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Fly ash addition into anaerobic digestion of biowaste: effects on biomethane production and fertilizing properties of the digestate

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ABSTRACT

The effects of adding fly ash into anaerobic digestion process were investigated by evaluating the biomethane production of biowaste with and without ash addition and fertilizing properties of the digestate. The addition of fly ash at specific quantities (2.5–7.5 g of ash per kg) increased the probability that methane production with ash addition was higher than without, while at higher ash doses (10–23.3 g kg^{-1}) the effect for methane production was negative. White mustard (*Sinapis alba* L.) was used as an experimental crop, and the responses followed were seed germination, plant growth, and number of flowers. Potential accumulation of heavy metals and nutrient uptake of the plants were also analyzed. Fly ash addition also enhanced the growth of mustard, with the highest ash addition of 23.3 g kg^{-1} leading to approximately 30 % increase in biomass growth. Germination rate was increased by the fly ash addition from 74 % in the digestate fertilized soil to 82–84 % with ash. Flowering of the mustard plants was affected similarly, where ash addition increased the flowering rate to 59 % compared to 33 % with digestate addition. Analysis of the growing media and the mustard plants showed no significant increase in heavy metal concentrations due to addition of digestate or ash. Fly ash was hence demonstrated to be a viable additive to anaerobic digestion process by increasing biomethane production and benefitting fertilizer quality of the digestate, when used in specific quantities.

1. Introduction

The need for efficient food production is becoming increasingly important as the human population is continuously growing [1]. As increasing amounts of fertilizers are needed to ensure good crop yields, the availability of mined fertilizers is raising concerns. The European Union has listed mined fertilizer materials such as phosphate and phosphate rock as critical raw materials since they are essential to agriculture and their production is restricted only to certain countries [2]. This has brought about the need to develop alternative, recycled fertilizers from e.g. local side streams and wastes.

Anaerobic digestion (AD) is a well-known biological process where microorganisms break down organic material in the absence of oxygen, producing biogas and digestate. Most common applications of AD are found in wastewater treatment plants, and in treatment of side and waste streams from agriculture. Due to restrictions on landfilling, AD is becoming a more popular treatment process for separately collected municipal biowaste in Europe. Recently, there also has been a growing interest on the AD process due to the need of replacing natural gas as a fuel. In addition to producing energy in the form of biogas, AD process preserves the soluble nitrogen; even part of the protein nitrogen is degraded to soluble nitrogen. This results in the residual sludge from AD, i.e. digestate, being rich in nutrients [3,4]. These digestates are still mainly underutilized even though recycled fertilizer products are becoming more interesting due to geopolitical changes.

Addition of fly ash into the anaerobic digestion process has been reported previously [5–7] with several benefits for the AD process. Trace elements present in fly ash, including Fe, Mn, Mo, Cu, Zn, Se, Co, and Ni, can enhance the stability of the AD process and enable an increased loading rate [5,7]. Further positive effects of ash addition have been

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reported, including not only the supply of alkalinity, but also the absorption of inhibitory compounds that can hinder the AD process [6]. The annual global production of fly ash is 0.75–1 billion metric tons with less than 30 % utilization rate [8], underlining the importance of finding alternative uses for fly ash. When fly ash is used in the AD process, virgin raw materials otherwise used as acidity controlling agents are replaced by a recycled alternative.

Digestates have a potential to be used as fertilizers in plant production and have been shown to increase the growth of many plant species [9,10]. There is also an indication that minor addition of some nutrients (P, S, and B) could increase the growth and improve the quality of plants in peat-based growing medium fertilized with digestate [11]. However, Holatko et al. [12] observed that sulfur and biochar as additional amendments did not result in better plant growth although many soil properties were affected. Yet, there is an indication that addition of fly ash to the sewage sludge digestion process can further improve fertilization properties of the digestate [13], but studies using separately collected municipal biowaste as a feedstock are currently lacking.

The objective of this study was to investigate whether different amounts of fly ash added to the AD process affect the biogas production potential, and the responses of plants growing in the digestate fertilized soil. Here, separately collected municipal biowaste was used as a feedstock material in biomethane potential (BMP) experiments followed by digestate used as a fertilizer in a growing trial. This study provides new information on using larger ash amounts to the AD process as well as uses separately collected municipal biowaste as a feedstock, which is a highly relevant feedstock and currently lacking from literature.

2. Materials and methods

2.1. Biomethane potential experiments with ash additions

Fly ash was added into the two separate BMP experiments. The digestates from the first experiment were used for a plant growing trial described in chapter 2.2. For the first experiment, separately collected municipal biowaste from Jyväskylä (Mustankorkea LTD), Finland, was used as a feedstock. The used fly ash was from a combined heat and power (CHP) plant in Kuopio, Finland, using different wood-based materials (56 %) and peat (44 %) as fuel (wood and peat ash: WP).

In the second experiment, separately collected municipal biowaste from Forssa (Envor Group LTD), Finland, was used as a feedstock. Three different types of fly ashes from the CHP plant in Saarijärvi, Finland, were added: ash from peat (P), wood (W), and mixture of wood (75 %) and peat (25 %) (WP).

Plastic bags and other coarse materials (e.g. bones) were sorted out manually before the experiments. Biowaste was then macerated into a paste-like substrate using a knife-mill (Retsch GmbH, Haan, Germany). The mixed material was stored in a freezer (−20 °C) and thawed in a refrigerator (4 °C) prior to the BMP experiments. Fly ashes were stored in a refrigerator (4 °C) prior to the experiments.

Inoculum for the first BMP experiment was obtained from Mustankorkea LTD and for the second experiment from Envor Group LTD biogas plant, both treating separately collected municipal biowaste at a mesophilic temperature (37 °C). Inocula were sieved to remove the coarse material before further treatment.

