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INTERESPAÇO
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EVALUATION OF THE POTENTIAL OF FOOD WASTE DIGESTATE IN LETTUCE SEEDS: germination and growth

AVALIAÇÃO DO POTENCIAL FERTILIZANTE DO DIGESTATO DE RESÍDUOS ALIMENTARES EM SEMENTES DE ALFACE: Germinação e desenvolvimento

EVALUACIÓN DEL POTENCIAL FERTILIZANTE DEL DIGESTATO DE RESIDUOS ALIMENTICIOS EN SEMILLAS DE LECHUGA: germinación y desarrollo

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ABSTRACT

The goal of this study was to determine if digestate from food waste can be used as biofertilizer in lettuce growth. Four germination tests were carried out with samples from liquid digestate produced with organic loadings rates of 6 and 8 kg TVS (total volatile solids) m^{-3} d^{-1}, as well as digestate that has been accumulated (D-AC) and a dry digestate (D-D). In the germination tests the dilutions of 5, 10, 25, 50 and 100% were tested. A pot experiment was performed using sandy soil and lettuce seeds in six different treatments: D-D, D-AC, mineral fertilizer (NPK), D-AC with NPK at a 1:1 ratio (D-AC+NPK), D-D and NPK at a 1:1 ratio (D-D+NPK), and a control. The higher seed growth was at 5% and 10% dilutions in all germinations tests. The results for the pot experiment showed best performance of the treatments NPK and D-AC+NPK. This result is a good indicative that digestate has potential to diminish the use of mineral fertilizers in agriculture.

Keywords: Anaerobic Digestion; Organic Waste; Germination Test; Biofertilizer; Waste Valorization.

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RESUMO

O objetivo deste estudo foi determinar se o digestato derivado de resíduos alimentares pode ser usado como biofertilizante no crescimento da alface. Quatro testes de germinação foram realizados com amostras de digestato líquido produzido com cargas orgânicas de 6 e 8 kg STV (sólidos totais voláteis) m^{-3} d^{-1}, bem como digestato que foi acumulado (D-AC) e um digestato seco (D-D). Nos testes de germinação foram testadas as diluições de 5, 10, 25, 50 e 100%. Foi realizado um experimento em vasos utilizando solo arenoso e sementes de alface com seis tratamentos diferentes: D-D, D-AC, fertilizante mineral (NPK), D-AC com NPK na proporção de 1:1 (D-AC + NPK), D-D e NPK na proporção de 1:1 (D-D + NPK), e um controle. O crescimento das sementes foi maior nas diluições de 5% e 10% em todos os testes de germinação. Os resultados do experimento com vaso mostraram melhor desempenho dos tratamentos NPK e D-AC + NPK. Este resultado é um bom indicativo de que o digestato tem potencial para diminuir o uso de fertilizantes minerais na agricultura.

Palavras-chave: Digestão Anaeróbia; Resíduos Orgânicos; Teste de Germinação; Biofertilizante; Valorização de Resíduos.

RESUMEN

El objetivo de este estudio fue determinar si se puede utilizar el digestato derivado de residuos alimenticios como biofertilizante en el desarrollo de lechuga. Se realizaron cuatro pruebas de germinación con muestras de digestato líquido producido con cargas orgánicas de 6 y 8 kg STV (sólidos totales voláteis) m^{-3} d^{-1}, así como digestato acumulado (D-AC) y un digestato seco (D-D). En las pruebas de germinación se probaron diluciones de 5, 10, 25, 50 y 100%. Se realizó un experimento en macetas utilizando suelo arenoso y semillas de lechuga con seis tratamientos diferentes: D-D, D-AC, fertilizante mineral (NPK), D-AC con NPK en la proporción de 1: 1 (D-AC + NPK), D-D y NPK en la proporción de 1: 1 (D-D + NPK), y un control. El crecimiento de las semillas fue mayor con diluciones del 5% y 10% en todas las pruebas de germinación. Los resultados del experimento en macetas mostraron mejor desempeño de los tratamientos NPK y D-AC + NPK. Este resultado es un buen indicativo de que el digestato es potente para disminuir el uso de fertilizantes minerales en la agricultura.

Palabras clave: Digestión Anaeróbica; Residuos Orgánicos; Prueba de Germinación; Biofertilizante; Valorización de Residuos.

INTRODUCTION

In Brazil, about 50% of the total solid waste collected are organic material (SNIS, 2019). Most of this material has as its final destination the landfills, causing the emission of greenhouse as well as lixiviate production. One option to treat the organic waste is through anaerobic degradation processes (CASTILHOS, 2003; CHARLES et al., 2009). Currently biogas production in Brazil is still not very representative compared with other sources of energy production (FREITAS, 2019). However, the country has great potential to produce it using biomass of organic waste from different sources (FERREIRA et al., 2015).

In the process of anaerobic digestion, microorganisms convert complex organic matter, such as carbohydrates, proteins and lipids in methane and less complex composts are carbon dioxide, ammonia and others (CHERNICARO, 2007). The typical anaerobic digestion systems operate with one stage only, with all four phases occurring simultaneously, however, for highly degradable organic waste it can cause some operational problems (BOUALLAGUI et al., 2009; JIANG, 2012).

Anaerobic digestion of organic waste that occurs in digesters with high organic loading rate need to maintain control over the parameters such as temperature, agitation and pH. Systems that operate in two physically separate phases provide greater stability to the process, as they allow greater control of the optimal pH conditions necessary for the development of each group of microorganisms that are present in the process (LIU et al., 2006; ARIUNBAATAR et al., 2014).

The benefits of two-stage methanization systems include the use of a smaller reactor, the operation with higher organic loading rates and shorter hydraulic retention times (HRT), as well as increased methane production and the generation of a nutrient-rich by-product that is called digestate (ASLANZADEH et al. 2014).

