

Review

Fertigation with domestic wastewater: Uses and implications

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The use of wastewater in agriculture is an alternative means of reducing wastewater release into water sources. This process, known as fertigation is an opportunity to make use of organic matter and other nutrients in wastewater for agricultural productivity. The presence of organic matter in these effluents serves as biofertilizer. Since 19th century in Britain, fertigation had been widely accepted due to the scarcity of water, the large amount of sewage produced and the concern about the final destination of wastewater. However, salinization, contamination of soil and agricultural products with the microbial pathogens and presence of heavy metals may limit the use of wastewaters in agricultural system. Thus, the plantation of agronomic varieties that do not have direct use as human food are optional cultivars, which may not have implications of fertigation with domestic wastewater. Fertigation with wastewater will be a nutritive support for the cultivation of *Brachiaria brizantha* cv Marandu in cerrado soil that is considered as low fertility soil. Soil microorganisms are fertility indicators. For example, nitrogen fixing bacteria (NFB) and arbuscular mycorrhizal fungi (AMF) has great potential to assist in the development of plant varieties under irrigation with domestic wastewater. These microorganisms are good parameters to be used in the analysis of changes that occur when sewage is applied into the soil. Furthermore, microbes contribute to the structuring of the soil, degradation of organic matter and nutrient availability to the biotic component of soil. Despite the limitations of fertigation with domestic sewage, it is a great alternative to use wastewater for nutritional enrichment to the soil that will be used for planting of agricultural crops of commercial interest.

Key words: *Brachiaria brizantha*, agriculture, salinization, diazotrophic bacteria, mycorrhizal fungi.

INTRODUCTION

Water is a natural resource that is essential to life, economic and social development. Although found in

abundance in the country, it has its quantity and quality compromised, especially in metropolises that generate

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Table 1. Vegetable Crops produced using wastewater from the sewage.

| Crops | Irrigation | Source |
|----------------------------------|----------------------|--|
| Olive | Wastewater | Petousi et al. (2015) |
| Melon | | Almeida Silva et al. (2012) |
| Sunflower | Domestic wastewater | Nobre et al. (2010), Gloaguen et al. (2010), Bezerra et al. (2014) |
| Mombaça grass | | Silva(2010) |
| Copaifera langsdorffii (Copaiba) | | Celentano Augusto et al.(2003) |
| <i>Eucalyptus grandis</i> | | Celentano Augusto et al. (2007) |
| Castor beans | | Ribeiro et al. (2012) |
| Corn | | Gloaguen et al. (2010), Nathan et al. (2012), Biswas et al. (2015), Malafaia et al. (2016) |
| <i>Amaranthus sp.</i> | | Silva et al. (2011) |
| <i>Jatropha curcas</i> | Santos et al. (2006) | |
| Cotton | Sewage treated | Sousa Neto et al. (2012) |
| Sugar cane | | Freitas et al. (2013) |
| Bean | | Rebouças et al. (2010) |
| Pumpkin | | Oliveira et al. (2013) |
| Cucumber | | Azevedo and Oliveira (2005) |
| Pepper | | Sousa et al. (2006) |

daily million per cube meters of wastewater. With the recent water crisis in some countries there is increase effort and investments towards the recovery of wastewater for direct consumption and other human activities. The sewage treatment has been seen as an extremely mature technology from the standpoint of human health and environmental impact (Batstone et al., 2015). Proper final destination and the treatment is a major concern of the individual, environmentalists and governments (von Sperling, 2005). Due to the large amount produced and its physical-chemical-biological properties, there is a need for appropriate treatment and disposal of the domestic sewage (Silva, 2010).

The use of wastewater in agriculture is an alternative way of preventing direct release of wastewater into the water bodies. This process has been called fertigation due to the presence of organic matter in effluents to be used as fertilizer (Rodrigues et al., 2009; Batstone et al., 2015). According to Silva et al. (2012), the fertigation is a technique of application of nutrients in the plant by irrigation water. In addition, the utilization of sewage in agriculture can minimize environmental damage caused by the release of these effluents into water bodies (Santos et al., 2006; Silva et al., 2012). Agriculture is the human activity that consumes more water on average 70% of all volume (Duarte et al., 2008, Almeida Silva et al., 2012, Christofidis, 2013, Alderson et al., 2015). In regions of dry climate, the irrigation uses up to 85% of water resources (Duarte et al., 2008, Paulino et al., 2011). In Brazil, this consumption is around 61%

(Paulino et al., 2011).