BMP experiments were carried out in mesophilic (37 °C) conditions using automated testing equipment (Bioprocess Control LTD, Sweden). At least three replicates of 500 mL borosilicate test bottles were used for each sample (four to five bottles in experiment 1 and three bottles in experiment 2) which were mechanically mixed (84 rpm) for 1 min per hour. The biowaste/inoculum volatile solids (VS:VS) ratio and the amount of ash added varied depending on the experiment (Table 1). The added ash amounts were based on previous literature (e.g. Refs. [14–19]) and preliminary experiments, where 3.3, 16.7, 33.3 and 50 g L^{-1} of two different ashes were added (data not shown). Distilled water was added to the bottles after the sample and the inoculum to achieve the desired liquid mass. All bottles were buffered with $NaHCO_3$ (3 g L^{-1}, Merck KGaA, Darmstadt, Germany) and flushed with N_2 to obtain anaerobic conditions. The duration of the experiments was 28 days for experiment 1 and 49 days for experiment 2. The volume of methane was determined with water displacement after CO_2 of the produced biogas was absorbed in a 3M NaOH solution (assuming that volume of other compounds is negligible). Methane yields were converted into normal conditions (0 °C, 101.32 kPa) according to the ideal gas law.

Table 1
Biowaste/inoculum VS:VS (volatile solids) ratios, liquid mass (g) and ash dosage (g/L) used in the two BMP experiments.

Experiment	Biowaste/inoculum VS:VS ratio	Total liquid mass used (g)	Ash dosages (g/L)	Sample abbreviations (Experiment number_Ash type[a]_Ash dosage)
1	0.25	300	0	1_0
			10	1WP10
			16.7	1WP16.7
			23.3	1WP23.3
2	0.34	400	0	2_0
			2.5	2P2.5; 2W2.5, 2WP2.5
			5	2P5; 2W5; 2WP5
			7.5	2P7.5; 2W7.5; 2WP7.5

[a] Ash type abbreviations: W = fly ash from combustion of wood, P = fly ash from combustion of peat, WP = fly ash from combustion of mixture of wood and peat.

2.2. Growing trial

The plant growing trial was carried out between May and June 2022 in a greenhouse at the Department of Biological and Environmental Science of the University of Jyväskylä. The temperature was maintained between 20 and 24 °C, while air humidity was not controlled. The growing period was set for five weeks, and the average daylight duration was approximately 19 h. White mustard (*Sinapis alba* L.), hereafter mustard, was used as an experimental crop, and seeds were provided by Impecta Fröhandel, Julita, Sweden. Seed viability was ensured before the experiments by sowing a test batch of 20 seeds.

A mixture of commercial packed compost mix (Kekkilä Garden, Lapinneva, Finland) and sand (grain size 0–2 mm; Fescon Oy, Hyvinkää, Finland) was used as a low nutrient growing medium, in a dry mass ratio of 20:80 of compost mix and sand. The commercial compost mix from Kekkilä Garden contained peat, clayey sand, wood bark and manure-based compost and it was sieved through a 10 mm mesh before use. The low nutrient growing medium contained approximately 8 % of organic matter, which is typical in Finnish arable soils.

The digestates from the first BMP experiment, with ash dosage of 0, 10, 16.7, and 23.3 g kg_{ww}^{-1} were added as fertilizers to the low nutrient growing medium to prepare the growing media GM0, GM10, GM16.7, and GM23.3, respectively. The growing media were prepared separately by mixing thoroughly 1600 g of low nutrient growing medium and 80 g of each digestate in a plastic bucket. The added amount of digestate was chosen as 5 wt-% in relation to the total wet weight, based on preliminary experiments. A control (C) of the low nutrient growing medium with no fertilizer addition was also prepared and included in the trial.

100 g of the growing medium, or control, was weighted to each plastic pot (area of 55 mm × 55 mm) with holes at the bottom. A piece of paper towel was put at the bottom to prevent growing medium from draining through the holes. After four days of settling time, 15 mustard seeds were sown in each pot at 2 mm depth, with each treatment containing 15 replicate pots (Fig. 1.). The pots of each treatment were placed in their own plastic container (600 mm × 300 mm) with water to prevent potential flow of nutrients between the pots of the different treatments. To maintain optimal development conditions for seeds and plantlets, water was added in containers and a transparent plastic cover was placed over them until seed germination. Irrigation was carried out

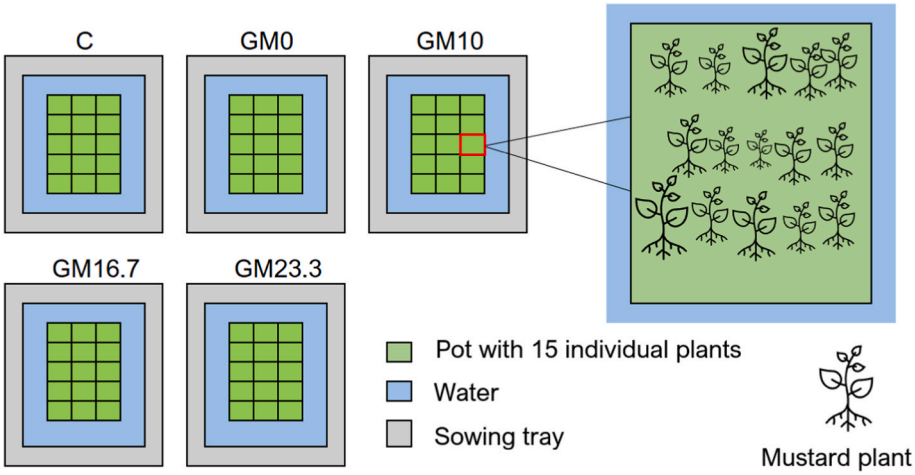

Fig. 1. Layout of the growing trial with five treatments: Control (C) and treatments containing digestate: GM0 (ash 0 g kg^{-1}), GM10 (ash 10.0 g kg^{-1}), GM16.7 (ash 16.7 g kg^{-1}), and GM23.3 (ash 23.3 g kg^{-1}).

by adding water to the containers to ensure constant water supply throughout the growing trials. The pots were randomly placed in their own treatment container and the positions of the pots and containers were rotated regularly.

