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CONCENTRATION OF SELECTED PLANT NUTRIENTS AND TARGET ELEMENTS FOR PHYTOREMEDIATION AND PHYTOMINING IN BIOGAS DIGESTATE

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Abstract: Germanium (Ge) and rare earth elements (REEs) are of increasing interest in phytoremediation and phytomining research. These elements are present in almost all soils and soil-grown plants contain considerable concentrations of these elements in their biomass. The process chain of phytomining involves i) the accumulation of target elements in harvestable plant biomass (phytoextraction), ii) production of bioenergy by burning or biogas production, and iii) the recovery of the elements from bioenergy residues. Additionally, the use of ashes and bio-digestion waste as substitutes for synthetic fertilizers offers a great chance to close the nutrient cycle. However, current research in phytomining mostly focuses on the optimization of the phytoextraction process and there is very little information available in the literature on the chemical behavior of target elements for phytomining during the process of energy production. Therefore, the aim of the present study was to assess the concentrations of selected plant nutrients (P, Fe, Mn, Zn, Cu, Ni, Mo, Co) and target elements in phytoremediation (As, Cd, Pb, Cr) and phytomining (Ge, REEs) in digestates from anaerobic fermentation and evaluate the effect of the fermentation process on the enrichment of the elements. In batch reactor experiments shoot biomass of the energy crop *Phalaris arundinacea* was anaerobically fermented. Major and trace element concentrations in the initial mixture of the plant material and inoculum as well as the resulting digestates were determined by ICP-MS. Concentrations of macro and micro nutrients in the digestates decreased in the order P > Fe > Zn > Mn > Cu > Ni > Mo > Co. The concentrations of potentially toxic trace elements were highest for Pb and decreased in the order Pb > Cr > As > Cd. The digestates contained considerable concentrations of REEs and Ge. Specifically, concentrations of REEs were higher than most of the toxic elements except of Pb. The fermentation process led to a significant increase of Zn, Pb, As, REEs and Ge while P, Fe, Mn, Mo and Cd were not affected. For Ni and Cr we observed a significant depletion of the digestates. Most probably the increasing concentrations result from carbon losses during microbial decomposition of the biomass and consequently an enrichment of the elements relative to the organic matrix. These results clearly show that optimization of the biogas production process offers a great opportunity to enhance the efficiency of the process chain of phytomining; however, the processes involved need to be understood and remain field for further studies.

Keywords: Phytoremediation, phytomining, biogas digestate, *Phalaris arundinaceae*, nutrient cycle, germanium, rare earth elements, heavy metals

1. INTRODUCTION

The energy-demanding communities are rapidly growing with increasing human population, modernization and urbanization. As a consequence it is generally expected that energy requirements will rise by 53% till 2030 which could vastly raise the cost of energy and is expected to reach at uttermost by 2050

(Khan & Dessouky, 2009). With the fastly depleting conventional fossil fuels (Shaahid & Elhadidy, 2007) and growing outcry against the carbon emissions the exploration of renewable energy sources is continuously increasing. The use of Biogas as a renewable energy resource has been rapidly increasing as, "biogas plants provide a fully sustainable and integrated system for resource and environmental management that offer governments a multipurpose technology option for fulfilling a cluster of policy needs" (Lukehurst et al., 2010). According to German Biogas Association (2019) by the year 2018 there were 9,444 biogas plants in Germany with the capacity of 4,953 MW of installed electricity. Biogas is fundamentally a mixture of CH_4 (40-75 %), CO_2 (15-60 %) in volume, H_2S (1–2 %), N_2 (0–1 %), H_2 (0-1%), and traces of CO and O_2 produced through anaerobic digestion (Baciocchi et al., 2013) of organic matter. In biogas plants anaerobic digestion is an established procedure to naturally breakdown the organic matter in the absence of oxygen for the production of biogas and digestate (Lukehurst et al., 2010).

