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# Innovative substrates for sugarcane seedling production: Sewage sludges and rice husk ash in a waste-to-product strategy



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### ABSTRACT

Recent advances in the sucroenergy production chain include a new way of planting sugarcane based on a method of seedling propagation which requires a significant amount of substrate. Since the sucroenergy sector has been noted as having cleaner energy production, it is imperative that sustainable substrates suitable for the production of sugarcane seedlings be developed. Therefore, the main objective of this study was to develop innovative substrates with solar-dried sewage sludges and rice husk ash to compose substrates for sugarcane seedling production. Batches of sewage sludges were collected in open drying beds from three different municipal wastewater treatment plants from the Rio Grande do Sul State, Brazil: from Passo Fundo city (treated by anaerobic digestion), from Rio Grande city (aerobic digestion) and from Santa Maria city (aerobic digestion). Rice husk ash was obtained from a rice processing industry in the Pelotas industrial region in southern Brazil. The content of trace elements and the pathogenicity of pure sewage sludge (SS) were analyzed. The nutrient content of SS and rice husk ash (RHA) was determined. Twelve substrates with differing ratios of SS, RHA, and vermiculite were formulated to evaluate the subsequent development of sugarcane seedlings. Chemical and physical attributes were determined in all substrates and compared to a commercial substrate. The experiment was conducted in a greenhouse using mini-setts of sugarcane of the RB867515 genotype. Fifteen days after planting the seedlings were evaluated for stalk diameter, shoot height, shoot dry weight and root, and the Dickson quality index was determined. Sewage sludge showed low levels of heavy metals and pathogenic organisms and high contents of nutrients, especially nitrogen, phosphorus and micronutrients such as Zn and Cu, showing promising suitability as a substrate component for seedling production. In general, substrates of all tested proportions of SS and RHA promoted greater shoot and root dry weight and a superior Dickson Quality Index than the commercial substrate used as reference, except for the formulation containing 87.5 % SS. The viability of combining sludges from municipal wastewater treatment plants and rice husk ash into one product was confirmed using a set of biometric attributes and nutrient tissue contents obtained from the production of sugarcane seedlings.

# 1. Introduction

Economic and environmental concerns, along with a worldwide depletion of natural resources, has led to a great amount of research and case studies into the bioeconomy, with direct recycling being one of the most innovative forms of reusing residues and by products. However, despite existing examples, many European Union countries (EstebanGutiérrez et al., 2018), as well as low to middle-income countries such as Brazil (Deus et al., 2017), continue to use landfill sites as the predominant disposal solution for organic solid wastes (Lim et al., 2016). This demonstrates that despite the existence of many viable solutions, such strategies need to be simplified or improved in order to be effectively and widely applicable.

Among the urban organic solid wastes, sewage sludge (SS) is one of

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the most important as it is produced continuously and on a large scale worldwide. According to Glab et al. (2018) and Kacprzak et al. (2017), the European Union alone generates around 12 million tons of SS every year, with Germany being the largest producer, followed by United Kingdom and France. Conversely, China and United States produce around 9 and 8 million tons per year, respectively. Promising examples of direct recycling can be found in Norway, where agricultural use comprises more than 90 % of the total generation of SS (Xu, 2014), and in France and Belgium, where agricultural use involves around 60 % of the total SS produced (Wang et al., 2008). Sewage sludge has been recognized as a valuable source of macronutrients (nitrogen, phosphorus, calcium and sulfur) and micronutrients (copper and zinc) to plants (Mohamed et al., 2018; Kacprzak et al., 2017; Samara et al., 2017; Abreu-Junior et al., 2017; Zoghlami et al., 2016), as well as a way to improve the water retention capacity of sandy soils (Glab et al., 2018). Among the many attributes of SS is its ability to promote morphological and ecophysiological parameters in sunflower seedlings (Mohamed et al., 2018), as well as wheat, ornamentals (Lopes et al., 2018; Samara et al., 2017) and in the development of eucalyptus plants in unfertile soils (Abreu-Junior et al., 2017). Other studies have highlighted the viability of SS as a substrate component, mainly due to its use in forest seedling production (Ibrahim et al., 2019; Siqueira et al., 2018; Caldeira et al., 2018; Santos et al., 2014; Gomes et al., 2013).

Rice husk ash (RHA) is also considered as a valuable organic waste, being generated from the burning of rice husks for energy production in low-efficiency production plants and/or in the rice milling industry (Teixeira et al., 2019). In Brazil, the production potential of RHA is around 0.44 million tons per year, with the immediate effect of its application in agricultural soils being as an amendment (Islabão et al., 2016), used to increase the soluble silicon and phosphorus content in the soil (Teixeira et al., 2019). Previous studies have documented RHA's role as a soil conditioner and as a substrate component and some commercial products containing RHA are available for purchase in the local market. Since RHA is relatively light and inert to hydration, it promotes high aeration space, which contributes towards making blends lighter and with a higher air-water ratio (Fermino et al., 2018).

It can be seen then, that the recycling of solid organic waste for agricultural activities should be therefore encouraged (Meena et al., 2019; Alvarenga et al., 2016). In Brazil, the sucroenergy sector stands out as having cleaner energy production, recycling sugarcane wastes as soil mulching, and second-generation biofuel production such as biogas and ethanol (Morato et al., 2018; Cortez et al., 2018). Recent advances in the sucroenergy production chain include a new method of planting sugarcane fields. Up until mid-2010, sugarcane production still utilized the conventional planting system involving large pieces of stalk (setts) planted into the soil. However, in 2012 a method was developed involving the propagation of seedlings in substrates using mini-setts from a single bud (Landell et al., 2012), aimed at reducing seedling volume and labor costs, and guaranteeing better crop homogeneity (Lee et al., 2007).

On the other hand, this new planting method requires a significant amount of substrate, resulting in an additional cost to the farmers. Moreover, commercially available substrates are quite expensive and developed mainly for ornamental and forest species, making them not satisfactorily suitable for use in production of sugarcane seedlings.

Therefore, we hypothesized that SS can be combined with other locally available wastes such as RHA to be used as a medium for the production of sugarcane seedlings. This strategy could also contribute towards controlling waste generated from the rice production industry and sewage treatment activities, by increasing the reutilization of waste products, and consequently reducing the impact of environmental pollution. Additionally, the development of this type of product for agricultural purposes could encourage sustainable supply chains and potentially reduce food and energy production costs.

In this context, the main objective of this study was to develop suitable substrates with solar-dried sewage sludges and rice husk ash for sugarcane seedling production. For this, the specific aims were: (i) to contrast measured pathogenicity and trace elements of domestic sewage sludges to legislative values; (ii) to evaluate the nutritional potential of domestic sewage sludges as raw material for plant growth media; (iii) to verify the adequacy of main physical and chemical attributes of the substrates formulated with sewage sludge from aerobic and anaerobic treatment processes; (iv) to evaluate the agronomic performance of substrates formulated with increasing sewage sludge rates and rice husk ash in the production of sugarcane seedlings.

