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Shifting of the microbiology in biogas fermenters digesting renewable resources – causes and consequences

Conventionally, chemical analyses of fermenter contents are performed for the indirect assessment of the microbiological status but results are often difficult to be interpreted. Analysis of the microbiological fermenter composition can help to identify problems early and to specify them. Methanogenic archaea are often the primary limiting factor in biogas production. With molecular biological methods the population composition of methanogenic archaea has been determined in monodigestion of grass and maize silage. Methane productivity was highly related to the concentration of methanogens. The composition of the methanogenic population varied at different conditions.

Keywords
Biogas, microbiology, archaea, methanogens

Abstract
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In mono-fermentation of renewable resources performance problems have been observed, but reasons were not found immediately. Common causes of process disturbance are excessive feeding, micronutrient deficiency or toxicity of substrates. Using examples we will show how population analysis allows to identify deficiencies which was not possible by conventional chemical and physical methods. The presented molecular biological methods allow to analyse the microbiological events. This can serve the plant operator as an additional basis for decision making.

In methane production various microorganisms are involved and need to interact optimally with each other so that a maximum biogas production can be achieved. Basically the Bacteria accomplish the first steps of the fermentation of organic matter, the last step, methanogenesis, is performed exclusively by certain Archaea [1]. The methanogenic Archaea, which perform the last step of biogas production, are particulary sensitive and thus were examined as a priority.

Operation of biogas fermenters and determination of the methanogenic Archaea

In the frame of the joint project IBMN [2], financed by the Agency for Renewable Resources, experiments were conducted in six fermenters (B1-B3; C1-C3). Fermentations were run at mesophilic (38 °C) and thermophilic (55 °C) conditions with maize- or grass silage as mono-substrates in single-stage 32 L flow-through fermenters with daily feeding. Biogas production and composition and the concentration of methanogens were analysed. In addition macro- and micro elements were quantified at distinct stages. Organic acids, pH- and TVA/TIC-values also were determined. Dependent on the process status a defined amount of trace elements (TE) was supplemented [2].

To analyse the composition of methanogens by polymerase chain reaction (PCR) based techniques, a selected gene segment of the key enzyme for methanogenesis was amplified. The increased DNA was directly cloned and sequenced. Methanogenic Archaea were identified by comparison of the sequences with the database of NCBI (National Center of Biotechnology). Alternatively, the amplified (double stranded) DNA was converted by the SSCP-method into single stranded DNA [2; 3; 4] which folds in a sequence-depending structure. In the following electrophoresis DNA fragments moved at different speed (**figure 1**). Bands were cut from the gel, DNA was eluted and processed as described above.

The concentration of the methanogenic Archaea in the sample was determined by quantitative PCR (qPCR) [2; 3; 5]. The qPCR method is based on PCR, but an additional dye is supplied

to the qPCR-mixture. This dye is fluorescing in different intensities correlating with the amplified amount of DNA present. Comparison with a defined DNA standard enables to quantify the methanogenic DNA.

Trace element deficiencies in mono-digestion of maize silage

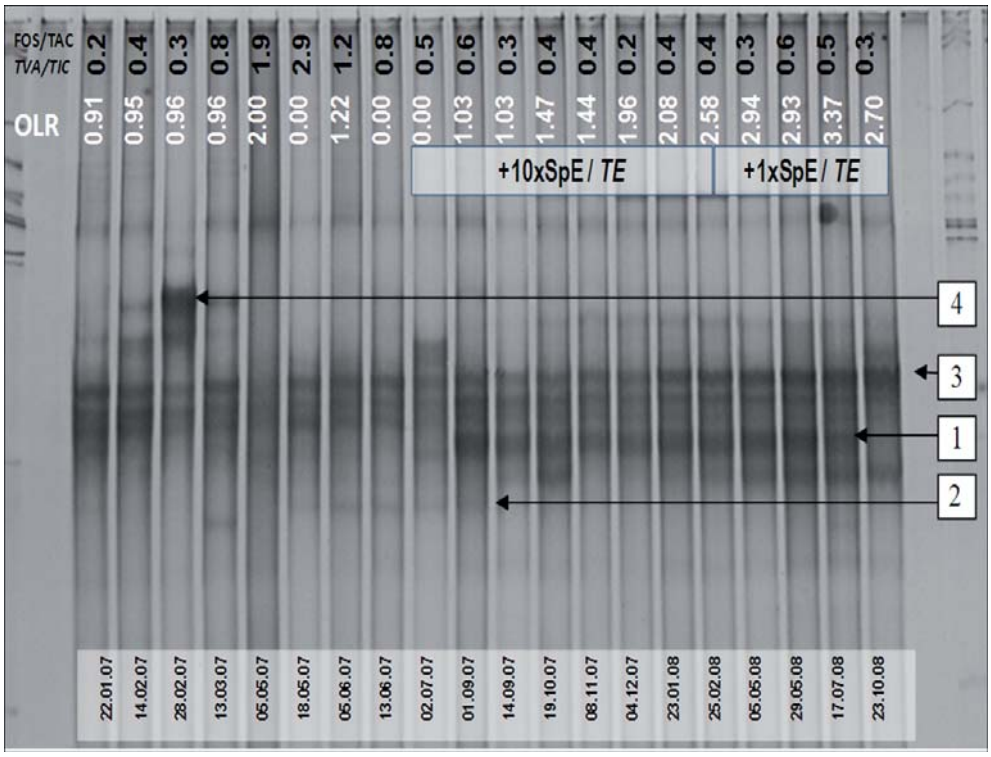
The SSCP-profiles (**figure 1**) indicated that the archaeal populations were differently composed during the experimental period. At higher feeding-rates and low TVA/TIC-values and with trace element supplementation two intense bands appeared (arrows 1 and 3). In contrast, in acidified fermenters a faint fragment was detected in the lower part of the gel (arrow 2). Band intensities were also decreased in acidified fermenters. Band 4 could only be detected once, it was hence considered as an artefact.