In relation to plant nutrition, several studies show positive effects of wastewater irrigation on the productivity of agronomic crops (Johns and McConchie, 1994; Al-Nakshabandi et al., 1997; Hespanhol, 2002; Silva et al., 2012; Biswas et al., 2015; Petousi et al., 2015; Malafaia et al., 2016). The importance of the sewage treated in the supply of nutrients compared with mineral fertilization has been shown by several authors (Table 1). However, the use of domestic wastewater for irrigation can cause physical, chemical and microbiological changes in soil; with contamination of agricultural products (Fonseca et al., 2007; Souza et al., 2011; Batista et al., 2010; Oliveira et al., 2014; Petousi et al., 2015). In this case, it would be of interest to use wastewater for the production of grasses; these are the crops that have no direct consumption for humans but ruminant animals.

The pasture is the main source of food for ruminants and more economical than the concentrated ones (Maranhão et al., 2010). But, the ecosystems of pastures are complex and have a number of biotic and abiotic components that interact with each other in different ways (Maranhão et al., 2010). Silva et al. (2012) showed that the productivity of the grass, *Panicum maximum*, increased with wastewater dose applied. According to them there was no change in the chemical composition of the grass irrigated with sewage when compared to control. The result of Silva et al. (2012) also shows the potential use of wastewater in fertigation of forage

intended for animal consumption. However, they did not analyze the profile of the microbial community before and after the application of sewage into the soil.

The microorganisms, especially nitrogen-fixing, nitrifying and solubilizing phosphates have great potential to assist in the development of grass under irrigation with domestic wastewater. Furthermore, these microorganisms contribute to soil structure and the availability of nutrients to the community of organisms living in the soil. Thus, studies of the microbial community may be an important factor to check the potential of fertigation damage with domestic wastewater.

A BRIEF HISTORY OF WASTEWATER REUSE IN AGRICULTURE

With the large increase in urban population, it has become a serious problem of environmental pollution by releasing untreated sewage directly into the water body. An alternative way to ease this problem is the incentive for the application of raw sewage in agriculture in rural areas that became known as sewage farms (Védry et al., 2001). In the late nineteenth century, there were about 50 of these farms in Britain (Santos, 2004). In the following century many projects of these farms were abandoned due to population growth and urbanization of rural areas (Silva, 2010). In US, the development of programs for wastewater reuse started in the first half of the twentieth century (Asano and Levine, 1996). In 1912, began the use of wastewater in ornamental ponds and also for fertigation of lawns (Tchobanoglous et al., 2003). In 1932, treatment plant of wastewater was built (Metcalf and Eddy, 1991). Also in 1960, it implemented the dual system of water supply, which supplied with wastewater the demand of irrigation of golf courses, parks, cemeteries and cleaning of public places (Metcalf and Eddy, 1991). In Brazil, the use of wastewater for irrigation is relatively recent; started with the application of vinasse in cane sugar plantation, around 1970. However, fertigated areas have grown a lot especially in regions producing fruits and vegetables (Lima et al., 2005).

Currently, available water resources are not sufficient to adequately supply the world's population. According to Rijsberman (2006), in the twentieth century, the world population has tripled while water consumption has increased in six-folds. In Brazil, water scarcity is quite visible, especially in the northeast region located in the semiarid. This region is characterized by presenting short rainy season, high temperature and high rate of evapotranspiration (Sousa Neto et al., 2012). In addition, the water crisis the country is facing should increase the use and treatment of domestic wastewater for various human activities, including direct consumption and agricultural uses. The largest water reservoirs intended for human consumption, are in recent years, below the level considered optimal and many Brazilian states are already using for human consumption the called dead

space.

The potable water used to address the basic needs of the world's population, is increasingly scarce; requiring management policies of water resources, to minimize the impact generated by human activities (Sousa Neto et al., 2012). It is estimated that two-thirds of the world population will be affected by water scarcity in the coming decades (Nobre et al., 2010; Alderson et al., 2015). In this context, the development of technology for wastewater reuse is very important.

