The valuation of malnutrition in the mono-digestion of maize silage by anaerobic batch tests

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ABSTRACT

Anaerobic digestion is a technology which is used to produce methane from organic solids and energy crops. Especially in recent years, the fermentation of energy crops has become more and more important because of increasing costs for energy and special benefits for renewable energy sources in Germany. Anaerobic bacteria require macro and micro nutrients to grow. Absence of these elements can inhibit the anaerobic process significantly. In particular mono-substrates like maize or certain industrial wastewater often cannot provide all required nutrients. For this reason this research investigates the influence of substrate and trace elements on anaerobic digestion in detail. Different agricultural anaerobic biomasses are analysed with special regard to their trace element content. Based on these results, the influence of three trace elements (iron, cobalt, and nickel) on anaerobic digestion was studied in anaerobic batch tests at different sludge loading rates and for different substrates (maize and acetate). Biogas production was found to be 35% for maize silage and up to 70% higher for acetate with trace element dosage than in the reference reactor.

Key words | digestion, energy crops, micro nutrients, trace elements

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INTRODUCTION

The renewable resources act was established in Germany in 2000. Since then, with governmental aid a lot of rural biogas plants were built, in which energy crops are used as substrate for the anaerobic digestion. In most cases energy crops were mixed with manure or other co-substrates to enhance the biogas-production and to stabilize the pH. By contrast, mono digestion plants use energy crops in mono-digestion only. Experiences with these plants showed that mono-digestion is more sensitive than digestion of a substrate-mix.

The anaerobic metabolism takes place in four steps with specific enzymes and bacteria: hydrolysis, acidification, acetonogenesis and methanogenesis. Enzymes are very essential for the anaerobic metabolism. As enzymes are specialised for a certain degradation process, a lot of different enzymes are necessary for the anaerobic degradation (Fuchs 2007). Enzymes contain metals like cobalt, nickel, iron, zinc, molybdenum, and tungsten. Therefore, a lack of these elements influences the conversion considerably (Osuna *et al.* 2002; Zandvoort 2005). If any required enzyme is limited, the total process may be disturbed. High temperature, pH value variation or the presence of inhibiting ions like cyanids, or heavy metals can influence the anaerobic processes negatively. But enzyme activity can also increase as a result of other inorganic salts (Mudrack & Kunst 2003).

The DWA (German Association of Water, Waste and Wastewater, formerly ATV (1990)) pointed out that in particular a lack of trace elements for methanogenic bacteria, which convert hydrogen and organic acids to biogas, can reduce the total anaerobic degradation rate. The kinetics of the methanogenic enzymes is well analysed. Important trace elements are for example cobalt, nickel, iron, zinc, and molybdenum.

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In mono-digestion plants, the availability of nutrients is insufficient as these substrates do not provide adequate amounts of trace elements. Table 1 shows the composition of three typical energy crops in comparison to the nutrient demand of micro-organisms. A lack of certain elements like calcium and iron can destabilize the anaerobic digestion process and reduce biogas production.

The main objective of this work is to investigate the effect of trace elements on anaerobic digestion of maize silage (corn cob mix, CCM) in detail. For this reason, the biomass concentration of different anaerobic digestion plants is analysed and the influence of nutrient dosage is investigated in anaerobic batch tests.

METHODS

Substrates and inoculum

The content of different biomasses from several anaerobic plants was analysed first by ICP-MS (Inductively Coupled Plasma Mass Spectrometry) which is a type of mass spectrometry that is highly sensitive and capable of the determination of a range of metals.

Ten different anaerobic digestion plants are selected to investigate the connection of plant operation, substrate characteristics and trace element concentration–one municipal sludge digester, one co-digestion plant, five mono-digestion plants with energy crops as substrate only, and three agricultural co-digestion plants with energy and manure as substrate (Figure 1).

