Production of organic fertilizer based on sewage sludge cultivated with grass under an aeration system[1]

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ABSTRACT

The cultivation of grasses in sewage sludge (SS) with aeration has the potential for stabilization of sludge organic matter in the production of organic fertilizer. Thus, the objective of this work was to evaluate the cultivation of *Urochloa brizantha* and *Pennisetum purpureum* under aerated system on the chemical and biological attributes of SS to obtain a matured organic fertilizer. The study was conducted in a randomized block design in a 2x2+2 factorial scheme with four replications. Factors consisted of cultivation of *P. purpureum* or *U. brizantha* in SS, with or without intermittent aeration. Control treatments were SS without cultivation, with or without aeration. Sewage sludge was collected in layers and the chemical and microbial attributes were evaluated. There was no significant difference between treatments for grass dry matter. The C/N ratio was not matched with organic fertilizer stabilization due to similar losses of C and N (~7%). However, microbial activity was reduced in the presence of plant cultivation demonstrating improvement in the properties of the organic fertilizer produced. Through uni- and multivariate analysis, organic fertilizer produced from SS aerated and cultivated with *P. purpureum* showed matured organic matter.

Keywords: biodegradation; biosolid; organic matter; residue recycling.

INTRODUCTION

The high volume of sewage sludge (SS) produced at the wastewater treatment plants (WWTP) has burdened the disposal of this waste in landfills, since its management may reach 60% of the operating costs of a WWTP (Godoy, 2013). An economically and environmentally alternative would be the disposal of SS in farming and forestry areas as a conditioner of the soil and/or organic fertilizer, therefore improving the physical, chemical and biological characteristics of the soil, as the SS has considerable concentration of nutrient and organic matter (Tontti *et al.*, 2017; Melo *et al.*, 2018).

However, SS may contain contaminants such as heavy metals (Oliveira *et al.*, 2018), toxic organic compounds (Alvarenga *et al.*, 2017), and pathogens (Oliveira *et al.*, 2019). In addition, it may not present stabilized organic matter, therefore, it needs some technique for its maturation. In Brazil, the agricultural and forestry use of SS is regulated by the National Council of the Environment (Conselho Nacional do Meio Ambiente - CONAMA) thought the resolution 498/2020 (Brasil, 2020a). This resolution establishes limits of heavy metal and pathogens organisms, classifying the SS, where, in which culture, and how it can be applied. And there are suggestions of how the organic matter of SS can be stabilized and contaminants reduced.

As an alternative, the SS cultivation technique would provide rapid stabilization of organic matter and degradation of toxic organic compounds (Alvarenga *et al.*, 2017). With this technique, studies reported that *Pennisetum purpureum* had a great development cultivated directly in SS (Alvarenga *et al.*, 2018); phytoextracted As (Alvarenga *et al.*, 2019); reduced Zn concentration bound

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to organic matter and increased residual Zn (Oliveira *et al.*, 2018); and reduced chlorobenzenes concentration in the SS after 150 days of cultivation (Alvarenga *et al.*, 2017).

Based on that, the cultivation of *Pennisetum purpureum* and *Urochloa brizantha* in SS presents potential due to the rusticity of these grasses, their fasciculated root system, and adequate efficiency in atmospheric CO_2 fixation for plant biomass production (Silva *et al.*, 2005; Flores *et al.*, 2012). In addition, scientific studies report the phytoremediation potential of Zn, Cd (Zhang *et al.*, 2010) and Cs (Kang *et al.*, 2012) for *P. purpureum* and picloran for *U. brizantha* (Braga *et al.*, 2016).

Another factor that may contribute to reducing the presence of contaminants and stabilization of organic matter would be SS aeration during cultivation. This process is known as aerated static pile composting, where the organic waste is disposed on a perforated air injection pipe, with no need for material revolving (Ekinci *et al.*, 2017). With this process, studies have reported a reduction of the toxic potential of heavy metals (Cai *et al.*, 2007a; Golbaz *et al.*, 2020) and removal of polycyclic aromatic hydrocarbons (Cai *et al.*, 2007b) in sewage sludge.

The hypothesis of this study is that the cultivation of grass in sewage sludge intercropped with its aeration may stabilize the organic matter of the SS, providing the production of a better-quality organic fertilizer. Thus, the objective was to evaluate the interference of the cultivation of *P. purpureum U. brizantha* under an aerated system on chemical and biological attributes of SS to obtain a matured organic fertilizer.

MATERIAL AND METHODS

The experiment was conducted at the Institute of Agricultural Sciences (Instituto de Ciências Agrárias - ICA) of the Universidade Federal de Minas Gerais (UFMG), Montes Claros campus, from November 2016 to January 2017. According to the Köppen's classification, the predominant climate in the region is the Aw – tropical savannah with rainy summer and dry winter. Rainfall of 253 mm and maximum and minimum temperatures of 32.2 and 20.7 °C were recorded over the experiment period, respectively (INMET, 2017).

The study was carried out in a randomized block design and four replications, in a 2 x 2 + 2 factorial scheme, consisting of two plant cultivations, with *P. purpureum U. brizantha* cv. Marandu (planting density of 50 plants per m²), combined with the absence or presence of aeration (0.14 m^3 min^{-1} m^{-3}) of sewage sludge. The two additional treatments corresponded to the presence or absence of sewage sludge aeration without the cultivation of plants in the SS. The experimental plots, made up of 0.7 x 0.7 x 0.55 m maideirits boxes, were filled up to 0.5 m high with sewage sludge from WWTP-Vieira, Montes Claros-MG.

