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Bioavailability of essential trace elements and their impact on anaerobic digestion of slaughterhouse waste

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ABSTRACT

Slaughterhouse waste is an energy rich feedstock suitable for anaerobic digestion processes. However, chemical characterization showed a deficiency in essential trace elements which are critical for optimal performance of the process. Hence this study investigated the degree of bioavailability of trace elements in four semi-continuous lab-scale AD tests accepting slaughterhouse waste under mesophilic conditions (38 °C) and a moderate organic loading rate of 2.2 kg/m^3 d. Parameters, such as volatile fatty acid (VFA) concentration, COD removal rate and specific methane yield were compared to the results of sequential extraction analysis. The highest methane yield (250–275 Nm^3/t COD), lowest accumulation of VFA (<500 mg/l) and high COD removal rate (75–80%) was obtained when the total concentration of 11.4 mg/l Ni, 25.4 mg/l Co and 4.8 mg/l Mo was present in the reactor, of which 62% of Ni and Co, and 68% of Mo were bioavailable for microbial uptake. Based on these results it can be recommended that a supply of 2.5 g/t Ni, 3.5 g/t Co, 0.6 g/t Mo and 0.05 g/t Se provide optimal conditions for anaerobic digestion of slaughterhouse waste.

1. Introduction

Optimal supply of trace elements is a prerequisite for microbial growth and metabolism in anaerobic digestion (AD) processes. Consequently, deficiency causes limitation of the activity of the microbial consortium [1]. This is because, methanogenesis, the final metabolic pathway during AD, involves the participation of various metal-rich enzymes such as carbon monoxide dehydrogenase/acetyl-CoA synthase (Cdh) or methyl coenzyme M reductase (Mcr). These enzymes catalyse key metabolic steps and require sufficient supply of Fe, Ni and Co [2,3]. The exact amount of required trace metals of AD may vary depending on the involved microbial species and their methanogenic pathway, but there are some general tendencies. Fe is the most abundant element, followed by Ni and Co, and smaller amounts of Mo, W and Zn. Fe is part of Fe–S clusters, which are used for both, electron transport and/or catalysis. Ni is either bound to Fe–S clusters or in the active centre of the co-factor F430. Co is present in cobamides involved in methyl group transfer. Mo and W are non-covalent bound to co-factors molybdopterin and tungstopterin, which catalyse two electron redox reactions (reduction of CO_2 to formate by formate dehydrogenase (FDH)) [3]. Furthermore, Mo also plays a crucial role in the syntrophic propionate oxidation [4].

In numerous research studies the positive impact of trace element supplementation on anaerobic digestion processes was demonstrated. It was reported that productivity, bio-methanation rate and hence process stability were significantly improved. However, uncertainty about the exact concentration of essential elements that need to be present in a well operating bio-process remains. Suggested values cover a wide spectrum of concentrations comprising a range of more than three decimal powers [5–7].

A major factor of influence is that presence of high metal concentrations does not necessarily imply that microorganisms are also able to take them up and incorporate them into catalytic centres of their enzymes [8]. Microorganisms are using two ways for metal uptake, a fast, passive and unspecific one, driven by the

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chemi-osmotic gradient across the cell membrane as well as a slow, active and high specific one [9]. Beside uptake as free ions, metals can also be taken up in the form of complexes, such as vitamin B12 [10], co-citrate [11] or bound to siderophores [12]. Trace metals present in the digester undergo complex physic-chemical processes. Their form whether existing as free ions, complex bound or as precipitates depends on various parameters, such as pH, alkalinity, and presence of sulphuric compounds and excreted soluble microbial products (SMPs)[13–15]. To overcome deficits, a broad spectrum of commercial mineral supplementation products are available, designed to address the specific needs for trace elements. On the other hand, addition of trace metals also implies the risk of overdosing, in this way causing toxic effects on the microbial consortium of anaerobic digestion [4]. High heavy metal concentrations may also limit the proper use of digestate as fertilizer and can cause environmental pollution.

Adequate supplementation requires appropriate knowledge on the bioavailability of the considered elements. However, on this specific issue little knowledge is available because typical measurement of trace metals present in anaerobic fermenter content usually comprise total concentration and hence does not provide adequate information about its bioavailability and toxicity [16].

In order to get such distribution patterns, sequential extraction techniques need to be applied. The speciation, i.e. the distribution into different fractions (free, adsorbed, precipitated, and unavailable) indicates their availability for metabolic activity [17,18]. Only a few studies investigated trace elements in AD processes using such sequential extraction methods [15,19,20]. The authors demonstrated, that depending on the matrix bioavailable fractions can substantially differ from the total concentration of the trace elements.

