Environment, Development and Sustainability (2024) 26:15361–15379
https://doi.org/10.1007/s10668-023-03253-6

Addition of iron ore tailings to increase the efficiency of anaerobic digestion of swine manure: ecotoxicological and elemental analyses in digestates

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Received: 9 July 2021 / Accepted: 13 April 2023 / Published online: 1 May 2023

Abstract
The anaerobic digestion process (AD) occurs via series of biochemical reactions, producing biogas as a renewable fuel and digestate, a rich by-product in trace elements (TEs), which has been directly disposed on soils as organic fertilizer. The application of mineral additives can result higher biogas production and CH_4 yields in AD process; however, the chronical effects of these additives in digestates have not been reported in the literature. In this way, this research aims to analyze the chronic effects of digestates from swine manure (SD) and swine manure with iron ore tailings as additive (SDIOT) on survival and reproduction of *Enchytraeus cryptfew*. The fresh samples were collected from sequential batch processes conducted at mesophilic conditions. The TEs and pH ranges in feedstock and digestates were determined in fresh matter (mg L^{-1}), including analyses of heavy metals and nutrients, in which ranges concentrations were discussed according to biogas and CH_4 productions, and compared with previous data of literature. These analyses were followed by chronic tests of digestates on reproduction

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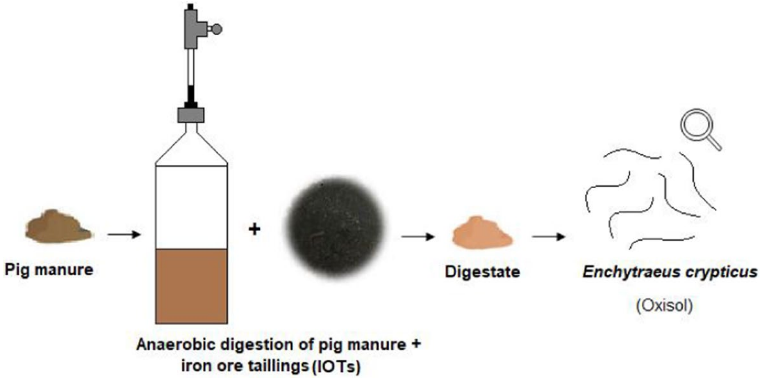
of *E. crypticus* in Oxisols, in which tests performance met the validity criteria stablished according to ISO 16387 standard. The means of treatments were compared using the Holm–Sidak method, and the means of control groups were compared using *t* test ($\alpha = 0.05$). The chronic effect on survival and reproduction of organisms might have occurred through synergistic effect of metals in digestates; however, more analyses must be needed to understand the toxicity effect of these products, specially under different climate conditions.

Graphical abstract



Article Highlights

- The application of swine manure resulted in a chronic effect to *Enchytraeus crypticus*.
- The synergistic effect of metals might have effects on organisms survival.
- Available fractions of metals and chemical properties of soils must also be evaluated.
- Climate change might influence on the speciation of metals within the soils.

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Keywords Digestates · Anaerobic digestion · Swine manure · Iron ore tailings · Ecotoxicological tests

1 Introduction

The optimization of anaerobic digestion process (AD) through the addition of trace elements (TEs) from active minerals has contributed to higher biogas volumes and CH_4 yields (Wang et al., 2017). According to Demirel and Scherer (2011), the addition of TEs such as iron (Fe), nickel (Ni), cobalt (Co), selenium (Se), zinc (Zn), copper (Cu), molybdenum (Mo), and tungsten (W) is essential for the growth and spread of methanogenic organisms. Due to the complexity of access to TEs and different ranges of concentrations, some researchers have suggested the use of additives in mineral forms, which contain mixed forms of these elements, as long as they are suitable for the metabolism of microorganisms and maintain an effective digestion process (Choong et al., 2016; Wang et al., 2017). In this way, some authors have reported the use of iron ore, such as hematite and magnetite, as promoting additives to increase CH_4 gas yield and latency reduction in the process (Liu et al., 2015; Qian et al., 2018; Wang et al., 2017).

The process also produces digestates, rich in TEs, which can be applied as an organic fertilizers to the soils. The expanded use of these products becomes even more relevant when considering the intensification in cattle ranching and agricultural production, which is responsible for roughly 14.5% of all greenhouse gas (GHG) emissions. Furthermore, higher concentrations of nitrogen (N) and phosphorous (P) on farming operations result in the increased pollution of water bodies (Sakadevan & Nguyen, 2017; Sigurnjak et al., 2019). According to Logan and Visvanathan (2019), a paradigm change is needed to refocus AD process optimization as not only biogas generation, but also as an "integrated biogas-digested output optimization." Thompson et al. (2020) analyzed the efficiency of hydrothermal pre-treatment in the anaerobic digestion of pelagic *Sargassum* sp. for biogas and fertilizer recovery. This research exposes the concept and practice of biorefineries based on marine algae, providing that mercury (Hg) and arsenic (As) remediation takes place in order to improve the quality of the digestate, ensuring compliance with environmental and soil quality regulations. This line of research opens the pathway for the identification of TEs in digestates and the application of ecotoxicological tests using soil organisms to thoroughly analyze the toxicity of these products in terrestrial environment.

