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EINSATZ VON SPURENELEMENTEN BEI DER VERGÄRUNG VON NACHWACHSENDEN ROHSTOFFEN IN BIOGASANLAGEN

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Abkürzungsverzeichnis

ATP	Adenosintriphosphat
B	Bor
Br_{oTS}	Raumbelastung bezogen auf organische Trockensubstanz
CCM	Corn cob mix
CH_4	Methan
Co	Kobalt
CO_2	Kohlendioxid
DM	Dry matter
EDDS	Ethylendiamindibernsteinsäure
EDTA	Ethylendiamintetraacetat
Fe	Eisen
H_2S	Schwefelwasserstoff
HBT	Hohenheim Biogas Yield Test
HRT	Hydraulic retention time
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IDS	Iminodibernsteinsäure
Mo	Molybdän
MSM	Maximal substrate specific methane yield
Na	Natrium
NADP	Nicotinamidadenindinukleotidphosphat
NawaRo	Nachwachsende Rohstoffe
NH_3	Ammoniak
Ni	Nickel
ODM	Organic dry matter
OLR	Organic loading rate
S	Schwefel
Se	Selen
VFA	Volatile fatty acids

1 EINLEITUNG

Die Bedeutung von Spurenelementen im Biogasprozess

Biogas ist ein sehr vielseitig nutzbarer erneuerbarer Energieträger, der aus Energiepflanzen oder organischen Reststoffen gewonnen wird. Die Produktion von Biogas aus Biomasse durch mikrobielle anaerobe Konversion ist ein fest etablierter Wirtschaftszweig, dessen Zukunft von der Wirtschaftlichkeit des Herstellungsprozesses abhängt. Aus diesem Grund sind die Maximierung der Gasausbeute sowie die Stabilisierung der Gärung wichtige Forschungsziele. Dies kann zum einen durch eine ausgereifte Biogastechnik und zum anderen durch die Optimierung der komplexen mikrobiologischen Prozesse, die an der Biogasproduktion beteiligt sind, erreicht werden.

Der anaerobe Prozess der Biogasbildung wird immer von den primären Gärern eingeleitet, welche die Biomasse in organische Säuren und Alkohol umwandeln. Diese Produkte werden durch acetogene Bakterien und schließlich durch methanogene Mikroorganismen weiter verarbeitet. Acetogene Bakterien und Methanogene leben dabei in einer engen Symbiose. Die freigesetzten Abbauprodukte und die aus den Stoffwechselprozessen gewonnene Energie werden für den Aufbau und Pflege ihrer eigenen Vitalfunktionen genutzt.

Das Wachstum und die Stoffwechselrate der an dem Prozess der Biogasbildung beteiligten Mikroorganismen hängen entscheidend von einer optimalen Versorgung mit Nährstoffen, Mineralien, Vitaminen und Spurenelementen ab (Bryant, 1979). Sowohl die Nährstoffe, als auch deren erforderliche Mengen sind spezifisch für die jeweilige Art der Mikroorganismen. Eine Unterversorgung der Mikroorganismen mit diesen Nährstoffen führt zu geringeren Stoffwechselraten und kann damit, bei gleichbleibender Substratzufuhr zum Fermenter, zu einer Destabilisierung des Prozesses und einer stark verminderten Methanproduktion führen (Lemmer, 2011).

Über den Nährstoffbedarf der hydrolytischen, acidogenen und acetogenen Bakterien im Biogasprozess ist bis heute nur sehr wenig bekannt. Dagegen war der Mineralstoffbedarf der zu den Archaen gehörenden Methanogenen bereits Thema zahlreicher Forschungsarbeiten. Umfassende Erfahrungen zu deren komplexen Nährstoffanforderungen konnten in den vergangenen Jahrzehnten, insbesondere bei der Abwasserreinigung, gewonnen werden.

Der Bedarf der methanogenen Mikroorganismen an Makroelementen hängt hauptsächlich von deren Wachstumsrate sowie der Zusammensetzung der bakteriellen Biomasse ab (Lettinga, 1995). Im Vergleich zu vielen Bakterien ist die Biomassebildung der Methanogene aufgrund des langsamen Wachstums jedoch gering, woraus sich auch ein relativ niedriger absoluter Bedarf an Makroelementen ableiten lässt. Die Mikroorganismen erhalten den benötigten Kohlenstoff im Wesentlichen aus dem zugeführten Substrat und nutzen diesen zum Aufbau der Zellstruktur. Stickstoff ist vor allem für die Proteinbiosynthese notwendig. Schwefel ist ein essentieller Bestandteil wichtiger Aminosäuren und auch ein wichtiger Nährstoff für das Wachstum von Methanogenen. Untersuchungen haben gezeigt, dass die Zufuhr von Schwefel eine positive Wirkung auf die Aktivität der Methanogene hat. Andererseits reduziert Schwefel die Bioverfügbarkeit von Spurenelementen durch die Bildung von unlöslichen Metallsulfiden (Gonzales–Gil et al., 2003; Zandvoort et al., 2005). Der Phosphatgehalt ist entscheidend für die Bereitstellung der Energieträger ATP und NADP für den Stoffwechsel. Einige Methanogene benötigen Natrium, um ATP für ihr Wachstum bilden und die hydrogenotrophe Methanbildung durchführen zu können (Perski, 1981).

Neben Makroelementen und Vitaminen sind auch Spurenelemente essentiell für die methanogenen Mikroorgansimen. Über die genaue Bedeutung vieler Spurenelemente kann aber oft nur eine unzureichende Aussage getroffen werden. Einigkeit besteht jedoch darüber, dass die Spurenelementkonzentration im Fermenter für den Biogasprozess von entscheidender Bedeutung ist (Bauer et al., 2009). Vier wesentliche Aufgaben der Spurenelemente in der anaeroben Vergärung konnten bestätigt werden. (1) Mikronährstoffe sind an vielen wichtigen enzymatischen Reaktionen beteiligt, da sie essentiell für den Aufbau der Coenzyme bzw. Cofaktoren sind (Bauer et al., 2009). (2) Spurenelemente werden von verschiedenen Nährstoffen als Bindungsatom genutzt. (3) Als dritte Aufgabe kann die Hemmung der Sulfidtoxizität im Fermenter genannt werden. (4) Als letzte Eigenschaft führen Mikronährstoffe zu einer allgemeinen Biomassestimulation (d.h. einer verbesserten Reproduktion der Mikroorganismen) (Takashima und Speece, 1989). Bei allen Vorgängen sind Spurenelemente grundsätzlich nur in gelöster und nicht in gebundener Form für die Mikroorganismen verfügbar (Bischofsberger et al., 2005).

Derzeit werden als essentielle Spurenelemente für das Wachstum und den Stoffwechsel der Methanogene Eisen, Nickel, Kobalt, Selen, Molybdän, Bor und Wolfram erachtet (Jarell, 1988).

Die Bedeutung von Eisen bei der anaeroben Konversion von Biomasse ist vielfältig. Aufgrund der hohen Konzentration im Vergleich zu den übrigen Mikronährstoffen kommt Eisen eine besondere Bedeutung bei der Sulfidfällung zu (Woo, 1993). Bei den Stoffwechselprozessen der Methanogene dient es als Transport-System bei der Reduktion von CO_2 zu CH_4. Eisen kann in Stoffwechselprozessen sowohl als Elektronenakzeptor als auch als Elektronendonor wirken und somit die Rolle eines Energieträgers erfüllen (Lacy, 1987).

Kobalt und Bor sind essentielle Spurenelemente für acetogene Bakterien und Methanogene. Kobalt wird als Zentralatom in Enzymen von Corrinoiden und Vitamin B12 verwendet (Jarell, 1988).

Molybdän katalysiert die Oxidations- und Reduktionsreaktionen von CO_2 und ist notwendig für die Bildung unterschiedlicher Enzyme. Selen kann ein wachstumslimitierender Faktor für einige Methanogene sein, da es für die Bildung von Aminosäuren wie Selenocystein erforderlich ist (Chasteen, 2003).

Die Besonderheit der Methanogene ist ihr Bedarf an Nickel, das für das Wachstum von Bakterien nur eine untergeordnete Rolle spielt (Diekert, 1980). Nickel verbessert das Wachstum von Methanogenen wie *Methanobacterium bryantii* und hilft dabei, ihre Zellmembran und damit ihre strukturelle Stabilität aufrecht zu erhalten (Jarell, 1988). Nickel ist auch an der Bildung von unterschiedlichen Enzymen wie Urease und Hydrogenase beteiligt und ist das zentrale Atom des Cofaktors F430, der essentiell für den letzten Schritt der Methanbildung ist (Diekert, 1980; Hausinger, 2001).

Bei allen Spurenelementen ist deren Konzentration von entscheidender Bedeutung (Bauer et al., 2009). Ist die Konzentration der vorhandenen Spurenelemente zu gering, so können die für den Stoffwechsel benötigten Enzyme und Coenzyme nicht mehr in ausreichenden Mengen gebildet werden. Das führt dazu, dass die Leistungsfähigkeit der Methanogene abnimmt und der Methangehalt des Biogases und der spezifische Methanertrag deutlich sinken. Andererseits können zu hohe Spurenelementgehalte die mikrobielle Aktivität hemmen oder sogar zum Erliegen bringen (Bischofsberger et al., 2005).

Wenn ein Spurenelementdefizit in dem Gärsubstrat einer landwirtschaftlichen Biogasanlage festgestellt wird, können handelsübliche Spurenelementlösungen verwendet werden, um dieses Defizit auszugleichen. Diese flüssigen oder granulierten Mischungen erhöhen die Konzentration der Mikronährstoffe in den Fermentern. Erfahrungen aus der Praxis zeigen, dass nach einem solchen Defizitausgleich die Stoffwechselrate der Methanogene sehr schnell zunimmt und die im Fermenter akkumulierten Säuren in kurzer Zeit zu Biogas umgesetzt werden. Aufgrund der Tatsache, dass es sich bei den meisten Spurenelementen um sogenannte Schwermetalle handelt, sollten aus ökologischen Gründen die zugesetzten Mengen so gering wie möglich gehalten werden.

Ziel der Arbeit

Aus Erhebungen im Praxismaßstab ist bekannt, dass die Konzentrationen von Makro- und Mikronährstoffen im Gärsubstrat landwirtschaftlicher Biogasanlagen in einem weiten Bereich schwanken, selbst wenn diese ausschließlich mit Wirtschaftsdünger und nachwachsenden Rohstoffen beschickt werden. Darauf aufbauend ist die Zielsetzung der Arbeit zweigeteilt. Zum einen sollten die Ursachen und Folgen der Konzentrationsunterschiede ermittelt werden. Zum anderen sollte geprüft werden, ob durch eine Komplexierung der Mikronährstoffe die Zugabemengen, im Vergleich zur derzeit üblichen Zugabe in Salzform, reduziert werden kann, ohne dass biologische Prozessstörungen auftreten.

Zum Erreichen der Ziele wurden die Untersuchungen in folgende Bereiche gegliedert:

- Untersuchungen zu Ursachen und prozessbiologischen Folgen unterschiedlicher Mikro- und Makronährstoffkonzentration im Gärsubstrat landwirtschaftlicher Biogasanlagen
- Laboruntersuchungen zu optimalen Konzentrationen von Spurenelementen im Gärsubstrat und der Möglichkeit, die notwendigen Zugabemengen durch den Einsatz von EDTA zu reduzieren
- Laboruntersuchungen zum Einsatz unterschiedlicher biologisch abbaubarer Komplexbildner zur Verringerung des Spurenelementeinsatzes.

Zum Erreichen des ersten Teilziels wurde eine Datenerhebung an landwirtschaftlichen Biogasanlagen durchgeführt. Die Fermenterproben wurden auf Spurenelementkonzentration und Fettsäurengehalt hin untersucht und die erhaltenen Daten statistisch analysiert.

Die weiteren Untersuchungen zur Komplexierung der Mikronährstoffe wurden im Biogaslabor der Landesanstalt für Agrartechnik und Bioenergie durchgeführt. Basis der Untersuchungen bildeten verschiedene unterversorgte Gärsubstrate von Biogasanlagen. Durch die Zugabe der Nährstoffe in unterschiedlichen Konzentrationen und Formulierungen konnte deren Einfluss auf den Gärprozess ermittelt werden.

In den folgenden Abschnitten werden die einzelnen Teilziele gesondert vorgestellt und erläutert.

2 PUBLIKATION1: MINERAL SUBSTANCES AND MACRONUTRIENTS IN THE ANAEROBIC CONVERSION OF BIOMASS: AN IMPACT EVALUATION

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Abstract

Optimal nutrient supply in the digester is essential for efficient biogas production. The aim of this study was to analyze the effects of various micro- and macronutrients on the process stability by a field test. The digestates of 25 biogas plants across the federal state of Baden-Württemberg, Germany, were investigated. Collected data including trace elements, macronutrients, and volatile fatty acids (VFA) concentrations, as well as the organic loading rate and hydraulic retention time were subjected to statistical analysis. High variations in the concentrations within the different biogas plants were observed. Statistically significant effects of substrate constituents and process parameters on the stability of the anaerobic digestion process were found. Several micro- and macronutrients and the relationships between these elements, as well as the process parameters propionic acid, acetic acid, and acetic acid equivalent were tested. Ni, Mo, and S had a consistent statistically significant effect, while the organic loading rate and Se only showed an effect limited to the acetic acid concentration and the acetic acid equivalent. No statistically significant effect could be shown for Fe, Co, and Na. Most of the significant interactions between the tested elements contained Ni, Fe, and Co. This shows that a balanced relation between the concentrations of these elements is of greater importance than the presence of individual elements for a digester to be able to operate at high organic loading rates and maintain low VFA concentrations.

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Research Article

Mineral substances and macronutrients in the anaerobic conversion of biomass: An impact evaluation

Optimal nutrient supply in the digester is essential for efficient biogas production. The aim of this study was to analyze the effects of various micro- and macronutrients on the process stability by a field test. The digestates of 25 biogas plants across the federal state of Baden-Württemberg, Germany, were investigated. Collected data including trace elements, macronutrients, and volatile fatty acids (VFA) concentrations, as well as the organic loading rate and hydraulic retention time were subjected to statistical analysis. High variations in the concentrations within the different biogas plants were observed. Statistically significant effects of substrate constituents and process parameters on the stability of the anaerobic digestion process were found. Several micro- and macronutrients and the relationships between these elements, as well as the process parameters propionic acid, acetic acid, and acetic acid equivalent were tested. Ni, Mo, and S had a consistent statistically significant effect, while the organic loading rate and Se only showed an effect limited to the acetic acid concentration and the acetic acid equivalent. No statistically significant effect could be shown for Fe, Co, and Na. Most of the significant interactions between the tested elements contained Ni, Fe, and Co. This shows that a balanced relation between the concentrations of these elements is of greater importance than the presence of individual elements for a digester to be able to operate at high organic loading rates and maintain low VFA concentrations.

Keywords: Anaerobic digestion / Biogas / Macronutrients / Micronutrients

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1 Introduction

The considerable advantage of anaerobic fermentation of organic source materials is biogas production, which can be used as a source of energy [1]. Biogas results in an incomplete stepwise conversion of biodegradable substances into CH_4, CO_2, NH_3, and H_2S via a syntrophic interaction between fermentative and acetogenic bacteria and methanogens [2]. The large number of microorganisms that participate in this biomass conversion break down the organic substance to use the building materials and energy thus released to build up and maintain their own vital functions.

Microorganisms participating in the anaerobic conversion of biomass require optimized milieu conditions to achieve high metabolic rates. Especially, acetogenic bacteria and methanogens are very sensitive to changes of pH and temperature values, the presence of inhibitors or oxygen, as well as inhibitory effects of the substrate. A high organic loading rate (OLR) or a sudden increase of it leads to an accumulation of volatile fatty acids (VFA) and finally to a pH breakdown. Also an inadequate temperature control and an increased NH_3 concentration are documented causes for process instability and malfunction [3].

The anaerobic fermentation and the growth of the microbial communities involved in the biogas process are dependent on the optimal supply of nutrients [4]. The quantities required are specific to the respective variety. An optimal nutrient supply in the digester is targeted, as insufficient microbiological activity can jeopardize the process stability of a biogas plant and lead to strong cuts in production and yield.

The macronutrient requirements are mainly assessed based on bacterial composition and growth yield and biomass composition [5]. The requirements are usually low, due to the fact that not much biomass is produced. The nutrient ratio is generally of C:N:P:S = 600:15:5:1 [6]. The microorganisms essentially

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Abbreviations: OLR, organic loading rate; **VFA**, volatile fatty acids obtain their carbon from the substrate supplied and use this to build up their cell structure. Nitrogen is needed above all for protein biosynthesis. Sulfur is a necessary constituent of important amino acids and also an essential nutrient for the growth of methanogenic bacteria. Studies showed that a supply of sulfur has a positive effect on the methanogenic activity and is also an important factor for the bioavailability of micro elements, as it may cause the formation of insoluble metal sulfides [7, 8]. In some cases, various sulfur compounds also serve as redox partners in electron transport. The sulfur content of methanogenic bacteria was reported to be unusually high when compared to aerobic microorganisms [9]. The phosphate content is crucial for providing the energy carriers ATP and NADP in the metabolism. Some archaebacteria require sodium to form ATP, for their growth and for carbon dioxide reduction to methane [10].

The production of biogas, just like all biotechnological processes, uses microorganisms and is therefore highly dependent on the presence and the concentration of micronutrients for an optimal performance [11]. There is still little information about the nutrient requirements of the hydrolytic, acid fermenting, and acetogenic bacteria, but qualitative determinations of the nutritional needs and stimulations of methanogenic archaea have been performed and reviewed in detail [12]. Trace elements important for the growth of microorganisms are iron, nickel, cobalt, selenium, molybdenum, and tungsten [6]. The micronutrients demand of various methanogens has already been documented [13].

Iron is an essential trace element for anaerobic microorganisms in regard to its function as a bonding element in the sulfide precipitation and reduction of the sulfide toxicity in the digester [14]. Methanogenic archaea require a transport system for the reduction of CO_2 to CH_4 and iron can fulfill the role of an energy carrier [15]. Iron is also the constituent of many enzymes [14, 16].

