Reactors temperature affect enrichment and chemical fractionation of plant nutrients, potentially toxic trace elements and economically valuable elements in digestate from anaerobic digestion

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Abstract

This study investigated the effect of mesophilic (37°C) and thermophilic (55°C) digestion on the enrichment and fractionation of plant nutrients (P, Mn, Fe), potentially toxic trace elements (Cd, Cr, Pb, As, Cu, Ni, Co, Zn) and economically valuable elements (Ge, rare earth elements REEs). Batch experiment was conducted with the substrates of *Phalaris arundinacea* and inoculum for 40 days and the latter digestate was collected for further analysis. Digestate from selected reactors was filtered through 0.2 µm syringe filters to separate dissolved elements from the particulate. The solid digestate was extracted with ammonium acetate (pH 7 and pH 5) to determine the extraction of mobile/exchangeable and acid soluble elements, respectively. In fresh digestate, element concentration increased by more than 20 to 100% especially Ge (94%) and REEs (119%) and pH (6.5–7.9) was significantly higher compared to mesophilic (6.1–7.5). In dried digestate, thermophilic digestion showed increased enrichment of Fe, Co, Cu, Zn, Cr, As, Cd, Pb and especially Ge (193%) and REEs (90%) compared to mesophilic indicating a strong enrichment in thermophilic digestion. Considering both operating conditions 5% of elements were present in the liquid, less than 30% were exchangeable and acid soluble and more than 70% were stabile bound into solids. Thermophilic conditions significantly increased the portion of dissolved and labile-bound elements in the digestate. Reactor temperature offers a promising way to use digestate as a secondary raw material for element recovery in the spirit of phytomining, which contributes to a "cascade use" of digestate in the circular economy.

Keywords: Thermophilic digestion, Nutrients, Valuable elements, chemical fractionation, Digestate

1. Introduction

The increasing consumption of energy and rapidly diminishing fossil fuel resources worldwide push scientists to look into alternative, sustainable, and renewable energy sources that could replace fossil fuels and thus mitigate negative climate change impacts. However, due to growing political, social, and environmental concerns, both academic and industry groups have seen a large increase in recent years in research into significant energy. [1]. Bioenergy is a viable substitute for conventional energy (fossil fuel) obtained from biological sources such as wood, crops, or animal feces [2]. According to the European Biogas Association's (EBA) most recent statistical report on the state of European biogas, published in 2021, the total number of biogas facilities operating in Europe was 18,774 and biogas is expected to account for 30 to 40% of total gas consumption in Europe by 2050 to reduce the dependency on conventional energy and the reduction of conventionally produced energy, i.e. fossil fuels [3]. The reduction of conventional energy resources is both in line with Europe's growing efforts to curb carbon emissions and economic reforms. The biogas sector in Germany delivered 19.7 % of electricity from renewable energies in 2021 [4].

Biomass from bioenergy crops is now globally the largest potential source of renewable energy, accounting for roughly 10% of the world's primary energy supply. Bioenergy crops are expected to yield up to 273–1381 EJ/yr by 2050, according to estimates [5]. At present, many scientists are focusing to increase biogas production by exploring new suitable crop species, as well as by improving biomass yield [6]**.** Dedicated energy crops and agricultural co-products that can be used to produce bioenergy are examples of bioenergy crops. Moreover, fast growth, a big volume of biomass, high planting density and harvest frequency, strong biotic and abiotic stress tolerance, and minimal input requirements are all features that make a plant species desirable as a prospective bioenergy crop. Many edible plants, such as corn, wheat, sugarcane, and rape seed, were initially employed as bioenergy crops since they can also be used for food and compete for fertile land and resources with food crops, resulting in high food costs and a danger to food security. Switchgrass (*Panicum virgatum*), *Phalaris arundinacea*, *Medicago sativa, and Pennisetum purpureum* are examples of bioenergy crops that have been engineered to overcome the limits of first-generation biofuels. They may grow on marginal lands and produce lignocellulosic biomass for biofuels and biogas [7]. In particular, the use of perennial bioenergy crops such as *Phalaris arundinacea* and *Miscanthus giganteus* contributes to the

