Biogas production from mono-digestion of maize silage–long-term process stability and requirements

M. Lebuhn, F. Liu, H. Heuwinkel and A. Gronauer

ABSTRACT

Biogas production from mono-digestion of maize silage was studied for more than one year in six continuously stirred, daily fed 36 L fermenters. Chemical and microbiological parameters were analysed concomitantly. The reactors acidified already after 8 months of operation at a low organic loading rate (OLR) of $2 \, g \, VS * (L * d)^{-1}$. The TVA/TAC ratio was the most reliable parameter to indicate early process instabilities leading to acidification. A TVA/TAC threshold of 0.5 should not be exceeded. After acidification and recovery of the fermenters, propionic acid was no reliable parameter anymore to indicate process failure, since values far below the threshold of $1 \, g * L^{-1}$ were obtained although the process had collapsed.

The acidified reactors recovered better, showed greatly improved stability and allowed a higher OLR when a trace element (TE) cocktail was supplemented. Hydrolysis was obviously not process-limiting, results indicated that methanogens were affected. The most limiting element in long-term mono-digestion of maize silage turned out to be cobalt, but data obtained suggest that molybdenum and selenium should also be provided. TE supplementation should be designed specifically in order to meet the actual needs. TE availability for the biocenosis appears to be a key issue in biogas production, not only in mono-but also in co-digestion processes.

Key words | biogas, long-term process stability, mono-digestion of maize silage, trace elements

M. Lebuhn
F. Liu
H. Heuwinkel
A. Gronauer
Bavarian State Research Center for Agriculture,
Institute for Agricultural Engineering and Animal
   Husbandry,
Vöttinger Straße 36,
85354 Freising,
Germany
E-mail: *michael.lebuhn@lfl.bayern.de*

INTRODUCTION

Anaerobic digestion (AD) has been used already some thousand years ago to convert organic matter to energy (biogas) and fertilizer (digest). With the shortage of fossil fuels and the perception that greenhouse gas emissions from combustion of fossil fuels is a major factor of the global climate change scenario, AD is gaining more and more attraction worldwide, in particular if renewable (organic) energy carriers are converted (Greenfield & Batstone 2005). Today, biogas production is one of the most promising biotechnologies to produce sustainable energy and energy carriers such as methane. If applied properly, AD can significantly contribute to minimize dissipation of fossil energy resources and greenhouse gas emissions (Verstraete *et al.* 2005).

Electricity from biogas accounted for roughly 1% of total electricity production in Germany in 2006, and even about 2.5% in the Federal State of Bavaria. Respective research was intensified concomitantly and the knowledge on process management increased. In the last years the number of biogas plants digesting or co-digesting energy crops has enormously increased in Germany. Particular interest was devoted recently to mono-digestion of energy crops and products thereof such as maize and grass silages, since many farms do not have animal husbandry. In addition, high biogas and energy yields can be obtained. However, mono-digestion processes and their requirements are much less understood, and respective management strategies currently are trial-and-error based. Evidence for process deficiencies particularly in long-term operation is rising in practice as well as from lab and technicum scale experiments (e.g., Jarvis *et al.* 1997; Preissler *et al.* 2007).

We are therefore investigating at which conditions and how long mono-digestion of energy crops is feasible. In July 2006, We started up six mesophilic (38°C) semicontinuously stirred lab-scale biogas reactors fed daily with renewable resources only. After switching to feeding maize silage as the only substrate we observed first symptoms of acidification after ca. 8 months of operation although the organic loading rate (OLR) was relatively low. After repeated feeding stops and resumes at very low OLR, no improvement of methane production and acidification was seen. Feeding was stopped for complete degradation of built-up volatile fatty acids (VFA) and thereafter supplemented with a trace element cocktail in order to investigate causes of the decreased reactor performance, and to develop options to optimize mono-digestion of energy crops for biogas production.

We identified thinning out of certain trace elements as a key problem in long-term mono-digestion of maize silage for biogas production. In this paper we describe how addition of apparently deficient trace elements in mono-digestion of maize silage led to process recovery and even improved reactor performance, and figure out which trace elements should essentially be supplied.