Cobalt is also an essential trace element for acetogens and methanogens [16]. It is used mainly as a central atom in corrinoids and vitamin B12 enzymes. Molybdenum is a necessary micronutrient for the synthesis of various anaerobic microorganisms [16]. It usually catalyzes the oxidation and reduction reactions of CO_2 and plays an important role in the formation of enzymes. Selenium has a stimulatory effect on numerous methanogens [16]. It plays an important role in the formation of proteins such as selenocysteine or selenomethionine, which are required by some methanogenic archaea for the oxidation of hydrogen. For these archaea, an insufficient selenium concentration may be a growth-limiting factor [17].

The methanogens are a special class of bacteria, with special nutrient requirements, nickel being a good example for this, as it is not essential for bacteria growth [18]. The addition of nickel enhances the growth of methanogens such as *Methanobacterium bryantii* and plays a role in the structural stability of some methanogenic bacteria by helping maintain the cell membranes [16]. In the biogas process, nickel is involved in the formation of several enzymes [19]. The cofactor F_{430} contains nickel as a central atom and is essential for the final step of the methane formation [18]. However, excessive use of Ni has an inhibitory effect on the methanogenesis [13].

The concentration in which these elements are present and their availability for the microorganisms are of critical significance [20]. Minimum and maximum concentration levels for each component have been documented, which limit or inhibit the microbial metabolic process if undercut or exceeded [21]. A deficiency of these trace elements is expected to lead to a reduced performance of the anaerobic digestion [22]. This leads to an accumulation of VFA, a decrease of the biogas yield, and in extreme cases to direct limitation of the maximum loading rate [23]. The deficits can be ascertained in the fermenting substrate of a digester and compensated individually for each biogas plant by adding technical mineral substance mixtures. By that the process generally stabilizes clearly within a few days together with the breakdown of the accumulated acids and the loading rate can be increased again or further. Moreover, the degree of substrate exploitation is frequently improved [24].

Of great importance for a good performance of the anaerobic fermentation process is the concentration of VFA. VFA are key intermediates in the metabolic pathway of methane production and have a significant effect on the methanogenesis. In high concentrations, the terminal fermentation products of the acidogenesis can cause microbial stress, affect the loading, efficiency and stability of methane production, affect the degradation rate of acetic acid, drastically reduce the methane yield, ultimately leading to failure of the fermentation process [25, 26].

The strong effect of nutrients on VFA and the relation between the production of VFA and concentration of nutrients in the digester was investigated in several studies. In the case of biogas production from Napier grass, the addition of a mixture containing Ni, Mo, Se, S, and Co leads to a decrease of acetate, butyrate, and propionate concentrations below the detection limits and increased the methane production by approximately 40% [27]. The addition of a complex trace element solution to a laboratory-scale experiment with maize and rye silage prevented the accumulation of fatty acids and allowed an increase of the feeding rate [28]. Suboptimal nutrient concentrations in UASB plants produced high VFA concentrations. The addition of a nutrient mixture led to a rapid improvement of the reactor performance [29].

A strong relation exists between the OLR and the methane production. Research on this subject revealed that an increase of the OLR leads to a decrease of the methane yield and a parallel increase of VFA concentrations [30].

Several studies on large number of biogas plants revealed high fluctuations of the nutrients concentration. Analyses of the fermenting substrate of over 700 different biogas plants revealed differences in the contents of individual elements by the factor of 200 [31]. The investigation of trace element concentrations in the digesters of 10 biogas plants across Europe revealed great variations covering a range of 1–2 orders of magnitude [22].

The objective of the study presented here was to demonstrate significant statistical effects of nutrients and of the OLR on the stability of the anaerobic digestion process of several biogas plants in practical operation in Germany. The nutritional elements examined were micronutrients (Ni, Fe, Co, Mo, and Se) and macronutrients (Na and S). The analyzed process parameters were propionic acid, acetic acid, and the acetic acid equivalent. Effects of single nutrients, as well as the relations between these elements were determined.

2 Materials and methods

2.1 Investigated biogas plants

Twenty-five different biogas plants in practical operation geographically dispersed across the federal state of Baden-Württemberg in Southern Germany were selected for this study. The digester contents were sampled and examined. The complete technical data of the plants were also surveyed and a documentation and analysis of the operational data was carried out. The information submitted by the biogas plant owners was subjected to a thorough plausibility check for process parameters like the OLR and the hydraulic retention time (HRT). The complete sampling process was repeated after three months. A detailed description of the selected biogas plants is presented in Table 1. In the case of four plants, samples were collected from both digesters, which added the number of viable samples up to 58. All samples were considered independent and the gathered data were subject to an unpaired analysis for a better overview of the effects in the digesters.

2.2 Sample collection and analysis

Samples obtained from all digesters were immediately cooled down and then sent to an external laboratory for analysis. Concentrations of macronutrients and trace elements, as well as the fatty acids content were determined.

The types of fatty acids present in the samples, as well as their concentration were determined using the HPLC method. A total of 2.5 g of the sample were purified by removal of suspended particles and proteins. Sulfric acid was added in a 1:1 ratio and 0.5 mL of this mixture was tested in the HPLC (Knauer, Germany). During this chromatographic separation method, the liquid sample passes under high pressure through a separating column and the retention time is measured by a UV detector and an index of refraction detector. The different retention times of each detected substance allow their identification by comparing them with existing standards and the determination of their concentration in the sample.

The determination of trace elements was carried out using inductively coupled plasma optical emission spectroscopy. The samples were prepared using a disintegration method, where 10 mL nitric acid and 2 mL hydrogen peroxide were added to the sample (dry residue at 105°C) that was then heated for 20 min and boiled for another 20 min. After cooling, the sample was transferred in a 100-mL volumetric flask with distilled water and then tested in the optical emission spectrometer (ICAP 6000 Series, Thermo Scientific, USA). The concentration of each element present in the sample was determined in relation to the wave length of its optical emissions.

2.3 Statistical methods

The analysis of variance (ANOVA) was conducted to assess possible effects on propionic acid concentration, acetic acid concentration, and on the acetic acid equivalent of eight different variables. These variables include the OLR, two macronutrients (Na and S) and five micronutrients (Ni, Co, Mo, Se, and Fe).

ANOVA was performed using the general linear model (GLM) procedure of the SAS/STAT® software. In order to assure the validity of statistical tests using the GLM procedure, the normality of the residuals was first checked using normality tests with no problematic results. The MODEL statement was used to analyze the dependent and independent variables. The only independent variable that is not continuous and therefore a class variable is the biogas plants variable that was declared in the CLASS statement. This article considers three models for the data described. Each model tests the effects of the single variables, as well as the effect of the interactions between them on each of the three described process parameters. The MEANS procedure was used to describe all variables to be analyzed and the CORR procedure was used to test existing relationships between some parameters.

3 Results and discussion

3.1 OLR and HRT distribution and correlation

The measured OLR related to the organic dry matter in the digesters lay between 1.4 and 7.3 kg m^{-3} day̅ and the HRT between 36 and 131 days. The distribution of the two process parameters is presented in Fig. 1.

To better understand the relationship between these two process parameters, Pearson's correlation coefficient matrices were calculated. Statistical analysis revealed a significant relationship between the OLR and the HRT ($r = -0.74$, $p<0.0001$). This indicates a negative linear relation between these two variables and the fact that 55% of the variability in one variable can be explained by variance in the second variable.

Additionally, the relation between the two parameters and the manure quota in the digesters was evaluated. A significant relationship between OLR and manure quota ($r = -0.53$, $p \leq 0.05$), but no significant relationship between HRT and the manure quota ($r = 0.38$, $p \geq 0.05$) was observed. This result does not dispute a relation between manure quota and HRT, but in this case for the given data this was not proven.

3.2 Statistical analysis

All analyzed variables were described using the MEANS procedure. The arithmetic means and the standard deviations of each effect specified in the statement and contained in the model were computed, as well as the minimal and maximal values. The description of the data is presented in Table 2. The null hypotheses for the analysis were that none of the variables or the interactions between them has an effect on the selected process parameters.

3.2.1 Propionic acid

In the case of propionic acid, the overall *F*-statistic is highly significant ($F = 5.38$, $p<0.0001$) at the 95% level indicating that the model as a whole accounts for a statistically significant portion of the variation in the propionic acid concentration. The

Table 1. Description of the selected biogas plants.

Plant nr.	Total substrate input in relation to fresh matter per day (kg FM/day)	Animal manure (%) a)	Feedstock (%) b)	Digester capacity (m^3)	Electric capacity (kW el)	OLR (kg m^{-3} day^{-})	HRT (day)	Steps
1	9550	Pig slurry (40.8), solid manure (1.6)	Maize silage (52.4), grass silage (5.2)	900	233	2.1	94	1
2	15, 626	Cattle slurry (36.8), solid manure (1.6)	Maize silage (46.4), grass silage (9.6), grain (2.8), WPCS (2.8)	766	464	4.7	49	2
3	39, 200	Pig slurry (20.4), solid manure (19.9)	Maize silage (59.7)	3400	472	2.7	87	2
4	7070	Solid manure (6.7), cattle slurry (4.2)	Grass silage (43.8), maize silage (41.1), WPCS (4.2)	617	223	3.0	87	2
5	24, 400	Pig slurry (24.6), cattle slurry (24.6)	Maize silage (32.8), WPCS (12.3), grass silage, (4.1), grain (1.6)	1925	244	2.7	73	1
6	13, 610	Solid manure (23.5)	Grass silage (69.4), maize silage (4.9), grain (2.2)	1500	423	2.2	100	2
7	10, 000	Cattle slurry (35.0)	Grain (50.0), maize silage (15.0)	1100	300	4.5	50	1
8	7800	Cattle slurry (48.7), solid manure (2.6)	Grass silage (48.1), maize silage (0.6)	880	81	1.8	100	1
9	9690	Cattle slurry (36.1)	Maize silage (42.6), grass silage (21.3)	655	209	2.7	68	2
10	13, 899	Pig slurry (29.5), cattle slurry (9.9), solid manure (1.6)	Maize silage (46.8), grass silage (12.2)	740	303	3.3	53	2
11	18, 700	Cattle slurry (15.0), solid manure (8.0)	Maize silage (45.5), grass silage (24.1), sun flower silage (5.3), grain (2.1)	1000	335	5.1	51	2
12	22, 010	Cattle slurry (40.4), solid manure (0.2)	Grass silage (31.0), maize silage (11.9), grain (8.5), CCM (8.0)	800	763	7.3	36	2
13	65, 000	No addition	Maize silage (71.0), WPCS (15.3), CCM (9.2), grass silage (3.0), grain (1.5)	3600	1,320	6.0	50	2
14	17, 000	Cattle slurry (35.3), solid manure (14.7)	Grass silage (27.6), WPCS (22.4)	880	171	4.1	52	1
15	22, 300	Cattle slurry (44.8), solid manure (6.7)	Grass silage (17.9), WPCS (17.0), maize silage (13.6)	900	226	4.6	42	1
16	31, 600	Cattle slurry (28.5), solid manure (6.3), poultry dung (0.9)	Maize silage (45.9), grass silage (14.2), grain (2.6), CCM (1.6)	1200	513	6.5	38	2
17	37, 000	Pig slurry (43.2)	Maize silage (24.3), WPCS (13.5), grass silage (10.8),grain (8.2)	2520	494	3.5	68	2
18	18, 700	Cattle slurry (80.2)	Grass silage (19.8)	930	281	2.9	50	1
19	29, 380	Cattle slurry (71.8), solid manure (5.2)	Maize silage (11.3), grass silage (5.3), WPCS (3.5), grain (2.9)	1408	186	2.2	46	2

Table 1. Continued

Plant nr.	Total substrate input in relation to fresh matter per day (kg FM/day)	Animal manure (%) [a]	Feedstock (%) [b]	Digester capacity (m^3)	Electric capacity (kW el)	OLR (kg $m^{-3}day^{-}$)	HRT (day)	Steps
20	33, 500	Cattle slurry (29.8), solid manure (7.5)	Maize silage (44.8), WPCS (17.9)	2250	869	3.4	67	2
21	3200	Solid manure (40.6), cattle slurry (37.5)	Grass silage (21.9)	420	21	1.4	131	1
22	5150	Cattle slurry (34.0), solid manure (7.8)	Grass silage (58.2)	980	56	1.5	120	2
23	19, 370	Cattle slurry (45.9), solid manure (0.3)	Grass silage (27.1), maize silage (15.2), CCM (5.8), grain (3.5), sun flower silage (2.2)	800	515	2.7	48	2
24	20, 000	Pig slurry (27.5), solid manure (4.0)	Maize silage (49.0), grain (12.0), grass silage (7.5)	1310	822	4.7	66	2
25	5000	Cattle slurry (60.0)	Grass silage (40.0)	400	38	2.0	80	1

[a] Animal manure percentage of total substrate input in relation to fresh matter per day.
[b] Feedstock percentage of total substrate input in relation to fresh matter per day.

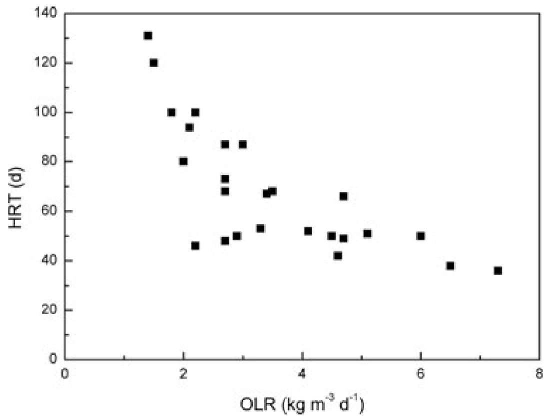

Figure 1. Distribution of the hydraulic retention time (HRT) and the organic loading rate (OLR) of the analyzed biogas plants ($N = 25$).

R^2 statistic indicates that the model explains 90% of the variation in the analyzed variable. Table 3 shows the output of the statistical test containing the results of the effect of all nutrients and only the significant interactions between them. The results of ANOVA revealed that the OLR has a significant effect on the propionic acid concentration. Among the two studied macronutrients, S has a significant effect at the 95% level while Na has none, as does the interaction between the two variables. In the case of the five trace elements considered in the model, Se showed the highest significance ($F = 41.10$, $p<0.0001$). The effect of Ni and Mo on the process parameter is also significant at a 0.05 level. Co and Fe have no significant effect on the propionic acid concentration, but both are present in several interactions with a significant effect. Other significant interactions between the trace elements contain up to four different trace elements.

Table 2. Descriptive statistics of the variables used in the analysis of variance.

Variable	Label	*N*	Mean	SD	Minimum	Maximum
PA[a]	Propionic acid	58	194.60	490.93	0.00	3109.00
HAc[a]	Acetic acid	58	532.43	854.53	0.00	4362.00
HAc eq[a]	Acetic acid equivalent	58	719.12	1195.37	0.00	5733.00
OLR[b]	Organic loading rate	58	3.40	1.50	1.40	7.30
Na[c]	Sodium	58	0.23	0.11	0.04	0.60
S[c]	Sulfur	58	0.44	0.07	0.31	0.67
Ni[d]	Nickel	58	6.23	3.31	1.56	16.34
Fe[d]	Iron	58	2639.88	1576.27	786.03	7783.67
Co[d]	Cobalt	58	1.71	1.23	0.47	8.10
Mo[d]	Molybdenum	58	3.05	1.08	1.18	6.25
Se[d]	Selenium	58	0.49	0.31	0.00	1.43

N, number of observations; Mean, mean value; SD, standard deviation;
[a] Concentration of volatile fatty acids in mg kg^{-1}
[b] Organic loading rates in kg ODM ($m^3 \times$ day) $^{-1}$
[c] Macronutrients concentration in % DM
[d] Micronutrients concentration in mg kg^{-1} DM

3.2.2 Acetic acid equivalent

For the acetic acid equivalent, the overall *F*-statistic is significant ($F = 3.85$, $p = 0.0007$) at the 0.05 level. According to the R^2 statistic, the model accounts in this case for 86% of the variation in the acetic acid equivalent. Table 4 presents the output of the statistical test containing the results of all tested effects, as well as all the significant interactions. In the case of the acetic acid

Table 3. Values of the analysis of variance for the selected variables and the propionic acid concentration.

Source	*F*-value	Pr > *F*
Se	41.10	<0.0001
Mo × Se	23.67	<0.0001
OLR	15.70	0.0007
S	14.29	0.0010
Co × Se	11.27	0.0028
Co × Mo × Se	10.81	0.0034
Mo	9.46	0.0055
Fe × Co × Mo × Se	8.97	0.0067
Ni	8.07	0.0095
Ni × Fe × Mo	5.71	0.0258
Ni × Co × Se	5.67	0.0263
Na	3.47	0.0760
Co	0.29	0.5982
Fe	0.10	0.7537

Table 4. Values of the analysis of variance for the selected variables and the acetic acid equivalent.

Source	*F*-value	Pr > *F*
Ni	19.75	0.0002
Ni × Co	11.44	0.0027
S	10.91	0.0032
Se	8.78	0.0072
Co × Mo × Se	7.52	0.0119
Mo	6.73	0.0165
Ni × Fe × Co × Mo	6.27	0.0202
Fe × Co	5.83	0.0245
OLR	5.81	0.0247
Ni × Co × Se	5.59	0.0272
Co × Se	5.16	0.0332
Ni × Fe	4.83	0.0387
Ni × Fe × Co	4.80	0.0393
Ni × Fe × Co × Se	4.79	0.0395
Ni × Fe × Se	4.50	0.0455
Co	1.65	0.2120
Na	0.74	0.3997
Fe	0.58	0.4525

equivalent, OLR has a significant effect for ($F = 5.81, p = 0.0247$) at a 95% level. For the macronutrients, the null hypothesis can only be rejected for S. Among the trace elements, Ni has the highest effect. Se and Mo also have a significant effect on the acetic acid equivalent, while Co and Fe have no effect on this process parameter. Most interactions that have a significant effect according to the statistical test used in this study contain Ni, Fe, and Co and up to four elements.