development of low-input cropping systems for bioenergy purposes. In addition, it can also enhance biodiversity, present potential for phytoremediation and erosion control at the same time, boost soil organic carbon, carbon sequestration in soil and mediate water flow and nutrient content [8]. Some of these plant species have been recently described as accumulators of potentially toxic trace elements and economically valuable elements such as germanium (Ge) and rare earth elements (REE) [9–11], and thus, element recovery from plant biomass would contribute to an environmentally friendly, cost-effective raw material production (phytomining) [12–15]. Phytomining strategies are being pursued by several enterprises and scientific research groups. Several plant species have been identified as effective hyperaccumulators of various elements. Some ferns, for example, can accumulate REEs at concentrations of 0.1 percent or higher in their dry biomass. *Dicranopteris dichotoma* is one of these ferns, and it is well-known for its high REE and potentially toxic element (PTE) accumulation capacity [16]. Many plant species, such as *Amaranthus retroflexus, Polygonum aviculare*, *Gundelia tournefortii*, *Noea mucronata*, and *Scariola orientalis*, have been found to naturally accumulate high concentrations of PTEs such as As, Cd, Cu, Co, Mn, Ni, and Zn [17,18]. After the accumulation of elements in aerial plant parts and harvest of the plants, it is critical to deal with polluted plant residue, which contains some important components, throughout the phytomining and phytoremediation processes [19]. Obtaining valuable products from biomass could increase the commercial viability of phytoremediation for contaminated soils [13,20], but is viable only if the plant biomass is processed in a way that makes the residues exploitable for subsequent extraction methods. This includes the enrichment of the valuable and reduction of compounds in concert with optimization of bioenergy production. Besides burning biomass [21–23], for energy followed by metal recovery from the ash [24–27] and pyrolysis [28–32] the biomass can be converted anaerobically to produce biogas in parallel with a digestate which can later be used for raw material recovery and thereafter returned to the soil as organic fertilizer.

Anaerobic digestion (AD), is an established procedure to naturally break down organic matter in the absence of oxygen for the production of biogas and digestate [33]. Co-digestion of both animal manure and crop wastes has proven to be a successful technique for AD stability and efficient biogas generation [34]. Nowadays, the majority of biogas plants are run at mesophilic (36–38 °C) conditions and the processes

during biogas production were extensively described in the existing literature; however thermophilic digestion conditions (55 °C) are increasingly considered. Due to the increased activity of fermentative microorganisms, thermophilic (AD) has been used to facilitate the breakdown of resistant cellulose, although the procedure often results in a higher concentration of free ammonia nitrogen (FAN) and the formation of volatile fatty acids (VFAs) [35,36]. Most studies dealing with thermophilic AD reported the impact of temperature on process parameters, gas yield and process stability [37]. However, there are only a few papers [38,39] available that compare tests carried out in similar ways under thermophilic and mesophilic conditions concerning energy crops and information on the suitability of the process for phytomining purposes is scarcely available in the literature. Phytoremediation in combination with bioenergy generation is a relatively new topic and most research did not consider the whole spectrum of elements contained in biomass ranging from plant nutrients, over potentially toxic trace elements, to valuable elements. Moreover, the current literature did not consider accumulator plants/crops for bioenergy production and/or has not examined metal fate in bioenergy plants or biogas reactors [40].

Therefore, this study primarily focuses on the effect of batch digestion under both thermophilic 55 °C conditions and mesophilic 37 °C conditions on biogas production, and on the enrichment of plant nutrients (P, Fe, Mn), potentially toxic trace elements (Cd, As, Pb, Cr, Zn, Cu, Ni, Co) and economically valuable elements (Ge, REE). Moreover, we aimed to determine the distribution of elements in the liquid and solid phases of the biogas residue. For this purpose, we used the perennial grass species *Phalaris arundinacea*, (reed canary grass) as biomass and bioenergy feedstock, because it is a large, fast-growing, high-biomass-producing plant with a high capacity for trace element bioaccumulation, particularly of germanium and rare earth elements [9,10,41]. This study will contribute to an optimization of the fermentation process towards the production of a highly enriched “bio-ore” representing the basis for later metallurgical processes for element recovery and fertilizer production. Considering the large density of biogas plants in Europe and the widespread occurrence of Ge and REE in soils at average concentrations of 2 mg/kg (Ge) and 165 mg/kg REE [42,43] and particular enrichment of these elements in Baltic soils [44], this process chain could indicate a technological innovation for the renewable global energy, raw material and circular system.

2. Material and methods

2.1 Experimental setup and characterization of plant biomass

The batch anaerobic digestion experiments were carried out in 2 L glass bottles with a working volume of 1.5 L placed in a thermostat to maintain a constant temperature. Bioreactors were connected to the biogas collection bags via tubes, where gas counters were installed to calculate the volume of the produced biogas. Biomass of *Phalaris arundinacea* (genotype PX9160014) was obtained from Deutsche Saatveredelung AG, Germany (DSV) and originated from a field site near Asendorf, Germany. The grass biomass was air-dried and ground to a particle size ≤ 1 mm in length to increase the surface area. The inoculum for the anaerobic digestion process consisted of fresh digestate collected from previous running experiments. Before the fermentation experiments the physicochemical characteristics, elemental composition and total concentration of elements of both the grass biomass and inoculum have been determined (Table 1). Total solids (TS) and volatile solids (VS) were measured according to VDI-RICHTLINIE [45]. The elemental compositions (C, H, N, S) of the grass and inoculum were measured by an elemental analyzer (vario MICRO Cube, elementar Analysen systeme GmbH). For the assessment of initial concentrations of plant nutrients (P, Mn, Fe) potentially toxic trace elements (Zn, As, Pb, Cd, Cr, Ni, Co, Cu) and valuable elements (Ge, REE), a homogeneous subsample of plant material and the inoculum were dried at 105°C for 24 hours. Triplicates of each of these samples were processed for trace element analysis as described below (Section 2.3). Compared to the plant biomass, the inoculum showed substantially higher concentrations of all investigated elements when the element concentrations were compared on a dry matter basis (Table 1). Also, it has to be noticed that the inoculum was characterized by relatively low TS (3.5%) compared to the grass biomass (89.5%), which strongly influenced the resulting total element concentrations of the initial biomass in the reactors (Table 1).

