Lebuhn, Michael and Gronauer, Andreas

Microorganisms in the biogas-process – the unknown beings

The importance of biogas production from agricultural products has recently largely increased in Germany. Biogas production is expected to cover a significant portion of energy supply. Consequently, research on the true motor of methane production, the microorganisms in the fermenter, was intensified. Important results are to be implemented by engineering to optimize the processes. In the current article, latest insights from micro- and molecular biology research are presented modifying established views on dominant microbial transformation pathways of biomass to biogas. Consequences for specifically optimized process design are discussed.

Keywords
Microbiology, molecular biology, biogas, methanogenesis, trace elements, renewable resources

Abstract
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2 figures, 7 references

Already 2,000 years ago, organic matter has been transformed by the microbiological process of anaerobic digestion to biogas, heat and digestate yielding energy and fertilizer. With the shortage of fossil energy carriers and the price explosion for energy supply biogas is further gaining attraction and importance nowadays. At the same time biogas can contribute significantly to the mitigation of greenhouse-gas net emissions when applied appropriately.

The majority of the currently ca. 4,000 biogas plants in Germany is operated with renewable resources (RR) because of the higher energy yield, frequently in co-digestion with animal manure. Maize, grass and cereal whole plant silages are the most important RR fed.

In order to achieve economically and ecologically reasonable operation of RR-biogas plants, maximum efficiency must be obtained. For this pupose, the process performing microbial community must be optimally composed and active, and for optimum microbial performance, a process-specific optimally designed engineering framework is essential. However, microbiology of biogas production from RR and microbiological possibilities of process optimization are insufficiently investigated. In parts knowledge has been adopted from waste and wastwater treatment without further proof.

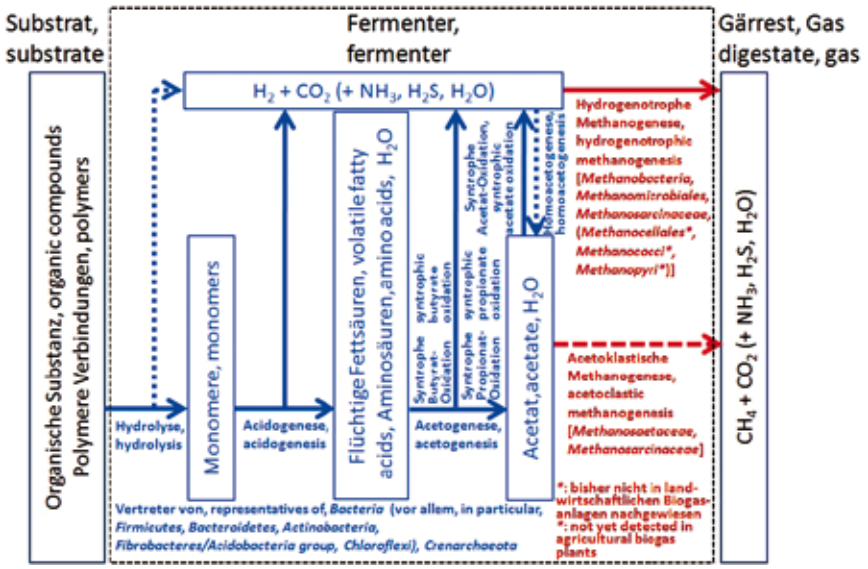
Not until the advent of molecular methods we are aware of the existence of three domains of life (*Archaea*, *Bacteria, Eukaryota*). The methane producing microorganisms rank among *Archaea*. The upstream conversion steps of organic matter are primarily carried out by *Bacteria* **(Fig. 1)**. Less than 1 % of the microorganisms participating in the anaerobic digestion process is known, and cultivation which would alleviate studying these microorganisms is particularly difficult in the anaerobic environment.

Moreover there is almost no experience in long-term ope-

ration of full-scale RR biogas plants. Most plants are operated as „black boxes“ after the principle of „trial-and-error“. Accordingly reports accumulate on process disturbance, problems in start-up or havaries particularly when RR are fed as single substrate (mono-digestion). For prevention or remedy some operators add “magic powders” as process additives according to the slogan “adding much helps much”. The consequences can be fatal for enterprise and environment.

Because of these reasons research on microbiology and on reasons of process disturbances has been strongly intensified also at the Institute for Agricultural Engineering and Animal Husbandry of the LfL. Some relevant results have already emerged which do not fit the established text book opinions on the dominant microbiological transformation pathways of biomass to biogas.

