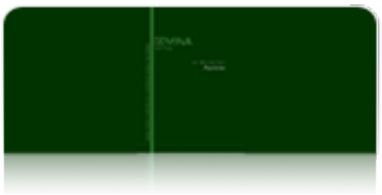

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Microbiota in swine wastewater treatment plant and area of Tifton 85 grass production

Microbiota da água residuária de suinocultura na estação de tratamento e área de produção de capim-tifton 85

Jaqueline Rocha Wobeto Sarto[1*]; Marcela Abbado Neres[2]; Samantha Mariana Monteiro Sunahara[3]; Caroline Daiane Nath[4]; José Renato Stangarlin[5]; Marcos Vinicius Mansano Sarto[6]

Abstract

The objective of this study was to evaluate the effects of biodigester treatment on the microbiological characteristics of swine wastewater (SW) at the production and storage sites, and to characterize and compare the microbiological composition of the soil, organic matter, and plants in the area of hay and haylage production. The area has been planted with Tifton 85 grass for eight years; and is exclusively intended for hay and haylage production, SW was used as the only fertilizer source. The experimental design was completely randomized with subdivided plotsin timeand five replications; the plots were the main areas of the SW *in natura* (affluent), the biodigester outlet (effluent), the storage pond, and the area of Tifton 85 production, which included the soil, plant residue on the soil surface (organic matter), and the aerial parts of the grasses with subplots in the rainy and dry seasons. The microbial count in the SW was reduced during the treatment process, with higher counts in the affluent, and lower counts in the effluent and storage pond. The SW treatment was efficient in reducing the microbial population. The populations of *Bacillus* and *Clostridium* were influenced by the season of the year, with larger populations during the rainy season (summer) than during the dry season (winter). The mold genera identified in the SW area were *Penicillium, Rhizopus, Fusarium, Helminthosporium,* and *Phoma.* The genera *Penicillium, Rhizopus, Fusarium, Cladosporium, Helminthosporium, Bipolaris, Phoma, Aspergillus,* and *Trichoderma* were found in the area of Tifton 85 production.
Key words: Forage. Manure. Microorganisms. Soil.

Resumo

O objetivo deste estudo foi avaliar a eficiência do tratamento do biodigestor nas características microbiológicas da água residuária de suinocultura (ARS), no local de produção até o local de armazenamento, bem como caracterizar e comparar a composição microbiológica do solo, matéria

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orgânica e planta, em uma área de produção de feno e pré-secado. A área de capim-tifton 85 foi implantada há oito anos e é destinada exclusivamente à produção de feno e pré-secado, utilizando como única fonte de adubação a aplicação da ARS. O delineamento experimental foi inteiramente casualizado com parcelas sub divididas, com cinco repetições, sendo as parcelas principais: ARS *in natura* (afluente), a ARS após a saída do biodigestor (efluente) e a ARS na lagoa de armazenamento; além da área de produção do capim-tifton 85: solo, resíduo das plantas na superfície do solo (matéria orgânica) e parte aérea da planta. As sub parcelas foram: a estação chuvosa e a estação seca. Os resultados mostram que a carga microbiana presente na ARS foi reduzida durante o processo de tratamento, com maiores contagens no afluente, e menores no efluente e lagoa de armazenamento. O tratamento da ARS foi eficiente na redução da população microbiana. A população de *Bacillus* e *Clostridium* é influenciada pela estação do ano, com maiores populações na estação chuvosa (verão) em relação à estação seca (inverno). Os gêneros de bolores identificados na área de produção da ARS foram *Penicillium, Rhizopus, Fusarium, Helminthosporium, Phoma.* Na área de produção do capim-tifton 85 foram encontrados os gêneros *Penicillium, Rhizopus, Fusarium, Cladosporium, Helminthosporium, Bipolaris, Phoma, Aspergillus, Trichoderme.*

Palavras-chave: Forragem conservada. Micro-organismos. Forragem. Solo. Planta.

Introduction

The production of swine wastewater (SW) has increased because of agricultural growth, and it can be used for replenishing soil fertility. However, if improperly disposed, it may compromise the quality of groundwater, result in the emission of polluting gases, and cause impacts on the health problems of soil organisms (PESSUTO et al., 2016). Therefore, effective and economical methods have been used to treat swine manure, and minimize waste for the recovery of bioenergy (ZHOU et al., 2016).