The disposal of agricultural waste as a soil fertilizer in agricultural areas is commonplace in Brazilian agriculture. However, there are no researches evaluating the effects of these residues on the reproductive activity of soil organisms. When considering digestates generated from the AD process with addition of trace elements, these studies are even rarer. In this way, this research becomes even more valid considering elevated risks to inhabitants and environment of IOTs waste disposal in the country—upstream tailing dam method. According to the National Water Agency of Brazil (ANA), the country accounts 492 tailing dams, in which 67% are concentrated in the State of Minas Gerais, where mining is the most expressive economic activity in the State (Duarte, 2008; ANA, 2019). These products are mainly composed of iron oxides, manganese, calcium, silicon, aluminum, sulfur, and phosphates; however, they can also contain heavy metals (HMs) (TEs density ≥ 5 g cm^{-1}) (Koller & Saleh, 2018; Yang et al., 2014).

The application of ecotoxicological tests to evaluate the quality of substrates and digestates might be a strategic method to evaluate if these products might be directly applied to the soils. Currently, farmers are used to directly spread the substrate and/or digestates on the soils as substitutes for mineral fertilizers. Although some treatments are necessary to adjust the ideal concentration of nutrients in the digestate for crops (e.g., NKP), the main requirements of quality can be found in these composts (Jensen, 2013; Głowacka et al., 2020; Pecorini et al., 2020). As a result, it enables the reuse of the generated waste and reduced operational costs related to the purchase of mineral fertilizers. Furthermore, the use of digestates can reduce energy used in the production of mineral fertilizers, since Haber–Bosch process accounts for most nitrogen used in agriculture, resulting 1–2% and 3–5% of the world's total energy consumption and world's natural gas consumption, respectively (EBA, 2021). However, previous researches that cannot guarantee soil contamination will not occur, since these products might also contain some HMs, such as Ni, Mg, Fe, Co, Pb, Cu, Cr, and Zn (Sager, 2007). In fact, some HMs are essential to plant growth and development; however, excess concentrations of these elements can be toxic for these organisms, influencing in the capacity of crops to uptake and accumulate higher concentrations of essential elements (Arif et al., 2016).

In this way, the implementation of fertilizers regulations are essential to regulate the effective use of these products in agribusiness. In Brazil, the Normative Instruction No. 61/2020 guarantees the sale of organic fertilizers and biofertilizers earmarked for agriculture (Brazil, 2020). These products can be organic raw materials derived from urban, industrial, and agroindustrial activities (Class B). They can include organic fraction of urban solid waste from conventional collection, sludge from sewage treatment plants, industrial and agroindustry sludge generated in wastewater treatment systems. These products might contain sanitary contaminants, since their authorized use be determined by environmental agency, resulting in a product of safe use in agriculture. The legislation lays down minimum contents of primary and secondary macronutrients (N, P_2O_5, K_2O, and Ca, Mg, S), and micronutrients (Ca, Mg, S, B, Cl, Co, Cu, Fe, Mn, Mo, Ni, Se, Si, Zn). In Europe Union, the fertilizing products are framed according to product function categories (PFCs), in which organic fertilizers (PFC 1A) shall contain organic carbon and nutrients (P_2O_5, K_2O, CaO, MgO, Na_2O, SO_3), being of solely biological origin, and contaminants threshold values are stablished to Cd, Cr VI, Hg, Ni, Pb, As, Cu, and Zn elements (EC, 2019).

Some authors have analyzed some deleterious effects of liquid swine manure on reproduction of *Enchytraeids crypticus* (*E. crypticus*) in some types of soil (Maccari et al., 2015). According to some researches, higher toxicity levels were reported in Cambisol when compared to Nitosol soil. The increased risk of environmental contamination in State of Santa Catarina (Brazil) was also reported in Cambisol when compared to Latosol and Luvissol soils due to availability and accumulation of Cu, Zn, and Mn elements (Mattias et al., 2010). Alves et al. (2018) reported that the diversity of edaphic fauna was also influenced by different doses of liquid swine manure and mineral fertilization, in which lowest diversity was observed to the highest dosage of liquid swine manure applied. According to the authors, the planning of application, including the frequency and volume, must be planned to minimize initial effects of applying this material on the soils.

Although several studies analyze the metals bioavailability in soils containing liquid swine manure, few studies inspect the effects of swine slurry as a biofertilizer in the reproduction of enchytraeids and no studies have been found investigating the effects of digestates of swine manure and mining waste on reproduction of *Enchytraeus crypticus*. The main contribution of this work is analyzing the chronic effect of digestates of swine manure digestate (SD) and swine manure containing iron ore tailings as additive (SDIOT) on survival and reproduction of *E. crypticus* by the application of residual by-products which might be used as organic fertilizers in agriculture. In this way, some deleterious effects from the behavior of decomposing organisms from micro/meso fauna can be previously analyzed, resulting in a less complex analysis when compared with other types of interaction between species or vertebrate species. The fresh samples were collected from Castro e Silva et al. (2021), in which sequential batch processes were conducted at mesophilic conditions. The TEs and pH ranges in feedstock and digestates were determined in fresh matter (mg L^{-1}), including heavy metals, primary and secondary nutrients. These analyses were followed by chronic tests of digestates on reproduction of *E. crypticus* in Oxisols, according to Maccari et al. (2015).

