

Agronomic performance and optimal ranges of attributes of substrates with biochar from anaerobic sewage sludge for black wattle (*Acacia mearnsii*) seedlings

Alex Becker Monteiro^{1*}, Adilson Luís Bamberg², Ivan dos Santos Pereira¹, Cristiane Mariliz Stöcker³, Luís Carlos Timm⁴

Received: 22 April 2020 / Accepted: 11 July 2021 / Published online: 21 August 2021

Abstract

Purpose The objective was to evaluate the agronomic performance of black wattle seedlings (*Acacia mearnsii*) grown on substrates with increasing proportions of biochar based on anaerobic sewage sludge (ASS-biochar). In addition, the present study proposed appropriate ranges for the main physical-hydraulic and chemical attributes of these substrates.

Method The evaluated substrates included ten formulations with increasing proportions of ASS-biochar, combined with a “standard” mixture (organic compost, carbonized rice husk and vermiculite), in addition to three commercial substrates (references). The experimental design was randomized blocks, with four replications and eight plants per repetition. The physical-hydraulic and chemical attributes evaluated in all substrate formulations were: wet and dry density, total porosity, aeration space, easily available water, buffering water, available water, remaining water, pH and electrical conductivity (EC). The agronomic performance of the black wattle seedlings was evaluated through plant height, length of the root system, neck diameter, dry mass of aerial parts, dry mass of roots and Dickson’s quality index.

Results Biochar from anaerobic sewage sludge increased the chemical and physical-hydraulic quality of the substrates, particularly within the proportion of 45-50% of the substrate formulations, and thus positively affected the growth parameters of the black wattle plants.

Conclusion The biochar produced from anaerobic sewage sludge is an efficient raw material to compose substrates for the production of seedlings. Transformation of anaerobic sewage sludge into biochar and its combination with other locally available raw materials can be considered a safe way of reusing this residue in agriculture.

Keywords *Acacia mearnsii*, Growing media, Biosolids, Agricultural substrates

Introduction

The treatment of sewage originating from household collection networks generates a semi-solid material called sewage sludge, and its safe recycling is still

a major challenge for the modern society (Bai et al. 2017). The anaerobic treatment process, characterized by biological degradation of organic matter, is widely used worldwide and generally consists of four successive stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrolysis is the stage where fermentative enzymes convert complex and undissolved material into less complex dissolved compounds. In acidogenesis, the dissolved compounds present in the cells of the fermentative bacteria are converted into several simpler compounds (volatile fatty acids), alcohols, lactic acid, carbon dioxide (CO₂), hydrogen (H₂), ammonia (NH₃) and hydrogen sulfide (H₂S). In acetogenesis, the products of digestion are converted into acetate, H₂ and CO₂, as well as into new cellular material. In methanogenesis, acetate, hydrogen, carbonate and methanol are

✉ Alex Becker Monteiro
alexbeckermonteiro@gmail.com

¹ Universidade Federal de Pelotas, Faculdade de Agronomia Eliseu Maciel, Departamento de Solos, Av. Eliseu Maciel, s/n, CEP 96050-500, Capão do Leão, Rio Grande do Sul State, Brazil

² Embrapa Clima Temperado, Rodovia BR 392, km 78, 9º Distrito - Monte Bonito, CEP 96010-971, Pelotas, Rio Grande do Sul State, Brazil

³ Universidade Federal de Pelotas, Faculdade de Agronomia Eliseu Maciel, Departamento de Fitotecnia, Av. Eliseu Maciel, s/n, CEP 96050-500, Capão do Leão, Rio Grande do Sul State, Brazil

⁴ Universidade Federal de Pelotas, Faculdade de Agronomia Eliseu Maciel, Departamento de Engenharia Rural, Av. Eliseu Maciel, s/n, CEP 96050-500, Capão do Leão, Rio Grande do Sul State, Brazil

converted into methane, CO₂ and new cellular material (Van Lier et al. 2008). Solid waste resulted from these steps is called anaerobic sewage sludge (ASS).

The large volume of ASS generated daily is a major problem for sanitation companies, and its use in agriculture has been the main recycling form worldwide (Tessaro et al. 2016), especially in countries with a large amount of available agricultural land, such as Brazil. Several authors suggest using ASS as a fertilizer or as a soil conditioner in the recovery of degraded land areas and as a component of substrates for seedlings production (Monteiro et al. 2019). Intrinsic characteristics such as high contents of organic matter, macro and micronutrients, porosity, water holding capacity and low costs make ASS an economically and environmentally sustainable raw material to formulate substrates for plants (Abreu et al. 2017; Siqueira et al. 2018), in particular for seedlings production (Monteiro et al. 2017).

However, different contaminants may occur in ASS, mainly pathogenic organisms at levels above those established by the laws that regulate the agricultural use of ASS in soils (Brazil: CONAMA 375/2006; USA: USEPA / 1993), therefore limiting its direct use in the soil (Nascimento et al. 2014). Additional processes to reduce pathogenic contaminants and vector attraction are currently requested by the Brazilian legislation, which include aerobic/anaerobic digestion, heating, composting, and lime stabilization. However, despite numerous studies demonstrating the low risk of ASS for environmental contamination after going through these processes, recent studies have pointed out the persistence of some contaminants (Murray et al. 2019; Teoh and Li 2020), which poses a potential risk of dispersal of pathogens (viruses, bacteria, protozoa, among others) sometimes not completely inactivated by the aforementioned processes. This raises doubts about the real level of safety provided for the environment by the direct application of ASS in the soil (Zuloaga et al. 2012).

In recent years, thermal inactivation has made pyrolysis an additional option for ASS treatment worldwide, both for its effectiveness in eliminating pathogenic microorganisms and for the agronomic potential of the solid product derived from this process (Yue et al. 2017; Ren et al. 2018). Pyrolysis carbonizes organic materials in the absence or in low concentrations of oxygen. With high temperatures (300 - 1000°C), pyrolysis result in a product rich in pyrogenic carbon called bio-

char (Lehmann and Joseph 2015; Rizwan et al. 2016). Biochar enhances the use of ASS in agriculture and reduces the costs of its disposal (Tang et al. 2019), thus, it has been widely studied as a soil conditioner and/or fertilizer (Yue et al. 2017; Frišták et al. 2018; Ren et al. 2018). However, studies on the use of ASS biochar as a substrate component for seedling production are incipient (Silva et al. 2017; Gonzaga et al. 2018). Thus, best plant responses to ASS-derived substrates require optimization of the main physical-hydraulic and chemical attributes.