Anaerobic digestion is an important economical alternative for the treatment of manure (KORZENIEWSKA et al., 2014; HAMAWAND, 2015; YAN et al., 2015). It is widely regarded as an optimal approach to organic waste treatment, since this process results in the production of sustainable energy and wastethat can be used as an agricultural fertilizer (POSCHL et al., 2010). Consequently, manure can be transformed into a valuable raw material (MATA-ALVAREZ et al., 2014).

According to Menezes et al. (2003), it is considered that the amount of wastewater produced per pig per day is 12 to 15 liters, depending on the productive phase. As a result, the use of SW has enabled the cultivation of fodder crops in some regions because ofitsabilityto totally or partially replace chemical fertilizers, and simultaneously, it can be used to resolve environmental problems.

The use of SW as a fertilizer for forage crops is a common andwell-established practice. Despite the abundance of literature on irrigation with SW (MULLER et al., 2013; MENEZES et al., 2003), most research studies focus on its effects on plant growth and development, although irrigation with SW may also pose risks to the forage quality of the crops. Therefore, it is of interest to characterize the microbiological composition of SW, since it affects the sanitary quality of forage, mainly because of the occurrence of pathogenic bacteria and molds that produce mycotoxins.

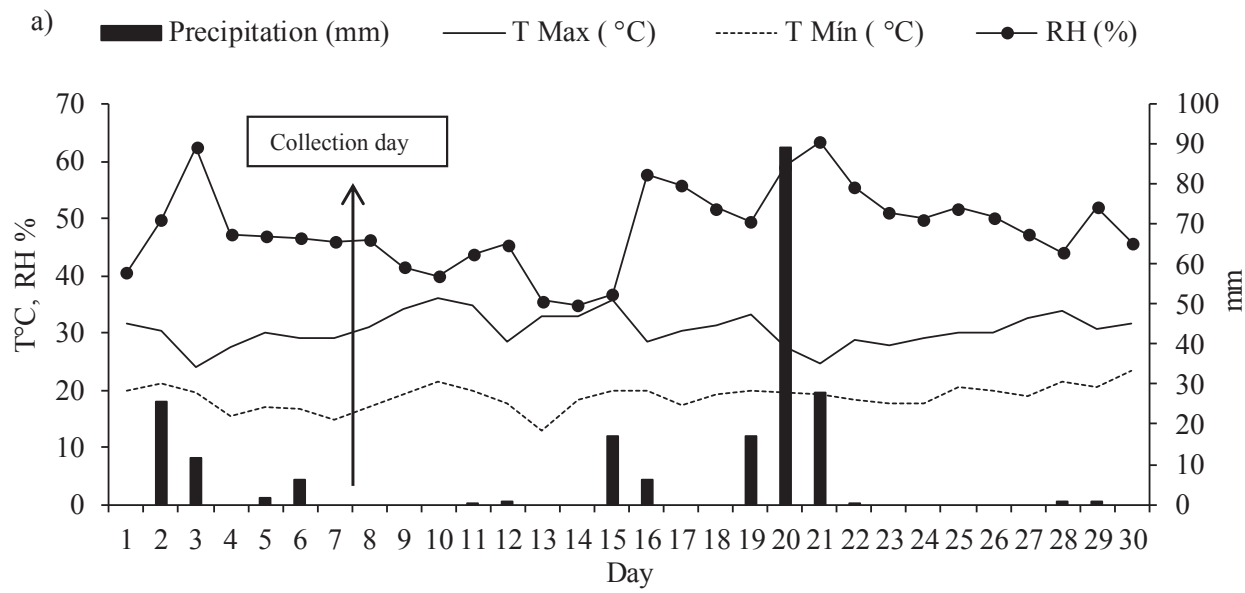
It is important to identify and characterize the genotypes of the predominant molds and other microorganisms that thrive during the stages of forage production, since the presence of large populations of pathogenic molds that produce mycotoxins, particularly, in hay compromises the health of the animals by the induction of carcinogenic and hepatotoxic effects, and mutagenesis (FREIRE et al., 2007).

