ISSN: 1696-8352

Anaerobic digestion of vinasse improves its use as a liquid fertilizer

Digestão anaeróbia da vinhaça melhora seu uso como fertilizante líquido

DOI: 10.55905/oelv22n1-027

Recebimento dos originais: 01/12/2023
Aceitação para publicação: 02/01/2024

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ISSN: 1696-8352

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ABSTRACT
The global demand for exploiting the potential of renewable biofuel production is increasing. When countries with large agricultural areas produce ethanol, they also generate a harmful byproduct called vinasse. Vinasse is a dark brown effluent that contains a high amount of organic matter, has an acidic nature, and is rich in mineral salts. Managing this effluent poses a challenge due to its significant volume, which ranges from 8 to 20 liters per liter of ethanol produced. Traditionally, raw vinasse has been applied directly to the soil as a liquid fertilizer. However, this practice, without proper guidance, can lead to soil degradation, micronutrient imbalance, pH change and excessive accumulation of organic matter. To resolve these issues and still use vinasse as a fertilizer, the process of anaerobic degradation of vinasse in biodigesters was analyzed. The resulting treated effluent, known as digested vinasse, was then compared to raw vinasse in terms of pollution parameters and the balance of minerals crucial for sugarcane cultivation. The results demonstrated a significant reduction in the organic load of vinasse, with a removal of chemical oxygen demand (COD) ranging from 66% to 85%. After applying the digested vinasse to sugarcane like cultivation soil, a noticeable decrease in the environmental impact caused by excessive organic matter was observed. Additionally, the study found that important nutritional elements such as sodium (Na) did not have any adverse effects or accumulate excessively when compared to calcium (Ca) and magnesium (Mg). In conclusion, the research demonstrates that the anaerobic degradation of vinasse in biodigesters can effectively reduce its organic load and mitigate the environmental impact associated with the direct application of raw vinasse to agricultural soils

ISSN: 1696-8352

Keywords: vinasse, anaerobic treatment, pollution indicators, microelements.

RESUMO
A procura mundial para explorar o potencial da produção de biocombustíveis renováveis está a aumentar. Quando países com grandes áreas agrícolas produzem etanol, eles também geram um subproduto nocivo chamado vinhaça. A vinhaça é um efluente marrom-escuro que contém uma elevada quantidade de matéria orgânica, tem natureza ácida e é rica em sais minerais. O gerenciamento desse efluente é um desafio, pois seu volume é expressivo, variando de 8 a 20 litros por litro de etanol produzido. Tradicionalmente, a vinhaça crua tem sido aplicada diretamente no solo como fertilizante líquido. No entanto, esta prática, sem orientação adequada, pode levar à degradação do solo, desequilíbrio de micronutrientes, mudança de pH e acumulação excessiva de matéria orgânica. Para resolver esses problemas e ainda utilizar a vinhaça como fertilizante, foi analisado o processo de degradação anaeróbia da vinhaça em biodigestores. O efluente tratado resultante, conhecido como vinhaça digerida, foi então comparado à vinhaça bruta, em termos de parâmetros de poluição e balanço mineral crucial para o cultivo da cana. Os resultados demonstraram uma redução significativa na carga orgânica da vinhaça, com a remoção da demanda química de oxigênio (CQO) variando de 66% a 85%. Após a aplicação da vinhaça digerida em solo de cultivo semelhante ao da cana-de-açúcar, observou-se uma diminuição notável do impacto ambiental causado pelo excesso de matéria orgânica. Além disso, o estudo constatou que elementos nutricionais importantes, como o sódio (Na), não têm efeitos adversos ou se acumulam excessivamente quando comparados ao cálcio (Ca) e magnésio (Mg). Em conclusão, a pesquisa demonstra que a degradação anaeróbia da vinhaça em biodigestores pode efetivamente reduzir sua carga orgânica e mitigar o impacto ambiental associado à aplicação direta da vinhaça bruta em solos agrícolas

Palavras-chave: vinhaça, tratamento anaeróbio, indicadores de poluição, microelementos.

1 INTRODUCTION

Vinasse is a dark brown wastewater that results from the distillation process of fermented sugarcane juice or molasses for the production of first-generation ethanol (Moraes et al., 2015). It is characterized by a low pH (4-5) due to the presence of organic acids such as acetic, butyric, iso-butyric, lactic, succinic, malic, among others (Fuess et al., 2018). Vinasse also contains an excess of macronutrients like sodium, potassium, magnesium, and phosphorus, and it has a high organic load, with COD levels ranging from 15,000 to 45,000 mg of O_2 per liter (López-González et al., 2017; Marcato et al., 2019; Salomon and Lora, 2009). According to the Organisation for Economic Co-

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operation and Development (OECD) and the Food and Agriculture Organization (OECD/FAO, 2018), Brazil is the largest producer and supplier of sugar and ethanol from sugarcane. The production of vinasse in traditional ethanol factories ranges from 8 to 20 liters per liter of ethanol (Boncz et al., 2012; Cabrera-Díaz et al., 2016; Cabrera-Díaz et al., 2017; Moraes et al., 2015; Ribas et al., 2009; Rocha et al., 2007; Salomon and Lora, 2009). With Brazil accounting for 50 percent of the global sugarcane-based bioethanol production (Pedro-Escher et al., 2016), proper management of this waste is a concern for producing countries. The National Supply Company of Brazil (CONAB), estimates that sugarcane production in the 2023/24 harvest should grow by 4.4% in relation to the 2022/23 cycle, reaching aroud 637.1 million tons (CONAB, 2023). Since the creation of the National Alcohol Program in 1975, the disposal of vinasse in water bodies has been prohibited by law since 1978 (BRAZIL, 1978). Vinasse, rich in potassium (2,000 mg/L), carbon (10,000-20,000 mg/L), and nitrogen (400 mg/L), is commonly applied directly to sugarcane fields as fertilizer at rates ranging from 50 to 200 m^3 per hectare (Christofoletti et al., 2013; Elia-Neto and Nakahodo, 1995; Fuess et al., 2017; Rodrigues Reis and Hu, 2017).

While the application of vinasse to the soil can have positive effects, such as increasing pH and cation exchange capacity (CEC), promoting stable aggregates, improving soil moisture, water retention, and supporting the development of soil microflora and fauna (Ferreira and Monteiro, 1987; Tejada et al., 2007), long-term application of vinasse can potentially harm the terrestrial and aquatic environment. Adverse effects may include the use of chemical amendments to raise soil pH, leaching of organic and inorganic compounds into groundwater, degradation of surface water quality, deterioration of soil structure, and unpleasant odors near distilleries (Pathak et al., 1999; Rolim et al., 2013; Christofoletti et al., 2013; Matos et al., 2013; Fuess and Garcia, 2014; Oliveira et al., 2013).

The application of the biorefinery concept has been expanding in the scientific community (Morais, Zaiat and Bonomi, 2015). In this sense, the application of Anaerobic digestion in ethanol production plants, for the treatment and use of organic residues, such

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as vinasse, is an alternative for the production of energy and also for the generation of by-products (Morais, Zaiat and Bonomi, 2015 ; Barros et al., 2016).

Anaerobic digestion is a biological symbiosis that promotes the conversion of complex organic compounds into a variety of chemicals, including methane, carbon dioxide, ammonia, water, sulfhydric acid, and biomass (Deublein and Steinhauser, 2008; Lettinga et al., 1996). This technology is widely used to process various organic waste, such as food waste (Zhang et al., 2011; Pavi et al., 2017), urban effluents (Braga et al., 2018) and agro-industrial waste (Janke et al., 2015; 2016a; 2016b; 2016c; 2016d; 2017; 2018). It can also determine the reduction of environmental pollution caused by the improper disposal of these residues (Xu et al., 2018). From agronomic aspect, this treatment process can reduces the organic content of waste without interfering with the inorganic nutrient content, maintaining the quality of the biofertilizer, generated as a bottom by-product of biodigestersfor. Regarding energy aspects, anaerobic digestion will generate Biogas as a final product, which can be used as renewable energy source (Ferraz Júnior et al., 2016; Moraes et al., 2015; Santos et al, 2019).