Therefore, the objective of this work was to evaluate the agronomic performance of black wattle seedlings (*Acacia mearnsii*) grown on substrates with increasing proportions of biochar based on anaerobic sewage sludge (ASS-biochar). In addition, the present study proposed appropriate ranges for the main physical-hydraulic and chemical attributes of these substrates.

Material and methods

The experimental study was conducted under controlled conditions in a greenhouse at Embrapa Clima Temperado - Terras Baixas Experimental Station, Capão do Leão, State of Rio Grande do Sul, Brazil (31° 49' 13" S and 52° 27' 50" W), from August 2017 to December 2017.

Obtaining the biochar

The ASS was collected at the Sewage Treatment Station of Passo Fundo, State of Rio Grande do Sul, Brazil (28°13'18" S and 52°22'7" W). After collection, a 0.1 m thick layer of ASS was spread in fiberglass boxes that remained inside an agricultural greenhouse covered with transparent plastic (200 mm) until reaching less than 20% of humidity. After drying, the ASS was submitted to the pyrolysis process with a partial supply of air and with the temperature ranging from 300 to 600 °C for three hours, while being maintained inside the pyrolysis chamber under atmospheric pressure (1 atm). After the pyrolysis, the ASS-biochar had its granulometry standardized in particles smaller than 2.0 mm by means of mechanical grinding and sieving. The biochar produced was characterized in relation to pathogenic, organic and inorganic contaminants, in full compliance with the maximum limits allowed by the Brazilian legislation: CONAMA Resolution No. 375/2006.

Treatments

The evaluated substrates included ten formulations with increasing proportions of ASS-biochar (S1-S10) and three commercial substrates (references: S11-S13) (Table 1). The formulated substrates (S1-S10) were composed of ASS-biochar and a “standard” mixture constituted by 33.33% commercial organic compost + 43.33% carbonized rice husk + 23.33% fine vermiculite (<3 mm) (3.3:4.3:2.3 m:m ratio). The commercial substrates (S11-S13) had the following compositions: composted agro-industrial organic waste obtained from seeds, bagasse and grape stalks, ashes, peat and carbonized rice husk (Commercial 1); mixture of pine bark, vermiculite, dolomitic limestone and macronutrients (Commercial 2); and a mixture of sphagnum peat, expanded vermiculite, carbonized rice husk, dolomitic limestone, agricultural plaster, NPK fertilizer and micronutrients (Commercial 3).

Table 1 Substrates formulated with ASS-biochar and three commercial substrates

Substrates	ASS-biochar	“Standard” mixture
	-----% (m:m) -----	
S1	10	90
S2	20	80
S3	30	70
S4	40	60
S5	50	50
S6	60	40
S7	70	30
S8	80	20
S9	90	10
S10	100	0
S11	Substrate commercial 1	
S12	Substrate commercial 2	
S13	Substrate commercial 3	

* “Standard” mixture = 33.33% commercial organic compost + 43.33% carbonized rice husk + 23.33% fine vermiculite (3.3:4.3:2.3 ratio).

Characterization of substrates

Physical-hydraulic characterization – These analyses were carried out at the Soil Physics Laboratory of “Embrapa Clima Temperado”, where the following variables were determined: wet density (WD), dry density (DD), total porosity (TP), aeration space (AS), easily available water (EAW), buffering water (BW), available water (AW) and remaining water (RW). The wet

and dry densities of the substrates were determined through the self-compacting method described by Brasil (2007). The variables TP, AS, EAW, BW, AW, and RW were determined according to De Boodt and Verdonck (1972).

Chemical characterization - The pH and EC of the substrates were determined according to MAPA/SDA Regulatory Instruction No. 17 of May 24, 2007 (Brasil 2007) recommended by the International Society of Horticultural Sciences (UNE-EN 13037 2012 and UNE-EN 13038 2012). Substrate samples were diluted in the proportion of 1:5 (substrate:water) and subjected to mechanical horizontal agitation at 40 cycles per minute for 60 minutes. Subsequently, the liquid phase of samples was filtered through quantitative filter paper (8 µm). The pH was determined with a pH meter model HI 2221 (HANNA Instruments, São Paulo - Brazil) while EC was determined in the same samples with a Tec-4MP bench meter (Tecnal Scientific Equipment's, São Paulo - Brazil).

Conduction of the agronomic experiment

The agronomic experiment was conducted under micro-sprinkler irrigation. The applied irrigation level was calculated every day based on the crop evapotranspiration (ETc) that was obtained from the product of crop coefficient (Kc) and potential evapotranspiration (ETP) that was calculated using the Benevides and Lopez equation (1970), whose values were obtained from temperature (°C) and relative humidity (%) sensors installed inside the greenhouse. The black wattle seeds used in the experiment were collected from adult trees in a commercial forest intended for wood and tannin production, located in Piratini, RS State, Brazil. The seeds presented a germinative power of 72%. Before sowing, seed dormancy was broken by immersing them in hot water (~ 80°C) for 3 minutes (Martins-Corder et al. 1999). Sowing was carried out on August 21st, 2017, in polypropylene tubes of 53 cm³, arranged in box trays with two seeds per tube, with subsequent thinning to maintain only one plant per tube.

The performance of plants were evaluated 120 days after sowing, in relation to the following variables: plant height, length of the root system, neck diameter, dry mass of the aerial parts, dry mass of the roots and Dickson's quality index. Plant height (cm) and length of the root system (cm) were measured with a graduated ruler while the neck diameter was measured with

a digital caliper. All these variables, as well as the dry mass of the aerial parts and the roots, were determined individually, in each of the eight seedlings of each plot. To obtain the dry mass, the plant tissues were dried out in oven at 65 ± 1 °C until reaching constant mass, followed by weighing in precision balance.

Experimental design and statistical analysis

The experimental design was in randomized blocks, with four replications and eight plants per repetition. The normality of the probability distributions of the data sets obtained in the characterization of all substrates and in the agronomic experiment was evaluated. The data sets were checked for the presence of outliers and subsequently subjected to an analysis of variance (ANOVA). The data of each response variable with significant treatment effects obtained from the F-test ($p < 0.05$) were subjected to a polynomial regression analysis. Statistical analyses were performed using Sigma-Plot software version 11.0.