MATERIAL AND METHODS

Biogas reactors and management

Experiments were carried out in six 1-stage, 1-phase 36 L (28–32 L working volume, reactors B1–B3 and C1–C3, Figure 1a) semicontinuously stirred and daily fed flow-through reactors at mesophilic (38°C) conditions with automated reading of gas production and composition. Fermenter housing of reactors B2, B2 and C1 was made of glass, and of reactors B1, C2 and C3 of V2A stainless steel. Reactors were started up in June 2006 with 50% water and 50% inoculum from 3.5 m³ pilot plants (Figure 1d) producing our standard biocenosis. For reactor start-up and adjustment of regular operation, emphasis was put on changing slowly the conditions (OLR, temperature) in small steps in order to provide enough time for adaptation of the biocenoses to the new conditions. Temperature was increased by a maximum of $0.3°C\,d^{-1}$, and OLR by $0.5\,g$ $VS\,(L\,d)^{-1}$ only if gas composition was stable and gas production stabilized after ca. 14 d. A mixture of maize (70%) and grass (30%) silages was fed first until we switched to mono-digestion of maize silage on August 02-2006.

Examples of the fermenter equipment at the Institute for Agricultural Engineering and Animal Husbandry are shown in Figure 1. Detailed descriptions of reactor concepts and management as well as analytical parameters and procedures are presented in Gronauer et al. (2006).

Chemical and physical parameters

Biogas production was measured continuously using Ritter milligas counters, and production rates were converted to STP. Gas composition (CH_4, CO_2, O_2, H_2, H_2S) was determined hourly using AWITE devices. Chemical analyses of silages and fermenter contents were performed based on German standard analytical methods for the analysis of water, wastewater, and sludges (1981)and for feedstuffs (1997). Parameters determined were TS, VS, pH, $NH_4{-}N$, volatile fatty acids C_{2-7} (VFA), TVA/TAC (total
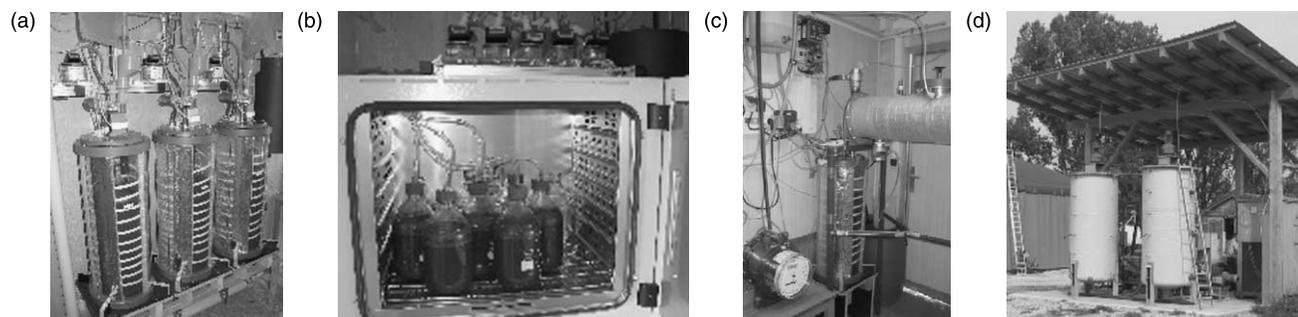

Figure 1 | from left to right: a) 36 L flow-through systems; b) 2 L batch systems; c) 2-stage flow-through systems; d) 3.5 m³ pilot plants.

volatile acids/total alkalinic carbonate, two-point titration method modified after De Haas & Adam (1995)), van Soest-fractions (NDF, ADF, ADL), Weender-fractions, macroelements (total C, N, P, K, Ca, Mg, S) by elemental analysis or ICP-OES, and trace elements (Fe, Ni, Cu, Co, Mn, Se, Mo, Zn, Al, B, Cd) from aqua regia dissolution by AAS or ICP-OES, following standard methods, and physical parameters (ambient temperature, air pressure). In parallel, methanogen populations were determined for selected samples using a direct PCR cloning and a PCR-single-strand conformation polymorphism (PCR-SSCP) approach (Bauer *et al.* 2008).