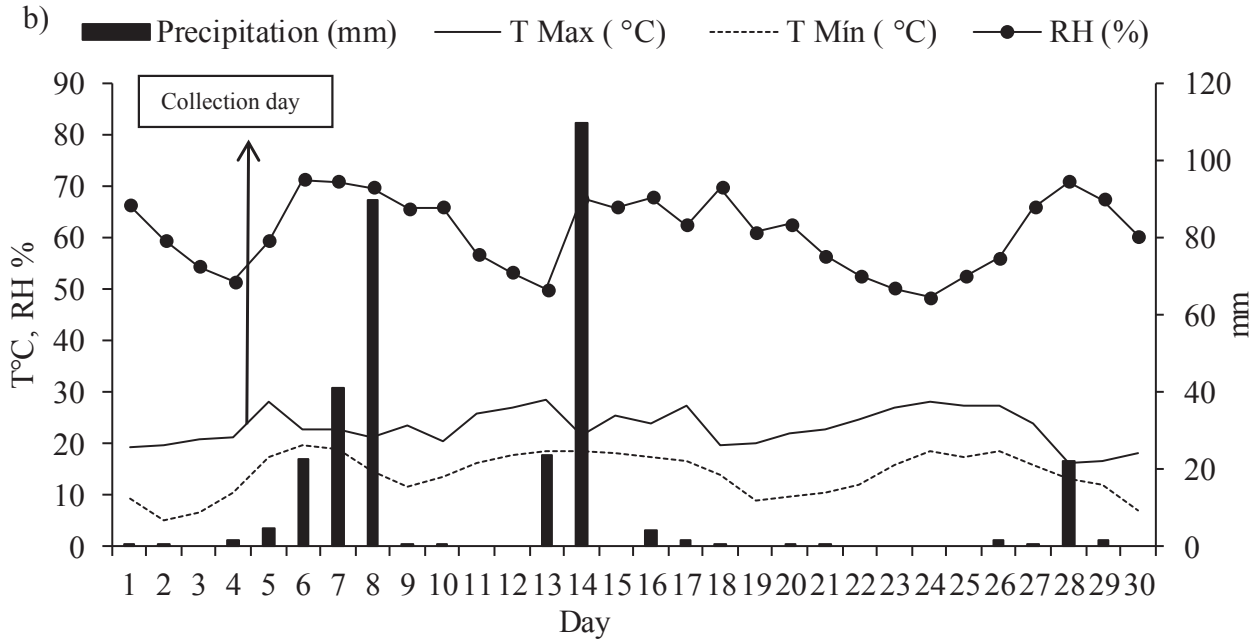
Few studies have focused on the microbiological characterization of SW that is utilized to irrigate the area of production of the hybrid bermudagrass, Tifton 85. Thus, our hypothesis was that the treatment process could reduce the microbial populations in SW, which differ according to the season of the year. Additionally, it is not known if the population of microorganisms that is present in SW remains in the soil, on the aerial parts of the Tifton 85 grasses and in organic matter after the irrigation of Tifton 85 with SW. The objective of this study was to evaluate the effects of biodigester treatment on the microbiological characteristics of SW as it is transported from the production to the application site, as well as to characterize and compare the microbiological composition of the soil, organic matter, and plants in the area of hay and haylage production.

Material and Methods

The experiment was carried out in Marechal Cândido Rondon, Paraná, Brazil, whose geographical coordinates are 24º33'40''S, 54º04'12''W, with an elevation of 420 m. According to Köppen the type of climate in this region was Cfa, which is described as a subtropical climate with well distributed rains during the year and hot summers. The climatic conditions at the collection stations are described in Figure 1.

Figure 1. Precipitation, maximum and minimum temperatures and relative humidity during rainy season (November, a) and dry season (June, b) in Marechal Cândido Rondon, Paraná, Brazil.