Anaerobic batch tests

The automatic anaerobic batch test (AABT) is an option not only to determine the kinetic parameters like biomass activity and degradability of substrate, but also to ascertain the influence of inhibiting compounds and the effect of nutrient or micro-nutrient deficiencies. The concept of the AABT is based on a specific CH_4 activity test (SMA-Test), in which the inoculum and substrate are mixed together in a batch reactor. During the AABT the gas production is measured online and recorded.

The anaerobic batch tests regarding trace elements is carried out in laboratory scale ($V = 1{,}000$ mL) with maize silage and acetate as substrates at different sludge loading rates. As the methanogenic micro-organism demand is

Table 1 | Nutrient requirement of anaerobic bacteria in comparison to their content in different mono substrates

	Calculated nutrient requirement	Nutrient content of average data from substrate: Maize silage[*,†]	Rye crops[*,‡]	Wheat crops[*,‡]
	[mg/kg COD_{in}]	[mg/kg COD]	[mg/kg COD]	[mg/kg COD]
Nitrogen	7,410	14,737	26,365	18,845
Phosphorus	1,710	2,411	3,925	4,164
Potassium	1,140	12,823	2,770	4,505
Calcium	456	2,082	346	352
Magnesium	342	1,534	1,270	932
Iron	205	111	28	56
Zinc	7	38	39	36
Manganese	2	32	28	35
Copper	1	4	5	4
Nickel	11	<1[§]	–[‖]	–[‖]
Cobalt	9	<1[§]	–[‖]	–[‖]
Molybdenum	7	<1[§]	–[‖]	–[‖]

[*]Keymer (2004).
[†]Egert (2005).
[‡]Schiemann (1948).
[§]Own data.
[‖]No data available.

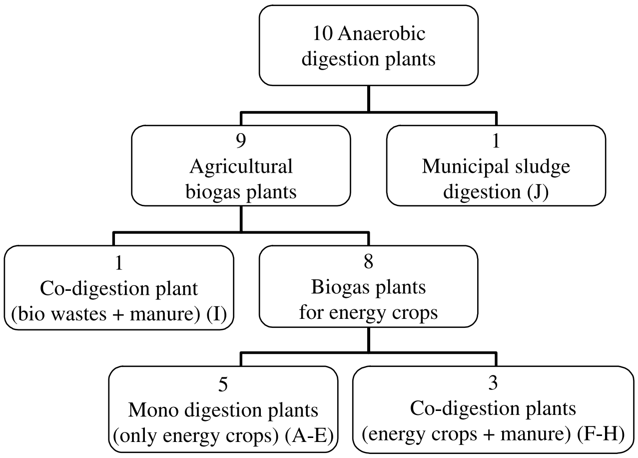

Figure 1 | Investigated anaerobic digestion plants.

regarded as decisive for the anaerobic degradation, their demand is used to determine the trace element dosage concentration. These micro-organisms have comparatively high demands for iron, nickel, and cobalt (Paulo *et al.* 2003). For this reason, these elements are examined in following investigations in particular. The dosage of micro nutrients is defined according to the recommendations of Lettinga *et al.* (1996) (Table 2).

Depending on sludge loading rate, different element quantities are added to the biomass as $FeCl_2{\cdot}4H_2O$, $NiCl_2{\cdot}6H_2O$, and $CoCl_2{\cdot}6H_2O$ in mix or separately. The element concentration in the batch reactor based on volatile suspended solids of biomass is summarised in Table 3.

RESULTS

Biomass analysis

Figures 2 and 3 show the ICP-MS-analysis results. The criteria for the order in use are the substrate composition of inoculum and the reactor operation time:

Table 2 | Required concentration of trace elements based on Lettinga *et al.* (1996)

