COMPOSTING AS ALTERNATIVE TREATMENT OF SOLID WASTES FROM LABORATORY ANIMAL CARE FACILITIES

[*Compostagem como alternativa de tratamento de resíduos sólidos de biotérios*]

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ABSTRACT - This study evaluated composting as a means of treating wastes from rabbits, guinea pigs, mice and hamsters from animal care facilities and its subsequent use for agricultural purposes. We built six compost windrows with 500kg solid wastes mixed with 221.65kg of cotton waste each, which gave a C:N ratio of nearly 30:1. Chemical, microbiological and parasitological analyses of the wastes and the composts were performed before and after treatment. Temperature and pH were measured inside the windrows throughout the experiment. The initial temperature of 28°C increased to a peak of 60°C and decreased to stabilization within approximately 100 days. The pH values oscillated between 6.5 and 8.0, the range indicated to assure pathogen removal and compost quality. At the end of the experiment, over 90% of *Escherichia coli*, *Salmonella sp*, protozoan oocysts and helminth eggs were efficiently eliminated in most of the composts. Chemical analyses detected suitable contents of macro and micronutrients and acceptable levels of heavy metals in the composts. We conclude that composting is an efficient method to treat the solid wastes produced by the studied species held in animal care facilities. It eliminates or reduces microorganism content, producing class B biosolids that can be used with restriction in agricultural practices.

Keywords: Environmental pollution, waste treatment, laboratory animals.

INTRODUCTION

The use of solid wastes produced in animal care facilities for agricultural purposes is still uncommon in Brazil, although it is a feasible alternative from both an economical and environmental perspective. In order to achieve the quality required to be applied to crops, solid wastes must undergo stabilization and processing procedures to be converted into biosolids (David, 2002). Biosolid is the term designated to solid wastes that are used profitably and in an environmentally friendly manner in agriculture (WEF, 1996).

Biosolids are divided into two classes according to pathogen reduction. "Class A" biosolids have additional pathogen reduction during waste treatment and can be applied to crops without any restrictions. "Class B" biosolids have lower pathogen reduction and can be used with restrictions on large crops, reforestation and other areas with controlled contamination risk (David, 2002; Fernandes, 2000).

Pathogen contamination is indicated by the presence of certain organisms such as coliform bacteria. Coliforms are used to indicate and assess contamination because they normally inhabit mammalian intestines, are easily identified and generally occur in human feces in numbers ranging between 100 and 400 billion units per inhabitant per day (Silva et al., 2005).

Microorganisms are quantified in solid wastes using the most-probable-number method, which estimates the number of bacteria per sample weight (MPN/g). "Class A" biosolids must have fecal coliform content < 1 000 MNP/g total waste, *Salmonella sp* absent in a 10g sample of total wastes, enteric viruses < 1 plaque forming unit (PFU)/4g dry waste

and viable helminth eggs < 1 egg/4g total waste. "Class B" biosolids can contain fecal coliforms < 2 x 10^6 MPN/g total waste and viable helminth eggs < 10 eggs/g solid waste (Brasil, 2006).

Many studies have described a range of solid waste treatments and applications that focus on microorganism removal. In general, there are two systems for the production and stabilization of composts: aerobic composting, in windrows or piles, and anaerobic biodigestion (Jiménez & Garcia, 1989), which offers the additional advantage of energy generation and fertilizer production.

Orrico et al. (2007) showed the importance and efficiency of composting for pathogen reduction (99.99%). They found that total and fecal coliform content (MPN) in the material initially introduced in the windrows was significantly different to that obtained at the end of the process.

Compared to plant wastes, the characteristics of animal wastes are more suitable for composting. Rabbit wastes are very rich in nitrogen, phosphorus and potassium. They also contain calcium, sodium, magnesium, sulfur and low levels of other elements. The average yearly feces production of a rabbit ranges from 80 to 100 Kg, and is one of the main organic substrates recommended for lettuce crop. However, it contains high levels of pathogens, including zoonotic agents (Andrade, 1996).

Considering the environmental impacts caused by the direct application of animal wastes to the soil, this material must be prepared for proper disposal and to minimize the negative environmental effects. Animal carcass and feces residue indeed carry a number of microorganisms and parasites that are commonly pathogenic to humans and other animals. Animal care wastes must therefore undergo proper treatment to offset their undesirable characteristics, explore their potential and guarantee their sanitary quality, especially if they are integrated to a watering system. Thus, the present study evaluates composting as a method for treating animal care wastes and the subsequent use of the compost produced for agricultural purposes, a rational and optimal use of these materials.