The soil was classified as eutrophic Red Latosol (EMBRAPA, 2013) and it exhibited the following chemical attributes: pH ($CaCl_2$) of 5.73; P (Mehlich-1) of 30.64 mg dm^{-3}; K (Mehlich-1) of 0.17 $cmol_c dm^{-3}$; Ca2+ (KCl 1 mol L^{-1}) of 7.41$cmol_c$ dm^{-3}; Mg^{2+} (KCl 1 mol L^{-1}) of 2.47 $cmol_c$ dm^{-3}; Al^{3+} (KCl 1mol L^{-1}) of 0.00 $cmol_c$ dm^{-3}; H + Al (calcium acetate 0.5 mol L^{-1}) of 3.14 $cmol_c$ dm^{-3}; SB of 10.05 $cmol_c dm^{-3}$, CTC of 13.19 $cmol_c dm^{-3}$; V of 73.2%, organic matter (Boyocus Method) of 15.04 g dm^{-3}; Cu concentration of 27.9 mg dm^{-3}; Zn concentration of 23.4 mg dm^{-3}; Mn concentration of 209.0 mg dm^{-3}; Fe concentration of 31.2 mg dm^{-3}.

The area was planted with Tifton 85 grass for eight years and is exclusively used for the production of hay and haylage and the only fertilizer source used is SW. SW was processed by anaerobic digestion, where by it was treated in a continuous-flow biodigester Canadian model, with a capacity of 3,200 m³ and 45 day of hydraulic retention time. The effluent was carried to a storage pond that was covered with a black plastic blanket, and had a capacity of 2,475 m³.

The SW pig farm was characterized as a confined unit for the production of breeding pigs, which had pig barn, and approximately 3,600 animals, including lactating sows, pregnant pigs, and piglets. The unit had pig barn that were categorized as nursery, maternity, and pregnancy rooms. The piglets remainedin the farm until they reached approximately the age of 30 days.

SW from several pig barn (affluent) was carried to the biodigester via concrete side channels and the facilities were cleaned daily. Upon leaving the biodigester, the produced SW was conveyed to the effluent tank and pumped into the storage pond with a capacity of 900 m^3, which was located in the hay and haylage production area.

Pumping was done once or twice a week, according to the amount of SW produced and the need for hay and haylage production. This was sprayed on the surface area of hay and haylage production using a coupled spraying equipment after 7 and 14 days of forage regrowth, at an average of 60 m^3 ha^{-1} per application. Due to the decanting process, prior to application, SW was homogenized with as praying equipment that was coupled to the tractor. The chemical analysis of SW by flame

atomic absorption spectrometry (FAAS) revealed the following composition: 0.80 g $kg^{-1}$of N, 0.96 g $L^{-1}$of K, 4.62 g $L^{-1}$of P, 0.64 g L^{-1} of Ca, 0.03 g $L^{-1}$of Mg, 0.87 mg $L^{-1}$of Cu, 0.45 mg L^{-1} of Zn 0.24 mg L^{-1} of Mn, and 5.61 mg L^{-1} of Fe.

The density, dry matter, pH, and temperature of SW in the rainy and dry seasons are described in Table 1. The bromatological composition of Tifton 85 bermudagrass at the moment of cut was 269.9 g kg^{-1} dry matter, 64.1 g kg^{-1} mineral matter, 133.9 g kg^{-1} crude protein, 705.9 g kg^{-1} neutral detergent fiber and 314.2 g kg^{-1} acid detergent fiber.

Table 1. Dry matter (DM), density, pH, and temperature of swine wastewater and ambient temperature at the site of production and the site of application in the rainy and dry seasons.

Rainy season					
Local	DM	Density	pH	Temp. (SW)	Temp. (ambient)
	%	g ml^{-1}		°C	
Affluent	0.46	0.94	8.20	25.40	34.00
Effluent	0.21	0.96	7.50	29.90	34.00
Pond	0.21	0.93	7.40	26.70	31.30
Dry season					
Local	DM	Density	pH	Temp. (SW)	Temp. (ambient)
	%	g ml^{-1}		°C	
Affluent	0.54	0.96	8.23	12.80	21.50
Effluent	0.27	0.96	7.84	18.60	21.00
Pond	0.16	0.95	8.14	15.00	18.80

The experimental design for the microbiological evaluation was completely randomized with subdivided plots and five replications, and the main plots were the area of the swine wastewater production, the biodigester outlet (effluent), the storage pond, the soil, residue of the plants on the soil surface (organic matter - OM) and the aerial parts (AP) of the grasses in the area of Tifton 85 production with subplots in the rainy season (RS) and dry season (DS). Microbiological analyses were carried out the day before the Tifton 85 grass was cut during two seasons, rainy and dry, after an average of 40 days of regrowth for the production of hay and haylage.

