.

Two reactor experiments were performed:

Experiment one: During this experiment, methane production was the only parameter used for indication of process stability. Samples for determination of the VFA concentrations in the reactors were taken on a daily basis. The samples were acidified with $30\,\mu l$ 17% phosphoric acid per ml, frozen at $-20\,^\circ C$ and not measured until the end of the experiment. The outline of the reactor experiment is given in Fig. 1. Two 4.5 l continuously stirred tank reactors (CSTR) with a working volume of 3.0 l [20] were used. One reactor functioned as a control reactor and the other, R1, as a test reactor. Both reactors were stirred by a propeller every third minute for 1 min at 100 rpm and operated at $53\,^\circ C$ with a HRT of 15 days. The reactors were inoculated with cattle manure that which had been digested in a stable lab-scale reactor at $53\,^\circ C$ and 17 days HRT. During start-up, the reactors were fed with 100 ml manure mixture per day. Full loading was applied from day 17 corresponding to $200\,ml\,d^{-1}$ and 2.3–2.5 g volatile solids $Ldigester^{-1}\,d^{-1}$. Both 1% (w vol^{-1}) MBM and 1% lipid mixture were added to the feedstock of R1 when the methane production had been stable for 1 week (day 29). At day 49 the addition of MBM and lipid mixture was increased to 2% of each substrate, and at day 66 both substrates were omitted from the feedstock.

Experiment two: Experiment one functioned as a template for experiment two. Thus, experiment two was a repetition of experiment one with the exception that propionate was used as the only parameter for indication of process stability. Samples for determination of VFA were taken and analyzed on a daily basis. Before start-up, the content of R1 was discarded and the reactor was re-inoculated with the same material as used in experiment one (Fig. 1). The reactor was named R2. The operation of the control reactor was continued as previously. Start-up, initiation of full loading and addition of 1% MBM and 1% lipid mixture were performed in the same manner as in experiment one. However, at day 50, MBM was omitted from the feedstock of R2 and at day 63, the lipid mixture was also omitted. At day 68 0.5% MBM and 0.5% lipid were added the feedstock. The motivations for the decisions concerning the loading of the reactor are discussed later.

2.3.1. Analytical methods

Total solids (TS), Volatile solids (VS), pH and ammonium content were determined using standard methods [21]. CH_4 production from the batch experiments was measured by gas chromatography using flame ionization detection. CH_4 and CO_2 production from the reactors and VFA were determined by gas chromatography using thermal conductivity detection. For manual VFA determination samples of approximately 15–20 ml of digested manure were withdrawn the reactors. A volume of $2 \times 1\,ml$ of the reactor content was acidified with $30\,\mu l$ 17% phosphoric acid, centrifuged at 10,500 rpm for 20 min, and analyzed on a gas chromatograph equipped with a flame ionization detector.