Anaerobic digestion can be used to stabilize several organic substrates, and around 20-95% of the organic matter is decomposed (MÖLLER and MÜLLER, 2012). The digestate can be used as organic fertilizer or soil amendment in agriculture (NKOA, 2014; CECCHI and CAVINATO, 2015; DOGAN-SUBASI and DEMIRER, 2016).

The use of digestate as fertilizer has the potential to decrease and/or avoid the use of mineral fertilizers, as it contains high levels of macro and micronutrients (MOLLER and MULLER, 2012). On the other hand, digestate's use must be associated with determining its composition and toxicity in order to avoid soil contamination.

The phytotoxicity of effluents and composts is determined in many countries through the seeds germination index, methodology that is widely used due to its quick response (DA ROS et al., 2018). Zucconi et al. (1981) reported that the index must be higher than 80% to indicate absence of phytotoxicity, although McLachlan et al. (2004) consider the index above 70% as a positive result.

In relation to plants, the use of lettuce (*Lactuca sativa*) for phytotoxicity analysis stands out, as it is a widely cultivated vegetable and consumed both by humans and animals. The lettuce seeds are excellent organisms for bioassays that use effluent treated by the most diverse processes, as they are highly sensitive to chemical stress, as well as have rapid germination and rehydration, ensuring the reproducibility of the assay (PELEGRINI et al., 2009; GIORGETTI et al., 2011; YOUNG et al., 2011).

Once the seeds germination is determined, pot experiments are also widely used for evaluation of digestate in agriculture, in order to verify the plants development and its biomass production. According to Nkoa et al. (2014), the effect of anaerobic digestates on crops can be analyzed *a posteriori* with respect to three types of control: unfertilized, undigested feedstock and mineral fertilizers. Those different treatments allow digestates performance to be compared with commonly used fertilizers.

The goal of this study is to determine if digestate from food waste can be used as biofertilizer in lettuce growth. To achieve that goal four different germination tests with lettuce seeds were performed, testing diverse dilutions and compositions of digestate. For further investigation, six different treatments were applied in a pot experiment with the same type of lettuce in order to determine the species growth rate.

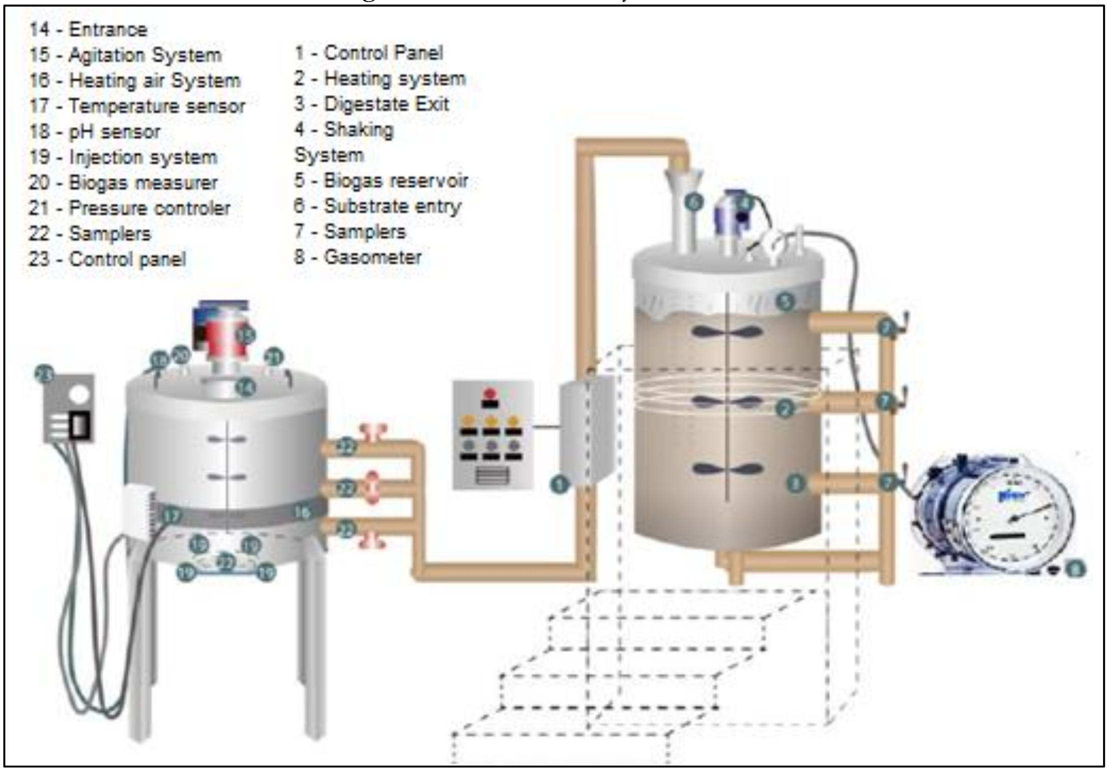
MATERIALS AND METHODS

Treatments characterization and description

The digestate was collected from a methanogenic reactor that belongs to a two stage methanization system. The pilot is located on Campus Trindade, of the Federal University of Santa Catarina (UFSC), in Florianópolis, Santa Catarina State, Brazil. The system operates in two stages, an acidogenic and a methanogenic. The first stage, acidogenic, and the second, methanogenic, are constituted by semi-continues digesters and operate with wet digestion, which means the total solids are lower than 10%. The system operates in mesophilic temperature, between 35-40°C.

As showed in Figure 01, the feeding starts at the acidogenic digester (left of the image) and after the first degrading of the substrate, the effluent goes to the methanogenic digester (right of the image). The digesters are fed in a daily basis according with the Organic Loading Rate (OLR) applied. In order to balance the C:N (Carbon:Nitrogen) ratio, the substrate is mixed in a the proportion of 95% food waste and 5% of gardening waste, which also comes from the University Campus. After the final process, the digestate is collected at the bottom of the methanogenic digester and the biogas is measured in the equipment.

Figure 1 – Methanization system scheme



Source: Malinowski (2021).

