Soil change induced by the application of biodigested vinasse concentrate, and its effects on growth of sugarcane

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Vinasse (or stillage) is a byproduct from ethanol production, which contains organic matter, K, N, and other plant nutrients that is regularly used as soil fertilizer. However, high transportation costs limits its application in areas far from distilleries. The possibility of biogas production from vinasse, and the direct or indirect advantages of its use, is a way to reduce costs due to its concentration. Biodigested vinasse concentrate (BVC) is an alkaline product that is very different from common vinasse. Therefore, the objective of this study was to compare the effect of BVC with common vinasse (CV) or KCl, with or without N fertilization, on soil fertility and growth and nutrition of sugarcane (Saccharum officinarum L.) plants. Plants were grown in pots containing Oxisol under different treatments and maintained for 60 d under greenhouse conditions; variables related to soil fertility, plant growth, and mineral nutrition were evaluated. It was observed that adding BVC induces higher soil pH (5.9 to 6.3) and lower potential acidity (13 to 10 mmolc dm-3) compared with KCl, and similar soil chemical changes to CV addition. Plants fertilized with BVC and N showed lower root dry matter (DM) (4.02 g) compared with those fertilized with KCl and CV (6.3 and 5.44 g, respectively). Plants fertilized with BVC have similar total DM (18.25 and 20.31 g) accumulation and nutritional conditions compared with those fertilized with CV and KCl. Plants fertilized with BVC had the highest Na accumulation (0.36 and 0.48 g plant⁻¹).

Key words: Biodigestion, plant growth, Saccharum spp., soil fertility, stillage.

INTRODUCTION

In conventional ethanol production from sugarcane (Saccharum officinarum L.), each liter of ethanol produced results in approximately 12 to 15 L vinasse as a byproduct (Franco et al., 2008). Vinasse has high water content, organic matter (OM), and potassium (K), but also contains nitrogen (N) and other mineral nutrients in low concentrations (Ribeiro et al., 2010). This byproduct is therefore regularly used as soil fertilizer in sugarcane fields with good results (Bebé et al., 2009; Britto et al., 2009; Silva et al., 2014). Due to its high water content, vinasse application in areas far from distilleries is limited by high transportation costs.

The possibility of using vinasse as a primary source for biogas production is a matter of interest since 1981, although the economic viability for different uses of the produced biogas is still a matter for discussion (Higa et al., 2014). Production and use of that energy source, or its indirect advantages (Szimanski et al., 2010; Solomon et al., 2011), is a way to reduce costs due to the concentration of biodigested vinasse. This concentration could make transport viable and the application of K and other nutrients found in vinasse in areas where these nutrients were withdrawn during the sugarcane harvest. However, vinasse biodigestion changes its original composition.

The first change occurs by adding alkaline compounds to raise pH to optimize the biological process of methane production (Döll and Forest, 2010). Biodigested vinasse is an alkaline product. Another change is the loss of organic matter and some N, which is clearly identified by the presence of CH₄, CO₂, and N₂ in the released biogas (Ueno et al., 2013).

Although there is no precise information in the literature about the changes produced by the concentration process, it is expected that the high temperature used for water evaporation, associated with the effect of the alkaline medium, induces other changes that still remain unclear. It is well known that the NH₄⁺ ion changes into NH₃ gas that easily volatilizes from the alkaline medium (Latifah et al., 2011).

These alterations lead to the hypothesis that part of the NH₄⁺ contained in the soil can be lost after biodigested vinasse concentrate (BVC) is applied, which could induce some differences in plant growth. Although vinasse contains a low N concentration, it is generally between 0.1 g m⁻³ (Silva et al., 2014) and 0.73 g m⁻³ (Carvalho...
et al., 2013) when applied at rates of 100 m³ ha⁻¹. These concentrations correspond to rates of 10 to 73 kg N ha⁻¹, which are considered as important values. Whether these amounts of N attributed to vinasse are relevant or not to sugarcane nutrition is still a matter for discussion (Franco et al., 2008). However, it has been shown that the balance between K and N is very important for sugarcane plant growth and ethanol production (Andrade Júnior et al., 2012).

Given the lack of studies on the use of BVC as a fertilizer, its effect on soil fertility and plant nutrition is unclear. Sugarcane growers are not sure about using this byproduct. More information is needed to support its use in sugarcane fields. Therefore, the objective of this study was to analyze the composition of BVC and compare its effect with common (in nature) vinasse (CV) and KCl application in the absence and presence of N fertilization on soil chemical changes, and the effects on growth and nutrition of sugarcane plants.