Fig. 1



Anaerobic degradation of organic matter (renewable resources, silages) in one-phase biogas process with participating systematic groups (taxa) of microorganism

At higher organic loading rate with renewable resources the pathway leading over syntrophic acetate oxidation and hydrogenotrophic methanogenesis is dominating

According to the current textbook opinion about 70 % of the biogas methane is formed from acetate and its cleavage (acetoclastic pathway) and 30 % from the reaction of hydrogen with carbon dioxide (hydrogenotrophic pathway) **(Fig. 1)**. This distribution is typical of high-rate reactors employed in wastewater purification with long retention times of the microorganisms (which can even be decoupled from the liquid retention time) and low acetate concentrations. In this environment, obligately acetoclastic methanosaetae can win through due to their high affinity to acetate although they can proliferate only slowly [1].

At higher organic loading rate with typically higher acetate concentration (> 60 mg * L^{-1}) the more quickly proliferating methanosarcinae take advantage. They are more versatile and can produce methane by the acetoclastic as well as by the hydrogenotrophic pathway. According to our experience this is the case (at least with maize silage) in the typical continuously stirred tank reactors already at an organic loading rate (OLR) of ca. 1.5 g VS * (L * d)$^{-1}$, probably mainly because methanosaetae are thinned out in flow-through operation due to their relatively long doubling time, and finally are diluted out completely.

In experiments with maize silage (mono-feeding) methanosarcinae were dominant at favourable environmental conditions (in particular optimum trace element supply, see below) and acetate concentrations up to 3 g * L^{-1} even at an OLR of 4 g VS * (L * d)$^{-1}$ [2, 3]. At higher concentrations of volatile fatty acids (VFA) due to lack of trace elements (see below), nearly only obligate hydrogenotrophic archaea (predominantly representatives of the orders *Methanobacteriales*, *Methanomicrobiales*) were recovered. In turn, methanosarcinae decreased strongly [2, 3]. Apparently acetate was barely split to CH_4 and CO_2, and methane was formed virtually exclusively via the hydrogenotrophic pathway **(Fig. 1)**. Hydrogenotrophic methanogens were dominating also in a full-scale biogas plant fed with maize silage and cattle manure [4]. The portion of methane formation from H_2 and CO_2 in biogas production obviously has been strongly underestimated until now.

Particularly interesting in this context are findings that acetate is increasingly transformed to H_2 and CO_2 via syntrophic acetate oxidation (SAO, **Fig. 1**) in the absence of methanosaetae as well as at higher VFA contents and higher ammonia concentrations (as calculated from ammonium) in the fermenter. Correspondingly methane is formed in accordance with our findings predominantly hydrogenotrophically [5]. The transfer of CO_2 and particularly H_2 from the producing SAO bacteria to the consuming hydrogenotrophic methanogenic archaea is running efficiently only if if both partners are located in very close vicinity. In this situation the methanogens can render possible the thermodynamically unfavourable production of H_2 and CO_2 by the SAO by withdrawing the products from the reaction equilibrium [6]. A consequence thereof is e.g. that on the one hand stirring is necessary to expose fresh substrate surfaces to the degraders, but on the other hand hydrogen transfer between the partners should not be disturbed by too intensive stirring. The whole process could break down as a consequence.

Such „syntrophies“, being similar to symbioses, have formed particularly in anaerobic environments in the evolution because the possible energy gain in anaerobic substrate degradation is considerably lower than with oxygen as terminal electron ac-

ceptor (aerobic respiration). Particularly the above mentioned oxidation of certain VFA can only be performed syntrophically above the limit of thermodynamically possible energy gain [6]. As a consequence the whole process is extremely efficient with respect to energy yield. Single steps, however, can proceed only very slowly without specific conditioning improvements. Our research emphasis is devoted to these unfavourable reactions, the „process bottlenecks“. Here the process chain can be optimized by engineering means.

Methanogenesis needs certain trace elements in an optimum concentration range

Basic microbiological research on methane formation could meanwhile reveal unique reaction pathways and the requirement of unusual trace elements such as cobalt (Co), nickel (Ni), molybdenum (Mo) and selenium (Se) in sub-processes of the anaerobic degradation chain. The heavy metals Co, Ni, Mo and Se are central components in the metabolism pathways of methanogenic archaea (e.g. electron transport chain, ATP production, methane formation, H_2 and CO_2 uptake, acetate cleavage, utilisation of methyl groups) or of essential cofactors involved in the correct and efficient function of the pathways.