For the germination tests, four digestate samples were used, which were collected in different times in the methanogenic digester: (i) D-OLR6: digestate from April 2019, when the methanization system operated with an organic loading rate of 6 kg TVS.m^{-3}.d^{-1} (TVS – total volatile solids); (ii) D-OLR8: digestate from June 2019, when the methanization system operated with an organic loading rate of 8 kg TVS.m^{-3}.d^{-1}; (iii) D-AC: accumulated digestate on the period between March and July of 2019; (iv) D-D: digestate collected from sample (iii) and dried at 65 ºC in a stove with forced air circulation.

As for the pot experiment, six different treatments were applied: (1) D-D: dry digestate, the same used in the germination test; (2) D-AC: accumulated digestate, also the same described above; (3) NPK: mineral fertilizer, to be compared with the digestates; (4) D-AC+NPK: liquid digestate (D-AC) mixed in a 1:1 ratio with mineral fertilizer; (5) D-D+NPK: dry digestate (D-D) mixed in a 1:1 ratio with mineral fertilizer; (6) control, without fertilization.

For the treatments with mineral fertilizer, it was used a combination to form NPK with urea (for N), triple superphosphate (for P) and potassium chloride (for K). For treatment NPK, before being applied the mineral fertilizer, liming was used in order to achieve a pH of at least 6.0, according to the recommendations for lettuce - *Lactuva sativa* (CQFS-RS/SC, 2016).

The soil sample used in the pot experiment was classified as Red-Yellow Argisol (EMBRAPA, 2018) and Typic Hapluldut (SOIL SURVEY STAFF, 2014). Soil samples were collected in the 0-20 cm layer. The chemical and physical characterization of these soil samples were made according to the methodology of Tedesco et al. (1995) and Embrapa (2017). Before the experiments take place, the soul was analyzed after the field sampling, to determine the amount of fertilizer to be used.

All digestate samples used for the experiments were submitted to physical-chemical analysis. For liquid digestate the analysis was based in the *Standard Methods for the Examination of Water and Wastewater* (APHA, 2005), as the dry digestate analysis was based on Tedesco et al. (1995) methodologies for organic waste. In liquid digestate samples, the pH was measured with electrometric method, acidity and alkalinity by the titrimetric method 2230B, the total Phosphorus by the Vanadomolybdate 4500C method, the total Nitrogen by acid digestion with sulfuric acid and hydrogen peroxide, followed by titration with H_2SO_4 0.025M; and the ammoniacal nitrogen by the titulometric method 4500C. Total solids (or dry matter) and volatile solids were measured by the heated plate evaporation method 2450 and the metals Calcium (Ca), Magnesium (Mg), Copper (Cu) and Zinc (Zn) by the method EPA 3010A, with determination in spectrophotometer of flame absorption.

Germination Tests Preparation

The assays were performed in the Seeds Laboratory of UFSC. All experiments were designed completely randomized. The germination tests for each organic loading rates from samples D-OLR6 and D-OLR8 were performed at most two days after the sample collection in the digester. The D-AC and D-D experiments were performed on the same day, resulting in a total of 92 samples for all three germination tests.

Each sample for the germination tests was assembled with 50 seeds of the curly type of lettuce (*Lactuva sativa*) and one gerbox germination boxes in square format with two filter papers (base and lid). The dilutions for all tests were 100% (pure digestate), 50%, 25%, 10% and 5%, as well a control of distilled water. Each treatment was replicated four times, resulting in a total of 24 samples per assay.

The liquid digestates (D-OLR6, D-OLR8 and D-AC) were used without any treatment, whereas the D-D was first extracted in distilled water in the proportion of 1:10, as showed in Luo et al. (2018). After dilution, the solid digestate was mechanically stirred for 1 hour at 4 rpm and then centrifuged at 4000 rpm for 20 minutes. Finally, the extract was collected to be used.

It was applied 6.25 mL of liquid per germination box, in order to wet both filter papers, and then the seeds were inserted one by one, with space between them. After the initial preparation, the germination boxes containing the seeds were transferred at random to the BOD incubation chamber and spread randomly.

The duration of the test, the day of seed counting, as well as maximum and minimum temperature and quality control followed the description of the Manual of Rules for Seed Analysis, published by MAPA (BRASIL, 2016), as regarded to lettuce seeds. It was applied a 12-hour photoperiod, starting at 07 a.m., and the temperature was maintained at 20 ºC.

After the seven days of testing, seeds were counted and the root lengths were measured with a digital caliper. At the end of each trial, the following calculations were performed to determine the germination index (GI), according to equations presented in Abdullahi et al. (2008) and Zucconi et al. (1981).

At first, the relative seed germination – RSG, Eq. (1), corresponding to the count of seeds that grew and actually germinated.

$$\mathrm{RSG} = \frac{\text{Number of germinated lettuce seeds (sample)}}{\text{Number of germinated lettuce seeds (control)}} \times 100 \quad (1)$$

Then the relative growth of the seeds radicles – RGR, Eq. (2) was determined by measuring with the digital caliper.

$$\mathrm{RGR} = \frac{\text{Total radicle lenght of germinated lettuce seeds (sample)}}{\text{Total radicle lenght of germinated lettuce seeds (control)}} \times 100 \quad (2)$$

At last the germination index (GI) was calculated, relating the Relative Seed Germination with Relative Growth of the Seeds Radicles - Eq. (3).

$$\mathrm{GI} = \frac{\mathrm{RSG} \times \mathrm{RGR}}{100} \quad (3)$$

The final germination indexes results were submitted to ANOVA and the mean values, when meaningful, were compared with Tukey HSD test with a 5% significance value, with the use of the software Statistica 8.0. Eventual outliers related to the seeds radicle length were deleted of the final count.

Pot experiment

This experiment was performed inside a greenhouse that belongs to the Microbiology, Imunology and Parasitology Deparment of UFSC. The experiment started at the end of July, ending at the end of September, resulting in 64 days of cultivation. The greenhouse is covered and work without temperature control, which varied between 16ºC and 31ºC, with an average of 25ºC during the experiment. The plants were submitted to a photoperiod between five and nine p.m., since it was winter season.