### 2. Materials and methods

### 2.1. Characterization of substrates based on different sewage sludges

Batches of SS were collected in open drying beds at three different municipal wastewater treatment plants from the Rio Grande do Sul (RS) State, Brazil. The first SS (SS-1) came from Passo Fundo city which serves about 50,000 inhabitants in the north region of RS (28°13'47"S and 52°26'46"W). The second SS (SS-2) came from Rio Grande city, representing about 100,000 inhabitants from Southern RS State (32°05'30"S and 52°11'19"W), and the third SS (SS-3) came from Santa Maria city, comprising about 200,000 inhabitants in the central region of RS State (29°43'35"S and 53°48'58"W). SS-1 was treated by anaerobic digestion, whereas SS-2 and SS-3 were treated by aerobic digestion. All collected SS contained 40-50% of total dry solids, obtained as a result of more than 90 days in the drying beds. Batches of SS were distributed in 0.1 m layers in fiberglass boxes placed inside agricultural greenhouses covered by transparent plastic (thickness = 200 □m) for sanitation and drying through solarization, which lasted for at least 60 days during the summer. Daily mean interior ambient temperatures of the greenhouses varied from 22 to 38 °C, with extreme values ranging from 10 °C (lowest) to 59 °C (highest), while daily solar radiation ranged between 400–600 cal cm<sup>-2</sup> day<sup>-1</sup>. Afterwards, the SS batches were sampled for laboratory analyses. Since SS-1 initially had a very low pH (3.4), 4% w/w of dolomitic limestone was added to increase the pH to 6.0. RHA was obtained from a rice processing industry in the Pelotas industrial region in Southern Brazil. This waste is widely available and is generated by the combustion of rice husks during thermal energy production. Vermiculite (VER), a sterile mineral raw material commonly used as a soil conditioner, was locally purchased and was used to compose the substrate formulations. In order to reduce the use of natural resources, the lowest proportion of vermiculite was used.

Twelve substrates with differing ratios of SS, RHA and VER (Table 1) were formulated and compared to a control substrate

### Table 1

Ratios of sewage sludges (SS), rice husk ash (RHA) and vermiculite (VER) used in substrate formulations.

Substrate Formulations	SS-1	SS-2	SS-3	RHA	VER
			%		
Substrate 1	87.5	_	_	6.25	6.25
Substrate 2	75	-	-	12.5	12.5
Substrate 3	50	-	-	25	25
Substrate 4	33	-	-	33	33
Substrate 5	-	87.5	-	6.25	6.25
Substrate 6	-	75	-	12.5	12.5
Substrate 7	-	50	-	25	25
Substrate 8	-	33		33	33
Substrate 9	-	-	87.5	6.25	6.25
Substrate 10	-	-	75	12.5	12.5
Substrate 11	-	-	50	25	25
Substrate 12	-	-	33	33	33

SS-1: anaerobic sewage sludge from Passo Fundo; SS-2: aerobic sewage sludge from Rio Grande; SS-3: aerobic sewage sludge from Santa Maria, RS State.

composed of the commercial substrate (main composition: peat + carbonized rice husk) commonly used in the region to produce forest seedlings. The particle-size distribution of SS, RHA, VER and the commercial substrate used in this experiment were determined by sieve analysis (see supplementary document for additional information).

### 2.2. Pathogenicity and chemical characteristics of the substrates

Samples of 100 % SS were sent to an accredited laboratory for analyses of pathogenicity and trace elements, and the results were compared to the maximum limits of contaminants allowed in substrates for plants (BRASIL, 2016). Additionally, samples of 100 % SS, 100 % RHA. 100 % VER and the commercial substrate were sent to the Laboratory of Soils and Substrates from the Federal University of Rio Grande do Sul for chemical characterization. Measurements of pH and electrical conductivity (EC) were carried out on samples of each substrate using the standard method of the Brazilian norm (BRASIL, 2007), recommended by the International Society of Horticultural Sciences (UNE-EN, 13037 and UNE-EN, 13038). In addition, the dry density (DD), total porosity (TP), aeration space (AS) and readily available water (RAW) were obtained from the water availability curves at the following potentials: 0, 1, 5 and 10 kPa, as determined according the methodology of Normative Instruction nº 17/2007 (BRASIL, 2007), described in detail by Fermino (2014). Analyses were performed at the Laboratory of Analysis of Substrates from the Agricultural Research Foundation of Rio Grande do Sul (FEPAGRO) in Porto Alegre, RS State.

# 2.3. Biometric attributes of sugarcane seedlings grown on formulated substrates

Experiments were conducted in a temperature-controlled greenhouse (15-40 °C), located in the city of Pelotas  $(31^{\circ}42'S \text{ and } 52^{\circ}24'W)$ , in Southern Brazil. The experiment utilized a Latin Square design with five replicates for each SS type associated with the four ratios of RHA and VER (Table 2). Mature sugarcane mini-setts (age 18 months) of the RB867515 genotype were placed in tubes of 180 mL into each formulation. For each SS type, luminosity (in the column) and sugarcane bud age (in the line) were adopted as local controls.

The buds sprouting period of each mini-sett was monitored, and at 50 days post-planting (the age when seedlings are typically transplanted), the following growth variables were measured: stalk diameter (SD, mm), shoot height (H, cm), shoot dry weight (SDW) and root dry weight (RDW) (dried in a forced-air oven at 65 °C for 72 h to a constant weight, expressed in g). The Dickson quality index (DQI) was obtained according to Dickson et al. (1960), where DQI = TDW / (H/SD + SDW/ RDW), in which TDW is the total dry weight (g). Seedling shoots were crushed and five samples per formulation were collected to determine nitrogen (N) content by dry combustion in an elemental analyzer (Truspec CN - LECO), and phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), cooper (Cu) and zinc (Zn) total content was determined following the methodology described by Tedesco (1985).

# 2.4. Statistical analyses

The analysis of experiment groups was performed in order to determine the most adequate formulation for the development of sugarcane seedlings. The ANOVA (F test, p < 0.05) was initially performed for each SS type, and from this the sum square of residues was obtained, which were then compared to verify the assumption of variance homogeneity between the experiments. Once verified, the ANOVA was performed considering the interaction between SS type and the different formulations. If there was an interaction effect, the degrees of freedom were unfolded and the effects of the formulation were compared within each SS type and vice versa. When no significant interaction was observed, a Tukey test (p < 0.05) was performed to compare the main effects of the formulations in each SS type. All data

#### Table 2

Pathogenic-related parameters and trace elements in pure sewage sludges (SS) in comparison with legislation limits and chemical composition of sewage sludges, rice husk ash (RHA), vermiculite (VER) and commercial substrate.