Addition of trace elements

In June 2006, we started up six mesophilic (38°C) semicontinuously stirred lab-scale biogas reactors fed daily with renewable resources only. After ca. 10 months of operation we observed first symptoms of acidification although the organic loading rate (OLR) was relatively low $(2.0–2.4\,\mathrm{g\ VS\,(L\,d)^{-1}})$. After a transient feeding stop, feeding was continued at a low rate $(1.0–2.2\,\mathrm{g\ VS\,(L\,d)^{-1}})$ but reactors acidified again with largely reduced methanation. Feeding was stopped this time for ca. $20–40\,\mathrm{d}$ depending on the degree of acidification and time needed for degradation of built-up volatile fatty acids.

From July 02-2007 on, $60\,\mu\mathrm{L}$ (1xTE) of a trace element (TE) cocktail was supplied to fermenters B1 and C1 (intermediary acidification), and $600\,\mu\mathrm{L}$ (10xTE) cocktail to fermenters C2 and C3 (severely acidified). TE addition was preceded by addition of 60 or $600\,\mu\mathrm{L}$ of a $\mathrm{FeCl_3\,6H_2O}$ $(1{,}453.23\,\mathrm{mg\,L^{-1}})$ solution to precipitate S^{2-}. Fermenters B2 and B3 (the least acidified) served as controls without TE supply. Feeding of fermenters B1, B2 and C2 was re-started on July 02-2007, with an OLR of $0.63\,\mathrm{g}$ $\mathrm{VS\,(L\,d)^{-1}}$, and of fermenters B3, C1 and C3 with an OLR of $0.52\,\mathrm{g\ VS\,(L\,d)^{-1}}$ on July 25-2007.

The TE cocktail consisted of the following compounds (in $\mathrm{mg\,L^{-1}}$): $\mathrm{FeCl_3\,6H_2O}$ (1,453.23), $\mathrm{CoCl_2\,6H_2O}$ (887.46), $\mathrm{MnCl_2\,4H_2O}$ (936.00), $\mathrm{NaMoO_4\,2H_2O}$ (262.34), $\mathrm{CuCl_2\,2H_2O}$ (0.242), $\mathrm{ZnCl_2}$ (1.69), $\mathrm{H_3BO_3}$ (18.88), $\mathrm{Na_2SeO_4}$ (25.00) and $\mathrm{NiCl_2\,6H_2O}$ (651.95). A single 1xTE addition supplied to the fermenter (in $\mu\mathrm{g}$): Fe (18), Co (13.2), Mn (15.6), Mo (6.9), Cu (0.005), Zn (0.049), B (0.198), Se (0.627) and Ni (9.66).

RESULTS AND DISCUSSION

Performance of the biogas reactors

Biogas production (not shown) in the six fermenters could be intensified steadily with the increased OLR for 294 days of operation until April 20-2007 (OLR 1.98, Figure 2). Specific methane production was highest (ca. $0.5\,\mathrm{L_{STP}\,(g\ VS)^{-1}}$) at OLR $0.91\,\mathrm{g\ VS\,(L\,d)^{-1}}$, and decreased at higher OLRs (not shown). Performance of the six fermenters was almost identical with methane contents typically between 50–55% until day 261 (March 17, 2007). Thereafter, biogas production of the fermenters diverged (C3 < C2, C1 < B3 < B1 < B2). First symptoms of acidification were obtained for fermenter C3 already on February 27-2007 (TVA/TAC > 0.5, Figure 2). A TVA/TAC ratio of > 0.5 has proved in practice to indicate acidification and compromised reactor performance (Effenberger *et al.* 2008). Subsequently, the other 5 fermenters also exceeded this threshold with reactor B2 being the least affected (Figure 2). Between May 05-2007 and June 05-2007, propionic acids values lay within $4.927\,\mathrm{mg\,L^{-1}}$ (C3) and $410\,\mathrm{mg\,L^{-1}}$ (B2). Concomitantly, biogas production was decreasing or low, and analyses of methanogens (Bauer *et al.* 2008) indicated decreased presence of these organisms in samples from May and June 2007 because stronger bands were obtained before and thereafter. Since acid production was intense, methanogenesis appeared to be impeded leaving the acids undegraded. Ammonia was not the reason, it was constant $(1–2\,\mathrm{g\ NH_4{-}N\,L^{-1}})$ throughout the experiments in all 6 fermenters.