3. Results and discussion

3.1. Characterization of process parameters at different full-scale biogas plants

The VFA concentrations in the eight different full-scale biogas plants are given in Table 1. The concentrations varied from 1.4 to 22.8 mM for acetate, 0.7–12.6 mM for propionate, 0.1–3.5 mM for isobutyrate, and 0.2–0.6 mM for butyrate. The range of the measured SMA was 4.9–204.3 $\mu mol\,g\,VS^{-1}\,h^{-1}$ for acetate, 9.3–28.6 $\mu mol\,g\,VS^{-1}\,h^{-1}$ for H_2/CO_2 and 0.5–12.8 $\mu mol\,g\,VS^{-1}\,h^{-1}$ for the control vials. In a previous experiment the SMA in three other centralized thermophilic biogas plants in Denmark was found to be 42–625 $\mu mol\,g\,VS^{-1}\,h^{-1}$ for
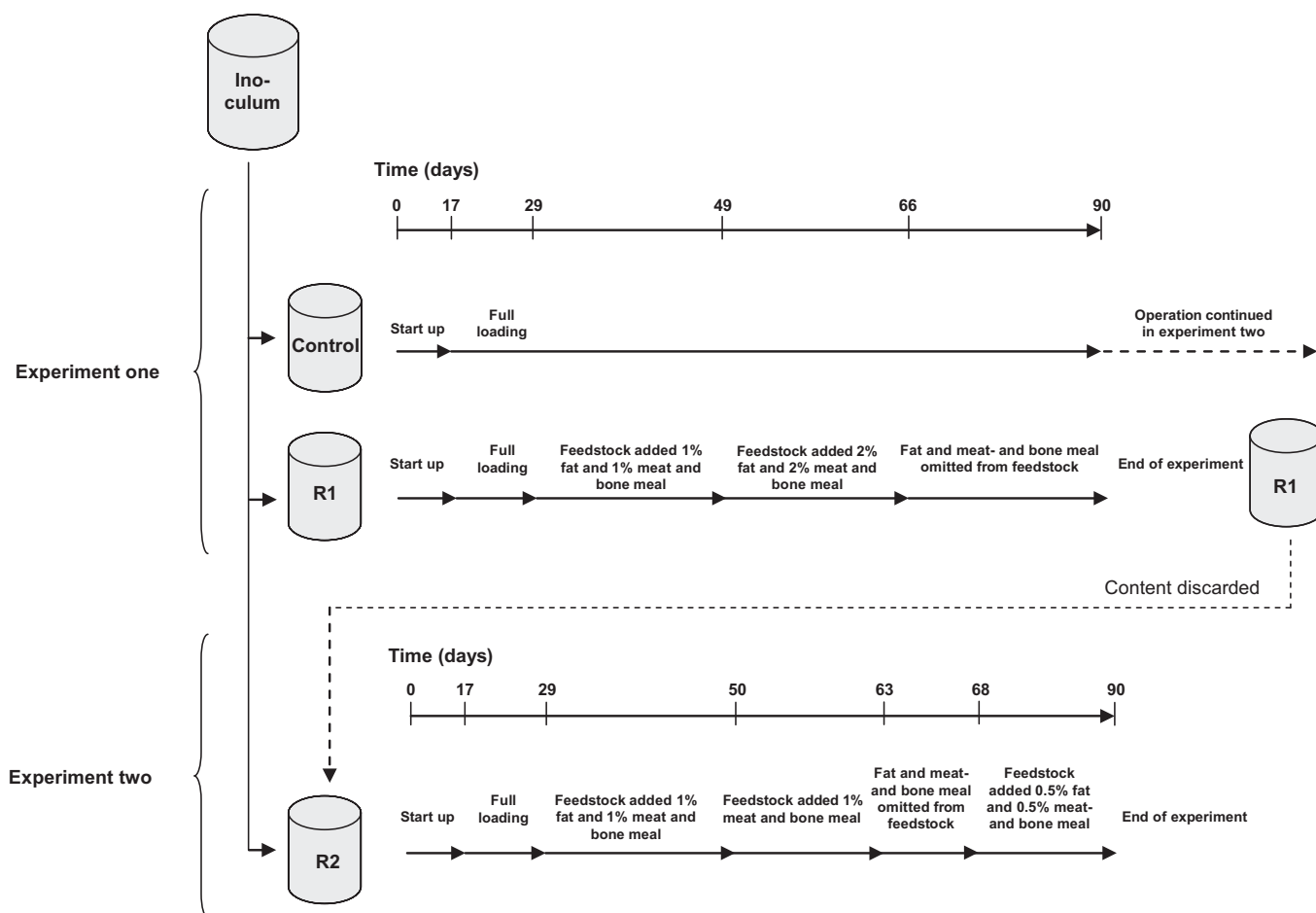
Fig. 1. Lab-scale reactor setup and operation.

Table 1
VFA levels, specific methanogenic activity (SMA) and rest potential of reactor samples from 8 full-scale biogas plants in Denmark

	VFA concentration						SMA		
	Ac., mM	Pro., mM	Isob., mM	But., mM	Total VFA, $g\,l^{-1}$	CH_4 potential, $l\,l^{-1}$	Acetate, $\mu mol\,g\,VS^{-1}\,h^{-1}$	H_2/CO_2, $\mu mol\,g\,VS^{-1}\,h^{-1}$	Control, $\mu mol\,gVS^{-1}\,h^{-1}$
Lintrup	22.8	3.7	0.5	0.4	1.64	11.9 ± 0.2	10.5 ± 1.7	10.2 ± 3.8	5.0 ± 1.4
Studsgaard	12.3	1.1	0.3	0.3	0.84	9.6 ± 0.1	7.8	17.2 ± 7.4	3.1 ± 2.5
Lemvig	8.4	1.6	0.2	0.2	0.62	5.7 ± 0.3	60.6 ± 6.3	9.3 ± 3.8	3.4 ± 1.3
Thorsø	6.9	2.5	0.2	0.2	0.59	3.8 ± 0.1	7.9 ± 1.1	28.6 ± 2.7	3.1 ± 0.6
Sinding	5.6	1.1	0.1	0.2	0.42	6.1 ± 0.3	204.3 ± 7.4	16.2 ± 5.7	8.45
Århus	1.4	0.7	0.1	0.2	0.14	1.7 ± 0.1	4.9 ± 1.0	17.2	2.4 ± 0.8
Vaarst	9.7	12.6	0.5	0.6	1.40	5.4 ± 0.1	11.6 ± 3.8	13.4	0.5 ± 0.3
Filskov	9.1	9.7	3.5	0.2	1.35	–	102.1 ± 3.3	25.6 ± 1.2	12.8 ± 3.0

