

## Evaluation of methodologies for chemical characterization of vinasse as a function of storage time

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### Abstract

The objective of this study was to evaluate efficient analytical methods of wet digestion using the official method of analysis, a mixture of nitric acid and perchloric acid ( $\text{HNO}_3 + \text{HClO}_4$ ), for the analysis of vinasse and also verify the effects of nutrients on stored vinasse. The analytical methods compared were: 1) Nitroperchloric ( $\text{HNO}_3$ ), 2) Nitric ( $\text{HNO}_3$ ); 3) Chloridric + Hydrogen Peroxide ( $\text{HCl} + \text{H}_2\text{O}_2$ ); 4) Sulfuric + Hydrogen Peroxide ( $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ ) and 5) Solubilization with 1 molar Chloridric acid ( $\text{HCl} 1 \text{ Mol L}^{-1}$ ). Phosphorus, potassium, calcium, magnesium and sulfur were determined over different storage periods, and these nutrients were analyzed at 5, 30, 90 and 150 days of storage, to verify the behavior of these nutrients over the time they are stored in laboratory conditions. The efficiency of the methods was evaluated by comparing the results of the nitroperchloric method obtained for each nutrient with the results obtained from the other methods. The results showed that the nitric ( $\text{HNO}_3$ ) and chloridric + hydrogen peroxide ( $\text{HCl} + \text{H}_2\text{O}_2$ ) digestions presented good to excellent precision equivalent to the nitroperchloric digestion ( $\text{HNO}_3$ ) for the analyzed nutrients. The solubilization method with 1 molar chloridric acid ( $\text{HCl} 1 \text{ Mol L}^{-1}$ ) presented a low accuracy and did not present the expected efficiency for quantification of any analyzed nutrient. With the exception of potassium contents, all the other nutrients analyzed in the study reduced at the end of the vinasse storage days.

**Keywords:** Humidity digestion, Macronutrients, Chemical composition.

### Resumo

O objetivo deste trabalho foi avaliar métodos analíticos eficientes de digestão úmida utilizando o método oficial de análise, uma mistura de ácido nítrico e ácido perclórico ( $\text{HNO}_3 + \text{HClO}_4$ ), para a análise da vinhaça e também verificar os efeitos dos nutrientes na vinhaça armazenada. Os métodos analíticos comparados foram: 1) Nitroperclórico ( $\text{HNO}_3$ ), 2) Nítrico ( $\text{HNO}_3$ ); 3) Clorídrico + Peróxido de Hidrogênio ( $\text{HCl} + \text{H}_2\text{O}_2$ ); 4) Sulfúrico + Peróxido de Hidrogênio ( $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ ) e 5) Solubilização com Ácido Clorídrico 1 molar ( $\text{HCl} 1 \text{ Mol L}^{-1}$ ). Fósforo, potássio, cálcio, magnésio e enxofre foram determinados em diferentes períodos de armazenamento, e esses nutrientes foram analisados aos 5, 30, 90 e 150 dias de armazenamento, para verificar o comportamento desses nutrientes ao longo do tempo em que são armazenados em condições de laboratório. A eficiência dos métodos foi avaliada comparando-se os resultados do método nitroperclórico obtidos para cada nutriente com os resultados obtidos dos demais métodos. Os resultados mostraram que as digestões nítricas ( $\text{HNO}_3$ ) e clorídrica + peróxido de hidrogênio ( $\text{HCl} + \text{H}_2\text{O}_2$ ) apresentaram precisão boa a excelente equivalente à digestão nitroperclórica ( $\text{HNO}_3$ ) para os nutrientes analisados. O método de solubilização com ácido clorídrico 1 molar ( $\text{HCl} 1 \text{ Mol L}^{-1}$ ) apresentou baixa acurácia e não apresentou a eficiência esperada para quantificação de nenhum nutriente analisado. Com exceção dos teores de potássio, todos os demais nutrientes analisados no estudo reduziram ao final dos dias de armazenamento da vinhaça.

**Palavras-chave:** Digestão de umidade, Macronutrientes, Composição química.

### Resumen

El objetivo de este estudio fue evaluar métodos analíticos eficientes de digestión húmeda utilizando el método oficial de análisis, una mezcla de ácido nítrico y ácido perclórico ( $\text{HNO}_3 + \text{HClO}_4$ ), para el análisis de vinaza y

también verificar los efectos de los nutrientes en la vinaza almacenada. Los métodos analíticos comparados fueron: 1) Nitroperclórico ( $\text{HNO}_3$ ), 2) Nítrico ( $\text{HNO}_3$ ); 3) Clorhídrico + Peróxido de Hidrógeno ( $\text{HCl} + \text{H}_2\text{O}_2$ ); 4) Sulfúrico + Peróxido de Hidrógeno ( $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ ) y 5) Solubilización con Ácido Clorhídrico 1 molar ( $\text{HCl} 1 \text{ Mol L}^{-1}$ ). Se determinó fósforo, potasio, calcio, magnesio y azufre en diferentes periodos de almacenamiento, y estos nutrientes se analizaron a los 5, 30, 90 y 150 días de almacenamiento, para verificar el comportamiento de estos nutrientes a lo largo del tiempo de almacenamiento en condiciones de laboratorio. La eficiencia de los métodos se evaluó comparando los resultados del método nitroperclórico obtenidos para cada nutriente con los resultados obtenidos de los otros métodos. Los resultados mostraron que las digestiones nítrica ( $\text{HNO}_3$ ) y clorhídrica + peróxido de hidrógeno ( $\text{HCl} + \text{H}_2\text{O}_2$ ) presentaron de buena a excelente precisión equivalente a la digestión con nitroperclórico ( $\text{HNO}_3$ ) para los nutrientes analizados. El método de solubilización con ácido clorhídrico 1 molar ( $\text{HCl} 1 \text{ Mol L}^{-1}$ ) presentó una baja precisión y no presentó la eficiencia esperada para la cuantificación de ningún nutriente analizado. Con excepción de los contenidos de potasio, todos los demás nutrientes analizados en el estudio se redujeron al final de los días de almacenamiento de la vinaza.

**Palabras clave:** Digestión por humedad, Macronutrientes, Composición química.

## 1. Introduction

Vinasse is a residual liquid, a product of the syrup during the distillation of alcohol fermentation liquor from sugar cane (South America) or sugar beet (Europe), also known regionally as restilo and vinasse; it is a by-product of alcohol production (Baffa et al., 2009; Poveda, 2014).

Brazilian distilleries produce between 7 to 15 liters of vinasse per liter of ethanol, depending on the size of the installation and the technology used (Salomon, 2007). In Brazil, approximately 250 million m<sup>3</sup> of vinasse are generated per harvest (Poveda, 2014), with this, the use of vinasse to fertigate sugarcane fields has been the solution used to allocate the enormous volume produced, promoting changes in chemical and physical properties of the soil, as an increase in the hydrogenionic potential (pH), increase in nutritional availability and in the cation exchange capacity (Bebé et al., 2009; Delhadj et al., 2013).