It can be found in that plant, reactors in which the anaerobic biological treatment of sewage is performed. Next, the wastewater from these reactors goes to aerobic biological filters. In the final stage of sewage treatment, the decantation process takes place, and the water can be discharged to the watercourse. The sewage sludge is centrifuged and thermally dried at 350 °C for 30 min (6-8% humidity). As the composition of sewage sludge may vary depending on different periods of the year, characterization analyses of the byproduct were performed before the experiment was set up (Table 1). The concentration of the heavy metal detected (Cu, Zn, and Pb) by the analysis was lower than the maximum concentration established by the CONAMA resolution 498 (Brasil, 2020a).

The aeration system consisted of a 1.5-hp, 1,150-rpm engine and a sirocco fan with a flow rate of 2.7 m^3 min^{-1}. The fan outlet was adapted and branched into 0.5 mm x 0.35 m fork-shaped 25 mm-PVC pipes with 12 holes of 10 mm diameter, spaced by 0.08 m, at the bottom of the experimental plots and covered by a shade screen to avoid clogging the holes. The aerated experimental plots received intermittent (30 min on/off) airflow of 0.14 m^3 min^{-1} per m^3 of sewage sludge, obtained according to the power and flow of the ventilation system. This flow was regulated with the aid of an anemometer and a ball valve at the entrance of the air of the plot.

P. purpureum was planted by using 10-cm long cuttings containing a bud. The planting of *U. brizantha* was done by using seedlings produced in commercial substrate, as the seeds did not show proper germination and uniformity when planted directly in the sewage sludge, in a previous test. Both grasses were planted with 0.14 x 0.14 m spacing at 0.05 m depth, with a density of 50 plants per m², totaling 25 plants per plot. Plots were irrigated over cultivation with supply water to keep moisture close to the field capacity (26% of the volumetric humidity).

After 90 days of grass cultivation, when *U. brizantha* began to emit inflorescence, the four central plants of the plot were collected and separated into aerial part and roots, in order to estimate dry matter production. And samples of sewage sludge in the layers 0-10, 10-20, 20-30, 30-40 and > 40 cm were collected. The plant and SS samples were dried in an air forced circulation oven at 65 ºC until constant weight, with the determination of the dry mass of the plant parts.

The following analyses were carried out in SS samples: pH in $CaCl_2$ 0.01 mol L^{-1}, organic C, total N, cation exchange capacity (CEC), and calculations of C/N and CEC/C ratios (Alcarde, 2009); microbial activity through the method of basal (BR) and cumulated respirometric (CR) rate (Silva *et al.*, 2007a); microbial biomass carbon (MBC) (Silva *et al.*, 2007b), with a Kc value of 0.197 (Sparling & West, 1988); and calculation of metabolic (qCO_2) and microbial (qMIC) quotients (Silva *et al.*, 2007a, b).

The data obtained from the grass analyses were submitted to the confidence interval test at 5% probability. The use of analysis of variance, in this case, was not possible because of the low number of experimental units, below 20, as suggested in the literature (Pimentel-Gomes, 2009). The data obtained from the sewage sludge analyses were subjected to analysis of variance, with treatment unfolding in orthogonal contrasts, by applying the F test at 0.05 probability. Contrasts were determined to compare treatments with and without cultivation (C1), the presence and absence of aeration in uncultivated SS (C2), the cultivation of *U. brizantha* and *P. purpureum* in sewage sludge (C3), the presence and absence of aeration of sewage sludge cultivated with *U. brizantha* (C4) and the presence and absence of aeration of sewage sludge cultivated with *P. purpureum* (C5). For the 0-50 cm layer, the Tukey multiple comparison test ($p \leq 0.05$) was performed. Correlation analysis was performed by using Pearson's test ($p \leq 0.05$). For these analyses, software RStudio (R Core Team, 2017) was used. Multivariate analysis was performed through principal component and cluster methods, with the aid of SAS software (SAS Institute Inc., 2002).

RESULTS AND DISCUSSION

Production of P. purpureum and U. brizantha grown in sewage sludge

Production of the dry matter of the aerial part (DMAP), root (DMR) and total (DMT) of *P. purpureum* and *U. brizantha* were similar between treatments with or without aeration in sewage sludge after 90 days of cultivation (Table 2). However, the DMR of *P. purpureum* was 2.1-fold greater than that of *U. brizantha*. The higher volume of *P. purpureum* roots may represent higher tolerance of the grass to sewage sludge contaminants and to the rise in substrate temperature in the initial phase of cultivation. The average total biomass of *P. purpureum* corresponded to 68 g per plant, with a density of 50 plants per m², were considered lower than that obtained by Oliveira *et al.* (2018), who found a total biomass of 150 g per plant, with a density of 25 plants per m², at 90 days of cultivation in sewage sludge.

Chemical attributes of cultivated sewage sludge

The pH of the SS was reduced when cultivated (C1) with *P. purpureum* (C3) and in the presence of aeration (C5) compared to without cultivation, cultivated with *U. brizantha* and without aeration, respectively (Table 3). SS acidification in these treatments was related to the release of H^+ ions through degradation of organic matter and N nitrification by microorganisms during the SS stabilization process and through exudation of organic acids by plants (Souza *et al.*, 2012; Villanueva *et al.*, 2012), especially in aeration treatments. The difference between treatments was more evident for WANC and WAPP, where this treatment had 0.31 pH units lower than WANC (Table 3). This result reinforces the hypothesis of the importance of grass cultivation in the SS stabilization process, especially the *P. purpureum*. Shen *et al.* (2001) observed

Table 2: Dry matter of the aerial part (DMAP), root (DMR) and total (DMT) of *P. purpureum* and *U. brizantha* grown in sewage sludge

Treat.	DMAP	DMR	DMT
	g per plant		
WAPP	49.3±22.7	22.2±12.4	71.5±20.7
NAPP	43.6±33.6	20.1±10.4	63.7±26.9
WAUB	35.6±37.3	9.5±4.5	45.1±24.9
NAUB	51.7±30.2	10.5±5.2	62.3±21.0

Averages followed by confidence interval ($p \leq 0.05$). Treat. – Treatments; WAPP – SS aerated and cultivated with *P. purpureum*; NAPP – SS non-aerated and cultivated with *P. purpureum*; WAUB – SS aerated and cultivated with *U. brizantha*; NAUB – SS non-aerated and cultivated with *U. brizantha*.