**MATERIALS AND METHODS**

The experiment was conducted under greenhouse conditions in Jaboticabal (21°14’24” S, 48°17’20” W; 583 m a.s.l.), São Paulo, Brazil. Treatments consisted of three K sources: BVC, CV, and KCl (all applied at rates equivalent to 450 kg K₂O ha⁻¹), along with a control (no K application); these treatments were combined with two N levels (0 and 30 kg ha⁻¹). The experimental design was completely randomized with a 4 × 2 factorial scheme with four replicates for a total of 32 experimental units. Each experimental unit consisted of a 20 dm³ capacity plastic pot containing 18 dm³ soil where one sugarcane seedling was transplanted.

Before seedlings were transplanted, soil was limed to reach 60% of base saturation. After 60 d of liming, soil fertility was determined following indications by Raij et al. (2001), and values were: pH (CaCl₂) 5.7; OM 4 g dm⁻³; resin: 12 mg P dm⁻³; 0.9 mg K dm⁻³, 16 mg Ca dm⁻³, 7 mg Mg dm⁻³, potential acidity (H⁺Al): 11, sum of bases (SB): 23.9, CEC (cationic exchange capacity) 34.9 mmol dm⁻³, and V (base saturation) 68%.

Along with soil preparation, sugarcane seedlings (`IACSP93-3046`) were prepared by planting mini-cuttings (5 cm long) containing one bud each in disposable cups filled with 500 cm³ sand. The sand was moistened daily with tap water, and seedlings were kept under this condition for 60 d to deplete nutrients in the cuttings. Following the recommendation for fertilization when establishing sugarcane (Raij et al., 1997), 14.06 g superphosphate, corresponding to 180 kg P₂O₅ ha⁻¹, was incorporated into the soil before planting. For N treatments, 0.85 g urea (equivalent to 30 kg N ha⁻¹) was also incorporated into each pot. The most uniform seedlings were then selected, removed from the sand, and one seedling was planted in each pot. Potassium fertilizers were applied to the respective treatments 10 d after the planting date. The K rate applied by CV and VC follows the recommendation by Rossetto et al. (2008) when applying it to the total area. This rate is more economic and equivalent to three times more than what is recommended for mineral fertilizer applied to the rows, corresponding to a rate of 450 kg K₂O ha⁻¹. To meet this recommendation, 0.196 dm³ BVC, 2.0 dm³ CV, and 9.7 g KCl were applied to the soil of the respective treatments. Water was added to each pot with the calculated amounts of BVC and KCl to be applied to complete 2.0 dm³ to prevent moisture differences among treatments. The chemical characteristics of BVC, CV, and diluted BVC are shown in Table 1.

Plants were cultivated for an experimental period of 60 d with daily irrigation with tap water. The amount of water applied was determined by weighing a few pots to estimate moisture loss and adding enough water to adjust them to approximately 60% soil water holding capacity. The following parameters were evaluated at the end of the experimental period: plant height (corresponding to the length between soil surface and insertion of the youngest fully expanded leaf, leaf +1), leaf number, tiller number, and leaf area. Leaf area was estimated by the formula: LA = L × W × 0.75 × (N + 2) where L is the length and W is the greatest width of the 3rd fully developed leaf, 0.75 is the shape factor, and N is the number of fully expanded leaves with at least 20% green area added to a correction factor of 2 following the methodology proposed by Hermann and Câmara (1999). After these evaluations, leaf +1 was collected from each experimental unit and the midrib was removed and prepared to determine mineral nutrient content. Plants were then collected and packaged in previously identified paper bags.

Soil was passed through a sieve; a homogeneous soil sample from each pot was taken and prepared to determine fertility. The rest of the soil with roots was removed from the sieve with a jet of water to obtain a clean root system. Roots were washed again in water with detergent (0.01% v/v) and then with tap water, packaged in identified paper bags, and prepared to determine dry weight. All plant samples were dried at 65 to 70 °C until constant weight and dry weight were determined. The DM of each plant sample was ground separately with a Wiley mill and passed through a 20-mesh sieve. The ground material was subjected to acid digestion to determine nutrient levels (AOAC, 1990).