Values are given as triplicate with standard deviations ($\pm$).

acetate, 103–199 for $H_2/CO_2\,\mu mol\,g\,VS^{-1}\,h^{-1}$ and 54–169 $\mu mol\,g\,VS^{-1}\,h^{-1}$ for the control series [23]. The VFA concentrations were in the same range as in the present experiment. The samples in the previous experiment had been handled in the same manner as in the present experiment and the data show that the biomass activity

of different biogas plants differs significantly. The methane potential of the reactor effluents was in the range from 1.7 to $11.9\,l\,l^{-1}$. The high biogas potential of the effluent of the reactors in Lintrup and Studsgaard was a sign of low efficiency of the process. However, no correlation between the VFA level, the biomass activity and the rest potential
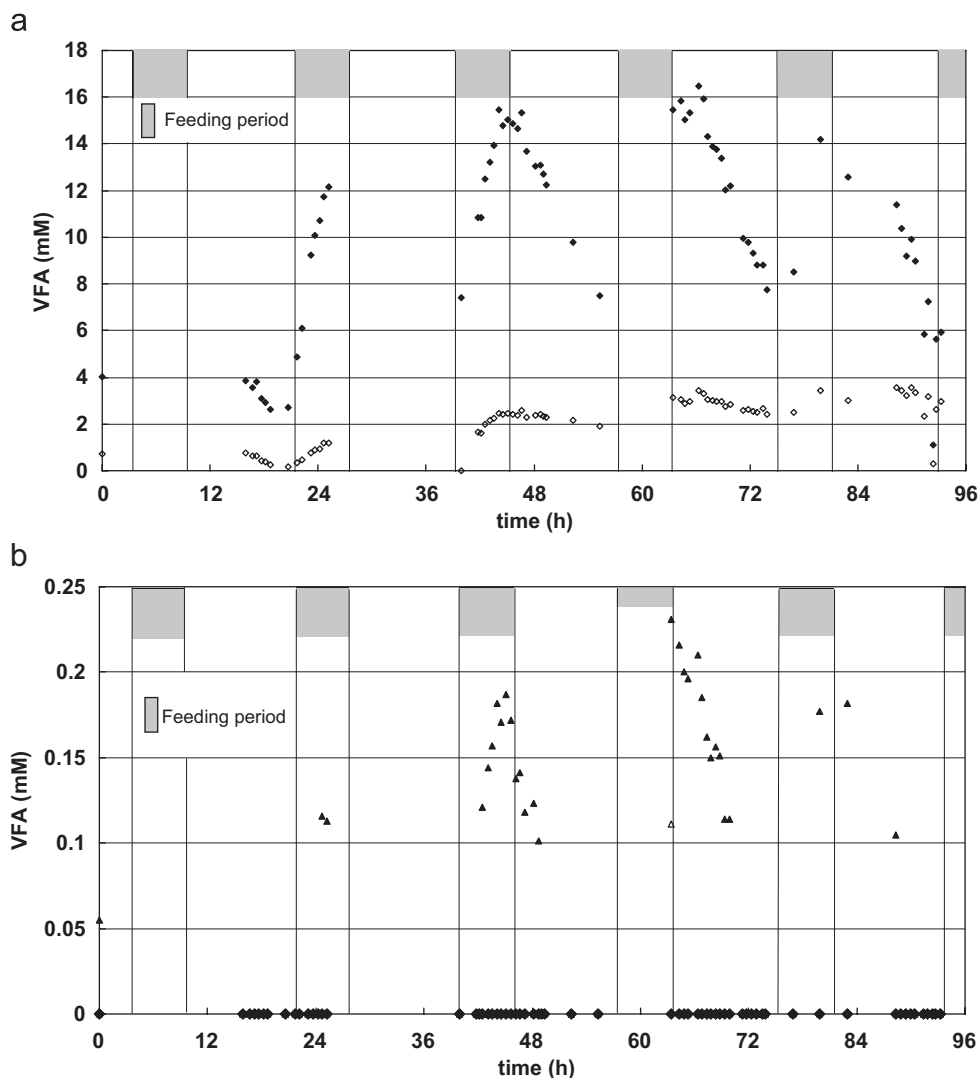
a

b

Fig. 2. VFA online monitoring at the Lintrup biogas plant. VFA dynamics during normal operation. (a) ♦: acetate, ◇: propionate; (b) ♦: isobutyrate, ◇: butyrate, ▲: isovalerate, △: valerate.

could be found. This shows that random samples only give a limited characterization of the state of the biogas process.