##Table 1##

Before starting the experiment, the inoculum was placed in a water bath at 37 °C or 55 °C, respectively, for 24 hours to activate the microbial activity and acclimatize the microbes. Subsequently, 47 g of the grass and 1447 mL of the inoculum was filled in the reactor bottles resulting in a substrate to inoculum ratio of 1.2 (on VS basis). Reactors filled with inoculum only served as a control. Based on the TS content of the inoculum and grass and the analytical data on trace element concentrations in grass and inoculum, respectively, the total element content of the reactor and the initial element concentrations were calculated (Table 1). Initial concentrations largely reflected the element concentrations in the inoculum and total element loading in the fermenters decreased in the order P > Fe > Zn > Mn > Cu > Ni > Pb > Cr > REE > As > Co > Cd > Ge showing a considerable amount of REEs together with P, Zn, Mn and Cu which could be also economically relevant for raw material and fertilizer production.

In an above-mentioned manner, thermophilic reactors were set up with 10 replications and mesophilic reactors were set up with 16 replications. Thermophilic and mesophilic fermentation was done in parallel; however, due to limited reactor capacities, the whole experiment was done in two steps. Initially mesophilic and thermophilic fermentation was done in four replications (thermophilic) and nine replications (mesophilic) which was repeated a second time for quality control. Due to the high variability of element concentrations in the highly inhomogeneous inoculum with the large portion of different particulate and liquid constituents, in each run, the element concentrations in the inoculum were separately analysed (Table 2). Initial element concentrations in the inoculum were not significantly different between mesophilic and thermophilic experiments except for Cd which showed 43% higher concentrations in inoculum in thermophilic experiments.

##Table2##

Throughout the experiment, the collected gas was analyzed daily with a laboratory gas analyzer (Milli Gas counters MGC-1 Ritter, Germany) the composition of CH_4, O_2, and H_2S as well as the pH of the reactors was monitored. After 40 days of experiment biogas production decreased and the process of digestion was stopped. The resulting digestates were collected in PET bottles and stored in a refrigerator at 4 °C until further processing the following day. The digestate of each reactor was homogenized and split into three sub-

samples. Firstly, the fresh digestate, second, the digestate that was dried at 60 °C (48 h) and finally a sample that was dried at 105 °C (24 h) for the determination of total element concentrations in the digestate.

2.2 Distribution of elements in digestate

Twenty grams of fresh digestate (mixture of liquid and solid) from six randomly selected mesophilic batch fermenters and six thermophilic reactors were filtered through 0.2 µm syringe filters to separate dissolved elements from colloidal or particulate element forms. The obtained filtrate was tenfold diluted with deionized water, stabilized with HNO_3 supra and stored at 4°C until being analyzed. 1 g of the 60°C dried and homogenized digestate was weighed into 50 ml tubes and mixed with 50 ml of solutions to extract elements from two operationally defined fractions in sequence. This included the extraction of mobile/exchangeable elements by shaking with 1 M ammonium acetate (pH 7) for 24 h (fraction 1) and acid soluble elements by shaking with ammonium acetate buffered to pH 5 for 5 h (fraction 2). After each extraction step, the samples were centrifuged at 4000 rpm for 10 min. The supernatants were collected in 15 ml centrifuge tubes, acidified with HNO_3 supra and stored at 4°C until being analyzed. Residual concentrations were calculated as the difference between total concentration and the sum of fractions 1 and 2.

2.3 Determination of element concentrations

Solid samples of grass biomass and dried digestates were processed in a microwave digestion system. According to Krachler et al. [46], 0.1 g of samples were weighed into Teflon vessels, mixed with 0.2 ml ultra-pure H_2O, 1.9 ml HNO_3 (65%) and 0.6 ml of 4.9% hydrofluoric acid and subsequently heated to 200 °C for 1 hour in the microwave digestion system (MLS-ETHOS plus, MLS GmbH, Dorsten, Germany) [9,47]. The dissolved samples were transferred into 15 mL centrifuge tubes and stored at 4 °C until being analysed by ICP-MS. Concentrations of P, Mn, Fe, Ni, Co, Cu, Zn, As, Pb, Cd, Cr, Ge, Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu in the acidified filtrates, extraction solutions and microwave digestates were determined by inductively-coupled plasma mass spectrometry (model X Series 2, Thermo Fisher Scientific, Dreieich, Germany), using 10 µg L^{-1} rhodium and rhenium as internal standards. Calibration solutions (0.01

– 100 μg L^{-1}) were prepared by adequate dilution of a multi-element stock standard solution (Merck). Accuracy was checked by analysis of the certified reference material NCSZC73032. The results deviated by less than 14% from certified values.