MATERIAL AND METHODS

This study was approved by the Committee on Animal Research Ethics from the UFMG, protocol number 183/07.

Solid wastes from rabbits, guinea pigs, mice and were collected. After surveying the laboratory population to plan the subsequent analyses, we collected and weighed the solid wastes, consisting of excrement and bedding, for three weeks. The wastes were separated for each animal species and weekly waste production was estimated for each individual.

Four cages of each species were randomly selected and their solid wastes were collected for quantification. The samples were weighed and oven-dried at 105°C for 24h. After this period the samples were weighed again and the total solid content was calculated using the formula: total solids (%) = (final weight/initial weight) x 100.

One waste sample of each species was taken to the Laboratory for Organic Waste Analyses were performed to determine pH in $CaCl_2$ 0.01 mol L^{-1}, humidity at 65ºC, total organic carbon, total N, P_2O_5, K_2O, CaO, MgO, S and equivalent $CaCO_3$ (Tedesco et al., 1985).

Six windrows were constructed to analyze the aerobic composting process. Each windrow contained 500 Kg of solid wastes from all the species studied at relative proportions of production and 221.65kg of cotton wastes to produce a C:N ratio close to 30:1. We protected the top and the sides of the windrow with jute sacks to prevent the compost from being dried up by sunlight exposure. We monitored pH and temperature daily. The composts were watered daily with tap water until they were completely wet. The piles were turned every 15 days throughout the 100-day experiment period.

We collected around 500g samples from each ingredient and from the mixed compost at the beginning and at the end of the experiment for microbiological and parasitological analyses. Aseptic techniques were used to avoid cross-contamination among the windrows.

Parasitological analyses tested for *Escherichia Coli* and *Salmonella sp* according to the procedures described by WHO (1996). After individual homogenization, 10g of each sample was placed in a plastic flask with 30 mL of a 10% solution of formaldehyde to preserve the eggs, cysts and oocysts. To identify these elements, the samples were added of water to 1L and subjected to the solid precipitation protocol for 12h. Next, we applied, the *Bailenger* method, modified by Ayres & Mara (1996) and recommended by WHO (1989). The number of eggs, cysts and oocytes was estimated using an optical microscope (10x and 40x) and *McMaster* chambers. The mean number of elements counted in the two chambers was multiplied by the volume (mL) of the final precipitate and divided by

0.30 (the summed volume of the chambers, 0.15 mL each) and multiplied by 1000 to obtain the final count per kilogram of compost (Ayres & Mara, 1996).

To evaluate composting efficiency we used the equation ordinarily applied to assess removal efficiency: EF = 100 (Co - Ce)/Co, where Co is the number of fecal coliforms and helminth eggs of protozoan cysts in the samples before composting and Ce is the number of these elements after composting.

RESULTS AND DISCUSSION

The weekly production of solid wastes in the animal care facility is shown in Table 1.

The waste content produced by the rabbits is in accordance with other studies. Table 1 shows that annual waste production reaches 86.4kg, corroborating Parodi (2007). Studies about waste production by the other species investigated in the present study were not found.

Chemical, microbiological and parasitological analyses characterized the ingredients used in the composting. Chemical characterization of the solid wastes from each animal species before and after composting is shown in Table 2.

Rabbit wastes showed the lowest total solid content. This occurred because these wastes were composed of feces and urine alone whereas wastes of the other species also contained bedding.

Volatile solids decreased in the composts, probably because organic mass was gradually mineralized over composting. Kiehl (1985) considered that an initial C:N ratio between 20:1 and 50:1 results in a dry matter reduction of 50% during composting. However, this is directly associated to the quality of the material used in composting, and higher reductions are expected in easier-to-degrade substrates or in those with lower fiber content.

Based on earlier studies, we used cotton wastes to obtain a C:N ration close to 30:1. When the mixture of plant matter and animal wastes produces this C:N ratio, the composting windrow is likely suitable for promoting rapid decomposition (Costa 1985). According to Matos et al. (1998), a C:N of 12 by the end of the process indicates compost maturation. In our study the C:N ratio was high even after the entire composting process, probably because the ingredients used contained high bedding content. However, this did not avoid compost maturation.