In the present study slaughterhouse waste with extremely high total nitrogen concentrations (TKN 9 g/kg) was used as the sole substrate in four semi-continuous lab-scale mono-digestion experiments. In earlier investigations it was shown that efficient digestion of this specific substrate is strongly dependent on trace metal supplementation [21]. The purpose of the current work was to study the impact of different levels of trace elements on process performance and to apply a sequential extraction technique aiming to link bioavailability of single elements to process improvement.

2. Materials and methods

2.1. Origin of feedstock and inoculum

The feedstock applied in all experiments derived from a large abattoir located in Austria which processes 800,000 heads of pig and more than 50,000 heads of cattle per year. For the continuous fermentation tests, material was taken from the storage tank after thermal hygienisation (70 °C, 1 h). This material serves as feedstock for the on-site operated biogas plant (mesophilic process at 38 °C). The samples were kept at 4 °C until use.

The inoculum derived from a previous 6l lab-scale experiment using the same feedstock as described above. To keep the microbial consortium viable after finishing this earlier experiment, feeding was continued with a moderate organic loading rate (OLR) of 0.5 kg VS/m^3 d. At start-up of the experiments the inoculum was transferred to the 1 l lab-scale digesters.

2.2. Experimental set-up

2.2.1. Semi-continuous fermentation tests

Four semi-continuous fermentation tests (SPU 0–3) were conducted using 1 l reactor vessels with a working volume of 800 ml. Three openings in the vessel (two in the top, one in the bottom) served as gas outlet and for feeding and sample taking, respectively. To maintain constant operation conditions the reactors were placed in an incubation room with a fixed temperature, 38 ± 0.5 °C. Manual feeding was done once a day seven times per week by substitution of part of the reactor content with fresh substrate. A constant organic loading rate (OLR) of 2.2 kg VS/m^3 d was applied over the complete operation period of 4 months. The reactors were continually mixed at 250 rpm.

Samples were analysed once a week (every Thursday) for the following parameters: volatile fatty acids (VFAs), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia nitrogen (NH4-N), total solid (TS) and volatile solid (VS). After 33 and 91 days samples were taken for sequential extraction (see Section 2.2.3).

Biogas quantity was continuously measured with high precision gas counters (MGC-1 V3, Ritter®, Germany) and recalculated to standard conditions. The gas composition (CH_4, CO_2, H_2S and H_2) was determined twice per week by GC. For this purpose biogas samples (approx. 15 ml) were taken from the head space of the reactors. Duplicate measurements were made using a GC-TCD gas chromatograph (5890 Hewlett Packard Series II). It consists of a single split-splitless injection port, a HP-Plot Q column and a thermal conductivity detector (TCD). Helium was used as carrier gas. Temperature and pH in the reactor were controlled with a standard multi parameter instrument (Multi 340i, WTW).

2.2.2. Routine analyses

TS, VS and COD of substrate and reactor content were analysed according to standard methods DIN DEV 38 414 part 2, DIN DEV 38 414 part 3 and DIN DEV 38409-H41-1, respectively. Samples for TKN analysis were digested in a block-digestion unit K-437 (Büchi®) connected to a Scrubber B-414 at 420 °C followed by subsequent titration conducted via a distillation unit K-370 with built-in titrator (Büchi®). The standard method according to VDLUFA was slightly modified using NaOH instead of MgO for pH adjustment. In order to avoid NH_3 losses, samples were kept on ice before starting the NH4-N analysis.

VFAs were determined after protein removal by Carrez precipitation according to standard method DIN 38 414-19 by HPLC (Agilent®; column COREGEL 87H, ICE Ion 300; solvent H_2SO_4 (0.01 M) using the following setting: flow rate 0.05 ml/min; temperature 65 °C; detector systems: multiple wavelength detector (MWD) and refractive index detector (RID).

2.2.3. Trace elements

Trace elements (TE) were added in different amounts to the continuous fermentation tests, termed SPU 1–3. Test SPU 0 served as reference and did not receive supplementation.

Three trace element solutions (A–C, corresponding to tests SPU 1–3) with increasing amounts were prepared (Table 1). The initial supplementation rate was 10 μl solution per day per l working volume in all the reactors. From day 34 on the daily rate was raised to 50 μl/l working volume. The respective volume of TE solution was mixed into the substrate immediately before feeding.

Table 1
Composition of trace element solutions (A–C) applied in the semi-continuous fermentation tests.

Element	Solution A [mM]	Solution B [mM]	Solution C [mM]
Ni	26.3	78.8	236.4
Co	39.1	117.3	352.0
Mo	4.3	12.8	38.4
Zn	8.8	26.3	78.8
Cu	2.3	7.0	21.0
Se	0.4	1.3	4.0