Germination was recorded at the emergence of plantlets and recounting was done a week later. After five weeks, the total number of individual plants (i.e. germination-%) and the number of flowering plants in each pot were counted and plant shoots were cut right above the soil surface. Plants from each pot were put in individual paper bags and dried at 70 °C overnight and weighted using an analytical balance. Roots of the plants were not collected.

2.3. Chemical analyzes

Total solids (TS) and VS were analyzed from biowaste and inocula for BMP experiments described in standard SFS 3008 [20]. Nitrogen, phosphorus, potassium, pH, total dry mass, and conductivity of digestates and fly ashes were analyzed at the University of Jyväskylä and in a commercial laboratory (Metropolilab, Finland).

Digestates, fly ashes, and mustard plants were analyzed for Al, Ba, Ca, Fe, K, Mg, Mn, Na, P, Si, Ti, As, Cd, Co, Cr, Cu, Ni, Pb, Rb, Sr, V, and Zn with ICP-OES (Avio 500, PerkinElmer) and ICP-MS (NexIon 350D, PerkinElmer) after digestion with microwave oven (CEM Mars6 I-wave). For quality control purposes, all microwave digestion runs included three blank samples and three replicates of selected reference material (Montana Soil NIST SRM 2710A for digestates, Trace Elements in Coal Fly Ash NIST SRM 1633c for fly ashes, and Tomato Leaves NIST SRM 1573a for plants, supplied by the National Institute of Standards and Technology). The digestate samples were ground to ≤0,2 mm particle size prior to digestion, which was based on a method described by Kane and Hall [21]. Fly ash was dried at 105 °C overnight and digested according to procedure described by Perämäki et al. [22]. The dried mustard plants were ground in a mortar to achieve a fine particle size. The digestion procedure for plants included weighing 0.1–0.3 g of the sample to a microwave vessel, addition of 5 ml of nitric acid (67 %, puriss p.a., Sigma Aldrich), 3 ml of ultrapure water (resistivity of 18.2 MΩ cm, PURELAB ultra, Elga LabWater), and 50 μl of hydrofluoric acid (40 %, EMSURE ISO, Merck). After 30 min, the samples were treated in a microwave oven (Mars 6 iWave, CEM) at 200 °C with 20 min ramp time and 10 min hold time.

2.4. Statistical analyzes of BMP experiment results

The daily methane production for each substrate and inoculum alone was estimated using Bayesian hierarchical modelling (e.g. Ref. [23]). The model structure is essentially the same as in Rasi et al. [24], who were the first to propose it to overcome the dilemma of how to account for the uncertainty in the results when there is a need to subtract the methane production of pure inoculum from the methane production of the substrate of interest. It was assumed that the observed amount of methane produced by substrate j on day i in bottle k ($y^{obs}_{i,j,k}$) follows a lognormal distribution common for all bottles k:

$$y^{obs}_{i,j,k} \sim logN\left(\log\left(\mu_{i,j}\right) - 0.5\log\left(c^2_{i,j}+1\right), \log\left(c^2_{i,j}+1\right)\right) \quad (1)$$

where $\mu_{i,j}$ is the expected methane production and $c_{i,j}$ the coefficient of variation of the methane production by substrate j on day i on the real scale. At this point of the calculation, methane production covers total production of the substrate and inoculum.

The expected methane production $\mu_{i,j}$ was given log-normal prior distribution with an expected value of 100 and a coefficient of variation 2 on the real scale. The daily coefficient of variations for methane production, $c_{i,j}$, were assumed to follow hierarchically a Beta($\gamma\eta$, $(1-\gamma)\eta$)-distribution with expected value γ and dispersion parameter η having uniform prior distributions:

$$\gamma \sim U(0.01, 0.5) \quad (2)$$

$$\eta \sim U(0.01, 1000) \quad (3)$$

All prior distributions in the model were chosen to be minimally informative.

A posterior predictive distribution for the daily methane production in a new, unknown bottle of substrate j was estimated by assuming that this new bottle shares the same hierarchical parameters as the observed bottles:

$$y^{pred}_{i,j} \sim logN\left(\log\left(\mu_{i,j}\right) - 0.5\log\left(c^2_{i,j}+1\right), \log\left(c^2_{i,j}+1\right)\right) \quad (4)$$

Predictive cumulative methane production, $y^{predTOT}_{j}$, was calculated by taking a sum of the production over the study period of length *Ndays*:

$$y^{predTOT}_{j} = \sum_{i=1}^{Ndays} y^{pred}_{i,j} \quad (5)$$

This model structure was fitted independently to all datasets of substrate specific methane production. As an exception, the inoculum in experiment 2 was estimated by assuming that the coefficients of variation have common hierarchical parameters with those parameters of inoculum in experiment 1. This change was made since one of the three original bottles of inoculum in experiment 2 was discarded, and thus

Table 2
Posterior predicted methane production with mean and 90 % probability interval, probabilities of higher methane production from samples with ash addition, duration of experiment, and number of bottles.

Sample abbreviations[a]	Posterior predicted production ($NmLCH_4/gVS_{added}$). Mean and 90 % probability interval			Probability that production with ash is higher than without ash addition
Experiment 1				
1_0	429	391	467	NA
1WP10	402	377	428	11 %
1WP16.7	395	363	427	7 %
1WP23.3	379	355	404	1 %
Experiment 2				
2_0	694	638	751	NA
2P2.5	716	639	789	66 %
2P5	715	671	755	70 %
2P7.5	789	724	851	97 %
2WP2.5	735	650	818	76 %
2WP5	789	729	847	98 %
2WP7.5	754	653	842	82 %
2W2.5	809	737	887	98 %
2W5	905	833	972	100 %
2W7.5	823	777	868	100 %

[a] Sample abbreviations: NA = not available, 1 = experiment 1, 2 = experiment 2, W = wood ash, P = peat ash, WP = wood-peat ash, latter numbers specify ash dosages (g kg^{-1}). More detailed abbreviations specified in Table 1.

estimation based on data from two bottles only would have resulted in unrealistically high uncertainty.