Trace element	Concentration [mg/kg COD_{in}]
Fe	205
Ni	11
Co	9

- The fraction of organic fertilizers (like liquid manure) in inoculum at a reactor start-up increases from A to E. Inoculum for reactor A was only biomass from an operating biogas plant. For reactor B and C the allotments of manure in inoculum are about 17% and 20% and the part of biomass from an operating biogas plant 50% and 80% respectively. The inoculum of reactor D and E is very similar, but these reactors differ in their time of operation (D:7 months, E:4 months). The Figures 2 and 3 show that the concentration of the elements Na, B, Ni, Cu, Zn, and Mo in mono digestion biomasses is higher with an increasing portion of manure in the inoculum, because manure contains important nutrients and micro-organisms.
- The importance of the organic fertilizers can also be found for co-digestion plants. These plants use liquid manure as substrate in addition to energy crops. In reactor H about 50% of substrate is liquid pig manure. In reactor F and G the portion of manure is only about 10% of total substrate.
- The plants I and J have a very high diversity in their substrate composition which also results in high trace element concentrations in these biomasses. In comparison, the concentration of important trace elements as Co, Ni, and Zn is very low in biomass A.

In conclusion, the results obtained in these investigations show that the concentration of certain micro and macro nutrients depends on following factors:

- substrate composition
- operation time (for mono-digestion plants as it influences the part of inoculums in biomasses)
- portion of manure in substrate (for co-digestion plants)

Manure and substrate diversity result in increasing nutrient concentration in the biomass which also can have a positive influence on the anaerobic process. Preißler *et al.* 2007 showed that manure as inoculum can improve the process stability of digestion significantly. A lot of biogas plants use manure for initial inoculation. As this manure is discharged after a certain time period, an influence and inhibition of the anaerobic systems occurs often a year or more after initial operation. One reason for a biological failure of the system after a certain time period is a lack of trace elements which can be provided by manure. But it has

Table 3 | Specific trace element concentration in batch reactor depending on sludge loading rates

	Concentration mg Fe/g VSS		Concentration mg Ni/g VSS		Concentration mg Co/g VSS	
	1 × Fe Normal	2 × Fe Double	1 × Ni Normal	2 × Ni Double	1 x Co Normal	2 x Co Double
SLR = 0.38 kg COD/kg VSS	0.08	0.16	0.004	0.008	0.003	0.007
SLR = 0.5 kg COD/kg VSS	0.10	0.20	0.005	0.01	0.004	0.009

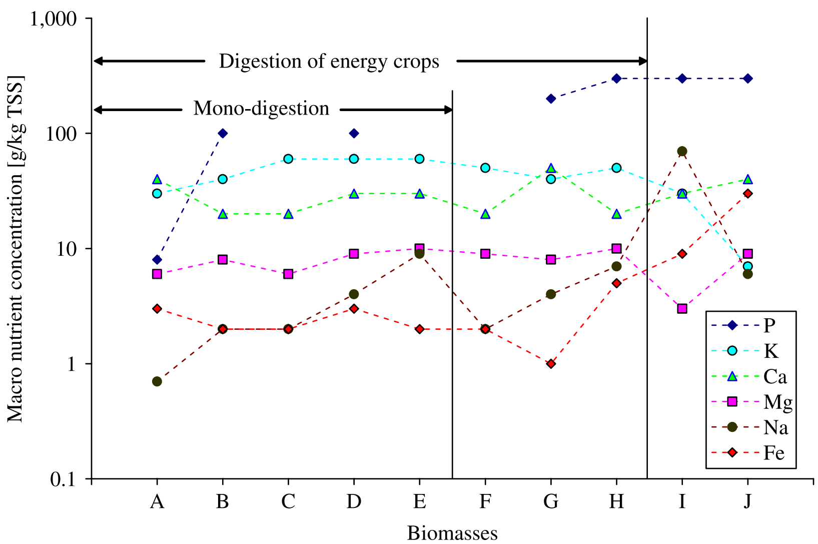

Figure 2 | Macro nutrients concentration in different biomasses [g/kg TSS].

