Originally published as:

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Early warning indicators for process failure due to organic overloading by rapeseed oil in one-stage continuously stirred tank reactor, sewage sludge and waste digesters

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ABSTRACT

Early warning indicators for process failures were investigated to develop a reliable method to increase the production efficiency of biogas plants. Organic overloads by the excessive addition of rapeseed oil were used to provoke the decrease in the gas production rate. Besides typical monitoring parameters, as pH, methane and hydrogen contents, biogas production rate and concentrations of fatty acids; carbon dioxide content, concentrations of calcium and phosphate were monitored. The concentration ratio of volatile fatty acids to calcium acted as an early warning indicator (EWI-VFA/Ca). The EWI-VFA/Ca always clearly and reliably indicated a process imbalance by exhibiting a two- to threefold increase 3 to 7 days before the process failure occurred. At this time, it was still possible to take countermeasures successfully. Furthermore, increases in phosphate concentration and in the concentration ratio of phosphate to calcium also indicated a process failure, in some cases, even earlier than the EWI-VFA/Ca.

Keywords
Anaerobic digestion, early warning indicator, process stability, over-acidification

List of abbreviations
CaO   calcium oxide
Ca   calcium
EWI   early warning indicator
LCFA   long chain fatty acid
OLR   organic loading rate
PAO   phosphate accumulating organism
PO_4 phosphate
VFA   volatile fatty acids
VS   volatile solids

1.   Introduction

Biogas formation is a complex process that depends on a large number of interactive factors. Despite many decades of research, many aspects are not completely understood. Therefore, full-scale biogas reactors are still regarded as "black boxes" and are often operated at suboptimal organic loading rates (OLRs) to prevent process failures. Hence, one-stage continuously stirred tank reactors in commercial waste treatment plants are typically operated at OLRs of 1 to 4.5 kg VS m^{-3} d^{-1} (Bischofsberger et al. 2005, Ahmad et al. 2011, FNR 2005). Developing a comprehensive understanding of this process is the key to optimize the reliability of the plant performance and the economy. It will help to increase the eco-balance of the entire process, because the failure risks will be decreased considerably.

Process failures, such as the excess accumulation of fatty acids, can be provoked by organic overloads as well as by different inhibitors (e.g. heavy metals, sulfides, ammonia and other toxic substances) (Pender et al. 2004, Tada et al. 2005, Chen et al. 2008, Braun et al. 1981, Karakashev et al. 2005). When an inhibitor exceeds its critical concentration, typically the methanogens are inhibited first. This results in an accumulation of acetic acid, an increase in the hydrogen partial pressure and a decrease in the methane content. If the hydrogen partial pressure exceeds 0.1 mbar (Haper and Pohland 1986), then acetogenic bacteria are inhibited which leads to an accumulation of propionic acid. Propionic acid further inhibits the methanogens. Due to the inhibition of both acetogenesis and methanogenesis, the products of the hydrolysis and acidogenesis (e. g. long and short chain fatty acids) begin accumulating. Hence, the pH and the gas production rate decrease further. This process failure will subsequently be called "over-acidification" in this article.

Process monitoring parameters, such as the biogas production rate and the gas composition, are measured at 60 to 70 % of all full-scale biogas plants in Germany. By the time the parameters indicate a process failure, it is often too late to stabilize the process efficiently (Schüsseler 2008). In full-scale plants, the fatty acid concentration is primarily monitored after a process failure, while the hydrogen partial pressure is not monitored at all. A survey of 400 full-scale biogas plants in Germany revealed that the redox potential was determined in the reactor only in two biogas plants (Weiland 2008).

In several studies, various parameters (e.g. the redox potential, the volatile fatty acid (VFA) concentration, the hydrogen partial pressure and the ratio of volatile fatty acids to the total amount of inorganic carbon (determined by titration and referred to as the FOS/TAC value)) have been investigated in search of a suitable early warning indicator for process failure due to the accumulation of VFAs (Allmann et al. 2007, Boe 2006, Chynoweth et al. 1994, Pind et al. 2003, Prechtel et al. 2006, Rieger and Weiland 2006). In each case, either the parameters were not suitable as an early warning indicator or they required uniform conditions in the substrate matrix and feed to allow for a reliable interpretation. Because the substrate matrix of most waste digesters varies widely in its composition, the methane content is quite variable due to fluctuations in the energy content of the substrate mix, thereby making it difficult to detect a decrease in the methane yield due to an imminent process failure.

The aim of this work was to develop universal early warning indicators (EWIs) that are reliable, easy to measure and allow sufficient time to undertake the appropriate countermeasures successfully.

In previous experiments, the addition of calcium by the supply of CaO contributed significantly to the recovery of a biogas formation process after an excess accumulation of fatty acids, and this concept was identified as an efficient measure to stabilize the biogas formation processes (Kleyböcker et al. 2012). The calcium formed insoluble salts with long chain fatty acids (LCFAs), and the amount of phosphate that was very likely released from the phosphate accumulating organisms (PAOs) during the VFA uptake after their accumulation. The precipitates aggregated with the microorganisms. These aggregates provided more favorable conditions for the acetogenesis and the methanogenesis. In addition, LCFAs seemed to adsorb on the surface. Thus, the fatty acid concentration decreased significantly, and the biogas formation process recovered. From this concept, it follows that the decrease in calcium concentration may indicate the beginning of a process failure due to the accumulation of VFAs and the subsequent calcium precipitation with phosphates and LCFAs. Taking into account the nature of VFAs as process indicators, the ratio of VFAs to dissolved calcium was chosen to investigate its applicability as an EWI. Furthermore, the phosphate concentration was monitored because the amount of phosphate released by the PAOs during the accumulation of fatty acids may also serve as an EWI.