Four replicates were also used for each of the six treatments, resulting in 24 pots. The pots of 3.6 L volume were distributed at random above a bench inside the greenhouse. The amount of fertilization applied was calculated from the previous analysis of the soil, based on the demands for lettuce culture described in the Manual of Adubation and Liming (CQFS-RS/SC, 2016). The quantity was calculated from contents of nitrogen, phosphorus and organic matter present in the soil used.

During the pot experiment, the lettuces were irrigated with water every time the soil was dry. At the end of the 64 days, the aerial and radicular parts of the plants were collected and the fresh mass was measured. After this measurement, all tissues were dried at an oven in 65-70 °C, inside paper bags, until the mass was constant. After the drying period, the dry mass of all tissues was weighted. Also, in the same day that the experiment ended, the soil was removed from the pots. After a drying period on a covered location, the pH of the soil from each sample was measured, according to Tedesco et al. (1995).

The mass results were submitted to analysis of variance (ANOVA) and the mean values, when meaningful, were compared with Tukey HSD test with a 5% significance value. Relationships among physical-chemical characteristics were analyzed by principal component analysis (PCA) using Statistica 8.0 software (only for the three liquid digestates). PCA was used in order to group variables and analyses the correlation between these groups. This correlation between the variables forms components that receive values according to the proximity of the variables in each component.

This analysis allows visualizing the degree of similarity between the areas and which variables influenced the pattern. Only the first two axes (PC1 and PC2) were used as they were considered sufficient to explain the data, despite the fact that it is a two-dimensional graph (MOITA NETO and MOITA, 1998).

RESULTS AND DISCUSSION

Composition of digestate

Digestates heterogeneity is what justify the importance of determine its composition before being used in agriculture, to avoid any contamination (ZIRKLER et al. 2014). Sheets et al. (2015) demonstrated that in their review, showing that the macro and micronutrients variability in the digestate can lead to different necessities of fertilization.

The composition for all digestates is presented in Table 1. ANOVA showed significant difference for the average of some parameters, which will be discussed on the sequence. For total solids, DLR-8 and D-AC showed similarity at the Tukey test HSD ($p<0.05$) while the D-OLR6 was different. On the other hand, volatile solids were similar for the first two treatments (D-OLR6 and D-OLR8), with lower values compared to D-AC.

Other interesting aspect is how different digestates composition is for the dry digestate. The nutrient contents presented values that were higher than the liquid digestates, with results from 40 to even 400 times higher for total nitrogen, copper, zinc and total phosphorus. The pH was also much higher for this treatment when compared to others.

Table 1 – Composition of digestates

Parameters	Types of digestate			
	D-OLR6	D-OLR8	D-AC	D-D
pH (H_2O)	6.48 C	6.32 D	6.82 B	8.86 A
Total solids – TS (%)	0.66 B	1.19 A	1.13 A	NE
Total Volatile solids (% TVS)	49.2 B	50.0 B	65.5 A	NE
Total alkalinity (mg.L^{-1} $CaCO_3$)	1740 C	2610 B	4820 A	NE
Electric conductivity – Ec (mS.cm^{-1})	5.05 C	8.37 A	8.30 B	NE
Total P (mg.L^{-1})	55.25 C	123.21 B	49.0 C	22454 A
Total Nitrogen (mg.L^{-1})	291.7 D	1001.8 B	570.7 C	23333 A
N-NH_4^+ (mg.L^{-1})	242.8	465.9	229.8	NE
Ca (mg.L^{-1})	32.0 C	187.5 A	102.6 B	NE
Mg (mg.L^{-1})	6.1 C	20.5 A	10.9 B	NE
Cu (mg.L^{-1})	0.06 D	0.34 B	0.17 C	9.98 A
Zn (mg.L^{-1})	0.05 D	0.85 B	0.23 C	46.9 A
COD (mg.L^{-1})	10143 C	43005 A	24710 B	NE
TOC (%)	48.43 A	46.46 B	38.2 C	NE

Source: Author. Subtitles: D-OLR6: digestate from organic loading rate of 6 kg TVS.m^{-3}.d^{-1}; D-OLR8: digestate from organic loading rate of 8 kg TVS m^{-3} d^{-1}; D-AC: accumulated digestate during a five months period; D-D: dry digestate. NE: not evaluated or measured. COD: Chemical Oxygen Demand. TOC: Total Organic Carbon. Statistics: Means in each column followed by the same capital letter on a line do not differ for Tukey test ($p<0.05$).

Between treatments, the accumulated liquid digestate (D-AC) presented a value slightly lower than the others, but still inside the values recommended for methanogenic bacteria, between 6.0 and 8.0 (CHERNICARO, 2007). All of the digestates presented different pH values, and D-OLR8 presented the lowest. The more alkaline pH in the solid digestate was also observed by other authors such as de La Fuente et al. (2013), who evaluated the liquid and solid fractions of digestate produced in a biogas plant that treats bovine manure, finding the values of 7.6 for liquid digestate and 8.9 for solid digestate. The more alkaline pH of the solid digestate can affect the soil and the availability of nutrients for plants (DE LA FUENTE et al., 2013). Also, lettuce is established at an ideal pH of 6.0 (CQFS-RS/SC, 2016), which indicates that pH values that are much higher or lower than this can impair its development.

The pH is related to another parameter which is alkalinity. The total alkalinity was lower in the D-OLR6 digestate, but still within the recommended range for anaerobic digestion that is between 1000 and 5000 mg. L^{-1} (METCALF and EDDY, 2014). Although the value of the digestate D-AC is higher, alkalinity is still in the range considered normal. The authors Akhiar et al. (2017), testing eleven liquid digestates originating from different substrates, observed that co-digestion with food residues contributed to higher values of alkalinity compared to fruit and vegetable digestates only.

