A novel in-situ sampling and VFA sensor technique for anaerobic systems

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Abstract A key information for understanding and controlling the anaerobic biogas process is the concentration of Volatile Fatty Acids (VFA). However, access to this information has so far been limited to off-line measurements by manual time and labour consuming methods. We have developed a new technique that has made it possible to monitor VFA on-line in one of the most difficult media: animal slurry or manure. A novel in-situ filtration technique has made it possible to perform microfiltration inside the reactor system. This filter enables sampling from closed reactor systems without large scale pumping and filtering. Using this filtration technique together with commercially available membrane filters we have constructed a VFA sensor system that can perform automatic analysis on animal slurry at a frequency as high as every 15 minutes. The VFA sensor has been tested for a period of more than 60 days with more than 1000 samples on both a full-scale biogas plant and lab-scale reactors. The measuring range covers specific measurements of acetate, propionate, iso-/n-butyrate and iso-/n-valerate from 0.1 to 50 mM (6–3,000 mg).

Keywords Anaerobic manure treatment; in-situ filtration; on-line measurement; VFA

Introduction Volatile Fatty Acids (VFA) are some of the most important intermediates in the anaerobic biogas process. Conversion of the VFA through the acetogenic and acetoclastic step into methane and carbon dioxide is the most important conversion in the biogas process. It is well recognized that monitoring the specific concentration of VFA can give vital information on process status (Ahring et al., 1995; Alonso, 1992; Cobb and Hill, 1991; Hickey and Switzenbaum, 1991; Hill and Bolte, 1989; Mosche and Jordenning, 1999; Pind et al., 1999; Rozzi et al., 1997). Focus has been applied especially on the isoforms of butyrate and valerate as fast indicators of changes in the process balance (Ahring, Sandberg, and Angelidaki, 1995; Cobb and Hill, 1991; Hill and Bolte, 1989; Hill and Holmberg, 1988), whereas propionate and butyrate levels are known to increase if the hydrogen level increases to inhibiting levels (Ahring et al., 1995; Mosche and Jordenning, 1999; Öztürk, 1991). A very complex interaction of inhibition, substrate affinity and pH dependency leads to the conclusion that a more complex evaluation of the VFA necessitates access to many and frequent VFA measurements. VFA is easily measured using GC or HPLC, provided that all particulate matter has been removed from the sample. However, when dealing with waste treatment in anaerobic processes, the presence of particulate matter is often high. Only a few attempts for development of on-line VFA monitoring systems are reported in the literature. (Ryhiner et al., 1993; Slater et al., 1990; Zumbusch et al., 1994), but the methods all suffered from membrane fouling and have not been used for more studies or use.

To our knowledge, no on-line VFA measurement technique has been validated on full-scale anaerobic systems treating solid wastes at present. In this presentation we present a new sample preparations system consisting of: first a prefiltration performed in-situ by a
rotating filter (Danish patent application number PA 2000 01014) then a ultrafiltration and finally acification of the sample prior to GC analysis. The system (Danish patent application number PA 2000 01013) can perform on-line sample filtration and preparation using only approximately 2 ml of sample for a single measurement.