In this context, this study aimed to compare the effects of the use of digested vinasse in comparison with raw vinasse applied to the soil as fertilizer with a view to a more complete analysis of the polluting potential of the application without technical criteria of this industrial effluent from the sulco-alcohol industry. Analyzes tried to corroborate data on soil fertility and health with pollution and environmental impact criteria.

2 MATERIALS AND METHODS

2.1 SOIL SAMPLES

Soil samples were collected in a region of sugarcane cultivation (Paraná, Brazil). The soil used is classified as Dystrophic Red Latosol - according to the Brazilian classification -3rd categorical level, a type of soil that, under natural conditions, has low fertility and the physical properties allow the infiltration and redistribution of water and oxygen (Santos et al., 2018). The Köppen climatic classification for the region is the Cfa type (humid subtropical climate), with precipitation, maximum temperature, minimum

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temperature and relative humidity of a ten-year average (2008-2018) equivalent to 158 mm, 28.5 ºC, 18.1 ºC and 69.62%, respectively (INMET, 2018).

The soil collection was layered fractional (0-5, 5-10, 10-20, 20-40, 40-60 and 60-80 cm depth), which were submitted to manual fragmentation. The samples were air-dried in a greenhouse experiment and sieved to 2.0 mm following the protocol of Teixeira et al. (2017). Regarding the sorption complex (pH, Ca, Mg, K, Na, Al and H + Al), the sum of the bases (SB) was calculated:

$$SB = Cas + Mgs + Ks + Nas \quad (1)$$

Potential cation exchange capacity (CEC) at pH 7.0

$$CEC = SB + H + Al \quad (2)$$

Base saturation (V)

$$V = (SB / CEC)x100 \quad (3)$$

Higher V values indicate better chemical conditions for agricultural soils (Santos et al., 2018).

2.2 PHYSICO-CHEMICAL CHARACTERIZATION OF VINASSE

Vinasse was collected from a sugar and ethanol-producing plant, naturally cooled and stored at 4 °C. Natural vinasse samples were analyzed for the following parameters, pH and turbidity. Apparent and true color (Platinum-Cobalt Standard Method). For nitrogen (N) concentrations the Persulfate Digestion Method was used and for the determination of phosphorus pentoxide (P_2O_5) the Acid Persulfate Digestion Method (APHA, 1998). Chemical demando of Oxygen (COD) (Method 5220 D ,), biochemical oxygen demand (BOD) (Method 5210 B - APHA, 1998), total solids (TS) (Method 2540 B-APHA, 1998), volatile solids (VS) and fixed solids (FS) (Method 2540 E - APHA, 1998),

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and K_2O (potassium oxide), CaO (calcium oxide), MgO (magnesium oxide) and Na_e (sodium) (Methods 3030 D and 3111 - APHA, 1998).

2.3 ANAEROBIC DIGESTION (AD)

For anaerobic digestion, a 1.2 L batch reactor was used. The system was operated in sequential batches with a working volume of 1.0 L. The temperature was kept in the mesophilic temperatures (35 ± 2 ºC), according to Andreoli et al. (2003) and 60 rpm stirring speed. The inoculum used was obtained from an active anaerobic bioreactor.

To feed the biodigester, COD was taken as a basis for the consumption of organic matter. COD concentration was analyzed every 3 days, and after analysis, corrected to a final concentration of 10 g • L^{-1}. The reactor was maintained on this feeding regime for 90 days. Every three days, digested vinasse (DV) was collected for later application in the soil.

COD removal efficiency was calculated by Eq. 4.

$$E_f = \left[\frac{(COD_i - COD_t)}{COD_i}\right] x\ 100 \quad (4)$$

where,

E_f is the efficiency of the AD (%); COD_i is the initial concentration ($g \cdot L^{-1}$) and COD_t is the concentration in time "t" after three days ($g \cdot L^{-1}$).

2.4 SOIL COLUMNS

The experiments for the application of Raw Vinasse (RV) and Digested vinasse (DV) were carried out in in soil columns of polyvinyl chloride (PVC) tubes (100 cm long x 15 cm in diameter), installed inside a greenhouse experiment. Columns were arranged vertically on a 10 cm high support structure, closed at the bases with aluminum screens (18 mesh) and plastic plates to avoid soil losses. Soil deposition was performed in the columns gradually, taking into account the soil density of the region (Moreira et al., 2014) to represent the same sequential order of the profiles in the ambient condition

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of the initial soil layers, until completing the height of 0.8 m. Columns was prepared in triplicate for each treatment: soil that received raw vinasse (SRV) and soil that received digested vinasse (SDV). The application of RV and DV was carried out manually. The soil samples were collected 60 days after application of wastewaters and analyzed on the same day.

The Maximum Dosage (MD) of wastewater was calculated in the soil columns, based on the methodology applied by the São Paulo State Environmental Company (CETESB, 2015), according to Eq. 5. This methodology determines the limit dosage of sugarcane vinasse in soils, according to the initial K_s content and K_2O (RV and DV). The calculations were considered for the soil characteristics of the dystrophic Red Latosol used in the region for sugarcane cultivation, in terms of CEC and K_s content (see in supplementary material – Table S1) and concentration of K_2O of the waste waters (Table 1).

$$MD = \frac{[(0.05\ x\ CEC - K_s)\ x\ 3{,}744 + 185]}{K_2O} \quad (5)$$

where,

MD is the maximum dosage of application of the wastewater ($m^3 \cdot ha^{-1}$); CEC is the Cation Exchange Capacity (total) at pH 7.0 to a depth of 0 to 0.8 meters ($cmol_c \cdot dm^{-3}$); K_s is the average concentration of potassium in the soil to a depth of 0 to 0.8 meters ($cmol_c \cdot dm^{-3}$); 3,744 is the correction factor to convert the results of CEC and K_s expressed in $cmol_c\ dm^{-3}$ to kg of K_2O, on a volume of 8,000 m^3 (1 ha x 0.8 m); 185 is the average mass extracted by the culture of sugarcane (kg of $K_2O \cdot ha^{-1} \cdot crop^{-1}$); and K_2O is the concentration of potassium oxide in the wastewaters (kg of $K_2O \cdot m^{-3}$).

The SRV and SDV soil collection occurred with the aid of a 10 cm diameter pipe to facilitate the depth marking and was carried out after 60 days of the application of either RV or DV wastewaters for the complete mobility of the wastewaters in the six layers. The soil layers were evaluated on the same day of collection, by the determination of the parameters of pH, Ca_s, Mg_s, Na_s, K_s, Al, H+Al and organic matter.

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2.4.1 Quantification of Environmental Impacts Indicators

Maximum dosage (MD) of RV and DV were used to account for the organic load applied to the soil for raw vinasse (AOL_{RV}) and digested vinasse (AOL_{DV}) and populations with equivalent pollution potential for raw vinasse (EPP_{RV}) and digested vinasse (EPP_{DV}). The AOL_{RV} and AOL_{DV} were determined by the Eq. 6 and 7, respectively, by methods established by Fuess et al. (2018). Indicators were used both to determine the EPP_{RV} and EPP_{DV}, using the Eq. 8 and 9, adapted from von Sperling (2007).

$$AOL_{RV} = MD_{RV}\ x\ BOD_{RV} \quad (6)$$

$$AOL_{DV} = MD_{DV}\ x\ COD_{DV} \quad (7)$$

$$EPP_{RV} = \frac{AOL_{RV}}{(BOD_{sweage}\ x\ WCP\ x\ CR\ x\ CP)} \quad (8)$$

$$EPP_{DV} = \frac{AOL_{DV}}{(COD_{sewage}\ x\ WCP\ x\ CR\ x\ CP)} \quad (9)$$

where,

AOL_{RV} and AOL_{DV} are the organic load applied to the soil for raw vinasse (RV) (kg BOD·ha^{-1}) and digested vinasse (DV) (kg COD·ha^{-1}), respectively; MD_{RV} and MD_{DV} are the maximum dosage of RV and DV, respectively (m^3·ha^{-1}); BOD_{RV} and COD_{DV} are the concentration of BOD and COD of RV and DV, respectively (kg·m^{-3}); and EPP_{RV} and EPP_{DV} are the populations with equivalent pollution potential for RV and DV, respectively. In the Eq. 8 and 9, the variables BOD_{sweage}, COD_{sweage}, Water Consumption per Person (WCP) have values of 0.3 kg BOD·m^{-3}; 0.6 kg COD·m^{-3}; 0,17 m^3·hab^{-1}·d^{-1} for a midsize city. The sewage flow/water flow Return Coefficient (RC) and the sugarcane Crop Period (CP), for the same city, correspond to 0,8 and 210 days (7 months), respectively (CONAB, 2018; Fuess et al., 2018; Moraes et al., 2014; USEPA, 2012; von Sperling, 2007; WHO, 2006).