Anaerobic digestion of organic material involves at least four process stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Methane is formed by a group of bacteria subsequently and necessary process stage following acetogenesis. Methanogenesis generally consists of two pathways: acetoclastic, i.e. the breakdown of acetic acid in methane and carbon dioxide and hydrogenotrophic, i.e. the formation of methane from hydrogen and carbon dioxide. However, some bacteria and archaea particularly especially represent the strains involved in the hydrogenotrophic pathway (Nettmann et al., 2010). Anaerobic digestion process occurs in two forms. One is batch anaerobic digestion and another is continuous anaerobic digestion. In the former, the digester is used to see the methane potential in particular substrate and the latter is used to see the biogas at pilot scale level, respectively. After that, the final product of anaerobic digestion, in addition to methane-rich biogas, is digestate.

Digestates are usually used as organic fertilizers due to presence of high contents of plant nutrients (N, P, K and essential trace elements) (Świątczak & Cydzik-Kwiatkowska, 2018) and usually have very good fertilizing properties. Therefore, biogas digestate turns to be a good candidate to replace inorganic fertilizers, high-quality compost, and soil improvers, provided that benefits for society in overall and for the environment in specific, as well as helping to preserve limited natural resources such as fossil resources of mineral phosphorus (Tambone et al., 2010). The nutrients in the digestate depend on the feedstock which is used in anaerobic digestion process. The feedstock used in this study is energy crop. The nutrients play a vital role for stability of anaerobic digestion process. The optimal balanced concentration of biogas digesters need C:N:P:S (~600:15:5:1) (Fricke et al., 2007) as well as other macronutrients (K, Na, Ca and Mg), trace metals (Fe, Zn, Mn, B, Co, Ni, Cu, Mo, Se, Al, W and V) (Schattauer et al., 2011) and vitamins (Aquino & Stuckey, 2003) to support microbial growth. A lack of nutrients has to be avoided, but excessive nutrient contents as well as a number of non-essential, potentially toxic elements (As, Pb, Cd, Cr) can generate inhibitory effects (Chen et al., 2008; Demirel, 2011). The required concentration of trace elements in the digester increases as along with the growth of bacteria (Zhang et al., 2003). Besides these nutrients anaerobic digestates from plant biomass may also contain some economically valuable elements such as germanium (Ge) and rare earth elements (REEs), which are of increasing interest in phytomining research. Typical steps of phytomining involve the accumulation in a plant species, harvesting the biomass and recovery of target elements from bioenergy residues (Wiche & Heilmeier, 2016). According to Wiche & Heilmeier (2016) plant species from the functional group of grasses are considered to accumulate high concentrations of Ge. In particular, in reed canary grass (*Phalaris arundinacea*) concentrations of Ge can reach up to 1 mg/kg Ge and 1 mg/kg REEs, respectively, and consequently these elements may also accumulate in digestate. However, until recently there is no report available in the literature dealing with concentrations of Ge and REEs in fermentation residues of anaerobic digestion. The aim of the present study is to i) give an overview over the elemental composition of digestates from anaerobic fermentation of biomass of soil grown *Phalaris arundinacea* with special focus on selected plant nutrients (P, Mn, Co, Ni, Fe, Mo), target elements in phytoremediation (Pb, As, Cd and Cr) and phytomining (Ge, REEs) research, and ii) evaluate the effect of the fermentation process on the enrichment of elements in the digestate. The outcome of this study will improve our general understanding on the behavior of target elements during the fermentation process and will be the basis for the development of a sustainable phytomining of Ge and REEs.