**Methods**

**Sensor system**

A schematic layout of the sample preparation system is shown in Figure 1. The rotating prefilter (1) has a pore size of 60 µm and an effective area of 25 cm². The prefilter is placed *in-situ* in the biogas reactor or in pipelines connected to a full-scale reactor. The hold-up volume between the cleaning valves (6) is 55 ml and is initially flushed for 30 seconds with prefiltered medium prior to running a recirculation pump (5). The recirculation pump increases the flow and pressure to 1 m/sec and 0.8–0.9 atm in an ultramembrane (4): UFP-100-E-4A membrane (A/G Technology Corporation) 100,000 NMWC, area 420 cm². The hold-up volume (approximately 20 mL) of the ultramembrane cartridge is flushed with filtered permeate for 6 minutes prior to sampling. The permeate is returned through a non-return valve (8) while the flushing is performed, thus minimising the sampling amount removed from the reactor system. Equal amounts of sample and 1% (w/v) phosphoric acid (15) are then mixed by a peristaltic pump (10) and (11). This sample mixture is first removed by (12), flushing all previous sample hold-up volumes and afterwards passes through a mini-filter (17), capturing possible precipitate formed in the sample mixture. An open sample vial or container (18) is flushed with the sample mixture, allowing degassing of carbonate. Overflow from the vial (18) is removed by an overflow pump (13). The sample is then transferred by an autoinjector syringe to a GC and (Shimadzu GC-14A). After injection, the flow-cell (18) and mini-filter (17) are back-flushed with neutralising liquid (16) to a waste container by (12) and (14). Finally, the flow-cell and tubes are emptied by (12) and the system is now ready for the next sample. Validation and testing of the individual parts of the sensor system was performed in both laboratory and full-scale application and is described in the following.

**Laboratory tests**

4.5 L reactors with active volumes of 3.5 L were used for the test. The reactor design was as previously described (Angelidaki and Ahring, 1993). *In-situ* filtration (60 µm pore size) was tested on the set-up shown in Figure 2. Filter flux was determined as function of tem-

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**Figure 1** Schematic illustration of the sample preparation system used for on-line VFA analysis in biogas systems. 1: rotating prefilter placed *in-situ*. 2: sampling port for control extraction by syringe (prefiltered samples). 3: peristaltic pump. 4: ultramembrane. 5: recirculation pump. 6: three-way valve for bypassing cleaning fluid for regeneration of the ultramembrane. 7: sampling port for control extraction by syringe (ultrafiltered samples). 8: nonreturn valve. 9: backflushing pump. 10 and 11: linked peristaltic pumps pumping equal amounts of ultrafiltered sample and 1% (w/v) phosphoric acid (15), respectively. 12: waste pump. 13: overflow pump. 14: pump backflushing with neutralising liquid (16). 17: minifilter. 18: flow-cell (vial)
perature (7–38°C) and concentrations of solids (35–106 g TS/L) on digested cattle slurry. Differential pressure varied from 0.05 to 0.15 atm, with increasing difference at higher fluxes. Maximum flux was estimated as the flux obtained just prior to filter constipation, observed as a rapid and irreversible pressure drop. Complete VFA sensor tests were conducted on a similar reactor at thermophilic temperatures (reactor TS of 55 g/L). Total solids were determined according to standard methods (APHA-AWWA-WPCF, 1975) and VFA as previously described (Ahring et al., 1995).

**Full-scale test**

The complete system and the sample preparation system were validated while conducting a reliability test on a full-scale biogas plant (Snertinge Biogas Plant, Denmark). Samples were taken from a recirculation loop servicing two reactors operating in the temperature range from 37–45°C with a reactor TS content ranging from 44 to 48 g/L. Membrane breakthrough was tested for each sample by backflushing the outer membrane cartridge with 20 ml of distilled water (9 in Figure 1), thereby ensuring that VFA from the previous sample would not significantly influence the VFA measurement of the new sample. More than 1000 samples were taken and analysed automatically during a period of 2 months. Control samples from reactor, prefiltered and ultrafiltered media were taken periodically for validation of the sensor system.

**Results and discussion**

**In-situ filtration**

The requirement for a simple and preferably in-situ microfiltration led to the construction of a small rotating filter. This unique filter construction occupies only 12–20 cm³ (length: 4–6 cm and diameter: 2–3 cm) inside the reactor system and can be placed in laboratory reactors and even standard piping in full-scale plants. A 60 µm pore size filter was used for all tests. The filter could supply a flux between 1,000 and 4,000 L m⁻² h⁻¹ depending on temperature, rotating speed and concentration of TS when used on cattle slurry. TS concentration up to 106 g TS/L was tested with success.
The filter could supply 2,000 L/(m³·h) at normal reactor conditions: TS lower than 50 g/L and temperatures higher than 30°C. The prefiltered manure had a TS content of 24–25 g/L when the reactor was running at normal conditions. An increase in prefiltered media TS was observed when the reactor TS was artificially increased up to 106 g TS/L, but it never exceeded 35 g/L.

