Behavior of trace elements and micronutrients in manure digestate during ozone treatment

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Abstract

Digestate treatment techniques have recently been proposed as a strategy to increase the ultimate biogas yield from dairy manure and to improve the digestate quality as an organic fertilizer. These studies however rarely take the trace elements (TE) and nutrient partitioning into account. This study focusses on ozone treatment (5 – 40 g O₃ kg⁻¹ Total Solids (TS)) as a digestate treatment technique to control the concentration of TE and nutrients in the liquid phase of the digestate. Controlling the TE and nutrient concentrations in the liquid and solid digestate can improve the agronomic value of dairy manure digestate. The ozone concentration of the gas stream entering reactor was 48.53 g O₃/Nm³ or 3.4% w/w O₃ in O₂-gas. The experiments were repeated using pure oxygen gas to investigate its influence. The results from ozonation and oxygenation of the dairy manure digestates revealed that O₃ treatment up to 40 g O₃ kg⁻¹ TS did not have a more pronounced effect on the biochemical parameters compared to supplementation of pure O₂. Ozonation of the digestate and the supernatant showed that the TE concentration in the liquid phase followed a parabolic profile. The observed initial increase in this parabolic profile was explained by the release of TE from the organic matter to the supernatant causing an increase in TE concentration, followed by a decrease due to precipitation of TE as hydroxides and sulfides, due to the increasing pH and sulfur concentrations.

Keywords: Digestate disintegration, ozone, trace elements, nutrients, digestate quality

1. Introduction

Organic fertilizers contain carbon and nutrients that originate solely from a biological origin, excluding fossilized organic materials (European Commission, 2016). Organic fertilizers, derived from manure can replace inorganic ammonium nitrate fertilizer or nitrogen – phosphorus – potassium (NPK) fertilizers by increasing the soil organic carbon content and soil productivity more efficiently than inorganic fertilizers (Sun et al., 2019). However, the direct spread of manure onto soils has been shown to contribute to greenhouse gas emissions and increase the chance of agricultural runoffs that pose eutrophication risks in water bodies. Several manure management options were introduced to replace the traditional direct application of manure as a fertilizer. Currently, biological conversion (i.e. composting, anaerobic digestion (AD)) and thermochemical conversion processes (i.e. pyrolysis) have been used to reduce the organic content of manure slurries and increase their potential for land applications (Font-Palma, 2019; Möller and Müller, 2012).

AD is a sustainable process that produces digestate which can be safely used as a fertilizer. However, digestates usually contain high organic content due to the rate-limiting step of hydrolysis (Azman et al., 2015). Therefore, digestate treatments (e.g. ozonation, ultrasonication) are recently used to valorize the residual organic content of the digestates and change their physicochemical properties (Lindner et al., 2015). Ozone treatment is a promising technique that solubilizes particles and inactivates biomass (Cesaro et al., 2019; Isazadeh et al., 2014). Ozone causes oxidation of organic molecules by two distinct pathways: i) a direct reaction pathway at low pH (<4) which is relatively slow and selective and ii) an indirect reaction pathway at high pH (>7) by hydroxyl radicals which is relatively fast with lower selectivity (Alaton et al., 2002; Hoigné, 1997; Prado et al., 1994). Hydroxyl radicals can oxidize organic matter but may react with carbonate (CO_3^{2-}) and HCO_3^- to produce $\cdot\text{CO}_3^-$ radicals (Crittenden et al., 1999; Weeks and Rabani, 1966). Therefore CO_3^{2-} and HCO_3^- ions eliminate the indirect pathway by radical scavenging under non-acidic conditions (Chiang et al., 2006). Ozone treatment of digestate has been mainly investigated for increasing the biodegradability of digestates and the effect of ozone treatment on the physicochemical properties of digestate is usually overlooked. For example, Cesaro et al. (2019) showed that ozone treatment on digestate was a more efficient disintegration technique in terms of COD release when applied to a biologically more active digestate (i.e. with lower hydraulic retention time; HRT) compared to a more matured digestate in which no COD disintegration was found (Cesaro et al., 2019). On the other hand, Somers et al. (2018) observed that for the digestate of HRT of 17 days and a pH of 8.0, ozone treatment by $30 \text{ gO}_3 \text{ kg}^{-1} \text{ TS}$ did not yield significant COD-solubilization or improve the biogas production (Somers et al., 2018). In this respect, the effect of ozone treatment on physico-chemical properties of digestates is a still remarkable research question that needs to be answered.

TE and nutrients are valuable components of manure digestate. The composition and the level of TE in manure digestate can vary with the input material, seasonal and geographical changes (Kupper et al., 2014) but the main source of TE and nutrients are derived from animal feed as they are used as growth supplements (Bolan et al., 2004; Upreti et al., 2009). These TE can be transferred to the soil and via crops further through the food chain. For example, applying cattle manure to soils can increase the final manganese and zinc concentrations in corn (Bolan et al., 2004). Hence, utilization of organic fertilizers is regulated by European legislation, which dictates that cadmium, hexavalent chromium, mercury, nickel and lead in organic fertilizers should not exceed a concentration of $1.5 \text{ mg kg}^{-1} \text{ TS}$, $2 \text{ mg kg}^{-1} \text{ TS}$, $1 \text{ mg kg}^{-1} \text{ TS}$, $50 \text{ mg kg}^{-1} \text{ TS}$, and $120 \text{ mg kg}^{-1} \text{ TS}$, respectively (European Commission,

2016). For this reason, the fate and behavior of TE and nutrients for each manure management practice should be evaluated.