3.2.3 *Acetic acid*

Given the significance of the overall test ($F = 4.06$, $p = 0.0005$, $R^2 = 87\%$), the effects of the variables on the acetic acid concentration were examined. The output of the statistical test containing the results of all analyzed variables and only the significant interactions between these variables is presented in Table 5. The ANOVA for acetic acid concentration revealed that the OLR has

Table 5. Values of the analysis of variance for the selected variables and the acetic acid concentration.

Source	*F*-value	Pr > *F*
Ni	23.86	<0.0001
Ni × Co	15.14	0.0008
Ni × Fe × Co × Mo	15.10	0.0008
S	8.02	0.0097
Fe × Co	7.82	0.0105
Ni × Fe × Se	7.73	0.0109
Ni × Fe	5.77	0.0252
Co × Mo × Se	5.61	0.0270
Fe × Mo	5.37	0.0302
Ni × Fe × Co × Se	5.15	0.0333
Mo	5.03	0.0353
Ni × Fe × Co	4.79	0.0395
Ni × Co × Se	4.71	0.0411
OLR	2.74	0.1123
Co	2.40	0.1352
Fe	1.52	0.2300
Se	1.52	0.2300
Na	0.20	0.6582

no significant effect. In the case of the macronutrients, only S has a significant effect at the 0.05 level ($F = 8.02$, $p = 0.0097$). There is no significant effect of Na or of the interaction effect between the two elements. When considering the micronutrients, the null hypothesis can only be rejected for Ni and Mo, as Fe, Se, and Co show no effect on this process parameter. Significant interaction effects between these elements are revealed mostly for Ni, Fe, and Co.

As expected, the analysis of acetic acid concentration and acetic acid equivalent revealed similar results and effects, as well as significant impacts of roughly the same interactions between the trace elements. In the case of the propionic acid concentration, this study demonstrated less significant effects of the tested elements and the relations between them. The results highlight the importance of the macronutrient S, which showed for each case an effect on the VFA concentration in the samples. Sodium in turn showed no effect and neither did the interaction between sodium and sulfur. The analysis of the data has also demonstrated the impact that the OLR has on the biogas process stability and on the VFA concentration for the sampled biogas plants.

Previous studies on different substrates and digester types describe the strong effect of trace elements presence and trace elements mixtures addition on the production and concentration of several VFA [27–29]. The study presented here described the effects of single micronutrients, as well as the effect of interactions between them. In the three statistical models for the analyzed parameters, Ni and Mo were the only variables and Co-Mo-SE and Ni-Co-Se the only interactions that had a consistently statistically significant effect. This confirmed that nickel has a highly significant effect on the VFA concentrations in digesters and thus an effect on the stability of the biogas process. Also a combined effect of Ni especially with Fe and Co was revealed. The null hypothesis can also be rejected for molybdenum. Mo showed a statistically significant and positive impact on all three analyzed process parameters and therefore also on the stability in the digesters. For Se, an impact on the acetic acid equivalent and propionic acid concentration was described, but no effect on the acetic acid concentration. In the case of the 25 sampled biogas plants, the general idea of the null hypothesis must be accepted for Fe and Co, as both failed to show an effect on the investigated parameters, although both were contained by most significant interactions between the trace elements.

3.3 Distribution of nutrient concentrations

The nutrient concentrations determined from the digesters of the selected biogas plants are represented graphically in Figs. 2 and 3. The concentration of nickel varies over a large range from 1.56 to 16.34 mg kg^{-1}. These values are significantly higher than the values described and evaluated by Schattauer et al. in an analysis of 10 biogas plants across Europe [22]. According to the patent of Oechsner et al., the values are in the target range, but mostly lower than the described optimal concentration [32]. The nickel concentrations are the highest among the analyzed trace elements. Co and Mo concentrations also vary widely from 0.47 to 8.10, respectively, 1.18 to 6.25 mg kg^{-1}. For both micronutrients, the values are up to 5, respectively, 12 times higher than the values documented by Schattauer et al. [22] but well in the range recommended by Oechsner et al. [32]. Selenium concentrations covered a range from 0.00 to 1.43 mg kg^{-1}, higher than previous recorded values. Sodium concentration ranges from 0.36 to 6.04 g kg^{-1}, while sulfur was determined in concentrations ranging from 3.05 to 6.68 g kg^{-1}, considerably higher than previously documented. With a mean of 2.64 g kg^{-1}, the iron concentrations vary between 0.79 and 7.78 g kg^{-1}, mostly well in the recommended range.

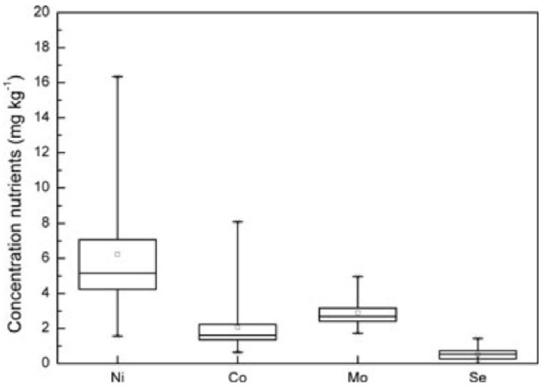

Figure 2. Distribution of trace element concentrations in the selected biogas plants based on the five number data summary. The median, upper, and lower quartiles; the minimum and maximum data values; and the mean value are indicated.

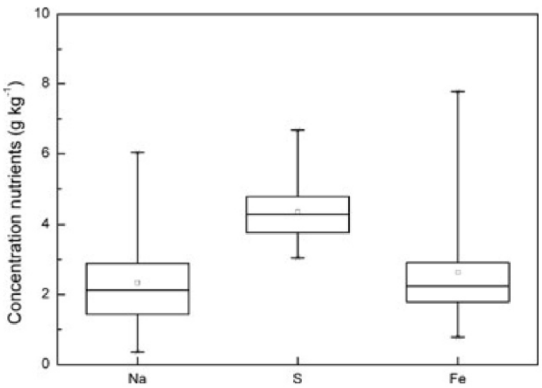

Figure 3. Distribution of nutrient concentrations in the selected biogas plants based on the five number data summary. The median, upper, and lower quartiles; the minimum and maximum data values; and the mean value are indicated.

4 Concluding remarks

Our findings support the hypothesis that several trace elements and macronutrients have a strong statistically significant effect on the stability of the biogas process as measured by short-chain fatty acid concentrations analyzed in selected working agricultural biogas plants, based on a feedstock of energy crops and manure. This study confirmed the results found in literature on the distinct differences and extremely high variations in the mineral substance contents of different digester samples. Our analysis is limited to the data gathered from the 25 selected biogas plants and utilizes a relatively small sample size, so that future efforts may attempt to generalize our findings at a regional and at a national scale. This paper does, however, add to the body of evidence on trace elements and macronutrients benefits and makes an argument for continued supply of these elements to assure a stable and optimal biogas production process.

Practical application

In 2011, almost 7000 biogas plants were operated in Germany. To achieve an economic benefit, a highly stable and efficient biogas production is very important. To assure an efficient biomethanization, the technology of the production plants, as well as the fermentation process itself has to be improved. Biogas results from incomplete anaerobic mineralization of organic source materials. A large number of microorganisms participate in this biomass conversion. These microorganisms require various mineral substances for their metabolism and growth. Therefore, optimal nutrient supply in the digester is essential for efficient biogas production. The aim of this study was to analyze the effects of various micro- and macronutrients on the process stability by a field test. Several trace elements, macronutrients, their relationships, as well as the process parameters propionic acid, acetic acid, and acetic acid equivalent were investigated.

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The authors have declared no conflict of interest.

5 References

[1] Rilling, N., Anaerobic fermentation of wet and semidry garbage waste fractions, in: Jördening, H.-J., Winter, J. (Ed.), *Environmental Biotechnology. Concepts and Applications*, WILEY-VCH Verlag, Weinheim 2005, pp. 355–373.

[2] Gallert, C., Winter, J., Bacterial metabolism in wastewater treatment systems, in: Jördening, H.-J., Winter, J. (Ed.), *Environmental Biotechnology. Concepts and Applications*, WILEY-VCH Verlag, Weinheim 2005, pp. 1–48.

[3] Lyberatos, G., Skiadas, I. V., Modelling of anaerobic digestion—a review. *Global Nest: the Int. J.* 1999, *1*, 63–76.

[4] Bryant, M. P., Microbial methane production—theoretical aspects. *J. Anim. Sci.* 1979, *48*, 193–201.

[5] Lettinga, G., Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek* 1995, *67*, 3–28.

[6] Weiland, P., Biogas production: current state and perspectives. *Appl. Microbiol. Biotechnol.* 2010, *85*, 849–860.

[7] Gonzalez-Gil, G., Jansen, S., Zandvoort, M. H., van Leeuwen, H.P., Effect of yeast extract on speciation and bioavailability of nickel and cobalt in anaerobic bioreactors. *Biotechnol. Bioeng.* 2003, *82*(2), 134–142.

[8] Zandvoort, M. H., Van Hullebusch, E. D., Gieteling, J., Lettinga, G. et al., Effect of sulfur source on the performance and metal retention of methanol-fed UASB reactors. *Biotechnol. Prog.* 2005, *21*(3), 839–850.

[9] Speece, R. E., Anaerobic biotechnology for industrial wastewater treatment. *Environ. Sci. Technol.* 1983, *17*(9), 416–427.

[10] Perski, H. -J., Moll, J., Thauer, R. K., Sodium dependence of growth and methane formation in *Methanobacterium thermoautotrophicum. Arch. Microbiol.* 1981, *130*(4), 319–321.

[11] Zandvoort, M. H., van Hullebusch, E.D., Fermoso, F. G., Lens, P. N. L., Trace metals in anaerobic granular sludge reactors: bioavailability and dosing strategies. *Eng. Life Sci.* 2006, *6*(3), 293–301.

[12] Zhang, Y., Zhang, Z., Suzuki, K., Maekawa, T., Uptake and mass balance of trace elements for methane producing bacteria. *Biomass Bioenergy* 2003, *25*, 427–433.

[13] Demirel, B., Scherer, P., Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass Bioenergy* 2011, *35*, 992–998.

[14] Woo, G. J., Wasserfallen, A., Wolfe, R. S., Methyl viologen hydrogenase II, a new member of the hydrogenase family from *Methanobacterium thermoautotrophicum. H. Bacteriol.* 1993, *175*, 5970–5977.

[15] Lacy, D., Negash, B., Basvapatna, S. R., Paul, J. W., Bacterial methanogenesis and growth from CO_2 with elemental iron as the sole source of electrons. *Science* 1987, *237*, 509–511.

[16] Jarrell, K. F., Kalmokoff, M. L., Nutriential requirements of the methanogenic archaebacteria. *Microbiology* 1988, *34*, 557–576.

[17] Chasteen, T. G., Bentley, R., Biomethylation of selenium and tellurium. *Microorganisms and plants. Chem. Rev.* 2003, *103*, 1–25.

[18] Diekert, G., Klee, B., Thauer, R. K., Nickel, a component of factor F430 from *Methanobacterium thermoautotrophicum. Arch. Microbiol.* 1980, *124*(1), 103–106.

[19] Hausinger, R. P., Karplus, P. A., Urease, in: Wieghardt, K., Huber, T. R., Poulos, T. L., Messerschmidt, A. (Eds.), *Handbook of Metalloproteins,* Wiley Verlag, West Sussex 2001, pp. 867–79.

[20] Callander, I. J., Barford, J. P., Precipitation, chelation, and the availability of metals as nutrients in anaerobic digestion. I. Methodology. *Biotechnol. Bioeng.* 1983, *25*, 1947–1957.

[21] Bischofsberger, W., Dichtl, N., Rosenwinkel, K.-H., Seyfried, C.F., *Anaerobtechnik*, Verlag Springer: Berlin Heidelberg 2005, pp. 65–84.

[22] Schattauer, A., Abdoun, E., Weiland, P., Plöchl, M. et al., Abundance of trace elements in demonstration biogas plants. *Biosyst. Eng.* 2011, *108*, 57–65.

[23] Preißler, D., Lemmer, A., Monovergärung von Grüngut - Teilprojekt Einfluss von Schwefel auf den Gärprozess und Eignung verschiedener Eisensalze zur Schwefelwasserstoffreduktion im Biogas, Stuttgart-Hohenheim: Universität Hohenheim, Landesanstalt für Agrartechnik und Bioenergie, Endbericht zum Forschungsprojekt, 2010.

[24] Lemmer, A., Biogaserzeugung, in: Bajohr, S., Graf, F. (Eds.), *Biogas. Erzeugung, Aufbereitung, Einspeisung,* Oldenbourg Industrieverlag GMBH 2011, pp. 75–118.

[25] Wang, Q., Kuninobu, M., Ogawa, H. I., Kato, Y., Degradation of volatile fatty acids in highly efficient anaerobic digestion. *Biomass Bioenergy* 1999, *16*, 407–416.

[26] Ren, N. Q., Wang, B. Z., Hydrogen-production technology using organic wastewater fermentation—theory and method. *Science Technology Press* 1994, cited by Wang, Y., Zhang, Y., Wang, J., Meng, L., Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. *Biomass Bioenergy* 2009, *33*, 848–853.

[27] Wilkie, A., Goto, M., Bordeaux, F. M., Smith, P. H. Enhancement of anaerobic methanogenesis from napiergrass by addition of micronutrients. *Biomass* 1986, *11*(2), 135–146.

[28] Abdoun, E., Weiland, P., Optimization of monofermentation from renewable raw materials by the addition of trace elements. *Bornimer Agrartechnische Berichte* 2009, *68*, 69–75.

[29] Goodwin, J. A. S., Wase, D. A. J., Forster, C. F., Effects of nutrient limitation on the anaerobic upflow sludge blanket reactor. *Enzyme Microb. Tech.* 1990, *12*(11), 877–884.

[30] Lindorfer, H., Corcoba, A., Vasilieva, V., Braun, R. et al., Doubling the organic loading rate in the co-digestion of energy crops and manure—a full scale case study. *Bioresource Technol.* 2008, *99*(5), 1148–1156.

[31] Lindorfer, H., Verbesserung der Wirtschaftlichkeit von Biogasanlagen durch die Optimierung der Fermenterbiologie; manuscript of oral presentation. Fachtagung Biogas der Arbeitsgruppe Biogas beim TBV e.V. on 28 May 2009, Jena.

[32] Oechsner, H., Lemmer, A., Ramhold, D., Mathies, E. et al., Verfahren zur Biogaserzeugung. Europäische Patentanmeldung EP 1 997 901 A2, 2008.

3 PUBLIKATION 2: EFFECT OF ETHYLENEDIAMINETETRAACETIC ACID (EDTA) ON THE BIOAVAILABILITY OF TRACE ELEMENTS DURING ANAEROBIC DIGESTION

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Abstract

The uptake of essential trace elements by methanogenic bacteria can be obstructed by precipitation in the presence of sulfides and carbonates. The objective of this study was to investigate whether the bioavailability of trace elements, and therefore the methane yield, can be improved through the use of complexing agents. Research showed that the use of EDTA as a complexing agent in the biogas process increases the solubility of essential metals and enhances their bioavailability. If the substrate of a biogas digester has a low content of trace elements, solutions of elements essential for the methanogenic bacteria have to be added to the process. If these metals are complexed with EDTA prior to their supply, the necessary amount can be reduced by up to 75 % compared to the non-complexed metals. Therefore, it would be advantageous for environmental and economic reasons to complex trace elements prior to their addition to the biogas process.

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Effect of ethylenediaminetetraacetic acid (EDTA) on the bioavailability of trace elements during anaerobic digestion

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HIGHLIGHTS

- Trace elements precipitation endangers the methane yield.
- EDTA increased the bioavailability of trace elements.
- Reductions of trace elements concentrations were compensated by EDTA.
- EDTA showed significant positive effects when added to heavily undersupplied substrates.
- EDTA addition to biogas processes is advantageous for environmental and economic reasons.

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ABSTRACT

The uptake of essential trace elements by methanogenic bacteria can be obstructed by precipitation in the presence of sulfides and carbonates. The objective of this study was to investigate whether the bioavailability of trace elements, and therefore the methane yield, can be improved through the use of complexing agents. Research showed that the use of EDTA as a complexing agent in the biogas process increases the solubility of essential metals and enhances their bioavailability. If the substrate of a biogas digester has a low content of trace elements, solutions of elements essential for the methanogenic bacteria have to be added to the process. If these metals are complexed with EDTA prior to their supply, the necessary amount can be reduced by up to 75% compared to the non-complexed metals. Therefore, it would be advantageous for environmental and economic reasons to complex trace elements prior to their addition to the biogas process.

1. Introduction

The production of biogas by anaerobic digestion from organic materials is a well-investigated and well-established process. Biogas is a highly versatile source of renewable energy, either based on energy plants or organic residues. Crucial for the future of the biogas technology is the cost effectiveness of the production process; therefore the maximization of gas yields and stabilization of the fermentation process is an important research goal. This can be achieved through a fully-developed biogas technology and by monitoring and optimization of the highly complex microbiological processes involved in the production of biogas. The anaerobic biogas formation process is always started by the primary fermentating bacteria, which convert the biomass into organic acids and alcohol. These products are further processed by acetogenic bacteria and finally by methanogenic archaea. Acetogenic bacteria and the methanogens live in symbiosis by breaking down organic substance to use the building materials and energy thus released to build up and maintain their own vital functions. It has been determined that the productivity of microorganisms decisively depends on the availability and optimal supply of nutrients, vital minerals and trace elements. This results in an improved gas formation rate and sufficient microbiological activity to ensure the process stability and to prevent strong cuts in production and yield [1].

Methanogens are an exceptional class of bacteria, with special nutrients requirements. Comprehensive research of the wastewater treatment process over the past few decades offered essential information about these complex nutrients requirements. Trace elements essential for the growth and metabolism of the methanogens are iron, nickel, cobalt, selenium, molybdenum, boron and tungsten [2].

