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Composting of Pig Effluent as a Proposal for the Treatment of Veterinary Drugs

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Abstract

Pig farming currently occupies a prominent place in the southern states of the Brazil, owning approximately 50% of the national squad, estimated at 42 million pig heads. However, the swine activity contributes significantly to the generation of environmental impacts on the environment. Recently, the greatest need for animal protein has exerted pressures on the current animal production system and one of the alternatives has been to the use of veterinary medicines, which have several uses ranging from therapeutic use, preventive in the treatment of various diseases and as growth promoters. Its indiscriminate and uncontrolled use is currently endangering the environmental balance of producing sites through effluent contamination. Many producers have been using contaminated slurry as a biofertilizer. In this sense, further studies on techniques and processes of treatment of organic effluents contaminated by veterinary drugs are necessary. Alternative low-cost and environmentally viable treatment systems are needed to minimize the entry into the environment of these contaminants. Therefore, the composting process that can be defined as a process of aerobic microbial decomposition of organic matter and nutrient recycling can be an alternative for the treatment of effluents contaminated by veterinary drugs.

Keywords: antibiotics, contamination, impacts, human health, resistance, resistance

1. Introduction (Composting)

The composting technique emerged around the year 1920, when a researcher named Albert Howard developed the process in India [1]. This process happens naturally next to the environment, where the biological degradation of the compounds occurs [2], this technique can still be characterized as a process of treatment of different types of residues and origins, among them (urban, industrial, forestry and agricultural), where a diverse population of microorganisms (bacteria, fungi) act [3].

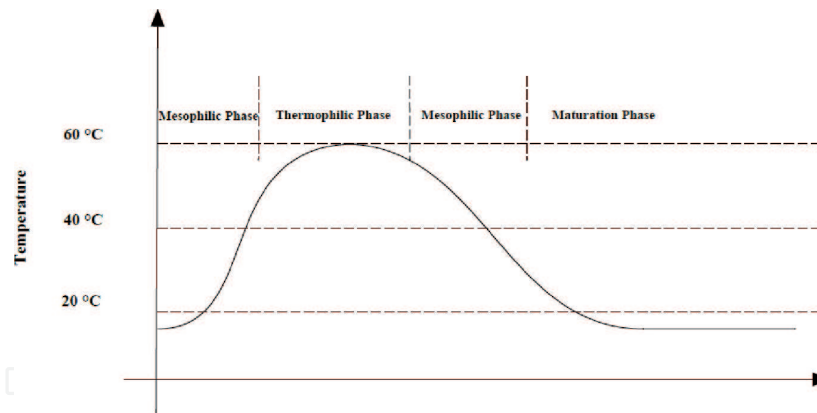


Figure 1. Composting phases according to the temperature in the system. Source: Adapted from [3].

During the composting process, four fundamental stages for the biodegradation of the compounds occur: initial mesophilic phase, thermophilic, mesophilic cooling and maturation. In the initial phase, mesophilic, the decomposition process accelerated by the gradual increase of existing microorganisms, as well as the increase in temperature. In the thermophilic phase, the temperature of the composting system can reach more than 60°C, resulting from the action and thermophilic microorganisms, generating heat and water vapor. In the mesophilic phase of cooling, the most resistant compounds degraded by mesophilic microorganisms, which after this degradation eventually decrease their activity. Finally, the maturation phase, where the decomposition is low or zero and where the humic acids are released, generating by end, a stabilized compound [1]. The temperature profile during the composting phases are showing in **Figure 1**.

During the composting phases, CO₂ detachment and heat release occur in the composted material (mesophilic phase), reaching the temperature peaks, where the vapor (thermophilic phase) is generated, which also contributes to inertization in the system, all based on the metabolism of microorganisms [2].

Composting has several benefits for the management of waste produced by pig producers, as it can eliminate up to 90% of the effluent volume [4]. The process also has numerous advantages: minimizes the generation of greenhouse gases, reduces the proliferation of vectors, reduces odors, has environmental technical feasibility, elimination of costs for disposal and transport of waste, technical feasibility to expand the current pig production systems [2–5].

2. Physical-chemical composting parameters

For the composting process to succeed, some aspects such as heat transfer, air-flow, steam and finally moisture balance must be observed [6]. The main parameters to be evaluated during the composting process are: oxygen/aesthetics; pH; Moisture content; Temperature; C/N ratio, in addition to the presence of microorganisms [6].