The preparation of the samples for microbiological analysis consisted of a previous dilution through the collection of 25 g of sample for soil, organic matter, and plant. The samples of affluent, effluent and storage pond were conditioned in sterile bottles, refrigerated, and transported to the laboratory when the initial dilution was obtained. Microbial populations were determined by selective culture techniques: 25 g of samples were added to 225 ml of sterile distilled water. From the solution obtained,1 ml was pipetted with dilutions ranging from 10^1 to 10^9 using test tubes for dilution water containing 9 ml of sterile distilled water.

The counts of molds and yeasts, *Clostridium, Bacillus* and enterobacteria were performed according to Beuchat and Cousin (2001), Labbe (2001), and Vanderzant and Splittstoesser et al. (1992), respectively, during the rainy season and the dry season. The results were obtained in selected dilution, and expressed as log of CFU g^{-1}. The molds

were isolated by inducing mycelial growth on potato dextrose agar medium through the induction of sporulation or by direct isolation of the spores (reproductive structures) of the pathogens from the collected samples (FERNANDEZ, 1993).

For the observation under a stereoscopic microscope (or a magnifying glass), semi-permanent slides of all the fungal structures, both in the symptomatic material and in the culture medium, were prepared. These structures were transferred onto a microscope slide using a needle or stylus, stained with lactophenol cotton blue, covered with a coverslip, sealed with enamel, and observed under an optical microscope for the identification of molds with the aid of specific identification keys (SAMSON et al., 1995).

Data were subjected to analysis of variance using the SISVAR program (FERREIRA, 1998), at 5% level of significance. When significant differences between the treatments for the variables under study were detected, they were compared by the Tukey's test at the same level of significance. The average values were calculated for the identification of mold genera, and subjected to descriptive analysis.

Results and Discussion

The populations of *Clostridium* and enterobacteria were larger in the affluent than in the effluent, storage pond, AP, OM, and soil ($P<0.05$) (Table 2). However, for *Bacillus,* the same did not occur. There was a gradual decrease in the microbial counts among the production site (affluent), biodigester outlet (effluent) and storage pond, which demonstrated that the SW sprayed on the soil (storage pond) had lower counts than the material in natura (affluent), thereby demonstrating the efficiency of the SW treatment. There was no decrease in microbial counts except for that of *Bacillus* in the dry season.

Schmidt et al. (2003) verified a lower diversity of microorganisms in the effluent than in the affluent, and demonstrated the tendency of decrease in the diversity of microorganisms throughout the manure treatment system. This lower population of microorganisms in the effluent possibly implies the disappearance of the bacteria that were which did not survive the different environmental conditions of the ponds (CHO; KIM, 2000).

Table 2. Population of bacteria in swine wastewater at the site of production, the site of application, during the rainy season and the dry season.

	Bacillus		*Clostridium*		Enterobacteria	
	Log CFU g^{-1}					
Local	RS	DS	RS	DS	RS	DS
Affluent	6,78bA	4,59bB	12,55aA	8,21aB	7,35aA	7,21aA
Effluent	7,98aA	4,60bB	10,73bA	6,77bB	4,89cB	5,77bA
Pond	5,72dA	4,73bB	10,72bA	6,18cB	4,85cA	4,62cB
AP	7,77aA	3,67cB	10,58bA	5,70dB	5,72bB	5,97bA
OM	5,84cdA	5,61aB	6,98cA	6,81bB	5,93bA	5,91bA
Soil	6,15cA	4,64bB	6,06dA	6,69bB	0,00dB	2,94dA
$CV(\%)_1$	2,31		2,06		2,51	
$CV(\%)_2$	2,80		2,27		3,66	

Values followed by the same letter, lowercase in the columns and uppercase in the line, do not differ by the Tukey's test at 5% level of significance.
CV(%)1: and CV(%)2: coefficients of variation of plot and subplot respectively
AP: aerial part, OM: organic matter, RS: rainy season, DS: dry season and CV: coefficient of variation.