After a 3 d famine period, feeding of reactors B1–C2 was resumed by OLR $1.22\,\mathrm{g\ VS\,(L\,d)^{-1}}$, but further OLR increase led to acidification again (Figure 2). After a period of 21 d without feeding and degradation of built-up VFA, propionic acid was below $100\,\mathrm{mg\,L^{-1}}$. On July 02-2007, supply of the TE-cocktail was started (Figures 2 and 3) concomitantly with feeding (OLR $0.63\,\mathrm{g\ VS\,(L\,d)^{-1}}$) of the least affected reactors B2, B1 and C2. Reactors B3, C1 and C3 were fed (OLR $0.52\,\mathrm{g\ VS\,(L\,d)^{-1}}$) again first on July 25-2007. Reactors B2 and B3 served as non-TE-supplemented controls.

Figure 3 shows the development of specific methane production of the six fermenters. The methanogenic biocenosis reacted immediately and strongly on the supplied
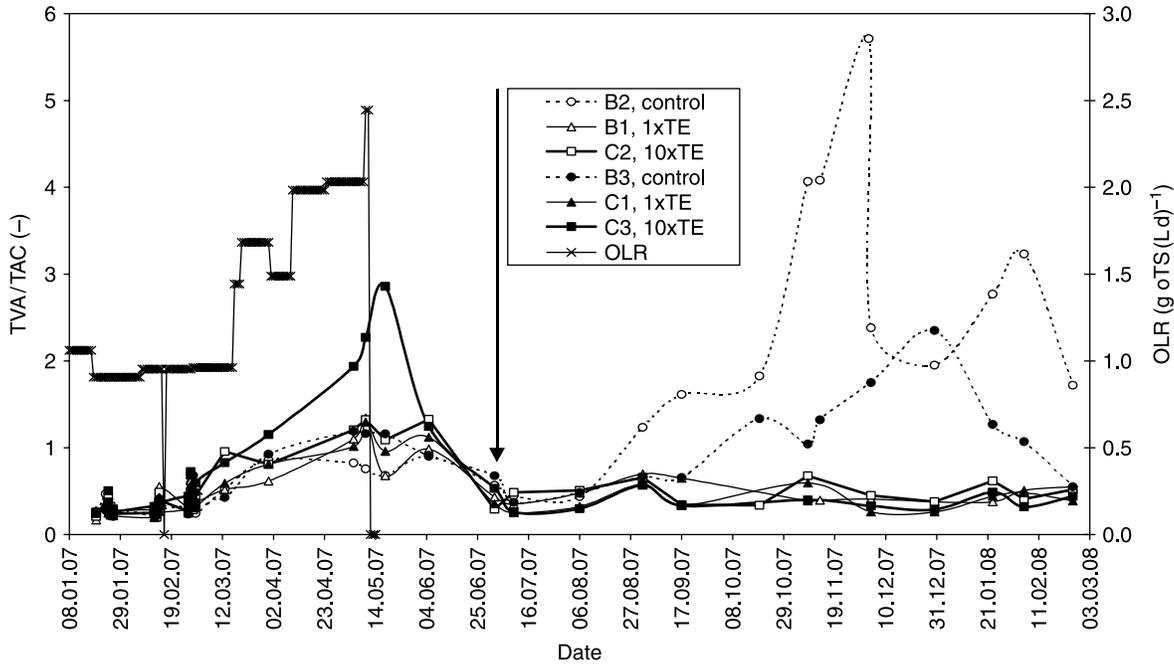
Figure 2 │ Development of TVA/TAC ratios before and after daily supply with trace elements. OLR data are shown only until the first feeding stop because OLR differed thereafter for the six fermenters. The arrow marks the date (July 02-2007), supply of the trace element (TE) cocktail (see text) was started.
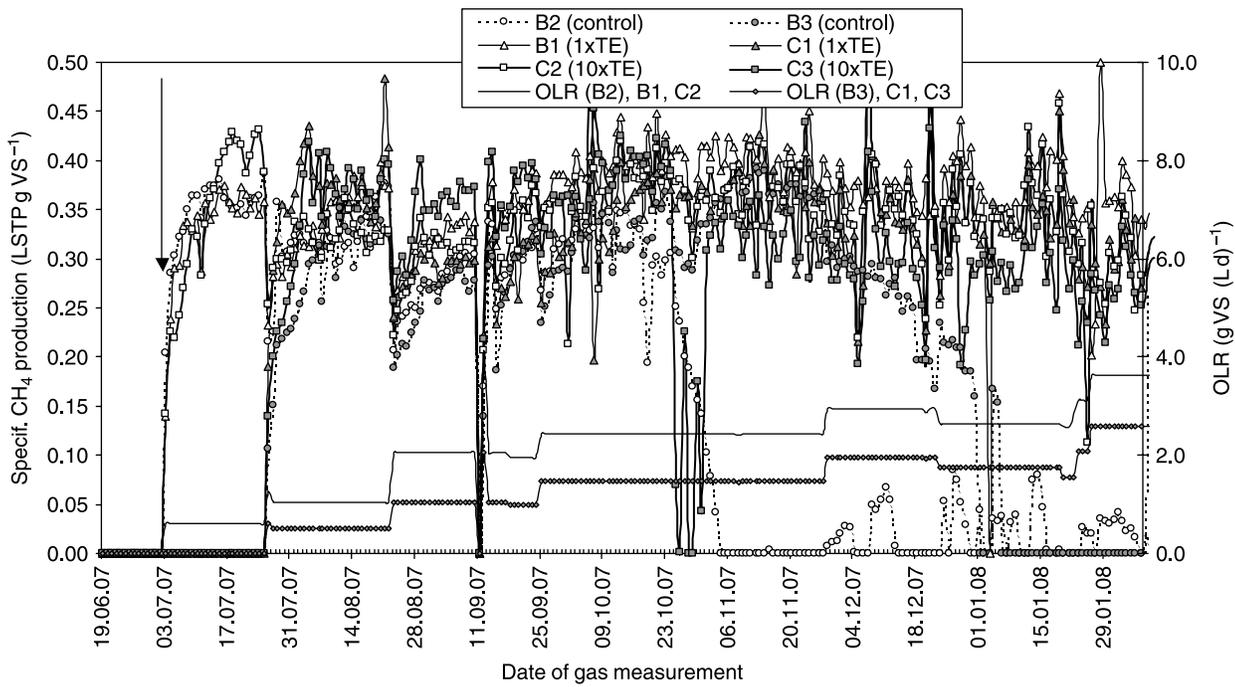