Iron functions as a binding element in sulfide precipitation [3] and is also used in the transport system of the methanogens for

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the reduction of CO_2 to CH_4. Iron acts both as an electron acceptor and as an electron donor, fulfilling the role of an energy carrier [4]. Cobalt and boron are essential trace elements for acetogens and methanogens. Cobalt is used as a central atom in corrinoids and vitamin B12 enzymes [2]. Molybdenum catalyzes the oxidation and reduction reactions of CO_2 and is necessary for the formation of enzymes. Selenium can be a growth-limiting factor for some methanogenic bacteria, as it is required for the formation of amino acids such as selenocysteine [5]. The distinct feature of the methanogens is their requirement of nickel, as it is not essential for bacteria growth [6]. Nickel enhances the growth of methanogens like *Methanobacterium bryantii* and helps maintain their cell membranes and thus their structural stability [2]. Nickel is also involved in the formation of several enzymes such as urease and hydrogenase and is the central atom of the cofactor F_{430}, which is essential for the final step of methane formation [6,7].

Demirel and Scherer [8] reviewed the micronutrient requirements of agricultural biogas digestion systems operated with energy crops and manure, concluding that limited amounts of data are currently available about the trace element requirements and supply of agricultural biogas plants. The reviewed studies showed that a lack of nutrients can lead to process instability and disruption of the energy production in agricultural biogas digesters. The addition of single trace elements, as well as additions of trace element combinations, produced significant effects on anaerobic digestion. The addition of trace elements is most important when energy crops are used as sole substrates for the production of biogas [9].

If the trace elements are not available in sufficient concentrations, the enzymes and coenzymes needed for metabolism are no longer properly formed, which leads to a decrease of the performance capability of the methanogenic microorganism populations [1].

If a trace element deficit is ascertained in the fermenting substrate of a full scale digester, commercially available trace element solutions are used. These solutions increase the concentration of micronutrients in the digester, thus substantially improving the metabolic rate of the methanogens, which subsequently leads to a better conversion of the organic acids and to higher substrate specific methane yields. Due to the fact that most trace elements are heavy metals, the added amounts should be kept as low as possible. An improvement of the bioavailability of micronutrients would reduce the required amounts and thus reduce the pollution of the ecosystems on which the digestate is applied.

However, the analysis of the concentration of nutrients in the digester only determines the absolute concentration. This cannot give precise information about the bioavailability and mobility of the trace elements for the uptake by methanogenic bacteria, as the availability is difficult to measure [10]. Bioavailability is affected by the presence of sulfide, carbonate and phosphate in the digester, as these chemical ions can lead to the precipitation of free metal ions, making them unavailable for microbial uptake. Other factors are metal ion concentration, pH value, redox potential and metal chelation by organic and inorganic agents [10,11]. Strategies were developed to improve the bioavailability of trace elements in the biogas process, one being the addition of complexing agents. They prevent metal ion removal and increase the soluble fraction of the metals, thus preventing precipitation. Gonzalez-Gil et al. [12] suggested the use of yeast extracts, while Callander and Barford [10] proposed the use of organic chelating agents.

Polyamino carboxylic acids, such as ethylenediaminetetraacetic acid (EDTA), are used in all media to modify the concentration of metal ions by preventing their precipitation and offering a possibility to control their concentrations [10].

EDTA, an anthropogenic hexadentate chelating agent, has been used for many years as a stabilizer or sequestrant in many industrial fields, such as the pulp and paper processing industry, wastewater treatment, medicine, the food industry, metal treatment, detergents manufacturing and the textile industry. It has the ability to form strong soluble complexes with a wide variety of metals. EDTA is a recoverable and reusable chelating agent with great potential. It is also the most efficient complexing agent used for phytoremediation of heavy metal contaminated soils, as it increases the bioavailability and plant uptake of metals in the soil [13].

The use of EDTA in the biogas process is currently not a common practice. In the present study, laboratory batch assays were used to evaluate the effects of EDTA addition and the addition of EDTA complexed ions instead of ion salts to different substrates in the presence of different initial trace element concentrations on the biological methane formation process. The objective was to determine and describe the effect of EDTA in different concentrations on the bioavailability of trace elements and to elucidate whether its presence in biogas digesters fed with energy crops and manure would allow a reduction of trace elements additions without affecting the methane yield. As a result, the accumulation of Co, Ni, Se and other ions essential for methanogenic bacteria in the effluent of biogas digesters can be avoided and these residues can be used as fertilizers without causing environmental problems.

2. Material and methods

This study was composed of two batch experiments carried out in the biogas laboratory of the State Institute of Agricultural Engineering and Bioenergy, Stuttgart. For this study, two fermentation substrates taken from full scale biogas plants were used as inoculi. The low trace elements concentrations of the substrates allowed the testing of several trace elements solutions in optimal and reduced concentrations, the addition of EDTA in different concentrations and the replacement of nickel and cobalt salts with EDTA complexed forms.

2.1. Biogas and methane analysis

The Hohenheim Biogas Yield Test (HBT) was used to carry out the batch experiments in this study. It is a well established and feasible laboratory batch test, developed at the University of Hohenheim (Patent No. 10227685, 20.01.2005) according to the VDI-Guideline 4630 [14] and it is used to evaluate and compare the methane production of different substrates, as well as their biodegradability. The HBT is based on 100 ml glass syringes (flask sampler) used as digesters and gas storage. The flasks are fitted into a motor-driven rotor responsible for mixing the substrate. The whole unit is placed into an incubator, which ensures the required temperature and environment for the experiments. Three replications were tested for each variant [15].

The amount of produced biogas is recorded with an accuracy of 1 ml and the methane percentage is measured by a gas transducer AGM 10 (Pronova Analysetechnik, Germany), with a non-dispersive infrared (NDIR) sensor. Temperature and atmospheric pressure are also measured each time, so the values can be corrected to standard conditions.

2.2. Materials and experimental design

The experiments were based on two different substrates taken from full scale biogas plants with low trace elements concentrations. Both agricultural biogas plants were fed with renewable raw materials and only a small amount of manure. At both stations the increasing concentrations of volatile fatty acids were monitored during the weeks before sample collection. The substrates were analyzed at the ISF GmbH laboratory according to their trace elements concentrations and fatty acids content. As presented in [16], concentrations of trace elements were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) and the fatty acids, as well as their concentrations, were determined using the HPLC method.

The optimal trace elements concentrations in this study were taken from a patent based on an earlier study at the University of Hohenheim [17], and are as follows (expressed in mg kg^{-1} DM – dry matter): for Ni 8, for Co 1.8, for Fe 2400, for Mo 4, for Se 0.5 and for B 25. Compared to the values given in the patent, the target value for nickel was reduced from 10 to 8 mg kg^{-1} DM. This followed unpublished results of new studies on trace elements requirements of methanogens, carried out by the same research group. The initial nutrients content and the concentrations of the volatile fatty acids of the digester substrates are presented in Table 1.

The chemicals used for the micronutrient solutions were nickel(II) chloride, cobalt(II) chloride, iron(III) chloride, boric acid, sodium selenite and ammonium heptamolybdate. EDTA was used as ethylenediaminetetraacetic acid disodium salt, as NiEDTA $(NH_4)_2$ with 5% Ni and $CoEDTANa_2$ (5% Co).

2.3. Experimental design

Thirty grams of the fermentative substrate was added to each flask. For the reference samples, 2 ml of water was added to the substrate. For all other variants, a mixture of 1 ml of the respective trace elements solution and 1 ml of the respective EDTA solution were added. Dried, ground maize was also added as substrate to all tested flasks to be converted into methane. Based on the characteristics of each substrate, 0.2 g maize was added to the substrate 1 samples and 0.1 g to all samples of substrate 2. Both experiments were carried out under mesophilic conditions at 40 °C. The duration of the first experiment was 70 days and the second experiment lasted 60 days.

The concentrations of the trace elements solutions added to each variant were calculated according to the results of the analysis for each substrate and the concentrations defined as optimal. For the variants with reduced trace elements concentrations, the reduction was calculated based on the necessary amount to be added to reach the optimal concentration.

2.4. Experiment 1. Effect of EDTA

Substrate 1 was used for this experiment and several variants were planned: a reference sample, a variant optimally supplied with trace elements and a variant with the Ni concentration reduced by 25%. Furthermore, in optimally supplied variants, Ni and Co chloride were replaced with Ni(II)EDTA and Co(II)EDTA. The Co(II)EDTA concentration was reduced by 25%, while in other variants only 10% and 50% of the optimal Ni concentration was added as Ni(II)EDTA.

The effect of EDTA as sodium salt on the bioavailability of the optimal trace elements concentrations and on reduced concentrations was also tested. The necessary EDTA amount was calculated based on the optimal iron concentration, the highest among the trace elements concentrations. Two different amounts of EDTA were added: a full amount (50 mg kg^{-1} DM) and an amount reduced by 25% (37.5 mg kg^{-1} DM).

For further analysis of the results, an interpolation was applied to calculate the time needed to produce 50% of the maximal substrate specific methane yield (MSM) in the different variants.

2.5. Experiment 2. Effect of EDTA concentration

For the second experiment, substrate 2 was used. The necessary EDTA concentration, Concentration 1 (C1), was determined based on the mol quantity of trace elements amount to be added for the optimal concentration (see Section 2.2). A second EDTA concentration, Concentration 2 (C2), was calculated by doubling the amount of C1. A total of 105 glass syringes were used for 35 different variants, each with three repetitions. The effect of EDTA was investigated by using the described three concentrations all through the experiment – C0 (no addition), C1 and C2. These concentrations were added to the control samples without micronutrients, to the optimally supplied variants and to the variants with a reduced trace elements concentration (by 50% and 75% of the required amount).

2.6. Statistical analysis

A statistical system was used to describe the effect of EDTA on the bioavailability of trace elements in the fermentation substrate based on its specific methane yield.

The SAS (SAS/STAT® software) PROC MIXED procedure was used to determine the statistical significance of the differences between the treatments (the addition of trace elements and the addition of EDTA) with their different levels (four trace elements and three EDTA concentrations). The MODEL statement was used to analyze the dependent and independent variables. This article considers a model for the data described. This model tests the effects of trace elements and EDTA addition in different concentrations on the specific methane yield of the substrate used in the trial. The means of the variables were compared using LSMEANS to evaluate differences at the 5% level of significance.

3. Results and discussion

The use of Ni(II)EDTA and Co(II)EDTA, and the addition of EDTA in salt form, were tested. EDTA complexes have high stability constants with several essential trace elements. The effect of random complexation of essential metals on the overall bioavailability of

Table 1
Volatile fatty acid and trace element concentrations of the fermentation substrates.

Substrate	Volatile fatty acids (mg kg^{-1})					
	Acetic acid	Propionic acid	i-Butyric acid	n-Butyric acid	i-Valeric acid	Total
1	5951	2877	471	206	660	10,165
2	1328	7138	1293	112	991	10,862
	Trace elements (mg kg^{-1} DM)					
	Ni	Co	Fe	Mo	Se	B
1	1.96	0.32	657.52	1.89	0.26	27.85
2	2.40	0.40	753.00	1.20	0.20	22.70
	8.00[a]	1.80[a]	2400.00[a]	4.00[a]	0.50[a]	25.00[a]

[a] Target values used throughout the article as optimal concentrations.

the trace elements present in the fermentation substrate was tested throughout the trial. The possibility of a reduction of the amounts of metals to be added in the presence of EDTA was also addressed. The results are based on the specific methane yield of the tested variants.

3.1. Experiment 1. Effect of Ni(II)- and Co(II)EDTA

A HBT was carried out to test the influence of Ni and Co on the stability of the biogas process and to improve their bioavailability by replacing Ni and Co chlorides with an EDTA complexed form. In this experiment, the maximal specific methane yield (ODM – organic dry matter) was 0.16 $m^3\,kg^{-1}$. The results of the experiment are presented in Fig. 1. 50% of the maximal specific methane yield (MSM), 0.08 $m^3\,kg^{-1}$ is marked by a line.

The reference sample reached a specific methane yield of 0.13 $m^3\,kg^{-1}$ and needed 45.8 days to reach half of the maximal specific yield. The reduction of Ni concentration by 25% brought a highly significant reduction of the methane yield, compared to the optimal nickel concentration. The optimally supplied variant produced 0.15 $m^3\,kg^{-1}$ and only needed 20.9 days to reach 50% of the maximal specific methane yield, while the Ni reduced variant needed 47.1 days and only reached 0.13 $m^3\,kg^{-1}$, confirming the effect of Ni on the stability of the process.

Nickel is an environmentally hazardous substance and research should focus on methods to reduce the amount of nickel added to the biogas process. The addition of nickel in an optimal concentration as chloride and as Ni(II)EDTA did not result in any differences in the final specific methane yield. Furthermore, the results show that a replacement of Ni chloride by Ni(II)EDTA is possible. The use of Ni(II)EDTA also allowed a high reduction of the amounts of nickel added to the biogas process, without having any negative effects on the process stability or the specific methane yield. Both reduced Ni(II)EDTA variants (by 50% and 90% of the amount to be added) slightly influenced the process, but reached a methane yield of 0.15 m^3 kg, just like the two optimally supplied variants, thus proving the strong positive effect of Ni(II)EDTA. These findings are in agreement with the results of Hu et al. [18], who confirmed that the presence of Ni(II)EDTA in wastewater can substantially increase the bioavailability of Ni. The use of Ni(II)EDTA brought very promising results, proving that complexes of individual metals can have a positive effect on the biogas process.

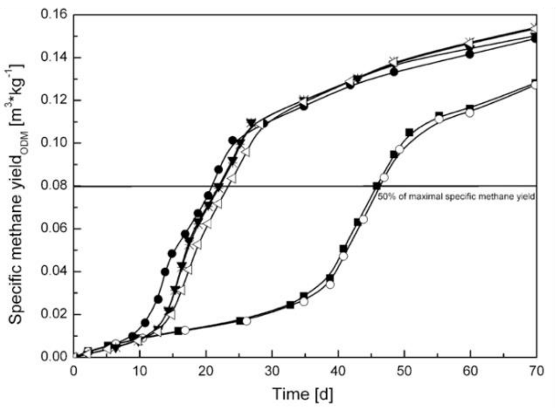

Fig. 1. Effect of Ni(II)EDTA addition on the substrate specific methane yield (ODM) in $m^3\,kg^{-1}$, HBT batch test, trial duration 70 days, mesophilic fermentation at 40 °C, STP, ■ reference sample, ● trace elements addition up to optimal concentration, ○ optimal trace elements addition – Ni reduced by 25%, ⚹ optimal trace elements addition – Ni as Ni(II)EDTA, ▼optimal trace elements addition – Ni(II)EDTA reduced by 50%, ◃ optimal trace elements addition – Ni(II)EDTA reduced by 90%.

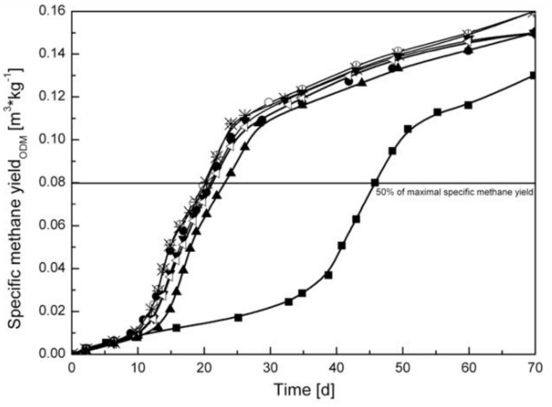

Fig. 2. Effect of EDTA addition on the substrate specific methane yield (ODM) in $m^3\,kg^{-1}$, HBT batch test, trial duration 70 days, mesophilic fermentation at 40 °C, STP, ■ reference sample, ● optimal trace elements addition, ○ optimal trace elements addition – EDTA (50 mg kg^{-1}), ⚹ optimal trace elements addition – EDTA (37.5 mg kg^{-1}), ▼ trace elements addition reduced by 25% – EDTA (50 mg kg^{-1}), ◃ trace elements addition reduced by 25% – EDTA (37.5 mg kg^{-1}), ▲ trace elements addition reduced by 50% – EDTA (50 mg kg^{-1}).

The addition of Co(II)EDTA showed no effect on the methane yield or process stability (data not shown).

3.2. Experiment 1. Effect of EDTA

The effect of EDTA is presented in Fig. 2. In this experiment, the maximal specific methane yield (ODM) was 0.16 $m^3\,kg^{-1}$ and 50% of the maximal specific methane yield (0.08 m^3 kg) is marked by a line.

The addition of EDTA, in two concentrations, to the optimally supplied variant with trace elements brought a slight improvement of the methane yield (0.16 $m^3\,kg^{-1}$) and process stability (MSM of 20.1 and respectively 20.4 days), but without affecting it significantly.

The supplied trace elements were reduced in the presence of EDTA in several concentrations by 25% and 50% of the amount to be added and compared with the optimal variant. Both reductions by 25% in the presence of the two EDTA concentrations, reached half of MSM in 21 days and a specific methane yield of 0.15 $m^3\,kg^{-1}$. The reduction by 50% with an EDTA addition of 50 mg kg^{-1} DM, reached half of the maximal specific methane yield in 23 days and a methane yield of 0.15 $m^3\,kg^{-1}$, just like the optimally supplied variant. The results showed that a high reduction of trace elements concentrations in the presence of EDTA is possible, without significant changes to the final specific methane yield and with a delay of only two days until reaching 50% of MSM. Based on the time needed to reach half of the MSM, it can be observed that all variants are more stable than the reference sample. This shows that the addition of EDTA to the biogas process also increased the stability of the process, not only the bioavailability of trace elements. These results confirm the findings of Callander and Barford [10] and Hu et al. [18,19] that complexing agents play an important role in improving the bioavailability of metals by facilitating the dissolution of metals from carbonates or sulfides, thus increasing their solubility. This could also have a high impact on the biogas plant entrepreneurs, who need trace elements supplements for their digesters.