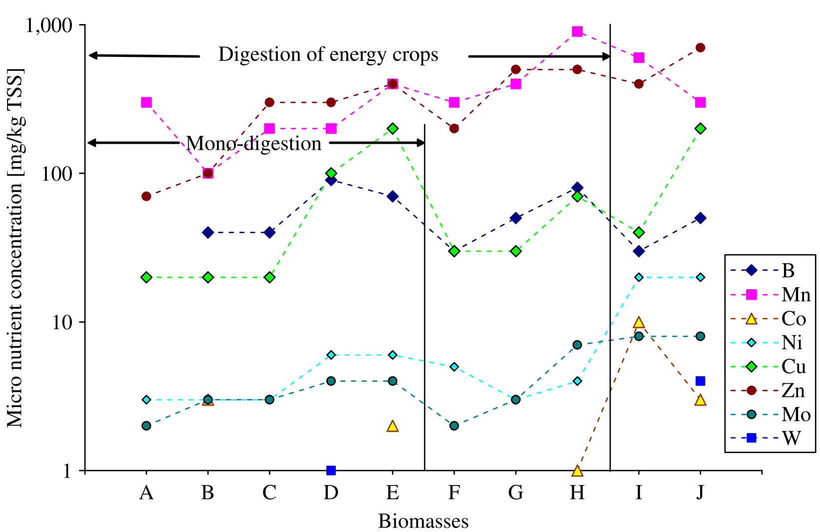

Figure 3 | Micro nutrients concentration in different biomass [g/kg TSS].

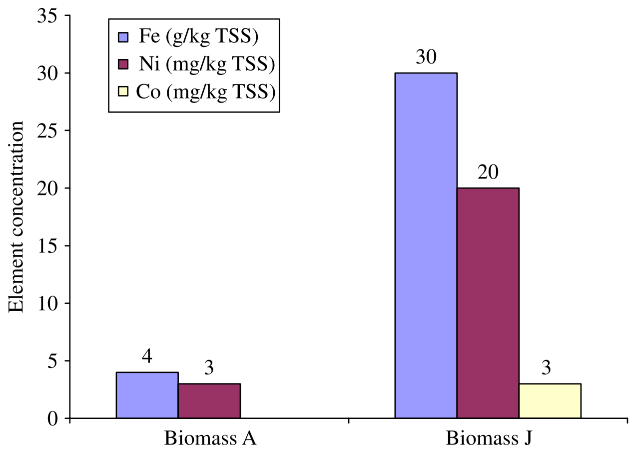

Figure 4 | Comparison of iron, nickel and cobalt concentration in biomass A (mono digestion of energy crops only) and biomass J (digestion of municipal sludge).

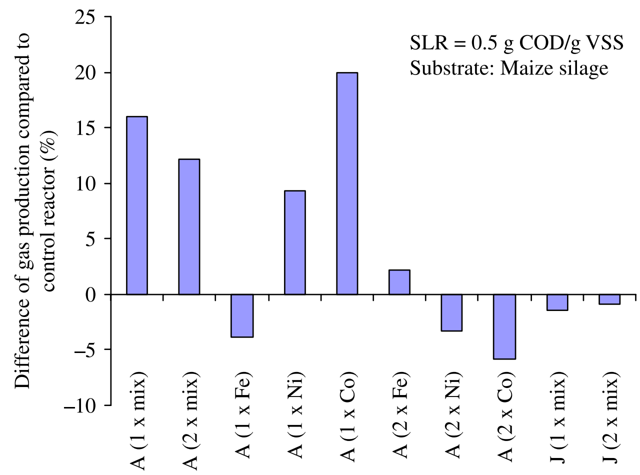

Figure 5 | Difference in gas production of batch tests with element dosage in comparison to control reactor (only substrate respiration) after 32 d.

to be kept in mind that manure also may inhibit the anaerobic process because of high NH_4^+ − concentration.

Anaerobic batchtests

At ISAH a lot of anaerobic batch tests were carried out to investigate COD degradation rates, the methane production of different biogas plants, and to determine the performance of the biomasses. The obtained data provides an overview of different biomass behaviours and possible inhibition factors as malnutrition. Comparisons of anaerobic batch tests from a mono-digestion plant and a co-digestion plant showed great differences in gas production. Further investigations with inoculum from a mono-digestion plant showed a positive aspect on nutrient solutions. Therefore, the influence of trace element dosage is investigated in detail for two different biomasses–biomass A from a mono digestion plant of energy crops, biomass J from a municipal sludge digester. Reactor A is operated at a volumetric loading rate of 3.9 kg VSS/(m^3·d) and a retention time of about 120 d. In contrast, the retention time in reactor J is about 19 d. As Figure 4 shows, the concentration of trace elements in biomass A is very low compared to biomass J, which seems to be sufficiently supplied.