2.4 Characterization of bacterial communities

After the experiment has finished (40 days), both reactor (mesophilic and thermophilic) digestion were sampled for DNA extraction. To achieve homogenous and representative samples, digestate was mixed and thoroughly collected in a 50 ml plastic tube and stored in a -80 °C refrigerator. The frozen samples were thawed before the total genomic DNA was extracted from the duplicate subsample using QIAGEN DNeasy Power Soil Pro kit according to the manufacturer's instructions. The amplifiability of the extracted DNA was checked by an 16S rRNA gene-based PCR and the concentration was examined with an Invitrogen Qubit Fluorometer. The following library preparation, Illumina sequencing, and 16S metagenomics analysis were done by Microsynth Seqlab, (Balgach Switzerland). Amplification of the 16S (V34 region) was performed with Nextera two-step PCR with purification and pooling of the libraries. The sequencing was a paired-end sequencing of 2 × 250 bp with MiSeq Ilumina. After demultiplexing, the trimming of Illumina adaptor was performed with cutadapt [48]. Merging, quality filtering, OTU construction and chimera removal (clustering with 97% identity) were executed with Usearch [49]. Taxonomy was assignment based on the RDP database [50]. From a total of 22,464.00 reads, 370 OTUs were obtained. We evaluated the prokaryotic communities by using the R packages "phyloseq" [51] and "vegan" [52] in the R software (R 2021-R version 4.1.2). Prokaryotic communities were normalized by rarefying to the lowest number of the sample sequence counts.

2.5 Data processing and statistical analysis

Based on the initial concentrations in the fermenters and the final concentrations in digestate the relative enrichment of elements in dried digestate was calculated as the percentage difference in initial concentrations in each reactor and final concentrations in digestate. This allows the correction for differences in initial element concentrations between mesophilic and thermophilic experiments originating from the inoculum

(Table 2) and the final evaluation of the element enrichment in reactors resulting from temperature changes. Additionally, based on the concentrations in the inoculum (Table 2), concentrations in the grass biomass and the amounts of biomass used in the reactors the amounts of elements in the fermenters before and after digestion were calculated. The element mass balances before and after digestion allowed the evaluation of element recovery and element losses during the fermentation process at different temperatures.

Differences in gas yield, total concentrations, the occurrence of elements in different operationally defined fractions, element enrichment and recovery at different temperatures were identified by multivariate analysis of variance (MANOVA) by using Statsgraphic Centurion 19. For each of the analyses, residuals were tested for homogeneity of variances by using Levene's test. In case of no significant differences in variances, significant differences between the mean values among different temperatures were identified by t-tests with Bonferroni adjustment. In case of significant differences in variances, significant differences between the median values among different temperatures were identified by the Kruskal Wallis test at $\alpha = 5\%$.

3. Results and discussion

3.1 Biogas production and biogas yield during batch anaerobic digestion

Daily biogas yields for mesophilic and thermophilic digestion of grass and inoculum are shown in Fig. 1. When comparing the gas yields between thermophilic and mesophilic conditions, thermophilic fermentation produced roughly 10% more biogas ($p < 0.01$) compared to mesophilic when the whole period of 40 days is considered. Most striking differences between the temperatures were especially observed at the beginning of the experiment. During the first 10 days of the stabilization process, thermophilic conditions produced roughly 40% more biogas compared to mesophilic digestion and this difference between the reactors operated at different temperatures decreased to 14% after 20 days and finally to 5% until the end of the experiment (day 20–40) (Fig. 1). After 20 days there was the asymptotic approval to a maximum gas production in both thermophilic and mesophilic digestion which indicated that most of the organic matter had been degraded by microbes. Compared to the co-fermentation of grass and inoculum, the inoculum alone produced only negligible amounts of biogas, which confirms the positive effects of co-fermentation with ligneous plant biomass (Fig. 1). Although it can be assumed that the reactors did not reach

the maximum biogas yield [53], the higher biogas volume achieved under thermophilic conditions are consistent with the findings of Dąbrowska et al. [54] and Kasinski [38]. The maximum biogas production rate under thermophilic conditions indicated that the substrate in the fermenter is digested at a relatively faster reaction rate, which was consistent with Kim et al. [55] and could be mostly explained by a higher substrate hydrolysis rate at 55 °C compared to 37 °C [56].

During fermentation at mesophilic temperature, the pH was between 6.1 and 7.5 (average 6.9 ± 0.3) while at 55°C the pH was significantly higher ($p < 0.001$) and ranged from 6.5 to 7.9 (average 7.6 ± 0.4). Similar results were observed by Song et al. [57] and Kasinski [38] during single-stage mesophilic and thermophilic digestion of municipal waste and sewage sludge. This increase in pH can be normally attributed to temperature-enhanced gas production where increased methanogenesis is accompanied by reduced production of volatile fatty acids and degradation of nitrogenous compounds which produces alkalinity [38,58].