Oxygen is directly interconnected with microbial activity, due to the composting process, being aerobic. The obtaining of oxygen from the composting system can be obtained by mechanical, physical or even forced aeration [7]. Its consumption and supply is tied, the humidity of the composting system, having optimum humidity in the range of 55%. The higher the microbial activity, the higher the demand for oxygen and the lower the humidity in the composting system [7]. Also for the author, systems with humidity below 40%, can inhibit the activity of bacteria, predominantly the higher activity of fungi.

The aeration has equal importance in the process, because with greater revolving and aeration of the system, the rate of decomposition of organic matter is increased. In his experiment of mechanized composting [5], he made the aeration during the injection of effluents in the trees and after 2 days, where he made only the revolving of organic matter.

Moisture in the composting system is of fundamental importance, being correlated with the rate of decomposition of organic matter by aerobic microorganisms. As already mentioned above, the ideal rate varies between 50% [3], and 55% [7]. Its control can be established by revolving, because at high humidity, anaerobiosis may occur in the system [3]. This, can also be controlled by the relationship between effluent injection and the amount of dry mass (DM). The author [5] used an initial rate of 1.47 liters of scum per kg (DM) in the first days up to a rate of 0.21 liters of scum per kg (DM) at the end of the experiment. Other aspects need to be observed, which are the phases of the composting process, mesophilic and thermophilic phase, both related to the elevation and decrease of temperature and water evaporation.

The state of organic matter decomposition can be measured from the hydrogenic potential (pH) found in the composting system, because for each phase of the process, the pH variation will occur. The initial pH values can be in the order of 4–5 [7], this due to the release of mineral acids and carbonic gas, where at this time the bacteria acidophilus and fungi act in the decomposition of cellulose [3]. Subsequently, the pH can reach values between 7 and 12, due to the formation of organic acids by alkalophilic bacteria [7], when there is stabilization of the compound [3].

With the ease of measuring throughout the process, the temperature profile plays a crucial role in composting. The temperature can range from 10–70°C, mesophilic phase and thermophilic respectively [3]. Its elevation and decrease linked with humidity and microbial activity, as demonstrated in the temperature profile in **Figure 1**.

Another important role of this parameter is the inertization and maturation of the compound over time. Pathogen microorganisms have their inactivation from three days at temperatures higher than 55°C [8], but in composting cells where revolving often occurs; the minimum is 15 days with temperatures above 55°C [4]. Aspects such as raw material, composting system configuration, presence of microorganisms, moisture, oxygen, directly affect temperature. Still the temperature can define the amount of revolving of the composting system. In his work [1], he observed the temperature variation in a process of composting of cattle slaughter residues, and in another study, [4], in a process of composting of pig effluents; both studies presented maximum temperatures did not exceed 55°C. In relation to **Figure 3(a)**, it is also noted that when revolving the composting system, the internal temperature increases significantly. Temperature profiles vary from research to research, [5] observed 72°C in the composting system at 22 days of experiment, [2], observed values not exceeding 52°C, already [9], obtained the maximum temperature of 61°C.

To prepare the composting system it is necessary to note the Carbon/Nitrogen ratio. Both compounds are important in the process, where carbon acts as an energy source and nitrogen as a respirometric source of microorganisms [3], also acting, in cell growth, formation of proteins, amino acids and nucleic acids [2]. The C/N ratio has been observed by several authors [1–4, 6, 9–11], who evaluated that this ratio should not exceed 30/1 in the initial phase, because in high relationships, the degradation of the compounds is delayed, already at the end, when the compound is matured, the ratio may reach 10/1. In this process, much of the carbon is released into the atmosphere in the form of CO₂ [6], and nitrogen can be released, when the ratio is low, in the form of ammonia, characterizing bad odor [2].

3. Use of veterinary drugs

The indiscriminate use of veterinary medicinal products in animal husbandry, especially in pig farming, has become the gateway of these pollutants to the environment [12]. Antibiotics used since prevention, therapeutic use, helping in the treatment of diseases such as infections, diarrhea, still being able to act as growth promoters [13–16]. Currently, there is a great concern in the academic community, with residues derived from veterinary drugs, due to their potential contaminant, but also, by the non-absorption completely, by the animal organism. Several authors, such as [15–19] point out that on average 60% of the veterinary medicinal product dosed to animals are excreted through urine and feces [20], and the group of drugs most commonly used today are antibiotics [15, 21], and among them, tetracyclines, sulfonamides, lincosamides, β -lactamines and macrolides [22].