3.2. Online VFA measurement at a full-scale biogas plant

According to specifications from Lintrup biogas plant, the biogas potential of the reactor effluent represented approximately 38% of the total methane production of the plant at the time when the sample was taken. Due to the high biogas potential of the effluent together with the low SMA and the relatively high VFA concentrations in the reactors it was decided to install the online VFA sensor at Lintrup biogas plant. In contrast to the random samples, which only gave a momentary value of the VFA level, the online measurement gave a detailed profile of the VFA dynamics in the biogas reactor.

VFA levels at normal operation: The dynamics of the VFA during normal process operation with a reactor

feeding every 12 h for 6 h are given in Fig. 2. The figure shows that the acetate concentration increased significantly during the feeding periods. After the feeding of the reactor had stopped, the acetate concentration could still be high for up to 2 h, but the concentration generally dropped, during the 12 h when the reactor was not fed, to about the same level as before feeding. The fluctuations in the propionate concentration before and after the feeding were more moderate than for acetate. The average level of acetate and propionate showed a slight increase during the week (0–96) because "fresh" substrate was fed to the reactor while stored substrate was fed during the weekend and at the beginning of the week.

The concentrations of butyrate and valerate were except for the isoform of valerate below detection limits for the non-disturbed process. The concentration of iso-valerate was generally much lower than acetate and propionate, but showed the same dynamics as acetate according to the
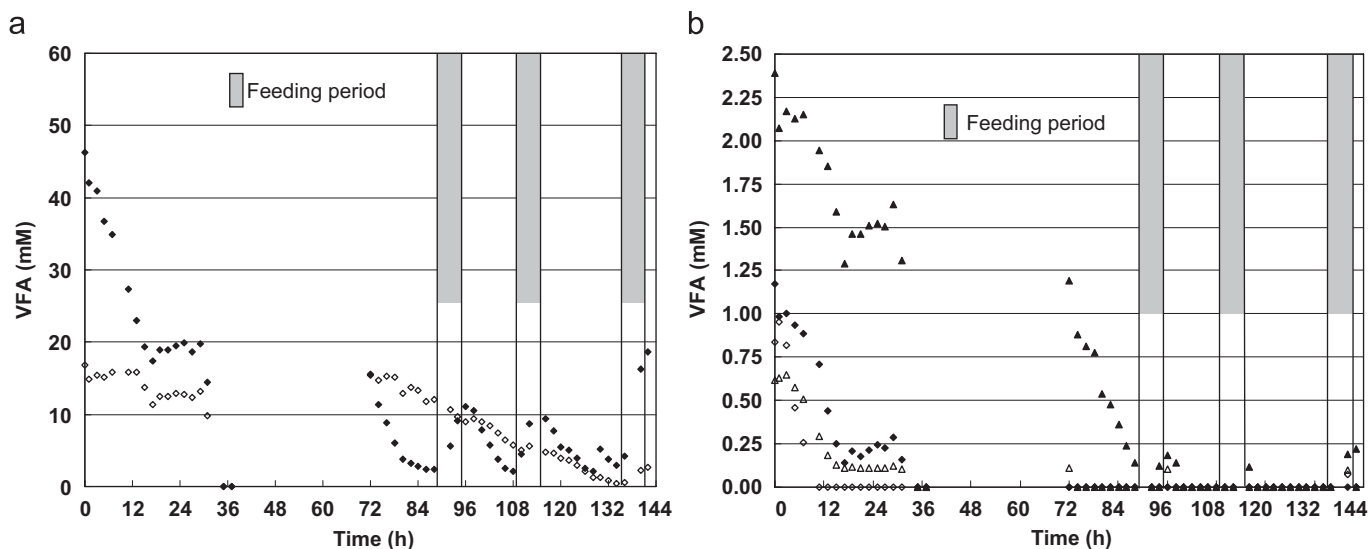
Fig. 3. VFA online monitoring at the Lintrup biogas plant. VFA dynamics during minor (a + b) process imbalances. (a) ◆: acetate, ◇: propionate; (b) ◆: isobutyrate, ◇: butyrate, ▲: isovalerate, △: valerate.

Table 2
Components of the manure mixture, lipid mixture and meat- and bone meal (MBM) used as feedstock in the lab-scale reactor experiments

	TS, %	VS, %	Total-N, mg g^{-1}	Ammonia-N, mg g^{-1}	Methane potential, ml g VS^{-1}
Manure mixture	4.54 ± 0.15	3.52 ± 0.13	4.5 ± 0.2	2.73 ± 0.1	398 ± 20.3
Lipid mixture	99.6 ± 0.03	99.6 ± 0.03	–	–	1145 ± 22.9[a]
MBM	95.9 ± 0.04	66.8 ± 0.27	95.4 ± 4.8	5.7 ± 0.2	534 ± 10.8

Values are given as triplicates with standard deviations (±).
[a] The theoretical methane yield of lipids is 1014 ml g VS^{-1} [22].

feeding periods. From the VFA pattern mainly five key values could be identified for indication of the process balance:

- The peak acetate concentration at the end of the feeding period (12–16 mM).
- The rate of change in acetate concentration after terminating the feed (−0.84 to −0.74 mM h^{-1}).
- The lowest acetate concentration before start of a new feeding period (2–4 mM).
- The average propionate concentration over the whole period (0.6–2.9 mM).
- The rate of change in propionate concentration after terminating the feed (−0.08 to 0.05 mM h^{-1}).