Results and discussion

Physical-hydraulic characterization of substrates

The increase in the proportions of biochar significantly influenced the physical-hydraulic characteristics of the substrates, with a linear increase in the wet and dry densities (Fig. 1A, 1B) and a linear reduction of total porosity (TP) (Fig. 1C) and aeration space (AS) (Fig. 1D). The results show that the obtained dry densities can be considered high according to the ideal range indicated by Fermino (2002), between 250 and 400 kg m⁻³ for dry density (DD) in tubes of up to 15 cm in length. The substrates with up to 40% biochar showed DD values within this recommended range, while the others showed higher values (Fig. 1B). Among the commercial substrates, S12 and S13 presented DD within the recommended range, however, S11 surpassed the indicated range for this cultivation system (Fig. 1B). Elevated DD will result in a decrease in TP, AS and in the space available for the development of the root system of the plants, in addition to affecting water storage, transportation and handling costs (Castoldi et al. 2014).

The TP values were reduced due to the increase in the proportion of biochar, however, the substrates with 20% biochar and S13 showed values within the

recommended range (80-90% - Fig. 1C). In relation to AS, a linear decrease was also observed as the proportions of biochar in the substrates were increased, thus, only substrates formulated with proportions between 70 and 100% of biochar had adequate volumes of AS (20-30% - Fig. 1D). Of the commercial substrates, only S13 had AS within the recommended range.

The linear reductions in TP and AS resulted from an increase in DD that occurs in response to the increasing proportions of biochar. Dry density is a physical property inversely proportional to porosity (Barros et al. 2019), evidenced by the significant correlation between DD and TP ($r = -0.91$, $p < 0.01$) and with AS ($r = -0.89$, $p < 0.01$). While TP is associated with macro and micropores, AS is mainly conditioned by the proportion of macropores in the substrate, and these parameters directly interfere with available water (AW) and remaining water (RW). In this regard, the present work suggests that the pyrolysis process reduces the volume of macro and micropores. Moreover, Monteiro et al. (2017) found an increase in total porosity, especially of micropores, in substrates with higher proportions of solarized SS.

Regarding easily available water (EAW) and AW, the different proportions of biochar had no effect on the studied substrates (Fig. 1E). Thus, all biochar-based substrates (S1 to S10) and commercial substrates (S11 to S13) showed EAW and AW values below the ideal range (20 - 30% - Fig. 1E; 24 - 35% - Fig. 1G). The buffering water (BW) increased linearly in response to the increase in the proportion of biochar in the formulation (Fig. 1F). However, only the substrates with 70 and 90% biochar and the commercial substrates S11 and S12 showed values similar to the recommended range (4 - 5% - Fig. 1F).

De Boodt and Verdonck (1972) state that the substrate should provide water to plants in the lowest possible energy state, as well as present sufficient amount of air in the plant-root zone. However, it can be seen that EAW, BW and AW were below the recommended values by the authors (Fig. 1). This low percentage of AW presented by substrates based on biochar (Fig. 1G) is related to the excess of macropores in the standard mixture, a fact that is expressed by the high AS (Fig. 1D) presented by the substrates formulated with the lowest proportions of biochar.

Regarding RW, the different proportions of biochar had no effect on it, however, the values obtained were within the recommended range (20 - 30% - Fig. 1H).

On the other hand, among the three commercial substrates, only the substrate S11 showed RW values within the recommended range, the others (S12 and S13) were higher. The RW, while being kept at higher water

potentials (above 10 kPa), represents the water from the micropores and can be considered poorly accessible to seedlings and young plants.

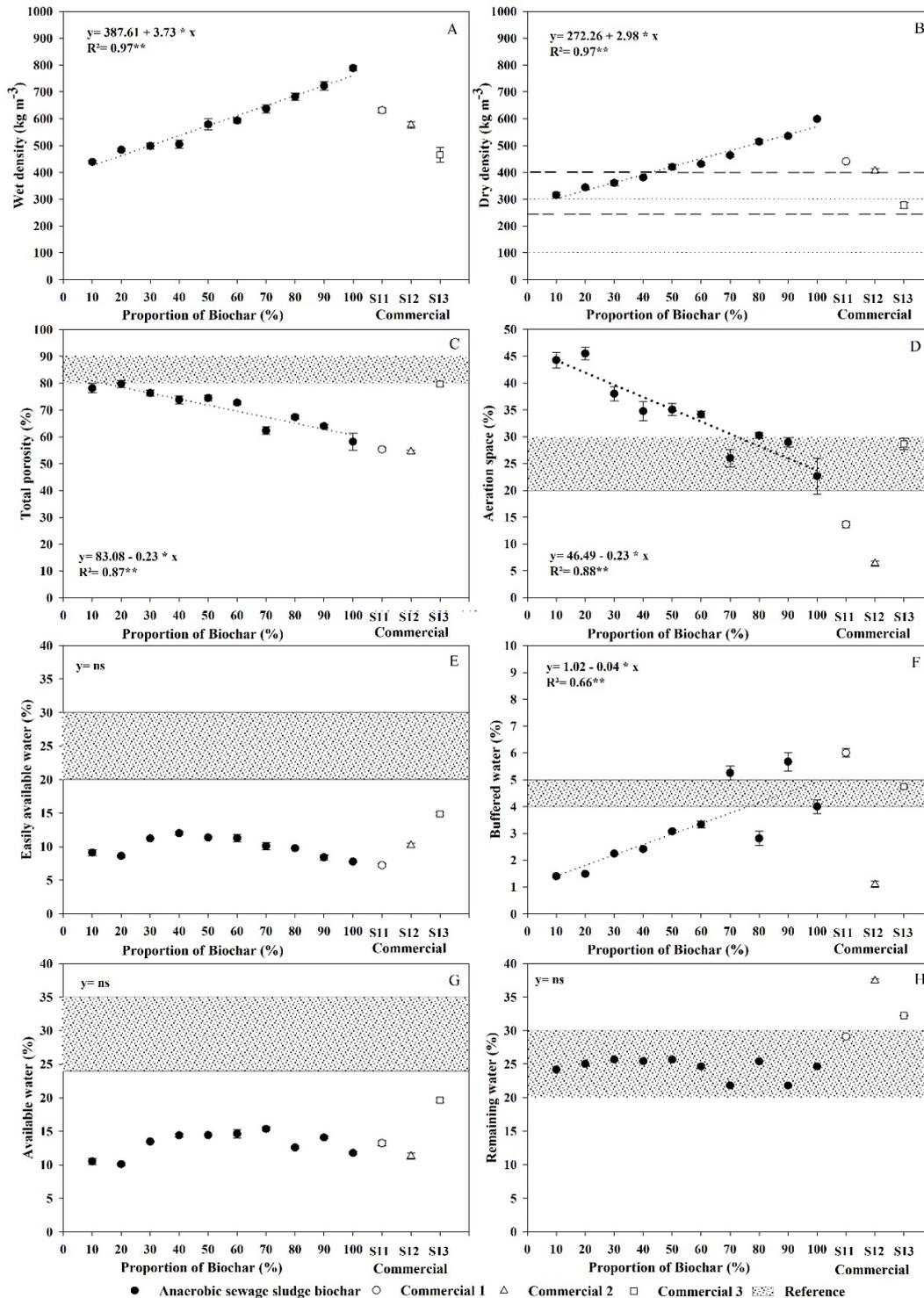


Fig. 1 Physical-hydraulic parameters in substrates with anaerobic sewage sludge biochar and three commercial substrates

ns: polynomial regression not significant at $p < 0.01$; *: significant at $p < 0.05$; **: significant at $p < 0.01$.