After their non-absorption by the body, veterinary drugs can reach soil and water resources [13, 16, 22] as unaltered substances or metabolites. The use of pig effluents as fertilizer may be emphasizing the spread and increase of antibiotic residues in the environment [12, 20]. Several studies have proven environmental contamination around the world through antibiotics, such as tetracycline in Brazil, Canada and China, [20, 23, 24], by sulfonamides in Bolivia, Czech Republic and USA, [25–27], macrolides in South Korea, United Kingdom and Hong Kong [28–30], fluroquinolones in Poland, Austria and Thailand [31–33], and kilane in China and Malaysia [25, 34].

In addition, to soil contamination by heavy metals [35], contamination of water resources [36], the application of biofertilizers, directly or indirectly in the soil [34], has caused change in the soil biotic community [37]. Another point that draws attention, and more significant is the resistance of microorganisms to antibiotics, several authors have found evidence of the resistance of microorganisms [38–42] thus enhancing the risk to human health [34, 38, 43].

The availability and use of pig effluent are justified by the size of the production chain. Brazil is among the four largest producers of pigs in the world, behind China, the United States and the European Union, with an estimated herd of 42 million heads, representing US\$ 1 billion annually in meat sales [44, 45]. The Southern Region of the country, where the states of Paraná, Santa Catarina and Rio Grande do Sul are located, account for 49.3% of the national production [46]. With a well-established production chain, a large amount of waste generated. Point out that a pig in the finishing phase can produce up to 7.6 liters of manure/day [47], often causing failures and overload in effluent treatment systems, which mostly treated by biological ponds.

Many of these existing environmental problems and pressures are due to traditional organic effluent treatment systems, widespread in pig-producing units, which are not efficient in the treatment of these pollutants [48]. Compliance with environmental laws, as well as the feasibility of managing waste produced in farms, generated the need for alternatives for the treatment of effluents.

With this, composting emerged as a treatment proposal, which is a natural process of nutrient recycling, through aerobic microbial decomposition of organic matter [4], under favorable conditions of temperature, pH, oxygen, humidity, presence of chemical substances, raw material and C/N ratio [3, 47], resulting in a material with relative stability and quality [49]. The advantages range from minimizing the volume of effluents of about 90% [4], reducing the emission of greenhouse gases and the proliferation of vectors. Another important factor is the technical feasibility, to expand the current pig production systems [2–5], showing be a practical proposal, low cost [23, 25, 50], still classified as a clean and viable method [28], for the correct management of waste. Another advantage is the inactivation and immobilization of pathogens, nutrients and veterinary drugs [13, 48, 51, 52], thus becoming a potential proposal for the treatment of veterinary antibiotics [23, 25, 48, 53].

Studies indicate that composting has potential in the treatment and decay of drug concentrations [3, 13, 48, 49, 51, 54–56]. Antibiotic residues such as florfenicol, sulfadimetoxin, sulfametazin and tylosin reached 95–99% decline in 21 days of composting [51]. Tetracycline, sulfonamides and macrolides had 99%, 96% and 95% decaine, respectively, through composting [53] in 35 days of testing. [48], they did not detect the presence of a group of sulfonamides (Sulfametazin (SMZ) and Sulfamethoxazole (SMX)) in the final compound.

4. Composting as a proposal for treatment of swine effluents

Technologies that seek to reduce residues of veterinary medicinal products (RMV), mainly veterinary antibiotics (AVs) found in organic and industrial effluents disposed as fertilizer in the soil is a necessity to minimize the environmental impacts generated by these compounds [25]. Traditional organic effluent treatment systems, widespread in pig-producing units, are not efficient in the treatment of these pollutants [48].

Among the various technologies and treatment systems for different origins and compositions of organic effluents, including pigs, composting has been shown to be a practical proposal, low cost [23, 25, 50], classified as a clean and viable method [28] for the correct management of waste. This technique can be developed as an alternative for the treatment of effluents in small properties, located in regions with high concentration of pigs and with little agricultural area available for final disposal [4], as well as proposal for treatment of veterinary antibiotics [23, 25, 48, 53].

Composting can be defined as a process of aerobic microbial decomposition of organic matter, being a natural process of nutrient recycling, used since ancient civilizations [4], under favorable conditions of temperature, pH, oxygen, humidity [47], presence of chemicals, raw material and C/N ratio [3], resulting in a material with relative stability and quality [49]. Treatment by composting reduces the volume of effluents, inactivates and immobilizes pathogens, nutrients and veterinary drugs [13, 48, 51, 52], and finally produces a by-product (substrate), with economic and agronomic value [49, 50]. **Figure 2**, shows the cycle of inputs and outputs during the composting process.