VFA levels following a process imbalance: The VFA concentrations following the process disturbance due to accidental overload of industrial waste are shown in Fig. 3. The incidents were characterized by a significant increase in all VFA concentrations. The biogas production decreased as well, but the data could not be obtained from the plant. The process disturbance recovered after 4 days of not feeding the reactor and was classified as a minor imbalance. The disturbance was reflected in the following VFA concentrations: acetate > 45 mM, propionate > 15 mM,

iso-valerate > 2.0 mM, iso-butyrate > 1.0 mM, butyrate > 0.75 mM, and valerate > 0.5 mM. Due to technical problems, no VFA measurement was performed from hour 36 to 72, but still a rather clear pattern of the VFA dynamics following the imbalance was observed. While the levels of acetate, iso-valerate, iso-butyrate, butyrate and valerate decreased following the first 12 h of not feeding the reactor, the propionate concentration stayed almost unchanged at 15 mM for more than 3 days. When regular feeding of the reactor was restarted after 3 days, the acetate concentration followed the same "steady-state" dynamics as seen in Fig. 2 and the concentration of butyrate, iso-butyrate, valerate and iso-valerate fell below detection limits. However, the decrease in the propionate concentration was much slower and rather independent of the feeding periods and it took 2 more days until the propionate concentration was below 5 mM. Therefore, the level and dynamics of the propionate concentration could best describe the normalizing of the process.

3.3. Regulation an optimization of the biogas process in lab-scale reactors

The composition of the various substrates with regard to TS and VS, total-N and ammonia-N content and methane

potential is given in Table 2. The methane potential of the substrates was determined in another experiment, by anaerobic degradation in batch vials [16]. The reactor performances, pH and VFA dynamics of the lab-scale reactors are illustrated in Figs. 4 and 5.

Experiment one: A stable process was obtained for R1 and the control reactor shortly after start-up, and from days 25 to 29 the performance of the reactors was between 256 and 272 ml g VS^{-1} corresponding to 0.62–0.65 LCH$_4$ Ldigester^{-1} d^{-1}. During the rest of the experiment the performance of control reactor remained stable at a level of 243–284 ml g VS^{-1}. An immediate steep increase in the methane production in R1 was observed from day 29 when the feedstock of the reactor was supplemented with 1% MBM and 1% lipid mixture. The production showed a peak at day 38, followed by a slight decrease and stabilization from days 43 to 49. Based on the stable methane production from days 43 to 49 the concentration of MBM and the lipid mixture was increased to 2% in order to increase the methane production. The methane production increased to a maximum of 1.43 LCH$_4$ Ldigester^{-1} d^{-1} at day 58 but the peak was followed by a rapid decrease and at day 66, the methane production in R1 was lower than in the control reactor despite the increased loading. Because of the process breakdown MBM and the lipid mixture were excluded from the lipid mixture from day 66, but the methane production rate continued to decrease until day 69 where it stabilized at 0.25 l digester^{-1} d^{-1}. No improvement in the methane production was observed before day 76, and it took 24 days before the production in R1 had reached the level of the control reactor (day 90).

At Danish full-scale biogas the operation of the plants is based on a "trial and error" strategy and the experience of the plant operator. In practice, this means that a random amount of organic matter is added the reactors and the biogas production is subsequently followed. The results of experiment one clearly demonstrate that this strategy is very vulnerable with regard to the indication of process imbalances. This was in particular illustrated from days 43–49. During that period the methane production of R1 showed a stabilization following a minor peak giving no indication of a process imbalance, while the increase in VFA concentration indicated the initiation of a an imbalance. As a consequence of the inadequate process control a further increase in the loading of the reactor was performed, resulting in an organic overloading and a breakdown of the process. The VFA concentration continued to increase significantly from day 49, but any indication of process imbalance by the methane production was not observed until approximately days 61–62, which was too late to prevent the process failure. The indication of the process imbalance by propionate occurred 12–18 days before any indication was given by the methane production.

Experiment one also demonstrated that pH is unsuitable as a single parameter for indication of process imbalance in reactors treating manure. Before the breakdown pH was stable and between 7.82 and 7.92, but during the breakdown no changes were observed until day 65 where pH started to drop until it reached a level of 7.57 at day 76 (Fig. 5a). Furthermore, it was demonstrated that the propionate/acetate relationship could not be used for indication of process imbalances since the propionate/acetate ratio during the breakdown was well below the limit of 1.4 suggested by Hill et al. [7] (Fig. 5c).