2.   Materials and Methods

Altogether, nine experiments (OA1 to OA9) were conducted to provoke process failures and to study the process recoveries. The OLR was increased until the VFA concentration increased and the biogas production rate decreased. In experiments OA7 to OA9, CaO was added to prevent the biogas formation process from a threatening failure.

2.1. *Laboratory-scale biogas digesters*

Each reactor contained 23 L of sludge (Fig. 1 in Kleyböcker et al. 2012). The reactors were maintained at 50 °C by a thermostat (Thermo Haake B7, Phoenix II) connected to a heating coil that was placed around the reactor. The sludge was mixed pneumatically using biogas at a flow rate of 150 L h^{-1} each day for 15 min before the samples were withdrawn and 15 min after the substrate was introduced. For the biogas recirculation, a KNF N86KTE membrane vacuum pump was used. For substrates, sewage sludge and rapeseed oil were used and were charged manually every day. The sewage sludge consisted of excess and primary sludge from a wastewater management plant applying EBPR (enhanced biological phosphorus removal). Rapeseed oil was chosen as a co-substrate due to its high LCFA contents, such as oleic acid (52-67 %), linoleic acid (16-25 %) and linolenic acid (6-15 %), and because LCFAs are frequently found in actual wastewaters (Komatsu et al. 1991). The OLR depended on the state of each experiment. While the amount of rapeseed oil varied between 0 and 9 kg VS m^{-3} d^{-1}, the daily sewage sludge load was nearly constant, with rates between 1.0 and 1.2 kg VS m^{-3} d^{-1}. The hydraulic residence time was between 20 and 23 days, depending on the OLR. The volume of the produced biogas was measured with a gas meter (Ritter TG05/5).
For the analysis of the digested sludge, samples were withdrawn at the reactor outlet. The biogas samples were withdrawn from a bypass of the gas pipe between the gas outlet and the gas wash bottle.
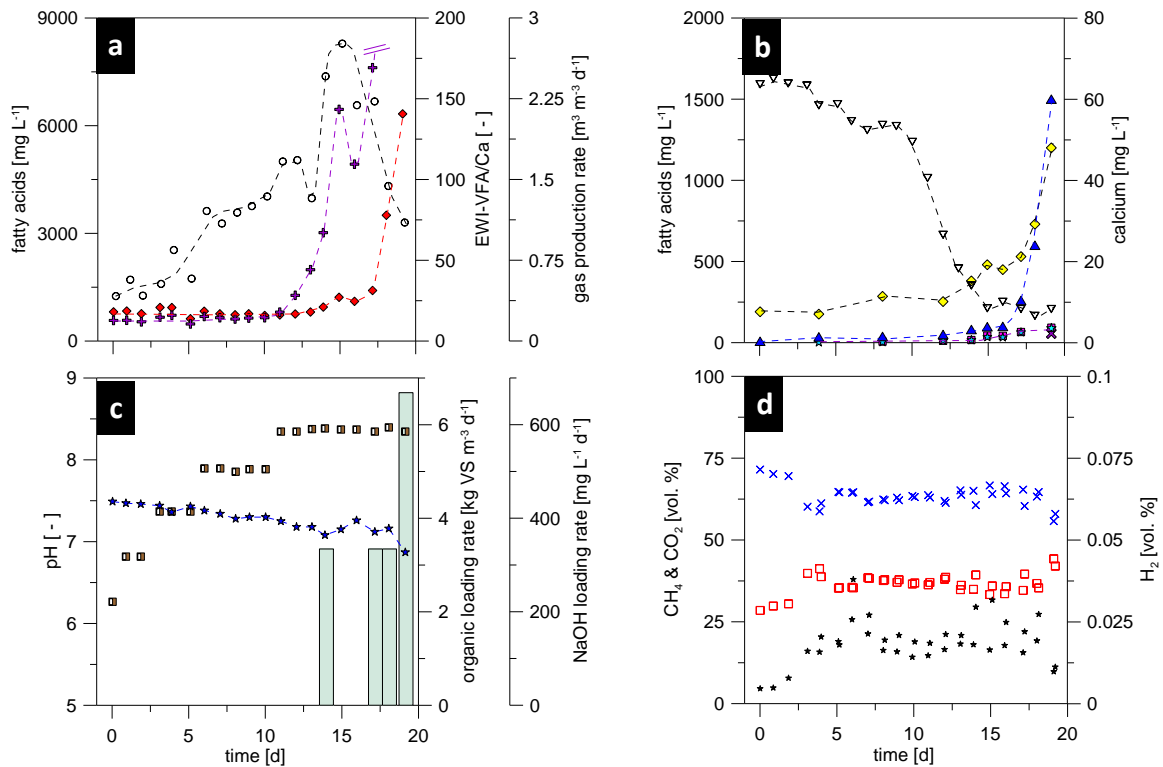
Fig. 1   Results of the experiment "with pH regulation by NaOH"

a   VFA concentration (◆), the gas production rate (O), and the EWI-VFA/Ca (✚) during an over-acidification provoked by rapeseed oil.

b   Concentration of volatile short chain fatty acids (acetic acid ◇ , propionic acid ▲ , butyric acid ■ , iso-butyric acid ✖ , valeric acid (★) and calcium (▽).

c   pH (★), organic loading rate (▣) and NaOH loading rate (▨).

d   Volume fractions of methane (✕), carbon dioxide (□) and hydrogen (★).