Table 1: Initial chemical and biological characterization of sewage sludge

Attributes	Unity	Value	Attributes	Unity	Value	Attributes	Unity	Value
pH $CaCl_2$	-	5.9	P	g kg^{-1}	7.8	Mn	mg kg^{-1}	120.0
OC	g kg^{-1}	323.0	K	g kg^{-1}	4.0	Ni	mg kg^{-1}	23.0
Total N	g kg^{-1}	32.0	Ca	g kg^{-1}	10.3	Pb	mg kg^{-1}	31.0
CEC	$mmol_c$ kg^{-1}	916.0	Mg	g kg^{-1}	1.6	BR	mg kg^{-1} h^{-1}	3.8
OM	g kg^{-1}	572.0	Fe	mg kg^{-1}	3,075.0	CR	mg kg^{-1}	68.0
TMW	g kg^{-1}	428.0	Na	mg kg^{-1}	618.0	MBC	mg kg^{-1}	10,715.0
C/N	-	10.0	Zn	mg kg^{-1}	283.0	qMIC	%	3.3
CEC/C	-	28.0	Cu	mg kg^{-1}	112.0	qCO_2	mg g^{-1} MBC h^{-1}	0.3

OC – Organic carbon; CEC – Cation exchange capacity; OM – Organic matter; TMW – Total mineral waste; BR – Basal respiration; h – Hour; CR – Cumulative respiration; MBC – Microbial biomass carbon; qMIC – Microbial quotient; qCO_2 – Metabolic quotient; Cr was detected (< 1.25 mg L^{-1}); Ba, Cd, and Mo weren't detected.

Table 3: pH in $CaCl_2$ 0.01 mol L^{-1} and organic carbon of sewage sludge cultivated with *P. purpureum* or *U. brizantha*, with or without aeration

Treataments / Contrasts	**pH in $CaCl_2$ 0.01 mol L^{-1}**						**Organic Carbon (g kg^{-1})**					
	0-10	**10-20**	**20-30**	**30-40**	**>40**	**0-50**	**0-10**	**10-20**	**20-30**	**30-40**	**>40**	**0-50**
	cm											
WANC	6.32	6.31	6.32	6.29	6.13	6.28 a	298	305	274	283	322	296 abc
NANC	6.19	6.20	6.17	6.21	6.12	6.18 ab	271	293	291	298	290	289 c
WAPP	5.84	5.91	5.94	6.11	6.15	5.97 b	304	320	314	322	290	310 a
NAPP	6.19	6.11	6.08	6.11	6.17	6.13 ab	293	302	276	296	342	302 abc
WAUB	6.10	6.04	6.29	6.35	6.32	6.22 ab	288	285	311	288	280	290 bc
NAUB	6.19	6.18	6.13	6.16	6.08	6.15 ab	314	305	305	314	302	308 ab
Average	6.14	6.13	6.16	6.21	6.16	6.16	295	302	295	300	304	299
C_1 = NS - CS	0.70^{*}	0.78^{*}	^{ns}	^{ns}	^{ns}	0.45^{*}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	-40^{*}
C_2 = WANC - NANC	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}
C_3 = PP - UB	^{ns}	^{ns}	-0.40^{*}	^{ns}	^{ns}	-0.27^{*}	^{ns}	^{ns}	^{ns}	^{ns}	51^{*}	^{ns}
C_4 = WAPP - NAPP	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	26^{*}	^{ns}	8^{**}
C_5 = WAUB - NAUB	-0.09^{*}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	^{ns}	5.3^{*}	^{ns}	-22^{**}	^{ns}
CV (%)	2.85	3.06	2.78	3.70	4.24	1.85	8.38	6.32	7.73	5.70	7.81	2.75

C_1, C_2, C_3, C_4 e C_5 – Contrasts 1, 2, 3, 4, and 5, respectively; NS – Non-cultivated SS (WANC; NANC); CS – Cultivated SS (WAPP; NAPP; WAUB; NAUB); WANC – SS aerated and non-cultivated; NANC – SS non-aerated and non-cultivated; PP – SS cultivated with *P. purpureum* (WAPP; NAPP); UB – SS cultivated with *U. brizantha* (WAUB; NAUB); WAPP – SS aerated and cultivated with *P. purpureum*; NAPP – SS non-aerated and cultivated with *P. purpureum*; WAUB – SS aerated and cultivated with *U. brizantha*; NAUB – SS non-aerated and cultivated with *U. brizantha*.; CV – Coefficient of variation; ns – Not significant at 0.05 probability by F test; *, ** – Significant at 0.05 and 0.01 probability by F test, respectively. Average values followed by the same letter are not significant at 0.05 probability by Tukey test.

that *P. purpureum* can exudate organic acids, such as oxalic acid, citric acid, pentanedioic acid, and phthalic acid, leading to a acidification of the rhizosphere zone.