The speciation of the TE and nutrients in the liquid or solid phase of manure digestate is affected by the total metal concentration, pH, the redox potential, complexation with organic substances, adsorption and precipitation processes (Möller and Müller, 2012). These processes make the metal speciation a complex system that can be difficult to predict. Only a few studies investigated the turnover of TE and nutrients such as calcium, magnesium in digestates (Massé et al., 2007; Möller and Müller, 2012). Insight into the speciation of TE and micronutrients after digestate treatment will allow for the implementation of these treatments to produce more tailor-made organic fertilizers. Digestate treatment strategies can increase the agronomic value of manure digestate by matching the TE concentration more closely to the nutrient requirements of crops (Massé et al., 2007). In this respect, ozonation can oxidize inorganic compounds. For example, ozonation of Fe^{2+} in water can result in the precipitation of $\text{Fe}(\text{OH})_3$ and Mn^{2+} can precipitate as $\text{MnO}(\text{OH})_2$. Arsenite (As^{3+}) can be oxidized to arsenate (As^{5+}) and H_2S and S^{2-} can be oxidized to SO_4^{2-} (Kim and Nriagu, 2000; Radjenovic et al., 2008; Reckhow et al., 1991). However, the effect of ozone on TE speciation in digestates is not extensively studied due to the different complex processes that occur within the digestate matrix.

The aim of this study is to investigate the dynamics of TE and micronutrients during ozone digestate treatment and to determine the effect of ozone on the biochemical parameters. In this scope, ozone treatment was applied to the digestate and its supernatant. The TE and micronutrient concentrations in the liquid phase were monitored before and after ozonation. The effect of oxygen gas in the $\text{O}_3 - \text{O}_2$ gas mixture was also investigated by oxygenating the digestate since oxidation might occur from both ozone and oxygen gas.

2. Materials and Methods

2.1 Digestate characteristics

The dairy manure digestate used in the ozonation experiments was collected from a farm-scale (100 m^3) digester in Rumst, Belgium which was fed daily with dairy manure. The anaerobic digester was operated at 37°C, with a hydraulic retention time (HRT) between 15 and 17 days (Table 1).

Table 1: Initial parameters of the dairy manure digestate used during the ozonation experiments. Footnotes represent expected concentration ranges in manure digestate.

Parameter	Unit	This study
pH	-	7.7 (± 0.0)
TS	%	7.1 (± 0.1)
VS	%	5.3 (± 0.1)
tCOD	g L ⁻¹	36.7 (± 0.3)
sCOD	g L ⁻¹	15.1 (± 0.6)
TAN	g-N L ⁻¹	2.3 (± 0.0)
Alkalinity	g-HCO ₃ ⁻ L ⁻¹	8.2 (± 0.2)
Al	g kg ⁻¹ TS	1.9 (± 0.1)
As	mg kg ⁻¹ TS	4.7 (± 0.6)
Ca	g kg ⁻¹ TS	20.6 (± 1.5)
Cd ¹	mg kg ⁻¹ TS	0.6 (± 0.0)
Co	mg kg ⁻¹ TS	1.4 (± 0.1)
Cu ²	mg kg ⁻¹ TS	51.2 (± 2.4)
Fe	mg kg ⁻¹ TS	1448.8 (± 71.3)
K	g kg ⁻¹ TS	47.1 (± 2.3)
Mg	g kg ⁻¹ TS	13.9 (± 1.3)
Mo	mg kg ⁻¹ TS	7.3 (± 0.4)
Na	g kg ⁻¹ TS	12.0 (± 0.9)
Ni	mg kg ⁻¹ TS	3.9 (± 0.1)
P	g kg ⁻¹ TS	11.6 (± 0.8)
Pb ³	mg kg ⁻¹ TS	7.2 (± 0.5)
Se	mg kg ⁻¹ TS	1.6 (± 0.2)
V ⁴	mg kg ⁻¹ TS	3.6 (± 0.1)
Zn ⁵	mg kg ⁻¹ TS	275.3 (± 24.0)

1 Expected concentration range in digestate is 0.2-0.3 mg kg⁻¹ TS (McBride et al., 2001, Bolan et al., 2004)

2 Expected concentration range in digestate is 21-139 mg kg⁻¹ TS (McBride et al., 2001, Bolan et al., 2004)

3 Expected concentration range in digestate is 2.1-4.1 mg kg⁻¹ TS (Sager 2007, Bolan et al., 2004)

4 Expected concentration range in digestate is 4.7-6.8 mg kg⁻¹ TS (Chen et al., 2005, McBride et al., 2001)

5 Expected concentration range in digestate is 86-191 mg kg⁻¹ TS (McBride et al., 2001, Bolan et al., 2004)

Manure digestate for the bubble size measurement was taken from a 140 m³ digester (HRT 23 to 47 days) in Temse, Belgium which was fed daily with dairy manure. The digestate from this digester had a TS content of 5.82 ± 0.02% and a Volatile Solid (VS) content of 3.88 ± 0.02%.