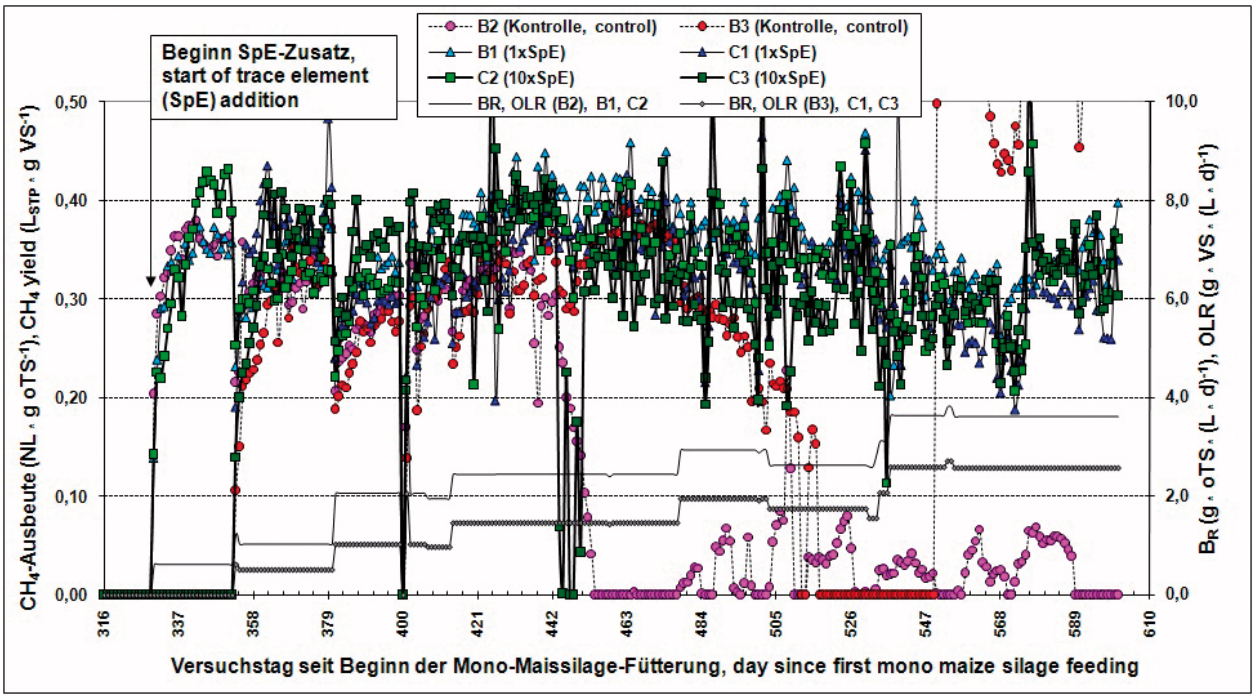
Other elements such as boron, vanadium, aluminium or tungsten are not always essential or their importance is disputed. Zinc and iron are important but their concentration in RR digesters is typically sufficient to sustain the process – particularly in co-digestion with animal manure. For other heavy metals such as arsenic, cadmium, quicksilver, silver and lead only toxic effects have been described hitherto.

Co, Ni, Mo and Se can also become toxic if they are present in too high concentration. This must be considered particularly in the context of further use of the digestate, e.g. if fertilizer or biowaste regulations are concerned. Specific optimum concentrations for the single trace elements have to be adjusted between minimum requirements and a sufficient or eventually maximum concentration.

It is known in the area of wastewater treatment for a long time that sufficient supply with trace elements is essential for effective methanation in the reactor. However, deficiencies in trace element supply has rarely been a topic considering the composition of the substrate streams, rather eventual toxic effects that can arise from (further use of) the sewage sludge. It must be considered in this context that the concentration of heavy metals increases in the digestate due to the mass loss occurring in biogas production. Heavy metals typically do not migrate into the gas phase.

The biogas scene was astonished when first reports appeared dealing with insufficient process performance or even havaries of RR biogas plants. After hardly a year of operation on average biogas production, methane content of the bioagas and degradation of volatile solids were reduced and the concentration of VFA strongly increased in the fermenter. A restart after transient feeding stop was successful only for a short time period.