2.2. Wet chemical and gas analyses

The temperature and pH were measured in the digested sludge samples with a WTW pH 340i, using a Sen Tix 41 pH electrode.

For the total solids (TS) and volatile solids (VS) analyses, the samples were dried at 105 °C in a drying chamber (Memmert) for 24 h and then burned at 550 °C (Nabertherm Controller B170). The sample weights were determined by a Sartorius CP220S-OCE weighing machine. The TS and VS were analyzed according to the German guideline DIN 38409-1.

The concentrations of VFAs (LCK 365), phosphate (LCK 350) and calcium (LCK 327) in solution were determined photometrically (Hach-Lange DR2800) after the samples had been centrifuged twice at 10,000 rpm for 10 min (Eppendorf Centrifuge 5804).

The phosphate concentration was not monitored from the beginning of this study because it was found to be an important parameter only after most of the experiments had already been conducted. Therefore, the phosphate concentration was only determined punctually in certain retained reference samples.

The volatile short chain fatty acid ($C_2H_4O_2$ to $C_5H_{10}O_2$) concentrations were determined by ion chromatography (IC) equipped with an AS11-HC column (DIONEX ICS 3000, CA, USA). Sodium hydroxide was used as the eluent. The gas composition was analyzed by gas chromatography (GC) (SRI 8610C; SRI Instruments, Torrance, USA). The GC was equipped with a thermal conductivity detector, a silica gel column and a 13X mole sieve column (SRI,

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USA) using argon as the carrier gas. The measured gas components were hydrogen, oxygen, nitrogen, methane and carbon dioxide.

3.   Results and Discussion

To test the early response to a process imbalance and the predictive accuracy of the developed EWIs, process failures were provoked by organic overloads using rapeseed oil in laboratory-scale reactors. The EWI-VFA/Ca was investigated under different process conditions: (1) with pH regulation using NaOH and (2) without pH regulation, (3) while preventing a process failure by the addition of CaO, (4) at different VFA concentrations, (5) at different OLRs and (6) at different calcium concentrations. Furthermore, the concentration ratio of phosphate to calcium (EWI-PO$_4$/Ca) and the phosphate concentration itself (EWI-PO$_4$) were compared to the EWI-VFA/Ca in two experiments: "with pH regulation using NaOH" and "without pH regulation".

3.1. Provoked accumulation of fatty acids by overloading, (1) with pH regulation using NaOH, (2) without pH regulation and (3) EWI-VFA/Ca while preventing a process failure by the addition of CaO

Fig. 1 shows the EWI-VFA/Ca during the accumulation of fatty acids compared to the gas production rate, the gas composition, the OLR and the VFA composition. The OLR was increased from 2.2 kg VS m^{-3} d^{-1} by a factor of three within 11 days by adding rapeseed oil, and the pH was held constant at a neutral value by the addition of NaOH. Consequently, the gas production rate increased up to 2.8 m³ m^{-3} d^{-1} over two weeks. Within the following two days, it decreased slightly to 2.2 m³ m^{-3} d^{-1}. On day 18, it decreased rapidly by approximately 30 %. At that time, the VFA concentration increased by a factor of three. The EWI-VFA/Ca started to increase slightly on day 12, and, one day later, it had increased by a factor of three. On day 15, the EWI-VFA/Ca had already increased by a factor of eight, while the gas production rate was still increasing.

As the OLR was increased, the dissolved calcium concentration decreased (Fig 1). After day 10, when the OLR was increased by a factor of three, the calcium concentration decreased more rapidly. The accumulated short chain fatty acids mainly consisted of acetic and propionic acids. Acetic acid was the first of the short chain fatty acids to accumulate; on day 14, the acetic acid concentration increased significantly to 380 mg L^{-1}. Propionic acid accumulated by a factor of three on day 17 to a final concentration of 1500 mg L^{-1}. The concentrations of butyric, iso-butyric and valeric acid increased only slightly and did not exceed 100 mg L^{-1}.

When the OLR was increased, the methane content declined slightly, while the carbon dioxide increased (Fig. 1). On days 3 and 20, significant decreases and increases were observed at each concentration. Between days 3 and 20, the methane content varied between 59 and 67 vol. %, and the carbon dixiode concentration ranged between 33 and 41 vol. %. The hydrogen content increased significantly until day 6, with a maximum value of 0.038 vol. %. Then, it decreased during the next 4 days, followed by fluctuations between 0.01 and 0.03 vol. %.