Another important factor in its application in agriculture is either by classical fertilization or by soil irrigation when planting sugarcane (Formagini, 2011; Godoi et al., 2019). It is a material with about 2-6% of solid constituents, where organic matter stands out, in greater quantity, and in mineral terms it presents a high amount of potassium and medium for calcium and magnesium (Rossetto, 1987). Highlighting the supply of potassium, which corresponds to about 20% of the total of organic and mineral compounds (Marques, 2006), in addition to nitrogen, calcium, magnesium and phosphorus in lower concentrations (Seixas et al., 2016). When it comes to environmental maintenance, as vinasse is used as a biofertilizer, an important characteristic when choosing the raw material for ethanol production is the sodium concentration, since, in sugarcane, this element has a low concentration in the vinasse when compared to sugar beet, in addition, high values of sodium are undesirable for the environment, as it is harmful to the soil and plants (Silva et al., 2007).

Thus, it is important to study its chemical components, given that in vinasse there are unbalanced and different amounts of mineral and organic elements (Silva et al., 2007). However, to carry out its chemical characterization, it is necessary to adapt the analytical methods to be used in the different mineralogical analyses. There is little scientific and technical material regarding analytical methods on the chemical constitution of vinasse. In addition, the recommended methods are often excessively complex or time-consuming, and dangerous, as proposed by Fort et al. (1939) and Payne (1968), where phosphates were determined by the common volumetric method, which is very limited due to some basic requirements for the success of a titration such as stoichiometry and/or reaction speed and turning point visualization.

In addition to sulfur and magnesium, also mentioned in the work by Fort et al. (1939) and Payne (1968), were filtered through an asbestos mat, which is a filter composed of asbestos fiber (extremely dangerous and contaminating substance) in its composition, to obtain the respective extracts.

Generally, the analytical techniques adapted for the analysis of sugar mill and distilleries residues are those already established for the analysis of other materials, such as leaves, where the decomposition of plant tissue, aiming at the determination of essential plant nutrients, is performed by wet route, through solubilization with nitric and perchloric acids (Carmo et al., 2000), being adapted for the analysis of vinasse.

Thus, this study aimed to evaluate different digestion methodologies and wet acid solubilization for the chemical analysis of vinasse, as a function of the storage time in the laboratory, defining: if any other digestion presents equivalence to the traditional method (Embrapa Official) of nitroperchloric decomposition and whether there are

nutrient losses over storage time under laboratory conditions.

## **2. Material and Methods**

### *2.1 Obtaining Vinasse*

The vinasse samples were obtained at power plant Nova União S/A, located in the municipality of Jandaia, Goiás state, Brazil, (17°16'13.11" S and 50°8'10.39" W), with an average elevation of 500 m. During collection, 500 mL plastic bottles were used. Samples were collected on the same day, in different bottles to be analyzed with different storage times. The bottles were hermetically closed and opened only at the time of analysis.

Vinasse samples were collected in the rainy season, on October 30, 2020, in two different locations: vinasse from the dam, known as the distribution pond, where it is stored for use in fertigation, and vinasse in the channel, where the vinasse is removal of containment tanks (dam) and launched in main channels, where the solution reaches the irrigation furrows opened between the rows of the cane field, with 4 repetitions of each sample being made for the different digestion methodologies.

### *2.2 Period of Analysis*

The collected samples were stored in the Soil and Foliar Tissue analysis Laboratory of the Goiano Federal Institute, Rio Verde Campus, Goiás, Brazil at room temperature, to simulate the real storage condition at the Plant. The analyzes were carried out from the freshly collected samples and at 30, 90 and 150 days after collection, with the storage period being evaluated to determine possible changes in the nutritional contents analyzed from the chemical composition of the vinasse.

The period of analysis of the collected vinasse samples were freshly collected samples, between the 7<sup>th</sup> and 16<sup>th</sup> of November 2020, after 30 days of storage between the 14<sup>th</sup> and 22<sup>nd</sup> of December 2020, after 90 days, being analyzed between the 25<sup>th</sup> of February to March 12, 2021, and lastly between March 18 and June 4, 2021, totaling 150 days of storage.

### *2.3 Experimental Procedures*

Digestion of vinasse and analysis of Calcium (Ca), Magnesium (Mg), Phosphorus (P), Potassium (K) and Sulfur (S) contents were tested in 4 different forms of wet digestion methodologies, namely: perchloric, sulfuric, nitric, chloridric and non-digested nitric digestion process performed using HCl conc. 1 Mol L<sup>-1</sup> for mineralogical determination and analytical processes as per (Table 1).

**Table 1.** Types of digestion, methodologies, and analytical processes performed for chemical analysis in the determination of Ca, Mg, P, K and S in vinasse samples collected in the dam and channel of power plant Nova União, Goiás state, Brazil.

Digestion	Methodology	Analytical process
Nítricoperchloric	(Carmo et al., 2000): Samples will be solubilized with nitric acid (65%) and perchloric acid (72%), proportion (3:1).	Aliquot of 2 mL of the vinasse was transferred to a digester tube added with 8 mL of acid solution. Then, the solution was heated gradually in a digester block to 120 °C, and then to 200 °C, until white vapors of HClO <sub>4</sub> were obtained, and the extract was colorless. After that time, the solution was cooled and 25 mL of distilled water were added.
Nitric	(Silva, 2009): Nitric acid P. A (65%)	Aliquot of 2 mL of the vinasse was transferred to digestion tubes, added with 10 mL of HNO <sub>3</sub> P. A. Then, the digestion was carried out in a digester block, gradually increasing the temperature to approximately 200 °C; until colorless, then the solution was cooled, and the solution was made up with 25 mL of distilled water. (Method adapted from the nitroperchloric method).
Sulfuric	Based on the oxidation of organic matter: H <sub>2</sub> SO <sub>4</sub> 98% (v/v) + H <sub>2</sub> O <sub>2</sub> 30% (v/v), (Kjeldahl method adapted for macronutrient analysis).	Aliquot of 2 mL of the vinasse was added with 3 ml of H <sub>2</sub> SO <sub>4</sub> P. A, and 1 mL of H <sub>2</sub> O <sub>2</sub> P. A, followed by a gradual digestion in a digester block, up to approximately 110 °C; the solution after digestion of the organic matter was cooled, becoming colorless, and soon after, it was completed with 25 mL of distilled water.
Chlorid	(It has no official methodology). It is used in aqua regia, but not insulated. Samples will be solubilized with HCl P. A (85%) + H <sub>2</sub> O <sub>2</sub> (v/v).	Aliquot of 2 mL of the vinasse was transferred to digestion tubes, added with 3 mL of HCl P.A, and 1 mL of H <sub>2</sub> O <sub>2</sub> P. A, followed by digestion at approximately 110 °C; until a colorless liquid was left, then the solution was cooled and 25 mL of distilled water was added. (Adapted nitroperchloric method).
HCl 1 mol L <sup>-1</sup>	(Silva, 2009): Chlorid acid conc. 1 mol L <sup>-1</sup> : 83 mL for 1 L	Aliquot of 2 mL of the vinasse was transferred to the test tube containing 25 mL of 1 Mol L <sup>-1</sup> HCL solution, the total mass was determined on an analytical balance. Then, the whole was heated for 15 min in a water bath at 80 °C, and after this time, the whole was shaken for 15 minutes in a Vortex shaker at 250 rpm. Then, the whole was cooled and the evaporated water was replaced until the initial mass, and soon after, it was filtered on qualitative filter paper.

The analytical determination for Ca, Mg, P, K and S was performed after the digestion step, submitted to the preparation of extracts 1 and 2. Extract 1 was obtained from the wet digestion process and from the solubilization process with HCl conc. 1 Mol L<sup>-1</sup> solution. Extract 2 was obtained from 5 mL of extract 1, and 20 mL of distilled water.