Pathogenic-related parameters and trace elements							
Parameters		SS-1	S	S-2	SS-3		Limit Value*
Thermotolerant coliforms $(MPN/g TDS)^1$		45	4	5	140		1000
Helminth eggs $(N^2/4 \text{ g TDS})^2 < 0.25 < 0.25 < 0.25$					).25	1	
Salmonella ssp. (MPN/10 g)		Absent	А	bsent	Absent		Absent
Arsenium (mg $kg^{-1}$ )		< 0.5	1.	.2	< 0	.5	20
Cadmium (mg $kg^{-1}$ )		< 0.03	0	.4	0.35		8
Chromium (mg kg $^{-1}$ )		1.41	4	.4	6.92		500
Mercury (mg $kg^{-1}$ )		< 0.02	0	.3	< 0	.02	2.50
Selenium (mg kg <sup>-1</sup> )		< 0.2	<	< 0.2	< 0	.2	80
Lead (mg kg <sup>-1</sup> )		4.88	7.	.4	11.5		300
Chemical composition							
Total content	SS-1	SS-2	SS-3	RHA	VER	Com	mercial subs.
Nitrogen - Kjeldahl (g kg <sup>-1</sup> )	29	54	57	1	18	40	
Phosphorus (g $kg^{-1}$ )	18	24	29	15	11	24	
Potassium (g kg <sup>-1</sup> )	3	3	2	3	4	3	

Phosphorus (g kg <sup>-1</sup> )	18	24	29	15	11	24
Potassium (g kg <sup><math>-1</math></sup> )	3	3	2	3	4	3
Calcium (g kg <sup>-1</sup> )	12	17	21	9	8	19
Magnesium (g kg $^{-1}$ )	7	6	7	10	27	8
Sulphur (g kg <sup>-1</sup> )	5	8	9	4	3	6
Sodium (g kg $^{-1}$ )	0.6	1.2	1.5	0.5	0.7	0.5
Cooper (mg kg <sup>-1</sup> )	120	134	124	101	76	157
Zinc (mg kg $^{-1}$ )	601	640	831	546	445	1100
Manganese (mg $kg^{-1}$ )	100	613	424	1400	767	417

 $^{1}$  MPN/g TDS Most probable number per gram of total dry solids;  $^{2}N^{o}/4$  g TDS: number of eggs in 4 g of total dry solids.

\* Brazil [24].SS-1: anaerobic sewage sludge from Passo Fundo; SS-2: aerobic sewage sludge from Rio Grande; SS-3: aerobic sewage sludge from Santa Maria, RS State.

analysis was performed using the statistical software R version 3.5.1 (2018).

## 3. Results

### 3.1. Characteristics of sewage sludge as plant substrate

Regarding the maximum values allowed for pathogenic microorganisms and trace elements in accordance with current Brazilian regulations (BRASIL, 2016), and comparing the limit values with experimental results (Table 2), we observed that SS did not exceed the allowable levels regardless of the wastewater treatment plant, considered as evidence for the efficiency of the stabilization process followed by solarization. On the other hand, the chemical composition of the different substrate components showed that SS under aerobic digestion (SS-2 and SS-3) presented higher nitrogen, phosphorus, calcium, sulphur, sodium, cooper, zinc and manganese content than SS under anaerobic digestion (SS-1) (Table 2).

As shown in Fig. 1 (a), sludge from anaerobic digestion (SS-1) even after liming presented a more acidic pH (5.6) than those from aerobic digestion (SS-2 and SS-3), which presented pH values naturally closer to neutrality (6.8 and 7.1, respectively). The VER showed a pH close to neutrality (6.4), whereas the RHA generally behaved as an alkalizing material, with a pH close to 9.0.

Despite its high pH, RHA was not able to cause changes in the pH of mixtures with SS-1 (Subs. 1–4). On the other hand, SS under aerobic digestion (SS-2 and SS-3) showed different behaviors when RHA was added. pH equal or greater than 8.0 were observed in the different formulations with SS-2 (Subs. 5–8), showing that the addition of RHA promoted an increase of pH in the substrate, which was originally 6.8

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a)



Fig. 1. Values of pH (a) and electrical conductivity (EC) (b) in the substrates with 100 % sewage sludge from Passo Fundo, Rio Grande and Santa Maria (SS-1, SS-2, SS-3, respectively), 100 % rice husk ash (RHA), 100 % vermiculite (VER), commercial substrate (Commercial), and different combinations of SS types with RHA and VER, namely Subs.1:87.5 %SS1 + 6.25 %RHA + 6.25 % VER, Subs.2: 75 %SS1 + 12.5 %RHA + 12.5 %VER, Subs.3: 50 % SS1 + 25 %RHA + 25 %VER, Subs.4: 33 %SS1 + 33 %RHA + 33 %VER, Subs.5: 87.5 %SS2 + 6.25 %RHA + 6.25 %VER, Subs.6: 75 %SS2 + 12.5 %RHA + 12.5 %VER, Subs.7: 50 %SS2 + 25 % RHA + 25 %VER, Subs.8: 33 %SS2 + 33 %RHA + 33 %VER, Subs.9: 87.5 %SS3 + 6.25 %RHA + 6.25 %VER, Subs.10: 75 % SS3 + 12.5 %RHA + 12.5 %VER, Subs.11: 50 %SS3 + 25 % RHA + 25 %VER, and Subs.12: 33 %SS3 + 33 %RHA + 33 % VER

# b)

# (Fig. 1 a).

Concerning electrical conductivity (EC), it was observed that among the components of the pure substrate, SS presented higher values  $(2.59-4.91 \text{ mS cm}^{-1})$  than RHA  $(0.14 \text{ mS cm}^{-1})$  and VER  $(0.03 \text{ mS} \text{ cm}^{-1})$  (Fig. 1b), possibly due to the higher nutrient concentration in sewage sludge (Table 4). Nevertheless, EC decreased when RHA and VER were added toSS-1 (Subs. 1–4) and SS-2 (Subs. 5–8) (Fig. 1b).

The physical characterization of the substrates showed that pure sewage sludge (100 % SS-1, 100 % SS-2, 100 % SS-3) had a dry density (DD) between 414 and 494 kg m<sup>-3</sup>, whereas the DD of the commercial substrate, RHA, and VER were 297, 194 and 73 kg m<sup>-3</sup>, respectively. Consequently, increasing proportions of RHA and VER to the SS promoted a moderate reduction of substrate DD, with values of 321 kg m<sup>-3</sup> in the combination of 33 %SS-1 + 33 %RHA + 33 %VER, 270 kg m<sup>-3</sup> in the combination of 33 %SS-2 + 33 %RHA + 33 %VER, and 364 kg m<sup>-3</sup> in the combination of 33 %SS-3 + 33 %RHA + 33 %VER (Table 3). DD is an important characteristic since high substrate densities have direct implications on available space for root development, water storage and other pore-volume related characteristics, besides the cost of transport and handling of the material (Castoldi et al., 2014). Regardless of the combination of sludge (100 %, 87.5 %, 50 % or 33 %), DD values remained within the reference range for substrates (350 to 500 kg m<sup>-3</sup>) (Schafer et al., 2015). However, according to Kampf (2005), DD values should be defined according to the size of the seedling pot. Thus, commercial substrate and VER are suitable for multicellular pots, due to DD values among  $100-300 \text{ kg m}^{-3}$ , whereas most of the SS's combinations should be recommended for use in pots with 0.20-0.30 m height due to its DD values of between 300 and 500 kg m<sup>-3</sup> (Table 3).