Table 2
Specific methane yield (%) in presence of three EDTA concentrations (C0 – no addition, C1 and C2), with no trace elements addition (na) and optimal addition (opt); difference of means of tested variants.

Specific methane yield (%)						Difference of means	t Value
No trace elements addition			Optimal trace elements addition				
C0 na	C1 na	C2 na	C0 opt	C1 opt	C2 opt		
100	132	128	145	139	141	C0 na – C1 na	<0.0001
						C0 na – C2 na	<0.0001
						C0 na – C0 opt	<0.0001
						C0 opt – C1 opt	1.12
						C0 opt – C2 opt	0.29

Table 3
Specific methane yield (%) in presence of three EDTA concentrations (C0 – no addition, C1 and C2), with no trace elements addition (na), optimal addition (opt) and reduced addition by 50% and 75%, difference of means of tested variants.

Specific methane yield (%) Optimal and reduced trace elements addition								Difference of means	t Value
C0 na	C0 opt	C0 red50	C1 red50	C2 red50	C0 red75	C1 red75	C2 red75		
100	145	139	152	132	139	152	132	C0 opt – C0 red50	0.15
								C0 red50 – C1 red50	0.002
								C0 red50 – C2 red50	0.04
								C0 opt – C0 red75	<0.0001
								C0 red75 – C1 red75	<0.0001
								C0 red75 – C2 red75	<0.0001

3.3. Experiment 2. Effect of EDTA on trace elements additions to a heavily undersupplied substrate with high fatty acid concentrations

Substrate 2 (Table 1) was tested based on the results of the first experiment, which attested to the positive effect of EDTA on the bioavailability of trace elements in the biogas process. The scope was to further determine factors influencing the function of EDTA. The substrate was specially selected and was heavily undersupplied, with less than 1000 mg kg^{-1} DM trace elements and with a fatty acid concentration higher than 10000 mg kg^{-1} fresh matter.

The trial was again carried out with the HBT measurement system. For a better evaluation of the results, and differences between variants with various EDTA and trace elements concentrations, the specific methane yield of the reference sample was assumed to be 100% and all other variants were calculated relative to this value.

Reference samples were used to determine the effect of EDTA, in three concentrations, on the bioavailability of the trace elements already present in the substrate. A variant with trace elements and EDTA addition was used to determine the influence of EDTA on the methane yield in the case of optimal trace elements supply. The results are presented in Table 2.

The sole addition of trace elements had a highly significant ($p < 0.0001$) effect on the methane yield, improving it by 45%. The sole addition of EDTA to the substrate showed a highly significant positive effect ($p < 0.0001$) on the formation of gas and the total methane yield after a fermentation period of 60 days compared to the reference sample. The addition of EDTA in C1 and C2 increased the final specific methane yield by 32% and respectively 28%. The complementary addition of EDTA to optimally supplied variants only produced a methane yield comparable to the yield of the optimal variant. Therefore, the supplementary addition of EDTA had no positive effect on the bioavailability of trace elements.

Several variants with an addition of EDTA and reduced trace elements concentrations were tested. If an EDTA addition increases the bioavailability of trace elements, a reduction in the addition of trace elements should be enough to stabilize the biological conversion process. Therefore, combinations of reduced trace elements and complexing agent concentrations were examined. The results are presented in Table 3.

The fermentation substrate demonstrated a high dependency on trace elements. Already a reduction by 50% of the optimal trace elements addition led to lower methane yields compared to the optimal variant. A reduction of the trace elements addition by 75% led to similar low methane yields to the one achieved by the reference sample.

In the variants with a reduction of the addition of trace elements, EDTA had a considerably positive effect. Despite reducing the amount of trace elements in the fermentation substrate by 50% and 75% compared to the optimal variant, a complementary addition of EDTA in Concentration 1 brought significantly higher methane yields than the optimal variant without EDTA (Table 3).

The experiment suggested that the trace elements have a great influence on the methane yield. However, a reduction of the required trace elements amounts was compensated by the simultaneous addition of EDTA, which furthermore ensured the process stability and high methane yields. The experiment also showed that an overdose of the complexing agent can have negative effects on the process.

These results demonstrate that the characteristics (trace elements and fatty acid concentrations) of the selected fermentation substrate have a high and significant influence on the effect of EDTA in anaerobic digestion. This allowed a better understanding of the influence of complexing agents. The conclusions of statistical analysis of the results were, in the case of strongly undersupplied substrates with high fatty acids content, that the bioavailability of trace elements can be increased by the addition of EDTA and a reduction of the optimal concentration of trace elements by up to 75% is possible.

4. Conclusions

This paper demonstrated the advantages of the use of EDTA as a complexing agent in the biogas process, which is currently not a common practice. The effect of EDTA is influenced by several factors, the most significant of which is the substrate. The results showed that the addition of EDTA to heavily undersupplied substrates with high fatty acids content increases the methane yield by improving the bioavailability of metals. This would be advantageous from an environmental and economic point of view. Also, the addition of EDTA can achieve a higher methane yield of up to 32%. The results provide a solution to minimize the addition of essential trace elements to the biogas process.

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References

[1] A. Lemmer, Biogaserzeugung, in: S. Bajohr, F. Graf (Eds.), Biogas. Erzeugung, Aufbereitung, Einspeisung, Oldenbourg Industrieverlag GMBH, 2011, pp. 75–118.

[2] K.F. Jarrell, M.L. Kalmokoff, Nutriential requirements of the methanogenic archaebacteria, Microbiology 34 (1988) 557–576.

[3] G.J. Woo, A. Wasserfallen, R.S. Wolfe, Methyl viologen hydrogenase II, a new member of the hydrogenase family from *Methanobacterium thermoautotrophicum*, J. Bacteriol. 175 (1993) 5970–5977.

[4] D. Lacy, B. Negash, S.R. Basvapatna, J.W. Paul, Bacterial methanogenesis and growth from CO_2 with elemental iron as the sole source of electrons, Science 237 (1987) 509–511.

[5] T.G. Chasteen, R. Bentley, Biomethylation of selenium and tellurium. Microorganisms and plants, Chem. Rev. 103 (2003) 1–25.

[6] G. Diekert, B. Klee, R.K. Thauer, Nickel, a component of factor F_{430} from *Methanobacterium thermoautotrophicum*, Arch. Microbiol. 124 (1) (1980) 103–106.

[7] R.P. Hausinger, P.A. Karplus, Urease, in: K. Wieghardt, T.R. Huber, T.L. Poulos, A. Messerschmidt (Eds.), Handbook of Metalloproteins, Wiley Verlag, West Sussex, 2001, pp. 867–879.

[8] B. Demirel, P. Scherer, Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane, Biomass Bioenergy 35 (2011) 992–998.

[9] E. Abdoun, P. Weiland, Optimierung der Monovergärung von Nachwachsenden Rohstoffen durch die Zugabe von Spurenelementen (Optimization of monofermentation from renewable raw materials by the addition of trace elements), Bornimer Agrartech. Ber. 68 (2009) 69–78.

[10] I.J. Callander, J.P. Barford, Precipitation, chelation, and the availability of metals as nutrients in anaerobic digestion. I. Methodology, Biotechnol. Bioeng. 25 (1983) 1947–1957.

[11] F.E. Mosey, J.D. Swanwick, D.A. Hughes, Factors affecting the availability of heavy metals to inhibit anaerobic digestion, Water Pollut. Control 70 (1971) 668–679.

[12] G. Gonzalez-Gil, S. Jansen, M.H. Zandvoort, H.P. van Leeuwen, Effect of yeast extract on speciation and bioavailability of nickel and cobalt in anaerobic bioreactors, Biotechnol. Bioeng. 82 (2) (2003) 134–142.

[13] L. Wu, Y. Luo, X. Xing, P. Christie, EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk, Agric. Ecosyst. Environ. 102 (2004) 307–318.

[14] VDI 4630, Fermentation of organic materials, in: Characterization of Substrate, Sampling, Collection of Material Data, Fermentation Tests, VDI Gesellschaft Energietechnik, 2006.

[15] H. Oechsner, D. Helffrich, Hohenheimer Biogasertragstest, Agrartechnische Forsch. 9 (3) (2003) 27–30.

[16] A. Vintiloiu, A. Lemmer, H. Oechsner, T. Jungbluth, Mineral substances and macronutrients in the anaerobic conversion of biomass: an impact evaluation, Eng. Life Sci. 12 (3) (2012) 287–294.

[17] H. Oechsner, A. Lemmer, D. Ramhold, E. Mathies, et al., Verfahren zur Biogaserzeugung, Europäische Patentanmeldung EP 1 997 901 A2, 2008.

[18] Q.-H. Hu, X.-F. Li, H. Liu, G.-C. Du, J. Chen, Enhancement of methane fermentation in the presence of Ni^{2+} chelators, Biochem. Eng. J. 38 (2008) 98–104.

[19] Q.-H. Hu, X.-F. Liu, G.-C. Du, J. Chen, Effect of nitrilotriacetic acid on bioavailability of nickel during methane fermentation, Chem. Eng. J. 143 (2008) 111–116.

4 PUBLIKATION 3: EFFECTS OF CHELATES ON THE BIOAVAILABILITY OF TRACE ELEMENTS DURING ANAEROBIC DIGESTION: COMPARISON OF EDTA, EDDS AND IDS

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Abstract

EDTA proved to have a significant effect on the bioavailability of trace elements during anaerobic digestion. The amounts of trace elements added to biogas plant digesters to ensure optimal concentrations were reduced in the presence of EDTA by up to 75 % without an effect on the methane yield. The objective of this study was to investigate the use of readily biodegradable complexing agents as an alternative to the persistent EDTA in an attempt to reduce the risk of environmental damage. The chelates tested in this study were EDDS and IDS and their effect on degradation kinetics and on the bioavailability of trace elements was determined. The use of EDDS in the anaerobic digestion showed no effect. IDS significantly reduced the time needed to reach the maximum methane yield and increased the bioavailability of metal ions by up to 58 %. Reductions of trace elements additions by up to 75 % were possible in the presence of IDS without negative impacts on the methane yield. IDS proved that it represents a valid alternative to EDTA and that it can be successfully used in anaerobic digestion processes.

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Article

Effects of chelates on the bioavailability of trace elements during anaerobic digestion: Comparison of EDTA, EDDS and IDS

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Abstract: EDTA proved to have a significant effect on the bioavailability of trace elements during anaerobic digestion. The amounts of trace elements added to biogas plant digesters to ensure optimal concentrations were reduced in the presence of EDTA by up to 75 % without an effect on the methane yield. The objective of this study was to investigate the use of readily biodegradable complexing agents as an alternative to the persistent EDTA in an attempt to reduce the risk of environmental damage. The chelates tested in this study were EDDS and IDS and their effect on degradation kinetics and on the bioavailability of trace elements was determined. The use of EDDS in the anaerobic digestion showed no effect. IDS significantly reduced the time needed to reach the maximum methane yield and increased the bioavailability of metal ions by up to 58 %. Reductions of trace elements additions by up to 75 % were possible in the presence of IDS without negative impacts on the methane yield. IDS proved that it represents a valid alternative to EDTA and that it can be successfully used in anaerobic digestion processes.

Keywords: bioavailability; anaerobic digestion; biogas; ethylenediamine-tetraacetic acid; ethylenediaminedisuccinic acid; iminodisuccinic acid

1. Introduction

The number of operational biogas plants in Germany has grown rapidly and surpassed 7,500 by the year 2012 [1]. Several substrates are used for the feeding of these plants, mostly energy plants, manure and organic residues. By means of the used substrates, macro and micro elements enter the digesters and serve as essential nutrients for the microorganisms involved in the production of biogas. Several types of microorganisms are responsible for the anaerobic fermentation and the production of the highly versatile source of renewable energy. Biogas results in a stepwise conversion of biodegradable substances into CH_4, CO_2, NH_3, and H_2S via a syntrophic interaction between fermentative and acetogenic bacteria and the methanogenic archaea [2].

The process of biogas production is induced by primary fermentating bacteria, which convert the biomass into organic acids and alcohol. Acetogenic bacteria followed by the methanogenic archaea further process these products. The large number of microorganisms that participate in this biomass conversion break down the organic source materials and use the building materials and energy thus released to build up and maintain their own vital functions. The anaerobic fermentation and the growth of the microbial communities decisively depend on the bioavailability and optimal supply of minerals and trace elements [3]. The required quantities are specific to the respective variety. An optimal nutrient supply in the digester is targeted, as insufficient microbiological activity can jeopardize the process stability of a biogas plant and lead to strong cuts in production and yield [4].

Most of the operating biogas plants in Germany use maize as substrate [5]. When energy crops are used as sole substrates for the biogas production, an addition of trace elements is essential [6]. When a micro nutrient deficit is ascertained in the influent of full-scale digester, commercially available trace element solutions are generally supplied. These solutions increase the concentration of micro nutrients in the digester, thus substantially maintaining an optimal reactor performance. The added concentrations should be kept as low as possible, as most trace elements are heavy metals.

Not all trace elements present in the digester are available for microbial uptake. Factors like the presence of sulfide, carbonate and phosphate in the digester, metal ion concentration, pH value and redox potential affect the bioavailability of free metal ions, making them unavailable for microbial uptake [7, 8]. A higher bioavailability of the micro nutrients would reduce the amount of trace elements solutions added to biogas plant digesters and also the possibility of environmental damage of the ecosystems on which the digestate is applied. A possibility to increase the availability is the addition of chelating agents, which form complexes with metal ions and increase metal solubility. This prevents their removal and improves the chance for organisms to take up trace elements [7].

EDTA is the best known chelating agent and has the ability to form highly stable complexes with most metal ions. It has been the choice for many years in industrial fields, such as wastewater treatment, medicine, the pulp and paper processing industry, metal treatment, the food industry, detergents manufacturing and the textile industry.

Due to its strong complexing ability for different metals, it is also the most effective chelating agent used for phytoremediation of heavy metal contaminated soils, as it increases the bioavailability and plant uptake of metals in the soil [9]. However, the use of EDTA in phytoremediation is not without risks. Its non-biodegradable properties, as well as the environmental persistence could lead to environmental damage [10]. This triggered over recent years a search for replacement chelating agents that are equally efficient and readily biodegradable. Several environmentally friendly alternatives for EDTA have been proposed, including ethylenediaminedisuccinic acid (EDDS) and iminodisuccinic acid (IDS). The use of these complexing agents in improving the metal uptake by plants and limiting the leaching of trace elements from soils, as well as their use in the pulp and paper processing industry and detergents manufacturing has become a new and promising field of research [11-13].

The use of EDTA in the biogas process is currently not a common practice. Vintiloiu et al. [14] showed in a previous study that EDTA can significantly improve the bioavailability of the trace elements present in the fermentative substrates of agricultural biogas plants and that the required amounts to be added for a stable process can be reduced by up to 75 % without significantly affecting the methane yield.

The objective of this study was to investigate the impact of the readily biodegradable complexing agents EDDS and IDS on the bioavailability of micro elements in the biogas process, and to compare their effect with the effect of the persistent EDTA. A laboratory batch assay was used to evaluate the effects of EDTA, EDDS and IDS addition to a fermentative substrate in the presence of different initial trace element concentrations on the biological methane formation process. The goal of this study was to determine and describe the effect of EDTA, EDDS and IDS in different concentrations on the bioavailability of micro elements, and to determine whether their presence in biogas digesters fed with energy crops and manure would allow a reduction of trace elements additions without influencing the methane yields. As a result, the accumulation of the heavy metals essential for methanogenic archaea in the effluent of the digesters could be avoided and these residues could be used as fertilizers without causing environmental damage.

2. Material and methods

This study was composed of a batch experiment carried out in the biogas laboratory of the State Institute of Agricultural Engineering and Bioenergy, Stuttgart. A fermentation substrate from a full-scale biogas plant was used as inoculi. The characteristics of the substrate (low trace elements concentrations (less than 1,000 $mg^{*}kg^{-1}$ DM) and high fatty acids concentration (over 10,000 $mg^{*}kg^{-1}$)) allowed the testing of several micro elements solutions in optimal and reduced concentrations, as well as the addition of EDTA, EDDS and IDS in two different concentrations.

2.1 Biogas and methane analysis

The batch experiment in this study was carried out with The Hohenheim Biogas Yield Test (HBT). The HBT is a feasible and well established laboratory batch test, developed at the University of Hohenheim (Patent No. 10227685, 20.01.2005) according to the German standard for anaerobic digestion experiments (VDI-Guideline 4630) [16]. It is used to evaluate and compare the methane production of different substrates, as well as their biodegradability. 100 ml glass syringes (flask sampler) are used as digesters and gas storage. These glass syringes are fitted into a motor-driven rotor, which is responsible for mixing the substrate. The whole unit is placed into an incubator, thus ensuring the required temperature and environment for the experiments. Three replications were tested for each variant [15].

The methane percentage is measured by a gas transducer AGM 10 (Pronova Analysetechnik, Germany) with a non-dispersive infrared (NDIR) sensor, while the amount of produced biogas is recorded with an accuracy of 1 ml. Temperature and atmospheric pressure are also measured each time, so the values can be corrected to standard conditions.

2.2 Materials and experimental design

The experiments were based on a fermentation substrate with low trace elements concentrations from a full-scale biogas plant fed with a small amount of manure and renewable raw materials. The increasing concentrations of volatile fatty acids were monitored during the weeks before sample collection. The substrate was analyzed at the ISF GmbH laboratory and the micro elements concentrations and fatty acids content were determined. The trace elements concentrations were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES). The samples were prepared using a disintegration method, with nitric acid and hydrogen peroxide addition to the sample (dry residue at 105°C). The sample was then heated for 20 min and boiled for another 20 min and, after cooling, transferred in a 100 mL volumetric flask with distilled water. The concentration of each element present in the sample was determined in the optical emission spectrometer (ICAP 6000 Series, Thermo Scientific, USA) in relation to the wave length of its optical emissions. The types and concentrations of fatty acids present in the samples were determined using the HPLC method. After purifying the sample by removal of suspended particles and proteins, sulfuric acid was added and the mixture was tested with a HPLC (Knauer, Germany). The retention time of the liquid sample during this chromatographic separation method is measured by a UV detector and an index of refraction detector. A comparison of the obtained retention times of each detected substance with existing standards allows their identification and the determination of their concentration in the sample.