The determination of Ca and Mg contents was obtained from an aliquot of 0.5 ml of extract 2 + 2.5 mL of aqueous strontium chloride solution (48.7 g for 1 L<sup>-1</sup>) (v/w) and 23 mL of distilled water. The reading was carried out in an atomic absorption spectrometer. Phosphorus was determined by colorimetry using molybdate blue methodology. An aliquot of 1 mL of extract 2 + 20.5 mL of distilled water, 2.5 mL of an aqueous solution of molybdate (solution: 1 g of bismuth subcarbonate, 136 mL of H<sub>2</sub>SO<sub>4</sub>, 20 g of ammonium molybdate) (v/w) was used to 1 L and 1 mL of ascorbic acid solution (0.04 g in 25 mL of distilled water) (v/w). The solution was

then homogenized in a Vortex equipment, and then left to rest for 20 min. After this period, the reading was performed in a UV-Vis spectrophotometer at a wavelength of 725 nm.

The Potassium was determined in a flame photometer, with 1 mL of extract 2 + 24 mL of distilled water. The solution was homogenized in a Vortex equipment for 1 min, and then the reading was performed. The sulfur determination was performed by turbidimetry, from a 10 mL aliquot of extract 2 + 1 mL of an aqueous solution of conc. 6 N and 0.01 mg BaCl<sub>2</sub> P. A. Then, the solution was stirred for 1 min in a Vortex equipment, and after 5 min, the reading was performed in a UV-Vis spectrophotometer at 420 nm.

The analysis of the chemical composition of dam and channel vinasses, in relation to the different digestion methods in an open system and at different storage times, were submitted to analysis of variance, and the means were compared by the *Tukey's* test, at a significance level of 5 % for the digestions and by the regression test, for the storage time.

#### 2.4 Nitrogen analysis

Nitrogen content was determined by the wet solubilization technique, followed by steam distillation and finally by titration for quantitative quantification of NH<sub>4</sub>. Sulfuric solubilization (H<sub>2</sub>SO<sub>4</sub> + catalysts) converts plant tissue proteins, and amino acids into N-NH<sub>4</sub><sup>+</sup> which is distilled and then complexed in boric acid solution with mixed indicator. Titration was performed using standardized aqueous solution of H<sub>2</sub>SO<sub>4</sub>.

In the wet digestion with H<sub>2</sub>SO<sub>4</sub>, 2 mL of vinasse + 1 g of the digester mixture containing K<sub>2</sub>SO<sub>4</sub> and CuSO<sub>4</sub> conc. (10:1) + 10 mL H<sub>2</sub>SO<sub>4</sub> P. A. in the digester tube. Then, the tubes were transferred to the heating block with gradual heating up to 350 °C. The end of the reaction is given by obtaining a greenish colored liquid. Then, the tubes were cooled, and soon after, 40 ml of distilled water was added. 10 mL aliquot was transferred to another digester tube containing 10 mL of an aqueous conc. 40% Mol L<sup>-1</sup> and 0.5 mL of an aqueous solution of NaOH conc. 0.025 Mol L<sup>-1</sup>. The digested solution was transferred to the nitrogen still, and into a 125 mL *Erlenmeyer* flask containing 25 mL of a 2% aqueous solution of H<sub>3</sub>BO<sub>3</sub> (v/w) and 0.5 mL of a 1% diphenylamine solution (v/w). The digested content was distilled to obtain the product about 45 ml, and then it was titrated with aqueous conc. 0.01 Mol L<sup>-1</sup> and the turning point was determined when the wine color was obtained.

In the wet digestion with H<sub>2</sub>SO<sub>4</sub> + H<sub>2</sub>O<sub>2</sub> 2 mL of vinasse + 1 g of digester mixture of K<sub>2</sub>SO<sub>4</sub> and CuSO<sub>4</sub> conc. (10:1) + 3 ml of H<sub>2</sub>SO<sub>4</sub> P.A and 1 mL of a 30% (v/v) aqueous solution of H<sub>2</sub>O<sub>2</sub> in the digester tube. Then, the tubes were transferred to the digester block, with gradual heating up to 350 °C. The end of digestion occurred when the solution showed a greenish color. After cooling, 50 mL of distilled water was added and homogenized for 1 min in a Vortex equipment. Then, the digester tube was transferred to a nitrogen still, where 10 mL of an aqueous solution of NaOH conc. 40% L<sup>-1</sup> (v/w), and at the outlet of the retort a 125 mL *Erlenmeyer* containing 25 mL of an aqueous solution of H<sub>3</sub>BO<sub>3</sub> 2% L<sup>-1</sup> (v/w) was added. Then, the sample was distilled until obtaining 45 mL. Afterwards, titration with an aqueous solution of conc. 0.01 mol L<sup>-1</sup> (v/w). The turning point occurred with the change of color to wine tone.

The results were submitted to analysis of variance and means were compared by *Tukey's* test at a significance level of 5% for the two analyzed digestions, and by regression test for storage time.

#### 2.5 Organic matter (OM), moisture and pH analysis

The organic matter was determined by the volumetric method, based on the oxidation of OM, when in contact with sulfuric acid and potassium dichromate, and subsequent titration for dosage with a standard solution of ammoniacal ferrous sulfate (Bezerra et al., 2011). The moisture was determined by the oven method, which is based on the removal of humidity by heating, being 24 h at a temperature of 105° C.

The pH was determined by the sample method of the pHmeter (potentiometric method), where after the meter is calibrated by buffer, the electrode is immersed in the sample and the reading is taken. Data were submitted to regression analysis.

### 3. Results and Discussion

No effect was observed between digestion *versus* time interactions, with a positive effect between the isolated treatments. Thus, the results for digestion and storage time were presented and discussed separately.

3.1 Chemical characterization of nutrients: Calcium (Ca), Magnesium (Mg), Phosphorus (P), Potassium (K) and Sulfur (S) for the vinasse of the dam and canal

The analysis of the chemical composition of the vinasse from the Power plant Nova União dam, with the different open system digestion methods were described in (Table 2).

**Table 2.** Evaluation of the different methods of acid digestion in an open system in the extraction and quantification of nutrients for the analysis of the chemical composition of the vinasse collected in the power plant Nova União dam.

Digestions	Ca	Mg	P	K	S
	g L <sup>-1</sup>				
Nitroperchloric (control)	0.65 a	0.13 a	0.017 a	1.78 a	0.47 b
Nitric	0.65 a	0.12 a	0.015 a	1.58 a	0.46 b
Sulfuric	0.65 a	0.13 a	0.016 a	1.37 ab	1.10 a
Chloridric	0.64 a	0.12 a	0.015 a	1.43 a	0.45 b
HCl conc. 1 Mol L <sup>-1</sup>	0.27 b	0.04 b	0.010 b	0.94 b	0.19 c
CV (%)	17.55	24.93	18.83	28.68	36.17

The nutrients Ca, Mg and P showed similar behavior in the digestions: nitroperchloric, nitric, sulfuric and chloridric, superior between the digestions when compared to solubilization by digestion with HCl conc. 1 mol L<sup>-1</sup>. In relation to K the greatest quantity was observed in the digestions: nitroperchloric, nitric and chloridric in relation to the digestion by HCl conc. 1 mol L<sup>-1</sup>, similarity was observed between the sulfuric digestion with the other digestions.