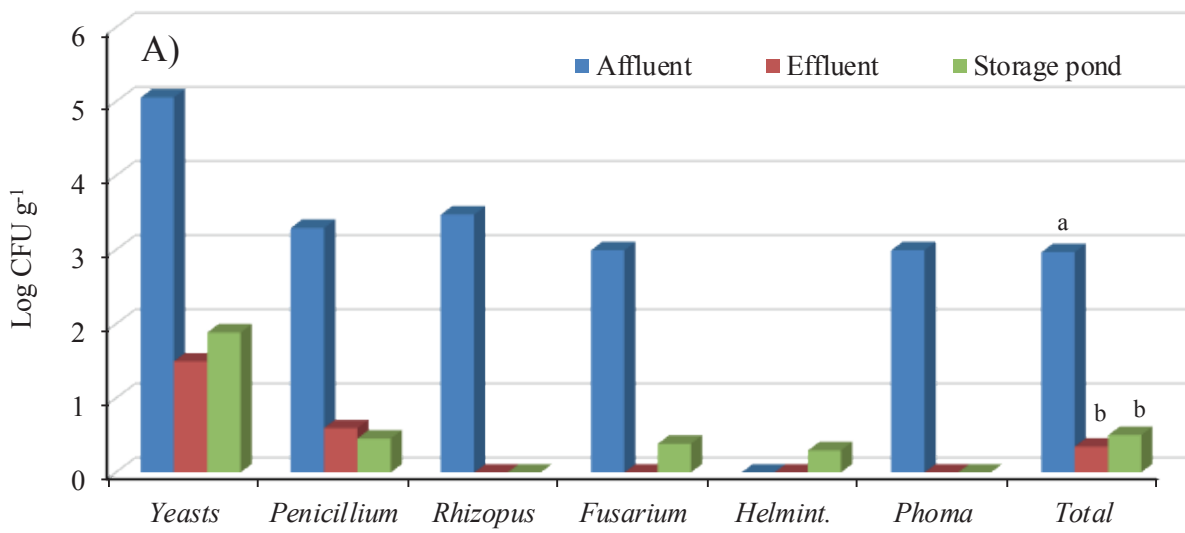
It was observed that larger populations of *Bacillus* and *Clostridium* were found in the affluent, effluent, storage pond, AP, OM and soil during the rainy season than during the dry season (Table 2), thus showing that high humidity and temperature could be the factors that influenced the growth of these microorganisms.

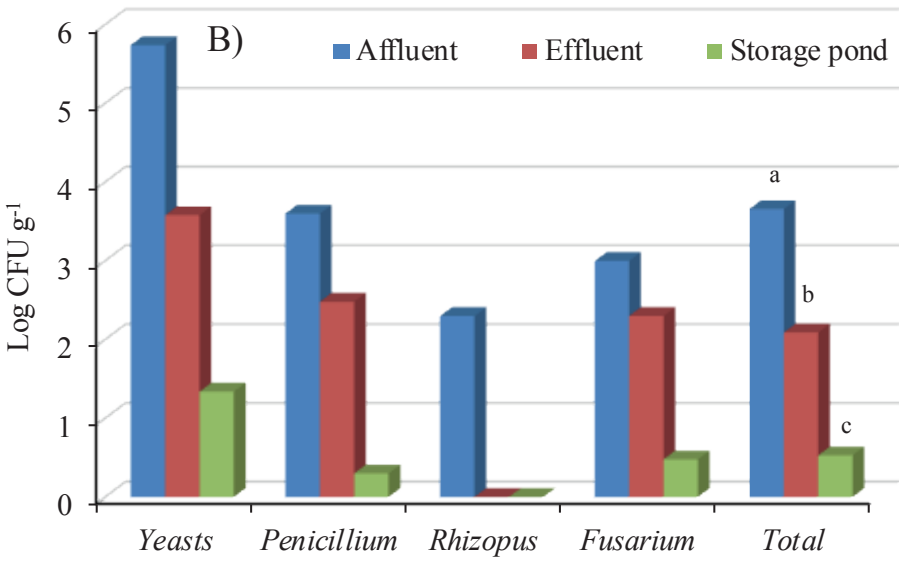
By comparing the values of SW in the storage pond and in the AP of Tifton 85 cultivation, it can be observed that the microbial load in SW remained on the aerial parts of the Tifton 85 grasses was able to increase according to the climatic conditions. Such an increase in the populations of *Bacillus* and *Clostridium* was observed, mainly during the rainy season (Figure 1a), when the temperature and relative humidity were more favorable to their growth. According to Blakeman (1981), most of the microorganisms present in the plants are found on the leaves at the base of the stem, where they are more protected from radiation, and humidity is higher.