The values of Ca, Mg, Zn and Cu followed the same pattern for all liquid digestates, with D-OLR8 presenting the higher values, followed by D-AC and D-OLR6. The digestate after drying, in addition to presenting a higher pH, also showed elevation and concentration of the nutrients nitrogen (N), phosphorus (P), copper (Cu) and zinc (Zn). In the soil, Cu and Zn, as well as other heavy metals, can increase their content when the medium undergoes acidification, so the contents may be higher than the concentrations necessary for the development of plants (ZHANG et al., 2012), and this form may be phytotoxic.

However, even with higher levels, these levels were still much lower than those found in the liquid and solid digestate fractions characterized by Tambone et al. (2017). The authors characterized complete digestates and separated liquid and solid fractions, collected from thirteen biogas plants that treat different mixtures of substrate from swine, cattle and energy crops, finding values on average from 44.6 to 71.1 $mg.kg^{-1}$ TS for Cu and 205-353 $mg.kg^{-1}$ TS for Zn.

Some parameters are also related to digestion biostability, as the COD. The chemical oxygen demand was higher for the D-OLR8, followed by D-AC and D-OLR6. During the process, the amount of organic matter and undigested carbon content is due to the decomposition of easily degradable carbon compounds in the digesters (STINNER et al., 2008). The authors Albuquerque et al. (2012b) suggest a relationship between COD and NTK less than 1.5 g C/g N as indicative of a stable digestate. The C/N ratio of D-AC, which is 5.8, showed that it is possible that the digestate could be not completely stable after being collected on the methanogenic digester.

Germination Tests results

In Table 2 the results for the RSG (relative seed germination) and for the RGR (relative growth of the seeds radicles) of each treatment and dilutions are presented, as well as the statistics for Tukey test ($p<0.05$).

The RSG was the same for all liquid digestates at 5, 10, 25 and 50% dilutions ($p<0.05$), what demonstrates that almost the same number of seeds grew for all of them. The statistic formed the same groups for all liquid digestates in these dilutions. The dry digestate, on the other hand, showed higher growth only with 5% and 10% dilutions, being lower with 25% and none at all with 50 and 100%. In 10% dilution D-D was also in the same statistical group as the D-OLR8, but only at 5% dilution it was at the same group as the other digestates ($p<0.05$).

The RGR, on the other hand, shows the lettuce seeds growth. For this parameter, at 5 and 10% dilutions, all liquid digestates were in the same statistic group, presenting the same results ($p<0.05$). However, for 25 % and 50%, D-OLR6 presented the better results, followed by D-OLR8 and D-AC. Again, the dry digestate presented the worst results.

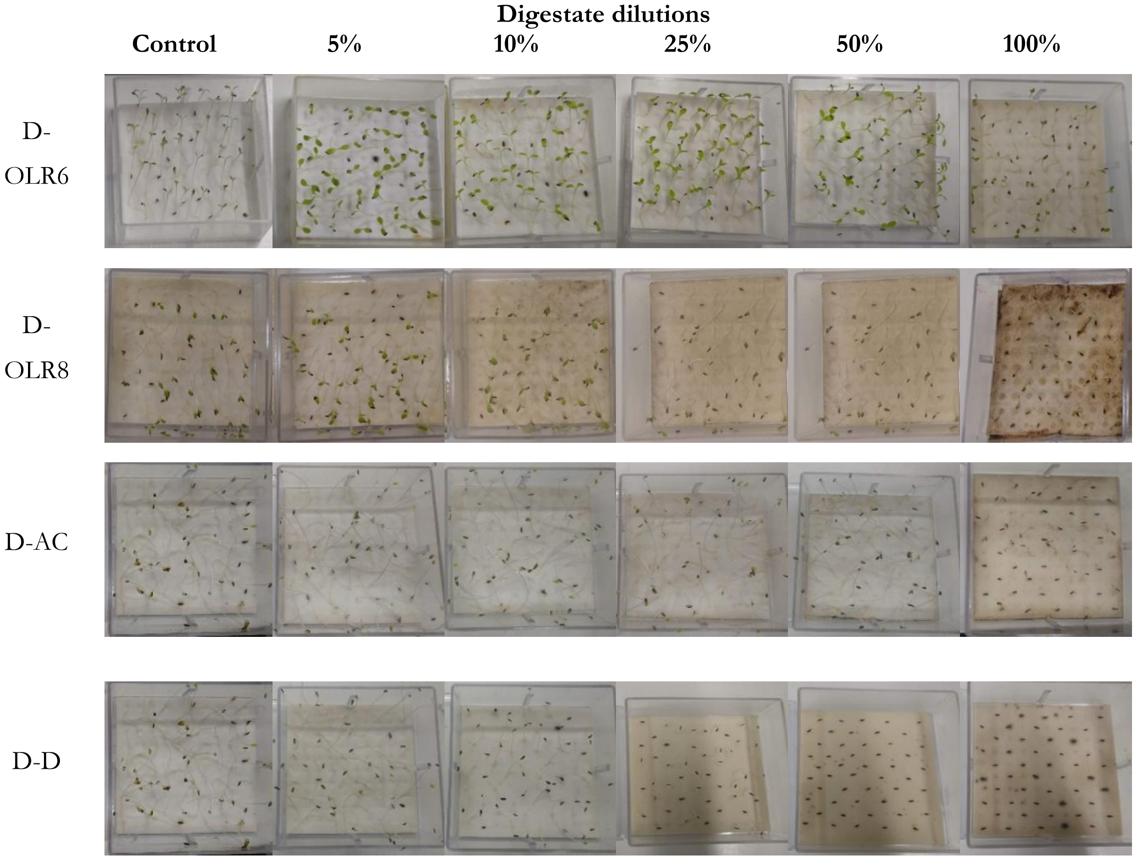
Table 2 – Relative seed germination (RSG) and relative growth of the seeds radicles (RGR) for all treatments and dilutions