Batch tests in series are carried out with biomass A and J at sludge loading rates of 0.5 g COD/g VSS for maize silage and acetate and additionally of 0.38 g COD/g VSS for acetate. Each test was prepared three times concerning

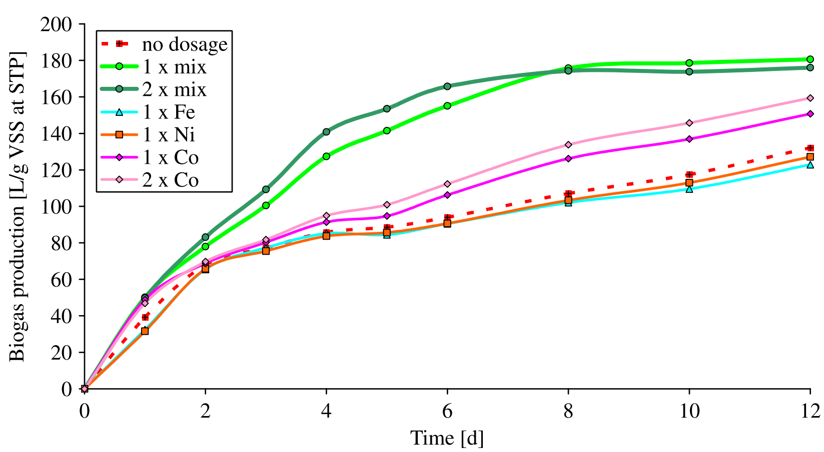

Figure 6 | Gas production in different anaerobic batch tests with maize silage at SLR = 0.5 g COD/g VSS.

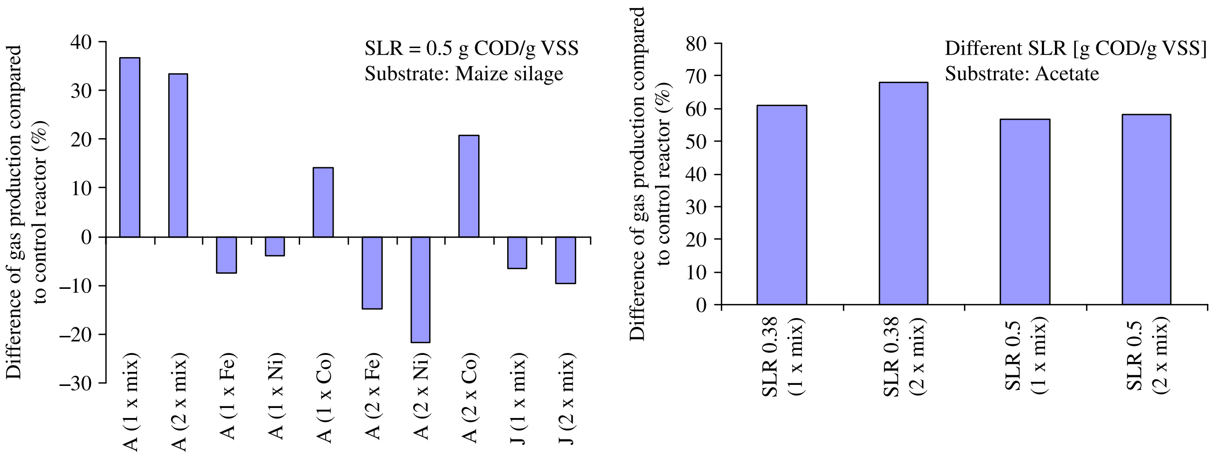

Figure 7 | Difference in gas production of batch tests with element dosage in comparison to control reactor (only substrate respiration) at day 12 after second addition for mais silage and acetate.

failure minimisation. The results for maize silage after 32 d are shown in Figure 5. Concerning biomass A, the first results show that especially cobalt has a significant effect on anaerobic digestion. In comparison, in biomass J a sufficient amount of trace elements seems to be available. So the addition of trace elements does not have an effect on gas production.