In relation to the mold and yeast counts, the SW area was observed to exhibit various genera of molds, such as *Penicillium, Rhizopus, Fusarium, Helminthosporium*, and *Phoma,* in addition to a large population of yeasts, which favor the rapid decomposition of silage and haylage in particular (Figure 2).

In the areas of SW (affluent, effluent, and storage pond), larger populations were obtained in the affluent during the rainy and dry seasons, with a mean of 2.97 Log CFU g^{-1} and 3.66 Log CFU g^{-1}, respectively. There was a reduction in the counts and populations of the molds and yeasts because of the process of SW production, whereby they were reduced to less than 2 Log CFU g^{-1} in the effluent and 0.5 Log CFU g^{-1} in the storage pond. Despite the diversity of the molds found in SW, this indicated that the SW that was sprayed on the Tifton 85 grasses was not an initiator of the high microbial counts in hay, haylage or silage that would be produced subsequently. Rammer et al. (1997), and Anderson and Christie (1998) classified the risk of using liquid fertilizers for forage crops as low.

Figure 2. Molds and yeasts in the areas of swine wastewater production during the rainy season (A) and the dry season (B). Values followed by the same letter do not differ by the Tukey's test at 5% level of significance.



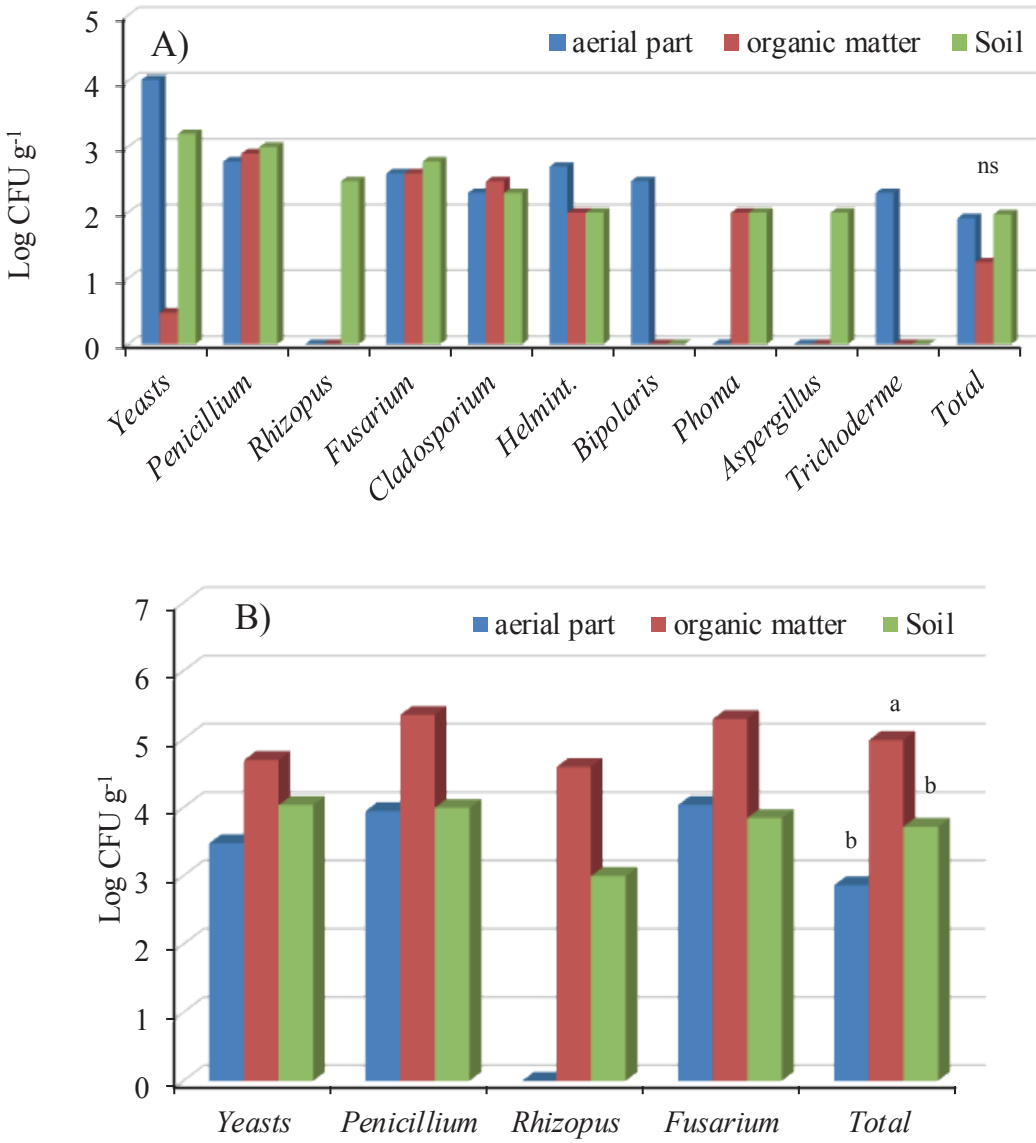


*Helmint.: Helminthosporium.

Nine genera of molds were isolated during the rainy season: *Penicillium* (16,89%), *Rhizopus* (4,83%), *Fusarium* (15,53%), *Cladosporium* (13,78%), *Helminthosporium* (13.04%), *Bipolaris* (4.83%), *Phoma* (7.78%), *Aspergillus* (3.89%), and *Trichoderma* (4.48%), in addition to yeast (14,98%) (Figure 3). Variations in the mold genera were observed between the dry and rainy seasons. However, *Penicillium, Rhizopus,* and *Fusarium* persisted during the dry season and the rainy season. Molds such as *Penicillium, Fusarium,* and *Aspergillus* favor the decomposition of hay bales, and pose a risk to animal health, mainly since they persist after days of storage. According to Belém (1994), these are the dominant mold genera that have a high ability to produce mycotoxins.