All combinations of SS with RHA and VER showed a total porosity (TP) of close to or higher than  $0.70 \text{ m}^3 \text{ m}^{-3}$ , and were similar or slightly greater than the commercial substrate, which was taken as a reference. The SS-1 and RHA combinations showed an aeration space (AS) of around  $0.20 \text{ m}^3 \text{ m}^{-3}$ , higher than the commercial substrate, whereas the RHA combinations with SS-2 and SS-3 showed AS around  $0.10 \text{ m}^3 \text{ m}^{-3}$ , lower than commercial substrate (Table 3).

Available water content (AWC) was below the ideal range  $(0.25-0.35 \text{ m}^3 \text{ m}^{-3})$  for all substrates tested, including the commercial, agreeing with Schaefer et al. (2015) who observed that most commercialized substrates in the South of Brazil (75.3 %) provide insufficient AWC, directly impacting the frequency of irrigation. Under these conditions, farmers may increase their risk of losing plants if irrigation management is not automated or frequently checked. In the present study, the RHA addition of 33 % in Subs. 4 promoted an increase of readily available water (RAW) from 0.41 m<sup>3</sup> m<sup>-3</sup> (100 % SS-1) to 0.54 m<sup>3</sup> m<sup>-3</sup> (33 % SS-1), while in SS-3 the RHA addition promoted a decrease of RAW from 0.71 m<sup>3</sup> m<sup>-3</sup> (100 % SS-3) up to 0.62 m<sup>3</sup> m<sup>-3</sup> (Subs 9) (Table 3).

### Table 3

Mean values of dry density (DD), total porosity (TP), aeration space (AS), available water content (AWC), readily available water (RAW) in substrates composed by 100 % sewage sludges (SS-1, SS-2 and SS-3), 100 % rice husk ash (RHA), 100 % vermiculite (VER), different combinations of SS with RHA and VER, and commercial substrate taken as reference.

Substrate Formulations	DD kg m <sup>-3</sup>	$TP m^3 m^{-3}$	AS	AWC	RAW
Commercial Substrate	297 ± 2.67	$0.66 \pm 0.01$	$0.15 \pm 0.05$	$0.04 \pm 0.04$	$0.46 \pm 0.02$
100 % SS-1	$468 \pm 3.38$	$0.69 \pm 0.06$	$0.22 \pm 0.12$	$0.07 \pm 0.10$	$0.41 \pm 0.01$
100 % SS-2	414 ± 11.99	ND	ND	ND	ND
100 % SS-3	494 ± 1.59	$0.80~\pm~0.01$	$0.05 \pm 0.01$	$0.04 \pm 0.01$	$0.71 \pm 0.02$
100 % RHA	$73 \pm 2.00$	$0.90 \pm 0.02$	$0.03 \pm 0.01$	$0.04 \pm 0.01$	$0.83 \pm 0.01$
100 % VER	$194 \pm 1.09$	$0.70 \pm 0.01$	$0.04 \pm 0.02$	$0.14 \pm 0.02$	$0.52 \pm 0.01$
Subs 1: 87.5 %SS-1 + 6.25 %RHA + 6.25 % VER	$486 \pm 3.00$	$0.69 \pm 0.03$	$0.20 \pm 0.09$	$0.07 \pm 0.09$	$0.43 \pm 0.08$
Subs 2: 75 %SS-1 + 12.5 %RHA + 12.5 %VER	$477 \pm 10.62$	$0.67 \pm 0.06$	$0.19 \pm 0.08$	$0.05 \pm 0.08$	$0.43 \pm 0.06$
Subs 3: 50 %SS-1 + 25 %RHA + 25 %VER	$421 \pm 2.66$	$0.72 \pm 0.07$	$0.18 \pm 0.07$	$0.05 \pm 0.05$	$0.48 \pm 0.09$
Subs 4: 33 %SS-1 + 33 %RHA + 33 %VER	$321 \pm 5.61$	$0.73 \pm 0.05$	$0.14 \pm 0.05$	$0.06 \pm 0.04$	$0.54 \pm 0.09$
Subs 5: 87.5 %SS-2 + 6.25 %RHA + 6.25 %VER	$394 \pm 9.84$	$0.70 \pm 0.03$	$0.08 \pm 0.02$	$0.09 \pm 0.03$	$0.53 \pm 0.02$
Subs 6: 75 %SS-2 + 12.5 %RHA + 12.5 %VER	$363 \pm 3.59$	$0.71 \pm 0.03$	$0.10 \pm 0.02$	$0.08 \pm 0.03$	$0.54 \pm 0.01$
Subs 7: 50 %SS-2 + 25 %RHA + 25 %VER	$327 \pm 16.61$	$0.72~\pm~0.01$	$0.08 \pm 0.03$	$0.10 \pm 0.01$	$0.54 \pm 0.01$
Subs 8: 33 %SS-2 + 33 %RHA + 33 %VER	$270 \pm 2.86$	$0.74 \pm 0.02$	$0.10 \pm 0.01$	$0.12 \pm 0.01$	$0.51 \pm 0.01$
Subs 9: 87.5 %SS-3 + 6.25 %RHA + 6.25 %VER	$428 \pm 0.36$	$0.75 \pm 0.01$	$0.10 \pm 0.01$	$0.03 \pm 0.01$	$0.62 \pm 0.02$
Subs 10: 75 %SS-3 + 12.5 %RHA + 12.5 %VER	$408 \pm 2.29$	$0.75 \pm 0.01$	$0.11 \pm 0.01$	$0.01 \pm 0.01$	$0.64 \pm 0.01$
Subs 11: 50 %SS-3 + 25 %RHA + 25 %VER	$391 \pm 3.35$	$0.77 \pm 0.00$	$0.10 \pm 0.01$	$0.03 \pm 0.01$	$0.64 \pm 0.01$
Subs 12: 33 %SS-3 + 33 %RHA + 33 %VER	$364 \pm 3.26$	$0.74~\pm~0.02$	$0.07~\pm~0.01$	$0.03~\pm~0.02$	$0.64~\pm~0.01$

SS-1: anaerobic sewage sludge from Passo Fundo; SS-2: aerobic sewage sludge from Rio Grande; SS-3: aerobic sewage sludge from Santa Maria, RS State. \*ND = not determined because the material did not present structural stability for the physical analysis.

# 3.2. Biometric attributes of sugarcane seedlings in sewage sludge-based substrates

The interaction between substrate formulation and SS type was observed for seedling height and bud sprouting period, and the results are presented for each formulation within each SS type (Table 4). Buds sprouted earlier (mean of 11 days) in formulations with sewage sludge from aerobic treatment (SS-2 and SS-3), and the seedlings had the greatest height (respectively, average of 100.45 and 125.3 cm) in relation to the formulations with SS from anaerobic treatment (SS-1), where the buds sprouted after 14 days and the seedlings had a mean height of 71 cm. In comparison to the commercial substrate, formulations with 50 and 33 % of SS-1 and all proportions of SS-2 and SS-3 presented superior seedling heights (Table 4). This fact can be attributed to the physical condition of these formulations, that is, while the SS formulations presented a TP equal or higher than  $0.70 \text{ m}^3 \text{ m}^{-3}$  and RAW equal or greater than  $0.48 \text{ m}^3 \text{ m}^{-3}$ , the commercial substrate presented a TP of  $0.66 \text{ m}^3 \text{ m}^{-3}$  and a RAW of  $0.46 \text{ m}^3 \text{ m}^{-3}$  (Table 3). Concerning stalk diameter (SD), shoot dry weight (SDW) and root

dry weight (RDW), no interaction between SS types and formulations

were observed, therefore, the isolated effects were presented (Figs. 2 and 3, respectively). In this sense, sugarcane seedlings grown on SS-1 and SS-2 respectively presented SD 26 and 15 % (Fig. 2a), SDW 54 and 34 % (Fig. 2b), and RDW 28 and 36 % (Fig. 2c) lower than seedlings grown on SS-3.