Chemical characteristics

The pH of the substrates had a quadratic response due to the increase in the proportions of biochar (Fig. 2A). The substrates with 60 to 90% ASS, in addition to S13, showed pH values within the recommended range for most crops, including black wattle (pH around 5.5) (5.5 - 6.5 - CQFS -RS/SC, 2016). On the other hand, the substrates formulated with the lowest biochar proportions (10 - 50%) had a higher pH than recommended (pH>6.5), probably due to the components of the “standard” mixture, formulated with organic compost, carbonized rice husk and fine vermiculite, with pH of 7.19, 7.83 and 6.63, respectively. According to Valeri and Corradini (2000), substrates with a very high pH can induce a deficiency of phosphorus, iron, zinc and copper in plants. On the other hand, the substrate with 100% biochar, as well as S11 and S12, had pH values below the ideal range for most cultures (Fig. 2A).

In several cases, anaerobic sewage sludge may present very acidic conditions (Pereira et al. 2020), with pH values from 3.5 to 4.0, as in the case of our study. After the pyrolysis, the resulted biochar usually presents a higher pH than before carbonization. In the present study, the pH of ASS biochar reached 4.5 (Fig. 2, with 100% of ASS biochar), insufficient for plant substrates. The addition of components with higher pH values, such as carbonized rice husks, provides an increase in the final pH of the substrates, fitting them into the recommended range for most crops (pH from 5.5 to 6.5). This was acquired with the addition of 50 to 90% of biochar, as recommended by Fermino et al. (2018). The

mentioned authors also evaluated the reuse of wastes as components of substrates to produce *Eucalyptus grandis* seedlings and observed that the combination of carbonized rice husk with other raw materials increased the final pH of the substrates. This fact corroborates the results obtained in the present study.

The values of electrical conductivity (EC) (dilution 1:5 - v:v) were increased linearly as the proportion of biochar increased (Fig. 2B). According to Cavins et al. (2000), the EC of substrates can be classified as very low (0.00 to 0.11 mS cm⁻¹), low (0.12 to 0.35 mS cm⁻¹), normal (0.36 to 0.65 mS cm⁻¹), high (0.66 to 0.89 mS cm⁻¹), very high (0.90 to 1.10 mS cm⁻¹) and extremely high (>1.10 mS cm⁻¹) for a dilution of 1:5 (v:v). The results indicate that substrates formulated with more than 40% biochar presented an extremely high EC. Substrates with a high EC can damage the roots and reduce or even prevent the absorption of water and nutrients, which makes this one of the main indicators that must be considered when choosing the substrate components (Cavins et al. 2000).

The high EC of the biochar is probably due to its high nutrient load, which generally results in a saline material (Fornes et al. 2015). The pyrolysis reduces the amount of moisture and volatile solids (Tang et al. 2018), particularly those containing organic phases of elements such as Nitrogen, Sulphur, Carbon and Oxygen, thus concentrating alkali metals in the mineral form, as observed by Monteiro et al. (2020a). The high concentrations of these elements are responsible for the higher electrical conductivity (EC) values of the biochar when compared to the other raw materials.

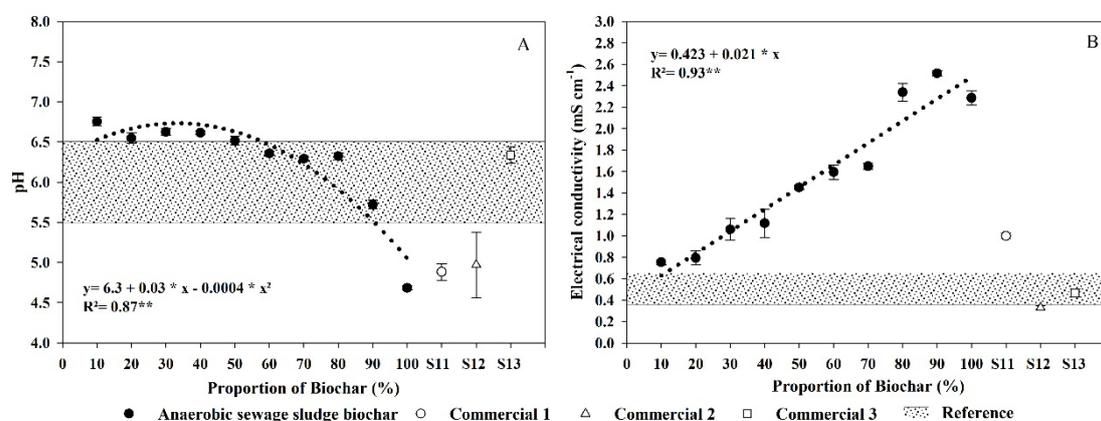


Fig. 2 Response of hydrogenionic potential (pH) and electrical conductivity (EC) in substrates with increasing proportions of anaerobic sewage sludge biochar and three commercial substrates

*: polynomial regression significant at $p < 0.05$; and **: significant at $p < 0.01$.

Agronomic parameters of black wattle (*Acacia mearnsii*)

The black wattle seedlings grown under a sprinkler irrigation system were significantly influenced by the proportions of biochar in the substrates (Fig. 3). For all growth variables, a quadratic response was obtained due to the increasing quantities of biochar in the substrates, and the maximum technical efficiency was estimated at 46, 46, 50, 50, 50 and 50% for plant height, length

of the root system (LRS), neck diameter, dry mass of the aerial parts (DMAP), dry mass of roots (DMR) and Dickson’s quality index (DQI), respectively (Fig. 3).