2.2 Ozonation and oxygenation experiment

The ozonation and oxygenation experiments were conducted under constant magnetic stirring (200 rpm, 2 mag AG, München, Germany), in a lab-scale glass reactor with an active volume of 600 mL. The glass reactor was connected by flexible plastic tubing to an ozone generator (Anseros, Tübingen, Germany). This generator was fed with pure oxygen gas (99.995%, Air Liquide, Schelle, Belgium) at a flow rate of 75 L h⁻¹. The ozone concentration of the gas stream entering the reactor was 48.53 g O₃ Nm⁻³ or 3.4% w/w O₃ in O₂-gas. The ozone off-gas was monitored (M465, Teledyne, San Diego, CA, USA) and antifoam (Silicon oil, Buchi, Flawil, Switzerland) was added at a concentration of 80 µL kg⁻¹ digestate, to prevent foaming during the experiments. It should be noted that silicon oil can be degraded to silicon oxides (SiOx) under the influence of ozone. Furthermore, UV/ozone treatment can remove the carbon of silicon oil, which thus results in a final elemental composition closer to SiO₂ (Ouyang et al., 2000).

The experiments were divided into three parts. The first part of the experiments (part I) consisted of ozonation experiments (5 – 40 g O₃ kg⁻¹ TS; triplicate) on the digestate (i.e. both solid and liquid phase). In part II, the influence of O₃ in the liquid phase, i.e. the supernatant, was investigated. The comparison between the results of part I and part II allowed to indicate the influence and role of the solid particles during digestate ozonation. For this purpose, the digestate was centrifuged (10 min at 26,200 g) and roughly filtered, and O₃ was subsequently applied (0.4 – 2.8 g O₃ L⁻¹) to obtain equal dosages as in part I. Due to the limited available volume of the supernatant, the ozonation was conducted once and analyzed in triplicate. In the last part, Part III, the influence of oxygen gas present in the O₃ – O₂ mixture was investigated. This was simulated by applying pure O₂ gas to the digestate. The dosages were expressed in oxygenation time, i.e. the time necessary to apply the ozone in part I (10.3 to 122.6 min; triplicate). Since the dosages and reaction times of part I, part II and part III are linearly correlated by design (Fig A.1 and Fig A.2 can be found in the supporting information), they can be expressed and discussed in terms of treatment levels (Table 2).

Table 2: Relationship between the treatment doses of the three experimental parts.

Treatment Level (-)		0	1	2	4	6	8
Part I	Mass ozone (g O ₃ kg ⁻¹ TS)	0	5	10	20	30	40
Part II	Concentration ozone (g O ₃ L ⁻¹)	0	0.4	0.7	1.4	2.1	2.8
Part III	Oxygenation time (min)	0	10.3	26.7	52.7	77.3	112.7

2.3 Biochemical parameters

pH, TS and VS were measured according to standard methods (APHA, 2005). Total alkalinity (titration until pH 4.3, expressed as g HCO_3^- L⁻¹) of the uncentrifuged sample was measured by titration with 0.5 M HCl. Soluble chemical oxygen demand (sCOD) and total chemical oxygen demand (tCOD) and supernatant COD were measured with Hach Lange (Mechelen, Belgium) test kits (LCK-014). Total ammonia nitrogen (TAN) was measured with Hach Lange test kits (LCK-302). Soluble fractions for sCOD and TAN measurement were obtained in four steps: centrifugation (10 min at 26,200 g), five times dilution of the supernatants, second centrifugation (10 min at 26,200 g) and filtration through a 0.60 μm filter paper (Macherey-Nagel, Düren). The second centrifugation on the diluted sample was necessary to prevent clogging on the filter paper.

Acetic acid was measured on the uncentrifuged sample by GC-FID equipped with an HPFFAP column (Agilent, Santa Clara, CA, USA). The column had a length of 30 m with an internal diameter of 0.25 mm and a film thickness of 0.25 μm . Helium was used as a carrier gas. The initial oven temperature was held at 45°C for 1 minute, which was heated up with an increment of 10°C min⁻¹ until 200°C. The split ratio was 30:1. The GC was equipped with a flame ionization detector (FID) operating at a temperature of 240°C. Prior to GC-analysis, acetic acid was extracted with diethyl ether (Sigma Aldrich, Saint Louis, MO, USA). 2-methyl valeric acid (Acros Organics, Geel, Belgium) was used as an internal standard.

2.4 Bubble size measurement

Dairy manure digestate (origin: Temse, Belgium) was used and the same set-up as during part I and part II (which used a different digestate with origin Rumst, Belgium) were repeated. The statistical distribution of the sizes of the ozone bubbles in the liquid of the reaction vessel was measured with a semi-automatic camera-based approach. A Canon (Tokyo, Japan) 7D single-lens reflex (SLR) camera, equipped with a Canon EF 100mm Macro f/2.5L IS USM lens was placed at approx. 30 cm distance of the reaction vessel. Automatic exposure was applied, yielding a shutter speed in the range of 1/80 to 1/256 seconds, and an aperture number of F4. 15. Images were taken at a resolution of 4.48 MP which were, approx. equally distributed between the liquid and the viscous experiment. Illustrative images can be found in the supplementary files. In order to measure these effects numerically, an image processing script was written, enabling to manually annotate every bubble in the images. A calibration image was taken under the same conditions in order to convert the annotated bubble diameters to metric dimensions. The average rising angle of the bubble stream was also measured from those images. Normal distribution test was done by the Kolmogorov-Simonov test. The Mann-Whitney test was used to determine if the difference in numeric mean bubble diameter were significant ($p < 0.05$). It has to be noted that, for this measurement, the aeration pebble was placed close to the glass reactor wall to facilitate the measurement during parts I, II and III.