The composition of the populations of methanogenic Archaea was dependent on the fermenter condition (**table 1**). At increased acetate concentrations and higher TVA/TIC-values, certain members of *Methanomicrobiales* were predominantly observed. Dominance of *Methanosarcinales* with *Methanobacteriales* appears to indicate a good fermentation process. Because of their typical occurrence in the high-throughput fermentation they may be suitable as bioindicators. The diversity of methanogenic Archaea populations was different at mesophilic and thermophilic conditions: The mean of the Shannon-indices was 1.4 at mesophilic and 0.6 at a thermophilic conditions. The lower diversity can be a reason for a higher susceptibility to stressors at thermophilic conditions.

In the fermentation of maize-silage an interdependence between the concentration of the methanogens and methane productivity rate was recognised (**figure 2**). The first fermenter acidification was caused by a low concentration of cobalt (< 0.03 mg • L^{-1}) and the second by lack of sodium (< 10 mg • L^{-1}). Both are essential elements for methanogens. After balancing the shortfalls of cobalt, methane productivity and the concentration of methanogens increased (**figure 2**). The decreasing concentration of the methanogens since February 2009 indicated the stress situation induced by sodium deficiency. Methanogens could not increase anymore but methane productivity still raised and then rapidly broke down. The process deficiency could be detected early by the development of the Archaea concentration.

Analysis of substrates used showed for some charges of maize silage a cobalt concentration < 0.01 mg • kg_{FM}^{-1} (in grass silages the mean cobalt concentration was about 0.06 mg • kg_{FM}^{-1}). The mean sodium concentration was in maize silage about 20 mg • kg_{FM}^{-1} (in grass silage it was about 150 mg • kg_{FM}^{-1}), but in some maize silage charges it was below 10 mg • kg_{FM}^{-1}. Other important trace elements like nickel, iron, selenium and molybdenum did obviously not fall below minimum required concentrations, thus quantitative threshold values could not be defined. In practice, primarily a lack of cobalt and sodium can be expected for mono digestion of maize silage. Fermenter

Fig. 1



SSCP-gel time series of a digester with monofermentation of maize silage (mesophilic) (TE, trace elements)

contents should therefore be analysed during the fermentation of trace element poor substrates from time to time to prohibit a lack of trace elements and process breakdown.

Ammonium toxicity in grass silage

With grass silage as the sole substrate the loading rate could not be increased above 2.5 g • L_{Fc}^{-1} • d^{-1} under mesophilic and not above 1.5 g • L_{Fc}^{-1} • d^{-1} under thermophilic conditions [2]. Free ammonia is a decoupler of ATP-synthesis and is toxic for all organisms. The shift of the chemical balance between ammonium and ammonia at higher temperatures in favor to free ammonia is probably the major reason for the problems with the digestion of grass silage especially at thermophilic conditions. The calculated ammonia concentrations were mostly above 1 g • L^{-1} (up to 2.7 g • L^{-1}). At these conditions the process was strongly inhibited. At mesophilic conditions, ammonia concentrations were mostly between 0.1 und 1.1 g • L^{-1}. At an increased loading rate, methane yield was higher at mesophilic than at thermophilic conditions. However, a clear relationship between the concentration of methanogens and ammonia caused process inhibition could not be found.

In fermentation of grass silage, inhibition of the process was observed although the TVA/TIC-value stood below the critical threshold of 0.7 [1]. Probably other steps in the biogas process, such as hydrolysis, acidogenesis and acetogenesis, were inhibited primarily. Particularly syntrophic bacteria which can gain only very few energy with their metabolism may be particularly sensitive to the decoupler of ATP-synthesis. Plant operators should pay more attention to the ammonia concentration when N-rich substrate is digested. Feeding stuff with a high protein content (trefoil, chicken dung) should be fed at a relatively low loading rate. Increasing the C/N ratio e.g. with maize can help to minimize ammonia toxicity.