For the analysis of sodification, it was used the data of the physicochemical characteristics of the wastewaters. Sodium/Calcium Ratio (SCR), Sodium Adsorption Ratio (SAR) and Exchangeable Sodium Percentage in the soil at depths of 0.0 to 0.8

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meters (ESP), were determined according to the Eq. 10, 11 and 12, respectively, as reported in Richards (1954).

$$SCR = \frac{Na_e}{CaO} \quad (10)$$

$$SAR = \frac{Na_e}{\sqrt{[(CaO + MgO)/2]}} \quad (11)$$

$$ESP = \ x\ 100 \quad (12)$$

where,

SCR and SAR are the sodium/calcium ratio and sodium adsorption ratio (dimensionless) of the wastewaters, respectively; Na_e, CaO and MgO are the concentrations of sodium, calcium and magnesium in the wastewaters (mEq·L^{-1}), respectively; ESP is the exchangeable sodium percentage of the soil to the depth of 0.8 m (%); and Ca_s, Mg_s, Na_s and K_s are the exchangeable calcium, magnesium, potassium and sodium contents of the soil to the depth of 0.8 m (cmol$_c$·dm^{-3}), respectively.

2.5 STATISTICAL ANALYSES

All studies were conducted, except the initial soil, in triplicates and the results are presented as replicate means, along with the standard deviation. The means were analyzed, with significance level of $p<0.05$ (95% confidence). The graphical representation was sketched through the OriginPro 9.0 software.

3 RESULTS AND DISCUSSION

3.1 CHARACTERISTICS OF THE INITIAL SOIL

The initial analysis of the soil allows the later verification of the accumulation or leaching of minerals and the presence of sand, silt and clay, 689, 42 and 269 g·kg^{-1}, respectively (Table S1), which allowed the framing of the soil in the "Sandy Clay Loam" textural class, according to the classification proposed by IBGE (2007) and Santos et al. (2018).

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The chemical attributes of the sorption complex with respect to the sum of bases (SB) varied from 1.59 to 0.69 $cmol_c \cdot dm^{-3}$. The values of CEC varied from 5.56 to 9.20 $cmol_c \cdot dm^{-3}$. The cations belonging to the SB had the highest value in Cas (0.67 $cmol_c \cdot dm^{-3}$) and the lowest in Ks (0.040 $cmol_c \cdot dm^{-3}$), both in the 40-60 cm layers. Al was the only element that underwent concentration gradient in the layers. In all the layers, the collected soil showed a low pH in $CaCl_2$. However, the potential acidity (H+Al) obtained its highest result in the 0-5 cm layer (8.36 $cmol_c \cdot dm^{-3}$) and lowest value in the 40-60 cm layer (3.97 $cmol_c \cdot dm^{-3}$) (Table S1). The results of the attributes of the sorption complex were close to other studies according to Oliveira et al. (2013) and Zolin et al. (2011), in which they had similar taxonomic and textual characteristics.

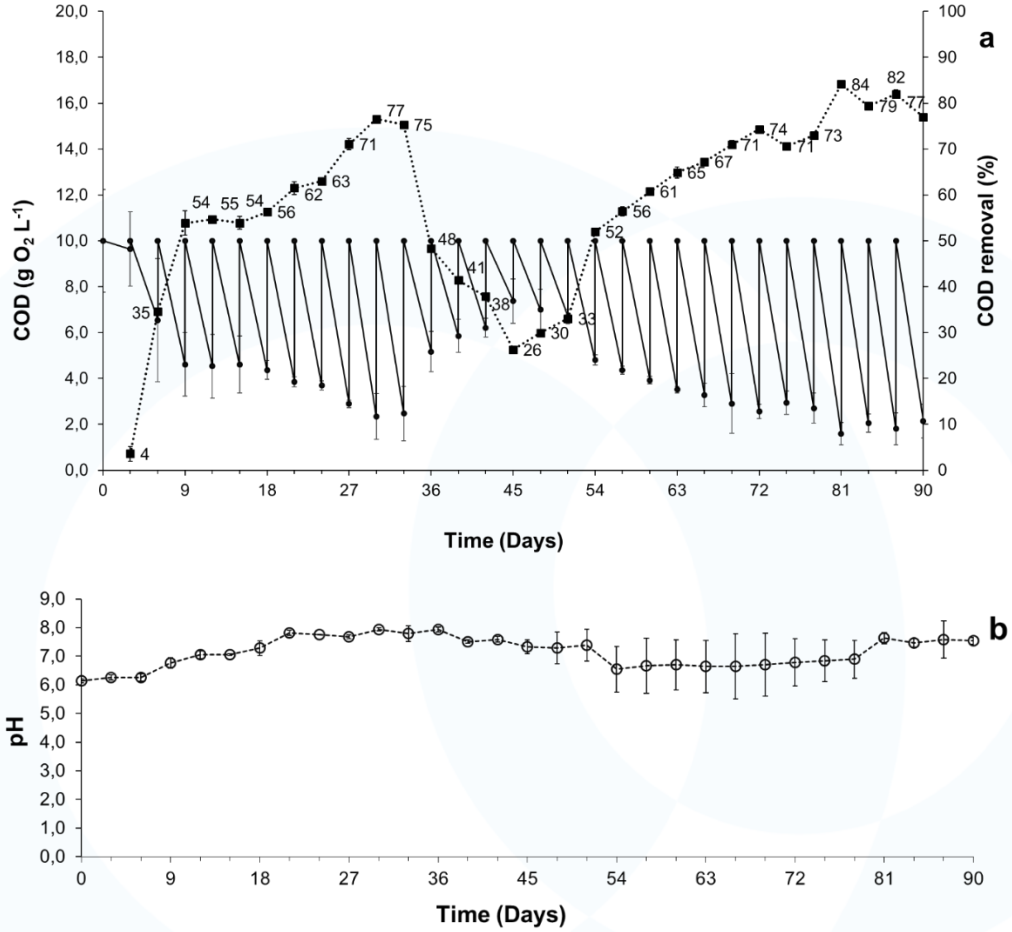
The highest content of organic matter in the soil (19.1 $g \cdot dm^{-3}$) occurred in the first layer, a behavior that was already expected, since it was a soil under native vegetation. Alves et al. (2015) obtained 18 $g \cdot dm^{-3}$ of organic matter in the 0-20 cm layer in Red-Yellow Latosol with 176 $g \cdot kg^{-1}$ of clay and in areas that were free from the application of sugarcane vinasse for more than 10 years. This value corroborates the study with Lourenço el al. (2019) identified that value of 21 $g \cdot dm^{-3}$ also for the 0-20 cm layer of a Red Latosol.

3.2 ANAEROBIC DIGESTION OF THE VINASSE

Fig. 1a shows that, in the first collection, the RV had COD_i of 10 $g \cdot L^{-1}$. After three days, there was a slight advance in the degradation of the RV, presenting an E_f of 4% ($COD_{3^{th}day}$ = 9.6 $g \cdot L^{-1}$). Six days after the operation, the conditions of the reactor improved, achieving a COD removal of 35% ($COD_{6^{th}day}$ = 6.5 $g \cdot L^{-1}$). From nine to twenty-four days of digestion, the treatment showed a significant change in its efficiency, with a range of E_f from 55% to 64% (COD_t from 4.6 to 3.7 $g \cdot L^{-1}$), respectively.