##Figure 1##

3.2 Effect of temperature on the fermenter microbiome

The prokaryotic communities that were involved in biomass conversion at mesophilic and thermophilic conditions were analyzed at the end of the fermentation process. We found that reactor temperature strongly steered community composition at the genus and phylum levels. The predominant phylum at both temperatures was *Firmicutes*, accounting for 35.3% and 74.4% of the communities at 37°C and 55°C, respectively (Fig. 2). Further predominant phyla at mesophilic conditions include Synergistes (10.8%), *Bacteriodetes* (8.3%), *Chloroflexi* (6.9%), *Proteobacteria* (5.0%) *Actinobacteria* (4.3%), and *Thermotogae* (3.3%), while at 55°C other bacterial phyla, besides Firmicutes, showed only relative abundances of less than 3% (Fig. 2). At both temperature conditions, the prokaryotic communities comprised a comparable large fraction - 18.5% and 14.7% at 37°C and 55°C, respectively.

Comparing the two digestion, it was clear that the temperature had a significant impact on the dominating bacteria and methanogens. *Firmicutes, Chloroflexi, and Bacteroidetes*, which metabolize organic molecules including

303 polysaccharides, monosaccharides, amino acids, glycerol, etc., were the most prevalent bacteria at the phylum level
304 [59,60]. The relative abundance *of Firmicutes, Chloroflexi, and Bacteroidetes* changed as the process advanced.
305 Among them, an increase in the relative abundance of *Bacteroidetes* and *Chloroflexi* was seen in mesophilic
306 digestion compared to thermophilic digestion and an increase relative of *Firmicute* abundance was observed in
307 thermophilic digestion. As is well known, as the process progresses, the digestible component changes, which leads
308 to variations in the bacteria that contribute to the decomposition of organics [61]. Therefore, the contents of the
309 biodegradation component may be connected to the change in the relative abundance of *Firmicutes, Chloroflexi*,
310 and *Bacteroidetes*. Similar findings were found in a prior work by Jiang et al. [62], which showed that the
311 abundance of *Firmicutes* slowly increased as lignocellulose degraded, whereas *Bacteroidetes*' abundance decreased
312 with the reduction of degradable substrates.
313
314 ##Figure 2##
315
316 3.3 Enrichment of elements in digestate depending on temperature
317
318 Literature on concentrations of essential elements (P, Mn, Fe) and potentially toxic trace elements (As, Pb,
319 Cd, Cr, Co, Ni, Cu, Zn) in digestate from anaerobic fermentation is extensive [54,63]. However, to our best
320 knowledge, there is no report available in the literature dealing with the fate of the elements during anaerobic
321 fermentation at different temperatures, especially concerning the economically valuable elements Ge and
322 REE. Initially, before the fermentation, the concentrations in all reactors decreased as mentioned above (Sec
323 2.1). The concentration pattern observed in our study is in good agreement with previous studies [54,64],
324 however, Ge and REEs represent a group of elements that are only scarcely reported in the literature, so a
325 comparison was not possible. The fermentation process at different temperatures substantially increased the
326 concentrations of all investigated elements in fresh digestate which still consisted of a large portion of water
327 (on average 4 % TS). More specifically, in fresh digestate the concentrations increased by 50% (P), 54%
328 (Mn), 128% (Fe), 69% (Co), 54% (Ni), 27% (Cu), 100% (Zn), 149% (Cr), 132% (As), 250% (Cd), 79%
329 (Pb), 94% (Ge) and 119% (REE) (Table 3) indicating a strong enrichment of Fe, Zn, Cr, As, Cd, Pb, Ge and

330 REEs in digestate following thermophilic fermentation, while P, Mn, Co, Ni and Cu were somewhat weaker
331 affected. Surely, concentrations of elements in fresh digestate are strongly influenced by changes in water
332 contents.
333
334 ##Table 3##
335
336 Digestate from thermophilic fermentations had significantly lower water contents (higher TS), most probably
337 due to higher water losses at elevated temperatures (Fig. 3). Thus, concentrations on a dry matter basis are
338 more reliable to evaluate the effects of temperature on element concentrations in digestate. Not surprisingly,
339 the concentrations in dried digestate from all reactors were a factor of roughly 20 higher (Table 3) compared
340 to the fresh digestate which still consisted of more than 96% of water (96.4 ± 1.2% TS, n = 28) (Fig. 3). In
341 dried digestate there was no significant difference in the concentrations of P, Mn, Co, Ni and Cu between
342 thermophilic and mesophilic digestate (concentrations increased by less than 30%). However, the
343 concentrations of Fe, Zn, Cr, As, Cd, Pb, Ge and REE increased significantly ($p < 0.01$) by 50% (Fe), 33%
344 Zn, 62% (Cr), 59% (As), 110% (Cd), 37% (Pb), 35% (Ge) and 31% (REE). Thus, thermophilic fermentation
345 equally affected the element concentrations in digestate, except Cd. It has to be noticed that the initial Cd
346 concentrations in thermophilic fermenters were already 43% higher than in mesophilic reactors which most
347 likely influenced the final concentrations in digestate (Table 2).
348
349 ##Figure 3##
350
351 The calculated enrichment rates which integrate the element concentrations in dried digestate after
352 fermentation relative to the initial concentrations in the reactors showed a relative enrichment of all
353 investigated elements following anaerobic digestion and this enrichment was affected by temperature (Fig.
354 4). More specifically, in mesophilic digestion the absolute enrichment of P, Mn, Co, Ni, Cu, Zn, Cr, As and
355 Pb was very similar and ranged from 41% to 52%, whereas the enrichment of Fe, Cd, REE and especially Ge
356 was markedly lower (31%, 35%, 30%, 14%) (Fig. 4). Compared to that, in digestate from thermophilic
357 fermentation the absolute enrichment ranged between 34% (Ni), 41% (Ge) to up to 86% (As). Higher