The above-mentioned biometric results culminated in the DQI of seedlings grown in SS-1 (0.33) and SS-2 (0.34) formulations, being up to 35 % lower than seedlings grown on substrates with SS-3 (0.51) (Fig. 2d). Concerning the formulations, we observed that except for root dry matter, all proportions among SS, RHA, and VER (notably the proportion of 33 %), promoted better biometric attributes when compared to the commercial substrate (Fig. 3a-d).

The physical conditions of the SS formulations, in detrimental of chemical characteristics, had a greater influence on the initial sugarcane development, evidenced when considering the nutrient contents present in the plant tissues. In other words, although SS-2 and SS-3 showed higher nitrogen (57 and 54 g kg<sup>-1</sup> respectively) and phosphorus (29 and 24 g kg<sup>-1</sup> respectively) concentrations in relation to SS-1 (29 and 18 g kg<sup>-1</sup> respectively), as shown in Table 4, the formulations with these SS did not promote greater concentrations of these nutrients

### Table 4

Sugarcane bud sprouting period and seedling height in different sewage sludge-based formulations and commercial substrate.

Substrate formulations	Sewage sludge type			
	SS-1	SS-2	SS-3	
	Bud sprouting (days)			
Commercial Substrate	$13 \pm 0.37$ <sup>ns</sup>	$10 \pm 0.37 c$	$10 \pm 0.68^{ns}$	
87.5 %SS + 6.25 %RHA + 6.25 %VER	15 ± 0.58 B	$12 \pm 0.71 \text{ aA}$	11 ± 0.60 A	
75 %SS + 12.5 %RHA + 12.5 %VER	$15 \pm 0.58C$	$12 \pm 0.40 \text{ aB}$	$10 \pm 0.40 \text{A}$	
50 %SS + 25 %RHA + 25 %VER	14 ± 0.51 B	$11 \pm 0.51 \text{ bA}$	11 ± 0.71 A	
33 % SS + 33 %RHA + 33 %VER	$15 \pm 0.51 \text{ C}$	$9 \pm 0.58$ cA	$10 \pm 0.24 \text{ B}$	
	Seedlings height (cm)			
Commercial Substrate	$58.2 \pm 2.1 \text{ b}$	46.4 ± 6.6 c	84.0 ± 6.1 b	
87.5 %SS + 6.25 %RHA + 6.25 %VER	55.6 ± 3.6 b C	90.6 ± 6.2 b B	123.0 ± 7.6 a A	
75 %SS + 12.5 %RHA + 12.5 %VER	$64.2 \pm 3.0 \text{ abC}$	83.4 ± 8.5 bB	123.8 ± 6.3 aA	
50 %SS + 25 %RHA + 25 %VER	74.2 ± 3.5 aC	$106.6 \pm 6.5 \text{ aB}$	118.4 ± 5.6 aA	
33 % SS + 33 %RHA + 33 %VER	88.6 ± 1.5 aB	121.2 ± 1.5 aA	$136.0 \pm 4.2 \text{ aA}$	

Means followed by the same lowercase letter in the column and capital letter in the row do not differ by Tukey's test ( $p \le 0.05$ ), comparing the different sewage sludge-based formulations and commercial substrate, respectively. ns: not significant.SS-1: anaerobic sewage sludge from Passo Fundo; SS-2: aerobic sewage sludge from Rio Grande; SS-3: aerobic sewage sludge from Santa Maria, RS State; RHA: Rice Husk Ash; VER: Vermiculite.



**Fig. 2.** Mean values of stalk diameter, shoot dry weight, root dry weight and Dickson quality index of sugarcane seedlings in substrates with anaerobic - Passo Fundo (SS-1) and aerobic - Rio Grande and Santa Maria (SS-2 and SS-3) sewage sludges. Means followed by the same letter do not differ by Tukey's test ( $p \le 0.05$ ).



Fig. 3. Mean values of stalk diameter, shoot dry weight, root dry weight and Dickson quality index of sugarcane seedlings on a commercial substrate and substrates with different proportions of sewage sludge, rice husk ash (RHA), and vermiculite (VER). Means followed by the same letter do not differ by Tukey's test ( $p \le 0.05$ ).

in the plant tissue (Table 5). In this sense, the physical attributes of a substrate are more important in the initial development of seedlings with direct effects on biometric parameters such as bud sprouting and rooting (Milner, 2001; Xavier et al., 2014). However, when the nutritional reserve of the bud is depleted the substrate must continue to provide the necessary nutrition for the seedlings (Landell et al., 2012).

Substrate which has adequate nutrition contributes to quicker production and better establishment of seedlings under field conditions, which in turn has a direct effect on sugarcane yield. This information converges with our SDW, RDW, SD and DQI results of the sugarcane seedlings developed in the substrates with SS in relation to the commercial substrate.

### Table 5

Nutrient contents in sugarcane seedlings grown in different sewage sludge-based formulations of substrates.