	Dilutions				
RSG	**5%**	**10%**	**25%**	**50%**	**100%**
D-OLR6	103.95 A	97.74 A	93.22 A	102.26 A	87.01 A
D-OLR8	99.44 A	90.96 AB	92.09 A	96.61 A	0 B
D-AC	101.04 A	97.92 A	96.88 A	95.31 A	0 B
D-D	95.31 A	81.77 B	54.17 B	0 B	0 B
	Dilutions				
RGR	**5%**	**10%**	**25%**	**50%**	**100%**
D-OLR6	86.95 A	77.88 A	95.19 A	81.63 A	24.74 A
D-OLR8	104.25 A	98.53 A	70.24 AB	44.23 B	0 B
D-AC	99.95 A	88.69 A	53.30 B	43.73 B	0 B
D-D	43.27 B	21.38 B	6.63 C	0 C	0 B

Source: Author. Subtitles: RSG – relative seed germination; RGR – relative growth of the seeds radicles. D-OLR6: digestate from organic loading rate of 6 kg $TVS.m^{-3}.d^{-1}$; D-OLR8: digestate from organic loading rate of 8 kg $TVS.m^{-3}.d^{-1}$; D-AC: accumulated digestate during a five month period; D-D: dry digestate. Statistics: Means followed by the same capital letter in the column do not differ by Tukey test for $p<0.05$.

The growth of lettuce seeds and roots can be seen in Figure 2. Only in the trial with D-OLR6 the seeds germinated in all dilutions tested, and for pure digestate (100%) the germination index was low: 21.52%. For the other digestates the germination did not occur with pure digestate (100%).

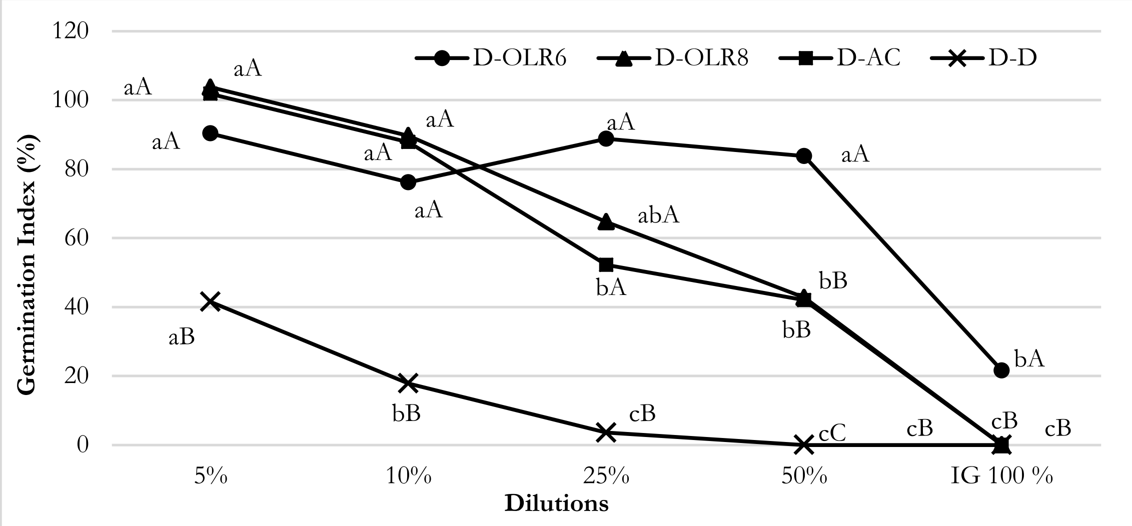
Figure 2 – Germination of lettuce seeds at each treatment and dilutions tested



Source: Author. Subtitles: D-OLR6: digestate from organic loading rate of 6 kg TVS.m^{-3} .d^{-1}; D-OLR8: digestate from organic loading rate of 8 kg TVS.m^{-3} .d^{-1}; D-AC: accumulated digestate during five months; D-D: dry digestate.

In Figure 3, the germination indexes (GI) are presented with statistical analysis (small letters represent difference between dilutions, and big letters between treatments). For all liquid digestates, the average of GI with 5%, 10% and 25% dilutions were statistically the same, for 5% significance. The values of GI for all liquid digestates in 5% and 10% dilutions were above 70%, so the digestate would be nonphytotoxic according to McLachlan et al. (2004).

Figure 3 – Germination indexes for four digestates at different dilutions



Source: Author. Subtitles: D-OLR6: digestate from organic loading rate of 6 $kgVS.m^{-3}.d^{-1}$; D-OLR8: digestate from organic loading rate of 8 $kgVS.m^{-3}.d^{-1}$; D-AC: accumulated digestate during five months; D-D: dry digestate. Statistics: Small letters present difference between dilutions, as big letters between treatments by Tukey test for $p<0.05$.

For D-OLR6, the germination indexes (GI) were the same ($p<0.05$) for all dilutions, except for pure digestate (100%), which was low. Those results are interesting, once higher digestate concentrations also helped seeds to germinate. For D-OLR8, dilutions of 5%, 10% and 25% were also the same, and close or higher that a 70% germination index. That result is very interesting, because even with digestate from different samples, the seeds germinated at a similar rate.

On the other hand, for the solid digestate in all concentrations the GI were less than 50% and showed no statistic relation with the other treatments. The germination index for this treatment presented the worst results, possibly due to the high load of nutrients as well as for the pH (Table 1), around 9.0, which is much higher than the 6.0 recommended for lettuce. For lower concentrations, as 5% digestate, the OLR-8 and D-AC presented a stimulating effect, with values above of the ones in the control. Pastor-Bueis et al. (2017) also find those results at a 10% dilution using digestate from a mix of fruits and vegetables as substrate, and using for the test seeds of lettuce, radish, cress, tomato and sweet pepper. At 20% dilution, the seeds were stimulated as well inhibited, depending on the species analyzed.

At dilutions of 50 and 100%, all digestates were phytotoxic. Coelho et al. (2018), testing digestates from eleven different biogas plants in Ireland and United Kingdom, and using seeds from cress (*Lepidium sativum)*, also found these results, with dilutions above 50% suppressing completely the seeds germination. That also happened in the work of Abdulahhi et al. (2008), which used digestate from a mix of food and green waste and radish (*Raphanus sativus L.*) seeds. In both researches, higher concentrations lowered the seeds germination. At this study, the seeds germination also decreased with higher digestate concentrations.