To investigate the accumulation of fatty acids without the addition of NaOH in contrast to the previously decribed experiment, the experiment "without pH regulation" was conducted. During a "normal operation" with an OLR of 3 kg VS m^{-3} d^{-1}, the fatty acid concentration was less than 1000 mg L^{-1} and the gas production rate ranged between 3 and 4 m³ m^{-3} d^{-1} (Fig. 2).
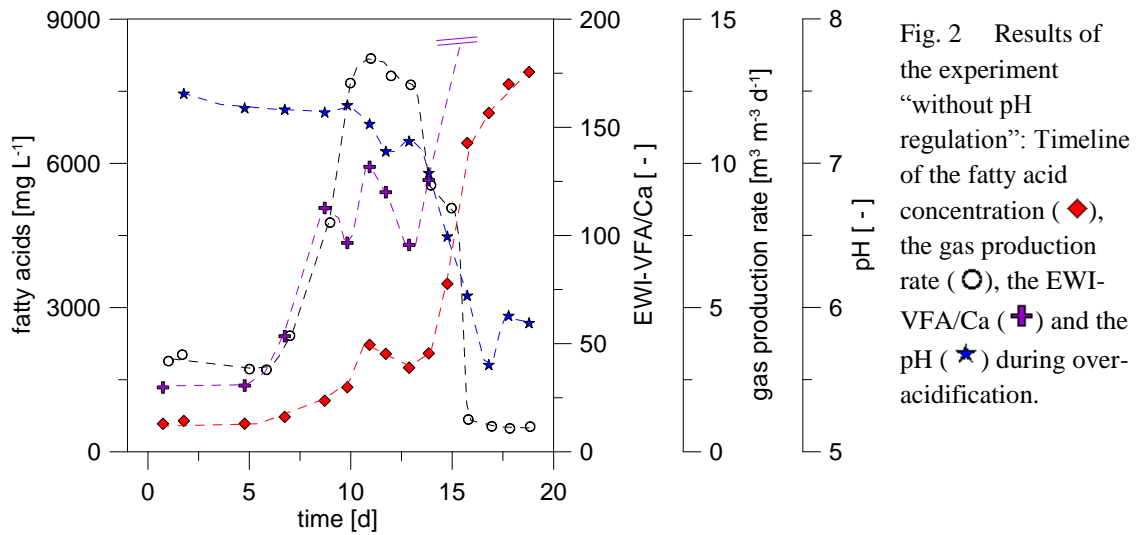
Fig. 2   Results of the experiment "without pH regulation": Timeline of the fatty acid concentration (◆), the gas production rate (○), the EWI-VFA/Ca (✚) and the pH (★) during over-acidification.
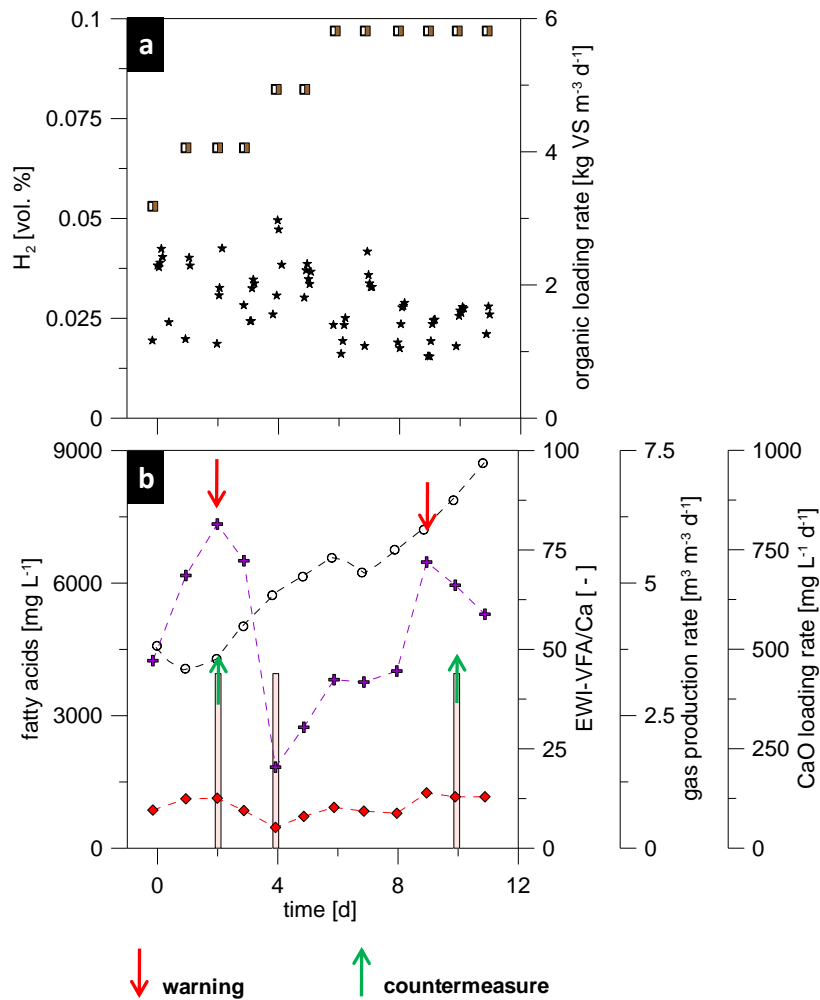

Fig. 3   Results of the experiment for "preventing a process failure by the addition of CaO".

a Hydrogen content (★) as the OLR (▣) was increased.

b Timeline of the EWI-VFA/Ca (✚) and the CaO additions (▦) as a countermeasure against a process failure as the OLR was increased. The concentration of fatty acids (VFAs) (◆) remained low and the gas production rate (○) increased.

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After the OLR was increased to 10 kg VS m^{-3} d^{-1}, the gas production rate increased to 12 m³ m^{-3} d^{-1} within 4 days, and the VFA concentration increased from 730 mg L^{-1} to 1063 mg L^{-1} on the first day. At that time, the EWI-VFA/Ca doubled from 53 to 113. The VFA concentration increased slowly, and the pH was at 7.0 or higher within the first two weeks. On day 14, the pH decreased to slightly less than 7.0 and the gas production rate decreased from 13 to 9 m^3 m^{-3} d^{-1}. The pH became more acidic and was 5.6 on day 17, while the gas production rate significantly decreased starting on day 15.