This treatment proposal has been shown to be effective in the management of organic waste from production processes confined to pigs, poultry and cattle, and has the potential to treat emerging organic pollutants (POEs) [57]. The decay of the concentration of veterinary medicinal products through composting has been researched by several authors [13, 48, 49, 51, 54–56], for different types of effluents and organic residues.

The decline of 27% OF CTC was observed in swine effluents [57] and 92% in poultry manure in a composting system for 42 days. When analyzing the decline of

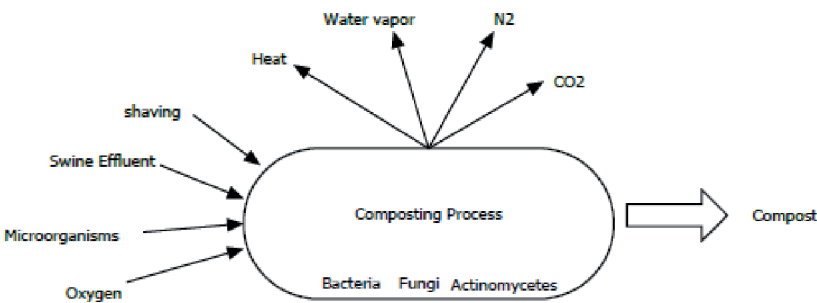


Figure 2.
Flow of entry and exits in the composting process. Source: Author (2017).



Figure 3. Mechanized composting system of swine effluents (a) [58], shaving bed after effluent injection and revolving (b) municipality of Concordia, SC.

4 antibiotics (florfenicol, sulfadimetoxin, sulfametazin and tylosin) [51] during the composting process of domestic effluents, approximately 95–99% of antibiotics were degraded after 21 days of testing. Antibiotic decline [53] was evaluated (tetracycline 96%, 99% sulfonamides and macrolides 95%) during the composting process, [48], after 35 days of bench-scale composting, they did not detect the presence of antibiotics from the Sulfonamide Group (Sulfametazin (SMZ) and Sulfametoxazole (SMX)).

Figure 3 shows one of the processes of composting of existing pig effluents, the mechanized one, which consists of mixing the waste produced by pigs in the rearing systems, with shavings, sawdust or straw in beds/beds [50].

However, this process as a proposal for the treatment of veterinary medicines of different classes, has yet been developed in the country, justifying the proposal, having presented good results in research already developed, its application becomes important in the search for new alternatives to minimize the potential environmental risks caused by these contaminants, since in contact with environmental matrices can be accumulated in the soil, as well as being leached for water resources [56].

5. Use of composting in the treatment of veterinary drugs

One of the determining points for the development of research and its scientific relevance is the potential for contamination by veterinary antibiotics. Currently the pig production chain in the south of the country is estimated at 20.5 million heads. Considering only the state of Rio Grande do Sul (7 million heads), and assuming that the main group of antibiotics, tetracycline [15, 21], which is given in the order of 400 mg/animal/80 kg. The medicated with the main antibiotic group, and 70% of the dose is excreted by urine and feces [18]. If this residue is deposited in current treatment systems, which can reduce its concentration by 50%, this would result in 0.140 g/tetracycline/animal, representing 0.98 tons of antibiotics that would be dumped into the soil annually along the effluent in the form of biofertilizer [59].

The search for technical alternatives for the treatment of pig manure contaminated with residues of veterinary drugs was decisive for the accomplishment of the

work, considering the size of the production chain in the country, due to the lack of research at the national level, but mainly in minimizing the potential damage, they can cause to the environment. The results observed in the research point a potential for chronic contamination and disturbances at the environmental level, but also at the social level, very expressive, but also point to the need for research aimed at the search for technological alternatives for the treatment of these residues, often left aside.

Based on the results obtained [58], it was observed that composting proved to be effective in the degradation of 19 veterinary drugs, divided into 8 groups. The decay/degradation rate ranged from 33.7–100% in 150 days. The antibiotics sulfatiazole, tetracycline and chlortetracycline showed 100% decay. The mean degradation of antibiotics was 97.2%, proving composting as a technique for the treatment of swine effluents contaminated by antibiotics, however, at the end of composting, some antibiotics presented residues in the order of milligrams per kg in the final compound. Therefore, further research on the behavior of these compounds during composting would elucidate whether these compounds are actually degraded or if they generate some kind of metabolites or other substance.