2.5 Elemental analysis

The following elements were analyzed: alkali metals: K, Li, and Na, alkali-earth metals: Be, Ca, Mg and Sr, Transition metals: Co, Cr, Cu, Fe, Mn, Mo, Ni, Ti, V and W, Poor metals: Al, Cd, Hg and Pb and non-metals: S, P, As and Se. The data of TE that was not used in the discussion, can be found in the supplemental files.

Prior to the elemental analysis, the samples were treated as follows: solid samples were obtained after centrifugation (10 min at 26,200 g) of the digestate and one washing step with demineralized water, followed by a second centrifugation (10 min at 26,200 g) and filtration through a 0.60 μm filter paper

(Macherey-Nagel, Düren). The solid samples were dried in the oven at 105 °C and stored in falcon tubes for analyses. Digestion of the samples was performed in a microwave oven (Multiwave Go, Anton Paar, Graz, Austria), using an acid mixture of 5.8M HF (40%, trace metal grade, Fisher, Geel, Belgium) and 3.66M HNO₃ (65%, Fisher, trace metal grade) and 1.14M HCl (30%, Fisher, trace metal grade). A temperature-controlled heating program was used: ramp to 180°C during 10 min and a 10 min hold time at 180°C. 30 mL of 4% H₃BO₃ was added and the sample was heated at 120°C (ramp time 10 min, hold time 5 min) to neutralize the excess HF. After digestion, samples were stored in acid cleaned polyethylene storage bottles (60 mL, Nalgene).

Liquid phase samples were obtained by centrifugation (10 min at 26,200 g), dilution of the supernatant, a second centrifugation (10 min at 26,200 g) and filtration through a 0.60 µm filter paper (Macherey-Nagel, Düren, Germany). Pretreatment of the liquid phase was conducted in a microwave oven (Multiwave Go, Anton Paar, Graz, Austria). 6 mL filtrate was transferred to the digestion vials and 2 mL HNO₃ (65%, Fisher, trace metal grade) was added and the sample heated to 180°C (ramp time 10 min, hold 10 min). After digestion, 10 mL deionized water (Milli-Q, Millipore Advantage) was added.

Inductively coupled plasma sector field mass spectrometry (ICP-SF-MS, Thermo Element II, Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the concentrations of TE. Indium was added as an internal standard (final concentration 1 µg L⁻¹). Quantification of trace metal concentrations was based upon calibration curves prepared from multi-element (Merck, Multi-element standards XIII, VIII) or single element (Johnson Matthey Specpure) standard solutions. Blanks and standard reference materials were included in each digestion batch to verify the accuracy of the extraction method. For the analysis of the solids, the standard reference material MESS3 (river clay sediment) was used. For the liquid phase, the reference samples SPS-SW1, SW2 and NIST 1640a were used as quality control samples. All elements analyzed were within the specified ranges with recoveries ranging from 95-105%.

2.6 Statistical methods and calculations

Relative changes were expressed in percentages and calculated as follows:

$$\text{Change (\%)} = 100 * (C_E - C_0) / C_0$$

(Eq 1.)

Where C₀ and C_E are the initial concentration and the concentration after treatment of a given parameter.

One Way Analysis of variance (ANOVA) was used to calculate if ozone or oxygenation had statistically significant effect on the analyzed parameters. Statistical significance was established at the $p < 0.05$ level. Principal Component Analysis (PCA) and the varimax rotation were used for interpretation of the biochemical data. PCA decomposes the dataset into a smaller number of orthogonal, principal components. In this way, it can be examined how many components are necessary to describe the data and which variables are correlated. Varimax maximizes the variance of loadings in each component. Hence, one variable that will load high on the first dimension (dim) will not load high on the second (Kaiser, 1958). Varimax rotation was performed in R© version 3.5.2 (2018-12-20) using package FactoMineR (Lê et al., 2008).

3. Results and discussion

3.1 Biochemical parameters

The effect of ozone on the digestate was investigated in three parts. In Part I, the digestate was ozonated while in part II only the supernatant of the digestate was ozonated. The supernatant was ozonated in part II to study the effect of the solid particles in digestate on the ozonation. Part III investigated the effect of the oxygen gas in the O₃/O₂ gas mixture (3.4% w/w ozone). This part followed the same design of part I but instead of ozone, pure O₂ gas was supplied to the digestate.

During Part I, ozone had a limited effect on the measured biochemical parameters, which can be due to the radical scavenging effect of HCO₃⁻ and CO₃²⁻ ions. There was no effect on sCOD and tCOD, which decreased by 1.3% and 6.4% ($p = 0.206$ and 0.086), respectively (Fig. 1). Similarly, during part III, no significant increase in sCOD was observed (4.3%, $p = 0.490$). These results indicated that the solubilization of organic material in digestate by ozonation was unlikely or very limited (1.3%). In Part II, the supernatant COD was measured to quantify the loss of total COD due to ozonation. The supernatant COD decreased significantly by 23.4% ($p = 0.000$) which was attributed to the clogging and adhesion of particulate matter to the aeration pebble. These results were supported by PCA analysis which indicates that the sCOD was not related to the treatment in both parts I and III (Fig. 2). These results are similar to previous experiments (Somers et al., 2018).