2 Methodology

2.1 Feedstock and digestates

Feedstock and digestate samples of swine manure digestate (SD) and swine manure containing iron ore tailings as additive (SDIOT) were obtained from Castro e Silva et al. (2021). The AD process was conducted in sequential batch reactors with varied IOTs dosages (0 kg m^{-3} IOT FM^{-1}, 1.56 kg m^{-3} IOT FM^{-1}, 3.12 kg m^{-3} IOT FM^{-1}, and 4.69 kg m^{-3} IOT FM^{-1}) under mesophilic conditions (35 °C) for a 45-HRT. The digestates were kept at temperatures between − 14 ºC/− 25 ºC and reached room temperature (20 ºC) to perform the analysis.

2.2 Elemental analysis and pH

The pH was analyzed according to standard methods for the examination of water and wastewater (APHA, 2017). The wet digestion was carried out through a combination of 5% HNO_3 65% v/v, 3% H_2SO_4 95 v/v, and 3% v/v HCl (VETEC) in 5% v/v fresh matter samples (FM) (PerkinElmer). The elemental analysis in digestates was determined by applying ICP-OES radial configuration and discussion of data was conducted according to biogas and CH_4 productions in AD process with IOTs as additive in laboratory scale reported in Castro e Silva et al. (2021).

2.3 Ecotoxicological tests

The chronic effects of digestates of swine manure (SD) and swine manure with iron ore tailings as additive (SDIOT) on survival and reproduction of *E. crypticus* were analyzed according to ISO 16387 (ISO, 2014). These organisms were chosen since they are

Table 1 Chemical parameters of natural soil (NS)

Parameter	Value	References
Cation exchange capacity	3.52 meq 100 g^{-1}	Oliveira (2017)
pH (H_2O)	5.52	
pH (KCl)	5.94	
Organic matter	11.06%	
Water retention capacity	53.46%	Determined in tests

Table 2 Experimental planning of bioassays

Experimental condition	EU[1]	Digestate concentration ($m^3\ ha^{-1}$)	No. replicas
I	Ct NS[a]	0	5
II	Ct AS[b]		
III	SD 15[c]	15	4
IV	M1.5 SDIOT 15[d]		
V	M3 SDIOT 15[e]		
VI	M4.5 SDIOT 15[f]		
VII	SD 30[g]	30	
VIII	M1.5 SDIOT 30[h]		
IX	M3 SDIOT 30[i]		
X	M4.5 SDIOT 30[j]		
XI	SD 45[k]	45	
XII	M1.5 SDIOT 45[l]		
XIII	M3 SDIOT 45[m]		
XIV	M4.5 SDIOT 45[n]		

[1]EU: Experimental unit

[a]Ct NS: Natural soil control

[b]Ct AS: Artificial soil control

[c]SD 15[c]: 15 $m^3\ ha^{-1}$ of 0 kg m^{-3} of IOT FM^{-1}

[d]M1.5 SDIOT 15: 15 $m^3\ ha^{-1}$ of 1.56 kg m^{-3} of IOT FM^{-1}

[e]M3 SDIOT 15: 15 $m^3\ ha^{-1}$ of 3.12 kg m^{-3} of IOT FM^{-1}

[f]M4.5 SDIOT 15: 15 $m^3\ ha^{-1}$ of 4.69 kg m^{-3} of IOT FM^{-1}

[g]SD 30: 30 $m^3\ ha^{-1}$ of 0 g kg m^{-3} of IOT FM^{-1}

[h]M1.5 SDIOT 30: 30 $m^3\ ha^{-1}$ of 1.56 kg m^{-3} of IOT FM^{-1}

[i]M3 SDIOT 30: 30 $m^3\ ha^{-1}$ of 3.12 kg m^{-3} of IOT FM^{-1}

[j]M4.5 SDIOT 30: 30 $m^3\ ha^{-1}$ of 4.69 kg m^{-3} of IOT FM^{-1}

[k]SD 45: 45 $m^3\ ha^{-1}$ of 0 kg m^{-3} of IOT FM^{-1}

[l]M1.5 SDIOT 45: 45 $m^3\ ha^{-1}$ of 1.56 kg m^{-3} of IOT FM^{-1}

[m]M3 SDIOT 45: 45 $m^3\ ha^{-1}$ of 3.12 kg m^{-3} of IOT FM^{-1}

[n]M4.5 SDIOT 45: 45 $m^3\ ha^{-1}$ of 4.69 kg m^{-3} of IOT FM^{-1}

ecologically relevant and abundant in many soils, easy to handle and cultivate in laboratory, having shorter cycle of life when compared to earthworms. Furthermore, they have high reproduction rate and are sensitive to contaminants (Maccari et al., 2015).

2.3.1 Test soils

The tropical artificial soil (TAS) and natural soil (NS)—Oxisol (Soil Survey Staff, 2014) were collected from the Center for Water Resources and Environmental Studies (CRHEA)—Engineering School of São Carlos, São Paulo, Brazil—22°10′09″ S 47°54′01″ W. The samples were dried in an oven at 60 °C for 24 h and sieved (2 mm) for soil defaunation. The chemical parameters of NS are shown in Table 1. The preparation of the TAS was adapted from Garcia (2004), using a mixture of 75% fine sand, 20% kaolin, and 5% powder of coconut fiber. The pH value of this control was approximately 6.0 ± 0.5

and the humidity was corrected to 60% of the water retention capacity of each soil, according to standard requirements.