The cultivation (C1) of *P. purpureum* (C3) with aeration (C4) increased the organic C (OC) concentration in the sewage sludge (Table 3). Also, in the 0-50 cm layer, WAPP increased by 6.9 and 7.3% the OC concentration in the SS in comparison to WAUB and NANC treatments, respectively. These results were attributed to the higher cycling capacity of organic compounds in the grass root system (Costa *et al.*, 2009) and the microbial efficiency in the conversion rate of SS carbon into stabilized organic compounds in aeration treatment, corroborating with the results of pH and higher root production by *P. purpureum*.

The presence of root system of plants, mainly *P. purpureum*, and aeration contributed to the increase of biological activity, leading to greater degradation of organic matter from sewage sludge. This could be related to the liberation of exudates in the substrate, enhancing microbial diversity and activity (Shen *et al.*, 2001; Carvalhais *et al.*, 2011). The most pronounced effect of *P. purpureum* may be due to the association of its roots with Enterobacter and *Pantoea agglomerans*, which act intensely in the degradation of humic substances and even toxic substances, such as polycyclic aromatic hydrocarbons (Videira *et al.*, 2012; Li *et al.*, 2015, 2016; Chikere & Fenibo, 2018; Umar *et al.*, 2018).

As a consequence, there was an average reduction by 7.4% in the OC concentration at the end of cultivation in relation to the initial sewage sludge (Table 1). But it was smaller in relation to other SS stabilization processes, with a reduction by 19% in revolving piles (Moretti *et al.*, 2015) and 10-25% in biological reactors (Yuan *et al.*, 2016; Awasthi *et al.*, 2018). However, the OC concentration obtained in this study is greater than the minimum threshold of 150 g kg^{-1}, established for the production and commercialization of organic fertilizer by the Ministry of Agriculture, Livestock and Supply (MAPA) in Normative Instruction (NI) No. 61 (Brasil, 2020b).

Nitrogen concentration in the sewage sludge was not influenced by the treatments ($p > 0.05$), however, a higher N concentration was found in the grass treatments (C1; Table 4), showing that they were able to maintain the N concentration in the SS. This may also be related to N uptake by root recycling and the association of grasses with bacteria capable of absorbing atmospheric N and incorporating it into sewage sludge, as observed by Silva *et al.* (2013) for *U. brizantha* and by Morais *et al.* (2012) for *P. purpureum*. Nitrogen losses caused by volatilization or leaching by the processes proposed in this work reached levels below composting in biological reactors, where a reduction by 16 to 23% in N concentration may occur (Yuan *et al.*, 2016; Awasthi *et al.*, 2018). Also, NI 61 states that the minimum concentration of N in organic fertilizer should be 5 g kg^{-1} (Brasil, 2020b), however, at the end of cultivation, an average concentration of 29.8 g kg^{-1} was obtained.

The cation exchange capacity (CEC) was influenced by grass cultivation treatments (C1), however, no difference was observed between treatments in the 0-50 cm layer (Table 4). Higher CEC values in cultivation treatments represent greater stability of sewage sludge organic matter (Zhang & Sun, 2016). The CEC showed a moderate and positive correlation with the OC ($r = 0.54$; $p < 0.05$) and N ($r = 0.49$; $p < 0.05$) concentration in the SS (Figure 1). This correlation is related to the negative charges existing in the C and N groups, such as carboxylic, esters, amines, amides, among others (Sanchez-Monedero *et al.*, 2018). It can explain the average reduction of 2.4% in the initial sewage sludge CEC (Table 1), due to the decrease in OC and N concentrations. However, it is noticed that this reduction was smaller than the losses in OC and N concentration (~7%), therefore, evidencing the formation of new functional groups, responsible for the bindings between negative charges of the surface of the cation sites (Sanchez-Monedero *et al.*, 2018).

The CEC/C ratio was negatively correlated ($r = -0.62$; $p < 0.05$) with the C/N ratio (Figure 1), so the lower the C/N ratio, the higher the CEC/C ratio of the SS, a condition that expresses a higher degree of maturity and stability of the organic fertilizer produced (Moretti *et al.*, 2015). No difference was found between treatments for C/N ratio; however, the contrasts showed greater SS stability in treatments without cultivation (C1), with *U. brizantha* cultivation (C3) and without aeration (C5; Table 5). Neither there were significant changes in this index compared to the initial sewage sludge (Table 1). This is the result of the equivalent losses of OC and N (~7%) that occurred at the end of grass cultivation, so the C/N ratio was not effective in evaluating the stability of the organic fertilizer produced. Even so, the values obtained in this work (average of 10.2) are below the maximum threshold of 20, established by IN 61 to obtain stabilized organic fertilizer (Brasil, 2020b).

The CEC/C ratio was higher in the treatments in which sewage sludge was cultivated with *U. brizantha* (C3) with aeration (C5) and *P. purpureum* without aeration (C4; Table 5), showing greater stability in these systems. At the end of cultivation, organic fertilizer obtained an average CEC/C ratio of 29.9, corresponding to an increase of 5.3% compared to that of the initial sewage sludge (Table 1). Lima *et al.* (2009) established a minimum CEC/C ratio of 17 for mature compost. An average of 76% higher than the recommended was obtained in this study. This index was more satisfactory compared to C/N ratio to determine the stability of organic fertilizer, due to the perception of changes in organic matter loads per OC concentration.