Figure 3 │ Specific methane production in the fermenters after the onset of trace element supply. In the legend (B2) and (B3) are in paranthesis because feeding was stopped on Nov. 04-2007 and Jan. 01-2008, respectively. The arrow marks the day of first addition of the trace element (TE) cocktail.

maize silage after the famine period (Figure 3). PCR bands for methanogens were strong in the sample from Sept. 01-2007 (C3, Bauer *et al.* 2008), and ca. 35 and ca. 17 L_{STP} biogas with a methane content between 52 and 54% were produced daily by the fermenters of group B2-B1-C2 and group B3-C1-C3, respectively (not shown). However, first symptoms of (re)acidification of fermenter B2 were obtained at this time, the TVA/TAC was higher than 1 (Figure 2), and propionic acid increased in contrast to the other fermenters for which no signs for compromised reactor performance were obtained yet. Although the TVA/TAC value for reactor B2 approached 6 in the end of November 2007 (Figure 2), the highest propionic values in this period for B2 was only 812 mg L^{-1}. However, longer VFA were strongly increased (not shown), suggesting that in addition to inhibited methanogenesis, syntrophs and β-oxidation were compromised. Propionic acid values below the threshold of 1 g L^{-1} (Effenberger *et al.* 2008) are obviously not always indicative of stable reactor performance.

First signs of (re)acidification of the second control fermenter (B3, no TE supply) were obtained in the second half of October 2007. The TVA/TAC ratio exceeded 1 (Figure 2), and concentration of some higher VFA, but not propionic acid, increased. Specific methane production was severely compromised in the following (Figure 3), TVA/TAC exceeded 2 (Figure 2), and propionic acid increased to values > 3 g L^{-1} in the beginning of 2008.

In contrast to the havaries of the control fermenters, no signs of acidification were obtained for the TE supplemented fermenters. Although the OLR could be increased (until now) to 3.62 g VS $(L d)^{-1}$ (Figure 3), a rate that could not be achieved before, the TVA/TAC was always below 0.6 (Figure 2), and propionic acid (and higher VFA) below 500 mg L^{-1}. These data indicate, that stable and effective reactor performance was achieved by the daily supply of the TE cocktail.