In the area of Tifton 85 production, including the AP of the grasses, OM and soil, no statistical difference (P>0.05) in the total mold and yeast counts during the rainy season was observed. However, during the dry season, there were higher counts of molds and yeasts in the OM than in the soil and the AP of Tifton 85 production (Figure 3). According to Mufatto et al. (2016), greater fungal population was observed in soil and dead plant material, predominantly *Penicillium* in all parts of the plant studied.

Figure 3. Molds and yeasts in the area of Tifton 85 grass production, aerial part, organic matter, and soil during the rainy season (A) and the dry season (B). Values followed by the same letter do not differ by the Tukey's test at 5% level of significance.



*Helmint.: Helminthosporium.

The most frequent mold genera in the soil were *Penicillium, Fusarium,* and *Rhizopus*. There is a great diversity of molds found in the soil, however, the most frequently isolated genera are *Mucor, Penicillium, Trichothecium,* and *Aspergillus,* followed by *Rhizopus, Zygorhynchus, Fusarium, Cephalosporium,* and *Verticillium* (ROITMAN et al., 1991).

Ames et al. (2014) obtained values lower than 30 CFU g^{-1}, and identified the mold genera *Penicillium, Cladosporium, Phoma, Fusarium* and *Diplococcium,* when they evaluated Tifton 85 before harvesting. Corroborating with the results obtained in this study on the aerial part of the Tifton 85 grasses. Similarly, Schocken-Iturrino et al. (2005) evaluated the genera *Aspergillus, Alternaria, Pithomyces, Curvularia, Fusarium, Cladosporium, Phoma, Epicoccum, Nigrospora,* and *Helminthosporium.*

Conclusions

The microbial count in the swine wastewater was reduced during the treatment process, with higher counts in the affluent, and lower counts in the effluent and storage pond. The biodigester treatment was efficient in reducing the SW microbial populations.

The populations of *Bacillus* and *Clostridium* were influenced by the season of the year, with larger populations in the rainy season (summer) than in the dry season (winter).

The mold genera that were identified in the area of SW production were *Penicillium, Rhizopus, Fusarium, Helminthosporium,* and *Phoma.* The genera *Penicillium, Rhizopus, Fusarium, Cladosporium, Helminthosporium, Bipolaris, Phoma, Aspergillus,* and *Trichoderma* were identified in the area of Tifton 85 cultivation.

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