In relation to S, the highest content presented was observed in the sulfuric digestion, this being overestimated in relation to the nitroperchloric (control), nitric and chloridric digestions, being the digestion with HCl conc. 1 mol L<sup>-1</sup>, the most underestimated content, due to the formation of ammonium sulfate during digestion with sulfuric acid.

The analysis of the chemical composition of the vinasse from the power plant Nova União channel, with the different open system digestion methods are described in (Table 3).

**Table 3.** Evaluation of the different methods of acid digestion in an open system in the extraction and quantification of nutrients for the analysis of the chemical composition of the vinasse collected in the power plant Nova União channel.

Digestions	Ca	Mg	P	K	S
	g L <sup>-1</sup>				
Nitroperchloric (control)	0.61 a	0.09 a	0.010 a	1.70 a	0.39 b
Nitric	0.59 a	0.09 a	0.010 a	1.71 a	0.38 b
Sulfuric	0.63 a	0.09 a	0.010 a	1.47 a	0.67 a
Chloridric	0.61 a	0.09 a	0.011 a	1.52 a	0.40 b
HCl conc. 1 mol L <sup>-1</sup>	0.15 b	0.03 b	0.009 a	0.58 b	0.20 c
CV (%)	20.68	36.51	29.03	29.89	22.10

The nutrients Ca, Mg, P and K showed similarity in the behavior of the digestions: nitroperchloric, nitric, sulfuric and chloridric, superior when compared to the digestion with conc. 1 mol L<sup>-1</sup>. In relation to S, the same was observed, that in vinasse 1, the highest content of this element was observed in the sulfuric digestion, presenting an overestimated content in relation to the other digestions, and the solubilization with HCl 1 mol L<sup>-1</sup>, was the highest content underestimated.

Comparing the HNO<sub>3</sub> digestion method, in the study by Ferreira (2014) with the results of the nutrient contents of the standard samples obtained by extraction with the nitroperchloric method, it was found that for the extractions of K and Ca nutrients in the analyzed samples, concentrations remained within the confidence interval. Also, in the study by Ferreira (2014) for the nutrients S, P and Mg, the accuracy of 70, 80 and 90% respectively, was found in relation to the nitroperchloric method.

The mixture H<sub>2</sub>SO<sub>4</sub> + H<sub>2</sub>O<sub>2</sub> is used in the digestion of plant tissue for the determination of total N, however it was not feasible to determine the S, as the digestion is done with fuming sulfuric acid to produce the oxidation, forming the ammonium sulfate, the which can form very high levels of S and overestimate the quantity of the sample under analysis (Araujo, 2019). In addition, concentrated sulfuric acid, when heated, oxidizes several elements, being itself reduced to SO<sub>3</sub>, elemental sulfur or H<sub>2</sub>S (Ferreira, 2014), which may justify an overestimated quantification of S, as observed in the two vinasses in this study.

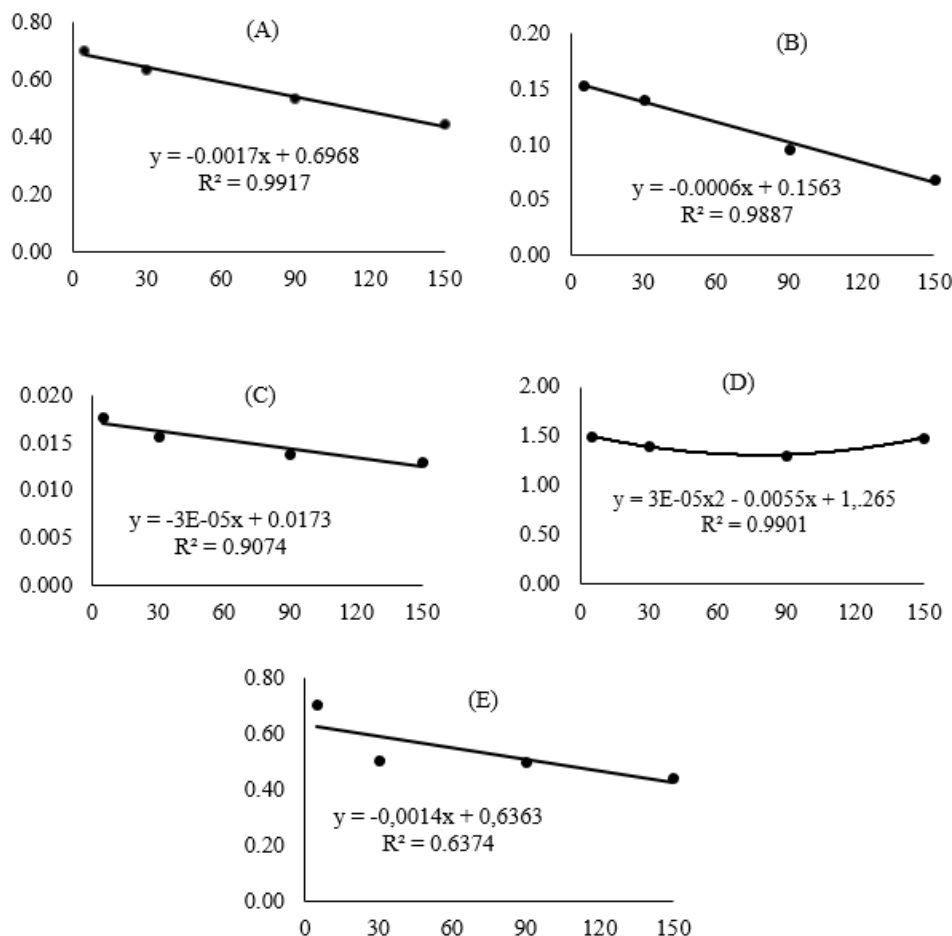
The method that combines the non-oxidizing acid, HCl with hydrogen peroxide, was efficient when compared to digestion using a mixture of nitric and perchloric acids. Mixtures of acids with hydrogen peroxide are particularly efficient in oxidation (Kingston et al., 1988), and (Krug, 2000), being equivalent to the most used method, showing efficiency in the quantification of nutrients in vinasse. The use of chloridric and sulfuric acid usually results in interference due to the formation of some poorly soluble chlorides and sulfates in some samples, but in this case of vinasse, it did not occur with HCl, but only with H<sub>2</sub>SO<sub>4</sub>.

Solubilization with HCl conc. 1 mol L<sup>-1</sup>, for all quantified nutrients: Ca, Mg, P, K and S, presented the best extraction quantitative for the two vinasses in this study. This can be explained by the fact that its oxidation power is lower when compared to nitroperchloric digestion (Perkin, 1973) and other digestions used, with the same behavior observed in the study by Nova et al. (2012).

Extraction with dilute acid solutions (methods with HCL 1 mol L<sup>-1</sup> without digestion), presents a disadvantage over this method because it presents partial extraction of nutrients, thus obtaining the lowest values for all nutrients in both contents of vinasse. This is easily discussed in the study by Ferreira (2014), where the researcher concluded that the use of the nutrient extraction method with HCl conc. 1 mol L<sup>-1</sup>, resulted in a lower extraction behavior on the concentrations of N, P, Ca and S, showing that the values were below the indicated confidence intervals.

Similar results to this study were also observed by Souza et al. (2012), the researchers observed significant superiority in the recovery of wet digestion with a mixture of nitric and perchloric acids. In general, the nitric and hydrochloric methods showed efficient precision.