COMPOSITION AND CHARACTERISTICS OF THE DOMESTIC SEWAGE

The domestic sewage mainly come from residences, commercial buildings, institutions or any building containing toilets, laundries and kitchens (Silva, 2010). It consists essentially of bath water, urine, feces, paper, food rest, soap, detergent and washing water of dishes and clothes (Jordão and Pessoa, 1995; Torres, 2004; von Sperling, 2005). These wastewaters are rich in nutrients with potential uses in irrigation (Alderson et al., 2015; Batastone et al., 2015; Kong et al., 2015; Malafaia et al., 2016). Furthermore, the characteristic of wastewater is function of the uses, which they are placed and vary with the climate, social and economic situation and people's habits (Kong et al., 2015).

The wastewater contains about 99.9% water. The remaining fraction comprises organic and inorganic solids as well as microorganisms (Santos et al., 2011; Batastone et al., 2015; Derry and Maheshwari, 2015). The organic substances found in wastewater consist of protein, carbohydrates, fats and oils, urea, surfactants, phenols and pesticides (Derry and Maheshwari, 2015; Petousi et al., 2015). Pesticides and other organic compounds used in agriculture are sources of pollution, toxicity to soil and aquatic microbiota (Felix et al., 2007).

The presence of nutrients such as nitrogen, phosphorus and potassium may be one of the great advantages of using wastewater in fertigation of agricultural crops (Batastone et al., 2015; Alderson et al., 2015; Malafaia et al., 2016). However, one of the major concerns is the application of excess nutrients and other contaminants into the soil. Mikkelsen et al. (1997) showed that the application of wastewater in soil for long periods may result in accumulation of excessive nutrients (eutrophication). Other potential risks to the environment are pollution of surface and groundwater, salinization, soil contamination with heavy metals and change displayed on the soil microbes (Gloaguen et al., 2010; Santos et al., 2011; Thongtha et al., 2014; Kong et al., 2015; Alderson et al., 2015; Petousi et al., 2015; Wang et al., 2015). These factors have negative effects on structure and macroporosity of soil, the microbial pathogens can also implicate the humans and animals (Alderson et al., 2015; Petousi et al., 2015). So, it is necessary to quantify the availability of nutrients in the soil, the nutritional

requirement for plants, the concentration of nutrients in the wastewater, and the effect on the microbial community that will contribute to soil fertility (Souza et al., 2011; Thongtha et al., 2014).

The inorganic matter of wastewater consists mainly of sand and minerals. The sand comes from washing water from the streets and underground waters (Jordão and Pessoa, 1995; Smith, 1996). Microorganisms found in sewage can be saprophyte, symbionts or parasites (Alderson et al., 2015; Derry and Maheshwari, 2015; Petousi et al., 2015). Some bacteria, viruses, protozoa and helminths are major groups of organisms of interest from the standpoint of public health (Sousa Neto et al., 2012). The origin of these pathogens in sewage is predominantly human, directly reflecting the health status of the population and the sanitation conditions of each region.

The coliform; *Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp. and *Citrobacter* sp. are indicators of faecal contamination, due to their presence in intestine of man and of other warm-blooded animals (Ribeiro et al., 2012; Petousi et al., 2015). This group of microorganisms is used to evaluate the quality of water intended for human consumption and to estimate the magnitude of pollution (Bitton, 1994). The determination of these microorganisms can be made by molecular biology technique, such as polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) and sequencing that exhibit a higher sensitivity when compared to culture techniques or the biochemical tests.

Therefore, the most aggravating in the domestic wastewater use in agriculture has been the health aspect. Such characteristics have limited the use of wastewater in agriculture (Sousa Neto et al., 2012). However, management and proper treatment of these waters can reduce these eminent risks. According to Rocha et al. (2003), after 54 days of application of treated sewage, fecal coliforms were not identified in the soil, and from 60 days, no positive sample with helminth eggs was found, despite the high level of initial contamination.