The pH increased in all parts, from 7.7 to 8.1 in part I ($p = 0.000$), from 8.1 to 8.4 in part II and from 7.5 to 8.2 in part III ($p = 0.000$). The higher initial pH in part II, compared to parts I and III, is probably the result of the supernatant preparation by centrifugation and filtration. From these results, it is clear that introducing a gas stream in the digestate matrix causes the pH to rise, regardless of the gas contains ozone or pure oxygen gas. This is supported by the PCA analysis that shows that the pH-vector is closely aligned to the treatments (i.e. ozone or oxygenation) in part I, II and III (Fig. 2). Such a pH increase can be the result of CO₂ stripping from the solution via the rising gas bubbles (Lei et al., 2007). Interestingly, the alkalinity in parts I and III decreased non-significantly from 8.2 to 6.6 g HCO₃⁻ L⁻¹ ($p = 0.125$) and from 8.4 at treatment level 1 to 8.2 g HCO₃⁻ L⁻¹ ($p = 0.915$) at treatment level 8. This is in contrast with the results of part II in which there was a significant alkalinity decrease from 8.8 to 7.9 g HCO₃⁻ L⁻¹ at treatment level 8 ($p = 0.003$). This occurrence indicates that ozone affects the alkalinity of the digestate to a greater extent than stripping with pure oxygen gas and that the alkalinity decrease is greater when digestate supernatant is treated, compared to digestate. Such a decrease was also observed by other authors during air stripping experiments (Bonmati and Flotats, 2003; De la Rubia et al., 2010; Limoli et al., 2016; Markou et al., 2017) which can be attributed to the stripping of ammonia, affecting the total alkalinity. In part I, TAN increased up to 7.5% at treatment level 1 and decreased by 6.8% at treatment level 8. ($p = 0.046$), which was similar to TAN reduction in part III (8.5%, $p = 0.170$). However, in part II the decrease in TAN was greater (19.1%; $p = 0.018$). This indicates that ozone has no greater effect on the TAN removal compared to oxygen gas and that a solid/liquid separation step can increase TAN stripping yields. Similar observations could also be made for acetic acid concentrations which did not significantly increase in both parts I and III. This indicates that ozone treatment on the digestate has no greater effect than oxygenation. However, after a solid/liquid separation step, the presence of ozone increased the acetic acid concentrations by 2.4 folds in part II from 208 to 501 mg L⁻¹ at treatment level 8 ($p = 0.000$). This increase can be related to the oxidation of various organic compounds such as carbohydrates, lignin, humic and fulvic acid-like substances in

the supernatant (Ben'Ko et al., 2013; Jiang et al., 2019; Mota et al., 2008; Schultz-Jensen et al., 2013; Travaini et al., 2016, 2013). However, the increase in acetic acid concentrations was limited to $293 \pm 11 \text{ mg L}^{-1}$ which could be due to radical scavenging effect of HCO_3^- and CO_3^{2-} that reduced the effect of ozone on the organic matter disintegration (Mehrvar et al., 2001; Weeks and Rabani, 1966). In parts II and III, PCA analysis also indicated that the acetic acid production was positively correlated with the ozone treatment, while no such indication was present for part I.

Figure 1: The effect of ozonation and oxygenation on the A: pH, B: COD, C: acetic acid, D: total alkalinity and E: TAN for Part I, Part II and Part III respectively

Figure 2: Varimax rotation for part I (A), part II (B) and part III (C) respectively.

3.2 Ozone bubble size and consumption rates

As discussed, there were clear differences between the effect of ozone gas on the TAN, acetic acid and total alkalinity when either the digestate (part I) or the supernatant (part II) was treated even though the same amount of ozone was dosed per digestate volume. These differences may be explained by the bubble sizes of the ozone/oxygen gas mixture. Removing the solid particles by centrifugation and subsequent filtration creates a supernatant with a lower viscosity compared to the digestate. Lower viscosity can create smaller average bubble sizes in the supernatant than in the digestate. This effect was measured by bubbling ozone gas through the manure digestate and its supernatant. It has to be noted that the digestate used in the bubble size measurements was sampled from a different reactor than the digestate used in the ozonation experiments. Therefore, the observed differences are restrictive for quantitative conclusions on the ozonation experiments, based on the bubble size measurements. However, qualitative trends are expected to be similar on both dairy manure digestates since they have similar composition. Hence, the trends observed here can be extrapolated to the ozonation experiments to support the hypothesis.

The diameter of 179 and 73 bubbles were measured for the digestate and the supernatant. The numeric mean bubble diameters were 0.95 mm and 0.53 mm and the median bubble diameters were 0.71 mm and 0.47 mm for parts I and II, respectively (Fig. 3). The differences in bubble diameter were statistically significant ($p=0.000$). Due to solid particles, the viscosity and the resulting superficial liquid velocity in part I, the bubbles were allowed to merge together, resulting in average greater bubble diameters (Ribeiro and Lage, 2004). Furthermore, the angle at which the bubbles leave the aeration pebble was higher in part II (39°) compared to the part I (11°). Indeed, the angle is the composition of the rotation speed of the liquid and the rising speed of the bubbles, which both are different in both experiments.

The differences in bubble diameters, and angles by which the ozone bubbles leave the aeration pebble, gave indications to the differences in reactor performance. Indeed, the gas-liquid volumetric mass transfer coefficient (kLa) is a reliable parameter by which two-reactor set-ups may be compared (Munasinghe and Khanal, 2010). The kLa is depended on the temperature, the bubble size, the gas retention time, mixing speed and mixing propeller design (Munasinghe and Khanal, 2010; Ungerman and Heindel, 2007). Although no quantitative measurements on the kLa could be performed, the bubble size results indicate that the kLa value in case of the supernatant was greater compared to the digestate during ozonation.