Regarding the community of microorganisms for Bacteria and Fungi [58], a great diversity in the level of phylum and genera observed in both kingdoms throughout the composting. Regarding phyla and genera of bacteria, 7 phylum and more than 70 genera of bacteria were observed over time (0, 15, 30, 45, 60, 75, 90, 120 2150 days). Fungal diversity at phylum and gender level was 2 phylum and 16 genera. This abundance and diversity may be related to the proposed identification methodology, new generation sequencing, which proved to be able to identify a wide range of the micro biota found during composting. In this context, a correlation between environmental variables and antibiotics with microorganisms was, also observed, proving through redundancy analysis that the main factors to have significance in the bacterial community were humidity, but not influencing the fungal community. Veterinary antibiotics (Tilcomisin and Ciprofloxacin) showed a positive correlation in the vast majority of bacteria genera, an effect not observed in fungi. In the fungal genera, the antibiotic Tilmicosin has a positive correlation with the genera (*Apiotrichum* and *Penicillium*) and Ciprofloxacin has a positive relationship with the genera (*Tricosporium*, *Parascedosporium*, *Petriella* and *Cryptococcus*).

6. Microbial communities of the composting process

The application of animal waste contaminated with residues of veterinary medicinal products has become the gateway to the expansion of several types of antibiotic resistance genes, caused by the indiscriminate use of antibiotics in the production of animal protein [60, 61]. In the composting process, there are different types of microorganisms, among them the predominance of bacteria, fungi and actinomycetes, divided into aerobic, thermotolerant and mesophilic [48], which are responsible for about 95% of microbial activity [3]. One of the most important parameters for the proliferation of these microorganisms is temperature, which should not exceed 65°C, for fungi and actinomycetes, and for bacteria, temperatures should be higher than 40°C [48].

Another important aspect is the presence of microorganisms, which are capable of contaminating the environment [9] including *E. coli* and other pathogens. Also according to the authors, during the experiment, carried out with composting of swine effluents, the average presence of 2 to 5 (\log^{10} NMP g^{-1}) of total coliforms was found.

It found 39 species of fungi in the composting process [50], many of which were identified only at the beginning or at the end of the experiment (**Table 1**).

Fungi identified at the beginning of the process	Fungi identified at the end of the process
<i>Absidia Hesseltinii</i>	<i>Alternaria alternata</i> *
<i>Alternaria alternata</i>	<i>Arthrotrrys sp.</i>
<i>Candid's aspergillus</i>	<i>Fumigatus aspergillus</i>
<i>Flavus aspergillus</i>	<i>Niger Aspergillus</i>
<i>Fumigatus aspergillus</i>	<i>Aspergillus sp.</i>
<i>Niger Aspergillus</i>	<i>Aureobasidium floccosum</i> *
<i>Aspergillus</i>	<i>Botryosphaeria sp.</i>
<i>Parasiticus aspergillus</i>	<i>Cephalosporium sp.*</i>
<i>Versicolor aspergillus</i>	<i>Cladosporium sp.</i>
<i>Aureobasidium sp.</i>	<i>Colletotrichum sp.</i>
<i>Bipolaris Maydis</i>	<i>Curvularia bezel</i>
<i>Botryodiplodia theobromae</i>	<i>Dreschlera sp.</i>
<i>Acremonium</i>	<i>Fusarium oxysporum</i> *
<i>Cephalosporium spp.</i>	<i>Gibberella sp.</i>
<i>Chaetomium globosum</i>	<i>Helminthosporium spp</i> *
<i>Cladosporium cladosporioides</i>	<i>Hysterium sp.</i>
<i>Clavata curvularia</i>	<i>Mycosphaerella sp.</i>
<i>Drechslera carbonum</i>	<i>Nigrospora sp.</i>
<i>Oxysporum fusarium</i>	<i>Septoria sp.</i>
<i>Solan's Fusarium</i>	<i>Stenocarpella Maydis</i>
<i>Graminearum fusarium</i>	<i>Tetraploa sp.</i>
<i>Moniliiform fusarium</i>	<i>Viride Trichoderma</i>
<i>Helminthosporium spp.</i>	
<i>Phaseoline macrophomine</i>	
<i>Monilinia sp.</i>	
<i>Mucor sp.</i>	
<i>Oryzae nigrospora</i>	
<i>P. funiculosum</i>	
<i>Penicillium citrinum</i>	
<i>Penicillium sp.</i>	
<i>Penicillium spp.</i>	
<i>Phoma herbarum</i>	
<i>Oryzae</i>	
<i>R. stolonifer</i>	
<i>Solani rhizoctonia</i>	
<i>Oligosporum rhizopus</i>	
<i>Synccephalastrum racemosum</i>	
<i>Harzianum Trichoderma</i>	
Source: [50] * genres that occurred throughout the process.	