2. MATERIALS AND METHODS

2.1. Experimental setup

The present research work was carried out in batch reactors consisting of 2 l glass bottles with 1.5 l working volume each as detailed in (Garuti et al., 2018). Plant samples of reed canary grass (*Phalaris arundinacea*, cultivar: PX9160014 and Lipaula) were collected from a field site near Freiberg, Germany. The inoculum (80% manure, 20% fodder and little grass) were collected from a biogas plant in Clausnitz, Germany. Later we cultivated this inoculum from our own which was residue of previosly batch test. Prior to the experiment, the particle size of the plant biomass was reduced to approximately 3 mm using a laboratory mixer. A subsample of the plant material and inoculum was used to determine the dry matter contents (total solids, TS) by drying at 105 °C for more than 24 h. Subsequently, a portion of the dried samples was used to measure the organic matter contents (VS) of grass, inoculum and digestate by heating the samples in the oven at 550 °C for 2 h. As a result, the plant material had a TS content of 88.9% and VS content of 93.8% of TS. The inoculum had TS content of 3.9% and VS content of 64.8 of TS. The digestate had a TS content 3.9% and VS content of 55.6% of TS. Initial trace element concentrations in the dried samples of the plant material and inoculum were measured according to Wiche & Heilmeier (2016) (see Table 1).

The plant material (24.11 g) and inoculum (1574 g) were mixed in the glass bolltles and incubated at 39°C in a thermostate room in duplicates. All reactors were stirred automatically. The pH values of samples were determined by a pH meter (TM 39, Sensortechnik, Meinsberg). The pH of the initial sample (mixture of grass and inoculum) was 7.7 and dropped to 7.4 at the end of the experiment. For monitoring the fermentation process gas production was continously measured (data not shown here). After 40 days the process of digestion was stopped due to decrease in the gas production because of low availability of substrate to microorganisms. After this process, the homogenized digestates were collected and stored in a freezer at -4 °C.

2.2. Element analysis in digestates

Concentrations of plant nutrients P, Mn, Co, Ni, Fe, Mo, Zn, target elements for phytoremediation (Pb, Cr, Cd, As) and phytomining (Ge, REEs) were analysed before and after the batch anerobic digestion process. The digestates were dried at 105°C and ground to a fine powder. A subsample of 100 mg powdered material of grass, inoculum and digestate was mixed with 0.9 ml 65% HNO_3, 0.2 ml H_2O and 0.6 ml HF and heated to 180 °C in a microwave using teflon vessels (Ethos plus 2, MLS) according to Krachler et al. (2002) and Wiche & Heilmeier, (2016). All samples were processed in duplicate. Concentrations of Ge, La–Lu, Y, Sc, P, Mn, Co, Ni, Fe, Mo, Zn, Pb, Cr, Fe, Cd, As in resulting solutions were measured by ICP-MS (Inductively coupled plasma mass spectrometry) with rhodium and rhenium (10 µg/l) as internal standards (Wiche & Heilmeier, 2016). Accuracy was checked by analysis of the certified reference material NCS ZC73032 (China National Analysis Center for Iron and Steel, 2014). All results deviated by less than 10% from certified values.

2.3. Data processing and statistics

Initial concentrations in mixtures of grass and inoculum were calculated based on results of concentrations in plant biomass and inoculum considering the defined mixing ratios of the dried material. Differences between initial concentrations in the fermenter and final concentrations in the digestate were identified by a t-test for paired samples using IBM SPSS Statistics 25. For each of the analyses residuals were tested for homogeneity of variances by using Levene’s test. In case of significant differences in the variances between the selected groups a log-transformation was performed. At variance homogeneity, differences among the groups were reported at $\alpha = 0.1$.

3. RESULTS AND DISCUSSSION

3.1. Elemental composition of the digestates

Anaerobic digestion has been known for outstanding nutrient recycling and recovery from organic waste. In particular with regard to the plant nutrients (P, Fe, Zn, Mn, Cu, Ni, Mo, Co) the use of digestates can help reduce the use of inorganic fertilizers and improve soil structure and soil biology (Alburquerque et al., 2012; Möller & Müller, 2012;