Table 4: Total N and cation exchange capacity of sewage sludge cultivated with *P. purpureum* or *U. brizantha*, with or without aeration

Treataments / Contrasts	Total N (g kg^{-1})						Cation Exchange Capacity (mmol$_c$ kg^{-1})					
	0-10	10-20	20-30	30-40	>40	0-50	0-10	10-20	20-30	30-40	>40	0-50
	cm											
WANC	29.5	29.4	29.2	29.2	28.4	29.1 a	918	909	870	886	860	889 a
NANC	28.7	28.6	28.0	28.7	27.7	28.3 a	873	878	858	831	842	856 a
WAPP	29.1	29.2	29.0	31.5	32.1	30.2 a	912	925	921	877	872	901 a
NAPP	30.1	29.6	29.9	30.3	31.5	30.3 a	945	912	850	865	889	892 a
WAUB	29.7	29.9	30.2	30.9	30.7	30.3 a	932	918	885	904	912	910 a
NAUB	30.3	29.8	30.3	32.8	31.0	30.9 a	946	884	918	900	915	913 a
Average	29.6	29.4	29.5	30.6	30.2	29.9	921	904	884	877	882	894
C_1 = NS - CS	ns	ns	ns	-9.7*	-13.1*	-6.9*	ns	ns	ns	ns	-184*	-126*
C_2 = WANC - NANC	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C_3 = PP - UB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C_4 = WAPP - NAPP	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C_5 = WAUB - NAUB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	4.29	4.44	6.18	8.69	10.19	5.52	6.00	4.23	5.77	4.96	4.81	3.81

C_1, C_2, C_3, C_4 e C_5 – Contrasts 1, 2, 3, 4, and 5, respectively; NS – Non-cultivated SS (WANC; NANC); CS – Cultivated SS (WAPP; NAPP; WAUB; NAUB); WANC – SS aerated and non-cultivated; NANC – SS non-aerated and non-cultivated; PP – SS cultivated with *P. purpureum* (WAPP; NAPP); UB – SS cultivated with *U. brizantha* (WAUB; NAUB); WAPP – SS aerated and cultivated with *P. purpureum*; NAPP – SS non-aerated and cultivated with *P. purpureum*; WAUB – SS aerated and cultivated with *U. brizantha*; NAUB – SS non-aerated and cultivated with *U. brizantha*.; CV – Coefficient of variation; ns – Not significant at 0.05 probability by F test; *, ** – Significant at 0.05 and 0.01 probability by F test, respectively. Average values followed by the same letter are not significant at 0.05 probability by Tukey test.

Microbial attributes in cultivated sewage sludge

There was no significant effect of contrasts and between treatments for basal (BR) and cumulative (CR) respiration, however, a reduction of 48 and 57% was found in BR and CR (Table 6), respectively, at the end of cultivation compared the initial sewage sludge (Table 1). This result reflects the high maturation of the sewage sludge OM due to the reduction in microbial activity. The decrease in microbial activity, demonstrated in this work through the reduction of CO_2 evolution, indicates greater stability of the produced fertilizer at the end of the cultivation period (Nikaeen *et al.*, 2015), therefore, it is an important index in determining compost maturation.

The microbial biomass carbon (MBC) was higher in the treatments cultivated (C1) with *P. purpureum* (C3) and *U. brizantha* without aeration (C5; Table 6). The presence of the root system, especially *P. purpureum*, may have increased microbial biomass through exudate release (Shen *et al.*, 2001; Tian *et al.*, 2017), favoring the decomposition of organic matter and stabilization of sewage sludge. This result is corroborated by the moderate negative correlation (r = -0.48; $p < 0.05$) with the C/N ratio (Figure 1), as the higher the microbial biomass, the lower the C/N ratio. Moreover, there was a final reduction of 82% in MBC compared to the initial SS (Table 1), possibly due to the selection of microorganisms caused by the increased recalcitrance of humified organic matter (Alves *et al.*, 2011).

The high coefficient of variation in the results of the basal and cumulative respiration and in the microbial biomass C (Table 6) are in consequence of the high organic matter concentration of the sewage sludge. The method used was developed to soil (Silva *et al.*, 2007a, b), but it was adjusted to be used in this study and the results were as good as was expected for soil analysis.

The metabolic quotient (qCO_2) is an index that infers upon the efficiency of microorganisms in the use of OC, that is, how much C is lost as CO_2 per C incorporated microbial biomass, and the lower the value of qCO_2, the higher the efficiency of microbial activity (Vieira & Pazianotto, 2016). The qCO_2 was lower in the SS aerated and non-cultivated treatment (C2), SS aerated and cultivated with *P. purpureum* (C4), and SS non-aerated and cultivated with *U. brizantha* (C5; Table 7). Thus, it can be inferred that the microorganisms showed higher efficiency in the use of C in the aerated sewage sludge, except when cultivated with *U. brizantha*. It could be explained by a more favorable environment for micro-