Validation of representative VFA concentrations in the prefiltered slurry was documented by Figure 3. The average deviation of +2.85% on the filtered samples was not considered significant since it is based on a few samples obtained in the concentration range between 1 and 5 mM VFA. Later controls from full-scale test showed a good comparison of reactor samples and prefiltered samples with a factor of [prefiltrate] = 0.9922 · [reactor samples] and a regression factor of 0.9964 (R²) (data not shown).

The rotating filter showed high reliability during the laboratory and full-scale tests. The filter facilitates sampling, both manually and automatically, from biological systems that exhibit relatively high concentrations of particulate matter. The filter could be applicable not only in biogas reactors but all types of biological systems. Furthermore, the filtration technique could easily be applied to smaller pore sizes down to the ultrafiltration range dependent on the medium.

**Ultrafiltration and sample preparation**

A membrane unit with an average pore size of 100,000 NMWC was chosen for an ultrafiltration step on the basis of screening tests. With proper configuration of recirculation and pressure on the membrane it was possible to obtain a sample with 95% VFA recovery within 5 minutes. Membrane flux was observed over a period of 2 months testing (see Figure 4) with satisfying flux if it was cleaned periodically every 15–18 hours of use (minimal 38–45 hours exposure) or approximately every 200 samples. A declining recovery of the membrane flux was observed within the first 64 hours of use. Only hot tap water was used as cleaning agent during the first 64 hours. At 64.5 hours (indicated by the arrow in Figure 4) backflushing of the membrane at point (9) in Figure 1 was introduced during the cleaning procedure and the recovery increased to almost 95%. The same ultramembrane was used for more than 1,000 samples during the 2 months full-scale testing and additional 1,000 samples during 70 days running on lab-scale reactors before replacement of the ultramembrane was necessary. Therefore, it was concluded that the membrane employed could provide sufficient flux if periodical cleaning was employed. Cleaning was controlled manually during the present tests, but could easily be done automatically.

**Figure 3** Comparison of specific VFA concentrations measured in lab-scale mesophilic reactor. Reactor samples and samples taken from the recirculation loop of the rotating filter. The line is the best linear fit of the data.
After prefiltration and ultrafiltration the sample has to be transferred to GC by techniques insensitive to the presence of gas (carbonate), inorganic and organic components still present in the sample.

VFA data obtained from the full-scale tests showed a fixed and constant mixing of sample and 1% (w/v) phosphoric acid throughout the test period (Figure 5a). A comparison of the same data with controls taken from the sampling point showed that the sensor system had a recovery of 98.14% compared to the manual obtained sample (Figure 5b), based on best fit of the data. This is explained by not having 100% recovery in the ultramembrane where a similar recovery of 98.03% was found as the best fit (with 6 minutes recirculation

![Figure 4](https://iwaponline.com/wst/article-pdf/45/10/261/424857/261.pdf)

**Figure 4** Membrane flux test, during continuous testing of the VFA sensor system at a full-scale biogas plant. The flux is illustrated as a function of the accumulated time the membrane had been in use. The membrane was periodically cleaned with hot water (1 hour) when insufficient flux was observed. Arrow indicates when high pressure backflushing of the membrane was induced as part of the cleaning.

![Figure 5](https://iwaponline.com/wst/article-pdf/45/10/261/424857/261.pdf)

**Figure 5** Comparison of specific VFA concentrations measured at different points of the sensor system during a full-scale test at Snertinge biogas plant. Line is best fit of the data. a: VFA concentrations measured by VFA sensor and control samples taken from the ultrafiltered medium. b: VFA concentrations measured by VFA sensor and VFA measured in reactor samples.
before sampling). The recovery would statistically have been higher if the membrane had not been flushed for each sample. Comparison of VFA concentrations in samples taken directly form the reactor and samples taken from the prefilterate and ultrafiltered showed similar good linear data fit as in Figure 5 (data not shown).