temperatures did not affect the element enrichment of P, Mn and Ni. However, in thermophilic digestion, the element enrichment of Fe, Co, Cu, Zn, Cr, As, Cd, Pb, Ge and REE were further increased by 54% (Fe), 23% (Co), 31% (Cu), 64% (Zn), 66% (Cr), 71% (As), 76% (Cd), 36% (Pb), 193% (Ge), and 90% (REE) compared to reactors operated at mesophilic conditions (Fig. 4).

Generally, changes in total element concentrations in the homogenized and dried digestate can only take place when mass losses of organic matter lead to a relative enrichment of the elements in the fermentation residues while differences in enrichments among different elements might additionally indicate element losses with the gas stream or contaminations. Thermophilic fermentation produced significantly more biogas (see Section 3.1). Moreover, digestate from thermophilic digestion was characterized by significantly (28%) lower contents of combustible organic solids (VS) most likely resulting from enhanced microbial degradation of organics and enhanced methanogenesis at elevated temperature (Fig. 3).

##Figure 4##

It is reasonable that higher gas production and consequential losses of carbon, nitrogen, oxygen and hydrogen lead to the element enrichment in thermophilic digestate. Also, the element enrichment during fermentation is following Tian et al. [65] who demonstrated a heavy metal enrichment in fermenters due to substrate degradation. Nonetheless, substrate degradation alone would equally lead to an enrichment of all elements in the digester, which was not the case in our study where enrichment rates largely varied among different elements, especially concerning the low enrichments of Ge and REE in mesophilic fermenters and the strong enrichment of Cu, Zn, Cr, As, Cd, Pb, Ge and REE in thermophilic reactors while P, Mn and Ni were remained largely unaffected (Fig. 4). For Ge the literature indicates the formation of gaseous chlorides, fluorides, and hydrides that can escape the reactor together with the stream of biogas, especially at higher temperatures [66]. In particular, these element losses with the gas stream can strongly affect the enrichment of elements, especially of those elements with low initial concentrations such as Ge. However, thermophilic reactors were characterized by a substantially higher pH (Fig. 1) which normally also coincides with higher DOC concentrations in the digestate [38]. Dissolved organic carbon compounds can act as element chelators and might counteract the formation of element precipitates and the formation of gaseous compounds [67,68]. This could explain also the strong enrichment of Zn, Cr, As, Cd and Pb,

386 Ge and REE in our thermophilic reactors since these elements form very stable organo-element complexes under
387 neutral to alkaline conditions [69]. Concomitantly, low enrichment rates for P, Mn, Fe and Ni could result from the
388 immobilization/uptake of the elements in biofilms, precipitation with sulfate or phosphate in concert with the
389 formation of Fe-plaques on the fermenter surfaces that may also interact with other elements such as REE and
390 mask the element enrichment in the reactors [63,70,71]. The calculated element recoveries showed higher
391 recoveries in thermophilic than in mesophilic reactors (Table 4). Here, Fe, Cr, Ge and REEs exhibited significant
392 element losses (up to 27%) (Table 4), while in thermophilic reactors the losses for these elements ranged between
393 1.7% (Cr) to 14% (Fe) and losses for Ge (10%) and REE (13%), most probably due to the interaction with DOC at
394 high temperature.
395
396 ##Table 4##
397
398 Unfortunately, we did not consider these chemical forms in our study and thus our data does not allow
399 further interpretation. Moreover, previously published articles only report a few trace elements in the
400 digestate without considering relative enrichments [39,68] or comparing mesophilic and thermophilic
401 conditions [63]. Nevertheless, the direct comparison of element enrichment at different temperatures in our
402 study demonstrated the positive effects of thermophilic fermentation on the enrichment of a broad spectrum
403 of economically valuable elements in the digestate. The elucidation of the processes involved and
404 optimization of the fermentation process towards element enrichment remains a field for further research in
405 the future.
406
407 3.4 Fractionation of elements in digestate depending on temperature
408
409 In fresh digestate from mesophilic fermentation the dissolved element concentrations (F1) decreased in the
410 order P > Fe > Ni, Mn, Zn > Co, Cu > As > Pb, Cr > REE, Ge > Cd (Table 5). Increasing the temperature
411 from 37 °C to 55 °C significantly increased the concentrations of almost all elements considered in this
412 study, except Cu and Zn (Table 5). More specifically, the concentrations of dissolved elements calculated on
413 a dry matter basis increased by 168% (P), 257% (Mn), 411% (Fe), 240% (Co), 119% (Ni), 224% (Cu), 257%