Methane production in later part of the experiment was summed together to ease the computation, thus treated similarly as a single day of the experiment. In the experiment 1, this summation was done for days 10–28 and in the experiment 2 for days 14–49, respectively.

In each experiment, the predicted scaled methane production for each substrate without inoculum was calculated by subtracting the predicted inoculum production ($y_I^{predTOT}$) from the predicted substrate production including inoculum ($y_{S,j}^{predTOT}$) and by dividing the result with scaling coefficient (C^{gVS}):

$$R_j = \frac{y_{S,j}^{predTOT} - y_I^{predTOT}}{C^{gVS}} \quad (6)$$

As all estimated quantities are probability distributions expressed as a set of simulated values, the probability that a substrate A produced more methane than substrate B can be calculated by comparing pairwise the simulated values and setting up a vector that gets value 1 when A is larger than B and value 0 when otherwise. The probability can thus be obtained by taking a mean over the resulting vector of zeros and ones.

The model was fitted using MCMC sampling with JAGS 4.3.1 software (Just Another Gibbs Sampler; Plummer 2003) and the R runjags package (Denwood 2016). The model was run for 81,000 iterations with two chains, from both of which the first 1000 simulations was removed as burn-in. Convergence was diagnosed using Gelman-Rubin statistics [25].

2.5. Statistical analyzes of growing trial results

Each pot was regarded as an independent replicate and one-way ANOVA was used to analyze possible differences in plant variables between the treatments. Tukey's test was used for pair-wise comparisons. If the requirements for parametric tests were not met, a square root or logarithm transformation was employed. If the requirements were still not met after the transformations, the Kruskal-Wallis nonparametric test was used instead of ANOVA. A 95 % confidence interval was used in all statistical analyses. Pearson correlations were calculated for the elemental concentrations in the GMs and plant responses: germination, biomass, and flowering. Data processing was conducted using IBM SPSS Statistics (Version 26).

Bioaccumulation factors (BAF) for heavy metals were calculated [26] by dividing the heavy metal concentration in plant (C_{plant}, mg kg^{-1}, dry weight) by the concentration in soil (C_{soil}, mg kg^{-1}, dry weight):

$$BAF = C_{plant}/C_{soil} \quad (7)$$

BAF values higher than 1 were considered to indicate accumulation of heavy metals in plants.

3. Results and discussion

3.1. The effect of ash addition on methane potential of biowaste

From the BMP experiments, posterior predicted methane production was calculated, as well as the probability that methane production with ash addition is higher than without ash (Table 2). In the first BMP experiment with higher ash dosages, all samples with ash additions indicated negative effect on methane production potential compared to biowaste alone: when ash was added the mean of the predicted methane potential varied between 379 and 402 $NmLCH_4/gVS_{added}$ compared to 429 $NmLCH_4/gVS_{added}$ without ash addition. However, the differences were small between the samples especially with 90 % probability intervals. Further, the probability that methane potential with ash addition is greater than with biowaste alone, decreased from 11 % to 1 % with increasing ash dosage.

To find out the effect of lower ash dosage and differences with ash quality, the second experiment was conducted. In this experiment, a clear positive effect of ash addition on methane production potential was found: the mean of the predicted methane potential varied from 715 to 905 $NmLCH_4/gVS_{added}$ compared to 694 $NmLCH_4/gVS_{added}$ without ash addition.

Additionally, the cumulative methane production rate in this study was similar with all ash additions in both experiments compared to the biowaste alone (Supplementary material, Figs. S1 and S2), indicating that the ash addition did not affect the degradation rate. Due to the different biowastes and inocula in each of the two experiments, the experiments are not comparable with each other, only the ash additions in each experiment.

Table 3
Results from previously reported methane production experiments with ash additions: substrate, amount of ash additions, and increase in methane production.

Substrate	Ash addition	Increase in methane production	Ref.
Municipal solid waste	150 mg_{ash}/L	300 %	Guerrero et al. [16]
Municipal solid waste	50 mg_{ash}/L	28–96 %	Montalvo et al. (2016)
Municipal solid waste	25 mg_{ash}/L	200 %	Montalvo et al. [18]
Municipal solid waste	36 g_{ash}/gVS_{added}	350 %	Lo et al. [15]
Municipal wastewater	0.20 g_{ash}/gVS_{added}	2.5 l/kg VS d	Lo et al. [14]
Sewage sludge	<50 mg L^{-1}	70 %	Huiliñir et al. [28]
Winery wastewater	25 mg L^{-1}	18 %	Lauzurique et al. [27]
Winery wastewater	25 mg L^{-1}	75 %	Lauzurique et al. [32]

In previous studies presented in Table 3, the ash additions to methane production experiments have had a positive effect in methane production with ash dosages <150 mg L^{-1}. Although, in AD of winery wastewaters, Lauzurique et al. [27] observed a 20 % decrease in methane production with 100 mg L^{-1} ash dosage. Similarly, ash dosage of >250 mg L^{-1} in AD of sewage sludge has been reported to decrease methane production by up to 11 % [28]. These ash additions can be considered low compared to this study where up to 23.3 g L^{-1} was added. Yet, the methane potentials of all samples in this study were close to previously reported methane potential values from food or biowaste (e.g. Refs. [29–31]), indicating that the effect of fly ash on methane production was relatively low.