Experiment two: The performance of the control reactor during the second experiment is not illustrated in Figs. 4 and 5 but during the entire experiment the process was stable with a methane production of 250–280 ml g VS^{-1} and acetate and propionate concentrations of 13–20 and 4.1–6.2 mM, respectively.

During start-up and following the initiation of full loading, the performance of R2 was very similar to the performances of R1 and the control reactor in experiment one. The reaction pattern of R2 following the addition of 1% MBM and 1% lipid mixture to the feedstock was also the same as in R1. The increase in acetate and propionate concentrations shortly after the addition of 1% MBM and 1% lipid mixture was expected and reflected an imbalance between the acid-producing and acid-consuming microorganisms as a result of an increased hydrolysis/fermentation and possibly a short-term increase in H$_2$ partial pressure, a pattern that previously has been observed when the organic loading rate of a reactor has been rapidly increased [5]. Therefore, no changes in the operation procedure were made. However, the sudden increase in propionate concentration from days 47–50 was interpreted as an indication of a potential process imbalance because no changes in reactor operation were made during that period. At day 50, the ammonia-free ammonia concentration in the reactor was 0.66 g-N l^{-1} which according to Angelidaki and Ahring [11] is close to the inhibitory level, and MBM was omitted from the feedstock, in order to avoid a further increase in the ammonia level. As expected, the lower organic loading rate resulted in a lower methane production rate, but the methane yield and utilization rate stayed in the same range. The acetate concentration that also had increased from day 47 showed stabilization from days 50 to 62, indicating