The ANOVA was performed to detect significant differences (P < 0.05) among treatment means and, the

| Material    | pH  | C   | N   | P   | K   | Ca  | Mg  | S   | Na  |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| CV          | 4.00| 18.00|0.04| 0.05| 2.30| 0.34| 0.17| 0.04| 0.10|
| BVC         | 8.80| 15.00|1.48| 1.19| 23.75|3.54| 1.25| 0.98|14.58|
| Diluted BVC | 7.80| 1.46|0.14| 0.11| 2.30| 0.34| 0.12| 0.09| 1.41|
RESULTS AND DISCUSSION

The effects observed in the soil were changes in pH, K and Ca contents, potential acidity (H+Al), and cation exchange capacity (CEC) as a result of applying K sources. Small changes in the CEC value were also detected because of N application (Table 2). The pH was higher in treatments that received BVC and CV. Soil that received these treatments also showed a lower concentration of H+Al. The increase in pH caused by CV application was also reported by Bebé et al. (2009) and Britto et al. (2009). The same effect was also observed in this experiment for BVC (Table 2). The increase in soil pH induced by adding vinasse occurs because OM contained in the residue has a good reducing potential (Brito et al., 2009); when reacting in the soil, it can increase the pH by neutralizing the proton H+ in the soil solution. Biodigested vinasse concentrate had a lower OM concentration compared with CV, but its pH was approximately 8.0 once NaOH was added to the original vinasse to improve conditions and optimize the biological process of methane production (Döll and Forest, 2010), which certainly contributed to increasing soil pH.

Soil, which received BVC, CV, and KCl, showed higher K concentrations than soil in the control treatment. Soil Ca concentrations with the BVC treatment showed an intermediate value that was slightly lower than other K sources and higher than unfertilized soil.

The CEC values observed at the end of the experiment ranged between 32 and 37 mmol c dm⁻³; this is very close to the CEC value of 34.9 mmol c dm⁻³ initially obtained from the soil before treatments. In spite of that, soil that received BVC showed a significantly (P < 0.05) lower value than other treatments. This result seems to occur once the CEC value in this research was calculated by the sum of bases K, Ca, Mg, and potential acidity (H+Al); these values were slightly lower (some not significant at P = 0.05) for soil added with BVC and resulted in a lower CEC value. Given that part of the CEC value of tropical soils corresponds to variable charges coming from OM and that soil pH from the BVC treatment was higher than those from other K sources, it was to be expected that the CEC value for BVC would increase (Camargo et al., 1997), whereas the opposite result was found. Perhaps Na added to the soil by applying BVC might have influenced this result, and more appropriate studies are needed to confirm this effect and detect its possible cause. A low CEC value was also observed when N was applied (Table 2). Adding N induced greater plant growth (Tables 3 and 4) and improved nutrient absorption (Tables 6 and 7). Franco et al. (2007) explain that applying N can promote better macronutrient absorption by sugarcane because of synergism or improved root development, resulting in soil base depletion, which, in turn, can be reflected on the CEC value. The P, OM, and Mg contents, sum of bases (SB), and base saturation (V%) did not change (P > 0.05) because of the treatments (Table 2).

### Table 3. Effect of N levels on plant height, leaf area, and leaf dry matter of sugarcane plants.

| N levels | Plant height | Leaf area | Leaf dry matter |
|----------|--------------|-----------|-----------------|
| cm       | g plant⁻¹    | m² plant⁻¹| g plant⁻¹       |
| 0 kg ha⁻¹| 30.04b       | 0.11b     | 15.75           |
| 30 kg ha⁻¹| 32.07a       | 0.18a     | 15.75           |
| F test   | 8.97*        | 13.59*    | 10.20*          |

Different letters in columns indicate significant differences according to Tukey’s test.

**P < 0.01, nsP > 0.05 according to F-test.

### Table 4. Effect of interaction among K sources (BVC, KCl, and CV) and control (without K source) versus N levels, on root dry matter (RDM) and total dry matter (TDM) per plant of sugarcane.

| Variables | N levels | Control | KCl | CV | BVC |
|-----------|----------|---------|-----|----|-----|
| RDM, g    | 30 kg ha⁻¹| 6.28a   | 4.48b | 6.61| 4.9b |
| 0 kg ha⁻¹ | 30.04b   | 3.04b   | 6.61| 4.9b |
| F test    | 6.61     | 4.9b    | 0.79 | 5.49 |
| TDM, g    | 30 kg ha⁻¹| 22.10a | 23.46a| 21.26a| 15.59a |
| 0 kg ha⁻¹ | 20.72a   | 20.31a | 20.72a| 15.59a |
| F test    | 9.88     | 7.92   | 0.06 | 0.97 |

Uppercase letters compare means within the rows and lowercase letters within the columns. Different letters indicate significant differences according to Tukey’s test.