2.3.2 *Enchytraeus crypticus* bioassays

Polystyrene vessels were used as experimental units ($h = 4.2$ cm and $d = 3.5$ cm). The following concentrations: 15 m^3 ha^{-1}, 30 m^3 ha^{-1}, and 45 m^3 ha^{-1} were applied to 20 g soil (experimental conditions I—XIV) according to Maccari et al. (2015). Ten adult organisms, preserved in agar substrate with well-developed clitellum, were selected per replicate of each treatment. The experimental design was entirely random with four repetitions for the treatments and five repetitions for TAS and NS controls (Table 2), in which water content and pH were checked at beginning/end of experiments. The organisms were kept under controlled temperature (20 °C ± 2°) and photoperiod (16:8, light/dark) conditions. Distilled water was added once a week based on weight loss, and the organisms were fed once a week with ground oat flakes. The tests were left overnight after adding 96% alcohol/Bengal rose solution (1% v/v) for the coloring of organisms after 21 days of exposure. The survived organisms were handed sorted from experimental units to Petri dishes and counted under a stereo microscope.

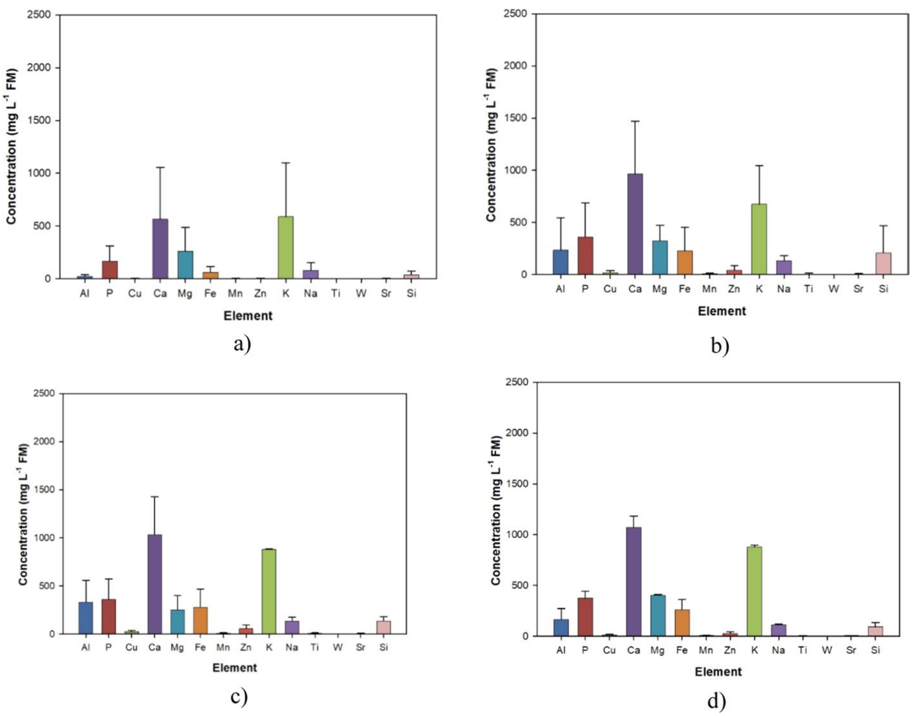

Fig. 1 Concentrations of elements in digestates with IOTs as additive for **a** 0 kg m^{-3} IOT FM^{-1}, **b** 1.56 kg m^{-3} IOT FM^{-1}, **c** 3.12 kg m^{-3} IOT FM^{-1}, **d** 4.69 kg m^{-3} IOT FM^{-1}

Table 3 Biogas and CH_4 productions in AD process with IOTs as additive in laboratory scale. *Source*: Elaborated from Castro e Silva et al. (2021)

IOTs concentration ($kg\ m^{-3}$ IOT FM^{-1})	Pressure (Pa)	Nm^3 biogas FM^{-1}	Nm^3 CH_4 FM^{-1}	pH
0	2,148.95	1.62×10^{-1}	2.81×10^{-2}	5.39 ± 0.07
1.56	2,470.10	1.62×10^{-1}	5.30×10^{-2}	5.90 ± 0.97
3.12	6,987.66	1.71×10^{-1}	5.81×10^{-2}	6.43 ± 1.02
4.69	18,703.97	1.95×10^{-1}	4.78×10^{-2}	5.28 ± 0.04

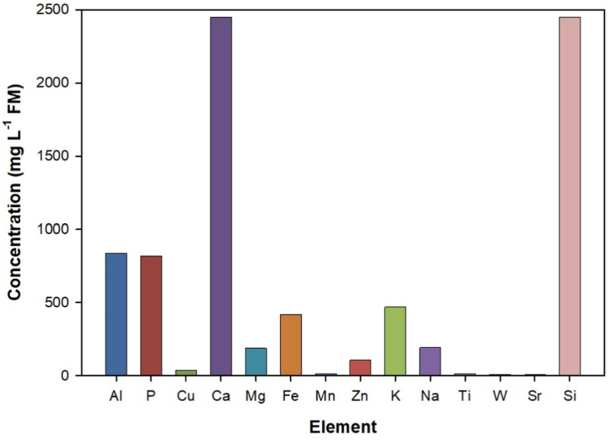

Fig. 2 Concentrations of elements in swine manure feedstock

2.3.3 Statistical analysis

The main hypothesis of this work is based on possible chronic effects of digestates of swine manure (SD) and swine manure with iron ore tailings as additive (SDIOT) on survival and reproduction of *E. crypticus*. The coefficient of variation (CV), average mortality rate, and the mean number of juveniles per experimental unit were verified according to ISO 16387 (ISO, 2014). The normality and homogeneity of the tests were checked according to Kolmogorov–Smirnov and Bartlett tests, respectively. The data were submitted to one-way ANOVA analysis and the means of treatments were compared using the Holm–Sidak method, while the means of both control groups were compared using the *t* test ($\alpha = 0.05$) via Sigma Plot 12.0 software (Systat Software, 2011).