Substrate formulations	Sewage sludge type				
	SS-1	SS-2	SS-3		
	Nitrogen (g kg $^{-1}$ )				
Commercial Substrate	$9.0 \pm 0.07  \mathrm{d}$	$7.9 \pm 0.08 c$	$6.4 \pm 0.06 c$		
87.5 %SS + 6.25 %RHA + 6.25 %VER	29.3 ± 0.11 aA	$23.8 \pm 0.12 \text{ aB}$	$17.6 \pm 0.05 \text{ aC}$		
75 %SS + 12.5 %RHA + 12.5 %VER	27.7 ± 0.05aA	22.1 ± 0.04 aB	$17.8 \pm 0.08 \text{ aC}$		
50 %SS + 25 %RHA + 25 %VER	21.4 ± 0.07 bA	23.5 ± 0.12 aA	$17.2 \pm 0.04 \text{ abB}$		
33 %SS + 33 %RHA + 33 %VER	$16.6 \pm 0.08c^{NS}$	$16.7 \pm 0.10b$	$14.3~\pm~0.01b$		
	Phosphorus (g kg <sup>-1</sup> )				
Commercial Substrate	$2.55 \pm 0.12 b^{NS}$	2.80 ± 0.33 c	$3.14 \pm 0.42 c$		
87.5 %SS + 6.25 %RHA + 6.25 %VER	$5.68 \pm 0.97 a^{NS}$	7.44 ± 0.74 a	6.54 ± 0.16 a		
75 %SS + 12.5 %RHA + 12.5 %VER	$4.05 \pm 0.77 \text{ ab}^{NS}$	3.47 ± 0.48 c	$3.02 \pm 0.74 c$		
50 %SS + 25 %RHA + 25 %VER	$4.97 \pm 1.64 a^{NS}$	$3.62 \pm 0.46$ bc	$5.13 \pm 0.47 \text{ b}$		
33 %SS + 33 %RHA + 33 %VER	$5.26 \pm 0.63 a^{NS}$	4.33 ± 0.64 b	$5.55 \pm 0.18 \text{ b}$		
	Potassium (g kg $^{-1}$ )				
Commercial Substrate	25.8 ± 0.99 a	$31.6 \pm 0.73^{ns}$	30.5 ± 1.52 a		
87.5 %SS + 6.25 %RHA + 6.25 %VER	12.9 ± 0.45 cC	29.0 ± 1.24A	$18.8 \pm 1.06 \text{ cB}$		
75 %SS + 12.5 %RHA + 12.5 %VER	$13.8 \pm 0.64 \text{ cC}$	30.6 ± 1.49A	24.2 ± 1.13 bB		
50 %SS + 25 %RHA + 25 %VER	13.4 ± 0.96 cC	29.7 ± 1.89 A	$23.9 \pm 0.92 \text{ bB}$		
33 %SS + 33 %RHA + 33 %VER	$20.4 \pm 1.29 \text{ bB}$	30.4 ± 0.67 A	$17.7 \pm 1.01 \text{ cB}$		
	Magnesium (g kg $^{-1}$ )				
Commercial Substrate	$3.88 \pm 0.20 \text{ c}$	$2.64 \pm 0.16 \text{ b}$	$2.70 \pm 0.13 \text{ b}$		
87.5 %SS + 6.25 %RHA + 6.25 %VER	$5.51 \pm 0.24 bA$	4.21 ± 0.29 aB	$4.89 \pm 0.21 \text{ aA}$		
75 %SS + 12.5 %RHA + 12.5 %VER	$6.02 \pm 0.22 \text{ abA}$	4.30 ± 0.32 aB	4.45 ± 0.22 aB		
50 %SS + 25 %RHA + 25 %VER	6.68 ± 0.10 aA	4.44 ± 0.13 aB	4.87 ± 0.27 aB		
33 %SS + 33 %RHA + 33 %VER	6.69 ± 0.18 aA	$4.20 \pm 0.08 \text{ aC}$	$5.07~\pm~0.08~aB$		
	Cooper (mg kg $^{-1}$ )				
Commercial Substrate	$3.00 \pm 0.12 \text{ b}$	3.91 ± 0.18 a	$0.85 \pm 0.20 \text{ b}$		
87.5 %SS + 6.25 %RHA + 6.25 %VER	5.35 ± 0.42 aA	2.42 ± 0.21 bB	$5.55 \pm 0.67 \text{ aA}$		
75 %SS + 12.5 %RHA + 12.5 %VER	4.90 ± 0.39 aA	3.33 ± 0.14 abB	5.74 ± 0.43 aA		
50 %SS + 25 %RHA + 25 %VER	3.91 ± 0.28abB	2.67 ± 0.16 bC	6.66 ± 1.31 aB		
33 %SS + 33 %RHA + 33 %VER	$3.25 \pm 0.38 \text{ bB}$	$1.98~\pm~0.05~\mathrm{bC}$	$6.11~\pm~0.32~aB$		
	Zinc (mg kg <sup>-1</sup> )				
Commercial Substrate	$22.39 \pm 1.61c^{NS}$	23.35 ± 6.00 b	$13.93 \pm 1.22 \ d$		
87.5 %SS + 6.25 %RHA + 6.25 %VER	$143.50 \pm 13.90 a^{NS}$	37.72 ± 2.19 a	37.94 ± 1.37 c		
75 %SS + 12.5 %RHA + 12.5 %VER	$138.60 \pm 8.90a^{NS}$	38.72 ± 4.32 a	56.96 ± 6.10 b		
50 %SS + 25 %RHA + 25 %VER	$127.76 \pm 8.83 a^{NS}$	39.29 ± 1.69 a	$38.71 \pm 2.16 c$		
33 %SS + 33 %RHA + 33 %VER	$58.70 \pm 36.16 b^{NS}$	38.10 ± 2.29 a	106.63 ± 2.76 a		

Means followed by the same lowercase letter in the column and capital letter in the row do not differ by Tukey's test ( $p \le 0.05$ ), comparing the different types of the sewage sludge and formulations substrate, respectively. NS: not significant. SS-1: anaerobic sewage sludge from Passo Fundo; SS-2: aerobic sewage sludge from Rio Grande; SS-3: aerobic sewage sludge from Santa Maria, RS State; RHA: Rice Husk Ash; VER: Vermiculite.

The aerobic digested sludges (SS-2 and SS-3), mainly because of their richer nutrient composition, released more nutrients and probably over a longer time when compared to sludge under anaerobic digestion (SS-1). Even so, at 50 days all the seedlings presented nitrogen content in their tissues close to or within the range considered suitable for the sugarcane crop under Brazilian conditions (between  $18-25 \text{ g kg}^{-1}$ ), except the commercial substrate.

In relation to P, K, Mg and Zn concentrations (Table 5), regardless of SS type and substrate formulation, sugarcane seedlings presented levels above those recommended for the culture, with values of  $1.5-3.0 \text{ g} \text{ kg}^{-1}$ ,  $10-16 \text{ g} \text{ kg}^{-1}$ ,  $1-3 \text{ g} \text{ kg}^{-1}$  and  $10-50 \text{ mg} \text{ kg}^{-1}$ , for P, K, Mg and Zn, respectively (Raij and Cantarella, 1996). Regardless of SS type, the foliar content of Cu for most of the formulations was found below the ideal range (between 6 and  $15 \text{ mg} \text{ kg}^{-1}$ ). On the other hand, compared to the commercial substrate, SS formulations presented higher concentrations of all of the evaluated nutrients, except for K and Cu.

No interaction between SS type and formulation was observed for Ca concentration; therefore the isolated effects were taken. The SS-1 derived substrates promoted the highest Ca concentration in sugarcane seedlings ( $3 g kg^{-1}$ ) (See supplementary document for additional information). Although a lower concentration of this nutrient was

observed in the foliar tissue of plants in SS-2 and SS-3 substrates, it was within the recommended range for the crop  $(2-8 \text{ g kg}^{-1})$ . Compared to the commercial substrate, substrates with 87.5 % and 33 % of SS promoted the lowest Ca concentrations in the shoot dry matter of the sugarcane seedlings.

### 4. Discussion

Sewage sludge showed potential as a substrate component for plants, according to Table 4, with similar or even higher levels of nitrogen (29 to 57 g kg<sup>-1</sup>) than other solid organic materials widely used in agriculture, such as poultry, turkey, pig and cattle manures. Likewise, P levels (18 to 29 g kg<sup>-1</sup>) were similar or superior than pig and cattle manures, whereas K (2 to 3 g kg<sup>-1</sup>) levels were lower than all the other organic materials commonly used in agriculture (CQFS, 2016). Tsutiya et al. (2002) mention that potassium concentrations are low in sewage sludges due to its high solubility in water, remaining in the liquid effluent and generally taken apart in the sewage treatment process.