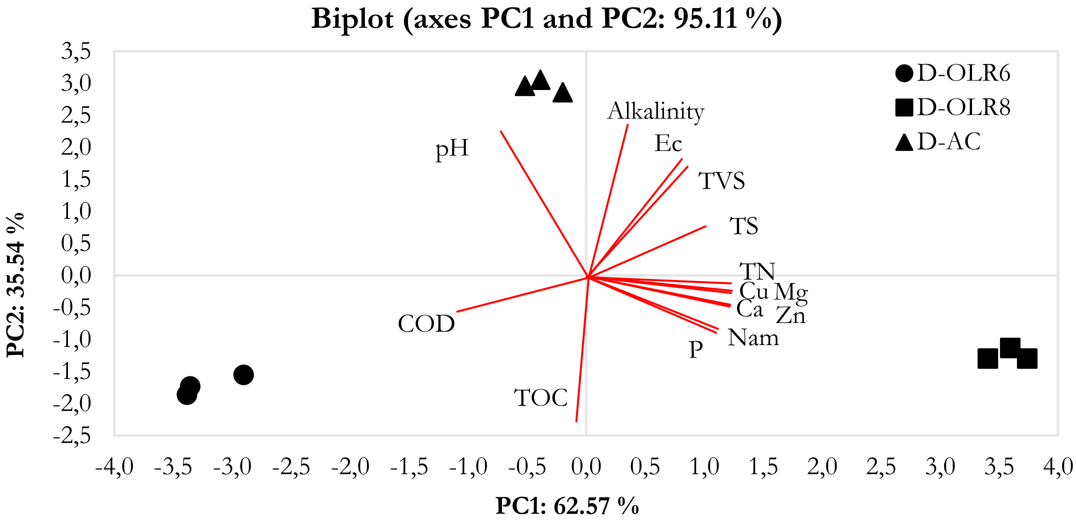
That same tendency is found in other works as the ones of Albuquerque et al. (2012), Pivato et al. (2016), Tigini et al. (2016) and Da Ros et al. (2018). Some parameters were evaluated by authors as possible causes of low germination indexes. Di Maria et al. (2014) evaluated digestate produced from food and vegetable waste performing a germination test with the extract. The germination index found was around 19.3% and it was related with high index of substances that are easily degraded, which could lead to a fast nutrient liberation for the crops.

The average of the germination indexes considering all dilutions tested at each treatment were 72.15% for D-OLR6; 62.38% for D-OLR8; 56.75% for D-AC and 12.55% for D-D. For that average it was observed an inverted correlation between electrical conductivity and high indexes for each treatment with liquid digestate, with R^2=0.93. In the studies of Coelho et al. (2018) and McLachlan et al. (2004), high concentrations of soluble salts were the main cause for phytotoxicity. This correlation was also observed in the work of Albuquerque et al. (2012), while testing cress and lettuce seeds. As a result, variations were found between digestates types and the species tested. Overall, it was observed that the concentration of 1% had better results, while pure digestate showed no germination for both species.

Relationship among physical-chemical traits

Principal component analysis (PCA) was conducted in order to determine relationships between digestates and their physical-chemical characteristics (Figure 4). All nutrients, represented by Total N, Ammoniacal N, P, Ca, Mg, Cu and Zn were correlated. Total Solids, Volatile Solids, electrical conductivity and alkalinity were also correlated. Regarding of the digestates, D-OLR8 was most highly correlated with all nutrients, while D-AC with pH and D-OLR6 with TOC and COD. Both COD and TOC were slightly correlated, what can be explained since both parameters are related with digestate's stability. D-AC was associated with pH since it had the highest value. D-OLR8 was associated with COD, and this digestate presented the highest value for this parameter. The D-OLR8 digestate presented the highest values for all nutrients, what is also showed in the analysis.

Figure 4 – Ordination diagram produced by principal component analysis of physical-chemical traits in liquid anaerobic.



Source: Author. Subtitles: PC – Principal Component. COD – Chemical oxygen demand; TOC – Total organic carbon; Ec – electrical conductivity; TS – total solids; TVS – total volatile solids; TN – Total nitrogen; Nam - ammoniacal nitrogen. D-OLR6: digestate from organic loading rate of 6 kg TVS.m^{-3} .d^{-1}; D-OLR8: digestate from organic loading rate of 8 kg TVS.m^{-3} .d^{-1}; D-AC: accumulated digestate during five months.

Results from the pot experiment

The soil used in this experiment presented the following composition: pH 5.20, clay, sandy, and silt constitution of 180, 750 and 70 g kg^{-1}, respectively, 7.0 mg dm-^{3} of P, 159.0 mg dm-^{3} of K, 26.60 g kg^{-1} of total organic carbon, 2.30 g kg^{-1} total nitrogen, 2.30 cmolc$_c$ dm-^{3} of Ca, 0.90 cmolc$_c$ dm-^{3} of Mg, 0.60 cmolc$_c$ dm-^{3} of Al, 40% base saturation, and 15% Al saturation. From those results, the digestate and the mineral fertilizers were applied based on fertilization needs for lettuce (CQFS-RS/SC, 2016).

The pH was also measured after the end of the pot experiments, with average values of 4.98 for treatment D-D, 5.46 for treatment D-AC, 6.29 for treatment NPK, 5.09 for treatment D-AC+NPK, 4.58 for treatment D-D+NPK and 4.73 for control. The third treatment showed difference from all others, with a 5% significance at the Tukey test. The low pH, presence of Al and lack of fertilization on the control could be responsible for lack of lettuce growth. The plants did not grow in treatment D-AC also, probably due to the high alkalinity (Table 1).