To test if the EWI-VFA/Ca provides an early enough warning to allow successful countermeasures to be undertaken, CaO was added when the EWI-VFA/Ca indicated a process imbalance (Fig. 3). The OLR was increased from 3.2 to 4.1 kg VS m^{-3} d^{-1} by increasing the daily rapeseed oil load, and the EWI-VFA/Ca doubled within 2 days. As soon as the CaO was fed, the EWI-VFA/Ca decreased, while the VFAs concentration remained low at approximately 900 mg L^{-1} and the gas production rate continued to increase. After the EWI-VFA/Ca tripled within another 5 days due to the increased OLR of 5.9 kg VS m^{-3} d^{-1}, more CaO was added. While the EWI-VFA/Ca decreased, the VFA concentration remained in the same range as before, and the gas production rate further increased to its expected level based on the OLR. During the experiment, the hydrogen content ranged between 0.015 and 0.05 vol. % and did not exhibit any distinct trends.

Although process instabilities resulting in a low methane yield and a low efficiency are often encountered in full-scale biogas plants, typical monitoring parameters, such as VFA concentration are determined only very roughly. However, the gas production rate is normally determined online at full-scale biogas plants. In the own experiments, the gas production rate typically increased due to a higher OLR before an over-acidification issue occurred. In experiments "with" and "without pH regulation", the EWI-VFA/Ca had already indicated a threatening fatty acid accumulation when the gas production rate was still increasing and was therefore not suitable as an early warning indicator. A decrease in the methane yield indicates a process failure, but its determination is time-consuming. In particular, if the substrate matrix changes daily, as it is common for waste digesters, then the methane yield varies due to the different energy content for each substrate. If the methane potential of a substrate is unknown, then it is not possible to determine the expected methane yield in time. Hence, the methane yield is less suitable to serve as a process controlling parameter. In addition, Walte et al. (2008) have reported that by the time the methane yield decreased, it was typically too late to take successful countermeasures.

In the experiment conducted "without pH regulation", the pH decreased to less than 7.0 when the EWI-VFA/Ca had already indicated an impending over-acidification since 5 days. Because the change in pH occurred much later than the change in EWI-VFA/Ca, the pH was not suitable for indicating a process imbalance at an early stage.
The own results are in agreement with the results of Anderson et al. (1982) and Weiland (2008). According to their reports, a high buffer capacity of the digested sludge (due to hydrogen carbonate and ammonium) can cause a delay in the pH decrease.

In the experiment conducted "with pH regulation", an accumulation of VFAs occurred even though the pH was held constant by the addition of NaOH. Acetic acid accumulated first due to the inhibition of the acetoclastic methanogenesis, followed by the accumulation of propionic acid due to the inhibition of the acetogens. Then butyric acid, valeric acid, and iso-butyric acid

accumulated. The concentration profiles of the short chain fatty acids indicated a process imbalance 4 days after the EWI-VFA/Ca had already shown a significant increase. In the other experiments, the EWI-VFA/Ca indicated an impending over-acidification two to six days earlier than the VFAs.

The own observation of the late VFA accumulation is different from the observations of Ahring et al. (1995). They observed that butyric acid and iso-butyric acid are particularly well-suited to be used as control parameters that indicate a process imbalance at a very early stage. Boe (2006) and Nielsen et al. (2007) reported that propionic acid could serve as an early warning indicator. Gallert and Winter (2008) also observed that propionic acid was the dominant acid and accumulated first. However, in the own experiments the increase in propionic acid did not serve as an early warning indicator.

Furthermore, Weiland et al. (2008) proposed that the concentration ratio of acetic to propionic acid would indicate a process imbalance, when it decreased to less than the critical value of one. In the own experiment, the ratio decreased to less than one on day 19, when the over-acidification was already completed and the EWI-VFA/Ca had already indicated an impending process failure 6 days before.

Marchaim and Krause (1993) found the increase of the concentration ratio of propionic to acetic acid independent of a critical value to be a satisfactory indicator for the beginning of an organic overload. In the own experiment, the ratio showed no significant increase before day 16. Moreover, Weiland et al. (2008) proposed that the critical value of propionic acid concentration was 1000 mg L^{-1}. This value was attained in the own experiment after an over-acidification was already completed, 6 days after the EWI-VFA/Ca already indicated a process imbalance.

The hydrogen content in the experiment conducted "with pH regulation using NaOH" significantly increased on day 6, which indicated a process failure, but the hydrogen content decreased afterwards. It increased again on days 14 and 15, but then decreased again, even though the system did not stabilize. When the over-acidification was completed on day 19, the hydrogen content was so low that a process failure could not be observed without including other process parameters. In the experiment conducted by "preventing a process failure by the addition of CaO", the hydrogen content ranged between 0.015 and 0.05 vol. % during the entire experiment although the process did not fail due to the introduction of CaO to the reactor. Although the process did not fail in this experiment, the hydrogen content was sometimes even higher than in the experiment previously discussed, in which the process failed. Hence, the experiment showed that the hydrogen content is not suitable as a universal early warning indicator for process failures because its concentration is clearly dependent on other circumstances.

This finding is not in agreement with the results of Prechtel et al. (2006) and Zosel et al. (2008), who used the long-term hydrogen concentration profile to successfully diagnose a process imbalance at an early stage. However, the results of Pind et al. (2003) are in agreement with the own results: they could not detect a long-term inhibition using only gaseous hydrogen as an indicator. Furthermore, they also could not stabilize the biogas formation process using the hydrogen content as a process-controlling parameter. Voolapalli and Stucky (2001) and Boe (2006) also did not recommend using hydrogen as the only monitoring parameter.