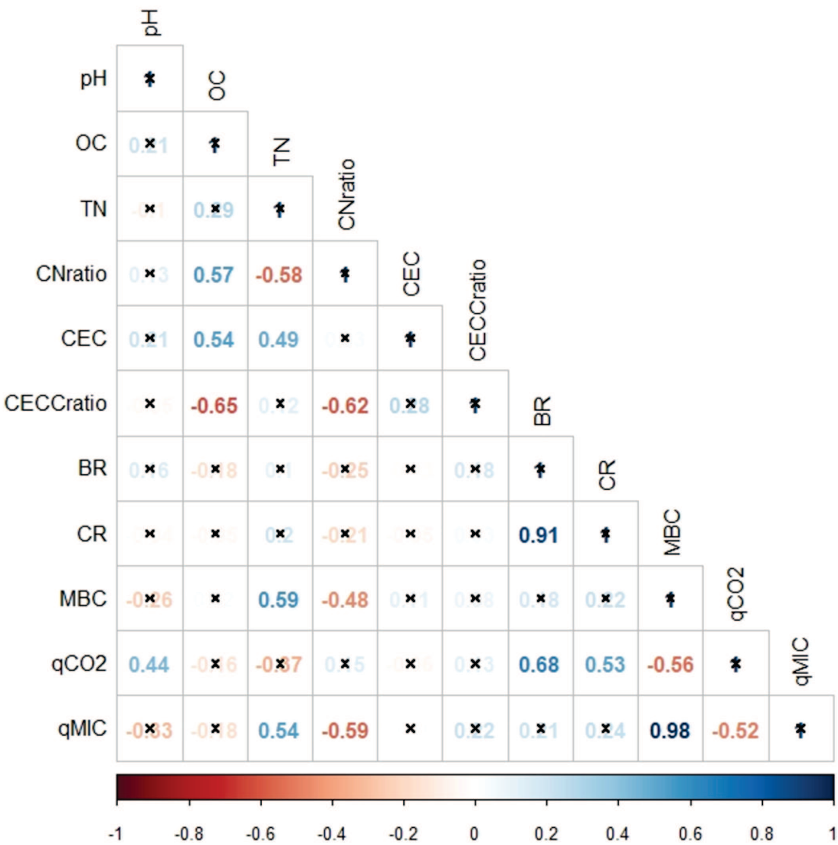

Figure 1: Pearson correlation between variables evaluated in sewage sludge after cultivation with *P. purpureum* and *U. brizantha* in the presence or absence of aeration Values with “x” were not significant at 0.05 probability by Pearson test. OC – Organic carbon; TN; Total Nitrogen; CEC – Cation Exchange Capacity; BR – Basal Respiration; CR – Cumulative Respiration; MBC – Microbial Biomass Carbon; qCO2 – Metabolic Quotient; qMIC – Microbial Quotient.

Table 5: C/N and CEC/C ratios of sewage sludge cultivated with *P. purpureum* or *U. brizantha*, with or without aeration

Treataments / Contrasts	C/N ratio						CEC/C ratio					
	0-10	10-20	20-30	30-40	>40	0-50	0-10	10-20	20-30	30-40	>40	0-50
	cm											
WANC	10.3	10.8	9.2	9.6	11.0	10.2 a	31.9	29.8	32.3	30.5	26.8	30.1 a
NANC	9.4	10.5	9.5	10.4	10.1	10.3 a	32.5	30.3	29.8	27.9	29.1	29.7 a
WAPP	10.2	11.0	11.0	11.1	10.0	10.7 a	30.1	29.3	29.4	27.3	30.2	29.2 a
NAPP	10.0	10.3	9.1	9.8	11.6	10.1 a	32.3	30.3	31.1	29.3	26.0	29.6 a
WAUB	9.6	9.6	10.9	9.7	9.4	9.7 a	32.4	32.3	28.7	30.5	33.0	31.4 a
NAUB	10.7	10.4	10.5	10.4	10.2	10.2 a	30.1	29.1	30.2	28.7	30.3	29.6 a
Average	10.1	10.4	10.1	10.2	10.4	10.2	31.5	30.2	30.2	29.0	29.2	29.9
C_1 = NS - CS	ns	ns	-4.0*	ns	ns	ns	ns	ns	ns	ns	ns	ns
C_2 = WANC - NANC	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C_3 = PP - UB	ns	ns	ns	ns	2.0*	ns	ns	ns	ns	ns	-7.1**	ns
C_4 = WAPP - NAPP	ns	ns	ns	ns	ns	ns	ns	-1.0*	ns	ns	ns	-0.4*
C_5 = WAUB - NAUB	ns	ns	0.3*	-0.7*	-0.8*	ns	ns	ns	ns	ns	2.7*	ns
CV (%)	9.00	7.49	9.68	7.45	8.11	6.51	11.33	5.7	9.73	5.91	7.25	3.91

C_1, C_2, C_3, C_4 e C_5 – Contrasts 1, 2, 3, 4, and 5, respectively; NS – Non-cultivated SS (WANC; NANC); CS – Cultivated SS (WAPP; NAPP; WAUB; NAUB); WANC – SS aerated and non-cultivated; NANC – SS non-aerated and non-cultivated; PP – SS cultivated with *P. purpureum* (WAPP; NAPP); UB – SS cultivated with *U. brizantha* (WAUB; NAUB); WAPP – SS aerated and cultivated with *P. purpureum*; NAPP – SS non-aerated and cultivated with *P. purpureum*; WAUB – SS aerated and cultivated with *U. brizantha*; NAUB – SS non-aerated and cultivated with *U. brizantha*.; CV – Coefficient of variation; ns – Not significant at 0.05 probability by F test; *, ** – Significant at 0.05 and 0.01 probability by F test, respectively. Average values followed by the same letter are not significant at 0.05 probability by Tukey test.

organisms activity due to the aeration of the SS and the presence of roots, especially of the *P. purpureum*, since its qCO_2 value tends to be lower than the other treatments. This result corroborates with those found for pH and OC (Table 3).

The microbial quotient (qMIC) index is used to infer the quality of OM, since the higher its value, the higher the lability of C, which is an appropriate environment for microbial growth (Vieira & Pazianotto, 2016). The qMIC was higher in treatments with grass cultivation (C1) and *U. brizantha* cultivation and without aeration (C5) compared to without cultivation and with aeration of the sewage sludge, respectively (Table 7). However, the SS aerated and cultivated with *P. purpureum* showed a tendency to have higher qMIC values, corroborating to the previous results found in this study. Thus, these treatments showed favorable environment for microbial development for decomposition of organic matter and maturation of SS. A reduction of 81% of the initial sewage sludge (Table 1) was found at the end of cultivation, which may have occurred due to the increase of more humified and recalcitrant organic material, reducing the lability of C.