Further, the results of this study indicated that the samples with ash from wood combustion had generally higher methane potential compared to samples with ash from peat combustion (Table 2). The mean predicted methane potential of the experiment with wood combustion ash dosage of 5 g L^{-1} (2W5) was greater than the potential of all peat combustion ash additions (2P2.5, 2P5, and 2P7.5) with probability of 98–100 % (Supplementary Material, Table S1). For the smaller wood combustion ash addition of 2.5 g L^{-1} (2W2.5), the probabilities were higher than 92 % when compared to peat combustion ashes at dosage of 2.5 and 5 g L^{-1}(2P2.5 and 2P5), indicating clear difference in favor of wood combustion ashes. When comparing wood ash containing samples 2W2.5 and 2W7.5 with the highest peat ash dosage containing sample 2P7.5, no clear difference in the methane production could be distinguished.

The quality of ash, particularly the amount of trace elements it contains, influences the degradation process. Some trace elements present in ash have been found to be beneficial for the AD process [5–7]. These trace elements are reported to be essential for interspecies electron transfer, which may contribute to the increased stability of the AD process [33,34]. The amount of trace elements in the ashes used in this study varied depending on the material being combusted in the powerplant (Table S2). The added amounts of selected trace elements from different ashes in each experiment ($mg_{trace\ element}$/L) are presented in Fig. 2 (all measured trace elements are presented in Supplementary Material Table S3). The found differences in methane production between wood and peat ash additions are probably due to the different trace element concentrations in the ashes (Fig. 2). For example, the peat ash additions added very little trace elements overall to the process. With the wood and peat mix and wood ash dosages in experiment 2, an increase in especially Zn concentration with increasing ash dosage was observed indicating that Zn concentration could be enhancing the methane production compared to peat ash additions. On the other hand,

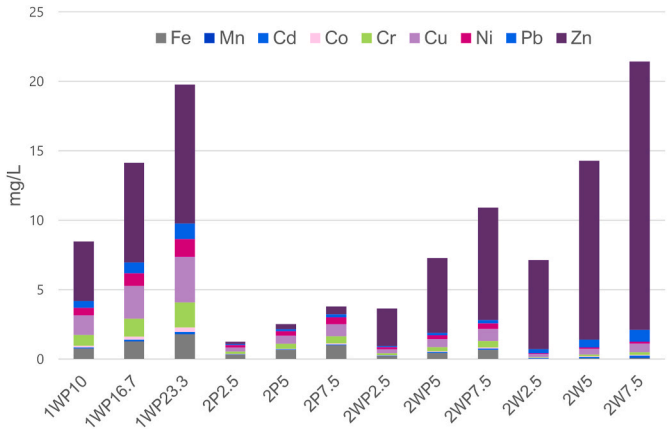

Fig. 2. The added concentrations of selected trace elements ($mg_{trace\ element}$/L) from different ashes in each BMP experiment. Sample abbreviations: 1 = experiment 1, 2 = experiment 2, W = wood ash, P = peat ash, WP = wood-peat ash, latter numbers specify ash dosages (g kg^{-1}). More detailed abbreviations specified in Table 1.

a similar effect cannot be seen in experiment 1, where the total trace element composition, biowaste, and inocula differs from that in experiment 2. Additionally, AD is a very complex process, including many biological reaction stages, such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis [35], each playing an important role in the total process. Microbiological analysis could have clarified the interpretation of the results for the two experiments even more, but the study of microbiological interactions was left out of the scope of this study.

In many cases, when biowaste is used as raw material for AD, some trace element additions are used to improve the degradation process. In this study, the concentrations of some trace elements were higher than trace element additions in the previous studies [31,34]. In addition, the combined effect of different trace elements influences overall process performance. For example, Moestedt et al. [5] concluded, that addition of Co alone can be detrimental for the process, while addition of Co along with Ni had positive effect on process performance. Huiliñir et al. [28] have summarized the concentration ranges of trace elements that are stimulating or inhibiting the anaerobic digestion process (Supplementary Material, Table S4). According to Huiliñir et al. [28] the amount of Cu was above the inhibitory concentration in 1WP23.3 and close to the inhibitory concentration in 1WP16.7, which may explain the lower methane production, although many other trace elements in these samples are in the stimulatory range. For Zn, there is wide variation of concentrations that are reported to be inhibitory; in this study some of those levels were exceeded in 2WP7.5, 2W5 and 2W7.5 but according to the results, the methane production was enhanced. This could also be due to the Fe concentration, which was at a stimulating level in the wood ash additions. When methane production potentials are presented as function of element concentrations added to the BMP experiments (Supplementary Material, Fig. S3), the two experiments differ clearly in methane production potential due to different elemental composition, biowaste, and inocula, but no clear correlations are observed in terms of trace element concentrations. In experiments such as presented here, where many trace elements are present simultaneously, it is difficult or impossible to differentiate the effect of each trace element on the process.

It has also been reported that mixtures of different heavy metals can have antagonistic or synergistic effects on the digestion process. For example, synergy with Ni and Zn is antagonistic [36]. In this experiment, the Ni concentrations were stimulating in all ash additions, but as the Zn concentrations varied, a clear antagonistic effect was not seen. This highlights the fact that the microbial community in the AD process has a huge variety of microorganisms that have different nutrient requirements. The temperature, loading rate, and feedstock influence nutrient needs and it is therefore not possible to recommend any general optimal nutrient concentrations [7]. It should be kept in mind that ash and trace element additions have also long-term effects on the anaerobic digestion process, e.g. the stable methane production [37,38] which is not seen in the BMP experiments.

3.2. Properties of growing media

Adding ash to the AD process can enhance the fertilizing properties of the digestate. For instance, the concentration of essential plant nutrients, such as calcium, has been shown to increase [39].The addition of ash to the digestion process can also affect the acidity of the resulting digestate, and thus the amount of nitrogen. However, it is important to note that ash may contain also harmful heavy metals, the concentration of which should be carefully considered. The prepared growing media were analyzed for chemical properties and selected elements with results presented in Table 4. The addition of digestate was shown to increase the pH of the growing medium (GM) from slightly acidic (5.6) to slightly alkaline (7.9). The amount of ash added did not influence the pH of the GMs, most likely due to the low amounts of ash added. An expected increase in organic matter due to digestate addition was observed in GMs in comparison to the control (~19 % vs 7.8 %). Nitrogen

Table 4
pH, dry matter, organic matter, specific weight, and concentrations of nutrients and harmful heavy metals (mg kg^{-1}, dry weight) in growing media. Abbreviations: control (C) and treatments containing digestate: GM0 (ash 0 g kg^{-1}), GM10 (ash 10.0 g kg^{-1}), GM16.7 (ash 16.7 g kg^{-1}), and GM23.3 (ash 23.3 g kg^{-1}), ND = not determined.