In general, the proportion of biochar that provided the greatest vegetative growth was around 50%, with proportions greater than 70% resulting in growth reduction, mainly with 100% biochar, which presented the worst performance among all substrates with biochar. Regarding the commercial substrates, S11 and S12 did not differ from the substrate with 100% biochar (Fig. 3). The

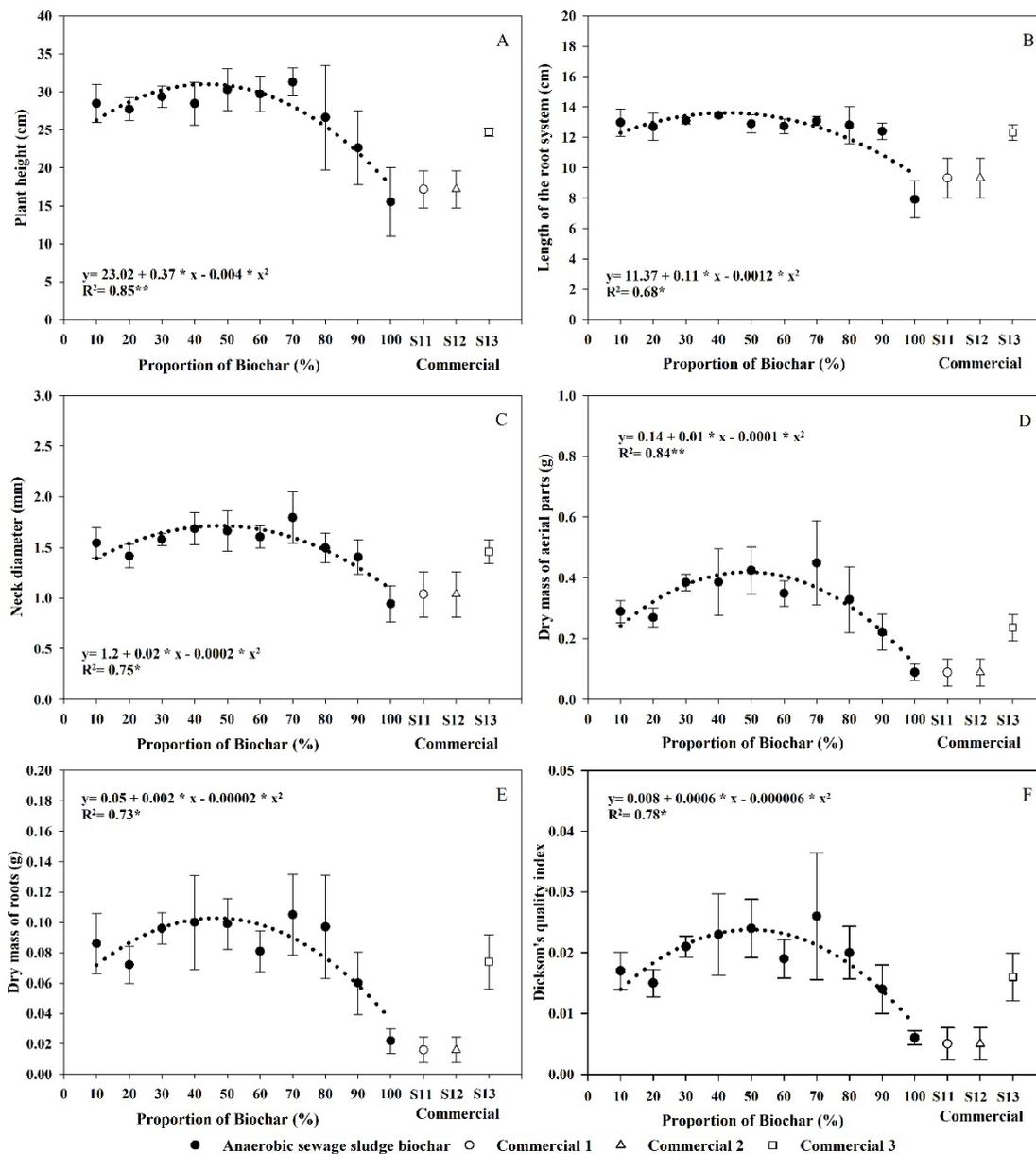


Fig. 3 Plant height, length of the root system, neck diameter, dry mass of the aerial parts, dry mass of the roots and Dickson’s quality index of black wattle (*Acacia mearnsii*) plants grown on substrates with anaerobic sewage sludge biochar and three commercial substrates

*: polynomial regression significant at $p < 0.05$; and **: significant at $p < 0.01$.

substrate S13, on the other hand, performed significantly better than the other commercial ones, being equivalent to substrates with 80 and 90% biochar (Fig. 3).

The agronomic results of this study are in accordance with those obtained for lettuce seedlings (Monteiro et al. 2020b), carried out with biochar made from aerobic sewage sludge and combined with other raw materials. The mentioned authors obtained the best results with 20% of biochar. In fact, the amount of biochar in the formulation is the key factor for agronomic performance, since it affects the pH, the EC, the physical conditions as well as the amount of nutrients supplied to the plants by the formulated growth media.

Physical-hydraulic and chemical characteristics of substrates versus morphological characteristics of black wattle

In general, the increasing proportions of biochar in the substrates significantly influenced the physical-hydraulic and chemical characteristics, which consequently affect the growth parameters of black wattle. The proportions of biochar that provided greater plant growth of black wattle were in the range of 40 - 70%. The main parameters that influenced the growth of black wattle seedlings, according to the correlation analysis, were BW, AW and RW (Table 2). Considering BW and AW, the effects were mainly due to BW, as AW is the sum of

EAW and BW, and EAW was not related to the growth variables (Table 2). In the sprinkler system, the water contents in equilibrium at pressures between 50 and 100 kPa were more important for plant growth, probably because these water contents were retained with an intermediate strength by the substrate, with less losses to the environment (evaporation) and at the same time easily accessible to the plants.

Another variable that influenced the growth of black wattle was RW (Table 2). In this case, the RW represents the water that, although present in the substrate, is difficult for plants to access. For this reason, there was a negative correlation between the RW and the growth parameters. That is, in substrates where the percentage of RW was higher, there was a reduction in the growth potential of the black wattle plants.

Although the chemical and physical-hydraulic aspects determined in the substrate formulations developed and evaluated in this study should be personalized for each crop, in general, it was possible to see that certain substrates with ASS-biochar mixed with carbonized rice husk, organic compost and vermiculite showed efficiency similar or superior to the three commercial substrates taken as references. This fact reveals the potential of using the ASS-biochar. The plant responses indicated that a proportion of around 50% of ASS-biochar in the substrate resulted in the best production of black wattle seedlings.