Multivariate analysis

The dendrogram was obtained from the cluster analysis by the Ward method (Figure 2A). It was observed the formation of four groups through likelihood: 1) SS non-aerated and non-cultivated (NANC); 2) SS aerated and non-cultivated (WANC) and SS non-aerated and cultivated with *P. purpureum* (NAPP); 3) SS aerated and cultivated with *U. brizantha* (WAUB); and 4) SS non-aerated and cultivated with *U. brizantha* (NAUB) and SS aerated and cultivated with *P. purpureum* (WAPP). The analysis suggests the increasing order of treatments that provided improvements in the characteristics of the organic fertilizer produced from sewage sludge. Thus, our results confirming that the introduction of grasses and aeration, mainly *P. purpureum*, promoted an increase in sewage sludge quality when compared to without cultivation and aeration, in agreement to other studies performed evaluating the effect of these techniques separately (Cai *et al.*, 2007a, b; Alvarenga *et al.*, 2017, 2018, 2019; Golbaz *et al.*, 2020). This result is also confirmed by univariate analysis, for example by microbial attributes (Tables 6 and 7), pH, and OC (Table 3).

By analyzing the biplot graph, it can be observed that the first (PC1) and second (PC2) principal components together represent 80.6% of the total data variability (Figure 2B). It is also possible to determine which variables had the greatest influence on treatments in multivariate analysis. Based on that, the lower values of CEC, TN, qMIC, and MBC attributes provided poorer quality of non-cultivated SS (NANC, WANC). For NAUB, the opposite was observed, where these same attributes were responsible for improving the characteristics of the SS in

Table 6: Basal (BR; mg CO_2 kg^{-1} h^{-1}) and cumulative (CR; mg CO_2 kg^{-1}) respiration and microbial biomass of sewage sludge cultivated with *P. purpureum* or *U. brizantha*, with or without aeration

Treataments / Contrasts	BR	CR	Microbial biomass carbon (mg kg^{-1})					
	0-50	0-50	0-10	10-20	20-30	30-40	>40	0-50
	cm							
WANC	1.70 a	23.0 a	1,432	1,075	1,947	1,818	2,830	1,820 a
NANC	2.38 a	32.3 a	1,515	1,661	2,012	1,201	1,700	1,618 a
WAPP	1.68 a	28.5 a	2,604	2,657	2,340	1,747	1,973	2,264 a
NAPP	1.72 a	25.4 a	2,482	1,432	969	956	2,936	1,755 a
WAUB	2.42 a	35.8 a	1,734	900	2,106	2,546	2,733	2,004 a
NAUB	1.86 a	31.3 a	2,079	1,754	2,884	1,203	2,194	2,023 a
Average	1.96	29.4	1,975	1,580	2,043	1,579	2,394	1,914
C_1 = NS - CS	ns	ns	-3,005*	ns	ns	ns	ns	ns
C_2 = WANC - NANC	ns	ns	ns	ns	ns	ns	ns	ns
C_3 = PP - UB	ns	ns	ns	1,435*	ns	ns	ns	ns
C_4 = WAPP - NAPP	ns	ns	ns	ns	ns	ns	ns	ns
C_5 = WAUB - NAUB	ns	ns	ns	-854*	ns	ns	ns	ns
CV (%)	40.1	33.9	35.8	41.4	85.7	57.8	38.6	41.0

C_1, C_2, C_3, C_4 e C_5 – Contrasts 1, 2, 3, 4, and 5, respectively; NS – Non-cultivated SS (WANC; NANC); CS – Cultivated SS (WAPP; NAPP; WAUB; NAUB); WANC – SS aerated and non-cultivated; NANC – SS non-aerated and non-cultivated; PP – SS cultivated with *P. purpureum* (WAPP; NAPP); UB – SS cultivated with *U. brizantha* (WAUB; NAUB); WAPP – SS aerated and cultivated with *P. purpureum*; NAPP – SS non-aerated and cultivated with *P. purpureum*; WAUB – SS aerated and cultivated with *U. brizantha*; NAUB – SS non-aerated and cultivated with *U. brizantha*.; CV – Coefficient of variation; ns – Not significant at 0.05 probability by F test; *, ** – Significant at 0.05 and 0.01 probability by F test, respectively. Average values followed by the same letter are not significant at 0.05 probability by Tukey test.

Table 7: Metabolic (qCO_2) and microbial (qMIC) quotients of sewage sludge cultivated with *P. purpureum* or *U. brizantha*, with or without aeration

Treataments / Contrasts	Metabolic Quotient (mg $C\text{-}CO_2$ g^{-1} MBC h^{-1})						Microbial Quotient (%)					
	0-10	**10-20**	**20-30**	**30-40**	**>40**	**0-50**	**0-10**	**10-20**	**20-30**	**30-40**	**>40**	**0-50**
cm												
WANC	0.93	1.69	0.79	0.81	0.70	1.03 a	0.49	0.35	0.70	0.67	0.88	0.62 a
NANC	1.25	1.26	1.50	1.39	1.17	1.51 a	0.57	0.58	0.67	0.41	0.60	0.57 a
WAPP	0.81	0.64	0.74	0.95	0.99	0.75 a	0.87	0.85	0.76	0.55	0.71	0.74 a
NAPP	0.88	1.50	1.30	1.13	0.64	1.04 a	0.86	0.49	0.33	0.33	0.87	0.59 a
WAUB	1.07	1.97	1.47	0.87	0.90	1.27 a	0.59	0.31	0.66	0.87	0.98	0.68 a
NAUB	0.87	1.00	1.43	1.29	0.84	1.01 a	0.67	0.59	0.96	0.39	0.74	0.66 a
Average	0.97	1.34	1.21	1.07	0.87	1.10	0.68	0.53	0.68	0.54	0.80	0.64
C_1 = NS - CS	ns	ns	ns	ns	ns	ns	-0.87*	ns	ns	ns	ns	ns
C_2 = WANC - NANC	ns	ns	ns	-0.58**	ns	ns	ns	ns	ns	ns	ns	ns
C_3 = PP - UB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C_4 = WAPP - NAPP	ns	-0.86*	ns	-0.18*	ns	ns	ns	ns	ns	ns	ns	ns
C_5 = WAUB - NAUB	ns	0.97*	ns	ns	ns	ns	ns	-0.28*	ns	ns	ns	ns
CV (%)	36.9	36.9	56.3	22.3	39.0	41.0	34.5	44.4	82.3	59.9	38.6	29.7