Analysis	Unit	Growing media:				
		C	GM0	GM10	GM16.7	GM23.3
pH		5.6 ± 0.1	7.9 ± 0.1	7.9 ± 0.1	7.9 ± 0.1	7.9 ± 0.1
Dry matter	%	95.28 ± 0.07	87.86 ± 0.12	88 ± 0.2	87.3 ± 0.2	87.1 ± 0.2
Organic matter	%	7.80	19.36	19.16	19	19.1
Specific weight	g/l	ND	640 ± 20	600 ± 20	630 ± 10	630 ± 20
Major nutrients						
N	mg/kg	586	778	795	807	810
P-tot	mg/kg	370 ± 50	450 ± 80	370 ± 20	390 ± 20	410 ± 60
P-sol	mg/kg	45 ± 13	84 ± 1	90 ± 1	103 ± 1	101 ± 1
K	mg/kg	7560 ± 510	8700 ± 550	8730 ± 580	8380 ± 400	8180 ± 220
Minor nutrients						
Ca	mg/kg	6160 ± 820	5770 ± 430	5840 ± 280	5710 ± 110	6030 ± 200
Mg	mg/kg	3020 ± 120	2830 ± 80	3110 ± 250	2990 ± 100	3080 ± 190
Trace nutrients						
Cu	mg/kg	9.1 ± 0.5	8.8 ± 0.8	9.2 ± 1.2	9.9 ± 1.4	10 ± 0.7
Zn	mg/kg	33.6 ± 2.3	33.7 ± 1.9	36.2 ± 0.6	36 ± 1.8	36 ± 1.7
Harmful heavy metals						
As	mg/kg	6.6 ± 0.3	1.7 ± 0.2	1.8 ± 0.3	1.6 ± 0.2	1.9 ± 0.2
Cd	mg/kg	<0.1	<0.1	<0.1	<0.1	<0.1
Cr	mg/kg	10.6 ± 0.6	10.9 ± 1.2	9.9 ± 1.9	9.6 ± 0.4	10 ± 0.8
Ni	mg/kg	3.9 ± 0.3	3.9 ± 0.3	4.4 ± 0.5	4.2 ± 0.2	4.4 ± 0.5
Pb	mg/kg	4.3 ± 0.1	6.4 ± 0.8	6.6 ± 0.6	6.3 ± 0.1	6.4 ± 0.4

concentration was also affected by the addition of the digestate, and 200–300 mg kg^{-1} more N was found in GMs compared to the control. Similarly, the concentration of K, soluble P and trace nutrient Zn increased due to the digestate addition.

Minor nutrients Ca and Mg were, however, found at similar or lower concentrations in the GMs compared to the control. There were no major differences in the concentrations of Cr, Cu, and Ni between the control and GMs, and Cd concentration was below the limit of detection in all samples. Interestingly, As concentration was lower in GMs compared to the control, which could be due to the lower pH of the control.

The safe use of fertilizers is ensured by European Union legislation Regulation [40] as well as national legislation. In Finland, the law on fertilizers [41] governs fertilizer use, and the safe levels of heavy metals have been established in a decree by the Ministry of Agriculture and Forestry[42]. For growing media, the maximum legal limits for harmful element are As 10, Hg 0.5, Cd 1.0, Cr 200, Cu 150, Pb 50, Ni 50, and Zn 300 mg kg^{-1}, which are not exceeded in any of our GMs. It should be noted that Hg was not analyzed since it's volatile and is not present in ashes formed in high temperature combustion processes.

Although this one-time addition of fly ash amended digestates showed no clear indication of heavy metal contamination in GMs, the possibility of long-term use resulting in ground water contamination and heavy metal accumulation should be considered in future studies to ensure the safe use of fly ash amended digestates. Additionally, the soil biological quality (soil microbiota and enzymatic activity) was not evaluated in this study but would be a valuable research focus in the future.

3.3. Germination, biomass, and flowering in growing trial

The highest germination rate of mustard seeds was found in the control, where 96 % of the seeds sprouted (Fig. 3A). In other treatments, germination rate varied between 74 and 84 %. Digestates with ash addition (GM10, GM16.7, GM23.3) induced better germination than the digestate without ash (GM0) (see test statistics: ANOVA, Krusal-Wallis, pair-wise comparison in Supplementary Material Tables S5 and S6). The higher variation observed in the growing media with digestate additions compared to the control was most likely due to a more heterogenous nature of the growing media. The lower germination rate in the growing media with digestate additions compared to control can be caused by the higher nutrient levels, which has been shown to decrease seed germination [43,44]. Indeed, negative Pearson correlation was found between N concentration and germination with 99 % confidence interval (Supplementary Material, Table S7). It can be concluded that addition of ash slightly improved seed germination when the digestate itself impaired it. Bauer et al. [13] received similar results on germination of bean seeds when they added fly ash to the digestion process using sewage sludge. In general, materials (e.g. compost or biochar) that are added to the growing medium fertilized with digestate tend to affect crop germination, either positively or negatively (see Ref. [10]).

All digestate additions increased the mustard biomass compared to the control (Fig. 3B), where the higher concentration of N in GMs could be a factor, with 586 mg kg^{-1} in control and 778–810 mg kg^{-1} in GMs (Table 4). Mustard biomass was also observed to increase with increasing ash content, which is most likely due to other factors than increase in N concentration, since this was minor between different ash additions. Similar responses were also found by Bauer et al. [13]: the highest amount of ash added to the digestion process induced the highest bean biomass. This was concluded to be due to the higher pH of the growing medium that tended to increase the availability of phosphorus from sewage sludge [13]. In our study, pH of the growing medium did not increase with increasing amount of ash added to the AD process. Hence, the increase in plant growth was most likely due to availability of nutrients mediated by other factors than pH.