(Zn), 314% (Cr), 900% (As), 100% (Cd), 263% (Pb), 18% (Ge) and 667% (REE) with the strongest increase for As and REE and the lowest for Ge (Table 5). Also, digestate from thermophilic digestion clearly showed higher concentrations of dissolved elements in thermophilic when the concentrations were calculated on a dry matter basis (Table 5). Moreover, in the ammonium acetate-soluble element fraction (F2) which largely integrates elements adsorbed onto particulate substances the concentrations of P, Mn Cu and Zn were significantly lower in thermophilic digestion compared to digestate from mesophilic digestion (Table 5) which suggests a release of the elements from adsorbed or precipitated element species into the liquid phase following an increase in temperature [39,68]. However, all other elements showed higher concentrations in F2 of the thermophilic reactor, except Cr, Ge and REE which were largely similarly concentrated in the digestates obtained at different temperatures (Table 5). In contrast, the concentrations of Ni, Cu, Pb and REE showed higher concentrations in F3 of mesophilic digestate, while P, Fe, As and Cd were more concentrated in thermophilic digestate and Mn, Cr and Ge were not affected by temperature (Table 5).

##Table 5##

Considering the percentage share of elements in the different fractions, digestate from both operating conditions contained on average less than 5% of the investigated elements in dissolved forms, less than 30% were present in exchangeable and acid soluble forms and more than 70% were present in the residual fraction (Fig. 5). The distribution of the elements in these fractions strongly varied depending on element specific chemical features and was affected by temperature. More specifically, in mesophilic, P (2%), Co (6%), Ni (7%), As (3%) and Ge (6%) showed the highest portion of dissolved forms, whereas the dissolved portion of other elements was substantially lower and ranged between 0.1% (Cu) and 0.8% (Fe). The solubility of Fe, Mn and Cr were very similar (0.5% – 0.8%) whereas Zn, Cd, and Pb showed 0.3% of dissolved elements only and Cu and REE were the least present in dissolved forms (less than 0.15%).

The application of ammonium acetate (pH 7) already solubilized 29% P, 20% Co, 18% Ni, 24% As and 10% Cd, while the percentage release of Fe, Cu, Zn, Cr, Pb, Ge and REEs was substantially lower (1–2 %). Additionally, ammonium acetate (pH 4) removed 45% P, Mn (%) 20% Co, 17% Ni, 43% Zn, 10% As, 47% Cd, 26% Pb and 10% REE indicating the presence of elements in the form of readily soluble phosphate and

sulfate precipitates and/or surface-complexation on organic matter. Moreover, more than 89% of Fe, Cu, Cr, Ge and REE were present in the residual fraction which suggests that these elements were still bound into organic structures or sparsely soluble oxides. In contrast, the residual fraction of Co, Ni, Zn, As, Cd and Pb were substantially lower (40–60%) and especially for P and Mn (less than 30%).

Compared to mesophilic digestions, thermophilic conditions increased the percentage share of all elements in the liquid phase; however, this increase was only statistically significant for Mn, Fe, Co, Ni, Cu, Cr, As and REE, while there was no significant effect on P, Zn, Cd, Pb and Ge at $\alpha = 10\%$. Arsenic, Cu, REE, Mn, Co and Ni responded the strongest and exhibited 422%, 315% 323%, 251%, 225%, and 160% more elements in solution compared to mesophilic respectively, whereas for Fe and Cr the increase was somewhat lower (138%, 96%). Thermophilic digestate still reflected the same element pattern observed in mesophilic digestion but up to five-fold higher concentrations and was characterized by considerable concentration of dissolved Co, Ni (roughly 20%), As (15%) and Ge (6%) while for all other elements the portion of soluble elements was below 1%). Similar results were also observed by Dabrowska [54] who found that Ni was significantly more soluble under thermophilic conditions compared to mesophilic conditions. Unfortunately, the pore size of the filters used in this study (0.22 µm) did not allow us to distinguish between truly dissolved and colloidal element forms that typically range between 1 nm and 1 µm.