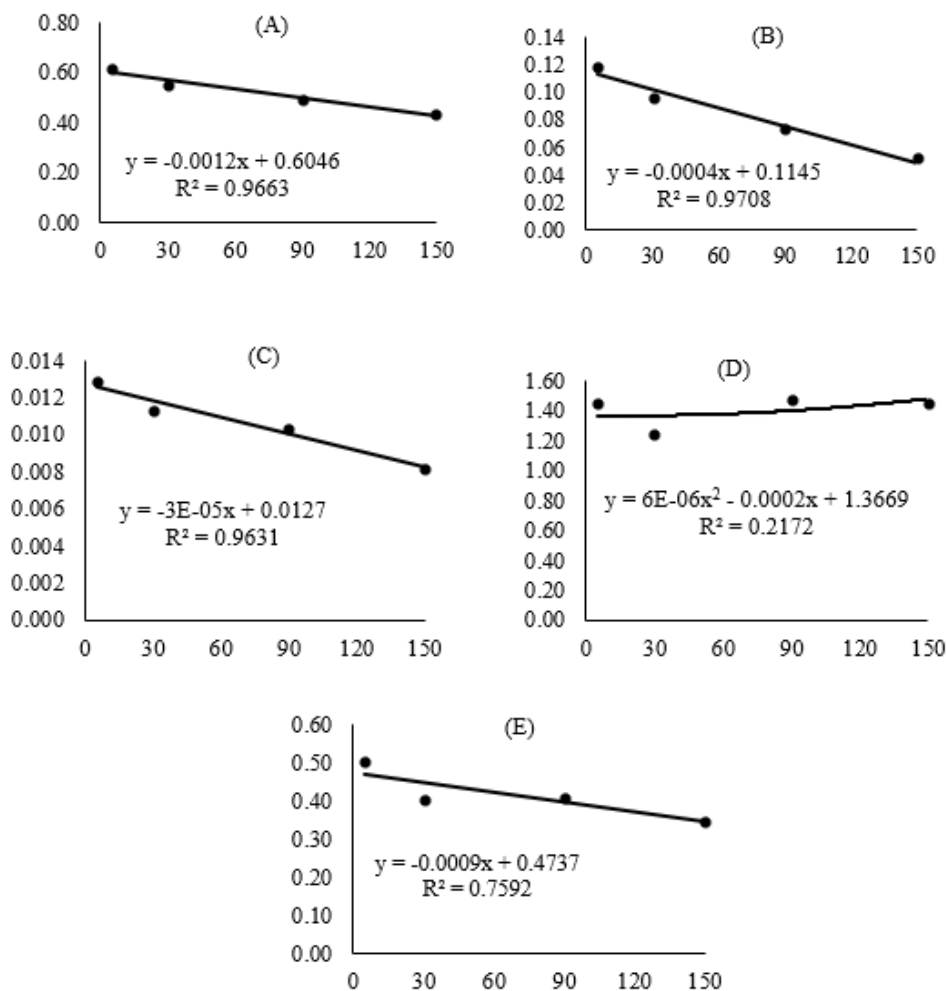
Regression analyzes referring to storage time (5, 30, 90 and 150 days) for vinasse collected in the dam at power plant Usina Nova União are described in (Figures 1 (A, B, C, D and E)).



**Figures 1.** Effect on the content of (A) Calcium (g L<sup>-1</sup>), (B) Magnesium (g L<sup>-1</sup>), (C) Phosphorus (g L<sup>-1</sup>), (D) Potassium (g L<sup>-1</sup>) and (E) Sulfur (g L<sup>-1</sup>) in vinasse collected at the power plant Nova União as a function of storage time between 5, 30, 90 and 150 days.

The nutrients Ca, Mg, P and S, presented a linear regression model, with a drop of 36.41%; 55.70%; 26.96% and 36.78% respectively, over the 150 days of storage. K presented a quadratic regression model, with a decrease at 30 and 90 days of storage, and a subsequent increase in content at 150 days of storage. Regression analyzes referring to storage time for vinasse collected in the canal at power plant Usina Nova União are described in (Figures 2 (A, B, C, D and E)).





**Figures 2.** Effect on the content of (A) Calcium (g L<sup>-1</sup>), (B) Magnesium (g L<sup>-1</sup>), (C) Phosphorus (g L<sup>-1</sup>), (D) Potassium (g L<sup>-1</sup>) and (E) Sulfur (g L<sup>-1</sup>) in vinasse collected in the power plant Nova União channel as a function of storage time between 5, 30, 90 and 150 days.

Ca showed a linear decrease of 29.55%, decreasing from 0.6149 g kg<sup>-1</sup> to 0.4332 g kg<sup>-1</sup> at the end of 150 days of storage (Figure 2 A). Nutrients Mg, P and S also showed a linear decrease of 56.48%; 35.93% and 31.14% respectively, (Figures 2 (B, C and E)). K showed a quadratic regression as seen in Figure 2 (D), with a decrease in content after 30 days of storage and increasing again in the levels in samples stored for 90 and 150 days.

All nutrients had their contents reduced after 30 days of storage, which suggests this cause, biological processes such as anaerobiosis that occur even in an environment with slightly reduced temperature during storage. According to Konzen (2006), anaerobiosis reduces the nutrient content.

Similar behavior was observed in the study by Gotardo (2017) which aimed to evaluate the magnitude of concentrations and evolution of cations, anions and free carbon in swine wastewater under anaerobic treatment. Their characteristics vary over time, the chemical species reduced or maintained their concentrations. The N, SO<sub>4</sub>, K<sup>+</sup> and Mg<sup>2+</sup> ions showed a reduction in their concentrations. This loss of nutrients is strongly linked to the volatilization and mineralization processes of the compounds.

In the study by Antunes et al. (2017), the researchers tested the effect of storage on the chemical characteristics of a compound, where they concluded that the storage of organic substrates for a period of 90 days was able to provide variations in pH values and macronutrient contents evaluated, and revealed that the storage promoted a reduction in the levels of organic N for the evaluated substrates.

This drop in nutrient content may have occurred due to ionic bonds made between mineral elements, throughout the storage period, forming stable molecules. These bonds are formed by the electrostatic attraction between ions

with opposite charges, with the definitive transfer of electrons (Fogaça, 2021), which makes it difficult to quantify these elements.

According to Saran (2021) the atomic concentration is determined by measuring the absorption or emission of radiation for each element. Being involved in this quantification of energy absorbed from the source, as a function of the vibration of the electrons of the elements in their ground state, for example:  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$ . Thus, the formation of stable compounds leads to an incomplete dissociation of the analyzed element, due to the formation of refractory compounds; such as Ca, in the presence of  $\text{SO}_4^{2-}$  or  $\text{PO}_4^{3-}$ , preventing their quantification.

Although decays in the contents of Ca, Mg, P and S nutrients are observed, the values obtained at the end of 150 days of storage still fit within the contents (maximum and minimum) expected for vinasse, according to the reference values presented in (Table 4).

**Table 4.** Minimum and maximum nutrient reference values for the chemical composition for unconcentrated fresh vinasse.