**P < 0.01, *P < 0.05, nsP > 0.05 according to F-test.

CV: common vinasse, BVC: biodigested vinasse concentrate.

### Table 2. Effect of K sources (BVC, KCl, and CV), control (no K source), and N levels on soil concentration of P, OM, K, Ca, and Mg, as well as on soil pH (CaCl₂), H⁺Al (potential acidity), sum of bases (SB), percentage of base saturation (V%), and soil exchange capacity (CEC).

| K sources | P-resin | OM | pH | K | Ca | Mg | H⁺Al | SB | CEC | V |
|-----------|---------|----|----|---|----|----|------|----|-----|---|
| KCl       | 41a     | 4a | 5.9b| 5.2a| 13ab| 6a | 13a  | 24a | 37a  | 65a|
| CV        | 38a     | 4a | 6.1ab| 4.7a| 13ab| 7a | 11bc | 24a | 35a  | 65a|
| BVC       | 41a     | 4a | 6.3a| 4.3a| 11b | 6a | 10c  | 32b | 37a  | 65a|
| Control   | 37a     | 3a | 5.9b| 0.6b| 15a | 8a | 12ab | 35a | 65a  | 65a|
| F-Test    | 0.4w    | 0.58w | 10.79w | 19.91w | 5.09w | 3.59w | 6.85w | 2.51w | 5.70w | 2.37w|
| N levels  | P-resin | OM | pH | K | Ca | Mg | H⁺Al | SB | CEC | V |
| 30 kg ha⁻¹| 42a     | 4a | 6.0a| 3.9a| 12a | 6a | 11a  | 22a | 33b  | 67a|
| 0 kg ha⁻¹  | 36a    | 4a | 6.0a| 3.6a| 13a | 7a | 12a  | 24a | 36a  | 67a|
| F-Test    | 2.7w    | 4.45w | 12.0w | 0.39w | 4.00w | 6.51w | 3.07w | 4.85w | 7.97w | 0.04w|

Different letters in the columns indicate significant differences according to Tukey’s test.

*P < 0.01, **P < 0.05 according to F-test.

K: potassium, CV: common vinasse, BVC: biodigested vinasse concentrate, N: nitrogen, P: phosphorus, OM: organic matter, Ca: calcium, Mg: magnesium, SB: sum of bases, V: base saturation, CEC: cationic exchange capacity.
The DM of plants grown in soil treated with BVC showed values similar to those of plants in soil with CV and KCl. However, N addition resulted in greater plant height, leaf area, and leaf DM, independently of the K sources used (Table 3). The effect ($p < 0.05$) of the interaction among K sources and N levels was observed for root DM (RDM) and total DM (TDM) per plant; means are compared in Table 4. Although no difference ($P > 0.05$) was detected in TDM, plants that received BVC had lower RDM production than other K sources, especially when its application was associated with N fertilizer. This result can be a consequence of increasing electrical conductivity of the soil solution (Santana et al., 2007) as a result of the amount of K$^+$ and Na$^+$ from BVC associated with other ions originating from N fertilizer. However, K sources did not differ ($P > 0.05$) for root DM production in the absence of N fertilization (Table 4). This indicates that the aerial part of the plants might have had a lower reduction than the roots, which leads to a nonsignificant difference ($P < 0.05$) in TDM. Plants fertilized with CV and BVC, independently of N levels, also showed values similar to TDM; this suggests that N from CV and BVC was enough to meet N plant demand until that time. Many studies have found that sugarcane has a low response to N fertilization. Nitrogen contributes approximately 1% of sugarcane TDM; however, N deficiency can cause a significant yield decrease (Franco et al., 2011). Leaf number and tiller number were similar ($P > 0.05$) among plants from studied treatments with a mean number of leaves and tillers of 16.5 and 2.6 per plant, respectively.