On the other hand, as pH of the soil was 5.2, it is evident that liming enhanced pH at treatment NPK. And, although lettuce did not grow in treatment D-AC, it also enhanced pH, what can demonstrate that it changes characteristics of the soil. In treatment D-AC+NPK the pH was just a little bit lower than in the original soil, but even if with no liming it maintained almost the same. The treatment with dry digestate, however, lowered the pH, what could also have contributed to less development of the lettuce.

The Al values where reduced only in the NPK treatment due to the effect of liming. The mineral treatment (NPK) treatment showed the lowest potential acidity value (H+Al) according to the data reported by Simon (2020). The other treatments showed similar Al values, since the SMP index that is used to calculate the amount of H+Al was similar between treatments, with values ranging from 6.3 and 6.5 (SIMON, 2020). Therefore, differences found in pH values are more related with C and N contents that are present in the D-AC and D-D treatments (Table 1). With the addition of organic waste rich in C and N, there is an increase in soil microbial activity, which may have accelerated the decomposition of organic matter, thus generating H^+ and acidifying the soil. As highlighted by Yan et al. (1996), when adding organic waste in the soil, microbial activity is intensified, possibly transforming the N by nitrification process, which is an acidifying process, justifying the pH drops, especially in the D-D treatment.

Table 3 shows average values for fresh mass (FM), dry mass (DM) and radicular mass (RM) at the end of the pot experiment, with statistical analysis. No mass was measured for treatments D-AC and control, because lettuce did not grow during the experiment. Although results using pure digestate were not positive, it is interesting to point out that the mix with mineral fertilizer showed results very similar to the use of mineral fertilizer alone (NPK), and also better than the control.

Table 3 – Average of fresh, dry and radicular mass at each treatment

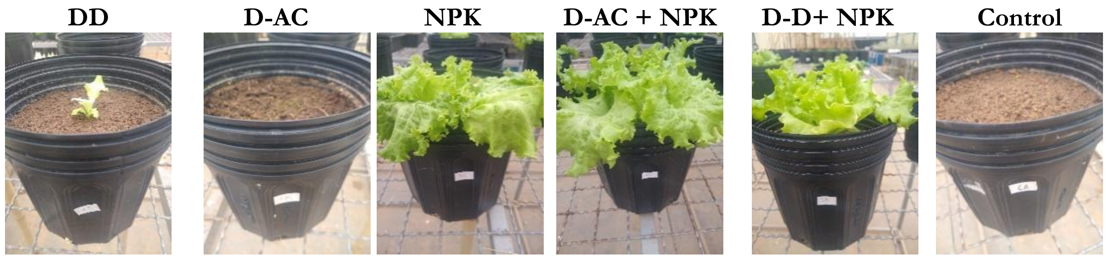
Treatment	FM (grams)		DM (grams)		RM (grams)	
NPK	22.0	A	3.0	A	0.85	A
D-AC+NPK	17.7	B	3.0	A	0.64	A
D-D+NPK	14.5	B	2.0	B	0.64	A

Source: Author. Subtitles: FM – fresh mass; DM – dry mass; RM – radicular mass. NPK: mineral fertilizer; D-AC+NPK: liquid digestate (D-AC) mixed in a 1:1 ratio with mineral fertilizer; D-D+NPK: dry digestate (D-D) mixed in a 1:1 ratio with mineral fertilizer. Statistics: Big letters represent difference between treatments (Tukey test for $p<0.05$).

When evaluation the fresh, dry and radicular masses, no statistical difference was found between the means in treatments NPK and D-AC+NPK. Treatment NPK is mineral fertilizer alone, as treatment D-AC+NPK is a mix between liquid digestate (D-AC) and mineral fertilizer. This result shows that the digestate could be used to lower the amount of mineral fertilizer used in agriculture, as it presented the same results as the use of mineral fertilizer alone. Also, the soil was limed in treatment NPK, and that was not made in treatments D-AC and D-AC+NPK, which showed values higher than the control, so the liquid digestate could be use also to enhance soil pH. Treatment D-D+NPK also presented good results, but not as good as NPK and D-AC+NPK. For D-AC+NPK, the standard deviations were higher, because lettuce grew differently in each pot, while at the other treatments they grew more similarly.

The results of the lettuce growth after the 64 days of experiment are presented in Figure 5. It is visible that treatments NPK, D-AC+NPK and D-D+NPK presented the better results, while treatment D-D showed only a little growth, as D-AC and control, no growth.

Figure 5 – Lettuce plants after 64 days pot experiment



Source: Author. Subtitles: D-D: dry digestate; D-AC: accumulated digestate; NPK: mineral fertilizer; D-AC+NPK: liquid digestate (D-AC) mixed in a 1:1 ratio with mineral fertilizer; D-D+NPK: dry digestate (D-D) mixed in a 1:1 ratio with mineral fertilizer; Control - without fertilization.

Other researchers also evaluated these parameters to verify digestate use as fertilizer. Barbosa et al. (2014) tested three diverse species (*Sida hermaphrodita, Zea mays L.* and *Medicago sativa L*) with digestate that comes from a biogas plant that uses corn silage as substrate. It was observed that plants grew higher than control for fresh and dry mass of the aerial parts, as for the radicular part no significant difference was found.

Some authors tested different dosages of digestate, as Chiconato et al. (2011) with cow dung digestate. No statistical difference was found for fresh and dry aerial tissue of the lettuce tested. In terms of fresh mass, the values were between 10 and 25 grams, while for dry mass it was between 1 and 4 grams, being the higher values for mineral fertilizer and the biggest dosage of digestate used. Comparing with this study, the results for fresh mass were very similar for treatments NPK and D-AC+NPK, with values between 14 and 27 grams.

CONCLUSION

The lettuce seeds grew better at lower concentrations of digestate and at lower organic loading rate at the digester, what highlights the importance of determining its composition. In the pot experiment with lettuce, when mixed with mineral fertilizer, the liquid digestate showed the same results as the mineral fertilizer alone, and both presented better results than control. That demonstrates the digestate has potential to be used in agriculture.

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