Dynamics of trace elements

TE concentrations determined for the maize silage fed were as follows (mg kg TS^{-1}, dataset not yet complete): Fe, 67.8; Mn, 14.6, Al, 20.0, Cd, 0.03, Cu, 5.4, Zn, 120.7, Co, < 0.35, Mo, 0.29, Ni, < 0.7, Se, < 0.35, Cd, 0.48, Sr, 8.7, Pb, < 0.7, As, < 3.5.

Data from the trace element analyses of fermenter contents (Figure 4) separated the elements and their dynamics in two groups. The first group included Co, Mo and Se, the second Ni, Fe, Mn, Al, Cu and Zn. Boron was always below the detection limit and cannot be discussed. Since corresponding trace element concentrations and developments were almost identical in the parallel setups B2-B1-C2 and B3-C1-C3, only one of the series is shown in Figure 4. Se (not shown) behaved similar to Co and Mo, and Fe, Mn, Al and Cu (not shown) similar to Zn and Ni (Figure 4).

As expected, Co, Mo and Se concentrations increased in the TS of the TE-supplemented fermenters whereas they remained unchanged in the controls at very low levels of ca. 0.02, 0.14 and 0.005 mg L^{-1}. The relative increase corresponded to the relative dosage supplied (Figure 4a, b). Bearing in mind the reactor performance dynamics, this suggests that these elements were taken up and immobilized in the methanogenic biomass apparently deficient of Co, Mo and Se. Jarvis *et al.* (1997) similarly concluded that the performance of grass-clover fed biogas reactors was compromised at Co- and Mo levels below 0.02 and 0.08 mg L^{-1}. Co was hence probably the most limiting factor in the reactors but Mo and Se can as well have contibuted to the impeded methanation of the maize silage. The 3 trace elements are essential components of corrinoids, formylmethanofuran dehydrogenase, selenocysteine or formate dehydrogenase (Blaut 1994; Deppenmeier *et al.* 1999; Ferry 1999). Without them, hydrogenotrophic metabolism and the terminal step of methanogenesis cannot work properly. Although the importance of Co for the process with a threshold of 0.02 mg L^{-1} appear to be clear, more data must be obtained for Mo and Se to figure out their relevance and threshold concentrations.

Data shown in Figure 4 suggest that Co and Mo concentrations approach a plateau of ca. 0.4 and 0.3 mg L^{-1}, respectively. We speculate that saturation was approached in the reactor at these concentrations, and active assimilation did not occur anymore. Further analyses are required to proove the assumption.