3 Results and discussion

3.1 Analysis of trace elements

The concentrations and standard deviations of trace elements in fresh matter ($mg\ L^{-1}$) and nutrients are shown in Fig. 1, in which correlation coefficients were 0.99 for all analytes approximately.

High concentrations of Ca, K, P, and Mg were found in digestate control, whereas higher concentrations of Al, Si, Fe, Zn, and Cu were found in digestates with IOT as additive. The scanning electron microscopy (SEM) via *energy dispersive spectroscopy* (EDS) was previously conducted by Castro e Silva et al. (2021). According to the authors, the IOTs contained a mixture of limonite ($Fe_2O_3.3H_2O$) and hematite (Fe_2O_3), in which the first one might be associated with hydrated iron oxides phosphates, colloidal silica, and hydrated aluminum oxides.

Elements such as Fe, P, K, Ca, Mg, Cu, and Zn elements are usually found in swine manure, constituting desirable characteristics of the substrate for agricultural use (Diesel et al., 2002; Miyazawa & Barbosa, 2015). In this way, Zn, Cu, and Mn are mainly important for animal nutrition, being used in feed formulation as mineral complexes, in which Zn is widely used in the formulation of piglet feed to eliminate gastrointestinal disturbances that might arise after weaning (Basso et al., 2012). The addition of these elements is beneficious for the application of manure as a feedstock in AD process, since they contribute to the growth and spread of microorganisms. The elements such as Fe, Cu, Zn, W, Co, Ni, Se, and Mo are the main constituents of the enzymatic system of some methanogens and act as important components of coenzymes and cofactors of systems, influencing on methane production phase (Mudhoo & Kumar, 2013; Scherer et al., 1983). The accumulated biogas and CH_4 productions, and pH values of digestates and standard deviations using IOTs as additive in AD process from Castro e Silva et al. (2021) are reported in Table 3.

Although lower pH values are reported in the batch processes, the addition of IOTs as additive contributed to higher values of pressure, biogas, and CH_4 productions in batch reactors. The application of IOTs resulted in higher biogas productions of 12.31% and 16.92% when compared to controls production, in which decreased values (3:1 and 9:1) were reported regarding the applied treatments. The application of 3.12 kg m^3 IOT FM^{-1} resulted in higher concentrations of Na, K, Al, Fe, Cu, Zn, Ti, and W, whereas higher contents of P, Ca, Mg, Mn, and Sr contents were found when applying 4.69 kg m^3 IOT FM^{-1} (Fig. 1c, d). Although this fact, it is necessary to stress that feedstock composition might also influence on HMs concentrations (TEs density ≥ 5 g cm^{-1}), such as Cu and Zn in digestates (Kupper et al., 2014). Figure 2 shows the concentration of elements in the feedstock of swine manure, in which high contents of Ca, Si, Al, and P were reported.

Some authors have reported that the variation of metals concentration might also be related with absorption and release process of chelated metals in manure (Chen et al., 2014; Sarker et al., 2019). It might explain the decreased concentrations of some elements in all digestates when compared to their contents in feedstock (Figs. 1a, 2). Furthermore, the pH might also influence on metal speciation, enabling the precipitation of Fe, Mn, and Al (Dong et al., 2013). The ideal pH range was found in the treatment containing 3.12 kg m^{-3} IOT FM^{-1}, which might also contributed to higher CH_4 production (Table 3), since pH range from 6 to 8 promotes the growing of methanogenic microorganisms (Chernicharo, 1997). Despite this performance, the treatment with highest IOTs addition (4.69 kg m^{-3} IOT FM^{-1}) resulted in the increase of 12.31% biogas content when compared to the addition of 3.12 kg m^{-3} IOT FM^{-1}. The main elements found in both treatments are reported in Fig. 1c, d.

Ahn et al. (2006) exposed that the addition of Ca and W in swine wastewater is essential to decrease the concentrations of total volatile fatty acids (VFAs) in AD processes. Some enzymes containing this element include formate dehydrogenase (FDH) and formylmethanofuran dehydrogenase (FMDH), which are necessary for energy conservation and carbon assimilation in anaerobic systems, coupling formyl group with menotafuran in methanogenic organisms (Plugge et al., 2009). Sr, W, and Ti were the scarcest elements in feedstock

(Fig. 2) and digestates control (Fig. 1a). The increased concentrations of these elements were reported as the IOTs additive was applied in AD process, in which W element showed the lowest concentrations for all treatments applied, resulting in higher concentrations of 0.97 ± 0.86–1.21 ± 0.61 mg L^{-1} FM in digestates containing 1.56 and 3.12 kg m^{-3} IOT FM^{-1} compared to 0.09 ± 0.08 mg L^{-1} FM of feedstock. These treatments corresponded to higher biogas contents and CH_4 yields (Table 3). Some authors reported the mix of metal oxides nanoparticles (Fe_2O_3 and TiO_2) might enhance methanogens organisms in digested cattle manure sludge (Farghali et al., 2019). According to the results, the highest biogas production was achieved by Ti supplementation of 500 mg L^{-1} (375 g inoculum/200 g feedstock/25 g water), also reaching one of the lowest cumulative H_2S yields during 30-day experimental period.