Elements	Reference values		
	Marques (2006)	Elias Neto & Nakahodo (1995)	Orlando Filho & Leme (1984)
	----- g L <sup>-1</sup> -----		
Phosphorus	0.001 – 0.021	0.009 – 0.018	0.009 – 0.050
Potassium	1.2 – 2.1	0.814 – 3.85	1.01 – 2.00
Calcium	0.13 – 1.54	0.071 – 1.09	0.13 – 0.76
Magnesium	0.2 – 0.49	0.047 – 0.25	0.066 – 0.31
Sulfur	0.6 – 0.76	0.79	-

### 3.2 Chemical analysis of nitrogen for dam and channel vinasses

The interaction Digestions *versus* Time was not significant for any of the analyzed vinasses. The Digestion and Time variables were significant in isolation for the dam's vinasse; thus, they were presented in an isolated way. And only the Digestion variable was significant for channel vinasse.

The N values obtained in the two methodologies used for the two vinasses are shown in (Table 5).

**Table 5.** Evaluation of the determination of nitrogen content by different wet solubilization techniques in the vinasses collected in the dam and channel of the power plant Nova União.

Methodology	Dam of the vinasse	Channel of the vinasse
		----- g L <sup>-1</sup> -----
Sulfuric ( $\text{H}_2\text{SO}_4$ )	0.26 b	0.33 b
Sulfuric + hydrogen peroxide ( $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ )	0.53 a	0.65 a

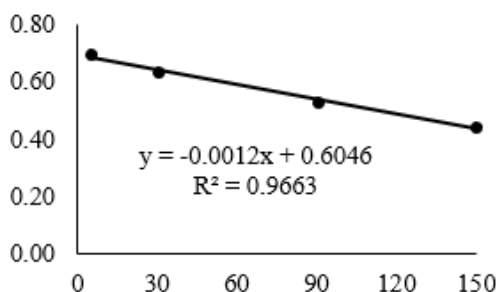
The sulfur methodology with hydrogen peroxide proved to be superior over the methodology that uses only  $\text{H}_2\text{SO}_4$ . According to the results obtained on the N contents of the two samples of vinasse, the values obtained by the  $\text{H}_2\text{SO}_4$  method incorporating  $\text{H}_2\text{O}_2$  were higher than those obtained by the  $\text{H}_2\text{SO}_4$  method, which suggests that the methodology using  $\text{H}_2\text{SO}_4$  solution added with  $\text{H}_2\text{O}_2$ , presents greater recovery de N. In the study by Araujo (2019) this tendency was also observed.

Method 2 uses  $\text{H}_2\text{O}_2$  to promote the complete oxidation of organic matter, which is the most used oxidizing agent, and has been shown to be the best among digestion methodologies that do not require sample pre-treatment (Ferreira, 2014).

Thus, the best methodology for the determination of N is sulfuric acid with hydrogen peroxide, which, also according to Soares (2013), the use of hydrogen peroxide, has been shown to be efficient in digestion processes.

### 3.3 Nitrogen content in relation to storage times of 5, 30, 90 and 150 days for the dam and channel vinasses

The regression analysis of variance for vinasse 1 showed a significant effect for the linear model, as described in (Figure 3).



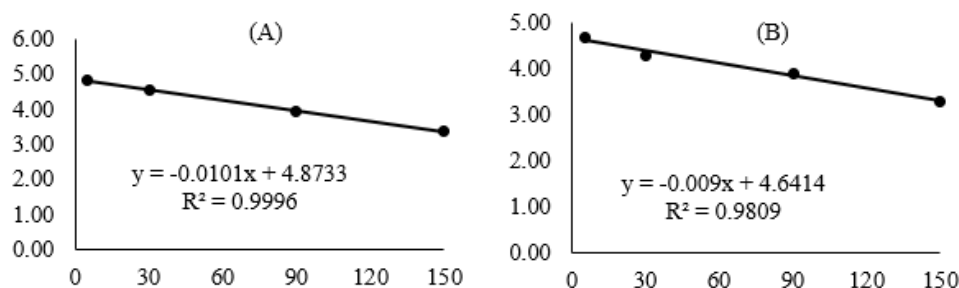
**Figure 3.** Regression of the analysis of variance of the Nitrogen content ( $\text{g L}^{-1}$ ) in vinasses collected at the power plant Destilaria Nova União dam as a function of storage time between 5, 30, 90 and 150 days.

A decrease in N contents was observed, which can be explained by the decrease in the pH of the sample, as, according to the pH of the environment, there is a predominance of ammonia nitrogen in its ionic form, that is, the non-toxic form of this element (Gomes, 2016).

The higher the pH, the greater the concentration of  $\text{N-NH}_3$ . This relationship of non-ionized ammonia with pH and temperature is due to the fact that the nitrification process tends to perform better in an environment with low alkalinity (Vidal, 2018). The relationship between pH, alkalinity and ammonia occurs from the content of accumulated organic matter (Vidal, 2018).

### 3.4 Analysis of pH, organic matter and moisture content

The regression analysis of variance for pH over the storage days for the dam vinasse showed a significant effect for the linear model, as described in (Figure 4 A), and for the channel vinasse also showed a significant effect for the linear model described in (Figure 4 B).

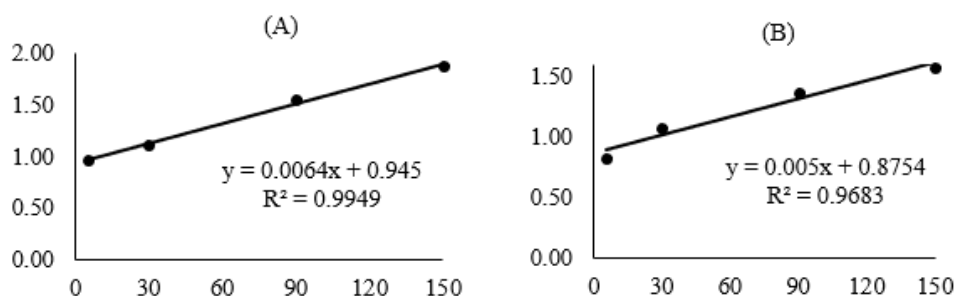


Figures 4. Regression analysis of *in natura* pH obtained by the potentiometric method using digital bench pH meter for (A) vinasse from the dam, and in (B) vinasse from the power plant Destilaria Nova União channel as a function of storage days between 5, 30, 90 and 150 days.

The two vinasses had an acidic pH. The initial pH = 4.84 for the dam vinasse, and pH = 4.67 for the canal vinasse, and at the end of 150 days of storage, there was an acidification in the pH values for both vinasses, with a value of 3.36 and 3.29 respectively.

According to Seixas (2006), the acidity of the effluent is mainly due to the presence of sulfuric acid that is added to the must during the fermentation stage, in addition to the presence of organic matter that is basically in the form of organic acids. Even though vinasse is an acid residue, it is capable of increasing the pH of soils, due to the reduction reactions (which consume  $H^+$ ) that occur after the application of vinasse; in addition to the neutralization of  $H^+$ ,  $Al^{3+}$  and  $Fe^{3+}$  in the soil by the negative charges of functional groups of organic matter provided by vinasse (Rosado et al., 2007; Cabral Filho et al., 2019).

The OM regression analysis of variance for vinasse 1 is shown in Figure 5 (A), and for channel vinasse in (Figure 5 (B)). Both regressions showed a significant effect for the linear model adopted.



**Figures 5.** Regression analysis of organic matter ( $g L^{-1}$ ) obtained by volumetric method in (A) vinasse from the dam, and in (B) vinasse from the power plant Destilaria Nova União channel as a function of storage days between 5, 30, 90 and 150 days.