##Figure 5 ##

However, due to the high DOC contents in the reactors, the concentrations of the investigated elements in the liquid phase are likely to be present in colloidal forms and complexes with low molecular weight organic compounds rather than true dissolved forms [72]. They reduce free metal ions in the process liquid, which can reduce precipitation [68,73–75]. Many studies could demonstrate that thermophilic conditions increase the DOC in the fermenters [38] and increased enzymatic substrate degradation, the release of the elements from cell and tissue structures, production of secondary metabolites in concert with the formation of soluble colloids and complexes [67] seems to be responsible for the increase in the solubility of the transition metals in thermophilic reactors, although an increase in pH typically reduces the solubility of cations in biological systems [76]. A strong increase in Fe solubility (Table 5), likely in the form of large-size colloids, can foster

the formation of mineral colloids which control the solubility of REE, Pb, Cr and Ge in natural waters [67] and possibly also influence the element solubility in the reactors (Fig. 4). Additionally, Ge and As may also occur in true dissolved forms with a positive relationship between solubility and pH [77]. Concomitantly, in thermophilic digestion, the exchangeable element fraction of Co, Ni and As increased by 33%, 64%, and 210% compared to mesophilic digestion while in thermophilic digestion P, Mn, Fe, Zn Cr, Cd, Pb, Ge and REE were significantly depleted in this fraction. Here, the strongest effects were observed for P, Mn, Cd and Pb (99%, 74%, 68% and 50% decrease) and relatively weak effects on REE, Cr, Cd, Fe, Ge, and Zn (decrease by 41%, 40% 29%, 20% and 16%). Also, acid-soluble Zn, Cr, Pb, Ge, and REE decreased by 25%, 57%, 53%, 27%, and 52% and in the residual fraction only As and Ni were significantly decreased with the strongest effects on As (93% decrease).

Owing to the degradation of organic matter at elevated temperature it is reasonable that Co, Ni and As firstly were transferred to the exchangeable pool where the elements are adsorbed to particulate organic matter, which is in equilibrium with the dissolved element fraction and leads to higher dissolved element concentrations in the solution. In contrast, a depletion of the adsorbed P, Mn Cd and Pb, REE, Cr, Fe, Ge and Zn could derive from the enhanced liberation of elements from organics into the solution until the elements were precipitated in the form of hardly soluble phosphates, sulphates and oxides that are best described by our acid soluble and particularly Cd, Pb, REE, Cr decrease in acid soluble and increase in residual fraction. the residual fraction. In fact, in thermophilic, the acid-soluble fraction of P and As significantly increased by 38% and 142%. Also, in the residual fraction the percentage share of Mn, Zn, Cd, Pb and REE was significantly higher by 24% (Mn, Zn), 79% (Cd), 21% (Pb) and 10% (REE), which supports our hypothesis and is consistent with the findings of Gonzalez-Gil et al [58] who state that high temperature leads to high pH and most likely high levels of sulfide due to the rising percentage of protein-rich feedstock. Such conditions can lead to an enrichment of heavy metals in the form of residual sulfide precipitates. Surely, the process is not yet fully understood and their study requires the use of isotope-labelled compounds that we methodologically did not consider in our study and remains a subject for future studies.

4. Conclusions

We demonstrated that anaerobic co-fermentation of plant biomass and inoculum enriches a large spectrum of elements, including Ge and REE in the digestate. This enrichment is related to the reactor temperature and gas production. Compared to mesophilic, thermophilic conditions increased gas production and increased the concentrations of Cu, Zn, Cr, As, Cd and Pb in digestate by more than 50%, depending on the element. Dried digestate from thermophilic reactors was characterized by 193% higher Ge and 90% higher REE concentrations. Mesophilic fermenters showed substantial element losses (up to 30%) that were significantly reduced at elevated temperatures (element recovery > 80%). The element concentrations in digestate depend on both, a temperature-dependent enrichment and the initial concentrations in biomass. In our study, we used biomass from an unpolluted experimental field site, which contained relatively low element concentrations. We emphasize that the element concentrations in biomass can be orders of magnitude higher, especially when hyperaccumulator species are used [10,11,78]. However, besides Ge, REE and plant nutrients, the fermentation also enriched several unwanted elements (e.g. As, Cd, Pb) which may impair the use of digestate from phytomining as organic fertilizers. The data obtained from the chemical fractionation procedure indicated an increased percentage share of dissolved and adsorbed elements, especially As, in the digestate from thermophilic reactors, while most of the Ge and REE (more than 94%) were retained in the residual fraction. This offers new perspectives for the removal of As from the anaerobic digestion residues by a simple liquid/solid separation and treatment of the digestate with neutral salt solutions before the digestate is subject to chemical element separation procedures and the extraction of P, Ni, Co, Ge and REE. This can be especially relevant for bioenergy production in arsenic-contaminated post-mining landscapes. The processes during element enrichment and chemical speciation depending on temperature are not yet fully understood. Nonetheless, we show that reactor temperature controls the element enrichment and chemical speciation and the optimization of the process offers a promising way to use digestate as a secondary raw material for element recovery in the spirit of phytomining which will contribute to the development of a sustainable renewable global energy and circular economy.

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