The increased kLa , as a result of smaller bubble sizes and the absence of solid particle, also resulted in significantly higher ozone consumption rates in part II, compared to part I. The ozone consumption rates were 0.02 ± 0.01 g O₃ min⁻¹ and 0.09 ± 0.01 g O₃ min⁻¹ for parts I and II, respectively. The overall ozonation absorption, defined as the amount of ozone absorbed over the amount of ozone produced by the ozone generator, was higher in part II ($78 \pm 6\%$) than in part I ($14 \pm 2\%$). This is a result of the greater surface area of the smaller bubbles, the differences in biochemical compositions and the exclusion of the solid matter in the supernatant.

The differences in bubble size observations also explain the higher TAN stripping yields observed in part II, as the increase in ammonia mass transfer from liquid to gas due to a higher surface area of the gas bubble (Lewis and Whitman, 1924). These results indicate that TAN stripping, a solid/liquid separation pre-treatment may enhance the stripping yields.

Figure 3: Bubble size distribution for $\square$ digestate and $\square$ digestate supernatant, respectively.

3.3 Trace elements

After ozone treatment of the digestate (part I) and the supernatant (part II), and after oxygenation of the digestate (part III), the TE and nutrient concentrations in the liquid phase were analyzed by ICP-SF-MS. Fig. 4 depicts the TE profiles of Cd, Cu, Fe, Pb, V and Zn, these were chosen for graphical representation because of their notable changes due to ozonation.

In part I, the concentrations of Mo, P and S concentration increased significantly with 270%, 68% and 36% respectively, while the Al concentration significantly decreased by 71% (Table 3). The concentration of Cd, Cu, Fe, Pb, V and Zn showed an initial increase to a maximum of 17%, 36%, 100%, 54%, 16% and 11%, respectively due to ozonation in part I (p- values in Table 3). These maximum increase in concentrations were measured at treatment level 1 for Cd, Cu, V and Zn, at treatment level 2 for Fe, and at treatment level 3 for Pb and V, respectively. Beyond these treatment levels, the concentration decreased again (Fig. 4). Ni and Se also showed a maximum concentration increase by 153% and 16% respectively, which were not significant (Table 3). For Cd, Cu, Pb, V, and Zn, the final concentration in Part I at treatment level 8 was 26%, 22%, 23%, 20% and 23%, respectively lower than the initial concentration of the untreated digestate. Fe, however, showed a final concentration which was 66% higher than the initial concentration (Table 3). Arsenic concentrations monotonically and significantly increased by 70%. No significant changes were observed for Ca, Co, K, Ni and Se. However, the P concentration nonsignificantly decreased by 29%, from 31.5 ± 3.9 to 22.4 ± 3.1 mg L⁻¹, at treatment level 2. Without the last measurement at treatment level 8 which was characterized by a high relative standard deviation of 31%, the p-value increases from 0.046 to 0.083. No outliers could be detected in the phosphorus data set. This indicates that the significance of the P increases by ozonation in part I could be a statistical type I error.

Table 3: Effect of ozone treatment on pH, alkalinity, trace elements and macro and micronutrient concentrations for part I, part II and part III respectively. p-values were calculated by one-way ANOVA analysis. Bold lines represent statistically significant changes.

Part I: Digestate ozonation									
	Treatment level	Unit	0	1	2	4	6	8	p-value
pH	Average	-	7.72	7.91	7.92	7.99	8.05	8.06	0.000
	SD	-	0.00	0.01	0.02	0.04	0.02	0.05	
S	Average	mg L ⁻¹	117.03	143.76	132.02	135.62	151.90	159.09	0.021
	SD	mg L ⁻¹	10.94	3.98	15.07	10.26	4.22	8.45	
Al	Average	μg L ⁻¹	2019.65	1295.75	1789.50	1468.05	625.40	578.80	0.000
	SD	μg L ⁻¹	398.33	140.47	242.80	172.90	84.43	49.03	
As	Average	μg L ⁻¹	18.00	21.10	18.75	25.25	28.00	30.60	0.039
	SD	μg L ⁻¹	1.70	2.73	3.70	3.27	3.73	5.80	
Cd	Average	μg L ⁻¹	7.75	9.05	8.40	8.75	6.35	5.70	0.000
	SD	μg L ⁻¹	0.28	0.68	0.05	0.28	0.52	0.15	
Cu	Average	μg L ⁻¹	436.50	593.10	459.65	573.75	402.00	341.10	0.009
	SD	μg L ⁻¹	12.30	37.00	65.27	21.50	87.30	62.30	
Fe	Average	μg L ⁻¹	8529.30	15252.25	17036.30	16727.05	16900.70	14204.35	0.000
	SD	μg L ⁻¹	420.80	402.93	1133.73	1515.83	328.83	2086.83	
Mo	Average	μg L ⁻¹	87.45	126.65	126.05	154.50	241.90	323.30	0.000
	SD	μg L ⁻¹	1.05	3.97	18.08	37.45	11.08	29.92	
V	Average	μg L ⁻¹	45.15	52.25	49.35	48.50	34.80	36.05	0.001
	SD	μg L ⁻¹	0.75	1.87	1.65	6.58	1.75	3.72	
Zn	Average	μg L ⁻¹	7091.05	7844.20	7074.85	7606.20	5169.45	5429.15	0.001
	SD	μg L ⁻¹	384.47	234.53	366.13	218.80	790.20	557.73	