Several trace elements solutions were added to the fermentation substrate throughout this study: An optimal concentration and concentrations reduced by 50 and 75 % of the required amounts to be added to reach the optimal concentrations. The optimal micro elements concentrations in this study, also used in a previous study [14], were taken from a patent (EP 1 997 901 A2, 2008) based on an earlier study at the University of Hohenheim [17], and are as follows (expressed in $mg^{*}kg^{-1}$ DM): for Ni 8; for Co 1.8; for Fe 2,400; for Mo 4; for Se 0.5 and for B 25. The target value for nickel was reduced from 10 to 8 $mg^{*}kg^{-1}$ DM compared to the values given in the patent. This followed unpublished results of new studies on micro nutrients requirements of methanogenic archaea, carried out by the same research group.

The chemicals used for the micro nutrient solutions were nickel(II) chloride, cobalt(II) chloride, iron(III) chloride, boric acid, sodium selenite and ammonium heptamolybdate. Three complexing agents were used in this investigation, as follows: ethylenediaminetetraacetic acid (EDTA) as ethylenediaminetetraacetic disodium dihydrate (VWR International, USA), ethylenediaminedisuccinic acid (EDDS) as (S,S)-Ethylenediaminedisuccinic acid trisodium salt (Sigma-Aldrich Co. LLC, USA) and iminodisuccinic acid (IDS) as iminodicuccinic acid tetrasodium salt (Kurt Obermeier GmbH, Germany).

2.3 Experimental design

The experiment presented in this study was carried out under mesophilic conditions at 40^{0}C and lasted for 60 days. 30 g of the fermentative substrate and 0.1 g dried, ground maize as additional substrate were added to all tested glass syringes to be converted into methane. Two milliliters of water was added to the substrate in the reference samples. A mixture of 1 ml of the respective trace elements solution and 1 ml of the respective EDTA, EDDS and IDS solution were added to all other variants.

The concentrations of the micro elements solutions added to each variant were calculated according to the results of the substrate analysis and the concentrations defined as optimal. The reduction of the amounts for the variants with reduced trace elements concentrations was calculated based on the required amount to be added to reach the optimal concentration.

2.4 Effect of complexing agents concentration

The necessary EDTA, EDDS and IDS concentrations, concentration 1 (C1), were determined based on the mol quantity of the trace elements amount to be added for the optimal supply (see Section 2.2). Concentration 2 (C2), the second concentration of the complexing agents was calculated by doubling the amount of C1. For the 28 different variants, a total of 84 flasks were used, each with 3 repetitions. The effects of the three complexing agents were investigated by using the described three concentrations all through the experiment – C0 (no addition), C1 and C2. These concentrations were used in the control samples with no micro nutrients addition, the optimally supplied variants and the variants with a reduced trace elements concentration (by 50 and 75 % of the required amount).

2.5 Kinetics of anaerobic digestion

Once the cumulative methane production curves were obtained over the course of the batch experiment, they were fitted to the modified Gompertz equation (1) that describes the cumulative methane production in batch assays assuming that the CH_4 production is a function of bacterial growth:

$$M = P \times \exp\left\{-\exp\left[\frac{R_m \times e}{P}\left(\lambda - t\right) + 1\right]\right\} \qquad (1)$$

where M represents the cumulative methane production (ODM) ($Nm^3 * kg^{-1}$), P the methane production potential (ODM) ($Nm^3 * kg^{-1}$), Rm the maximum daily methane yield (ODM) ($Nm^3 * kg^{-1} * d$), λ the duration of the lag phase, t the duration of the assay (d) and e is 2.71828. The values of P, Rm and λ are constants and can be estimated by the use of a non-linear regression [18, 19]. In order to assign a time to the maximum daily methane yield, the derivation of (1) was used. The derivation was fitted to the data by using the "Solver" function under the "Formula" menu in Microsoft Office Excel 2007 and obtaining the best values of P, Rm and λ. "Solver" uses as a method the Newton algorithm for the search of parameter values and the values of the starting parameters were directly replaced by the best estimates [20]. In the case of unrealistic results of the parameter values, limits could be defined according to the approach presented by Lay et al. [21].

Statistical analyses were performed to reveal the effects of the complexing agents on the methane yield and the differences among the variants by using the procedure PROC TTEST (SAS/STAT® software) at a significance level of ≤5 %.

3. Results

The addition of EDTA, EDDS and IDS to the anaerobic digestion of a fermentation substrate and their effect on the bioavailability of trace elements (already present in the sample and added to ensure optimal concentrations) was tested. In order to better determine and evaluate the effects of the tested complexing agents, the maximum daily methane yields were calculated with the modified Gompertz equation. All analyzed variants had a lag phase of approximately 20 days and the methane production was kept low during this acclimatization phase. This can be explained by the high fatty acids content and the low trace elements concentrations in the substrate. These conditions shifted the equilibrium in the system, favoring the fermentative acidogenic phase of the anaerobic digestion. The specific methane yields and the time assigned to the maximum daily methane production for all tested variants are presented in Table 1.

Table 1. Specific methane yields and time assigned to the maximum daily methane yield for all tested variants

Analyzed variant	Specific methane yield$_{ODM}$ (m^3*kg^{-1})	Time assigned to the maximum daily methane yield (d)
Reference sample	0.102	27.67
Reference sample EDTA C1	0.135	47.54
Reference sample EDDS C1	0.118	34.18
Reference sample IDS C1	0.133	27.35
Reference sample EDTA C2	0.131	36.47
Reference sample EDDS C2	0.074	32.82
Reference sample IDS C2	0.115	40.67
Optimal*	0.148	33.88
Optimal EDTA C1	0.142	26.36
Optimal EDDS C1	0.148	36.51
Optimal IDS C1	0.164	25.78
Optimal EDTA C2	0.144	37.55
Optimal EDDS C2	0.130	n.d.
Optimal IDS C2	0.168	26.85
TE -50%**	0.142	29.82
TE -50% EDTA C1	0.156	32.75
TE -50% EDDS C1	0.141	34.25
TE -50% IDS C1	0.163	28.36
TE -50% EDTA C2	0.135	31.72
TE -50% EDDS C2	0.113	n.d.
TE -50% IDS C2	0.155	25.38
TE -75%***	0.096	39.77
TE -75% EDTA C1	0.153	36.02
TE -75% EDDS C1	0.132	48.60
TE -75% IDS C1	0.154	28.36
TE -75% EDTA C2	0.139	31.72
TE -75% EDDS C2	0.086	n.d.
TE -75% IDS C2	0.170	26.47

* Trace elements supplied in optimal amounts
** Trace elements addition amount reduced by 50%
*** Trace elements addition amount reduced by 75%

3.1 Degradation kinetics of anaerobic digestion with and without EDTA

The effect of EDTA addition in two concentrations on the degradation kinetics in the methane formation process is presented in Figure 1.

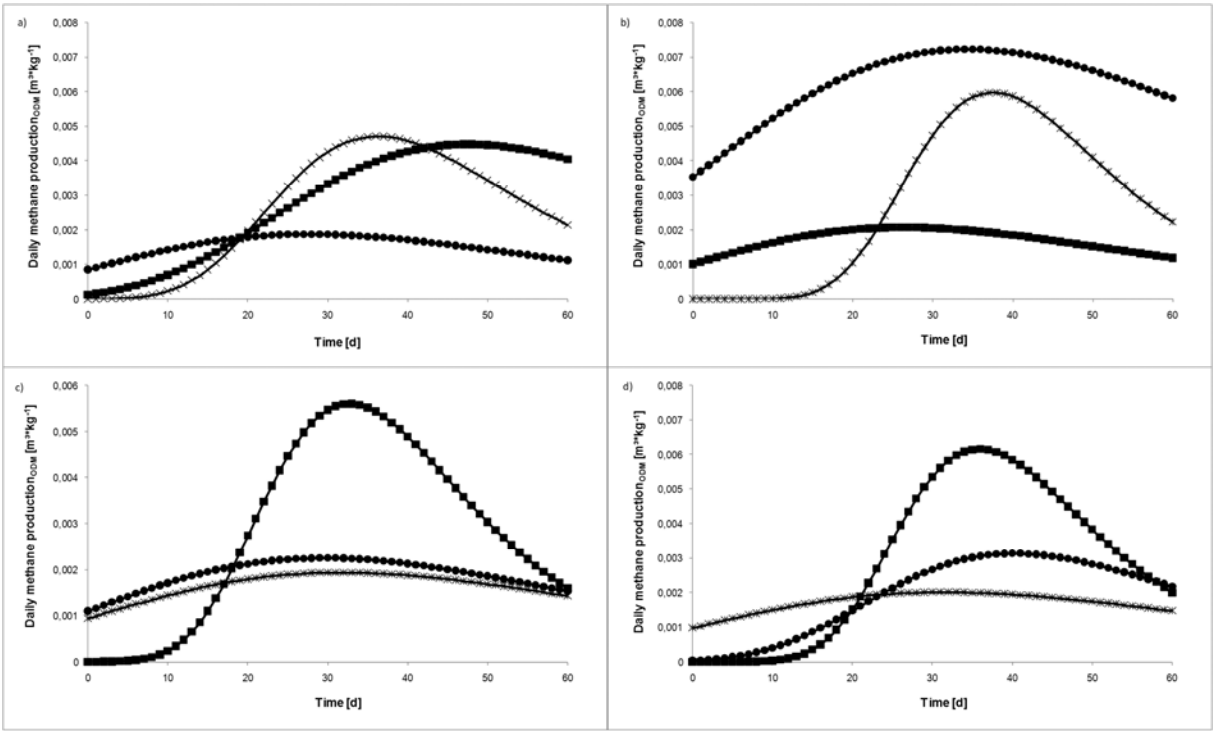

Figure 1. Daily methane production (ODM) in m³*kg^{-1} fit to modified Gompertz, (**a**) ⬢ reference sample, ■ EDTA addition C1, ✳ EDTA addition C2; (**b**) ⬢ optimal supply of trace elements, ■ EDTA addition C1, ✳ EDTA addition C2; (**c**) ⬢ amount of trace elements to be added reduced by 50 %, ■ EDTA addition C1, ✳ EDTA addition C2; (**d**) ⬢ amount of trace elements to be added reduced by 75 %, ■ EDTA addition C1, ✳ EDTA addition C2

The reference sample reached the maximum daily methane yield after 27.67 days and a specific methane yield (ODM) of 0.102 m³*kg^{-1}. The sole addition of EDTA in concentration C1 reached the maximum daily methane production in 47.57 days, while EDTA in the higher concentration only took 36.47 days. The use of the complexing agent may have delayed the daily methane production, but it managed to yield significantly higher methane amounts of 0.131 and respectively 0.135 m³*kg^{-1}.

When both EDTA and trace elements solutions were used, specific methane yields that did not differ significantly from the results of the variant with just the optimal trace elements supply were achieved. The optimal variant reached maximum daily methane yield in 33.88 days. The addition of EDTA in concentration C1 managed to decrease the number of days to reach the maximum daily methane production to 26.36, while the addition in concentration C2 increased the time to 37.55 days.

When the trace elements amount to be added was reduced by 50 %, no effect of the complexing agent on the degradation kinetics in any concentration was observed. However, EDTA in C1 increased significantly the methane yield and thus the bioavailability of the trace elements.

The use of the agent in both concentrations decreased the time needed to reach the maximum daily methane yield by 8 and respectively 4 days in the variants with addition of only 25 % of the required micro elements amount.

3.2 Kinetics of anaerobic digestion with and without EDDS

Figure 2 presents the effect of EDDS addition on the degradation kinetics in the biogas process.

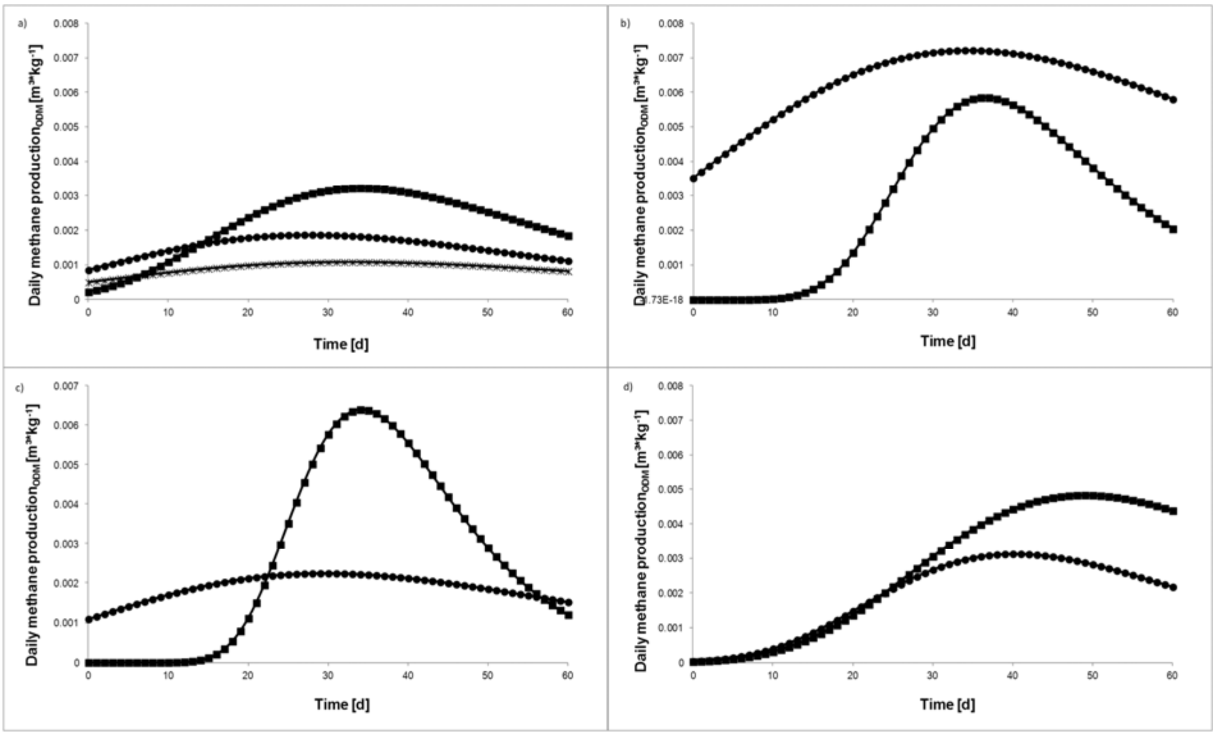

Figure 2. Daily methane production (ODM) in m³*kg-1 fit to modified Gompertz, (**a**) ● reference sample, ■ EDDS addition C1, ✳ EDDS addition C2; (**b**) ● optimal supply of trace elements, ■ EDDS addition C1, EDDS C2 addition n.d.; (**c**) ● amount of trace elements to be added reduced by 50 %, ■ EDDS

addition C1, EDDS C2 addition n.d; (**d**) ● amount of trace elements to be added reduced by 75 %, ■ EDDS addition C1, EDDS C2 addition n.d.

EDDS increased the time the maximum daily methane yield was reached compared to the reference sample by 8 and respectively 6 days, but also significantly increased the specific methane yield of the variants. No significant effect of the complexing agent on the degradation kinetics or the methane production was observed compared to the optimal supplied variant and the variant with reduced trace elements addition by 75 %. EDDS addition only decreased the number of days needed to reach the maximum daily methane yield by 4 days compared to the variant with 50 % reduction of micro elements addition.

3.3 Kinetics of anaerobic digestion with and without IDS

The effect of the use of IDS on the methane production is presented in Figure 3.

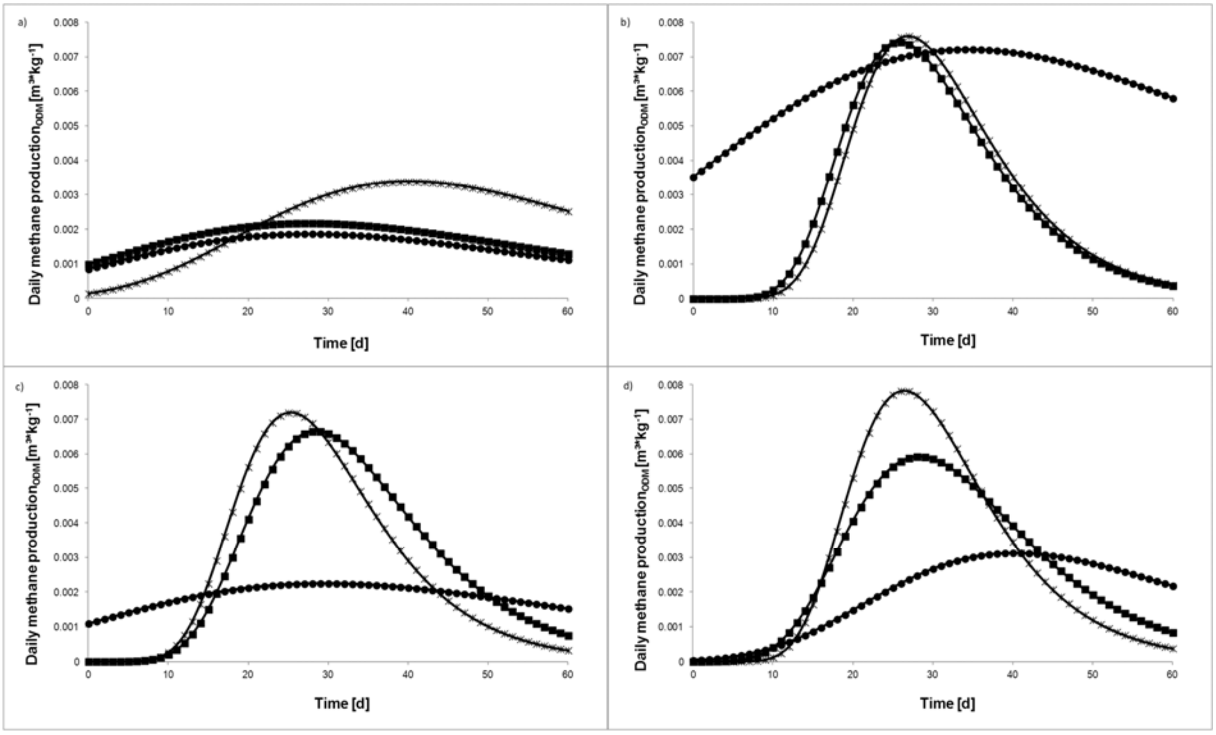

Figure 3. Daily methane production (ODM) in m³*kg-1 fit to modified Gompertz, (**a**) ● reference sample, ■ IDS addition C1, ✳ IDS addition C2; (**b**) ● optimal supply of trace elements, ■ IDS addition C1, ✳ IDS addition C2; (**c**) ● amount of trace elements to be added reduced by 50 %, ■ IDS addition C1, ✳

IDS addition C2; (**d**) ⬢ amount of trace elements to be added reduced by 75 %, ■ IDS addition C1, ✳ IDS addition C2

The sole addition of IDS showed no effect on the degradation kinetics compared to the reference sample, but increased the specific methane yield by 30 %. The variant with higher complexing agent concentration increased the time to reach maximum methane production by 13 days and only increased the methane yield by 13 %.