In Brazil, organic composts produced by stabilization processes such as anaerobic biodigestion and composting must meet reference values determined by the *Ministério da Agricultura Pecuária e Abastecimento* –MAPA (Ministry of Agriculture and Supply) to be commercialized (Brasil 1983). The values obtained for composts produced in the present study were close to the reference values provided by the MAPA. The cotton wastes obtained from the Santanense Textile Industry, Montes Claros – MG and used for composting contained 52.20% carbon and 2.54% nitrogen, representing a C:N ratio of 20.55.

The results of microbiological analyses related to fecal coliform count and *Salmonela sp* detection are shown in Table 3.

Table 3 shows that the highest fecal coliform contamination, with more than 1,100 MPN/g, was found in rabbit wastes, whereas in hamster and mouse wastes it was 210 MPN/g. The high coliform contamination in animal feces caused the high contamination in the initial compost as well. This reinforced the fact that animal care wastes must be subjected to treatments that decrease their microbial load. In addition, all the samples produced in the animal care facility were positive for *Salmonella sp*, making the use of fresh compost in the soil inappropriate (WHO, 1989).

Table 1. Weekly waste produced by the animal species in an Animal Care Facility.

Species	number of animals	Weekly wastes (kg)
Rabbit	100	160***
Mouse	3,500*	1,740
Hamster	100**	660
Guinea pit	200	69
Total	3,900	2,629

* 310 male breeders, 310 female breeders and 2,880 young mice.
** 20 male breeders, 20 female breeders and 60 young hamsters.
*** wastes composed of feces alone whereas in the other groups it included bedding as well.

Table 2. Chemical characterization of solid waste samples from an animal care facility before and after composting.

Variable	Individual wastes before composting				After composting in the windrows (W)							
	rabbit	mouse	guinea pig	hamster	W1	W2	W3	W4	W5	W6	Mean	SD
Total solids	28.72	66.60	60.32	77.20	38.30	35.90	40.10	32.40	39.20	32.80	36.45	3.30
pH in $CaCl_2$ 0,01 mol L^{-1}	8.50	8.60	8.30	8.70	6.60	6.70	6.70	6.80	6.70	6.90	6.73	0.10
Humidity at 65ºC (%)	62.70	34.40	25.50	20.00	60.10	55.30	49.30	53.20	51.10	56.90	54.32	3.95
Total Organic Carbon (%)	35.50	41.40	45.90	45.20	33.40	36.80	38.00	41.00	39.50	37.40	37.68	2.59
Total N (%)	0.77	1.40	1.04	1.02	0.77	0.73	0.98	0.83	0.91	1.02	0.87	0.12
P_2O_5 (%)	1.21	1.24	0.47	0.72	0.27	0.38	0.38	0.25	0.55	0.38	0.37	0.11
K_2O (%)	1.00	0.83	0.44	0.41	0.26	0.42	0.32	0.34	0.41	0.45	0.37	0.07
CaO (%)	1.16	1.09	0.49	0.65	0.88	0.92	1.06	0.81	1.39	1.22	1.05	0.22
MgO (%)	0.39	0.32	0.16	0.21	0.17	0.20	0.24	0.17	0.27	0.24	0.22	0.04
S (%)	0.13	0.15	0.07	0.07	0.07	0.08	0.08	0.06	0.09	0.10	0.08	0.01
C:N	46.10	29.57	44.13	44.31	43.37	50.41	38.77	41.83	43.40	36.67	42.41	4.74
equivalent $CaCO_3$ (%)	71.01	67.28	66.10	20.63	7.20	10.17	7.91	5.77	7.53	7.03	7.49	1.18

Note: Nutrient determination was performed in fresh samples. Organic carbon and equivalent $CaCO_3$ were determined in dry matter at 65 ºC.

Table 3. Microbiological analyses of the ingredients used in the composts before composting.

Ingredient	Occurrence of *Salmonella* sp/ 25 g		Most probable number of fecal coliforms/g
	Sample 1	Sample 2	Mean (N=6)
Rabbit wastes	Positive	Positive	> 1,100
Mouse wastes	Positive	Positive	210
Guinea pig wastes	Positive	Positive	1,100
Hamster wastes	Positive	Positive	210
Cotton wastes	Negative	Negative	<0.3
Bedding	Negative	Negative	< 0.3

Positive: detected growth; Negative: undetected growth

By comparing microorganism content before and after composting, we calculated the efficiency rate (EF) of microorganism removal (Table 4).