Part II: Supernatants ozonation									
	Treatment level	Unit	0	1	2	4	6	8	<i>p</i> -value
pH	Average	-	8.10	8.36	8.41	8.16	8.20	8.38	-
	SD	-	-	-	-	-	-	-	
HCO ₃ ⁻	Average	g L ⁻¹	8.79	8.34	8.03	8.24	8.03	7.93	0.003
	SD	g L ⁻¹	0.07	0.07	0.17	0.31	0.07	0.10	
Cd	Average	μg L ⁻¹	9.13	9.28	9.75	9.80	10.30	11.00	0.029
	SD	μg L ⁻¹	0.32	0.53	0.65	0.53	0.37	0.08	
Mo	Average	μg L ⁻¹	74.95	188.25	150.40	216.25	401.90	185.45	0.004
	SD	μg L ⁻¹	12.93	10.60	20.77	102.63	141.33	17.83	
V	Average	μg L ⁻¹	50.38	47.73	47.28	44.18	51.03	41.33	0.024
	SD	μg L ⁻¹	4.73	0.53	1.13	2.00	0.53	2.60	

Part III: Digestate oxygenation									
	Treatment level	Unit	0	1	2	4	6	8	<i>p</i> -value
pH	Average	-	-	7.47	7.54	7.97	8.08	8.16	0.000
	SD	-	-	0.01	0.03	0.02	0.01	0.01	
S	Average	mg L ⁻¹	-	130.95	154.53	153.40	159.78	171.53	0.051
	SD	mg L ⁻¹	-	14.30	1.90	13.80	8.23	5.61	
Cu	Average	μg L ⁻¹	-	414.20	509.85	598.40	621.53	663.80	0.011
	SD	μg L ⁻¹	-	47.07	32.60	29.23	64.28	66.63	
Fe	Average	μg L ⁻¹	-	9154.25	14981.70	19719.30	19703.90	22081.60	0.000
	SD	μg L ⁻¹	-	521.77	615.20	455.60	2175.77	442.03	
Ni	Average	μg L ⁻¹	-	44.95	60.05	51.70	60.75	64.40	0.040
	SD	μg L ⁻¹	-	1.98	9.17	2.62	1.75	6.52	
Pb	Average	μg L ⁻¹	-	42.10	49.35	54.20	52.85	61.80	0.042
	SD	μg L ⁻¹	-	1.98	3.70	2.78	8.38	1.75	
V	Average	μg L ⁻¹	-	48.15	56.80	57.45	58.70	58.15	0.017
	SD	μg L ⁻¹	-	4.30	0.38	1.25	2.62	2.27	

In part II, the digestate supernatant was ozonated. The only significant TE and nutrient changes can be found for Cd, Mo and V. Cd and Mo concentrations significantly increased by 20% and 147%, respectively at treatment level 8. Gagnon et al (2014) found a decrease in the Cd particulate metal concentrations with ozone treatment, which resulted in an increase of colloidal and preamable fractions (Gagnon et al., 2014). These findings can explain the increase in measured Mo and Cd concentrations. The smaller fractions of the particulate metals created by ozonation can penetrate the 0.60 μm pore size filter paper and thus increase in concentration after measurement with ICP-SF-MS. The decrease in V concentration by 18% can be due to the oxidation of V^{4+} which would then precipitate as V^{5+} (Nishimura et al., 2002). No significant changes were observed for Al, As, Ca, Co, Cu, Fe, K, Ni, P, Pb, S, Se and Zn. These insignificant changes in part II, compared to their significant changes in part I, indicate that the increased liquid phase TE concentrations in part I are a result of the interaction of the O_3/O_2 gas with the solid particles of the manure digestate. The effect of the O_2 in the O_3/O_2 gas on the TE concentrations was then differentiated in part III.

In Part III, Ca decreased significantly by 24% while Cu, Fe, Ni and Pb significantly increased by 60%, 141%, 43% and 47%, respectively at treatment level 8 without showing a local maximum. V concentrations significantly increase and showed a local maximum of 22% at treatment level 6, followed by a 21% increase from the initial concentration at treatment level 8. However, the differences between these two means were not significant ($p = 0.797$). P and S increased by 16% and 31%, respectively ($p = 0.212$ and 0.051 , respectively). No significant changes were observed for Al, As, Cd, Co, K, Mo, P, Se and Zn in part III. The TE parabolic concentration profiles measured in part I could not be replicated in part III with oxygenation, with the exception of V and Zn which were both not significant ($p = 0.797$ and 0.525 , respectively). This indicates that the changes in part I are due to the effect of ozone on the digestate instead of the oxygen gas.

The parabolic profile of TE during ozonation in part I implies that ozone causes a release of TE from the solid matter to the supernatant at a higher rate than the oxygen gas during part III. Furthermore, after a certain dose, the maximum amount of TE is released in the supernatant which is the local maximum of the profile. From this point on, the rate of TE precipitation is higher than TE solubilization, causing a decrease of TE concentrations in the liquid phase. The parabolic profiles of Cd, Cu, Fe, Pb, Se, V and Zn are prominent when second-order polynomial equations are fitted on the experimental results with statistically significant coefficients (table in Suppl. Files). All fits yield significant coefficients. The linear profile of these TE's during phase III indicates that this maximum concentration has not been reached.

Figure 4: TE profiles of Cd, Cu, Fe, Pb, V and Zn during Part I, Part II and Part III respectively.