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Figure 1. Parameters of (a) COD (g $O_2 \cdot L^{-1}$) and COD removal (%) (b) pH monitored during experimental anaerobic digestion.



Source: Data obtained by the authors

After 30-days, the system reached a high efficiency (E_f = 77%) (Fig. 1a). Despite this good performance, during the reactor feeding process, around the twenty-seventh day of operation, there was a problem of sealing in the system, resulting in the destabilization of the anaerobic condition and a decrease in the efficiency of the system, which reached 28% ($COD_{45^{th}day}$= 7.4 g·L^{-1}) on the 45th day of operation. However, nine days after maintenance (on the 54th day of operation), the system stabilized the consumption of organic matter and, during the interval of 63 to 90 days, the system returned to its microbiological activities, and was kept in a range of 66 to 85% E_f (COD_t from 3.5 to 2.1 g·L^{-1}) during such range of operation.

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The pH started with 6.1 and regularly had a gradual increase by 21 days reaching 7.8 (Fig. 1b). On the 54th day the pH was reduced to 6.5, increasing only after 81-days, when it remained constant until the end of the anaerobic system, with 90-days. According to Cioabla at al. (2012), the optimum pH range to anaerobic reactors is between 6.8 and 7.2; nonetheless, Andreoli et al. (2003) declare these systems can support operating at a range of pH at 6.5 to 8.0. Excepting the first three measurements, which consisted of the adaptation phase of the microorganisms, this range was kept throughout the treatment of the RV, explaining the good performance of the reactor. The follow up of the treatment of vinasse allowed us to realize that the COD and the pH are interrelated, because, as the COD decreased, the pH got closer to 7.0. At the finish AD experimente, with 90-days, the DV was physico-chemically characterized for its application into soil.

3.3 ANALYSIS OF RAW VINASSE (RV) AND DIGESTED VINASSE (DV)

Most of the parameters of the RV (Table 1) corroborate to the reported in the literature (López-González et al., 2017; Salomon and Lora, 2009; Santos et al., 2017), demonstrating that the material collected is representative. The pH of 4.5 emphasizes the acidity of the vinasse, possibly due to the sulfuric acid inserted before the fermentation stage by the sugarcane factories, as mentioned by Ensinas et al. (2009). The high values of turbidity, apparent color and true color, of 3,900 NTU, 29,967 uH and 6,900 uH, respectively, are due to melanoidins present in the vinasse, which are, according to Maillard (1913), Plavšić et al. (2006) and Wedzicha and Karupto (1992) natural compounds from dark brown amino acids and sugars, resulting from non-enzymatic browning reactions.

Table 1 - Physicochemical characteristics of the RV and DV.

Parameters	Raw vinasse (RV)	Digested vinasse (DV)	Removal (%)
pH	4.5 ± 0.02	7.4 ± 0.10	-
Turbidity (NTU)	3,900 ± 50	1,933 ± 104	50
Apparent Color (uH)	29,967 ± 252	16,159 ± 934	46
True Color (uH)	6,900 ± 50	5,617 ± 126	19
COD ($g \cdot L^{-1}$)	33.548 ± 0.083	2.175 ± 0.164	94
BOD ($g \cdot L^{-1}$)	14.933 ± 0.088	1.123 ± 0.051	92
TS ($mg \cdot L^{-1}$)	34,664 ± 311	9,998 ± 556	71

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VS ($mg \cdot L^{-1}$)	25,647 ± 818	2,911 ± 239	89
FS ($mg \cdot L^{-1}$)	9,017 ± 1068	7,087 ± 373	21
N ($mg \cdot L^{-1}$)	530 ± 46	415 ± 22	22
P_2O_5 ($mg \cdot L^{-1}$)	105 ± 5	85 ± 4	19
K_2O ($mg \cdot L^{-1}$)	3,431 ± 32	3,042 ± 30	11
CaO ($mg \cdot L^{-1}$)	684 ± 19	522 ± 8	24
MgO ($mg \cdot L^{-1}$)	765 ± 6	572 ± 9	25
Na_e ($mg \cdot L^{-1}$)	55 ± 4	32 ± 1	42

Source: Data obtained by the authors

The COD (33.548 $g \cdot L^{-1}$) and BOD (14.933 $g \cdot L^{-1}$) were extremely high and the COD/BOD ratio of 2.2 indicates the high biodegradability of the RV. Regarding the solid contents, the fractions of volatile solids (VS) and fixed solids (FS) (9,017 and 25,647 mg·L-1), which correspond to 74% and 26% of the total solids (TS), respectively, indicate a large amount of volatile organic acids required for the AD.

For the DV, it is noted that the pH value increased in relation to the RV, being close to neutrality. Polprasert (2007) states that this neutralization occurs due to the action of the hydrogenotrophic and acetoclastic *Archaea*, which deplete the products of acetogenesis (the initial phase of digestion), composed essentially of organic acids. After the treatment, the DV presented lower values of turbidity, apparent color and true color, showing removals of 50%, 46% and 19%, respectively.

The high removal efficiency of COD (94%) and BOD (92%) the efficiency of the AD process, within the expected removal pattern (Chernicharo, 2007). In the RV, the concentration of volatile solids was higher than the concentration of fixed solids, representing 74% of the total solids. However, after the biodegradation of the RV, volatile solids of the DV became smaller than the fixed solids, representing 29% of the total solids. Santos et al. (2017) also noted that the fixed solids are associated with the presence of salts in the vinasse and, therefore, they are little reduced by the AD. The removals of TS, VS and FS were 71%, 89% and 21%, respectively.

Looking at the aspect of the agronomic potential of biodigested vinasse the macronutrients N, P_2O_5, K_2O, CaO and MgO had a removal of 22%, 19%, 11%, 24% and 25%, respectively. According to Elia-Neto (2016), in the anaerobic treatment of wastewaters, only the parameters of organic origin are degraded, and the sugarcane

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vinasse is kept with nutritional characteristics but with a lower amount of COD and BOD, what may result in an improvement as biofertilizer. The composition of vinasse from cane-based fermentations depends on whether the feedstock was molasses alone or juice; the latter being of higher sugar purity than the former. In general, the main componential mineral compounds are potassium, calcium and sulfate ions, dead yeast cells, minerals from the metabolic process of microorganisms, alcohol and residual sugar, insoluble mineral compounds, and mineral volatile compounds (Meyer et al., 2011; Prasad and Shih, 2016).

Trace metal supplementation is one method to increase VFA utilization, and the inclusion of Fe was found to have a stimulatory effect on acetate utilization by methanogens (Speece, 1962). When Espinosa et al. (1995) increased the organic loading on a UASB reactor treating cane molasses stillage from 5 to 21.5 kg COD m^{-3} d^{-1}, an accumulation of VFAs, principally propionic acid, was observed. The addition of Fe (100 mg L^{-1}), Ni (15 mg L^{-1}), Co (10 mg L^{-1}), and Mo (0.2 mg L^{-1}) reduced the level of acetic acid and propionic acid significantly (1100 to 158 mg L^{-1} and 5291 mg L^{-1} to 251 mg L^{-1}, respectively). Although metals are available in the reactor at high total concentrations, the stimulating effects of these metals still can be observed, indicating that the metals can be present in a non-bioavailable form (Zandvoort et al., 2006).

The high efficiency of removal of COD (94%) and BOD (92%), the efficiency of the AD process, indicates that the trace elements were essential for the growth of anaerobic microorganisms.