The low pH value of SS-1 (Fig. 1a) is due possibly to an overload of organic matter in the anaerobic digester, which may have led to an increase in volatile acid concentration as a result of acidogenic cover

methanogenic bacteria, inhibiting the buffering effect of the medium and therefore causing a reduction in pH (Andreolli et al., 2014). When the different materials were combined with the SS types, it was observed that contrary to what was expected, an addition of RHA to SS-1 (Subs. 1–4) did not promote significant changes in substrate pH values, regardless of the proportion used, which remained between 5.52 and 5.64 (Fig. 1a), very similar to the pH that SS-1 had at 100 % proportion (5.58). The alkalinity behavior of RHA was not enough to counterbalance the pH of the anaerobic sludge, possibly due to the high acidification potential of this material. According to Chen et al. (2018), the pH range of the sewage sludge buffering mechanism can be due to carbonate dissolution (pH  $\geq$  6.2), cation exchange (5.5  $\leq$  pH < 6.2), organic buffering (4.2  $\leq$  pH < 5.5), aluminum hydroxide dissolution (3.8  $\leq$  pH < 4.2) and ferric hydroxide dissolution (pH < 3.8).

On the other hand, the pH increased when RHA was added to SS-2 formulations, agreeing with Fermino et al. (2018), who observed increased pH in substrates when carbonized rice husk was combined with other materials. In soils, Teixeira et al. (2019) and Islabão et al. (2016) also observed increased pH with additions of RHA. In SS-3 formulations (Subs. 9-12) the addition of RHA did not have an influence on the pH values of the substrate, ranging from 5.8 to 7.5 (Fig. 1a). The variations in pH among sewage sludges can be attributed to the stability of the organic compounds present in each of the materials, which can continue to decompose by aerobic or anaerobic processes, even after months of storage (Ferreira de Abreu et al., 2012). Despite these variations, pH values between 5.0 and 6.5 are considered suitable for most crops (Fermino et al., 2018; Schafer et al., 2015; Ferreira de Abreu et al., 2012). In this sense, the commercial substrate and VER covered this pH range, as well as SS-1 in its composition of 100 % and in all combinations with RHA and VER (Subs. 1-4), and the combinations of 50 %SS-3 + 25 %RHA + 25 %VER(Subs. 11) and 33 %SS-3 + 33 % RHA + 33 %VER (Subs. 12) (Fig. 1a).

Values of pH above 6.9 are commonly considered as extremely high for substrates (Kämpf, 2005). In turn, substrates with 100 % RHA and 100 % SS derived from aerobic digestion (SS-2 and SS-3) (Fig. 1a), as well as all combinations of SS-2 with RHA and VER (Subs. 5–8) and 75 %SS-3 + 12 %RHA + 12 %VER (Subs. 10), showed values close to or above this limit (Fig. 1a).

The EC results of different SS formulations (Fig. 1b) were similar to those observed by Mohamed et al. (2018); Samara et al. (2017); Zoghlami et al. (2016); Alvarenga et al. (2016), and Hernández -Apaolaza et al. (2005) in soils and/or in substrates. Combinations of RHA and VER with SS-3 (Subs. 9-12) did not present a pattern of change in terms of EC (Fig. 1b). Regardless of the combination of materials with SS, the majority of substrates presented an EC between 2 and  $4 \text{ mS cm}^{-1}$ , which is considered high (Kampf, 2005). However, the values agree with Alvarenga et al. (2015), who similarly observed that the compost samples (mixture of municipal solid waste compost, agricultural wastes compost and agricultural wastes and sewage sludge compost) and other sludges (agroindustrial sludge and municipal slaughterhouse sludge, pig slurry digestate) presented higher EC values than SS samples. In turn, Schafer et al. (2015), observed around 25 % of samples presenting  $EC > 1 \text{ mS cm}^{-1}$  while evaluating the chemical characteristics of substrates used in Southern Brazil. Such can be used to cultivate species that are tolerant to the concentration of ionized salts in the solution. Values of EC less than 1 mS cm<sup>-1</sup> were observed only in the commercial substrate and in substrates with 100 % of RHA and 100 % of VER (Fig. 1b).

The high DD values of SS (Table 3) concur with the studies conducted by Dede et al. (2012) and Higashikawa et al. (2010). A reduction of DD in the substrates was promoted by the addition of RHA, possibly due to the predominance of large size-particles (more than 50 % with diameter > 4.75 mm), according to the Supplementary document provided. On the other hand, the larger particles did not positively influence TP and AS when combined with SS. For example, in substrate with 100 % SS-1 the TP was found to be 0.69 m<sup>3</sup> m<sup>-3</sup>, which increased to  $0.73 \text{ m}^3 \text{ m}^{-3}$  when combined with 33 % RHA. However, this moderate increase in TP did not lead to an increase of AS in the substrate. On the contrary, there was a decrease from  $0.22 \text{ m}^3 \text{ m}^{-3}$  (100 % SS-1) to  $0.14\,m^3\,$   $m^{-3} with$  the combination of 33 %SS-1 + 33 %RHA + 33 % VER (Table 3), diverging from the results of Fermino et al. (2018) and Costa et al. (2017). At the same time, the substrate with 100 % SS-3 showed a TP of  $0.80 \text{ m}^3 \text{ m}^{-3}$ , but decreased to around  $0.75 \text{ m}^3 \text{ m}^{-3}$ when the RHA was added, regardless of the ratio used. Conversely, there was an increase in AS from  $0.05 \text{ m}^3 \text{ m}^{-3}$  (100 % SS-3) to around  $0.10 \text{ m}^3 \text{ m}^{-3}$  when RHA was added to the substrate with 50 %SS-3 + 25 %RHA + 25 %VER (Table 3). The different changes promoted by the addition of RHA to SS-1 and SS-3 are possibly due to the granulometry of both materials and the rearrangement between them. That is, while SS-1 showed a better distribution among the particle-size intervals (24.15 % between 4.75 - 2.00 mm, 21.10 % between 2.00 - 1.00 mm, 18.07 % between 1.00-0.50 mm, and 34.15 % < 0.50 mm), SS-3 showed 48.24 % of particles with diameters between 4.75-2.00 mm and the rest similarly distributed with diameters of between 2.00 - 1.00and > 4.75 mm. The same behavior may have occurred in combinations of RHA with SS-2, considering that the pure raw material (100 %) also had the majority of particles presenting a diameter between 4.75-2.00 mm (51.52 %), with a similar distribution between diameters 2.00 - 1.00 and > 4.75 mm (see supplementary document for additional information).

The results of AWC evidence that regardless of the proportion, RHA combined with SS-1 and SS-3 did not promote significant changes, varying between  $0.05 - 0.07 \text{ m}^3 \text{ m}^{-3}$  and  $0.01 - 0.03 \text{ m}^3 \text{ m}^{-3}$ , respectively (Table 3). In soil, Islabão et al. (2016) similarily did not observe an effect of RHA on AWC, even with RHA rates of up to120 Mg ha<sup>-1</sup>. Furthermore, the VER, well-known as a raw material that increases the water retention of substrates (Ferraz et al., 2005), did not improve the AWC in the substrates. The unsatisfactory drainage of the different substrates is further verified by the values of RAW up to 0.30 m<sup>3</sup> m<sup>-3</sup> (Table 3), considered unsuitable by De Boodt and Verdonck (1972). Schafer et al. (2015) likewise observed that most of the commercial substrates in general presented high unavailable water content to the plants.