3.2. EWI-VFA/Ca at different VFA concentrations, calcium concentrations and OLRs and summary of all conducted experiments

The accurancy of the prediction of the EWI-VFA/Ca was investigated at different VFA concentrations, calcium concentrations and OLRs. In all of the experiments, the EWI-VFA/Ca at stable process conditions increased by a factor of 2 to 3 to the first warning (Fig. 4), which was given 3 to 7 days before the process failed. When the process failed the EWI-VFA/Ca had already increased by a factor of 10 to 30. The VFA concentrations in a stable biogas formation process were less than 860 mg L^{-1}, while the VFAs concentrations ranged between 830 and 1610 mg L^{-1}, when the EWI-VFA/Ca indicated a process failure warning. When the process failed, the VFA concentrations exceeded 3500 mg L^{-1}.
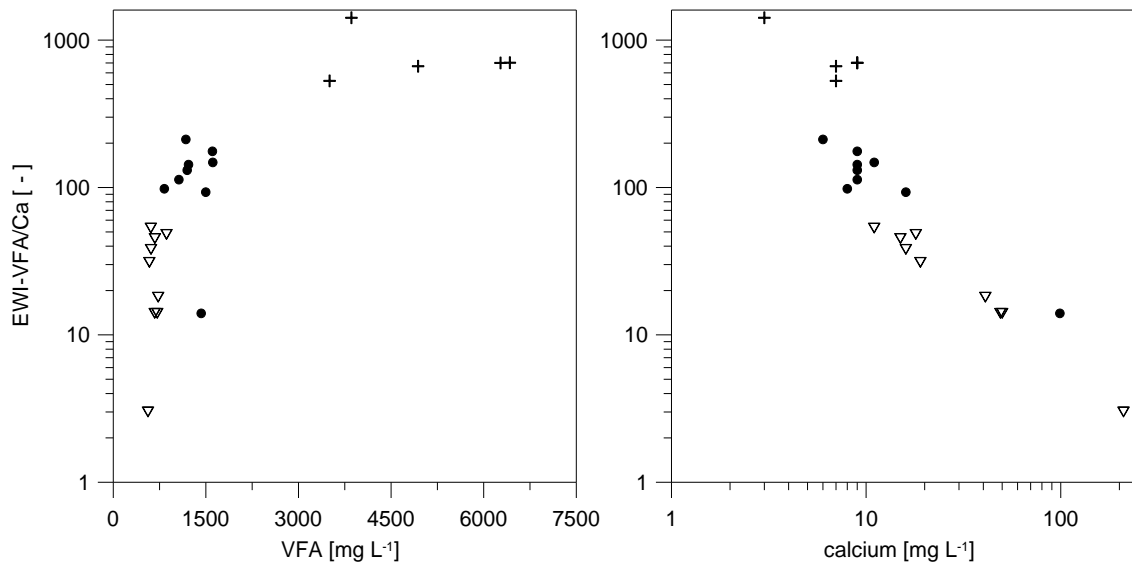

Fig. 4   EWI-VFA/Ca versus VFA concentration (left): ($\triangledown$) EWI-VFA/Ca during a stable process, ($\bullet$) EWI-VFA/Ca warning, ($+$) EWI-VFA/Ca during a process failure. EWI-VFA/Ca versus calcium concentration (right): ($\triangledown$) EWI-VFA/Ca during a stable process, ($\bullet$) EWI-VFA/Ca warning, ($+$) EWI-VFA/Ca during a process failure.

The dependency of the EWI-VFA/Ca on the calcium concentration in all of the experiments is shown in Fig. 4. While the calcium concentration varied over a wide range, between 11 and 200 mg L^{-1} under stable process conditions, the calcium concentration typically ranged from 6 to 16, when the EWI-VFA/Ca indicated a process failure warning. One exception was when the EWI-VFA/Ca indicated a process failure warning at a calcium concentration of 100 mg L^{-1}. In this experiment, the calcium concentration was higher than typical for the substrate matrix used due to a previous experiment with CaO additions. The EWI-VFA/Ca value was very low at 3 and increased to 14 by a factor of 4 at the warning time point. Although the absolute value of the EWI-VFA/Ca was very low compared to the values in the other experiments, the increase in the EWI-VFA/Ca that indicated a process imbalance was in the same range as in the other experiments.

Addtionally, the EWI-VFA/Ca was investigated over a wide range of OLRs between 1.2 and 10 kg VS m^{-3} d^{-1}. It was possible to apply the EWI-VFA/Ca regardless of the OLR. The EWI-VFA/Ca warned as soon as the process became instable, depending on the circumstances and the history of the reactor content (e.g. resulting in a certain high or low calcium concentration).

Tab. 1   Starting conditions for different parameters of the EWI-VFA/Ca experiments (maximum measured methane yield: the high methane yields are due to the high rapeseed oil loads).

Experi-ment	Methane yield [m³ (kg VS)$^{-1}$]	Calcium [mg L^{-1}]	VFA [mg L^{-1}]	OLR [kg VS m^{-3} d^{-1}]
OA1	0,68	19	585	3.0
OA2	0,70	11	609	3.0
OA3	0,64	50	713	1.2
OA4	0,18	41	727	5.9
OA5	0,69	15	674	2.2
OA6	0,79	210	562	2.3
OA7	0,81	18	863	3.2
OA8	0,79	49	671	4.9
OA9	0,58	15	611	8.7

Tab. 2   Overview of the conducted experiments regarding the EWI-VFA/Ca and the interval between the warning and the over-acidification.