Fig. 2



Methane yield and organic loading rate (OLR) of the 6 acidified/starved mono maize fermenters B1 – C3 after addition of a trace element (SpE) cocktail and feeding restart

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Imitated in a long-term trial in six 32 L laboratory flow-through fermenters in maize-mono operation the same phenomena arose. The fermenters acidified about 200 d after the start-up with our standard inoculum and daily feeding of maize silage at a relatively low OLR (< 2.4 g VS * (L * d)$^{-1}$). Reducing the feeding and feeding interruptions were not successful. Feeding was therefore stopped until excessive VFA were degraded to normal operation level. Since we supposed that lack of trace elements for methanogenic archaea was the reason of the process breakdown, a trace element cocktail was compiled [7] and added in different concentration to 4 of the fermenters with the maize silage when feeding was re-started (**Fig. 2**). Process breakdown was soon observed again for the 2 control fermenters (without TE addition) whereas with TE supply stable high performance was obtained (**Fig. 2**).

The causal chain is not yet completely analysed but first results point clearly to deficiencies of the "suspect" elements Co, Ni, Mo and Se. There is evidence meanwhile that lack of Co was the main reason for breakdown of methanogenesis.

According to ICP-OES or ICP-MS analyses of fermenter contents deficiency symptoms were clearly identified below ca. 20 µg Co * L^{-1} (ca. 300 µg Co * kg DM^{-1}). A concentration of about 50 µg Co * L^{-1} (ca. 750 µg Co * kg DM^{-1}) appears to be reasonable for stable operation. Since for selenium the corresponding concentration was about 5 times less, we recommend to adjust a concentration of about 10 µg Se * L^{-1} (ca. 150 µg Se * kg DM^{-1}) in the fermenter. For molybdenum the corresponding concentration was 10 fold and for nickel 40 fold higher than for cobalt. **Figure 2** also clearly shows that more (10 fold TE supply) did not lead to better performance. Methane yields were identical for both (1x, 10x) TE supply variants. It is solely important to adjust trace element concentrations within the optimum ranges!

Analyses of the fed maize silage pointed to negative bilances and deficiencies in long-term operation. The actual input with the silage was not sufficient to adjust the required levels in flow-through. The acidification can be explained in the following way: hydrolysis, acido- and acetogenesis were not affected and still active but the formed fatty acids could not be degraded due to the breakdown of terminal methanogenesis which was caused by trace element deficiency. As a consequence, VFA accumulated and an "acid jam" developed. Re-acidification after the re-start could only be prevented by reactivation of methanogenesis withdrawing the acids.

Regular analyses are important

According to other reports, co-fermentation with animal manure (or organic fertilizer) is not necessarily sufficient to adjust the desired trace element concentrations. Moreover, renewable resources to be fed can differ significantly in trace element and heavy metal contents according to their character, composition and origin, e.g. due to different site characteristics.

Consequently, regular analyses of the fed substrates (particularly when substrates and charges change) are important as well as a trace element balance and at about monthly analysis of the fermenter content in order to identify deficiencies in due time. This enables to define a well balanced trace element supplementation according to the actual fermenter state.

Furthermore it is suggested to analyse the fermenter status by molecular biology tools in order to assess if the desired composition of the microbial biocenosis and an adequate abundance (particularly of methanogenic archaea) is present. Results can indicate changes pointing out forthcoming difficulties in operation.

Future prospects

Concerning the analysis of methanogenic archaea, a number of questions still need to be adressed:

- Is the composition of the microbial population dependent on the nature of the fed substrates?
- Are organisms as detected by analysis of DNA also active in the fermenter according to their determined numbers?
- Can „biological markers" be identified for a good and a bad fermenter status, and is it sufficient to determine these marker organisms by quantitative PCR analysis?
- Which organisms are key players in important processes upstream of methanogenesis, and which organisms should be monitored in quantitative routine analysis?

Further important questions need to be adressed concerning the supply with macro- and micro-elements:

- Which (micro)elements are essential in which concentrations for which processes, and are these concentrations compatible with environmental constraints or requirements?
- Wich sustainable concepts can contribute to detoxification of ammonia?

Currently a process model implementing bilancing of trace elements is being developed. Optimum supply fo the process considering substrate contents is being identified on the basis of solid analyses. Concomitantly environmental requirements are being integrated. This approach should allow for an appropriate and sustainable agricultural application of the digestate.

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Authors

Dr. Michael Lebuhn is senior scientist at the Bavarian State Research Center for Agriculture, Institute for Agricultural Engineering and Animal Husbandry (LfL) and is leading the microbiology group in the LfL key activity biogas. E-mail: michael.lebuhn@lfl.bayern.de

Dr. Andreas Gronauer is coordinating the LfL key activity biogas and the research area environmental engineering in land use. Adress: Vöttinger Str. 36, 85354 Freising. E-mail: andreas.gronauer@lfl.bayern.de