3.4 CHANGES IN CHEMICAL ATTRIBUTES OF THE SOIL AND ENVIRONMENTAL IMPACTS AFTER VINASSE APLICATION

Combining the results of Cation Exchange Capacity (CEC) and Ks (Table S1) as well as the K_2O of the RV and DV (Table 1) and applying them in Eq. 5, was determined the maximum dosage (MD) of RV and DV to be applied into the soil, which corresponded to an irrigation of 370 and 418 $m^3 \cdot ha^{-1}$, respectively. As the application was carried out manually, the area from the base of the tubes was taken into account, resulting in a volume of 654 and 738 mL per soil column for RV and DV, respectively. In order to better

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visualize the changes in the chemical attributes of the soil after the application of RV and DV, the MD results obtained in order to draw the depth curves were used as a function of the initial soil concentration, SRV and SDV (see in supplementary material – Figure S2).

The pH (in $CaCl_2$) of the soil, after applying the wastewaters, increased only in the first three layers (Fig. S2a) and, in the others, it remained similar to the initial soil (average pH of 3.9). The pH of SRV increased 1.2, 1.0 and 0.2 in relation to the initial soil, corroborating the considerations to Ferreira and Monteiro (1987), that the vinasse has the property of increasing the pH of the soil. Such behavior may be related to both the introduction of basic ions (i.e. Ca_2^+ and Mg_2^+) commonly found in stillage and the consumption of H^+ ions in redox reactions conducted by microorganisms (Brito et al., 2009; Sivaloganathan et al., 2013). The pH of the SDV increased 2.5, 2.1 and 1.3 in relation to the initial soil, whose neutralization effect may be one of the advantages of applying DV, since it results in a reduction of the use of chemical correctives.

In general, salinization comprises the main adverse effect from the application of wastewaters to agricultural fields, so that several impacts may be observed: [i] reduction on the soil's osmotic potential, in order to unbalance the absorption of water and nutrients by the plants; [ii] generation of toxicity due to the presence of specific ions; and [iii] destruction of the soil structure by dispersing soil particles and clogging up pores (Tejada and Gonzalez, 2006; USEPA, 2012; WHO, 2006). Special attention should also be directed to the presence of sodium in stillage due to the occurrence of soil sodification (Fig. S2d), in which Na+ ions act as dispersive agents by disrupting soil aggregates, in order to clog soil pores up and reduce water infiltration (Richards, 1954; Tejada and Gonzalez, 2006; Tejada et al., 2007). This explains why the leaching of the salts was smaller in the lower layers, probably due to the clogging of soil pores due to the accumulation of salts in the upper layer (0-5, 5-10 and 10-20 cm).

Initially, the average of Cas was 0.46 $cmol_c \cdot L^{-1}$. With the use of the wastewaters, there was an accumulation of Cas in all layers. The highest Cas were observed in the first three superficial layers (0-5, 5-10 and 10-20 cm). In the SRV, for these same layers, the values of Cas were 4.33, 3.89 and 3.69 $cmol_c \cdot dm^{-3}$, and in the SDV, they were 5.00, 12.00, 4.53 and 3.32 cmolc·dm-3, respectively (Fig. S2b). The mean in the six layers for the

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SRV and SDV was 2.97 and 2.89 $cmol_c \cdot dm^{-3}$, respectively, showing that the effects on Cas after applying RV and DV were similar.

The average of the Mg_s in the initial soil was 0.34 $cmol_c \cdot dm^{-3}$. After the application of RV and DV (Fig. S2c), its concentration increased to 2.66 and 3.27 $cmol_c \cdot dm^{-3}$, respectively, both in the 0-5 cm layer. However, the concentrations in the SRV and SDV were close, along the depths, resulting in the average concentration for the six layers of 2.06 and 2.25 $cmol_c \cdot dm^{-3}$, respectively.

Fig. S2d shows that both soils (SRV and SDV) underwent significant changes in the Na_s along the depths, whose initial soil mean was 0.26 $cmol_c \cdot dm^{-3}$. In the SRV, the highest and lowest increase was found in the 0-5 cm layer (0.63 $cmol_c \cdot dm^{-3}$) and 20-40 cm layer (0.35 $cmol_c \cdot dm^{-3}$), whereas, in the SDV, these values are in the 0-5 cm layer (0.61 $cmol_c\ dm^{-3}$) and 60-80 cm layer (0.23 $cmol_c\ dm^{-3}$), respectively. The average concentration was 0.43 and 0.38 $cmol_c \cdot dm^{-3}$ for the SRV and SDV, respectively. The small accumulation of Na_s can be explained by the small Na_e, which were 55 and 32 $mg \cdot L^{-1}$ for RV and DV, respectively.

The pollution indicators of sodification (SCR, SAR and ESP) were analysed. For RV, these indicators resulted in 0.098, 0.43 and 7.6% (SRV), and for DV, it was 0.075, 0.29 and 6.7% (SDV). The change was low due to the small difference in Na_e detected in the RV compared to DV, which is only 23 $mg \cdot L^{-1}$, because the SCR and SAR of Brazilian soils cultivated with sugarcane are generally low, due to the small size of Na_e when compared to CaO and MgO. According to Silva et al. (2007) and Silva et al. (2014) wastewater irrigation with high of Na_e, in relation to CaO and/or MgO is unsuitable for the application into the soil. The values were well below the toxic limits established by the aforementioned authors, which is 3, 10 and 15%, respectively (Richards, 1954; USEPA, 2012; WHO, 2006).

The problems caused when SCR, SAR and ESP exceed these limits are varied. Tejada and Gonzalez (2006) and Tejada et al. (2007) stated that the soil sodification, it may increase the exchangeable sodium content in the soil and destroying its structure by the dispersion of the particles and pore clogging, reducing the rate of infiltration, allowing the surface runoff, favoring erosion and, consequently, degrading surface waters. The AD

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system has gained great advantage by not using dosage applications of Na-based alkalizing compounds such as NaOH and $NaHCO_3$, this alkalizing dosage applications may significantly increase sodium concentrations in vinasse, which may trigger negative impacts in soil.

In addition, a higher detachment of Nas can salinize groundwater or leach until it reaches the water table, being able to perceive the influence of the ESP indicator, which is directly proportional to the Nas parameter. That is, when looking at Eq. 12, it appears that an increase in Nas tends to increase the percentage of ESP.

Before the addition of the RV and DV, the average Ks was 0.056 cmolc·dm-3 (the lowest of all SB elements), being explicit by Fig. S2e that both the SRV and the SDV generated a sudden increase in the concentration of potassium in the soil, only in the superficial layer (0-5, 5-10 and 10-20 cm). For the SRV, the increase in relation to the initial soil in these layers was 3.05, 1.53 and 0.40 $cmol_c \cdot dm^{-3}$, and for the SDV, it was 3.88, 1.98 and 0.67 $cmol_c \cdot dm^{-3}$, respectively. The last three layers of the SRV (20-40, 40-60 and 60-80 cm) had an increase, compared to the initial soil, with concentrations of 0.049, 0.004 and 0.001 $cmol_c \cdot dm^{-3}$, as well as to the SDV, which were 0.075, 0.026 and 0.001 $cmol_c \cdot dm^{-3}$, respectively.

The concentration of potassium in the vinasse (K_2O) is, in most cases, higher than the mineral requirements. Therefore, the need to perform the control of how much vinasse is added into the soil is evident, so that the K_s does not exceed 5.0% of the CEC. This must be realized because, when this limit is reached, the application of vinasse will be restricted to the replacement of this nutrient as a function of the average extraction of the sugarcane crop, which is, 185 kg of K_2O per hectare per crop, as recommended by CETESB (2015). Despite the technical recommendation of the environmental agency of the State of São Paulo, there is no Brazilian legislation, up to now, that refers to K_2O as a contaminant of water. However, considering successive applications of vinasse, especially when adequate criteria for defining dosages and planning of the irrigation systems are not considered, a deterioration in the physico-chemical characteristics (i.e. agricultural potential) of the soil will be observed, also resulting in impacts on superficial water bodies and groundwater (Fuess and Garcia, 2014; Rolim et al., 2013).