For the two vinasses, there was an increase in OM over the days of storage. The percentage of OM was 93% for the dam stillage, and 89% for the channel stillage.

The study by Ebeling (2008) indicates that with increasing levels of organic matter, there is a reduction in pH values. The OM content showed a positive and significant correlation with the potential acidity, that is, the higher the OM content, the greater the acidity.

In studies carried out by Assad (2017) and Silveira (2015), where researchers report the presence of a group of microorganisms present in vinasse that act in the increase of OM contents, caused by its multiplication, where they transform mineral compounds into organic ones.

In aerobiosis, the immobilization of nutrients, such as organic matter, occurs. Microbial biomass immobilizes organic matter, and this OM immobilized by the microbial community can reach high values, but its recycling and release are faster than that of other fractions of OM in the soil, and as the microorganisms die, these are rapidly mineralized by the remaining microorganisms, releasing the immobilized nutrients in the process known as re-mineralization (Mary et al., 1996; Silva et al., 2021).

The variable moisture was not significant. The moisture content of the two vinasses remained around 98% during the entire storage period. Similar result on moisture content was observed in the study by Ferreira (2013), for vinasse analyzed with moisture content, around 96%. There was no significant difference in moisture content during the 150 days of storage, remaining constant.

#### 4. Conclusions

Nitric ( $HNO_3$ ) and chloridric ( $HCl + H_2O_2$ ) digestions are fair in all aspects, thus being used in place of the most used digestion, nitroperchloric.

In nitrogen determination, the sulfuric + hydrogen peroxide ( $H_2SO_4 + H_2O_2$ ) methodology becomes more recommended, as it has a quantification of nutrients, and requires a smaller volume of sulfuric acid, which generates economy and precision.

As for the storage time, in the two vinasses, the loss of nutrients was not significant, due to the fact that even after the decrease of the contents, over the 150 days of storage, the contents of Ca, Mg, P, and S, still were within the minimum expected limits for vinasse. The K did not decrease linearly over the days, not being influenced by the storage term.

The pH had a drop, that is, an acidification over the 150 days that the sample was stored, which can be explained by the increase in organic matter, and decrease in the N-NH<sub>3</sub> of the samples, because the lower the pH, the lower the concentration of N-NH<sub>3</sub>, and by the increase in the organic matter of the samples, which causes acidification of the medium. The moisture content of the two vinasses did not change over time, remaining close to 98%.

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## 6. References

- Antunes, L. F. S., Cruvinel, F. F., Souza, R. G., Silva, D. G., Correia, M. E. F. C. & Martelleto, L. A. P. (2017). *Effect of storage on the chemical characteristics of millicompost*. In VI Congresso Latino Americano, X Congresso Brasileiro e V Seminário do DF e Entorno, 13, 2017, Anais... Brasília: Cadernos de Agroecologia.
- Araujo, M. A. (2019). *Revisão bibliográfica: avaliação do método de Kjeldahl na determinação de nitrogênio e sua aplicação na análise foliar*. 2019. Uberlândia: Universidade Federal de Uberlândia, Uberlândia, 39 pp.
- Assad, L. (2017). Aproveitamento de resíduos do setor sucroalcooleiro desafia empresas e pesquisadores. *Ciência e Cultura*, 69(4), 13-16.
- Baffa, D. C. F., Freitas, R. G. & Brasil, R. P. C. (2009). O uso da vinhaça na cultura da cana de açúcar. *Nucleus*, 1(2009), 31-45.
- Bebé, F. V., Rolim, M. M., Pedrosa, E. M. R., Silva, G. B. & Oliveira, V. S. (2009). Avaliação de solos sob diferentes períodos de aplicação com vinhaça. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 13(6), 781-787.
- Belhadj, S., Karouach, F., El Bari, H. & Joute, Y. (2013). The biogás production from mesophilic anaerobic digestion of vinasse. *Journal of Environmental Science, Toxicology and Food Technology*, 5(6), 72-77.
- Bezerra Neto, E. & Barreto, L. P. (2011). *Análises químicas e bioquímicas em plantas*. Editora Universitária da UFRPE, Recife.
- Cabral Filho, F. R., Vieira, G. S., Silva, N. F., Cunha, E. S., Santos, L. N. S., Rodrigues, E. S., Cunha, F. N., Teixeira, M. B. & Soares, F. A. L. (2019). Sugarcane vinasse cations dynamics in Cerrado soils, Brazil. *Sugar Tech*, 21(1), 38-46.
- Carmo, C. D. S., Araujo, W. S., Bernardi, A. D. C.; Saldanha, M. F. C. (2000). *Métodos de análise de tecidos vegetais utilizados na Embrapa Solos*. Rio de Janeiro: Embrapa Solos
- Carneiro, C., Reissmann, C. B. & Marques, R. (2006). Comparação de métodos de análise química de K, Ca, Mg e Al, em folhas de erva-mate (*Ilex paraguariensis* St. Hil.). *Cerne*, 12(2), 113-122.
- Ebeling, A. G., Anjos, L. H. C. D., Perez, D. V., Pereira, M. G. & Valladares, G. S. (2008). Relação entre acidez e outros atributos químicos em solos com teores elevados de matéria orgânica. *Bragantia*, 67(2), 429-439.
- Ferreira, M. S. (2013). Avaliação bromatológica dos resíduos da industrialização da mandioca e seu aproveitamento em ração para animais ruminantes. *Revista Brasileira de Agropecuária Sustentável*, 3(1), 105-109.
- Ferreira, A. K. C. (2014). *Avaliação de métodos de análises químicas de nutrientes em tecido vegetal*. Universidade Federal Rural do Semi-Árido, Mossoró, 91 pp.
- Fogaça, J. R. V. (2021). *Exercícios Sobre Ligações Iônicas. Ligações Iônicas*. In Mundo Educação. (available at <https://exercicios.mundoeducacao.uol.com.br/exerciciosquimica/exercicios-sobre-ligacoes-ionicas-ligacoes-ionicas.htm>)
- Formagini, E. L. (2011). *Estabilização do pH na digestão anaeróbica da vinhaça utilizando bicabornato de sódio e uréia*. Universidade Federal de Mato Grosso do Sul, Campo Grande, 78 pp.
- Fort, C. A., McKaig Junior, N. (1939). *Comparative chemical composition of juices of different varieties of Louisiana sugar cane*. Technical Bulletin n.º 688. U.S.D. of Agriculture, Washington.