Unexpectedly and in contrast to the plausible dynamics for Co, Mo and Se, no or almost no increase of Ni and Zn (as well as of Fe, Mn, Al and Cu) was seen in the TE supplemented fermenters (Figure 4c, d), and a significant difference between supplemented and control fermenters in
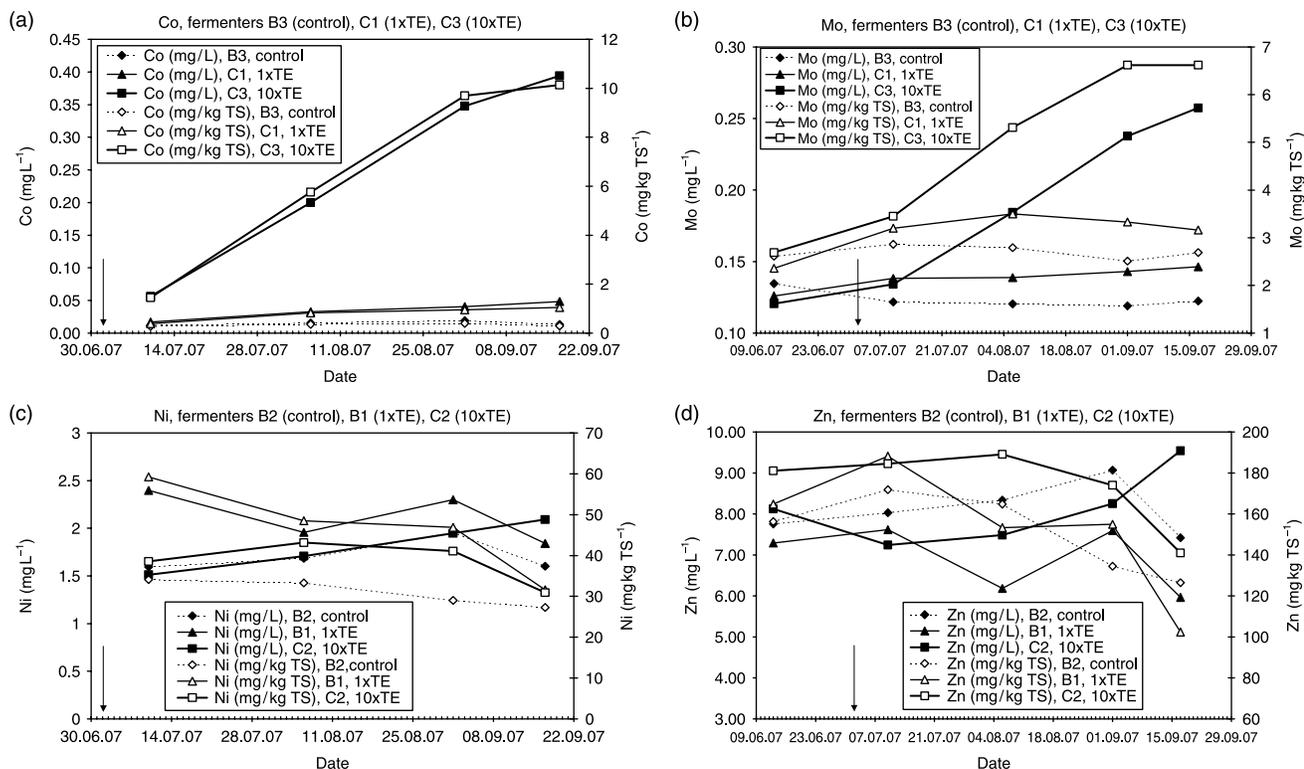
(a)

(b)

(c)

(d)

Figure 4 | Concentration of trace elements in total solids of the fermenters. The arrow marks the date (July 02-2007), supply of the trace element (TE) cocktail was started. The arrow marks the date (July 02-2007), supply of the trace element (TE) cocktail was started.

TE concentrations was not obvious. Comparison of the data from glass and stainless steel fermenters (not shown) confirmed that TE release from fermenter housings did not bias the data. Increased concentrations due to permanent TE supply should have been obtained in the flow-through systems, except that these elements were washed out preferentially or were immobilized in the upper fermenter layers and did not move to the outlet where samples were taken. Hints to explain the behaviour of Ni, Zn, Fe, Mn, Al and Cu are expected from the mass balances. However, this must be subject of a subsequent report since the respective dataset is not yet complete.

Ni, Fe and Zn are essential components of enzymes and cofactors participating in methanogenesis (Blaut 1994; Deppenmeier *et al.* 1999; Ferry 1999). Although we expected that at least Ni of the above mentioned 6 elements reacted by dynamics similarly as seen for Co, Mo and Se, this was not the case, and the relative high concentrations of Ni, Zn, Fe, Mn, Al and Cu determined in the fermenter TS do not favour the assumption that one of these trace elements was process-limiting.

Results obtained in this study show that hydrolysis is not always the limiting factor in anaerobic digestion of organic substrates. It was concluded in other studies that TE availability is a key factor in co-digestion with sludge (Noyola & Tinajero 2005) in mono-digestion of grass-clover (Jarvis *et al.* (1997), and even in co-digestion of manure and energy crops (Preissler *et al.* 2007). Taken together with our results, this suggests that TE availability is a key issue in biogas production. Particularly if substrates with low Co, Mo, Se and Ni contents are digested, TE concentrations in the fermenter should be determined at least 2 times per year to provide the basis for a specific supplementation, if this appears to be necessary.

CONCLUSIONS

Results of this study showed that process instability in biogas production from maize silage as the sole substrate can be due to deficiency of trace elements (TE) already at a

relatively low organic loading rate (OLR). Methanogens appear to be the most affected. The TVA/TAC ratio proved to be the most reliable parameter to indicate early process instabilities leading to acidification. Values above 0.5 are indicative of process instability. Monitoring propionic acid as the sole stability indicator is not sufficient, since low values can be obtained obtained at severe process failure.