A highly significant effect of the IDS addition was shown when compared with the results of the optimal supplied variant. In both concentrations, the complexing agent increased the specific methane yield by approximately 20 % and decreased the time needed for the maximum daily methane production by 10 days.

IDS addition caused in both concentrations significant increases of the methane production compared to the variants with reduced trace elements supply. In the case of the reduction by 75 %, IDS also decreased the number of days the variant needed to reach maximum methane yield by over 10 and increased the specific methane yield by 58 %.

4. Discussion

It was hypothesized that the use of readily biodegradable complexing agents in the biogas process can increase the bioavailability of trace elements present in the fermentative substrate of agricultural biogas plants or added to the digesters. A previous study by Vintiloiu et al. proved that the use of EDTA in the biogas process is feasible and the question was addressed, whether EDDS and IDS could represent environmentally friendly alternatives to EDTA [14].

The effects of the persistent complexing agent EDTA and of the readily biodegradable agents EDDS and IDS have been studied in several fields. Jones [13] analyzed the chelating agents in the paper and pulp industry and showed that for the complexation of transition metals, EDDS appears to be far superior to EDTA and IDS. Tandy et al. [11] studied the extraction of heavy metals from soils with the use of biodegradable agents and reported best results for EDDS for several metals. These findings are not in accordance with the results of this study, in which EDDS showed no effect when compared to the other chelating agents used.

When EDTA was used in this study at the same time as an optimally concentrated trace element solution, it showed a significant effect on the degradation kinetics of the process. In the variants with reduced trace elements addition amounts, the complexing agent significantly increased the specific methane yield, but did not influence the time needed to reach maximum daily methane production. EDDS showed no effect on the process kinetics and could only significantly increase the bioavailability of the trace elements already present in the substrate.

The use of IDS in this study caused significant increases of the specific methane yields in all tested variants and also a decrease of the time needed to reach the maximum daily methane yield both in the presence of an optimal trace element supply and when the elements addition was reduced by 75 %.

The higher concentration (C2) often inhibited the process, while concentration C1 proved to be feasible for the use in the biogas process.

The effects shown on the degradation kinetics by the complexing agents proved that even if the time needed to reach maximum daily methane yields was increased in some variants, it had no effect on the specific methane yield and the actual scope of these agents.

5. Concluding remarks

This paper demonstrated that complexing agents can be successfully used in the biogas process. The results showed that their addition to heavily undersupplied substrates with high fatty acids concentrations increases the methane yield by improving the bioavailability of metal ions, and thus the stability of the anaerobic digestion. EDTA can increase significantly the availability of trace elements present in fermentation substrates and the amounts of micro elements additions to the biogas process can be reduced by up to 75 %. This would be advantageous from an environmental and economic point of view. The problem of the persistent EDTA was solved by using readily biodegradable complexing agents. EDDS showed no influence in the methane production process and is therefore not recommended. IDS showed positive significant effects on the degradation kinetics of the process and increased the bioavailability of trace elements in all tested variants by up to 50 %. IDS proved to be a valid alternative to the use of EDTA in the biogas process.

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Conflicts of Interest

The authors declare no conflict of interest.

References and Notes

1. Jacobi, F.H.; Liebetrau, J.; Nelles, M. Flexibel und effizient – Lösungen für die Biogasanlage von morgen. 2013, Projekttag Bioenergie Berlin.
2. Gallert, C.; Winter, J. Bacterial metabolism in wastewater treatment systems. In: *Environmental Biotechnology. Concepts and Applications*, Jördening, H.-J., Winter, J., Eds.; Wiley-VCH Verlag, Weinheim, Deutschland, 2005, pp. 1–48.
3. Lemmer, A. Biogaserzeugung. In: *Biogas. Erzeugung, Aufbereitung, Einspeisung*, Bajohr, S., Graf, F., Eds.; Oldenbourg Industrieverlag GMBH, Deutschland, 2011, pp. 75-118.
4. Bryant, M.P. Microbial methane production—theoretical aspects. *J. Anim. Sci.* **1979**, 48, 193–201.
5. Weiland, P. Bundesweite Evaluierung neuartiger Biomasse-Biogasanlagen, 2007, 16. Symposium Bioenergie-Festbrennstoffe, Biokraftstoffe, Biogas, Bad Staffelstein, pp. 236-241.
6. Abdoun, E.; Weiland, P. Optimierung der Monovergärung von Nachwachsenden Rohstoffen durch die Zugabe von Spurenelementen (Optimization of monofermentation from renewable raw materials by the addition of trace elements), 2009 Bornimer Agrartechnische Berichte 68, pp. 69-78.
7. Callander, I.J.; Barford, J.P. Precipitation, chelation, and the availability of metals as nutrients in anaerobic digestion. I. Methodology, *Biotechnol. Bioeng.* **1983**, 25, 1947–1957.
8. Mosey, F.E.; Swanwick, J.D.; Hughes, D.A. Factors affecting the availability of heavy metals to inhibit anaerobic digestion, *Water Pollut. Control* **1971**, 70, 668–679.
9. Wu, L.; Luo, Y.; Xing, X.; Christie, P. EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk, *Agriculture, Ecosystems & Environment* **2004**, 102, 307-318.
10. Nowack, B. Environmental chemistry of amonipolycarboxylate chelating agents, *Environ. Sci. Technol.* **2002**, 36, 4009-4016.

11. Tandy, S.; Bossart, K.; Mueller, R.; Ritschel J. Extraction of heavy metals from soils using biodegradable chelating agents, *Environ. Sci. Technol.* **2004**, 38, 937-944.
12. Meers, E.; Rutens, A.; Hopgood M.J. Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals, Chemosphere **2005**, 58 1011-1022.
13. Jones, P.W.; Williams, D.R. Chemical speciation simulation used to assess the efficiency of environment-friendly EDTA alternatives for use in the pulp and paper industry, *Inorganica Chimica Acta* **2002**, 339, 41-50.
14. Vintiloiu, A.; Boxriker, M.; Lemmer A. et al. Effect of ethylenediaminetetraacetic acid (EDTA) on the bioavailability of trace elements during anaerobic digestion, *Chemical Engineering Journal* **2013**, 223, 436–441.
15. Oechsner, H.; Lemmer, A.; Ramhold, D.; Mathies, E. Verfahren zur Biogaserzeugung, Europäische Patentanmeldung EP 1 997 901 A2, 2008.
16. VDI 4630, Fermentation of organic materials. In: *Characterization of Substrate, Sampling, Collection of Material Data, Fermentation Tests*, VDI Gesellschaft Energietechnik, 2006.
17. Oechsner, H.; Helffrich D. Hohenheimer Biogasertragstest, *Agrartechnische Forsch.* **2003**, 9 (3), 27–30.
18. Nopharatana, A.; Pullammanappallil, P.C.; Clarke, W.P. Kinetics and dynamic modelling of batch anaerobic digestion of municipal solid waste in a stirred reactor. *Waste Management* **2007**, 27 (5), 595-603.
19. Budiyono; Widiasa, I.N.; Johari, S.; Sunarso. The kinetic of biogas production rate from cattle manure in batch mode. *International Journal of Chemical and Biological Engineering* **2010**, 3(1), 39 - 44.
20. Billo, E.J. Excel for Chemists: A Comprehensive Guide; John Wiley & Sons, Inc., 2001.
21. Lay, J.J.; Li, Y.Y.; Noike, T. A mathematical model for methane production from a landfill bioreactor treating the organic fraction of municipal solid wastes, *J. Environ. Eng. ASCE* **1998b,** 124 (8), 730-736.

5 GESAMTDISKUSSION

Das Hauptziel dieser Arbeit war es, den Effekt des Einsatzes von Spurenelementen in landwirtschaftlichen Biogasanlagen zu untersuchen und die Auswirkungen des Ausgleichs substratbedingter Mangelerscheinungen auf die Stabilität des Gärprozesses zu bestimmen. Dabei wurden mehrere Teilziele verfolgt:

1. Untersuchungen zu Ursachen und prozessbiologischer Folgen unterschiedlicher Mikro- und Makronährstoffkonzentration im Gärsubstrat landwirtschaftlicher Biogasanlagen
2. Laboruntersuchungen zu optimalen Konzentrationen von Spurenelementen im Gärsubstrat und der Möglichkeit, die notwendigen Zugabemengen durch den Einsatz von EDTA zu reduzieren
3. Laboruntersuchungen zum Einsatz unterschiedlicher biologisch abbaubarer Komplexbildner zur Substitution von EDTA.

Teilziel 1: Datenerhebung an Praxisanlagen und statistische Auswertung

Durch die Substratzugabe gelangen unterschiedliche Mikro- und Makroelemente in den Fermenter, deren Konzentrationen im Gärsubstrat einen großen Einfluss auf die Stabilität des biologischen Prozesses der Methanbildung haben. Zu geringe Konzentrationen der Elemente können den Metabolismus der Methanogene stören und dadurch den ganzen Prozess zum Erliegen bringen; dagegen können zu hohe Konzentrationen den Prozess hemmen. Dies führt zu einer Steigerung der Fettsäurekonzentrationen, zur Destabilisierung des Biogasprozesses und zur Beeinträchtigung des Methanertrages.

Teilziel 1 der vorliegenden Arbeit war es, Daten zu Konzentrationen von Mikro- und Makroelementen und zu wesentlichen Prozessparametern zu erheben und eventuelle Zusammenhänge und Effekte zu bestimmen. Hierfür wurden 25 unterschiedliche Praxisanlagen in Süddeutschland ausgewählt und deren Gärsubstrat auf ihren Makro- und Mikronährstoffgehalt hin untersucht. Die folgenden Elemente und Parameter wurden untersucht:

- Makroelemente: Natrium und Schwefel
- Spurenelemente: Nickel, Kobalt, Selen, Molybdän und Eisen
- Prozessparameter: Raumbelastung, Konzentrationen der Essig- und Propionsäure und das Essigsäureäquivalent.

Anschließend wurden die Auswirkungen der Nährstoffkonzentrationen auf die Prozessparameter mittels einer Varianzanalyse bestimmt.

Es wurde gezeigt, dass die Raumbelastung der untersuchten landwirtschaftlichen Biogasanlagen einen hoch signifikanten Effekt auf die Propionsäurekonzentration und auf das Essigsäureäquivalent hat. Es konnte jedoch kein Einfluss auf die Konzentration der Essigsäure festgestellt werden. Die Auswirkungen auf das Essigsäureäquivalent und auf die Propionsäure lassen sich dadurch erklären, dass das Propionat in der Berechnungsformel des Äquivalents enthalten ist und dieses damit direkt beeinflusst. Mit der Steigerung der Raumbelastung im Fermenter kann es dazu kommen, dass die Bildung der Säuren schneller erfolgt als deren Konversion zu Biogas. Die Folge ist ein Ansteigen der Propionsäurekonzentration. Dies erklärt den statistischen Zusammenhang zwischen der Raumbelastung und Propionatkonzentration. Diese Ergebnisse decken sich mit der Studie von Lindorfer (2008) in der gezeigt wird, dass ein Anstieg der Raumbelastung zu einer Abnahme des Methanertrages und im Weiteren zu einer Erhöhung der Fettsäurekonzentrationen führen kann.

Über die Analyse der Gärsubstratproben aus den Praxis-Biogasanlagen konnte kein Effekt von Natrium auf die Prozessstabilität nachgewiesen werden. Dagegen hatte Schwefel einen statistisch signifikanten Einfluss auf die Gärsäurekonzentration. Natrium ist nur für bestimmte Methanogene wichtig, während Schwefel einen großen Einfluss auf die Konzentration der Spurenelemente und deren Bioverfügbarkeit hat (Gonzales-Gil, 2003; Zandvoort et al. 2006).

Nickel und Molybdän hatten einen statistisch signifikanten Effekt auf alle untersuchten Prozessparameter, was die hohe Bedeutung dieser Spurenelemente für die Prozessstabilität belegt. Ein hoher Einfluss auf die Propionsäurekonzentration konnte auch für Selen bestimmt werden. Selen ist meistens nur in sehr geringen Mengen im Gärsubstrat vorhanden, dennoch konnte gezeigt werden, dass sogar diese kleinen Mengen die Fettsäurekonzentration beeinflussen können. Eisen und Kobalt hatten bei den 25 untersuchten Anlagen keinen statistisch signifikanten Effekt auf die Konzentration der Fettsäuren, obwohl beide Spurenelemente essentiell für die Methanogene sind. Die hohe Signifikanz der Interaktionen zwischen den Nährstoffen hat bewiesen, dass ein ausgewogenes Verhältnis zwischen den Konzentrationen dieser Elemente wichtiger ist als die Anwesenheit nur einzelner Elemente.

Andere Studien haben gezeigt, dass die Zugabe von Nährstoffen oder einer Nähstoffmischung zu einer Verringerung der Acetat und Propionat Konzentrationen führt und die Methanproduktion signifikant erhöht (Abdoun, 2009; Goodwin, 1990). Die Ergebnisse aus dieser Arbeit bestätigen den starken Effekt der Mikro- und Makronährstoffkonzentrationen auf die Konversion von Fettsäuren.

Im Rahmen der Untersuchungen konnten die Kenntnisse zur Bedeutung der Mikro- und Makronährstoffe im Biogasprozess erweitert und Ursachen für die unterschiedliche Versorgung der Anlagen mit diesen Elementen ermittelt werden. Diese Arbeit beschränkt sich auf Daten aus 25 untersuchten Anlagen und stützt sich damit auf eine relativ kleine Anzahl an Proben, so dass weitere Untersuchungen zur Absicherung der Ergebnisse durchgeführt werden sollten.

Teilziel 2: Effekte von EDTA auf die Bioverfügbarkeit der Spurenelemente

Die landwirtschaftlichen Biogasanlagen werden derzeit vorwiegend mit Mais beschickt (Weiland, 2007). Die Daten der 25 untersuchten Biogasanlagen belegen, dass Silomais im Vergleich zu Grassilage, Getreide-Ganzpflanzensilage oder Rübensilage i.d.R. nur sehr geringe Mineralstoffgehalte aufweist. Dadurch kommt es bei vielen Biogasanlagen oft zu Prozessstörungen und zu Methanertragseinbußen (Demirel und Scherer, 2011). Handelsübliche Spurenelementlösungen können dazu verwendet werden das Nährstoffdefizit im Gärsubstrat auszugleichen, was zu einer schnelleren Umsetzung der organischen Säuren führt. Die meisten Spurenelemente sind umweltgefährdende Schwermetalle, so dass die dem Biogasprozess zugefügten Mengen so gering wie möglich gehalten werden sollten. Durch eine höhere Bioverfügbarkeit der Spurenelemente könnte die benötigte Aufwandmenge und damit die Belastung der landwirtschaftlichen Flächen, auf denen der Gärrest als Dünger genutzt wird, reduziert werden.

Ziel der zweiten Versuchsreihe war es, eine Methode zu finden, mit der die Bioverfügbarkeit der Metallionen im Fermenter erhöht werden kann, um somit die benötigte Menge an Spurenelementen zu reduzieren. Dazu wurde der Komplexbildner Ethylendiamintetraessigsäure (EDTA) zugegeben und auf seine Wirkung hin getestet. EDTA wird in vielen Bereichen verwendet, um die Ausfällung von Metallionen zu verhindern. Es ist aber nur schwer biologisch abbaubar (Callander, 1983).

In verschiedenen Untersuchungen im Batch-Ansatz wurden die Effekte der Zugabe von EDTA in Salzform und als Nickelkomplex zu einem suboptimal versorgten Gärsubstrat getestet. Auch Spurenelementlösungen wurden in optimaler und reduzierter Konzentration zugegeben. Es konnte gezeigt werden, dass in Gegenwart von Nickel(II)EDTA die Konzentration des Nickels um bis zu 50 % reduziert werden kann, ohne dass dies einen negativen Einfluss auf den Methanertrag hatte. Da Nickel ein umweltgefährdender Stoff ist, hat die Zugabe als Komplex den Vorteil, dass geringere Mengen an Nickel mit dem Gärrest in die Umwelt freigesetzt werden.

Weitere Untersuchungen dieser Testreihe belegten, dass durch die ergänzende Zugabe von EDTA in Salzform die notwendigen Mengen sämtlicher essentieller Mikronährstoffe um bis zu 75 % reduziert werden konnten. Damit gehen die Ergebnisse der vorliegenden Arbeit konform mit den Untersuchungen von Callander (1983) und Hu et al. (2008), die belegen, dass der Komplexbildner EDTA die Bioverfügbarkeit der Metallionen stark verbessern kann. Eine Reduzierung der erforderlichen Spurenelementmengen kann durch die gleichzeitige Zugabe von EDTA kompensiert werden. Dadurch werden eine hohe Prozessstabilität und hohe Methanerträge gewährleistet.