- Godoi, L. A. G., Camiloti, P. R., Bernardes, A. N., Sanchez, B. L. S., Torres, A. P. R., Gomes, A. C. & Botta, L. S. (2019). Seasonal variation of the organic and inorganic composition of sugarcane vinasse: main implications for its environmental uses. *Environmental Science and Pollution Research*, 26, 29267-29282.
- Glória, N. A. & Orlando Filho, J. (1984). Aplicação de vinhaça: um resumo e discussão sobre o que foi pesquisado: primeira parte. *Álcool & Açúcar*, 14(1), 24-35.
- Gomes, N. A., Ribeiro, L. S., Almeida, M. V. A., Gomes, G. L., Monteiro, V. E. D. (2016). *Análise do comportamento do nitrogênio amoniacal produzido em um aterro experimental*. In VII Congresso Brasileiro de Gestão Ambiental Campina Grande/PB. Anais, Campina Grande
- Gotardo, R. (2017). Evolução e magnitude das concentrações de cátions, ânions e carbono no dejetos líquido de suínos em fase de terminação. *Revista em Agronegócio e Meio Ambiente*, 10(3), 849-871.
- Halvin, J. L. & Soltanpour, P. N. (1980). A nitric acid plant digest method for use with inductively coupled plasma. *Commun. Soil Science and Plant Analysis*, 11(10), 869-880.
- Isaac, R. A. (1980). Atomic absorption methods for analysis of soil extracts and plant tissue digests. *Journal Association of Official Analytical Chemists*, 63(4), 788-796.
- Jones Junior, J. B. & Case, V. W. (1990). *Sampling handling, and analyzing plant tissue samples*. In Westerman, R.L. (org). Soil testing and plant analysis. SSSA Book Series n° 3, Madison.
- Kingston, H. M. & Jassie, L. B. (1988). *Introduction to Microwave Sample Preparation - Theory and Practice*. ACS Professional Reference Book, Washington.
- Konzen, E. A. (2006). *Viabilidade ambiental e econômica de dejetos de suínos*. Embrapa Milho e Sorgo, Sete Lagoas.
- Krug, F. J. (2000). *Métodos de Decomposição de Amostras*. III Workshop sobre preparo de Amostras, São Carlos.
- Lelis Neto, J. A. (2008). *Monitoramento de componentes químicos da vinhaça aplicados em diferentes tipos de solo*. Universidade de São Paulo, Piracicaba, 89pp.
- Lyra, M. R. C. C., Rolim, M. M. & Silva, J. A. A. (2003). Topossequência de solos fertigados com vinhaça: contribuição para a qualidade das águas do lençol freático. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 7(3), 525-531.
- Longo, R. M. (1994). *Efeito da vinhaça in natura e biodigerida em propriedades de um solo cultivado com cana-de-açúcar*. Universidade Estadual de Campinas, Campinas, 111 pp.
- Marques, M. O. (2006). *Aspectos técnicos e legais da produção, transporte e aplicação de vinhaça*. In: Segato, S. V., Pinto, A. S., Jendiroba, E. & Nóbrega, J. C. M. (Org.). Atualização em produção de cana-de-açúcar. Ceres, Piracicaba, 369-375.
- Mary, B., Recous, S., Darwis, D. & Robin, D. (1996). Interactions between decomposition of plant residues and nitrogen cycling in soil. *Plant and Soil*, 181(1), 71-82.
- Nascimento, C. L. (2003). *Avaliação econômica do aproveitamento do vinhoto concentrado como fertilizante*. Universidade Estadual do Norte Fluminense, Campos dos Goytacazes.
- Nova, P. C. C. V., Arruda, E. J., Oliveira, L. C. S., Santos, G., Roel, A. R. & Stropa, J. M. (2012). Avaliação de método convencional e digestão úmida para determinação de níveis de Fe, Mn, Ni, Cu, Co, Mg, Zn, Ca, Mo e Se em amostras secas de tomates orgânicos (*Solanum lycopersicum L.*) por Absorção Atômica de Chama (FAAS). *Journal of Biotechnology and Biodiversity*, 3(4), 159-165.
- Payne, J. H. (1968). *Sugar Cane Factory Analytical Control*. Elsevier Publishing Co., Amsterdam.
- Perkin-Elmer (1973). *Analytical methods for atomic absorption espetrophotometry*. Perkin-Elmer Corporation, Norwalk.
- Rosado, T. L., Silva, C. M., Silva, A. L., Almeida, G. D., Passos, R. R. & Andrade, F. V. (2007): *Variação do pH em função da aplicação de doses de vinhaça em amostras de dois solos cultivados com brachiaria decumbes*. In: XI Encontro Latino Americano de Iniciação Científica e VII Encontro Latino Americano de Pós-Graduação, 2007, Anais... Universidade do Vale do Paraíba.
- Rossetto, A. J. (1987). Utilização agrônômica dos subprodutos e resíduos da indústria açucareira e alcooleira. In: Paranhos, S.B. (ed.). Cana-de-açúcar: cultivo e utilização. Fundação Cargill, Campinas, 435-504.

- Salomon, K. R. (2007). *Avaliação técnico-econômica e ambiental da utilização do biogás proveniente da biodigestão da vinhaça em tecnologias para geração de eletricidade*. Universidade Federal de Itajubá, Itajubá.
- Salomon, K. R. & Lora, E. E. S. (2009): Estimate of the electric energy generating potential for different sources of biogas in Brazil. *Biomass and Bioenergy*, 33(9), 1101-1107.
- Saran, L.M. (2021). *Espectroscopia de Absorção Atômica*. FCVA/UNESP Jaboticabal, 2021. (available at: <https://www.fcva.unesp.br/Home/departamentos/tecnologia/LUCIANAMARIASARAN/espectroscopia-de-absorcao-atomica-versao-final.pdf>)
- Silva (2009). *Manual de Análises Químicas de Solos Plantas e Fertilizantes*. Embrapa, Brasília.
- Silva, A. F. R., Brasil, Y. L., Koch, K. & Amaral, M. C. S. (2021). Resource recovery from sugarcane vinasse by anaerobic digestion – A review. *Journal of Environmental Management*, 295.
- Silveira, E (2015). *Vinhaça para gerar energia*. Revista Pesquisa FAPESP, São Paulo.
- Silva, G. M. A. & Orlando Filho, J. (1981). Caracterização da composição química dos diferentes tipos de vinhaça no Brasil. *Boletim Técnico PLANALSUCAR*, 3(8), 5-22.
- Silva, M. A. S., Griebeler, N. P. & Borges, L. C. (2007). Uso de vinhaça e impactos nas propriedades do solo e lençol freático. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 11(1), 108-114.
- Seixas, F. L., Gimenes, M. L. & Machado, N. R. C. F. (2016). Tratamento da vinhaça por adsorção em carvão de bagaço de cana de açúcar. *Química Nova*, 39(2), 172-179.
- Soares, H. R., Neto, E. B., Barreto, L. P., Lira, R. M., Lucena, E. H. L., Lima, N. S. & Silva, M. A. (2013). *Comparação de metodologias para determinação de n-total em tecido vegetal*. In: XIII Jornada de Ensino, Pesquisa e Extensão – JEPEX 2013 – UFRPE. Anais, Universidade Federal Rural de Pernambuco, Recife.
- Souza, D. M., Madari, B. E. & Sena, M. M. (2012). Aplicação de métodos quimiométricos na otimização da extração de Ca, Mg, K, Fe, Zn, Cu e Mn em folhas de braquiária. *Química Nova*, 35(1), 175-179.
- Tedesco, M. J., Gianello, C., Bissani, C. A., Bohnen, H. & Volkweiss, S. J. (1995). *Análise de solo, plantas e outros materiais*. Universidade Federal do Rio Grande do Sul, Porto Alegre.
- Vidal, F. S. & Nakao, C. J. H. (2018). *A relação entre o pH, a alcalinidade e a amônia na piscicultura*. Grupo Integrado de Aquicultura, 2018. also available at: <https://gia.org.br/portal/a-relacao-entre-o-ph-a-alcalinidade-e-a-amonia-na-piscicultura/>.
- Zasoski, R. J. & Bureau, R. G. (1977). A rapid nitric acid digestion method for multi-element tissue analysis. *Commun. Soil Sci. Plant Anal*, 8(5), 425–443.

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