Table 2 Correlation of the physical-hydraulic and chemical characteristics of the substrates with plant growth variables of black wattle seedlings (*Acacia Mearnsii*)

Characteristic of Substrate	Plant height	Neck diameter	LRS	DMAP	DMR	DQI
Physical-hydraulic characteristics						
WD	0.03 ^{ns}	0.29 ^{ns}	-0.13 ^{ns}	0.28 ^{ns}	0.17 ^{ns}	0.30 ^{ns}
DD	0.02 ^{ns}	0.28 ^{ns}	-0.12 ^{ns}	0.27 ^{ns}	0.16 ^{ns}	0.28 ^{ns}
TP	0.04 ^{ns}	-0.26 ^{ns}	0.24 ^{ns}	-0.23 ^{ns}	-0.11 ^{ns}	-0.26 ^{ns}
AS	0.02 ^{ns}	-0.29 ^{ns}	0.15 ^{ns}	-0.29 ^{ns}	-0.14 ^{ns}	-0.30 ^{ns}
EAW	0.06 ^{ns}	0.00 ^{ns}	0.36 ^{ns}	0.11 ^{ns}	0.04 ^{ns}	0.04 ^{ns}
BW	0.19 ^{ns}	0.51 ^{**}	-0.10 ^{ns}	0.50 [*]	0.35 ^{ns}	0.52 ^{**}
AW	0.21 ^{ns}	0.41 [*]	0.21 ^{ns}	0.49 [*]	0.30 ^{ns}	0.44 [*]
RW	-0.19 ^{ns}	-0.40 [*]	0.14 ^{ns}	-0.33 ^{ns}	-0.25 ^{ns}	-0.37 [*]
Chemical characteristics						
pH	0.12 ^{ns}	-0.12 ^{ns}	0.23 ^{ns}	-0.09 ^{ns}	-0.10 ^{ns}	-0.15 ^{ns}
EC	0.12 ^{ns}	0.33 ^{ns}	-0.02 ^{ns}	0.32 ^{ns}	0.15 ^{ns}	0.31 ^{ns}

LRS – length of the root system; DMAP – dry mass of the aerial parts; DMR – dry mass of the roots; DQI – Dickson's quality index; WD – wet density; DD – dry density; TP – total porosity; AS – aeration space; EAW – easily available water; BW – buffering water; AW – available water; RW – remaining water; pH – hydrogenionic potential; EC – electrical conductivity.

ns = Pearson's correlation coefficient not significant at $p < 0.01$ nor $p < 0.05$; *: significant at $p < 0.05$; **: significant at $p < 0.01$.

In addition, ASS-biochar can be considered a safe material, because after the pyrolysis process, in which the ASS is subjected to temperatures of 300 - 600 °C, the pathogenic contaminants are eliminated and at the same time there are no problems with inorganic contaminants, corroborating with similar studies found in the literature (Yue et al. 2017; Ren et al. 2018).

Even though the results indicate that several of the proposed substrate formulations have achieved similar and even superior performance to the tested commercial ones, it is believed that further studies should be conducted. There is a need to test new combinations with other materials and/or proportions of the same materials, as some chemical and physical-hydraulic parameters (pH, EC, Density, TP, AS, EAW, BW and AW) differed from those considered ideal in the literature (CQFS-RS/SC 2016; De Boot and Verdonck 1972; Fermino et al. 2018), indicating the possibility that the results obtained in the present study can be further maximized, customized or extended to a greater number of species.

Considering the plants that were more satisfactorily developed, ranges were proposed for the main physical-hydraulic and chemical characteristics of substrates, in particular for those more suitable for black wattle. Furthermore, appropriate ranges for a greater number of crops, with emphasis in growing conditions that also consider particularities of the different cultivation systems, should also be determined.

Recommended ranges for the physical-hydraulic and chemical characteristics of substrates for black wattle seedlings

Based on the performance of the black wattle plants and the corresponding physical-hydraulic and chemical characteristics observed in these substrates, significant improvements were made to update what is actually recommended by the literature (Fermino 2002; De Boedt and Verdonck 1972; CQFS-RS/SC 2016; Cavins et al. 2000). Therefore, new reference ranges were proposed for the physical-hydraulic (DD, TP, AS, EAW, BW, AW and RW) and for the chemical (pH and EC) characteristics (Table 3).

The DD values of the substrates that showed the best performance for the production of black wattle seedlings ranged from 420 to 460 kg m⁻³, being higher than those recommended by Fermino (2002), who recommends DD values between 250 and 420 kg m⁻³ for cultivation of seedlings in tubes up to 15 cm high. De Boedt and Verdonck (1972) proposed adequate ranges for a set of variables, including TP, AS, EAW, BW and RW. The present study set a specific interval for black wattle, which ranged from 60 to 75%, lower than the limits between 80 and 90%, recommended by De Boedt and Verdonck (Table 3). The AS interval that provided the best performance of black wattle seedlings ranged from 25 to 35%, being similar to the one indicated by the same authors, between 20 and 30%. The

Table 3 Recommended ranges for physical-hydraulic and chemical parameters for the cultivation of black wattle (*Acacia Mearnsii*) grown in substrates

Physical-hydraulic and chemical parameters of substrates	Suitable ranges for black wattle*	Reference ranges (Literature)
Dry density - DD (kg m ⁻³)	420 - 460	100 - 300; 250 - 400
Total porosity - TP (%)	60 - 75	80 - 90
Aeration space - AS (%)	25 - 35	20 - 30
Easily available water - EAW (%)	10 - 12	20 - 30
Buffering water - BW (%)	3 - 5	4 - 5
Available water - AW (%)	13 - 15	24 - 35
Remaining water - RW (%)	20 - 25	20 - 30
Hydrogenionic potential - pH	6.0 - 6.5	5.5 - 6.5
Electrical conductivity - EC (mS cm ⁻¹)	1.4 - 1.6	0.4 - 0.6

* Under controlled environmental conditions, with conical tubes of up to 15 cm in height, under micro-sprinkler irrigation

EAW values that provided satisfactory development of black wattle seedlings varied from 10 to 12% (Table 3), being lower than the recommended range of 20 - 30% proposed by De Boodt and Verdonck (1972). For BW, black wattle seedlings' growth was satisfactory in the range of 3 - 5%, similar to 5%, the value recommended by the mentioned authors. As the AW values represent the sum of the EAW and BW, the percentage of AW that provided the best performance of black wattle seedlings was in the range of 13 - 15%, being lower than the 20 - 30% interval recommended as ideal by the same authors (Table 3). Considering RW, the range that provided the best development of black wattle seedlings was between 20 and 25%, thus more restrictive than the range recommended, between 20 and 30% (Table 3).