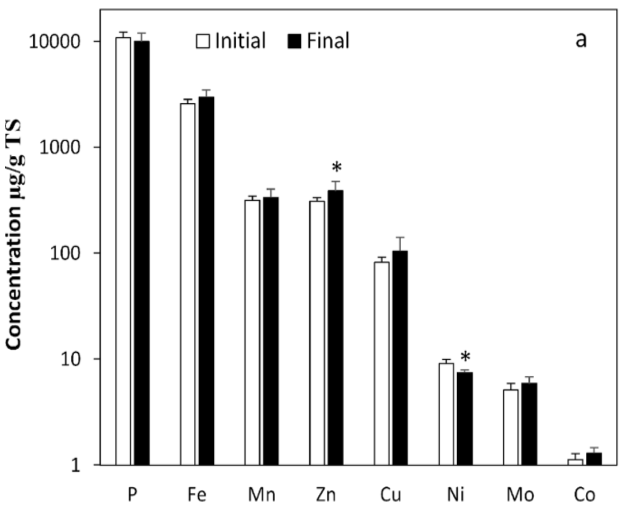
Table 1. Concentrations of selected major and trace elements in the plant biomass and inoculum used in the experiment. The dried plant material and inoculum was dried at 105 °C, ground to a fine powder and analyzed according to Wiche & Heilmeier (2016). REEs are calculated as the sum of 16 elements (Lanthanides +Y+Sc)

Substrate	P	Fe	Mn	Zn	Cu	Ni	Mo µg/g	Co	Pb	Cr	As	Cd	REEs	Ge
Grass	2340	205	37.6	35.9	5.1	10.9	2.6	0.2	0.4	14.9	7.3	0.1	0.3	0.2
Inoculum	13716	3416	412	402	109	8.4	5.9	1.4	19	9.1	2.38	0.9	8.7	0.5

Nkoa, 2014). In the present experiment we found that P showed highest concentration (up to 10 mg/g) (Figure 1a). Concentrations of essential trace nutrients decreased in the order Fe > Zn > Mn > Cu > Ni > Mo > Co reflecting the initial concentrations in the plant material and inoculum (Table 1). Generally, all toxic elements were below the recommended limits suggested by the Irish Bioenergy Association (2012). Therefore, the digestate can be used as biofertilizer. With regard to the target elements in phytoremediation and phytomining research concentrations of Pb was highest (18.0 µg/g) and decreased in the order Pb > REEs > Cr > As > Cd > Ge. In this experiment the digestate was from a mixture of manure and grasses. In the literature *Phalaris arundinacea* is described to be a plant species that typically shows very low soil-shoot-transfer of heavy metals (Deng et al., 2004; Polechońska & Klink, 2014) which might be the reason for the low concentrations measured in the shoot biomass of *Phalaris* plants obtained in this study (Table 1). Instead we found high concentrations of Ge and REEs in the plant biomass which where higher than Cd and Pb (Table 1). Accordingly, concentrations in the digestate reached up to 9.12 µg/g for REEs and 0.5 µg/g for Ge. Surprisingly, in our experiment, the inoculum showed highest concentrations of all considered elements which might be facilitated by an enrichment of the elements in the fermentations residue of earlier experiments which were used to inoculate the reactores with bacteria. These results clearly show that digestates may contain considerable quantities of elements which are of particular interest in phytoremediation and phytomining research. Surely the concentrations depend on the concentrations in inoculum, the plant material, operating conditions (temperature, pH, hydraulic retention time) as well as the mixing ratios of substrate and inoculum. Therefore, further experiments are needed to evaluate the elemental composition of the digestates as a result of different plant species, different inocula and mixing ratios, respectively.