Mixing the sample with phosphoric acid immediately caused CO₂ stripping. The waste pump (12) and flushing pump (13) had to be configured with 3 times higher flow than (11) and (10) to prevent pressure build-up and overflow because of CO₂ stripping (numbers referring to Figure 1). Likewise, the mixed sample had to stand in the vial or flush cell for 20–30 seconds before CO₂ had stripped off. The presence of other organic and inorganic compounds in the sample did not cause fouling of the tubes and sample preparation system. However, salts did precipitate when the samples were acidified, and had to be removed from the sample and system. Placing a mini-filter just prior to the flow-cell captured the precipitating salts. Backflushing with a neutralising liquid dissolved the salts, which subsequently were removed from the system. The liner used in the GC captured inorganic components that could not evaporate at the injector temperature employed. This liner was replaced periodically for every 200–400 samples. No effects on column retention, detector signal and reproducibility were seen during the full-scale test with more than 1000 samples. Calibration curves showed reproducibility throughout the test.

Accuracy below the standard range was tested using diluted standard solutions. All VFA could be measured with an accuracy of ±2% between 1–50 mM, ±5% between 0.1–1mM, ±10% between 0.05–0.1 mM and ±30% between 0.02–0.05 mM.

**Application of the sensor**

Dynamic response as a consequence of periodical feeding was seen for all specific VFA concentrations during laboratory tests. This dynamic response was very pronounced when temporarily changing the feeding from 4 times per day to 2 times per day as shown in Figure 6. Even though the accuracy of butyrate and valerate were ±30% below 0.05 mM,
the dynamic trends were very clear and reproducible. These dynamics were optimally detected by performing a measurement just prior to and after a feeding and an additional 2–4 times between feedings. Two distinct types of dynamic reactions were observed:

1. increase by feeding followed by additional increase within the next 2–3 hours before the level decreased again;
2. increase by feeding followed by almost linear decrease within the next 2–3 hours before bending off.

The first type would indicate a higher production than removal of the specific VFA. Whether initial inhibition of the degradation or simply a large amount of easily degradable organic molecules in the feed was the explanation for this reaction can only be speculated on. The second type indicates a high concentration of acetogenic bacteria capable of a fast removal of the specific VFA. This would indicate that no inhibition of these acetogens occurs. However, it is important to underline that this dynamic reaction occurs within a few hours after loading, which illustrates the strong need for monitoring with a relatively high frequency in sequence loaded reactor systems as previously shown by Pind et al. (1999).

By combining information about waste characteristics and history of the VFA measurements it would be possible to make a sophisticated evaluation of the state of the process. Increasing a specific concentration of VFA to a relatively high level would make it reasonable to assume negligible production as compared to the removal rate of this VFA (within a short time span). This would facilitate determination of specific kinetic parameters based on the linear removal rate at the beginning of the pulse. Studies of this special dynamic reaction during pulses of VFA on a periodically fed reactor system by VFA measurements as proposed are presented by Pind et al. (2001).

**Conclusion**

A novel rotating filter unit capable of *in-situ* filtration in reactor systems has been proven to work at high concentrations of TS and low temperatures. Flux capacities increased with increasing temperature, rotating speed, and decreasing TS concentrations. Optimal running conditions were a tangential speed higher than 1 m/sec and TS concentrations lower than 50 g/L, in the mesophilic to thermophilic temperature range. Complete recovery of dissolved VFA was seen in the filtered media. A commercial ultramembrane unit could be used for filtrating manure with periodical cleaning with hot water and backflushing. Membrane breakthrough could be obtained within 5 minutes at optimal conditions and was suitable for automatic measurements of VFA in biogas reactors. A sample preparation system of VFA analysis on manure was proven to work on both lab-scale and full-scale biogas reactors. The system could provide reliable data for more than 200 samples without service. Furthermore, the system could run for more than 100 days with periodically cleaning without replacement of units.

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**References**