Regarding the pH values of the substrates for black wattle in the micro-sprinkler irrigation system, the best performance of the seedlings was obtained in the range of 6.0 - 6.5, that is, within the recommended range (5.5 - 6.5) for most crops, as stated by CQFS-RS/SC (2016) (Table 3). As for the EC in substrates that provided satisfactory development for black wattle seedlings, the range varied between 1.4 and 1.6 mS cm⁻¹, being higher than those considered normal by Cavins et al. (2000), who recommend EC values in the range of 0.4 - 0.6 mS cm⁻¹.

Conclusion

The results demonstrate that the biochar produced from anaerobic sewage sludge is an efficient raw material to compose substrates for the production of seedlings. In this sense, the best agronomic efficiency was obtained within the proportion of 45-50% of ASS-biochar for the production of black wattle seedlings.

Under the micro-sprinkler system, the physical-hydraulic conditions of substrates that provided the best performance of black wattle were: 420 - 460 kg m⁻³ (dry density); 60 - 75% (total porosity); 25 - 35% (aeration space); 10 - 12% (easily available water); 3 - 5% (buffering water); 13 - 15% (available water); 20 - 25% (remaining water); 6.0 - 6.5 (pH); 1.4 - 1.6 mS cm⁻¹ (electrical conductivity).

This study opens new opportunities in sewage sludge use and can be considered one of the possible safe alternatives to various other methods of reusing this residue in agriculture.

Acknowledgement This study was financially supported by the Companhia Riograndense de Saneamento (CORSAN). The

authors are also thankful to Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) and Fundação de Amparo à Pesquisa Edmundo Gastal (FAPEG) for infrastructure and administrative support through the Technical Cooperation EMBRAPA/FAPEG/CORSAN, project number: 02.17.00.003.00.00.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Abreu AHM de, Leles PSS, Melo LA, Oliveira RR, Ferreira DHAA (2017) Characterization and potential of formulated substrate with biosolids in *Schinus terebinthifolius* Raddi. and *Handroanthus heptaphyllus* (Vell.) Mattos seedling production. Ci FI 27: 1179-1190.
<http://dx.doi.org/10.5902/1980509830300>
- Bai Y, Zang C, Gu M, Gu C, Shao H, Guan Y, Wang X, Zhou X, Shan Y, Feng K (2017) Sewage sludge as an initial fertility driver for rapid improvement of mudflat salt-soils. Sci Total Environ 578: 47-55.
<https://doi.org/10.1016/j.scitotenv.2016.06.083>
- Barros DL de, Rezende FA de, Campos AT (2019) Production of *Eucalyptus urograndis* plants cultivated with activated biochar. Rev Bras de Ciênc Agrar 14: e5649.
<https://doi.org/10.5039/agraria.v14i2a5649>
- Benevides JG, Lopez D (1970) Formula para el calculo de la evapotranspiracion potencial adaptada al tropico (15° N - 15° S). Agron Trop Maracay 20: 335-345
- Brasil Ministério da Agricultura, Pecuária e abastecimento (MAPA) (2007) Secretaria de Defesa Agropecuária (SDA). Instrução Normativa SDA N° 17 de 21 de maio de 2007 Métodos Analíticos Oficiais para Análise de Substratos e Condicionadores de Solos. Diário Oficial da União.
<https://www.diariodasleis.com.br/legislacao/federal/132159-analise-de-substratos-e-condicionadores-de-solos-aprova-os-mutodos-analuticos-oficiais-para-analise-de-substratos-e-condicionadores-de-solos.html>. Accessed 3 August 2017
- Castoldi G, Freiberger MB, Pivetta LA, Pivetta LG, Echer MM (2014) Alternativa substratos na produção de mudas de alface e sua produtividade no campo. Rev Ciênc Agron 45: 299-304.
<https://doi.org/10.1590/S1806-66902014000200010>
- Cavins TJ, Whipker BE, Fonteno WC, Harden B, Mccall I, Gib-