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The average from initial Al was 0.58 $cmol_c \cdot dm^{-3}$ and, after the use of the wastewaters, this trivalent cation had its value reduced mainly in the superficial layer (0-20 cm). In this layer, concerning the initial soil, Al from the SRV and SDV reduced to 0.25 and 0.26 $cmol_c \cdot dm^{-3}$, respectively (Fig. S2f). Regarding toxicity to plants, high concentrations of aluminum should be highlighted, since it may severely reduce the nutrients uptake by the plant roots due to structural modifications. Such negative effects are enhanced under acidic conditions (pH < 5.5), due to the higher solubility of aluminum, as well as of other metals in general (Fuess and Garcia, 2014; Petruzzelli, 1989).

In the potential acidity (Fig. S2g), an elevated difference in the soil was observed after applying the RV and DV, with the highest value of 7.75 cmolc·dm-3 (0-5 cm layer) for the SRV and 6.95 $cmol_c \cdot dm^{-3}$ (10-20 cm layer) for the SDV. The last three layers of the potential acidity had mean values of 4.73 and 4.70 $cmol_c \cdot dm^{-3}$ for the SRV and SDV, respectively. As discussed in Fig. S2a, the pH was further changed in the first two layers for the SRV and in the last three for the SDV.

The attributes belonging to the SB had a gradient from the highest to the lowest concentration along the depths of the soil (Fig. S2h), with the exception of Nas, which changed radically along the profile. Although K_2O was the macronutrient in the highest value of the wastewaters, the elements of the sum of base, exchangeable CaO and MgO, had a greater mobility along the layers.

The average CEC of the soil, for the six layers, increased from 6.92 $cmol_c \cdot dm^{-3}$ (initial soil) to 12 $cmol_c \cdot dm^{-3}$ (SRV and SDV) (Fig. S2i), resulting in an increase for both of approximately 5.1 $cmol_c \cdot dm^{-3}$. This condition may have occurred due to the fact that the average of Cas, Mgs, Ks, Nas and H+Al were very close in the six layers. Increase in the soil's Cation-Exchange Capacity (CEC), which improves its capacity in retaining and releasing nutrients to the plants, especially potassium, calcium and magnesium (Fuess and Garcia, 2014). However, high salt concentrations can produce adverse effects on the soil and osmotic potential could be generated. CEC is an extremely important factor, as it expresses the ions retention capacity of the soil.

The base saturation (V value), in relation to the average of the six layers of the initial soil (17%), increased mainly in the 0-5 cm layer, with values of 59 and 73% for

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SRV and SDV, respectively (Fig. S2j). The high values of V after applying the wastewaters are due to the considerable increase of the attributes belonging to the SB.

In relation to the organic matter (Fig. S2k), was verified that the SRV presented a considerable increase in relation to the average of the initial soil (11.5 $g \cdot dm^{-3}$), mainly in the last three layers, reaching a value of 38.76 $g \cdot dm^{-3}$ (60-80 cm layer). However, the organic matter of the SDV sharply decreased along the soil layers from 26.88 $g \cdot dm^{-3}$ (20-40 cm) to 6.94 $g \cdot dm^{-3}$ (60-80 cm). The organic matter average for SRV and SDV, in the six soil layers, was 31 and 21 $g \cdot dm^{-3}$, respectively. This difference of 10 $g \cdot dm^{-3}$, after applying RV and DV, can be explained by the high removal efficiency of COD, BOD and volatile solids by the AD.

From the bio-digestion process, there is a change in total organic carbon (TOC), observing a greater portion of dissolved organic carbon. According to Marques et al. (2012) the dissolved organic carbon shows temporal, spatial and depth variation, decreasing in the latter. According to Rolim et al. (2013), high organic loads, regardless of the culture to be applied, can be leached into groundwater, causing problems in the potability of the water. The use of raw vinasse in the fertirrigation of sugarcane has been studied for years, with the results indicating the adverse effects of its use on the environment (Marcato et al., 2019). Centurión et al. (1989) observed that high vinasse rates in soil led to undesirable effects, for example, sugarcane quality impairment in sugar production, groundwater pollution, and soil sodification. Pathak et al. (1999) and von Sperling (2007) state that the application of wastewaters from distilleries to rice crops and of domestic sewage to the soil is only beneficial when it has BOD of up to 1.0 and 0.4 $g \cdot L^{-1}$, respectively. Considering the BOD of the RV, which is 14.933 $g \cdot L^{-1}$, this value considerably exceeded the concentration suggested by the aforementioned researchers.

Although the soil has an excellent filtering power, the vinasse can reach and contaminate the water table through hydrological routes (Rolim et al. 2013). After the treatment, the BOD of the DV decreased to 1.123 $g \cdot L^{-1}$ and, consequently, the contents of the organic matter from the SRV in relation to the SDV. In this situation, the affected layers were the last two, with a significant difference between each other, and the concentration of organic matter was 24.42 $g \cdot dm^{-3}$ (40-60 cm) and 31.81 $g \cdot dm^{-3}$ (60-80

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cm). Both the SRV and the SDV had an increase of the organic matter in the superficial layer (0-20 cm). However, in the SRV, these values also exceeded in deeper layers, and it could cause damage in depths greater than 80 cm, with direct consequences in groundwater.

About to the inputs of organic matter (Eq. 6), the soil showed values of AOL_{RV} of 5,529 kg BOD·ha^{-1}. This high amount of easily degradable organic matter, continuously fed to the soil microbiota, can trigger numerous biological processes, especially the uncontrolled release of greenhouse gases. According to Moraes et al. (2014) and Oliveira et al. (2013), the excess of organic matter in the soil can stimulate microbial activity, which rapidly disrupts the levels of oxygen and generates conditions of anaerobiosis. However, in the course of digestion, the AOL value decreased in 83.6%, that is, the AOL_{DV} (Eq. 7) was 908 kg COD·ha^{-1} .

Organic overload can be harmful for the soil structure by dispersing soil particles and clogging up pores and promote reductions in dissolved oxygen (DO) concentrations and microbial activity, as well as stimulating the generation of unpleasant odors due to putrefaction (ie anaerobic consumption) of readily available organic carbons and of sulfides via sulfate-reduction (Fuees and Garcia, 2014). The environment generated by the application of raw vinasse in the soil creates an environment conducive to microorganisms to degrade organic matter, in such cases, local anaerobiosis is generated by the clogging up of pores, as poor aeration zones are created. Such impacts are mainly related to greenhouse gas emissions such as CH_4, N_2O, and CO_2, of which 99.8% belong to methane (do Carmo et al., 2013; Oliveira et al., 2013; Parsaee et al. 2019).

The EPP_{RV} was 645 hab·ha^{-1} (Eq. 8). These values are 8.2–9.2 fold higher than the pollution load for the largest population densities observed in Brazilian capital cities, such as Fortaleza (78 inhab·ha^{-1}), São Paulo (74 inhab·ha^{-1}), Belo Horizonte (72 inhab·ha^{-1}), and Recife (70 inhab·ha^{-1}) (IBGE, 2016). When going through the anaerobic treatment, it fell to 53 ha^{-1} (EPP_{DV}) (Eq. 6), and was 12.2 times lower than the EPP_{RV}. The controlled input of organic matter into the soil triggers numerous beneficial effects, such as the formation of stable soil aggregates and an increase in the moisture and CEC of the terrain (Fuess and Garcia, 2014; Tejada et al., 2007).

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4 CONCLUSIONS

In conclusion, the application of anaerobic digestion (AD) has proven to be a highly efficient system for reducing the organic matter present in sugarcane vinasse. The essential nutrients necessary for sugarcane development remained practically unchanged, while also improving the pH of the vinasse. Implementing bio-digestion as the primary treatment process for sugarcane vinasse in sugar plants could lead to more favorable scenarios in terms of reducing environmental problems and increasing sugarcane productivity. However, there are important environmental aspects to consider when applying wastewater to soil columns, such as the low removal of inorganic parameters (CaO, MgO, and K2O) from the vinasse. Therefore, it is crucial to establish preventive dosage criteria to ensure that the vinasse does not reach deeper soil layers. AD was unable to remove the salts present in the raw vinasse, and the sodification parameters remained similar for both the raw vinasse (RV) and the treated vinasse (DV).