FERTIGATION WITH DOMESTIC WASTEWATER IN AGRICULTURE

As already mentioned, one of the great advantages of using domestic wastewater in fertigation for crop productions is the presence of macronutrients such as nitrogen, phosphorus and potassium (Pescod, 1992; Alderson et al., 2015; Batstone et al., 2015; Malafaia et al., 2016). According to Souza (2005), the application of wastewater into the soil improves the physical, chemical conditions and fertility. In addition, wastewater reuses in agriculture recycle the nutrients and reduce the use of chemical fertilizers. This is another advantage of fertigation when wastewater is used (van der Hoek et al.,

2002).

Nitrogen is the nutrient that is mostly required by plants. This element however is almost entirely complexed in organic form, depending on the soil microbial biomass for its transformation and uptake by plants (Jenkinson and Ladd, 1981; Feigin et al., 1991). This biomass is susceptible to changes that occur in the soil and therefore act as a good indicator of the soil (Jackson et al., 2003).

The source of nitrogen of the soil is degradation and mineralization of organic matter, fertilizers and biological fixation of atmospheric nitrogen (Bloom, 2015). Most of the biologically fixed nitrogen comes from symbiosis plants and rhizobacteria, especially the family Rhizobiaceae including *Rhizobium* sp., *Mesorhizobium* sp., *Allorhizobium* sp., *Azorhizobium* sp. and *Bradyrhizobium* sp. (Vance, 1998; Rodrigues et al., 2013). This process has been used to evaluate the effect and application of sewage sludge in soil (Vieira, 2001; Vieira et al., 2004). Moreover, most of the nitrogen in wastewater is in organic form, which can be mineralized into soil by microbial enzymes with the liberation of ammonium and nitrate (Santos et al., 2006). These ions are then absorbed by plants or used by the soil organisms for the production of biomolecules.

Another nutrient of wastewater that benefits crop production is phosphorus (Thongtha et al., 2014; Batstone et al., 2015). Excessive amount of phosphate fertilizers on certain crops causes a reduction in productivity due to nutritional imbalance, because excess of phosphorus can reduce the availability of micronutrients such as copper, iron and zinc. Furthermore, high concentration of phosphorus can increase the precipitation of calcium in the effluent thereby increasing the sodium adsorption ratio (Feigin et al., 1991). The mycorrhizal fungi make phosphorus more available to the plants (Smith et al., 2010). This microorganism is a good indicator in the application of wastewater into the soil.

Several agronomic plants have been grown using fertigation with domestic wastewater (Table 1). However, the Mombaça grass (*Panicum maximum* cv. mombaça), castor, jatropha, eucalyptus and cotton produced by the fertigation process and there is no implication recorded, since they are not directly used for human food. Thus, the possible risks of contamination by pathogenic microorganisms of the wastewater are minimal or nonexistent.

Therefore, the wastewater is important sources of nutrients required by crops and studies have shown a productivity increase in beans and fruit trees (Oliveira et al., 2014). According to these authors, the use of wastewater in fertigation brings benefits to the environment and to farmers by reducing the costs of fertilizer application. Moreover, Silva et al. (2012) showed increased Mombaça grass productivity in function of the sewage dose applied. The results of this study are

interesting because it uses an agronomic crop that is not indirectly involved in human food and shows the application of fertigation in the soil of the cerrado.

PHYSICAL, CHEMICAL AND BIOLOGICAL RISKS OF IRRIGATION WITH WASTEWATER

One of the possible impacts of the agricultural use of wastewater can occur in groundwater (Silva et al., 2012). In arid areas with permeable soils and shallow, sewage treatment by disposal in soil, or even fertigation with wastewater can cause increases in groundwater level, contamination of these waters with pathogenic microorganisms, besides salinization/ sodification of soil, which has been a challenge (Fonseca et al., 2001; Gloaguen et al., 2010).