C_1, C_2, C_3, C_4 e C_5 – Contrasts 1, 2, 3, 4, and 5, respectively; NS – Non-cultivated SS (WANC; NANC); CS – Cultivated SS (WAPP; NAPP; WAUB; NAUB); WANC – SS aerated and non-cultivated; NANC – SS non-aerated and non-cultivated; PP – SS cultivated with *P. purpureum* (WAPP; NAPP); UB – SS cultivated with *U. brizantha* (WAUB; NAUB); WAPP – SS aerated and cultivated with *P. purpureum*; NAPP – SS non-aerated and cultivated with *P. purpureum*; WAUB – SS aerated and cultivated with *U. brizantha*; NAUB – SS non-aerated and cultivated with *U. brizantha*.; CV – Coefficient of variation; ns – Not significant at 0.05 probability by F test; *, ** – Significant at 0.05 and 0.01 probability by F test, respectively. Average values followed by the same letter are not significant at 0.05 probability by Tukey test.

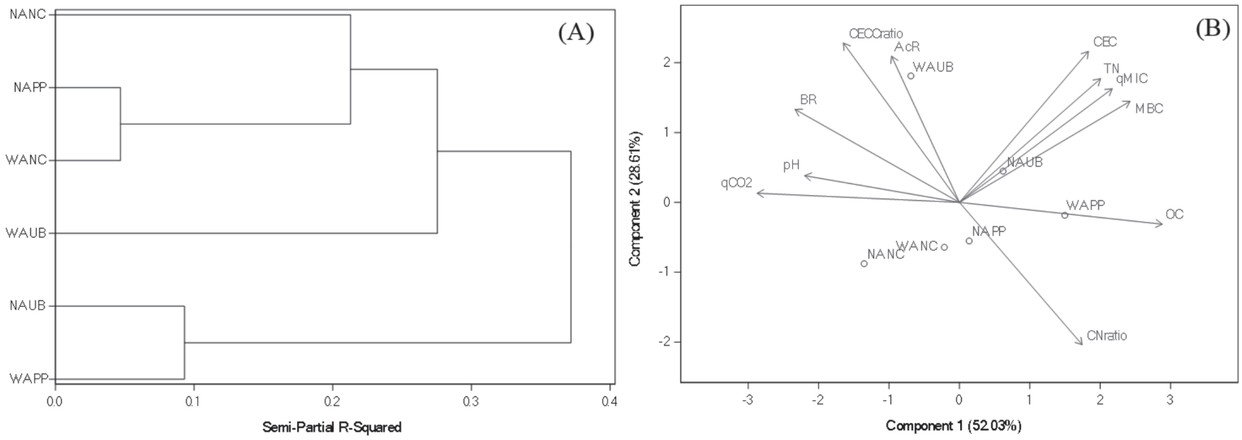

Figure 2: Dendrogram of the cluster analysis by Ward method (A) and biplot of principal component analysis (PCA) (B) of chemical and microbial attributes of sewage sludge cultivated with *P. purpureum* and *U. brizantha* in the presence or absence of aeration. WANC – SS aerated and non-cultivated; NANC – SS non-aerated and non-cultivated; WAPP – SS aerated and cultivated with *P. purpureum*; NAPP – SS non-aerated and cultivated with *P. purpureum*; WAUB – SS aerated and cultivated with *U. brizantha*; NAUB – SS non-aerated and cultivated with *U. brizantha*; OC – Organic carbon; TN; Total Nitrogen; CEC – Cation Exchange Capacity; BR – Basal Respiration; CR – Cumulative Respiration; MBC – Microbial Biomass Carbon; qCO2 – Metabolic Quotient; qMIC – Microbial Quotient.

this treatment. The C/N ratio was the most influential attribute on the NAPP treatment. The CEC/C ratio and the accumulated respiration were the attributes that influenced the WAUB treatment. The highest index value in the WAUB treatment induces a great stabilization of the sewage sludge, despite the high microbial activity, demonstrating that the organic matter is not completely matured in relation to the other treatments. The lower pH, basal respiration, and qCO_2 values and the highest OC concentration show the highest stabilization of organic matter of sewage sludge aerated and cultivated with *P. purpureum* .

Using both statistical methods (univariate and multivariate analyses), we can infer that was a higher quality of the organic fertilizer when the sewage sludge was aerated and cultivated with *P. purpureum* and lower when it was not aerated and cultivated. In addition, this technique can be used to produce an organic fertilizer with great features to be used as substrate for plants, source of nutrients, and soil conditioner, considering that the initial heavy metals concentration (Table 1) was below the established by the legislation (Brasil, 2020a).

CONCLUSIONS

The species *U. brizantha* and *P. purpureum* have potential for direct cultivation in sewage sludge. However, the *P. purpureum* root system provides better conditions for stabilization and maturation of the organic matter of sewage sludge.

The sewage sludge aerated and cultivated with *P. purpureum* provides the formation of organic fertilizer with greater maturation of the organic matter, enhancing the use of this by-product in farming and forest areas. Even though, further studies must be carried out to investigate the influence of cultivation and aeration of sewage sludge on heavy metals and organic pollutants.

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