Conclusions and perspectives

With molecular biological analysis of the microbial community it was possible to detect typically occuring Archaea at distinct fermenter conditions. Some identified microorganisms seem to be adequate as a biomarker, and their increase can inform the operator early of a beginning process inhibition. These phenomena are currently being analysed. A standardised application of the microbial analysis is possible and seems reasonable. The example of trace element deficiency demonstrated a dependency of the Archaea concentration and their population structure. There are also other factors such as free ammonia which can induce process inhibition. Tensides, too high stirring frequency and antibiotics are possible causes of process inhibition. Tensides promote foam formation and are antimicrobial agents, because they destroy the lipid double layer of the cell membrane. They also can destroy the anchorage of cells to the substrate and prevent substrate utilization. Such substances are used in animal husbandry. Intensive stirring also can destroy the sensible syntrophies. Additional research is required to shed more light on these fields.

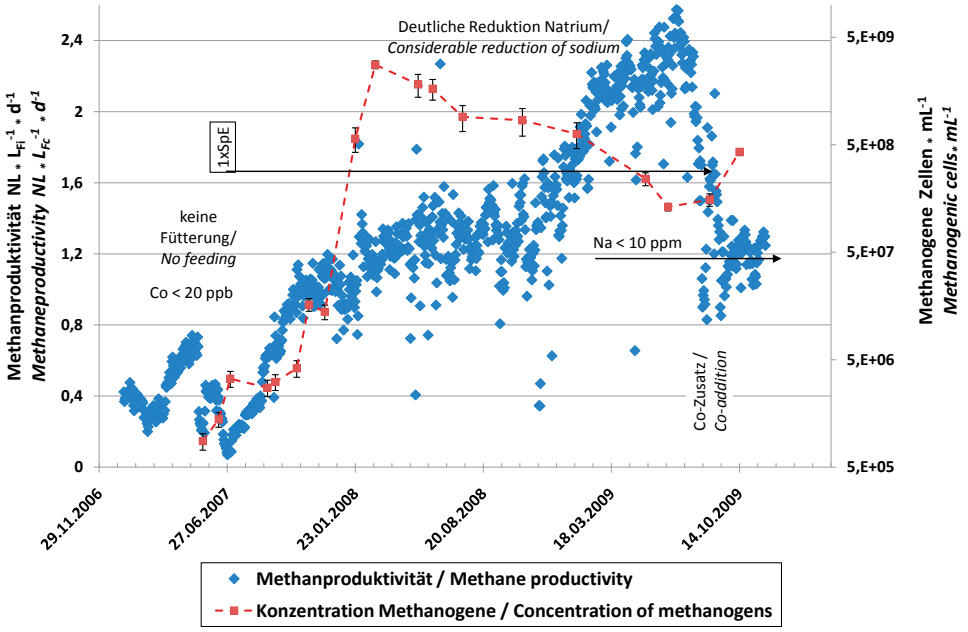
Table 1

Methanogenic archaea and characteristic data from monodigestion (mesophilic) of maize

Datum (Fermenter)/ Date (fermenter)	**25.02.08 (B1)[1)]**	**17.07.08 (B1)[1)]**	**26.01.07 (B2)[2)]**	**13.06.07 (B2)[1)]**	**13.06.07 (B2)[2)]**	**17.07.08 (B2)[1)]**	**17.7.08 (B2)[2)]**	**18.05.07 (C3)[1)]**	**23.10.08 (C3)[1)]**
pH [-]	7.7	7.9	8.0	7.7		7.5		6.3	7.6
FOS/TAC [-] *TVA/TIC [-]*	0.48	0.45	0.2	**0.9**		0.5		**2.9**	0.3
OLR [g oTS • L^{-1} • d^{-1}] *[g VS • L^{-1} • d^{-1}]*	3.6	4.5	0.9	0		1.1		0	2.6
Acetat [mg • L^{-1}] *Acetate [mg • L^{-1}]*	n.d.	1010	207	**2900**		601		**2621**	1087
M. bacteriales [%]	35	15	22	13	38	56	30	0	68
M. microbiales [%]	18	9	7	**87**	**46**	11	16	**69**	8
M. sarcinales [%] *(M.saetaceae) [%]*	47 (18)	67 (0)	70 (0)	0	15 (0)	33 (0)	45 (2)	31 (0)	24 (0)
Unbekannte Klasse I [%] *Unknown class I [%]*	0	9	0	0	0.2	0	0	0	0
Unbekannte Klasse II [%]/ *Unknown class II [%]*	0	0	0	0	0	0	9	0	0
Analysierte Klone/ *Analysed clones*	23	26	20	13	28	14	49	13	21

[1)] Direkte PCR Klonierung/*Direct PCR-cloning*.
[2)] PCR-SSCP Klonierung; unterstrichene Werte bezeichnen dominierende Populationen im Regelbetrieb. Fette Werte bezeichnen dominierende Populationen aus versäuerten Fermentern; Prozentwerte wurden unter Berücksichtigung von relativen Bandenintensitäten ermittelt; n.d: nicht detektiert.
PCR-SSCP-cloning; Underlined data mark dominant populations from regular operation. Bold data mark dominant populations from acidification. Percentage quotation was calculated from relative band intensities; n.d: not detected.

Fig. 2



Methane productivity and concentration of methanogenic archaea in the course of experiment (maize monodigestion, mesophilic, fermenter B1)

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