Some elements such Cu, Zn, and Se are necessary for the activation or functioning of many enzymes and coenzymes in anaerobic fermentation (Chen et al., 2014). Amaral et al. (2014) analyzed the Cu and Zn distribution in digestates from swine manure in 10 m^3 fiberglass upflow digester, in which feedstock concentrations were 63.58 ± 27.72 mg L^{-1} and 8.98 ± 3.99 mg L^{-1} for Cu and Zn at 36 (±2) °C. As also reported in this research (Figs. 1a–d, 2), the decrease in these elements concentrations in digestates could also be observed in three phases even with the increase in flow rates (HRT = 17.86 ± 0.10, HRT = 7.57 ± 0.06, HRT = 5.32 ± 0.03). The authors appoint the retention of these elements in bioreactors, evidenced by lower DM contents, resulting in high insoluble Cu and Zn sulfides.

According to Cabirol et al. (2003), the effect of Al on the microbial consortium of AD, especially regarding to methanogenic archaea, is not explored in the literature. As stated by Jackson-Moss and Duncan (1991), the maximum Al concentration of 2500 mg L^{-1} can be tolerated by acclimatized methanogenic bacteria. The concentration of this element in digestates was 8-folder decreased when 4.69 kg m^{-3} IOT FM^{-1} was used as additive in AD process, showing lower reduction when compared to the 42-folder decreased in digestate control regarding the swine manure feedstock.

Magnesium (Mg) is important to maintenance of biomass function, performing vital functions in ribosomes, membrane cells, and nucleic acids, and also promoting the optimization of CH_4 production (Ma et al., 2009; Romero-Güiza et al., 2016). Romero-Güiza et al. (2016) analyzed the effect of Mg as an inhibitor and activator of the AD process of swine substrate in 115 mL serum bottles (17 mL feedstock) at 37 °C. A lower inhibition was reported for 750 mg L^{-1} and 1000 mg L^{-1} concentrations, respectively. According to the authors, the stimulation or inhibition effects were not reported between the concentrations 40–500 mg L^{-1} of this element. As shown in Figs. 1a–d and 2, the Mg concentrations varied below the maximum concentration of the cited ranges.

Mn is essential in AD process, since it stabilizes the methyltransferase in CH_4-producing bacteria and contributes to redox reactions (Suárez et al., 2014). Mn is an essential element to plant development and its concentrations can vary between 50 and 55 mg L^{-1} in digestates (Tápparo et al., 2020). The concentrations of this element in swine manure feedstock and digestates were below the range value of the cited research, in which lower contents were reported according to the increase in IOTs as additive. The decrease in this element in AD process is also reported in the analysis of biogas production with magnetite powder addition conducted by Córdova et al. (2022); however, a high rate decrease was observed to the lowest concentration of additive.

K is a nutrient required for the growth of anaerobic organisms, in which optimal concentrations of CH_4 can be found when 400 mg L^{-1} of this element is added to the AD process (Cheng, 2017). Higher K concentrations were observed to digestates control

(589.07 ± 510.78 mg L^{-1}) (Fig. 1a) when compared to the concentration of the feedstock (Fig. 2). The means concentrations were higher when increased concentrations of IOTs as additive were applied (3.12 kg m^{-3} IOT FM^{-1} and 4.69 kg m^{-3} IOT FM^{-1}) compared to the content of this element in digestate control; however, higher standard deviations values for these treatments were observed (Fig. 1c, d). A previous research also reported the increase in this element in pig manure, chicken manure, and cow manure digestates (Doyeni et al., 2021). Na is essential for the growth of anaerobic bacteria due to its role in the formation of adenosine triphosphate, or in the oxidation of NADH, in which the range 100–350 mg L^{-1} is stablished as the optimal concentration in mesophilic AD system (Dimroth & Thomer, 1989). The concentration of swine manure feedstock and digestates (controls and treatments) were found according to the cited range, whereas increased Na concentrations were reported for digestates containing IOTs as additive (Fig. 1b–d).

P is required for normal growth of organisms and also reported as a valuable nutrient for agricultural use (Hafuka & Kimura, 2022). As the IOTs dosages were increased in AD process, the maximum percentages of P reduction were achieved (16.22%, 29.86%, and 46.10%, respectively). These results are in accordance with the study conducted by Zheng et al. (2013), whose higher efficiencies of P removal were achieved by applying higher amounts of iron scrap waste as additive in the AD process. As stated by the authors, the hydrolytic bacteria reduce the pH of the sludge to corrode the iron residue. The ferrous iron generated by iron-reducing bacteria precipitates P element; however, the adsorption of P in final residue is a probable mechanism to be solved, which can also be observed by the high reduction rate of P concentration in digestates controls and feedstock (Figs. 1a, 2).