TE can interact with organic matter in the manure digestate such as microbial cell walls (Kenney and Fein, 2011), organic sulphur compounds (e.g. organic thiol compounds) (Shakeri Yekta et al., 2014), soluble microbial products (SMP), organic acids (Aquino and Stuckey, 2006), organic substrates (e.g. yeast extract) (Gonzalez-Gil et al., 2003) and humic acids (HA) (Benke et al., 2008). The functional groups (COO^- , N=O , NO_2 , SO_2 , P-O and C=O) on the organic matter (e.g. cell walls) are responsible for metal adsorption or binding (Pradhan et al., 2007; Singh et al., 2003). Ozone can cause structural changes of proteins by aggregation due to crosslinking between amino acids or by fragmentation of the proteins (Davies, 2005). It is possible that ozone causes structural changes of the functional groups that cause desorption of the TE and thus increase their concentration in the liquid phase. Complexation reactions between TE and dissolved organic matter such as HA or dissolved inorganic matter are a second possible explanation of the increased TE concentrations in the liquid phase (Pandey et al., 2000). In the presence of HCO_3^- , CO_3^{2-} ions and sulfide ions, these complexation reactions can increase the soluble metal concentrations by up to a factor of 104. Ozone might increase the concentration of these complexation reagents by dissolving solid particulate matter (Callander and Barford, 1983;

Möller and Müller, 2012). However, no sCOD increase was observed. The decrease of TE concentrations from their local maximum value in part I at around treatment level 2, can be due to the increase in S concentrations and rising pH levels, via precipitation as sulfides, phosphates and hydroxides (Möller and Müller, 2012). Furthermore, as previously discussed, antifoaming agent as polysiloxanes can be degraded to silicon oxides (SiOx) by ozone treatment (Ouyang et al., 2000). A variety of different SiOx compounds, like for example, SiO₄⁴⁻ containing compounds, exists such as with Zn (Zn₂SiO₄) Al and Mg (Mg₃Al₂(SiO₄)₃), etc. Hence it is possible for precipitation with silicon oxides to occur. However, this could not be quantified as this would require knowledge of the Si concentration, as well as their oxidation state. Since Si was not included in the measurements during the different experimental parts, no interaction between Si and the measured TE's can be elucidated.

A second hypothesis is the oxidation of the TE by ozone, resulting in less soluble salts. For example, Fe²⁺ can be oxidized to Fe³⁺ and precipitate as Fe(OH)₃, As³⁺ can be oxidized to As⁵⁺, and V⁴⁺ can be oxidized to V⁵⁺ (Kim and Nriagu, 2000; Nishimura et al., 2002; Radjenovic et al., 2008; Reckhow et al., 1991).

As discussed above, the ozone treatment changes the TE and nutrient concentrations in the liquid phase of the digestate, which is important for the quality of organic fertilizers. For example, Zn plays an important role in organic fertilizers as it is necessary for vital metabolic processes such as protein synthesis and maintenance of the cell membrane integrity. Fe is, due to its limited bioavailability in soils, also an important element (Michalak et al., 2018). The manure digestate used in this study had Cd, Hg, Ni and Pb concentrations of 0.6 mg kg⁻¹ TS, 0.12 mg kg⁻¹ TS, 3.88 mg kg⁻¹ TS, and 7.15 mg kg⁻¹ TS, respectively. These concentrations are lower than the maximum concentration defined by the EU, and are in line with the maximum acceptable concentration of TE for organic fertilizers (Bolan et al., 2004; Chen et al., 2005; European Commission, 2016; McBride and Spiers, 2001; Sager, 2007; Yun et al., 2019). However, by carefully controlling the concentration of these TE in the liquid digestate, an added value can still be created for organic fertilizers by adjusting the TE concentration to the specific need of a certain crop (Massé et al., 2007). After a solid-liquid separation step, the solid digestate can be used as a phosphorus-fertilizer, while the liquid fraction is a good N-K fertilizer (Tampio et al., 2016). Hence, if a crop requires more N-K and TE, the final form of the fertilizer is better with the released TE in the liquid phase by ozonation. If the goal of digestate treatment is to increase the TE or heavy metal concentrations in the liquid fraction of the digestate for their use on soils as organic fertilizers, a specific ozone dose must be selected based on the desired TE and nutrients. If, however, the goal is to decrease the TE concentration in the liquid matter, a higher ozone dose (over 40 g kg⁻¹ TS) can be recommended for manure digestate. It may be interesting for future prospects to investigate other digestate treatment techniques (e.g. ultrasound, steam explosion, ...) on their effects on the TE and nutrient concentration in the liquid phase.

4. Conclusions

The results indicated that removing the particular matter (part II) before bubbling with an ozone/oxygen gas increased the TAN-removal efficiency (6.8% in part I versus 19.1% in part II) and acetic acid production, compared to ozonation or oxygenation of the total digestate (part III). The increase in pH (to pH 8.2) was not a result of the ozone treatment but a result of bubbling gas through the medium. All parts also showed a decrease in alkalinity during treatment. However, this was less

pronounced for part III, indicating an effect of ozone on the alkalinity. Hence, it must be concluded that ozone doses up to 40 g O₃ kg⁻¹ TS have limited effects on the biochemical parameters.

Ozonation of manure digestate initially significantly increased TE concentration of Cd, Cu, Fe, Pb, V and Zn, which was a result of desorption of TE from the organic material or increasing the concentration of complexation reagents. This initial increase was followed by a decrease from their maximum concentration which could be due to precipitation by increasing pH and S concentration or by oxidation of the TE.

Ozone can be used as a digestate treatment to adjust the TE and nutrient concentration in the digestate for increased agronomic value of the digestate, depending on specific requirements of different fertilizers.

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