In terms of biometric attributes of sugarcane grown on sewage sludge-based substrates, the observed shorter periods for bud sprouting and higher seedling heights seen in SS-2 and SS-3 formulations, is probably due to the greater amount of RAW observed in SS-2 ( $0.53 \text{ m}^3 \text{ m}^{-3}$ ) and SS-3 ( $0.64 \text{ m}^3 \text{ m}^{-3}$ ), in comparison to SS-1 ( $0.47 \text{ m}^3 \text{ m}^{-3}$ ), as presented in Table 4. According to Landell et al. (2012), the main favorable condition for sugarcane sprouting is the adequate availability of water, since it activates the enzymes and the production of hormones which control the cellular division and growth in buds and the root zone.

Besides water availability, a DD of between 100 and 300 kg m<sup>-3</sup>has been suggested as an important characteristic for bud sprouting (Xavier et al., 2014). In this sense, Table 3 showed that the SS-2 and SS-3 formulations presented mean DD values closest to this critical value (339 and 398 kg m<sup>-3</sup>, respectively), while in SS-1 formulations the mean DD value was 426 kg m<sup>-3</sup>. In spite of these higher DD values, total porosity (TP) values also remained high, with SS-2 and SS-3 formulations showing TP values of 0.72 and 0.75 m<sup>3</sup> m<sup>-3</sup>, respectively, while SS-1 formulations showed a mean value of 0.70 m<sup>3</sup> m<sup>-3</sup>. Regardless of the SS type, the proposed formulations presented a similar performance to the commercial substrate in relation to bud sprouting, evidencing that the mixture of wastes such as SS and RHA can generate suitable substrates which provide low resistance to the emergence of primary shoots (Cerqueira et al., 2015).

The physical and hydraulic characteristics presented by the SS-3 formulations (mean DD of 398 kg m<sup>-3</sup>, mean TP of 0.75 m<sup>3</sup> m<sup>-3</sup>, mean aeration space of  $0.10 \text{ m}^3 \text{ m}^{-3}$  and mean water remaining at 10 kPa of 0.64 m<sup>3</sup> m<sup>-3</sup>) promoted the best biometric attributes of the sugarcane

seedlings. It is also important to note that the average pH of the SS-3 formulations was 6.5, considered adequate for most cultures (Fermino et al., 2018; Schafer et al., 2015; Ferreira de Abreu et al., 2012), while SS-2 wasfound to be alkaline (pH equal or greater than 8.0), and SS-1 was acidic, with a pH close to 5.5 (see the supplementary document for additional information).

The best mean DQI values were found in the SS-3 formulations, similar to those observed by Cordeiro et al. (2019). This index measures seedling quality and the equilibrium between their biomass components, with higher index values indicating better seedling quality. DQI has been used to evaluate the quality of seedlings of different species such as Abelmoschus esculentus (Sarma and Nirmali, 2015). Campomanesia adamantium (Dresch et al., 2016). Eucalyptus grandis and Pinus elliottii (Binotto et al., 2010), also evidencing the strong correlation between the biometric variables and the condition of the substrates. For the present study, the DQI provided realistic results in regards to the influence of substrate components on the production sugarcane seedlings, demonstrating that the best agronomic performance was supplied by equal rates of solarized sewage sludge, rice husk ash and vermiculite. It was also observed that sugarcane seedlings grown on substrates with SS-3 presented the best DQI compared to SS-1 and SS-2. This behavior was mainly related to the chemical (pH), physical (TP and AS) and hydraulic (RAW) characteristics of the formulations containing this SS, which in turn, are related to the granulometry and consequent rearrangement with RHA and VER. It should be highlighted that this characteristic could easily be standardized for any SS through grinding and sieving, which would enable the use of sewage sludge from different origins as a raw material for substrates, as long as they comply with the current legislation regarding health and environmental safety.

Considering the differences of crop performance observed between the SS-2 and SS-3 formulations, all the SS-3 formulations (Subs. 9–12) presented pH values within the range considered adequate for sugarcane, while SS-2 formulations (Subs. 5–8) remained in the alkaline range (Fig. 1a). Additionally, the SS-3 formulations presented mean AS and RAW values higher than SS-2 formulations (Table 3). When combined, all these characteristics culminate in a superior development of the sugarcane seedlings using SS-3 formulations.

The performance of aerobic and anaerobic sludges presented in this study demonstrates that both are suitable for use in substrate in sugarcane seedling production. Nevertheless, aerobic sludges presented a higher potential than those from anaerobic digestion, mainly due to their nutrient content and response to pH adjustment. The physical properties of the substrates were less influenced by sludge type; therefore it is easier to be standardized by the control of particle-size distribution of sludges prior to the substrate formulation process. As a consequence, substrate producers should control the chemical characteristics of each sludge batch as well as standardize the granulometry in order to ensure appropriate and efficient products for use in the sucroenergy sector.

Finally, it is important to point out that the use of wastes from different companies for developing new valuable products is directly aligned with the principles of circular economy, which aims to reduce the use of non-renewable sources of raw material and eliminate waste by transforming it into new products through industrial symbiosis. By using SS and RHA for substrate production in the present study, we aim to contribute to the regional circular economy where products and residues could be interconnected in a synergic space with the joint end goal of cleaner sugarcane industry, to be used in sugar, ethanol, and bioplastics production.

### 5. Conclusions

Solar-dried sewage sludges produced under aerobic and anaerobic digestion presented low levels of pathogenic contaminants and trace elements and, therefore, showed a low risk to health and the environment.

Sewage sludges presented high levels of total macro-and micronutrients as well as suitable physical and hydraulic characteristics to be used as a raw material in plant growth media.

The tested proportions of SS, RHA, and VER in all substrates promoted better biometric attributes (shoot and root weight, and Dickson Quality Index) than the commercial substrate, except for those containing 87.5 % SS.

The development of sugarcane seedlings on media formulated with equal proportions of sewage sludge, rice husk ash and vermiculite showed the best agronomic performance, being similar to or better than a peat-based commercial substrate used as a reference.

The viability of combining sludges from municipal wastewater treatment plants and rice husk ash into one product were confirmed using biometric attributes and nutrient tissue contents obtained from the production of sugarcane seedlings.

# CRediT authorship contribution statement

Mariana Teixeira da Silva: Writing - original draft, Writing - review & editing, Validation, Visualization. Rosane Martinazzo: Funding acquisition, Supervision. Sérgio Delmar A. Silva: Supervision, Resources, Project administration. Adilson Luís Bamberg: Project administration. Lizete Stumpf: Formal analysis, Data curation. Maria Helena Fermino: Methodology, Resources. Thais W. Kohler: Investigation. Ester S. Matoso: Investigation, Conceptualization. Ricardo Alexandre Valgas: Software, Methodology.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2020.112812.

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