The effects of the wastewater application on soil chemical properties are pronounced only in the long term and depend on the physical composition, soil chemistry and of the climate conditions (Silva, 2010; Silva et al., 2012). Gloaguen et al. (2010) through a multivariate analysis established an inter-relationship between the negative effects of using wastewater for irrigation. According to them, the irrigation with treated wastewater is a technique used in agriculture to reduce water needs and the impact of nutrient loading in the freshwaters, but several negative effects have been recorded in the chemical properties (salinization/ sodification) and physical properties (changes in porosity and soil hydraulic conductivity) of soil. Their analyzes revealed six key factors such as, fine porosity (consisting of Na^+ and K^+), high porosity, ability of soil cation exchange (Ca^{2+} and Mg^{2+}), pH and texture of soil. The main conclusion of these authors was that the change of the distribution of soil pores (fine pores to large pores) caused by the application of wastewater leads to an increase of the water storage in soil and reduced drainage of salts. This finding confirms the salinization of the soil after use of wastewater and shows the ability of soil to retain water. So the most negative effect caused by the use of wastewater is the increase in salinization/sodification of soil that in long-term cause decreases in crop productivity. In addition, the quality of wastewater, the hydraulic conductivity of the soil, the organic matter content, soil drainage, the intervals between applications and the depth of the groundwater are also factors that determine soil salinity rate (WHO, 2004). The sodium adsorption in the soil particles can lead to colloidal dispersion and reduction in pore size (Gloaguen et al., 2010). However, this negative effect of sodium only occur if the concentration of Na^+ is higher than that of Ca^{2+} and Mg^{2+} and the electric conductivity of the soil saturation is very low (Silva, 2010). Furthermore, sodium or others salinization sources are persistent and usually their removal requires the use of treatment systems with high costs, as for example, cation exchange resins and reverse osmosis membranes (Toze, 2006). Wang et al.

(2015) showed that deionization capacity may be an alternative technique for desalination.

The management is also very important to prevent salinization of the soil. Silva (2010) and Silva et al. (2012) grow the *Panicum maximum* grass with different concentrations of sodium (75, 150, 225 and 300 kg ha⁻¹). According to them, sodium accumulation in leaf was proportional to the dose applied to the soil, and the cutting period. Their result shows that the sodium accumulation in the soil depends on the applied dose and of the plant. Thus, determination of soil holding capacity and absorption of sodium by microorganisms and plants are fundamental to the success of fertigation with sewage. In addition, recent studies have worried more in determining the microbial contamination of soil and agricultural product by pathogens contained in wastewater that with salinization/sodification (Alderson et al., 2015; Derry and Maheshwari, 2015; Petousi et al., 2015). However, the absence of microbial contamination was detected in the leaves and fruit of olive trees irrigated with domestic wastewater (Petousi et al., 2015).

Eutrophication is a pollution caused by excessive nutrient and biomass (Posadas et al., 2015; Kong et al., 2015). This anthropogenic contamination may be due to disposal of especially phosphorus in domestic sewage (Thongtha et al., 2014; Kong et al., 2015). Petousi et al. (2015) showed that there is a nutrient accumulation, particularly phosphorus and potassium, and salt in the soil after two years of application of sewage for irrigation olive trees. The high content of phosphorus causes serious problems in ponds, rivers and seas (Wang and Wang, 2009). Alternatively, to avoid the accumulation of nutrients in the soil, Thongtha et al. (2014) studied the ability of *Nelumbo nucifera* and *Cyperus alternifolius* plants and soil microorganisms to remove different phosphorus concentrations of the domestic sewage. They concluded that the phosphorus was absorbed from the soil by plants and by the community of microorganisms, particularly *Pseudomonas* sp. So, eutrophication can be ameliorated by local microbial community associated with phytoremediation. The phosphorous removal capacity and nitrogen from wastewater by microorganisms community has also been described in Kong et al. (2015).

Heavy metals such as Cd, Cu, Ni, Pb, As and Cr represent potential risk to living beings (Burchill et al., 1981). The accumulation of these metals can be observed after application of wastewater into the ground. However, the contamination by heavy metals can be removed also by plants (Alderson et al., 2015). Nathan et al. (2012) showed the capacity of *Zea mays*, *Commelina bengelensis*, *Helianthus annuuse*, and *Amaranthus hybridus* in bioaccumulation of Pb, Cu, Cd and Zn contained in wastewater. According to these authors, *H. annuuse* and *C. bengelensis* has the potential to remove Pb, Cu and Cd, while *Z. mays* and *A. hybridus* better absorb the zinc. Thus, these heavy metals may be

accumulated in food.

Therefore, due to these (mainly culture contamination with heavy metals and pathogens) the fertigation with domestic wastewater would be more interesting with cultivation of agronomic variety that not are used in human food. Our research group is developing the project of planting of *Brachiaria brizantha* grass in cerrado soil using domestic wastewater (item 7).