At the end of the experiment, most flowers were observed in the control, where 87 % of the individual plants were flowering; Fig. 3C. Growing media fertilized using digestates with higher ash additions (GM16.7 and GM23.3) had better flowering success compared to the digestate without ash (GM0): i.e. the more ash in the digestate, the higher level of reproduction in mustard. Differences between the growing media fertilized with digestates may be attributed to the nutrients brought in the ash. However, mustards in all digestate containing treatments flowered less well than in the control medium. Plants in the digestate treatments may have allocated more resources for growth than for reproduction. In sum, digestate reduced or retarded the flowering of the plants, but the addition of ash to the digestate slightly promoted it. It has previously been shown that when fly ash was applied to fields, increases in the yields - and often also in the seed production – were found in many agricultural crops [45]. Based on our results, it also seems that adding fly ash already into the AD process and using digestate as a fertilizer has at least somewhat the same effect.

Although addition of fly ash amended digestate shows beneficial effects on the germination, biomass growth, and flowering compared to digestate alone, the use of this type of fertilizer could face some regulatory challenges since fertilizer use is strictly regulated both at EU and national level. Additionally, a requirement for quality certification prior to widespread use would most likely be needed. This study provides a good starting point for future development of fly ash addition into the AD process.

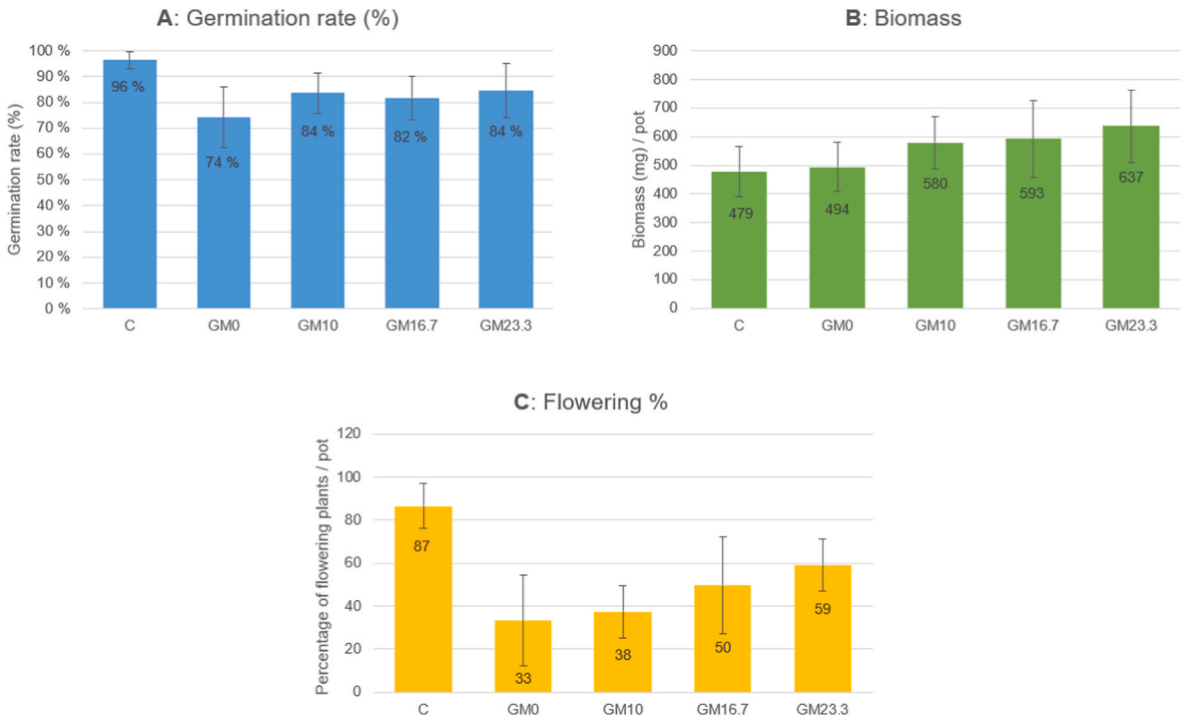

Fig. 3. A–C: Averages and standard deviations of mustard plants' **A:** germination rate (%), **B:** biomass (mg) per pot, **C:** percentage of flowering plants per pot. For each treatment, sample size was 15 pots (n = 15). Abbreviations: control (C) and treatments containing digestate: GM0 (ash 0 g kg^{-1}), GM10 (ash 10.0 g kg^{-1}), GM16.7 (ash 16.7 g kg^{-1}), and GM23.3 (ash 23.3 g kg^{-1}). Statistical tests (ANOVA, Krusal-Wallis, pair-wise comparison) are presented in Supplementary Material Tables S5 and S6.

3.4. Accumulation of nutrients and heavy metals in plants

Heavy metals and nutrients were analyzed from the mustard plants (roots excluded) at the end of the growing trial, and it was found that addition of digestate to the growing media increased the nitrogen concentration both in the growing media and in the plants when compared to control (Tables 4 and 5, statistical tests presented in Supplementary Material Tables S8 and 9). Additionally, concentration of K increased slightly in the plants when they were growing in fertilized GMs, while no clear differences were observed for P concentrations in plants. In future studies, root uptake and rhizosphere accumulation should also be taken into account, which were left out of the scope of this study.