After 32 days, an extra dose of trace elements and new substrates are added. The gas production is observed for 12 days again and is shown in Figure 6. The results illustrated in Figure 7 show the differences in gas production after 12 days for maize substrate silage at a sludge loading rate of 0.5 g COD/g VSS and for acetate substrate at different sludge loading rates compared to the control reactor.

The results of second batch test series show a lower effect of cobalt but still a high effect of trace element combination on gas production. Concerning cobalt, the micro-organisms seem to be better provided because the trace elements of the first dosage are still available. It seems that the interaction of different enzymes results in a demand of varied elements and that the effect can not only be related to a specific trace element. As the lower gas production for "2 × Fe" and "2 × Ni" show, too high concentration of certain trace elements even may result in inhibition because of overdose.

For acetate, batch tests were carried out at two different sludge loading rates (0.5 and 0.38 g COD/g VSS). The effect of trace element dosage was even higher compared to the tests with maize silage. As Figure 7 (right-hand side) shows, gas production was 68% higher for 0.38 g COD/g VSS and 58% for 0.5 g COD/g VSS than of the corresponding control reactor after 12 days. The effect of trace element dosage on anaerobic gas production is lower for higher SLR as the micro-organisms use the micro-nutrients within the substrate and convert the readily biodegradable first.

CONCLUSIONS

Trace element concentration can significantly influence the anaerobic digestion processes. Especially in anaerobic mono-digestion plants, a lack of trace elements results in reduced gas production. For this reason the concentration of trace elements of different biomasses was analysed and anaerobic batch tests carried out with trace element dosages at different SLR.

As a conclusion, the comparison of different biomasses concerning their trace element composition showed that the diversity of substrates and the allotment of manure in substrates have a significant influence. Furthermore, trace element concentration in biomasses depends on operation time for mono digestion plants and on the amount of manure in the substrate for digestion plants respectively. Concentrations found in sufficient provided biomasses can be considered as a general guideline for dosage recommendation. Nevertheless, it has to be kept in mind that too high concentrations can have a negative effect and cause inhibition, which has not been considered in detail in this research.

Two digestion plants (a mono digestion plant with low trace element concentrations in biomass, and a municipal sludge digester with high trace element concentrations) were selected for specific anaerobic batch tests. The batch tests were carried out with acetate and maize silage as substrate, and different trace elements concentrations for iron, nickel and cobalt were considered. The dosage concentration was determined based on Lettinga *et al.* (1996) related to substrate's COD-concentration (205 μg Fe/g COD, 11 μg Ni/g COD und 9 μg Co/g COD). The effect was investigated for a trace element combination as well as for each individual. For biomass of a municipal sludge digestion, the dosage does not have any effect on biogas production. In contrast, for biomass of a mono digestion plant (energy crops) the gas production can be improved significantly–with dosage of a trace element combination an increase in gas production of up to 35% for maize silage as substrate and up to 70% for acetate as substrate can be obtained compared to tests without trace element dosage. The dosage of the double trace element mixture does not improve the results significantly. For this reason, the investigated dosage concentration for Fe, Ni and Co is found to be a sufficient to improve and stabilize anaerobic digestion.

As the additions of trace element have such an influence on anaerobic digestion, the following issues have to be analysed in detail in further investigations.

- More biomasses have to be investigated to get a general idea about trace element concentration in different anaerobic digesters and to find more details concerning relationship between substrate, reactor operation and trace element concentration.
- The influence of other essential trace elements as molybdenum has to be analysed in anaerobic batch tests.
- The results should be used to investigate the effect of trace element addition to a large-scale anaerobic reactor.
- The knowledge can be applied for implementation in modelling and in kinetic equations.

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