RISKS OF THE IRRIGATION WITH WASTEWATER ON SOIL MICROBIAL COMMUNITY

The soil microbiota is mainly responsible for the decomposition of organic waste, cycling of nutrients and the energy flow within the soil (Vitousek et al., 2002; Gama-Rodriguez et al., 2005). Thus, it would be important to analyze the effect of using wastewater in irrigation on microorganisms that contribute to soil fertility. The arbuscular mycorrhizal fungi (AMF), the nitrogen-fixing bacteria, including the group of actinomycetes may be a good parameter to investigate the possible changes. In addition, these microorganisms can help to prevent the accumulation of nutrients in the soil, including phosphorus, nitrogen and heavy metals coming from domestic residual water

The AMFs are important component of the rhizosphere microorganisms in natural ecosystems (Verma et al., 2008). They are distributed in various habitats and forming mutualistic associations with extensive number of agricultural plant species and forest (Azcón-Aguilar et al., 1997; Oehl et al., 2011). This mutualistic symbiotic association formed between AMFs and plant roots is present in nature for over 400 million years and occurs in two-way transfer of substances (Pozo and Azcón-Aguilar, 2007; Smith et al., 2010). The AMF absorb the mineral nutrients such as phosphorus, zinc and copper ground water and share with the plant (Gosling et al., 2006; Smith et al., 2010). In exchange the plant provides carbohydrates from photosynthesis for AMF (Smith et al., 2010).

The AMFs contributes to the adaptation of the plants in different ecosystems, increases tolerance of plant to biotic and abiotic stress and, increase the uptake of water and nutrients by the plant (Taylor et al., 2001; Moraes et al., 2004; Berbara et al., 2006). In addition, these fungi contributed to the increased density of nitrogen-fixing bacteria in the rhizosphere, produce siderophores, increased the plant's resistance to heavy metals and reduce the population of nematodes in mycorrhizosphere (Neeraj, 2011). The AMFs are in phylum: Glomeromycota with seven genera: *Acaulospora*, *Archaeospora*, *Entrophospora*, *Glomus*, *Gigaspora*, *Paraglomus* and *Scutellospora* and about 210 species have already described in Invam (2015).

The traditional method used for the taxonomic and classification of AMF species is the comparison between

the morphological characteristics of the extracted soil spores (Moreira et al., 2007). However, analysis based only on the identification of such spores may be limited due to sporulation and be dependent on the particular characteristics of each type and their interaction with the environment; the spores obtained from a field sample may be in the decomposition process and these fungi are biotrophic (Rodríguez-Echeverría and Freitas, 2006). Thus, the use of molecular biology techniques has been increasingly employed for the identification of these species (Kramadibrata et al., 2000). This technique analyzes the AMF community in the soils and avoids the steps of cultivation and production of spores in trap crop (Kowalchuk et al., 2002). The DGGE has been used for analysis of AMF community. This technique allows the identification of these fungi in the root systems of plants, in soil samples or spores. Furthermore, the method has been tested on range of AMF isolates with ability to distinguish between the different species (Kowalchuk et al., 2002).

Microorganisms play an important role in plant growth and in soil fertility. They assist in decomposition, mineralization of organic compounds, assimilation and fixation of nitrogen (Vitousek et al., 2002; Gama-Rodriguez et al., 2005). Moreover, nitrogen of the microbial biomass is considered a readily available fraction to the plant (Gama-Rodrigues et al., 2005). Bacteria that fix nitrogen are called bacteria diazotrophic. They release large proportion of nitrogen into the soil rhizosphere (Wartiainen et al., 2008). The BNF is characterized by the reduction of atmospheric nitrogen to ammonia nitrogen. The process is performed by a variety of prokaryotic microorganisms. It is a process with high energy cost, sensitive to the presence of oxygen and has regulating post-transcriptional and transcriptional (Jenkins and Zehr, 2008; Hong et al., 2012). These microorganisms possess the nitrogenase enzyme encoded by *nif* genes that are located in the operon *nifHDK* (Zehr et al., 2003). Due to the presence of organism in most biological systems capable of fixing atmospheric nitrogen and containing highly conserved regions, the *nifH* gene has long been used in studies related to diazotrophic bacteria. Moreover, it has been reported that the phylogenetic analyzes of this gene are consistent with the phylogeny of the 16S rRNA (Zehr et al., 2003).