The organic load applied to the soil receiving raw vinasse (AOLRV) highlights the high amount of organic matter introduced into the soil, which can lead to various problems in terrestrial and aquatic environments. The equivalent pollution potential (EPP) indicates that raw vinasse represents extremely harmful wastewater. On the other hand, the sodification indicators (SCR, SAR, and ESP) do not report adverse effects of the treated vinasse (DV) due to the concentration of sodium (Na) being much lower than that of the metal oxides CaO and MgO. Furthermore, the acidity level of the soil pH that received the treated vinasse was higher than that of the soil receiving the raw vinasse, reducing the need for liming and chemical corrections that could harm the soil. Therefore, it can be concluded that treating vinasse in AD systems is a good alternative for its application to the soil, offering environmental and fertilizer benefits. However, careful consideration of the environmental aspects and the adoption of preventive measures are essential to ensure proper and sustainable use of vinasse.

ISSN: 1696-8352

ACKNOWLEDGEMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for supporting, and the Research Support Centers Complex of Maringá State University (COMCAP-UEM).

ISSN: 1696-8352

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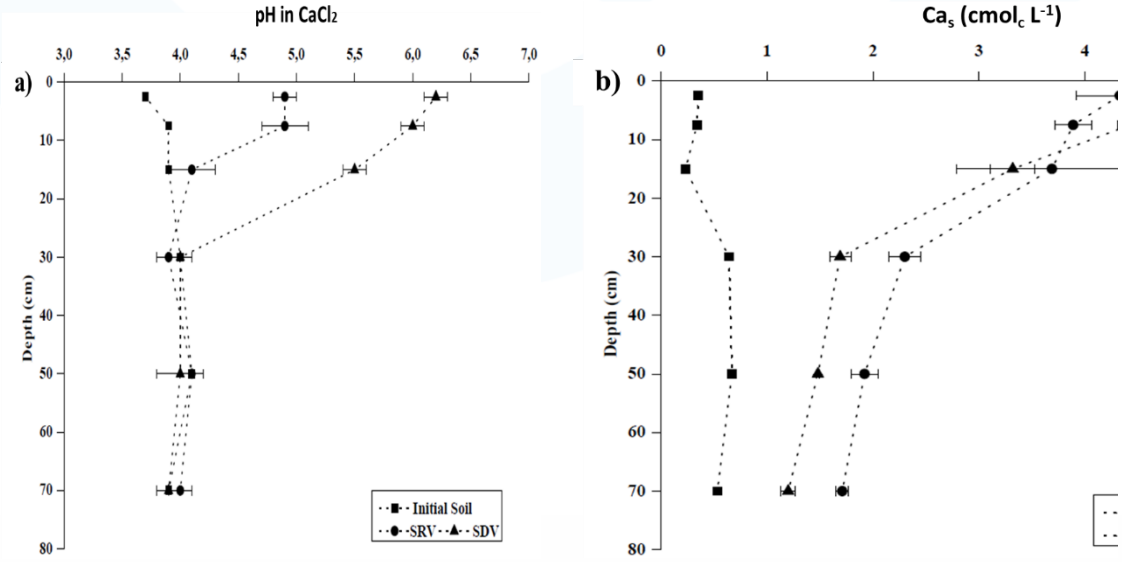
SUPPLEMENTARY MATERIAL

Table S1 - Physicochemical characteristics of the soil, without wastewater application (initial soil).

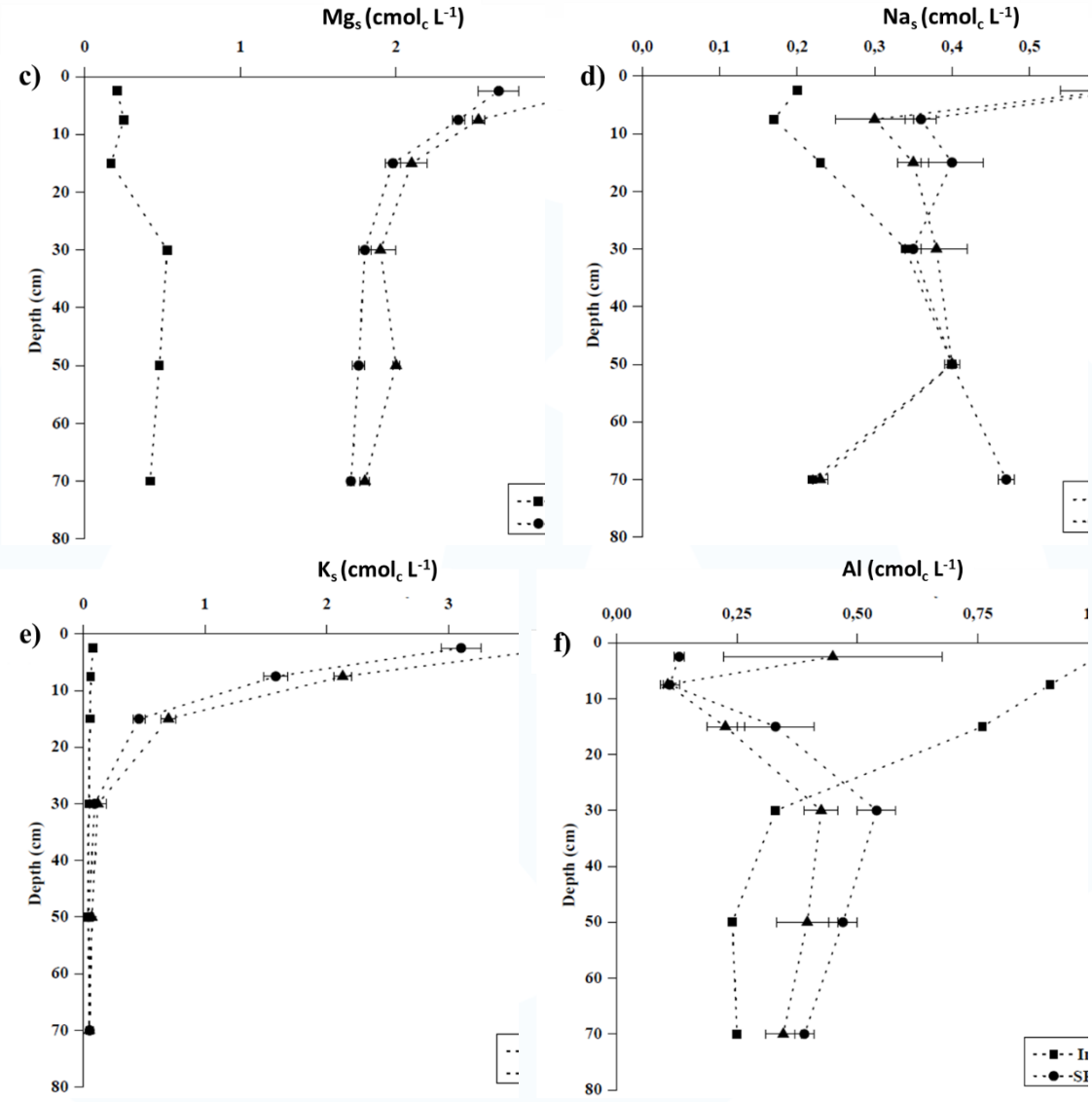
Soil layer (cm)	Sand	Silt	Clay	pH in $CaCl_2$	Ca_s	Mg_s	K_s	Na_s	Al	H+Al	SB	CEC	V	OM
	(g kg^{-1})				($cmol_c$ dm^{-3})								(%)	(g dm^{-3})
0-5	730	70	200	3.7	0.35	0.21	0.080	0.20	1.00	8.36	0.84	9.20	9.11	19.11
5-10	685	60	255	3.9	0.34	0.25	0.062	0.17	0.90	6.69	0.82	7.51	10.91	15.27
10-20	710	60	230	3.9	0.23	0.17	0.055	0.23	0.76	6.21	0.69	6.90	9.93	13.21
20-40	730	20	250	4.0	0.64	0.53	0.045	0.34	0.33	4.61	1.56	6.17	25.22	9.11
40-60	630	20	350	4.1	0.67	0.48	0.040	0.40	0.24	3.97	1.59	5.56	28.60	6.80
60-80	650	20	330	3.9	0.53	0.42	0.052	0.22	0.25	4.96	1.22	6.18	19.77	5.52
Mean ± SD	689 ± 42	42 ± 24	269 ± 59	3.9 ± 0.1	0.46 ± 0.18	0.34 ± 0.15	0.056 ± 0.014	0.26 ± 0.09	0.58 ± 0.35	5.74 ± 1.,61	1.12 ± 0.39	6.92 ± 1.30	17.26 ± 8.47	11.50 ± 5.27