Table 1.
Fungi identified during the process of composting of swine effluents with residues of treated seeds.

It is observed that of the total fungi, five species were found since the beginning of the process (*Alternaria alternata** *Aureobasidium floccosum** *Fusarium oxysporum** *Helminthosporium spp**). In a system of composting of poultry, waste found 3 phyls (kingdoms) in greater quantity: Betaproteobacteria; Firmicutes and Bacteria [49].

Evaluating the resistance of microorganism genes to the antibiotic Oxytetracycline (OTC) [13], observed the predominance in 95.3% of the bacteria found, with the following phyls (kingdoms) Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes and Proteobacteria, and that of these kingdoms, 50 generos (*Clostridium sensu stricto*, *Aquamicro Aquabium*; *Paenibacillus*; *Azoarcus*; *Jonesia*; *Gracilibacillis*; *Devosia*; *Celivibrio*; *Marinobacter*; *Tepidimicrobium*; *Ornithinibacillus*; *Paracoccus*; *Pelagibacterium*; *Turicibacter*; *Streptomyces*; *Leucobacter*; *Vulgatibacter*; *Steroidobacter*; *Bordetella*; *Chelatococcus*; *Trupera*; *Nonomuraea*; *Thermovum*; *Brumimicrobium*; *Caldalkalibacillus*; *Ornithinimicrobium*; *Jeotgalicoccus*; *Ureibacillus*; *Sphaerobacter*; *Saccharibacteria_genera_incertae_sedis*; *Pseudomonas*; *Corynebacterium*; *Dietzia*; *Clostridium XI*; *Sphingobacterium*; *Pusillimonas*; *Luteimonas*; *Flovobacterium*; *Actinomadura*; *Rhodopirellula*; *Verrucosispora*; *Nocardiods*; *Bacillus*; *Ammoniibacillus*; *Planifilum*; *Georgenia*; *Idiomamarina*; *Saccharomonospora* and *Thermobifida*) during aerobic composting of bovine effluent, as well as the increase in OTC resistance in some genders [13].

Also in relation to the resistance of microorganisms with antibiotics, [57] they state that CTC inhibited the growth of 12 soil bacteria at different concentrations. In addition to the increased intake of antibiotics in the environment, this can pose risks to human health, such as increased allergy to antibiotics and increased resistance to antibiotics, as many foods develop in places with inadequate effluent disposal and transfer a contaminated load.

Microorganisms such as *E. coli* have shown antibiotic resistance in several studies [58, 62–64]. *E. coli* resistance was tested from wastewater and wastewater treatment system in 24 antibiotics [64], distributed in 6 classes (Penicillins, Cephalosporins, Chynomas, AminoGlycosides, Sulfonamides and Tetracycline). The results showed that the groups of antibiotics with the highest resistance were, Penicillin; Cephalosporin; Kilonomonas; Sulfonamides and Tetracycline.

In another study [14] they found 14 tetracycline-resistant genes and three antibiotic resistance genes Sulfonamines, which modified ribosomal protection proteins, enzymatic inactivation proteins. These results can also be confronted by the high persistence and accumulation capacity that antibiotics have when they are in environmental matrices, especially in soils. Evaluated the persistence of 5 antibiotics in the soil (Tetracycline, Sulfametazin, Norfloxacin, Erythromycin and Chloramphenicol), where the highest rate of antibiotic adsorption in the soil was: Tetracycline > Norfloxacin > Erythromycin > Chloramphenicol > Sulfametazin, thus increasing the risk to the environment [61].

7. Conclusions

In the end, we can admit that the composting process presented itself as an alternative to the current treatment systems, since it combines, at the same time, the treatment of swine effluent, but it has the capacity to degrade antibiotic residues found in swine effluents, minimizing their effects. Impacts on the environmental matrices (soil and water), and still at the end, generate a product (compost) with agricultural potential superior to the use of effluents directly in the soil.

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