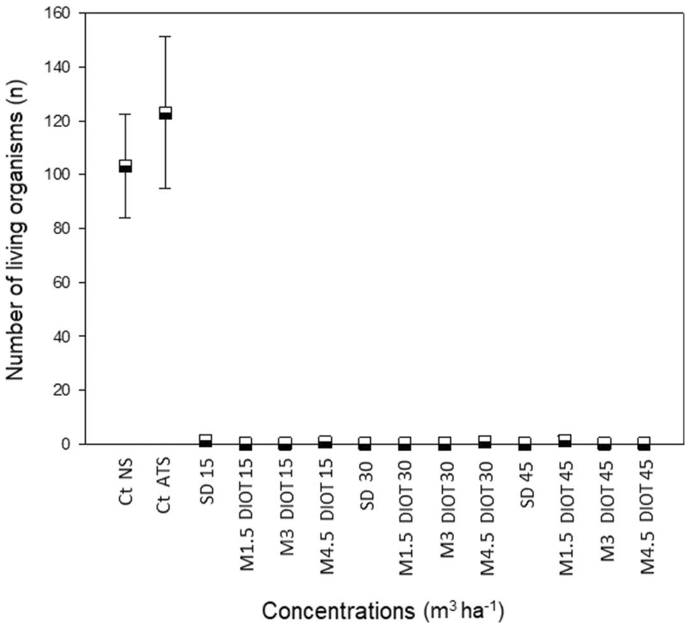

Fig. 3 Number of living organisms (m^3 ha^{-1})

Table 4 Statistical analysis of treatments

Treatment	n	*P* values		*P* < 0.05		
		Comparison with NS and AS	Comparison with each treatment	Comparison with NS and AS		Comparison with each treatment
Ct NS	122.80	–	0.231	< 0.001	–	+
Ct AS	103.00	0.231	–		–	+
SD 15	1.25	< 0.001	< 0.001	1.000	+	–
M1.5 DIOT 15	0	< 0.001	< 0.001	1.000	+	–
M3 DIOT 15	0.25	< 0.001	< 0.001	1.000	+	–
M4.5 DIOT 15	0.50	< 0.001	< 0.001	1.000	+	–
SD 30	0	< 0.001	< 0.001	1.000	+	–
M1.5 DIOT 30	0	< 0.001	< 0.001	1.000	+	–
M3 DIOT 30	0	< 0.001	< 0.001	1.000	+	–
M4.5 DIOT 30	0.75	< 0.001	< 0.001	1.000	+	–
SD 45	0	< 0.001	< 0.001	1.000	+	–
M1.5 DIOT 45	1.00	< 0.001	< 0.001	1.000	+	–
M3 DIOT 45	0.25	< 0.001	< 0.001	1.000	+	–
M4.5 DIOT 45	0	< 0.001	< 0.001	1.000	+	–

This section exposes a discussion of the ranges concentrations of TEs in the optimization of AD process with IOTs as additive compared to other types of mineral additives at mesophilic temperatures. Although the results promote the increase in literature data by applying IOTs as additive in AD process, it is necessary to stress these ranges might vary according to operational parameters, such as volume and type of digesters, temperature conditions, type and composition of feedstock, and homogenization degree of the sample. Furthermore, there is still a limited amount of data about the supply of nutrients in anaerobic digesters of animal excrements and agricultural waste involving the use of additives from iron wastes. This suggests that more research is needed concerning the diffusion of elements concentrations according to these factors, especially when using this type of as additive in AD process.

3.2 Toxicological effect of digestates

The control performance of *E. crypticus* met the validity criteria stablished by ISO 16387 standard (Appendix I). Figure 3 shows the number of living organisms according to the concentrations of digestates, in $m^3\ ha^{-1}$.

The proposed treatments resulted in a chronic effect on survival and reproduction of organisms. The application of digestates to Oxisols resulted in the death of organisms in all treatments applied. In this way, it was not possible to find a significant difference between these treatments (−), and the NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) values in NS and ATS could not be estimated. A significant difference could only be reported (+) when comparing the treatments with soil controls, in which the number of living organisms (*n*) and statistical analysis can be seen in Table 4.

Table 5 Treatments and pH ranges of bioassays

Treatment	ΔpH[1]
Ct NS	5.84 – 6.21
Ct AS	5.57 – 5.54
SD 15	5.55 – 6.82
M1.5 DIOT 15	5.48 – 6.93
M3 DIOT 15	5.53 – 7.14
M4.5 DIOT 15	5.50 – 6.70
SD 30	5.45 – 6.50
M1.5 DIOT 30	5.43 – 6.84
M3 DIOT 30	5.56 – 6.63
M4.5 DIOT 30	5.64 – 6.89
SD 45	5.66 – 6.89
M1.5 DIOT 45	5.40 – 6.28
M3 DIOT 45	5.42 – 6.70
M4.5 DIOT 45	5.36 – 6.80

[1] $\Delta pH = pH_{input} - pH_{output}$

The use of digestates from swine manure as fertilizers might result environmental problems, such as N_2O and NH_3 emissions, nutrients pollution, and soil contamination. Furthermore, the literature consensus is not found if the land application of these products might jeopardize agricultural productivity (Nkoa, 2014). On the other hand, the literature reports chemical fertility and small granular structure in Oxisols. In this way, the application of organic fertilizers might be beneficial for these soils, since these products can be available source of nutrients in short-term (e.g., N and P), enriching enzyme activities and microbial biomass (Albuquerque et al., 2012; Sousa & Lobato, 2003).

The toxicological effects vary according to the concentrations of organic matter and bioavailability of metals in the soil. As exposed in Table 1, the lower cation exchange capacity (CEC) of NS might be related with higher metals bioavailability, resulting in chronic effects on development and reproduction of the organisms. Maccari et al. (2015) reported some metals in manure can influence on survival, reproduction, bioaccumulation, and community profile of *Enchytraeus*. However, other authors reported that not only available fractions of metals might influence on these factors, but also physicochemical properties which might arise from interaction of the applied waste and soil (e.g., iron and aluminum oxides/hydroxides, competitive assorted ions, pH, CEC, water holding capacity, clay, and organic matter contents) (Kobetičová et al., 2010; Lock et al., 2006; Pinto, 2012; Violante et al., 2010).