Notes: CEC = cation exchange capacity; OM = organic matter; SB = sum of bases; V = base saturation.
Source: Data obtained by the authors

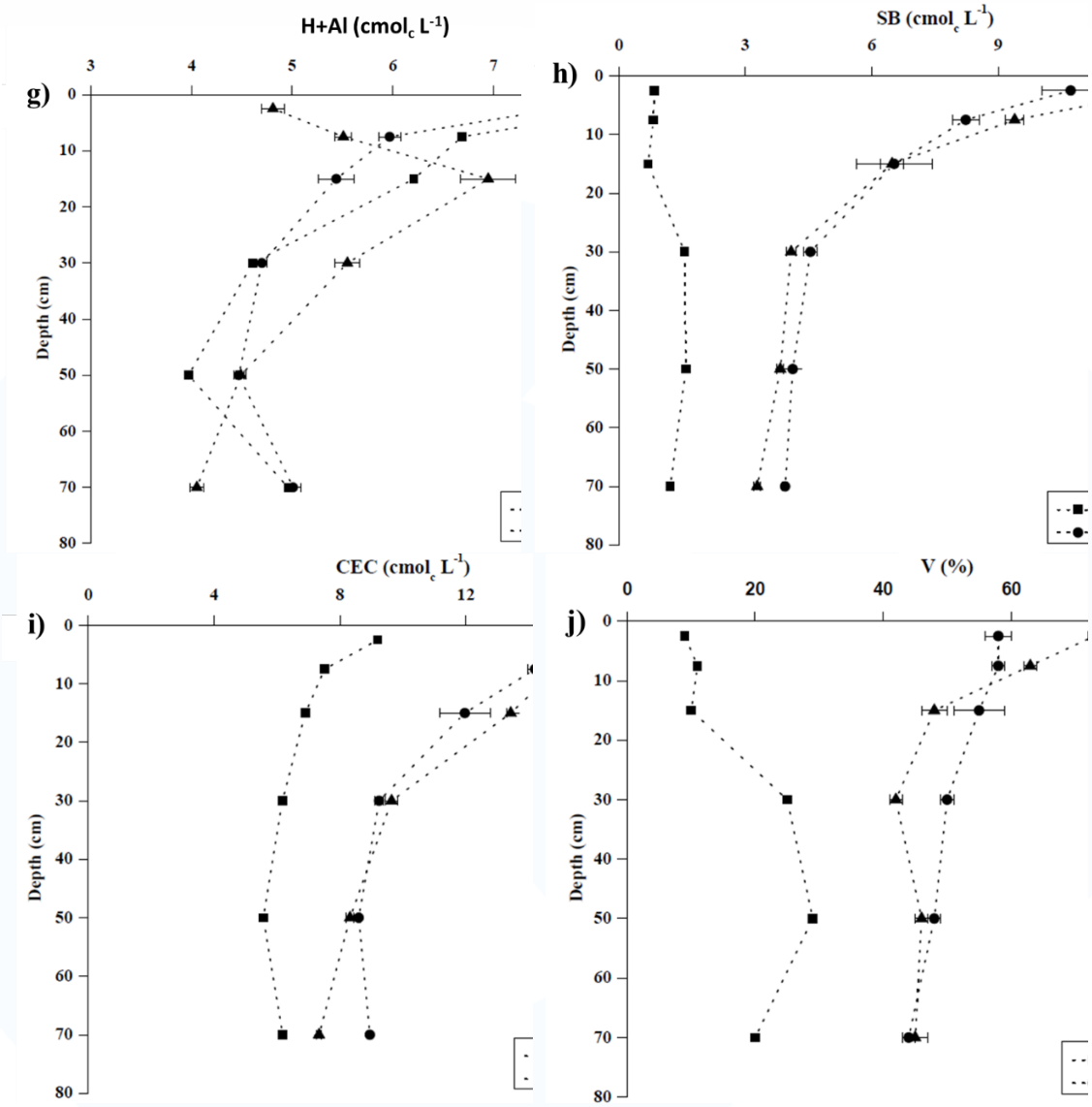
Figure S2. Concentration analyzes of the sorption complex and organic matter in the soil as a function of depth from 0 to 80cm.



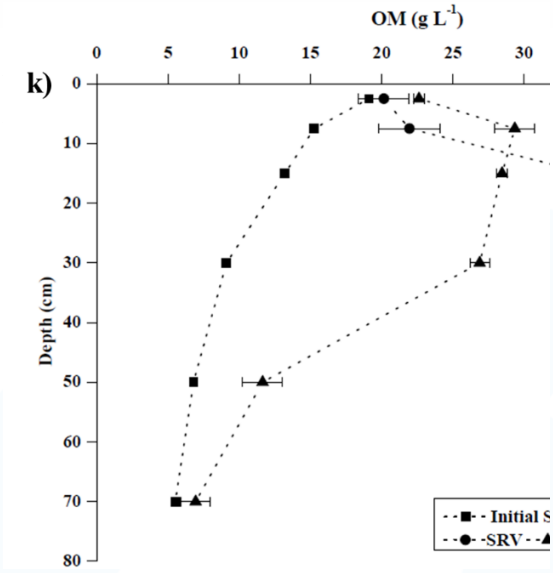
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(a) pH in $CaCl_2$; (b) concentration of Ca_s; (c) concentration of Mg_s; (d) concentration of K_s; (e) concentration of Na_s; (f) concentration of Al; (g) concentration of H+Al; (h) sum of bases (SB); (i) cation exchange capacity (CEC); (j) base saturation; and (k) organic matter (OM). Source: Data obtained by the authors.

NOMECLATURE

AD	anaerobic digestion	
AOL_{DV}	applied organic load into the soil for digested vinasse	
AOL_{RV}	applied organic load into the soil for raw vinasse	
BOD	biochemical oxygen demand	
	BOD_{sewage}	BOD of sewage
	BOD_{RV}	BOD of raw vinasse
CEC	cation exchange capacity	
COD	chemical oxygen demand	
	COD_{sewage}	COD of sewage
	COD_i	initial COD concentration
	COD_t	COD concentration in time "t"
	COD_{DV}	COD of digested vinasse
CP	sugarcane crop period	
DV	digested vinasse	
E_f	efficiency of the AD	
EPP_{DV}	populations with equivalent pollution potential for digested vinasse	
EPP_{RV}	populations with equivalent pollution potential for raw vinasse	
ESP	exchangeable sodium percentage in the soil	
MD	maximum dosage of the wastewaters	
	MD_{RV}	maximum dosage of raw vinasse
	MD_{DV}	maximum dosage of digested vinasse
N	Nitrogen	
P_2O_5	phosphorus pentoxide	
RC	sewage flow/water flow return coefficient	
RV	raw vinasse	

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SALTS	CaO	Effluent calcium oxide concentration
	Ca_s	Soil calcium concentration
	K_2O	Effluent potassium oxide oncentration
	K_s	Soil potassium concentration
	Na_e	Effluent sodium concentration
	Na_s	Soil sodium concentration
	MgO	Effluent magnesium oxide concentration
	Mg_s	Soil magnesium concentration
SAR	sodium adsorption ratio	
SB	sum of bases	
SCR	sodium/calcium ratio	
SDV	soil that received digested vinasse	
SRV	soil that received raw vinasse	
V	base saturation	
WCP	water consumption per person	