The diazotrophic bacteria can be epiphytic. Some strains of *Azospirillum* spp. live on the surfaces of roots, or endophytic such as *Azospirillum diazotrophicus*, *Azoarcus* spp., *Herbaspirillum* spp. and *Azospirillum brasilense* (James, 2000). These bacteria can also release compounds such as indole acetic acid which stimulates root growth, spore germination and the mycelial growth of mycorrhizal fungi (Vázquez et al., 2000). The actinomycetes belong to the phylum Actinobacteria and comprise the group of organisms with high content of guanine and cytosine in DNA

(Stackebrandt et al., 1997). The main representatives of this group are *Arthrobacter* sp., *Corynebacterium* sp., *Nocardia* sp., *Rhodococcus* sp., *Streptomyces* sp. and *Mycobacterium* sp. They are known as slow growing bacteria but produce antibiotics and fix atmospheric nitrogen (Dunbar et al., 2002).

The morphological and physiological analysis of these microorganisms present limitations; studies on these microorganisms must be carried out in combination with molecular biology techniques that allow for studying the microbial community including biotrophic microorganisms. The PCR-DGGE technique is currently a routine and reliable method for a quick analysis of microbial communities in the soil. It allows the comparison of large number of samples from different treatments (Van Elsas and Boersma, 2011). This technique can be used to monitor changes in the microbial community structure in response to environmental changes (Nicolaisen and Ramsing, 2002). The study of the microbial community may be performed by DGGE using markers such as 16S rDNA or 18S rDNA to evaluate the community of bacteria and fungi, or by using marker of functional genes for example, the *nifH* gene (Liang et al., 2008; Van Elsas and Boersma, 2011).

These molecular techniques are very important tools for the study of microbial community. Furthermore, analysis of the diversity of microorganisms can be used as an indicator of the quality of the soil because of their quick response to environmental changes (Tiedje et al., 2001). Thus, these techniques can contribute to the study of diazotrophic bacterial and AMF in the soil that will be used for *B. brizantha* planting fertirrigated with wastewater from sewage (item 7).

FERTIGATION OF GRASS IN THE CERRADO REGION

The application of fertigation with wastewater from sewage can be a good alternative for *B. brizantha* planting in soil of the cerrado. This process can reduce the use of drinking water for irrigation, application of fertilizers and deforestation for planting pastures. Silva (2010) showed the potential of irrigation with domestic wastewater in planting Mombaça grass in cerrado soil. According to him, the fertirrigated grass with wastewater had higher productivity than the control. The Cerrado region was marked by large investments for training pastures in the 1970s (Eiten and Goodland, 1979; Andrade et al., 2005). The degradation and abandonment of these areas favored the establishment of invasive plant species. Research shows that 70% of Brazilian pastures are in the process of degradation, mainly in the cerrado, which affects the productivity and sustainability of the national livestock. At the end of the first decade of this century, this degradation was around 70 million hectares (FAO, 2008). Thus, the development of technologies to recover areas of abandoned and degraded pastures such

as the reuse of wastewater can be an alternative to minimize the deforestation (Paulino et al., 2011). Besides to the low fertility, the cerrado soils are characterized by having high aluminum content and exchangeable manganese, low ability to retain potassium-based fertilizers and micronutrient deficiencies such as B, Cu and Zn (Malavolta and Kliemann, 1985). Furthermore, there is low level of organic matter in Cerrado soil (Adámoli et al., 1985). In this case, the nutrients in the wastewater may become available for plant growth in this type of soil. Thus, the use of wastewater for planting *B. brizantha* seems like a good alternative for the production of animal feed in the degraded cerrado soils. In addition, it reduces the use of drinking water for the same purpose.

CONCLUSION

Due to the recent water crisis in Brazil and the large amount of degraded soils, fertigation may be a viable alternative for wastewater reuse and recovery of soils. This recovery can be due to the nutrients of sewage that support the plant growth. Hence, further study will aim to produce *B. brizantha* in the cerrado soil using domestic wastewater for irrigation.

Conflict of Interests

The authors have not declared any conflict of interests.

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