We could demonstrate that efficient long-term methanation of maize silage is only possible if essential TE are provided. Supplementation should be designed according to the actual needs determined. Without TE supply, efficient biogas production without reactor acidification may be possible for ca. 8 months. TE availability determines the length of the time frame being at disposal for a stable process. This knowledge is essential particularly for biogas plant operators envisaging mono-digestion of silages or facing periodical lack of manure.

Although claimed in many reports, hydrolysis was obviously not limiting in the studied process. The most limiting element in long-term mono-digestion of maize (and grass) silage appears to be cobalt, but the data obtained suggest that molybdenum and selenium should also be supplemented. A higher OLR and stable performance was achieved in the TE supplemented variants.

ACKNOWLEDGEMENTS

REFERENCES

Bauer, C., Korthals, M., Gronauer, A. & Lebuhn, M. 2008 Methanogens in biogas production from renewable resources– a novel molecular population analysis approach. In: Proceedings of the Vth *International Symposium on Anaerobic Digestion of Solid Wastes and Energy Crops*, Hammamet, Tunisia, May 25–28, 2008.

Blaut, M. 1994 Metabolism of methanogens. *Antonie Van Leeuwenhoek* **66**(1–3), 187–208.

De Haas, D. W. & Adam, N. 1995 Use of a simple titration procedure to determine H_2CO_3 alkalinity and volatile fatty acids for process control in wastewater treatment. *Water SA* **21**(4), 307–317.

Deppenmeier, U., Lienard, T. & Gottschalk, G. 1999 Novel reactions involved in energy conservation by methanogenic archaea. *FEBS Lett.* **457**(3), 291–297.

Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung (German Standard Methods for The Examination of Water, Wastewater and Sludge) 1981 Wasserchemische Gesellschaft, Fachgruppe in der GDCh/in Gemeinschaft mit dem Normenausschuss Wasserwesen (NAW) im DIN e.V. (eds.), Weinheim, Wiley-VCH.

Die chemische Untersuchung von Futtermitteln (Chemical analyses of feedstuffs) 1997 Methodenbuch Band III, Mit 4. Ergänzungslieferung, VDLUFA-Verlag, Darmstadt.

Effenberger, M., Lehner, A. & Gronauer, A. 2008 Performance Figures of Agricultural Biogas Plants for (Co-)Digestion of Energy Crops. In: Proceedings of the Vth International Symposium on Anaerobic Digestion of Solid Wastes and Energy Crops, Hammamet, Tunisia, May 25 to 28, 2008, submitted.

Ferry, J. G. 1999 Enzymology of one-carbon metabolism in methanogenic pathways. *FEMS Microbiol. Rev.* **23**(1), 13–38.

Greenfield, P. F. & Batstone, D. J. 2005 Anaerobic digestion: impact of future greenhouse gases mitigation policies on methane generation and usage. *Water Sci. Technol.* **52**(1–2), 39–47.

Gronauer, A., Schlattmann, M., Speckmaier, M. & Metzner, T. 2006 Konzeptionierung, Erstellung und Betrieb einer Versuchsfermenteranlage zur Bearbeitung von Fragestellungen im Bereich Inputmaterialien und Mikrobiologie bei landwirtschaftlichen Biogasanlagen. Bayer. Landesanstalt für Landwirtschaft, ILT-Forschungsbericht, 198 pp. http://www.lfl.bayern.de/ilt/umwelttechnik/17837/bmbf_high.pdf

Jarvis, Å., Nordberg, Å., Jarlsvik, T., Mathisen, B. & Svensson, B. H. 1997 Improvement of a grass-clover silage-fed biogas process by the addition of cobalt. *Biomass Bioenergy* **12**(6), 453–460.

Noyola, A. & Tinajero, A. 2005 Effect of biological additives and micronutrients on the anaerobic digestion of physicochemical sludge. *Water Sci. Technol.* **52**(1–2), 275–281.

Preissler, D., Lemmer, A., Oechsner, H. & Jungbluth, T. 2007 Fermentation of forage maize silage without liquid manure. *Landtechnik* **62**(3), 160–161.

Verstraete, W., Morgan-Sagastume, F., Aiyuk, S., Waweru, M., Rabaey, K. & Lissens, G. 2005 Anaerobic digestion as a core technology in sustainable management of organic matter. *Water Sci. Technol.* **52**(1–2), 59–66.