Risks associated with the use of ash as a fertilizer in agriculture include heavy metals that may accumulate in plants from the soil [46]. Indeed, the accumulation of metals in plants is a common phenomenon [47], and fly ash contains heavy metals, such as Fe, Co, Ni, Cr, Pb, and Cd [48]. To assess the possibility of heavy metal accumulation, bioaccumulation factors (BAF) were calculated (Supplementary Material Table S10) showing no accumulation of heavy metals in plants expect for Zn in the control. Indeed, there were clearly higher concentrations of Zn in the plants grown in the control compared to the plants grown in the digestate fertilized growing media (Table 5). It is likely that the availability of Zn was higher in control due to its low pH, leading to elevated Zn concentrations in the plants. Basu et al. [45] have shown in field experiments that the high pH of ash can prevent heavy metals from accumulating in plant tissues or reduce the availability of heavy metals. For example, Cd does not accumulate in plants from alkaline soils but remains in the soil, unlike under acidic conditions [46]. In our study, concentrations of Cd were found to be below the limit of detection (0.02 mg kg^{-1}), and also below the maximum limit of 0.1 mg kg^{-1} (wet weight) allowed for edible leaf vegetables governed by the EU legislation [49]. For Pb, the permissible maximum limit in leaf vegetables is 0.3 mg kg^{-1} in wet weight, which also was not exceeded in any of the plants (Table 5). The analyzes indicated that neither ash nor the

Table 5
Average elemental concentration ± standard deviation in mustard plants (dry weight, n = 3). Abbreviations: control (C) and treatments containing digestate: GM0 (ash 0 g kg^{-1}), GM10 (ash 10.0 g kg^{-1}), GM16.7 (ash 16.7 g kg^{-1}), and GM23.3 (ash 23.3 g kg^{-1}).

Analysis	Unit	Plants from treatments:				
		C	GM0	GM10	GM16.7	GM23.3
C	wt-%	36.1 ± 3.4	41 ± 0.5	40.8 ± 0.6	40.2 ± 0.8	41 ± 1.2
Major nutrients						
N	wt-%	1.7 ± 0.2	3.2 ± 0.7	2.8 ± 0.5	2.7 ± 0.3	3.1 ± 0.3
P	wt-%	0.6 ± 0.06	0.63 ± 0.05	0.57 ± 0.04	0.57 ± 0.05	0.6 ± 0.05
K	wt-%	2.12 ± 0.18	2.41 ± 0.18	2.56 ± 0.22	2.49 ± 0.25	2.35 ± 0.25
Minor nutrients						
Ca	wt-%	2.04 ± 0.24	0.79 ± 0.14	0.85 ± 0.09	1.04 ± 0.25	0.96 ± 0.14
Mg	wt-%	0.64 ± 0.07	0.33 ± 0.04	0.34 ± 0.03	0.37 ± 0.04	0.36 ± 0.03
S	wt-%	0.8 ± 0.11	0.71 ± 0.07	0.7 ± 0.08	0.71 ± 0.08	0.61 ± 0.07
Trace nutrients						
Cu	mg/kg	5.62 ± 0.9	5.38 ± 0.88	5.24 ± 0.59	5.64 ± 0.88	5.01 ± 0.58
Zn	mg/kg	57.63 ± 6.75	31.12 ± 5.55	35.43 ± 4.53	35.11 ± 3.79	32.21 ± 2.64
Harmful heavy metals						
As	mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Cd	mg/kg	<0.02	<0.02	<0.02	<0.02	<0.02
Cr	mg/kg	3.04 ± 1.04	2.8 ± 1.28	2.6 ± 0.46	4.77 ± 2.1	4.18 ± 1.61
Ni	mg/kg	1.28 ± 0.61	0.83 ± 0.5	1.63 ± 0.34	2.61 ± 0.86	2.4 ± 1.48
Pb	mg/kg	0.35 ± 0.2	0.16 ± 0.1	0.13 ± 0.08	0.32 ± 0.24	0.21 ± 0.16

digestate significantly increased the heavy metal concentrations in the plants.

4. Conclusions

Fly ash can be used as an additive in anaerobic digestion to increase methane production and improve the quality of the digestate as a fertilizer, but too much ash can have the opposite effect. In this study, the fly ash dosage of 2.5–7.5 g L^{-1} had positive effect on methane production, while with dosage of 10–23.3 g L^{-1} the effect was negative although the overall results were within the range of previously reported biomethane potentials. It was also found that the raw material of the combustion plant has an impact on the ash quality and further on the methane production, with wood fly ash being more favorable than peat fly ash, most likely due to the different trace element concentrations in the ashes. The effect of adding trace nutrients in the form of ash has a complex effect on the AD process and is very case specific, depending on the type of ash and the raw material for AD, as well as the microbial community.

In the growing trial, the ash addition increased the growth of plants most likely by increasing the nutrient content of the growing media. However, germination and flowering decreased in all growing media containing digestate but increased with ash addition. It is likely that the plants with digestate addition allocated more resources to growth than to reproduction. Heavy metal concentrations in the plants were similar between different digestate and ash additions. Zn concentration was, however, clearly higher in the plants grown in the control compared to the plants grown in the growing media with digestate additions. This was likely due to the lower pH of the control.

Fly ash addition into AD process can have beneficial effects on biomethane production when the dosage is appropriate. Ashes are widely available and can also be used to adjust the alkalinity of the AD process. The digestate from the AD process can be used as a fertilizer as shown here for mustard plants, with increasing plant growth and beneficial effect on the availability of some elements. In addition, fly ash addition to the AD process did not lead to heavy metal accumulation in plants in this study.

CRediT authorship contribution statement

Siiri Perämäki: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition, Conceptualization. **Jari Haimi:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Saana Kataja-aho:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Tuuli Kuumola:** Writing – original draft, Investigation, Formal analysis, Data curation. **Pirje Palk:** Writing – original draft, Investigation, Formal analysis, Data curation. **Sylva Larsson:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Markku Vainio:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Henni Pulkkinen:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Saija Rasi:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Funding acquisition, Conceptualization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biombioe.2025.108259.

Data availability

Data regarding the anaerobic digestion experiments is available via the IDA service. Other data used in the study is available upon request.

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