Bei einer großen Anzahl der Biogasanlagen in Deutschland werden Spurenelementlösungen eingesetzt, um den Prozess zu optimieren und höhere Methanerträge zu erzielen (Jacobi, 2013). Nach der Vergärung gelangen diese Spurenelemente mit dem Gärresten auf landwirtschaftlich genutzte Flächen. Da einige der für methanogene Mikroorganismen essentiellen Nährstoffe keine Bedeutung für die pflanzliche Ernährung haben, sollten deren Zugabemengen zum Gärsubstrat soweit wie möglich reduziert werden. Die Tatsache, dass durch Zugabe von EDTA die Zugabemengen der Mikronährstoffe um bis zu 75 % reduziert werden können, ohne negativem Einfluss auf den Methanertrag, kann ökologische sowie auch ökonomische Vorteile mit sich bringen. Vor einer breiten kommerziellen Verwendung sind zur Absicherung der Ergebnisse jedoch weitere Untersuchungen notwendig.

Da EDTA üblicherweise nicht im Biogasprozess eingesetzt wird, gibt es nur unzureichende Informationen zu notwendigen Zugabekonzentrationen. Die Untersuchungen mehrerer Konzentrationen haben gezeigt, dass eine Überdosierung des Komplexbildners negative Auswirkungen auf den Prozess haben kann. Auch in diesem Bereich sind weitere Untersuchungen notwendig, um die optimale Aufwandmenge bestimmen zu können.

Im Rahmen der Untersuchungen konnte gezeigt werden, dass vom Gärsubstrat selbst ein sehr großer Einfluss auf die Effekte der EDTA-Zugabe ausgeht. Die Effekte des EDTA auf die Erhöhung der Prozessstabilität konnten insbesondere bei stark mit Nährstoffen unterversorgten Gärsubstraten nachgewiesen werden, die zudem bereits sehr hohe Fettsäurekonzentrationen aufwiesen. In diesen Fällen konnte die alleinige Zugabe von EDTA den Methanertrag des Gärsubstrates um bis zu 32 % erhöhen. Mit den durchgeführten Untersuchungen konnte gezeigt werden, dass die Eigenschaften Spurenelemente- und Fettsäurekonzentrationen des ausgewählten Gärsubstrates einen hohen und signifikanten

Einfluss auf die Wirkung von EDTA in der anaeroben Vergärung haben. Dies ist bei der Auswahl der Gärsubstrates für solche Testreihen zu beachten.

Teilziel 3: Effekte biologisch abbaubarer Komplexbildner auf die Bioverfügbarkeit der Spurenelemente

Die Ergebnisse der vorliegenden Arbeit zeigen, dass eine starke Reduzierung der Zugabe von Spurenelemente durch den Einsatz von EDTA möglich ist. In Teilziel 3 sollte untersucht werden, ob biologisch leicht abbaubare Komplexbildner den gleichen Effekt wie EDTA im Gärprozess haben können. Dadurch wird erwartet, dass die Belastung der Umwelt durch den schwer abbaubaren Komplexbildner reduziert werden kann.

In den weiteren Untersuchungen wurden daher zwei Komplexbildner mit ähnlichen Funktionen wie EDTA getestet. Aus der Gruppe der leicht biologisch abbaubaren Komplexbildner wurden Ethylendiamindibernsteinsäure (EDDS) und Iminodibernsteinsäure (IDS) ausgewählt. Diese gehören wie EDTA zu der Gruppe der Aminocarboxylate, gelten jedoch als umweltfreundliche Alternative zum weltweit absatzstärksten Produkt EDTA. Auch deren Einfluss auf die Bioverfügbarkeit der Spurenelemente und auf die Stabilität des Gärprozesses wurde untersucht.

Im Rahmen der Untersuchungen konnte festgestellt werden, dass von den getesteten Komplexbildnern IDS am besten geeignet ist, da es einen statistisch hoch signifikanten positiven Einfluss auf die Bioverfügbarkeit der Spurenelemente hatte. Dieser leicht abbaubare Komplexbildner stellt eine umweltfreundliche Alternative zu EDTA dar.

Die vorliegende Arbeit hat gezeigt, dass die Komplexbildner eine neue Anwendung im Biogasprozess finden können. Um diese Anwendung besser beschreiben und quantifizieren zu können, müssen in zukünftigen Untersuchungen die Einflüsse weiterer Faktoren (Sulfat-, Carbonat- und Phosphatkonzentrationen, Konzentration der Metallionen im Fermenter, pH-Wert) auf die Funktion der Komplexiermittel evaluiert werden.

Zusammenfassend konnte das Ziel dieser Arbeit, den Effekt des Einsatzes von Spurenelementen in landwirtschaftlichen Biogasanlagen zu untersuchen, erreicht werden und die Problematik der Unterschiede der Nährstoffmengen in den Biogasanlagen und deren Einfluss auf die Stabilität des Biogasprozesses und dem Methanertrag beschrieben werden. Mit der Komplexierung der Mikronährstoffe wurde eine Methode entwickelt, um die Bioverfügbarkeit der Metallionen im Fermenter der Biogasanlage zu verbessern und somit die Zugabemengen an Spurenelementen zu reduzieren.

6 ZUSAMMENFASSUNG

In Deutschland sind über 7500 Biogasanlagen in Betrieb, die überwiegend mit nachwachsenden Rohstoffen beschickt werden. Über die Substratzufuhr gelangen unterschiedliche Mikro- und Makroelemente in den Fermenter. Diese Elemente stellen essentielle Nährstoffe für methanbildende Mikroorganismen dar. Eine zu geringe Konzentration dieser Elemente kann die Bildung von Enzymen hemmen und dadurch den Metabolismus der Methanogene stören. Die, den Biogasprozess einleitenden, primären Gärer werden von diesem Mangel nicht inhibiert, so dass es zu einer Anreicherung der kurzkettigen organischen Säuren im Gärsubstrat kommt. Die Säureanreicherung kann bei einem Überschreiten der Pufferkapazität des Gärsubstrates zu einer Destabilisierung des Prozesses führen. Eine große Anzahl der landwirtschaftlichen Biogasanlagen verwendet daher handelsübliche Spurenelementlösungen, um das Nährstoffdefizit zu reduzieren, den Prozess zu optimieren und höhere Methanerträge zu erzielen. Nach der Vergärung gelangen diese Spurenelemente mit dem Gärrest auf landwirtschaftliche Flächen. Da die meisten Spurenelemente Schwermetalle sind, sollten die zugefügten Mengen so gering wie möglich gehalten werden, um die Umweltbelastung zu minimieren.

Ziel dieser Arbeit war es, die Ursachen für einen Spurenelementmangel in NawaRo-beschickten Biogasanlagen zu untersuchen. Es wurde auch geprüft, ob durch eine Komplexierung der Mikronährstoffe deren Verfügbarkeit für die Mikroorganismen gesteigert und dadurch die zur Stabilisierung des Gärprozesses notwendigen Zugabemengen reduziert werden können.

Im Rahmen der Arbeit wurde zunächst eine Datenerhebung an 25 landwirtschaftlichen Biogasanlagen durchgeführt, bei der die Gärsubstrate beprobt und die Konzentrationen von Makro- und Mikroelementen sowie unterschiedliche Prozessparameter untersucht und statistisch ausgewertet wurden. Im Speziellen wurde der Einfluss der Makroelemente Natrium und Schwefel, sowie der Spurenelemente Eisen, Nickel, Kobalt, Molybdän und Selen auf die Prozessparameter Propionsäure-, Essigsäuregehalt und Essigsäureäquivalent untersucht. Die Ergebnisse zeigten eine signifikant positive Korrelation zwischen dem Schwefelgehalt und den Gärsäurekonzentrationen. Dies ist dadurch begründet, dass Schwefel einen starken negativen Einfluss auf die Bioverfügbarkeit der Spurenelemente hat und dadurch die Fettsäurekonzentration beeinflusst. Für Natrium konnte dagegen kein Einfluss nachgewiesen werden. Mit steigender Nickel- und Molybdänkonzentration nahmen die Säuregehalte signifikant ab, während Eisen und Kobalt die Fettsäurekonzentration nicht beeinflussten.

Ergänzend wurden im Rahmen des Projektes Batch-Versuche zum Einfluss des Komplexbildners Ethylendiamintetraessigsäure (EDTA) auf die Bioverfügbarkeit von Metallionen durchgeführt. Es wurde gezeigt, dass durch den Einsatz von Nickel(II)EDTA die Nickelzugabemenge im Vergleich zur Zugabe in Salzform um bis zu 50 % reduziert werden konnte, ohne den Methanertrag negativ zu beeinflussen. Durch die alleinige Zugabe von EDTA zu einem suboptimal versorgten Gärsubstrat konnte der Methanertrag um bis zu 32 % erhöht werden. Beim Einsatz von Spurenelementen konnte durch die Zugabe von EDTA deren Zugabemengen um bis zu 75 % ohne negative Folgen für den Gärprozess verringert werden.

Da EDTA ein biologisch schwer abbaubarer Komplexbildner ist, wurde untersucht, ob biologisch leicht abbaubare Komplexbildner (Ethylendiamindibernsteinsäure (EDDS) und Iminodibernsteinsäure (IDS)) den gleichen Effekt haben können. IDS hatte im Rahmen dieser Untersuchungen einen statistisch hoch signifikanten positiven Einfluss auf die Bioverfügbarkeit der Metallionen, der den von EDTA übertraf. IDS stellt somit eine umweltfreundliche Alternative zu EDTA dar.

Durch den Einsatz von Komplexbildnern konnte die Bioverfügbarkeit der Metallionen im Fermenter erhöht und damit die Zugabemengen an Spurenelementen zum Ausgleich substratbedingter Mangelerscheinungen reduziert werden. Dies verringert die Belastung der landwirtschaftlichen Flächen, auf denen der Gärrest als Dünger genutzt wird. Die Untersuchungen zeigten, dass die Spurenelement- und Fettsäurekonzentrationen im Gärsubstrat einen wesentlichen Einfluss auf die Wirkung von Komplexbildnern im Biogasprozess haben.

7 SUMMARY

There are approximately 7,500 operational agricultural biogas plants in Germany and most of them are fed with renewable raw materials. During substrate addition, several micro and macro elements enter the digester. These elements are essential nutrients for the methanogens. If their concentration is too low, the production of enzymes can be inhibited, and thereby interfere with the metabolism of the methanogenic bacteria. The primary fermentating bacteria, which induce the biogas process, are not inhibited by this deficiency, which leads to an accumulation of short chain acids in the digestate. If the acid content exceeds the buffering capacity of the fermentative substrate, it can cause the destabilization of the process. A large number of agricultural biogas plants use commercially available trace element solutions to reduce the nutrient deficit, to optimize the process and to achieve higher methane yields. When the fermentation is complete, the digestate containing these trace elements is spread on fields as fertilizer. As most trace elements are heavy metals, the amounts added to the biogas process should be kept as low as possible in order to minimize the environmental damage.

The purpose of this study was to investigate the cause of trace elements deficiency in renewable raw materials fed biogas plants. It was also tested whether the chelation of the nutrients could increase their bioavailability for microorganisms and thus lead to a reduction of the amounts needed for the stabilization of the fermentation process.

The digestate from 25 agricultural biogas plants in Germany was sampled and data obtained for the concentrations of macro and micro elements, as well as for the concentration of different process parameters. The data was then evaluated statistically. Particularly, the influences of the macro elements sodium and sulfur and the trace elements iron, nickel, cobalt, molybdenum and selenium were assessed on the process parameters propionic acid, acetic acid and acetic acid equivalent. The results showed a significant positive correlation between the sulfur content and the concentration of the fatty acids. Sulfur has a strong negative influence on the bioavailability of trace elements and thereby affects the fatty acids content. Sodium showed no effect in this case. Increasing nickel and molybdenum concentrations led to a reduction of the fatty acids concentration, while iron and cobalt showed no effect.

Additionally, within the scope of the project, batch experiments were carried out with the objective to test the effect of the complexing agent ethylenediaminetetraacetic acid (EDTA) on the bioavailability of metal ions. It was shown that during the use of nickel as nickel (II) EDTA and not in salt form, the nickel addition could be reduced by up to 50 % without negatively affecting the methane yield. The sole addition of EDTA to an undersupplied substrate increased the methane yield by up to 32 %. When trace elements were also added, their amounts could be reduced by up to 75 % with no negative consequences for the fermentation process.

EDTA is a persistent chelating agent and so it was further tested, whether readily biodegradable chelating agents (ethylenediaminedisuccinic acid (EDDS) and iminodisuccinic acid (IDS)) could have the same effect. During the investigation, IDS had a high statistically significant positive effect on the bioavailability of the metal ions, which exceeded the effect of EDTA. IDS represents therefore a good alternative to EDTA.

The bioavailability of the metal ions in the digester was increased by the use of complexing agents, which made the reduction of the trace elements amounts needed to compensate for substrate-related deficiency symptoms possible. This reduces the pollution on the agricultural land on which the digestate is used as fertilizer. The investigation showed that the trace elements and fatty acid concentrations in the digestate have a significant influence on the effect of complexing agents in the biogas process.

8 LITERATURVERZEICHNIS

Abdoun E., Weiland P., Optimierung der Monovergärung von Nachwachsenden Rohstoffen durch die Zugabe von Spurenelementen (Optimization of monofermentation from renewable raw materials by the addition of trace elements), Bornimer Agrartech. Ber. 68 (2009) 69–78.

Bauer C., Lebuhn M., Gronauer A., Mikrobiologische Prozesse in landwirtschaftlichen Biogasanlagen, Direktmarketing & Digitaldruck Hallbergmoos, Freising-Weihenstephan 2009, S. 15-35.

Bischofsberger W., Dichtl N., Rosenwinkel K.-H., Seyfried C.F., Böhnke B., Anaerobtechnik, Springer Verlag Berlin, Heidelberg 2005, S. 23–86.

Bryant, M.P., Microbial methane production—theoretical aspects. J. Anim. Sci. 1979, 48, 193–201.

Callander I.J., Barford J.P., Precipitation, chelation, and the availability of metals as nutrients in anaerobic digestion. I. Methodology, Biotechnol. Bioeng. 1983, 25, 1947–1957.

Chasteen T.G., Bentley R., Biomethylation of selenium and tellurium. Microorganisms and plants, Chem. Rev. 2003, 103, 1–25.

Jacobi F.H., Liebetrau J., Nelles M., Flexibel und effizient – Lösungen für die Biogasanlage von morgen, 2013, Projekttag Bioenergie Berlin.

Demirel B., Scherer P., Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane, Biomass Bioenergy 2011, 35, 992–998.

Diekert G., Klee B., Thauer R.K., Nickel, a component of factor F430 from Methanobacterium thermoautotrophicum, Arch. Microbiol. 1980, 124 (1), 103–106.

Gonzalez-Gil, G., Jansen, S., Zandvoort, M.H., van Leeuwen, H.P., Effect of yeast extract on speciation and bioavailability of nickel and cobalt in anaerobic bioreactors. Biotechnol. Bioeng. 2003, 82(2), 134–142.

Goodwin, J.A.S., Wase, D.A.J., Forster, C.F., Effects of nutrient limitation on the anaerobic upflow sludge blanket reactor. Enzyme Microb. Tech. 1990, 12(11), 877–884.

Hausinger R.P., Karplus P.A., Urease, in: K. Wieghardt, T.R. Huber, T.L. Poulos, A. Messerschmidt (Eds.), Handbook of Metalloproteins, Wiley Verlag, West Sus-sex, 2001, pp. 867–879.

Hu Q.-H., Li X.-F., Liu H., Du G.-C., Chen J., Enhancement of methane fermentation in the presence of Ni^{2+} chelators, Biochem. Eng. J. 2008, 38, 98–104.

Jarrell, K.F., Kalmokoff M.L., Nutrientional requirements of the methanogenic archaebacteria, Microbiology 1988, 34, 557–576.

Lacy D., Negash B., Basvapatna S.R., Paul J.W., Bacterial methanogenesis and growth from CO_2 with elemental iron as the sole source of electrons, Science 1987, 237, 509–511.

Lemmer A., Biogaserzeugung, in: S. Bajohr, F. Graf (Eds.), Biogas - Erzeugung, Aufbereitung, Einspeisung, Oldenbourg Industrieverlag GMBH, 2011, pp. 75–118.

Lettinga, G., Anaerobic digestion and wastewater treatment systems. Antonie van Leeuwenhoek 1995, 67, 3–28.

Lindorfer, H., Corcoba, A., Vasilieva, V., Braun, R. et al., Doubling the organic loading rate in the co-digestion of energy crops and manure - a full scale case study. Bioresource Technol. 2008, 99(5), 1148–1156.

Perski, H.-J., Moll, J., Thauer, R.K., Sodium dependence of growth and methane formation in Methanobacterium thermoautotrophicum. Arch. Microbiol. 1981, 130(4), 319–321.

Takashima, M. und Speece, R.E., Mineral nutrient requirement for high-rate methane fermentation of acetate at low SRT. Res. Wat. Pollut. Control Fed. 1989, 61: S. 1645-1650.

Weiland, P. et al., Bundesweite Evaluierung neuartiger Biomasse-Biogasanlagen, 2007, 16. Symposium Bioenergie-Festbrennstoffe, Biokraftstoffe, Biogas; Bad Staffelstein, S. 236-241.

Woo G.J., Wasserfallen A., Wolfe R.S., Methyl viologen hydrogenase II, a new member of the hydrogenase family from Methanobacterium thermoautotrophicum, J. Bacteriol. 1993, 175, 5970–5977.

Zandvoort, M.H., Van Hullebusch, E.D., Gieteling, J., Lettinga, G. et al., Effect of sulfur source on the performance and metal retention of methanol-fed UASB reactors. Biotechnol. Prog. 2005, 21(3), 839–850.

Zandvoort, M. H., van Hullebusch, E.D., Fermoso, F. G., Lens,P. N. L., Trace metals in anaerobic granular sludge reactors: bioavailability and dosing strategies. Eng. Life Sci. 2006, 6(3), 293–301.

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