Some authors reported that the application of manure digestate can increase the pH of soils (Głowacka et al., 2020). As shown in Table 5, the pH increased in all the treatments applied to the soils. According to ISO 16387, the ideal range for the development of *E. crypticus* occurs between a pH range of 5.9 and 6.5, resulting higher number of juveniles between 4.8 and 6.5 range. Although some treatments showed pH > 6.5, these values indicate low variation from the appropriate range.

The synergistic effect of metals in digestates containing IOTs as additive and controls might also be taken into account concerning to the survival of organisms. Posthuma et al. (1997) analyzed the effects of Zn and Cu mixtures in enchytraeids. The authors reported that the Zn sorption was reduced by the presence of Cu element, whereas this phenomenon was inert for Zn addition. On the other hand, the Zn absorption from a soil solution was stimulated by the Cu element, where the absorption was not stimulated by Zn element. The studies involving absorption and inhibition analyses are needed to evaluate the interaction between these elements in digestates (Nutrição de Safras, 2019). Despite the proposed discussion, more analyses must be conducted to understand the toxicity effect of these products. In this way, the metals bioavailability in bioassays containing organisms with different exposure routes along soil speciation and discussed parameters could be determined. It also should be considered that higher air temperatures and reduced soil moistures might affect the toxicity of soils containing IOTs, since climate changes can influence on the bioavailability and bioaccumulation of metals on soils. Some authors reported the bioaccumulation of Cd, Pb and Zn in enchytraeids in drier conditions and higher temperatures (25 °C) (Barmentlo et al., 2017; González-Alcaraz & Van Gestel, 2015).

This study also opens a pathway to better understanding the behavior of trace elements in the AD process containing these types of additives. In this way, it would be possible to better understand their effect on this process and their availability to the crops by applying the digestates as organic fertilizers. The behavior and toxicity effect of these elements can be analyzed on different existing forms (exchangeable and acid soluble—F_1, reducible (F_2), oxidizable (F_3), and residual fraction—F_4 (Liu et al., 2018). The decrease in HMs toxicity can be obtained by applying thermal treatments such as gasification, pyrolysis, and hydrothermal processes, in which end products (e.g., biochars and hydrochars) might possibly be used as bio-based fertilizers in agriculture. Furthermore, valuable fertilizer components such as nutrients and trace elements might be found in these products, contributing to a bio-based economy, decreasing the energy consuming of mineral fertilizers production and negative impacts on environment (Abad-Segura et al., 2021; Celletti et al., 2021; Chojnacka et al., 2020; Mohamed et al., 2021; Sigurnjak et al., 2020). So far, the application of thermal treatments in digestates containing IOTs or mineral additives has not been found in the literature.

4 Conclusion

This work analyzed the main elements and chronic effects of digestates from swine manure (SD) and swine manure with iron ore tailings as additive (SDIOT) on survival and reproduction of *E. crypticus* in Oxisols. The elemental analysis of digestates showed high concentrations of Al, Si, Fe, Zn, and Cu in digestates containing IOTs as additive, whereas Ca, K, P, and Mg could be identified in digestates controls. The application of digestates to the soil resulted in the death of organisms in all treatments applied, resulting in a chronic effect of these residual by-products, in which a significant difference could only be reported when comparing the applied treatments with soils controls. The chronic effect might have occurred through synergistic effect of metals in digestates. This result will possibly influence on decomposition of organic matter, and consequently on the carbon cycle and nutrients in ecosystems. However, further studies are necessary to authenticate this information, specially under different climate conditions. The use of IOTs as additives can not only decrease the number of upstream tailings dam in Brazil, but also prevent failure accidents and reduce risks of environmental contamination. This study opens the pathway to better understanding the behavior of trace elements in the AD process containing these types of additives and the application of thermal treatments in digestates, which might influence on the behavior and toxicity effect of heavy metals in these products.

5 Appendix

Criteria	Values	References
Average mortality in controls (NS and ATS)	Ct NS—4% Ct ATS—6%	ISO 16387 (2014)
Number of juveniles in the controls considering the addition of 10 adults (with eggs in the clitello region)	≥ 50	
Coefficient of variation in controls (NS and ATS)	Ct NS—14.80% Ct ATS—23.44%	

Acknowledgements We thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and for the given support, evidenced by Master's scholarship granted to Hellen Luisa de Castro e Silva and Maria Carolina Triques and Doctoral scholarship granted to Dayane de Oliveira. The authors would also like to thank the National Council for Scientific and Technological Development (CNPq), for granting a productivity in research scholarship to Prof. Regina Mambeli Barros (PQ1D Process Number 303036/2021-4 and PQ2, Process Number: 303805/2018-8) and to Prof. Electo Eduardo Silva Lora (PQ1D). The authors would like to thank the Research Support Foundation of the State of Minas Gerais (Fundação de Amparo à Pesquisa do Estado de Minas Gerais, FAPEMIG; in English) for granting giving by Project: "Improvement of biogas energy potential from anaerobic (co)digestion of solid organic waste as an incentive to renewable energy sources: substrate pre-treatment and co(digestion) aiming at Hydrogen use" (Process N.: APQ-00568-21).

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