process stability. However, propionate concentration continued to increase and gave the opposite signal as the acetate concentration. Therefore, in order to avoid a breakdown of the process, as seen in experiment one, the lipid mixture was also excluded from the feedstock from day 63. This operation terminated the increase in propionate concentration and also resulted in a drop in acetate concentration. The methane production rate decreased further and the methane yield decreased as well due to the exclusion of the easy degradable lipid mixture. From day 68, 0.5% MBM and 0.5% lipid mixture were added into the feedstock in order to increase the methane production. This resulted in an immediate increase in acetate concentration which could give an impression of an impending imbalance. However, no
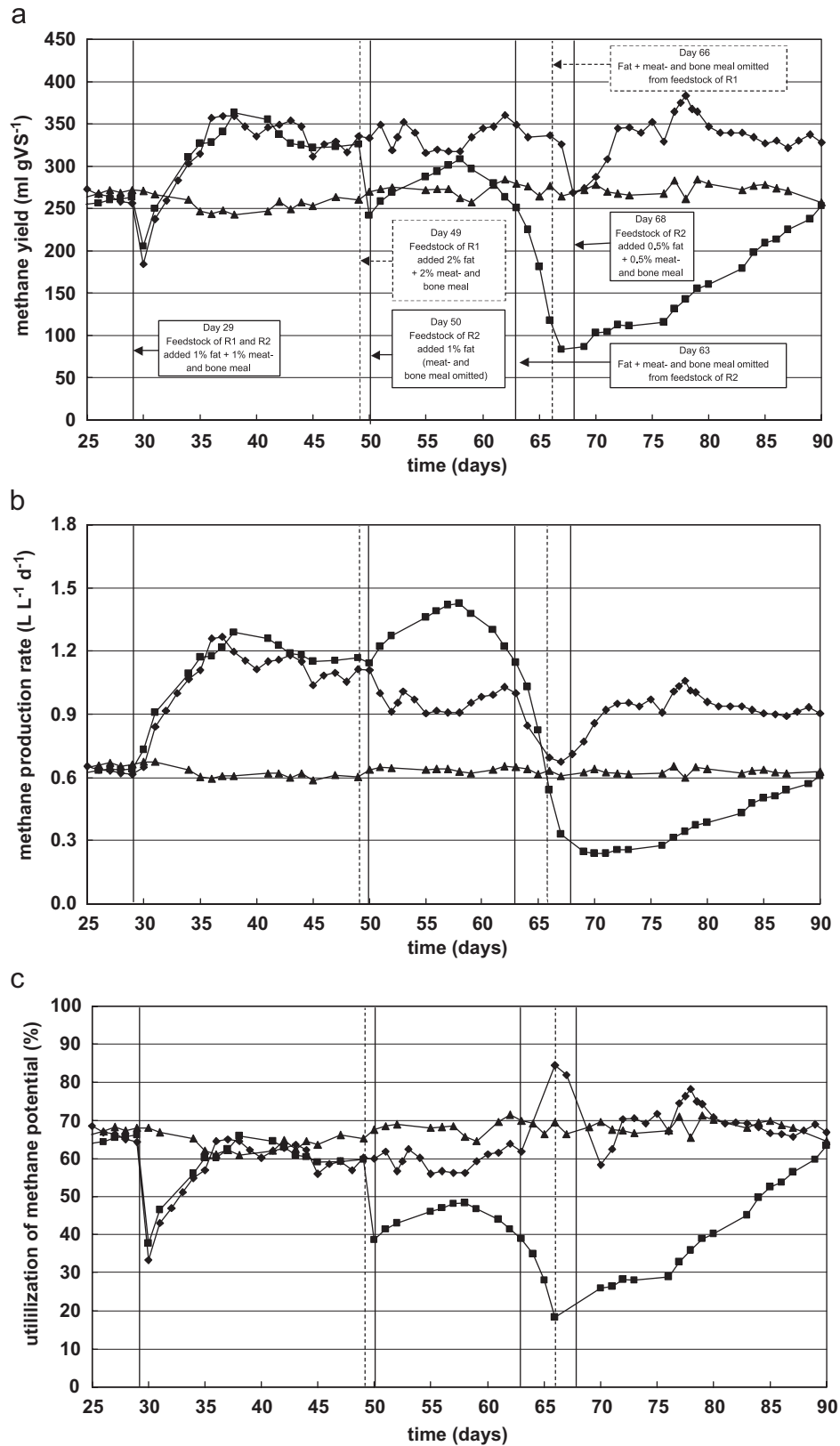
Fig. 4. Lab-scale reactor experiments. Reactor performances during start-up, full loading and addition of MBM and lipids. ■: R1, ◆: R2, ▲: the control reactor. (a) Methane yield; (b) methane production rate; (c) utilization rate of the biogas potential of the substrate. For calculation of the utilization rate during periods where lipid was added, the theoretical methane potential of lipids ($1014\,\mathrm{ml\,g\,VS^{-1}}$) was applied.
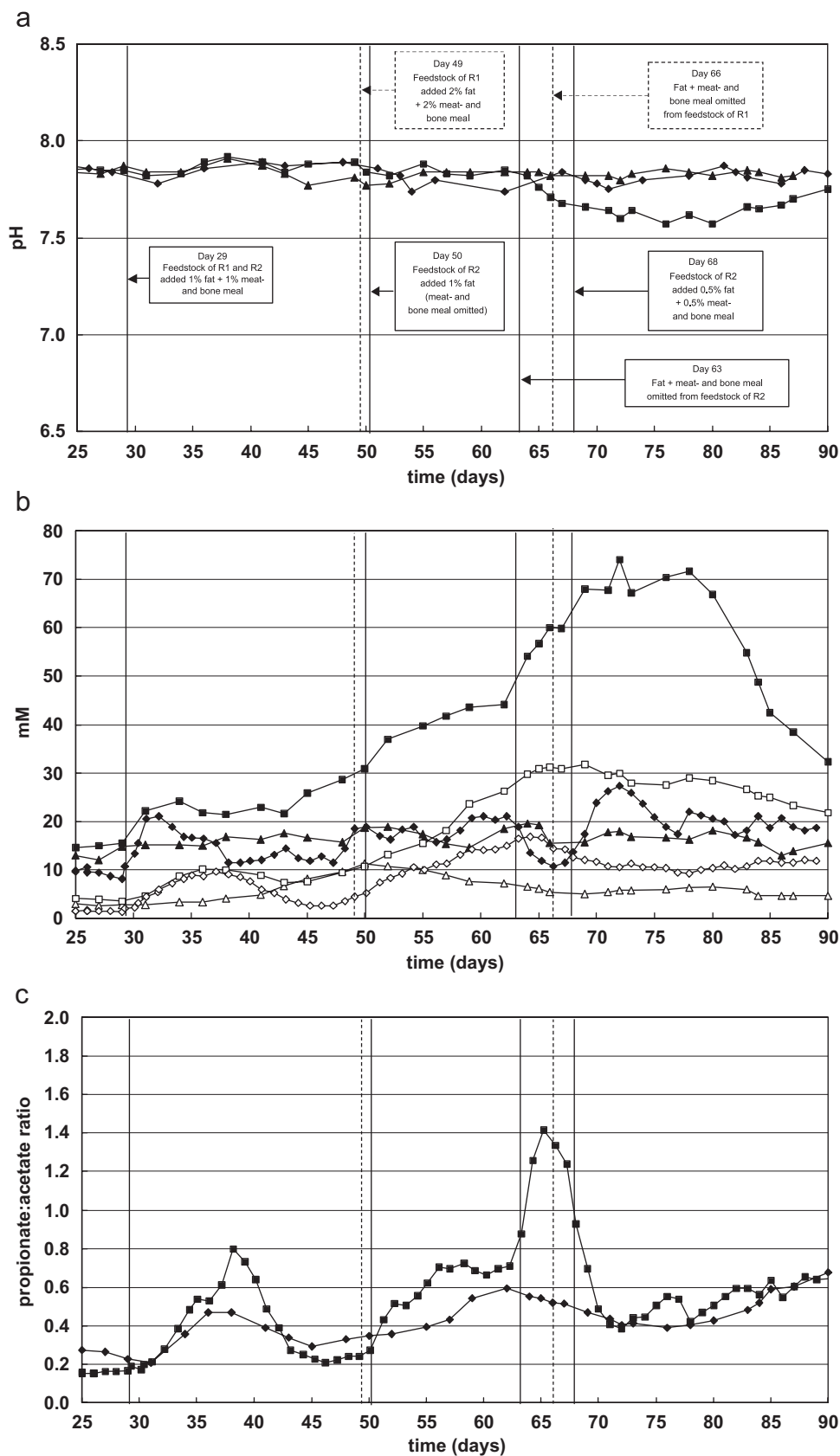
Fig. 5. Lab-scale reactor experiments. pH and dynamics of acetate and propionate during start-up, full loading and addition of MBM and lipids. (a) ■: pH in R1, ◆: pH in R2, ▲: pH in the control reactor; (b) ■: acetate concentration in R1, □: propionate concentration in R1, ◆: acetate concentration in R2, ◇: propionate concentration in R2, ▲: acetate concentration in the control reactor, △: propionate concentration in the control reactor; (c) ■: propionate:acetate ratio in R1, ◆: propionate:acetate ratio in R2.