Experi-ment	Interval between warning and over-acidification [d]	EWI-VFA/Ca [-] ideal state	EWI-VFA/Ca [-] warning	EWI-VFA/Ca [-] over-acidified
OA1	7	31	113	703
OA2	3	53	212	700
OA3	4	14	148	665
OA4	4	18	143	529
OA5	6	45	176	1418
OA6	7[a1]	3	14	18[a2]
OA7	-	48	72 - 95[b]	-
OA8	-	14	31 – 131[b]	-
OA9	-	41	60 – 98[b]	-

The initial conditions in the experiments regarding the methane yield, OLR, VFA concentration and calcium concentration are given in Tab. 1. Tab. 2 shows the EWI-VFA/Ca at stable process conditions and during the over-acidifications; it gives an overview of the intervals between the warnings and the failures. The intervals variied between 3 and 7 days. Under normal operating condictions indicated by low VFA concentrations and high methane yields, the value of the EWI-VFA/Ca was not greater than 53. The absolute value that indicated the onset of a process failure was approximately 100. At calcium concentrations ranging between 270 mg L^{-1} and 100 mg L^{-1}, the EWI-VFA/Ca warned already at 14. These high calcium concentrations, together with a high rapeseed oil loading rate (OLR: 5.9 kg VS m^{-3} d^{-1}), contributed to an increase in the solids content. A separation of the fluid and solid phases occurred. The process of biogas

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[a1,a2] The process of biogas formation was not completely over-acidified. Due to a high calcium concentration, the biogas production did not cease, but the methane yield was significantly reduced to 25 % of its expected value.
[b] The first value can already be interpreted as a warning. At the second value, the process was still stable, but preventive countermeasures were taken.

formation did not cease, but the methane yield was reduced to 25 % of its expected value. Optical analyses of the reactor content showed that the sludge was highly dewatered and the substrate could not be distributed adequately in the reactor. Consequently, zones had formed that were characterized by different microhabitats. For example, a 5 cm diameter large aggregate with different colored zones and pH gradients was observed in the reactor. The pH could be measuerd easily in the large aggregate compared to the small aggregates with diameters between 0.5 and 5 mm. In the core of the large aggregate, the pH was 7.75, in the next outer layer 6.86 and on its surface 5.62.

Contrary to the own findings involving the concentration profiles of hydrogen, methane and carbon dioxide, the EWI-VFA/Ca exhibited a clear trend that was dependent on the VFA accumulation at typical calcium concentrations found in this particular substrate matrix. The higher the magnitude of the VFA accumulation was, the more the EWI increased as the calcium concentration decreased. At very high calcium concentrations in the range of 100 to 270 mg L^{-1}, the value of the EWI was very low. Nevertheless, the magnitude of the increment during the process failure warning was the same as in the experiments with lower calcium concentrations. It should be noted that the extraordinarily high calcium concentration caused clumps of solids. Hence, to avoid the sludge dehydration that occurred in this experiment, the soluble calcium concentration should not exceed 100 mg L^{-1} under the conditions in the own experiments. If this condition is fulfilled, then the critical value of the EWI-VFA/Ca based on the used substrate matrix in the own experiments is 90 as a "rule of thumb". Independent of the critical value, the two- to three-fold increase in the EWI-VFA/Ca can be interpreted as a process failure warning. Thus, the value of the EWI-VFA/Ca is substrate-specific, while the two- to three-fold increase in its value is likely less dependent on the substrate matrix. Regarding the OLR magnitude, the EWI caused a process failure warning at several OLRs tested: hence, it was independent of the OLR magnitude.

In experiments OA7 to OA9, EWI-VFA/Ca values between 30 and 75 were interpreted as a process failure warning: nevertheless, the process could be held stable at values between 95 and 130. At this stage, it was still possible to prevent a process failure, either by adding CaO, as shown in the experiment "preventing a process failure by the addition of CaO", or by decreasing the OLR (data not shown). Hence, the process could be operated at an OLR of 5.9 kg VS m^{-3} d^{-1} with a fat fraction of 80 %, because the EWI-VFA/Ca warned early enough to take appropiate countermeasures. Loustarinen et al. (2009) increased the OLR of an reactor fed with sewage and grease trap sludge. They observed the highest methane yield at an OLR of 3.46 kg VS m^{-3} d^{-1} with a fat fraction of 46 %. At higher OLR the degradation was incomplete. Thus, the addition of CaO contributes significantly to the stabilization of the biogas formation process allowing for higher OLRs than without CaO addition.

With sewage sludge and rapeseed oil as substrates, the effect of process stabilization by CaO addition was mainly based on the precipitation and adsorption processes, together with the biofilm formation, which resulted in aggregate (with a size between 0.5 and 5 mm) formations as discussed in detail in Kleyböcker et al. (2012). Clearly, the microorganisms within these aggregates were protected from conditions such as high hydrogen concentrations, which were observed in the gaseous phase. Consequently, the degradation of propionic acid occurred even though, for this system, the hydrogen content was too high (> 0.01 vol. %) for the degradation according to Harper and Pohland (1986).

In contrast to the effect of CaO on the stabilization of the process of biogas formation, NaOH additions did obviously not contribute to a stable process as shown in the experiment "with pH

regulation using NaOH". Similar findings are described by Steinberg and Regan (2011) who also could not stabilze the biogas formation process within 25 days by only using NaOH after introduced shock loads of glucose to three differently inoculated biogas systems.