- son WC (2000) Monitoring and managing pH and EC using the PourThru extraction method. Horticulture Information Leaflet 590, New 7/2000. Raleigh: North Caroline State University, 17p
- CONAMA - Conselho nacional do meio ambiente (2006) Resolução Conama nº. 375, de 29 de agosto de 2006. Diário Oficial da República Federativa do Brasil. https://www.normasbrasil.com.br/norma/resolucao-3752--006_103464.html. Accessed 15 June 2017
- CQFS-RS/SC - Comissão de química e fertilidade do solo (2016) Manual de calagem e adubação para os Estados do Rio Grande do Sul e de Santa Catarina. 11. ed. SBCS/NRS: Porto Alegre. 376p
- De Boodt M, Verdonck O (1972) The physical properties of the substrates in: Floriculture. Acta Horticult 26: 37-44
- Fermino MH (2002) O uso da análise física na avaliação da qualidade de componentes e substratos. In: Encontro nacional de substratos para plantas. Campinas. IAC: 29-37
- Fermino MH, Araujo MM, Aimi SC, Turchetto F, Berghetti ALP, Zavistanovicz TC, Vilella JDM (2018) Reutilization of residues as components of substrate for the production of *Eucalyptus grandis* seedlings. Cerne 24: 80-89. <https://doi.org/10.1590/01047760201824022522>
- Fornes F, Belda RM, Lidón A (2015) Analysis of two biochars and one hydrochar from different feedstock: Focus set on environmental, nutritional and horticultural considerations. J Clean Prod 86: 40-48. <https://doi.org/10.1016/j.jclepro.2014.08.057>
- Fršták V, Pipiska M, Soja G (2018) Pyrolysis treatment of sewage sludge: A promising way to produce phosphorus fertilizer. J Clean Prod 172: 1772-1778. <https://doi.org/10.1016/j.jclepro.2017.12.015>
- Gonzaga MIS, Mackowiak C, Almeida AQ, Carvalho Júnior JIT (2018) Sewage sludge derived biochar and its effect on the growth and morphological traits of *Eucalyptus grandis* w. hill ex maiden seedlings. Ci Fl 28: 687-695. <https://doi.org/10.5902/1980509832067>
- Lehmann J, Joseph S (2015) Biochar for environmental management: An introduction. In: Biochar for environmental management. Routledge
- Martins-Corder MP, Borges RZ, Borges Junior N (1999) Photoperiodism and dormancy breakage of black wattle (*Acacia mearnsii* De Wild.) Ci Fl 9: 71-77. <https://doi.org/10.5902/19805098367>
- Monteiro AB, Pereira I dos S, Stöcker CM, Peres MA, Martinazzo R, Bamberg AL, Timm LC (2017) Substratos a base de lodo de esgoto solarizado e pirolizado para produção de mudas de alface. Congrega URCAMP - 14ª Jornada de Pós-Graduação e Pesquisa 14: 2571-2583. <http://revista.urcamp.tche.br/index.php/rcjgpg/article/view/678>. Accessed 5 April 2020
- Monteiro AB, Bamberg AL, Pereira I dos S, Stöcker CM, Timm LC (2019) Physico-hydric characteristics in substrates formulated with sewage sludge in the production of black wattle seedlings. Ci Fl 29: 1428-1435. <https://doi.org/10.5902/1980509834590>
- Monteiro AB, Pereira I dos S, Bamberg AL, Stöcker CM, Timm LC (2020a) Substratos for seedlings with sewage sludge and biochar. Ceres 67: 491-500. <https://doi.org/10.1590/0034-737x202067060009>
- Monteiro AB, Pereira I dos S, Bamberg AL, Stöcker CM, Ribeiro PL, Timm LC (2020b) Hydro-physical characteristics of substrates for lettuce production in floating growth system. Irriga 25: 629-640. <http://dx.doi.org/10.15809/irriga.2020v25n3p629-640>
- Murray R, Tien Y-C, Scott A, Topp E (2019) The impact of municipal sewage sludge stabilization processes on the abundance, field persistence, and transmission of antibiotic resistant bacteria and antibiotic resistance genes to vegetables at harvest. Sci Total Environ 651: 1680-1687. <https://doi.org/10.1016/j.scitotenv.2018.10.030>
- Nascimento AL, Sampaio RA, Zuba Junio GR, Fernandes LA, Crus SF, Carneiro JP, Barbosa CF, Lima NN (2014) Soil chemical attributes fertilized with sewage sludge stabilized by different processes and cultivated with sunflower. Biosci J 30: 146-153
- Pereira I dos S, Bamberg AL, Sousa RO, Monteiro AB, Martinazzo R, Silveira CAP, Silveira AO (2020) Agricultural use and pH correction of anaerobic sewage sludge with acid pH. J Environ Manage 275: 111203. <https://doi.org/10.1016/j.jenvman.2020.111203>
- Ren N, Tang Y, Li M (2018) Mineral additive enhanced carbon retention and stabilization in sewage sludge-derived biochar. Process Saf Environ 115: 70-78. <https://doi.org/10.1016/j.psep.2017.11.006>
- Rizwan M, Ali S, Qayyum MF, Ibrahim M, Zia-ur-Rehman M, Abbas T, Ok YS (2016) Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: a critical review. Environ Sci Pollut Res 23: 2230-2248. <https://doi.org/10.1007/s11356-015-5697-7>
- Silva IS, Mackowiak C, Minogue P, Reis AF, Moline EFV (2017) Potential impacts of using sewage sludge biochar on the growth of plant forest seedlings. Cienc Rural 47: e20160064. <https://doi.org/10.1590/0103-8478cr20160064>
- Siqueira DP, Carvalho GCMW de, Barroso DG, Marciano CR (2018) Lodo de esgoto tratado na composição de substrato para produção de mudas de *Lafloensia ghyptocarpa*. Floresta 48: 277-284. <http://dx.doi.org/10.5380/ufv.v48i2.55795>
- Tang S, Y Tang, C Zheng, Z Zhang (2018) Alkali metal-driven release behaviors of volatiles during sewage sludge pyrolysis. J Clean Prod 203: 860-872. <https://doi.org/10.1016/j.jclepro.2018.08.312>
- Tang Y, Alam MS, Konhauser KO, Alessi DS, Xu S, Tian W, Liu Y (2019) Influence of pyrolysis temperature on production of digested sludge biochar and its application for ammonium removal from municipal wastewater. J Clean Prod 209: 927-936. <https://doi.org/10.1016/j.jclepro.2018.10.268>
- Teoh SK, Li LY (2020) Feasibility of alternative sewage sludge treatment methods from a lifecycle assessment (LCA) perspective. J Clean Prod 247: 119495. <https://doi.org/10.1016/j.jclepro.2019.119495>
- Tessaro D, Sampaio SC, Castaldelli APA (2016) Wastewater use in agriculture and potential effects on meso and macrofauna soil. Cienc Rural 46: 976-983. <https://doi.org/10.1590/0103-8478cr20141648>

- UNE-EN 13037 (2012) Mejoradores de suelo y substratos de cultivo. Determinacion Del pH. Asociacion Espanola de Normatizacion y Certification-AENOR, Madrid. <https://www.une.org/encuentra-tu-norma/busca-tu-norma/norma/?Tipo=N&c=N0049121>. Accessed 03 March 2021
- UNE-EN 13038 (2012) Mejoradores de suelo y substratos de cultivo. Determinacion De La Conductividade Electrica. Asociacion Espanola de Normatizacion y Certification-AENOR, Madrid. <https://www.une.org/encuentra-tunorma/busca-tu-norma/norma/?c=N0049334>. Accessed 03 March 2020
- US Environmental Protection Agency (USEPA) (1993) Preparing sewage sludge for land application or surface disposal: A guide for preparers of sewage sludge on the monitoring, recordkeeping, and reporting requirements of the federal standards for the use and disposal of sewage sludge, 40 CFR Part 503. EPA/831B-93-002. https://www.epa.gov/sites/production/files/2015-03/documents/guide_on_monitoring_record_keeping_and_reporting.pdf. Accessed 25 March 2020
- Valeri SV, Corradini L (2000) Fertilização em viveiros para produção de mudas de Eucalyptus e Pinus. In: Gonçalves JLM, Benedetti V. (Ed.) Nutrição e fertilização florestal. Piracicaba, Brasil. pp 167-189
- Van Lier JB, Mahmoud N, Zeeman G (2008) Anaerobic wastewater treatment. Biological wastewater treatment, principles, modelling and design 1: 415-456. <https://doi.org/10.4324/9780203477397.ch15>
- Yue Y, Cui L, Lin Q, Li G, Zhao X (2017) Efficiency of sewage sludge biochar in improving urban soil properties and promoting grass growth. Chemosphere 173: 551-556. <https://doi.org/10.1016/j.chemosphere.2017.01.096>
- Zuloaga O, Navarro P, Bizkarguenaga E, Iparraguirre A, Vallejo A, Olivares M, Prieto A (2012) Overview of extraction, clean-up and detection techniques for the determination of organic pollutants in sewage sludge: A review. Anal Chim Acta 736: 7-29. <https://doi.org/10.1016/j.aca.2012.05.016>