The K, Ca, and S contents in plants were affected by K sources, independently of N levels (Table 5). Plant K contents were the same among treatments with BVC and CV. Treatments with CV and BVC were applied in equivalent amounts of K, and results suggest that the availability of K from BVC satisfies the initial crop demand and its K availability is similar to CV. Plants from BVC and CV treatments had lower Ca contents than those in the control treatment, possibly because of competition between cations, such as Na, Mg, and K, at the time of absorption. For plant S content, BVC did not differ from CV and KCl treatments, but these treatments induced lower plant S concentrations than the controls.

Nitrogen levels also affected leaf N, Ca, and S contents of sugarcane. Higher N and S contents and lower Ca content were observed with N application. These results agree with the report by Vieira et al. (2010) about the positive effect of N fertilization on leaf N and S concentration, while the positive effect on P and Mg concentrations was not confirmed (Table 5). The synergistic effect between S and N in sugarcane was also observed by Franco et al. (2007). It occurs because N and S are components of the amino acids cystine and cysteine, so the increase in N supply implies a greater use of S by the crop. There was no difference ($P > 0.05$) among treatments for P and Mg contents (Table 5). Equal amounts of all these nutrients were added, except N, in all the treatments during substrate preparation. Potassium accumulation was separately influenced by K sources and N levels (Table 6). Although BVC was no different than other K sources, it tended to induce less plant K accumulation. The lower K accumulation in plants with the BVC treatment can be explained by the fact that BVC contains much more Na than other K sources, and given an antagonistic relationship between Na and K, higher Na absorption could have reduced plant K absorption (Mansoori et al., 2014).

Higher K accumulation was observed in treatments with N application. It is possible that applying N can improve K absorption due to a synergistic effect on macronutrient absorption by sugarcane, which can increase nutrient accumulation in this plant (Franco et al., 2007). The effect ($p \leq 0.05$) of the interaction among K sources and N levels in N, Ca, Mg, and S accumulation was also observed (Table 7).

As expected, N fertilization induced greater N accumulation in plants. However, plants from the control treatment (no added K source) accumulated similar N amounts as in other K sources at each N level (Table 7). This suggests that the presence of K does not influence N absorption. Applying CV induced less N absorption by plants, which is reflected by no difference ($P > 0.05$)
Nitrogen fertilization induced plant Ca accumulation in the control treatment (without K addition) and plants in which N was associated with KCl. In all K sources, plant Ca accumulation did not differ (P > 0.05) in the absence of N fertilization. Data for S accumulation were similar to those obtained for Ca (Table 7). Potassium sources did not influence (P > 0.05) Mg accumulation in either the absence or presence of N, while N application increased plant Mg accumulation in the control treatment. Franco et al. (2007) reported that N application can increase nutrient accumulation by sugarcane because it improves the development of the root system. Contact between roots and nutrients increases, which could improve plant nutrient absorption despite the fact that P accumulation was not affected by treatments (P > 0.05) with an approximate mean of 0.04 g plant⁻¹. The effect (p < 0.05) of the interaction between K sources and N levels in Na accumulation was observed (Table 8). The BVC treatment induced higher plant Na accumulation because BVC has higher Na concentration than other K sources and the control (Table 1). These results are in accordance with information from the literature that reports Na as an element in which plant concentration increases due to salinity (Santana et al., 2007; Mansoori et al., 2014). This would be expected given added NaOH in the original vinasse to make pH suitable for the biodigestion process (Döll and Forest, 2010), which leads to high plant Na concentration from the BVC application.

Plants that received BVC showed the highest Na accumulation; however, N addition induced less plant Na accumulation in the BVC treatment. On the other hand, higher Na accumulation was detected in plants from other treatments that did not receive N addition. This result suggested that N application seemed to prevent Na absorption when BVC was used. Other authors have mentioned that N fertilization is used to attenuate the impact of salt stress in some species, and this also appears to be related to plant stress tolerance (Ehlting et al., 2007; Mansoori et al., 2014).

**CONCLUSIONS**

The addition of biodigested vinasse concentrate induces higher soil pH and lower potential acidity than KCl, and causes similar soil chemical changes as when common vinasse is added. Plants fertilized with biodigested vinasse concentrate and N showed lower root dry matter, but had total dry matter accumulation and nutritional conditions similar to plants fertilized with common vinasse and KCl. Plants fertilized with biodigested vinasse concentrate had higher levels of Na accumulation than common vinasse, KCl, and unfertilized plants.

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