3.3. EWI-PO$_4$/Ca and EWI-PO$_4$ as additional early warning indicators

The phosphate concentration (EWI-PO$_4$) and the concentration ratio of phosphate to the calcium (EWI-PO$_4$/Ca) indicated an imminent process failure by a threefold increase, which occurred 5 days before the increase in the EWI-VFA/Ca in the experiment "with pH regulation by the addition of NaOH" (OA4) (Fig. 5). In contrast, the EWI-VFA/Ca indicated the begin of a process failure 2 days earlier compared to both parameters in the previous experiment (OA3). Although the gridlines corresponding to the phosphate concentrations of the samples are not as close as those corresponding to the EWI-VFA/Ca, the figure clearly shows that the EWI-PO$_4$ and the EWI-PO$_4$/Ca can serve as additional indicators to the EWI-VFA/Ca. Regarding the EWI-PO$_4$, the one- to two-fold increase showed a process failure warning, while the two- to three-fold increase in the EWI-PO$_4$/Ca indicated an impending process imbalance.
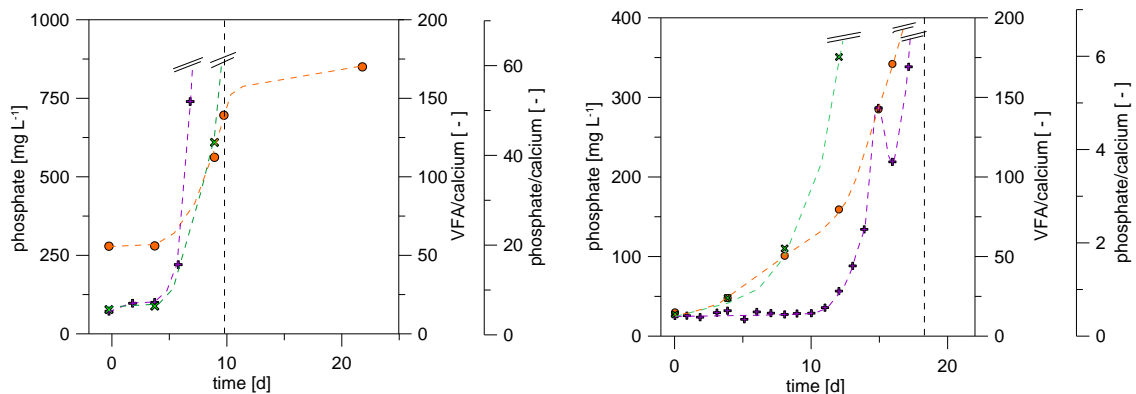

Fig. 5   Comparisons of the EWI-VFA/Ca (✚), EWI-PO$_4$/Ca (✕) and EWI-PO$_4$ (⬤): Experiment OA3 (left): The VFA/Ca ratio indicates a process imbalance earlier than the PO$_4$/Ca ratio and PO$_4$ and than the gas production rate (- - - significant decrease of 40 %), Experiment OA4 (right): The PO$_4$/Ca ratio and PO$_4$ indicate a process imbalance earlier than the VFA/Ca ratio and the gas production rate (- - - significant decrease of 50 %).

In addition to the EWI-VFA/Ca, the phosphate concentration (EWI-PO$_4$) and/or the ratio of phosphate to the calcium concentrations (EWI-PO$_4$/Ca) should be determined because in some experiments they indicated an impending process failure even earlier than the EWI-VFA/Ca. As they do not always warn earlier than the EWI-VFA/Ca, it is important to monitor them in addition to the EWI-VFA/Ca.

5.   Conclusions

The EWIs were well-suited for regulating the process operation and maintaining a stable process, because the warning time was sufficient to start successful countermeasures. After the determination of the EWIs for a stable process, monitoring of the EWIs is mainly required during a change in the substrate matrix to avoid process failure. Due to the application of cuvette tests to measure the concentrations of fatty acids, calcium and phosphate, there is no expensive technology necessary to determine the EWIs. The next step is to develop an

automatic system determining the EWIs online and giving an alarm to introduce countermeasures.

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Appendix

Additional figure for the reviewers:

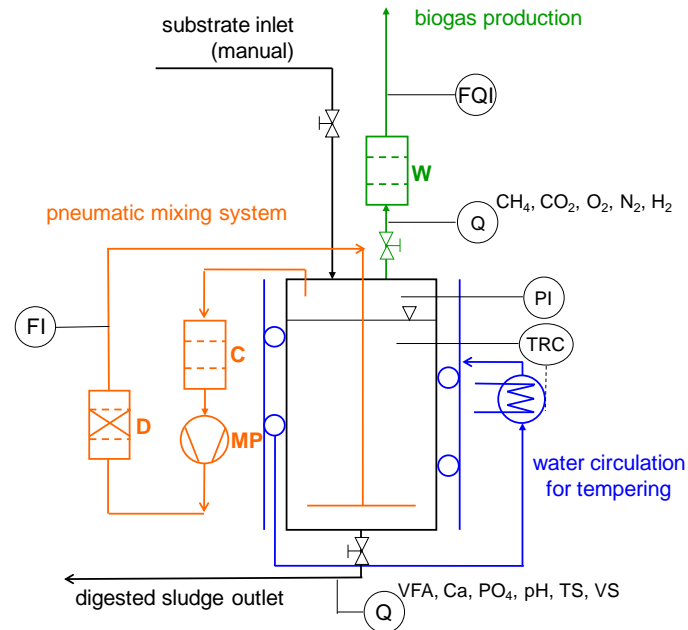In the text it is cited as Fig.1 in Kleyböcker et al. 2012.

Fig. A.1   **Scheme of a laboratory biogas reactor: condensate collector (C), membrane pump (MP), drying tower (D), wash bottle (W), quality (Q, if leading letter), quantity (Q, if non-leading letter), indicating (I), flow (F), controlling (C), pressure (P), temperature (T), recording (R).**