3.2. Enrichment of elements in digestates during the fermentation process

Some elements showed significantly higher concentrations in the digestate compared to initial concentration in the fermenter. Figure 1a shows a significant increase in Zn (388 µg/g) concentration in the digestate compared to initial concentration (307 µg/g). The concentration of Pb was a factor of 20 higher compared to initial concentration and As showed a significant increase compared to initial by a factor of 7. Moreover there was a significant increase in the concentrations of REEs and Ge in digestate (roughly a factor of 10 in case of REEs and 2 in case of Ge) (Figure 1b). In contrast, for Ni and Cr the digestate appeared to be depleted compared to the initial material, while there was no significant effect on P, Fe, Mn, Mo and Cd. Generally the enrichment of elements (Zn, Pb, As, REEs) could be due to mass losses /carbon losses of the organic material within the reactor through production of biogas. It appears reasonable that, differences in the enrichment behavior among the elements might result from element-specific chemical features causing different interactions with the microbes, uptake by the microbes and changes in chemical forms through chelation with released secondary metabolites (Cord-



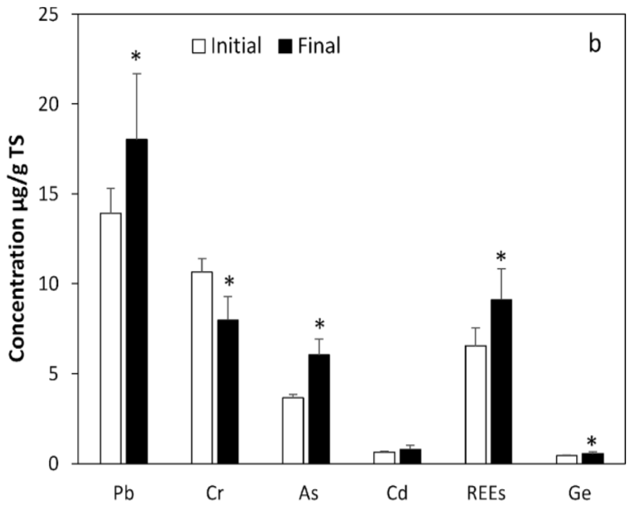


Figure 1. Initial (grass + inoculum) concentration and final (digestate) concentration of trace elements (a), heavy metals and valuable elements (b) on the basis of total solids (TS). Total concentrations of REEs were calculated as sums of lanthanides + Y + Sc. Each bar represents the mean ± sd of three replicates. An asterisk denotes significant differences between initial concentrations in the fermenter and final concentrations in the digestates for a certain element at $\alpha = 0.1$

Ruwisch, 2000; Schattauer et al., 2011). Possibly, during enzymatic degradation of the substrates the elements contained within cell structures are released to the external solution. Since these elements typically show a high affinity to organic matter they may be immediately adsorbed onto negatively charged dissolved and particulate compounds (Wiche et al. 2017).

However, based on the data obtained from this experiment it is still not clear why concentrations of Ni and Cr decreased and there was no effect on P, Fe, Mn, Mo, Cd.

Unfortunately, previously published articles exclusively report trace element concentrations in the digestate without comparison to initial levels and are mostly focused on nutrients and heavy metals (Zirkler et al., 2014) so that a comparison of our results with data from the literature is not possible. However, the enrichment of elements observed in this study generally offers new perspectives for a holistic optimization of the phytomining process chain. In particular, the efficiency of later metallurgical methods that aim to recover the elements as raw materials strongly depends on the the enrichment in bioenergy-residues (Brooks, 1998). Therefore further experiments on the effects of the initial chemical and microbiological properties of the plant material and inoculum as well as effects of mixing ratios and operating conditions (temperature, pH, hydraulic retention time) on the enrichment of target elements are needed. Moreover, the optimization of anaerobic digestion process for phytomining requires a sound understanding on the effect of operating conditions on the chemical binding forms of the target elements in digestates which remains field for further research.

4. CONCLUSION

We could demonstrate that biogas residues contain not only plant nutrients justifying their use as organic fertilizers but also considerable quantities of economically valuable elements such as germanium (Ge) and rare earth elements (REEs). Following the fermentation process some elements were significantly enriched (Zn, Pb, As, Ge, REEs), while others remained constant (P, Fe, Mn, Mo, Cd) or were depleted (Ni and Cr) compared to the initial material. Overall we conclude that optimization of the fermentation process offers a great opportunity to produce highly enriched “bio-ore” representing the basis for later metallurgical processes for element recovery.

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