Table 3
Total methane production in liters in R1 and R2 during the different operation periods

Period	Reactor		
	R1	R2	Control
Days 25–29	9.5	9.5	9.9
Days 30–49	68.8	64.8	37.1
Days 50–64	58.0	43.5	28.7
Days 65–90	29.8	67.8	46.8

changes were observed in the propionate concentration and from day 69 to the end of the experiments the concentration remained stable. The methane production of the reactor increased and from days 72 to 90 the methane production rate and methane yield were the same as during the period when 1% lipid was added into the feedstock. However, the utilization rate of the substrate was considerably higher than during any other period where MBM or lipid mixture was added.

In contrast to experiment one, a process imbalance was effectively avoided in experiment two because of the precise warning by propionate from days 47–63. Furthermore, when comparing the reactor performances of R1 and R2, the results show that propionate in general proved to be a good parameter for efficient regulation of the biogas process. During the first 50 days of the experiment, only small differences were observed in the methane production rate between R1 and R2 (see Fig. 4 and Table 3). From days 50 to 64, the methane production rate was significantly higher in R1 than in R2 because of the higher organic loading rate. However, the process in R1 was very ineffective and the methane yield and utilization rate was significantly higher in R2. The methane production rate, the methane yield and the utilization rate were highest in R2 from days 65 to 90 because of the breakdown of the process in R1 and the total methane production from days 50 to 90 was 27% higher in R2 than in R1. Thus, when comparing experiment one with experiment two as a whole, the digestion process was more stable and more efficient in R2 because of a more accurate regulation of the reactor operation.

Improving the strategy: In order to obtain a simple strategy for safe operation of Danish biogas plants, the potential of using either methane or propionate as single control parameters was examined in the present study. Propionate turned out to be the most reliable single parameter. However, for an improvement of the strategy, other parameters, especially hydrogen, should be considered. The thermodynamic conditions for the conversion of VFA can be calculated, by combining measurement of hydrogen with measurement of biogas production (CH_4 and CO_2), VFA and pH, Such analysis would result in an improved understanding of the biogas process during process imbalance. With respect to the results of the present study, thermodynamic analysis would have shown

whether the increase in propionate concentration, in experiment two from days 47 to 63, was caused by a thermodynamically inhibition of the propionate oxidation (caused by an increase in hydrogen partial pressure) or a kinetic inhibition of the propionate oxidizing bacteria. To our knowledge no control strategy including thermodynamic considerations has been presented for manure based CSTRs.

4. Conclusions

Online VFA monitoring at the Lintrup biogas plant enabled characterization of the specific process dynamics during stable and unstable operation. Following the imbalance caused by an accidental overload of the reactors with industrial waste, the concentration level and dynamics of propionate could best describe the renormalization of the process. In the lab-scale experiment, where two CSTRs were fed with a mixture of cattle and pig manure together with various concentrations of MBM and lipids, it was demonstrated that propionate is a key parameter for (1) indication of process imbalances in biogas plants treating complex organic waste and (2) for regulation and optimization of the biogas process. The results also showed that the methane production, pH or the propionate:acetate ratio cannot be used as a single reliable parameter for indication of process imbalances in biogas plants treating manure together with industrial waste.

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