The results show that *Salmonella sp* and coliform levels were significantly lower, with a removal index close to 100% in all the composting windrows. These results were different from those reported by Rocha et al. (2003). However, Pegorini et al. (2003), Almeida & Almeida (2005) and Silva et al. (2005) detected high microorganism removal indexes using similar to the ones we adopted. Orrico et al. (2007) showed the importance and efficiency of composting in decreasing total and fecal coliforms (MPN) in goat manure. These values differed by at least 99.99% from the beginning of windrow formation to the mature compost.

Removal and/or reduction of *Salmonella sp* and coliform content is important because it indicates sanitary quality of the test material. This bacterial group includes most pathogens, whose elevated counts indicate insalubrity.

Table 4. Pathogen removal efficiency index of composting of animal care wastes.

Composting windrow	Occurrence of *Salmonella sp*/25 g**			Most probable number of fecal coliforms/g **		
	before	after	EF* (%)	before	after	EF* (%)
1	Positive	Negative	100	210	<0.3	100
2	Positive	Negative	100	>1,100	<0.3	100
3	Positive	Negative	100	>1,100	15	99
4	Positive	Negative	100	1,100	<0.3	100
5	Positive	Negative	100	>1,100	11	97
6	Postive	Negative	100	>1,100	<0.3	100

Positive: detected growth; Negative: undetected growth
* EF= Removal efficiency index
** Mean results of three trials

The final composting product that will be used in soil must have low pathogen counts. This is especially important because if soil bacteria contaminate drinking water, farm animals are more likely to become ill, thereby decreasing productivity and possibly causing deaths.

After composting, contamination was under 1 000 MPN g^{-1}. The compost we produced can therefore be classified as a "Class A" biosolids, without restrictions according to the laws regulating organic fertilizers (Brasil 2006).

Tables 5 and 6 show the parasitological tests performed on the animal wastes at the beginning and at the end of composting. As shown in Table 5, wastes of all the animal species studied, except the hamsters, were contaminated with *Eimeria sp*. In addition, all the composting windrows were contaminated with a high helminth and protozoan load (Table 6). As a consequence, all the samples subjected to composting had high levels of helminth eggs and protozoan cysts. The detection of *Eimeria sp* in animal care wastes is important because it parasites both domestic and wild animals, causing persistent disorders.

Our findings indicate that post-treatment of the studied wastes is needed because the pathogens detected are zoonotic and may be transmitted between animals, thereby spreading the diseases. This is described by Long & Joyner (1984) and Silva et al. (2006) for oocysts and protozoans and by Doyle (2006), Scaini (2003), Anderson (2000) and Luca et al. (1996) for helminth eggs.

Table 5. Parasitological tests performed on guinea pig, mouse, hamster and rabbit wastes used for composting.

Material	Result
Guinea Pig wastes*	Negative for helminths and protozoans
	Positive for *Eimeria sp*
	Positive for *Eimeria sp*
Mouse wastes *	Positive for *Eimeria sp*
	Positive for *Eimeria sp*
	Positive for *Eimeria sp*
Hamster wastes*	Negative for helminths and protozoans
	Negative for helminths and protozoans
	Negative for helminths and protozoans
Rabbit wastes*	Positive for *Eimeria sp*
	Positive for *Eimeria sp*
	Positive for *Eimeria sp*

* mean value from 3 trials

Table 6. Removal efficiency index for eggs and oocytes in animal care wastes after composting.

Composting Windrow	Helminth eggs /4 g feces **			Protozoan oocysts/4 g feces **		
	before	after	EF* (%)	before	after	EF (%)
1	7 280	1080	85.16	7 840	20	99.74
2	5 640	160	97.16	7 280	0	100
3	14 280	380	97.34	14 760	40	99.72
4	12 680	40	99.68	3 800	0	100
5	11 920	0	100	8 640	0	100
6	14 160	60	99.57	13 680	40	99.71

*EF: Removal efficiency index
** mean of 3 trials

The results we obtained corroborate data from other studies that tested composting ingredients with different characteristics. Correa et al. (2007) used sludge with 4.7 viable helminth eggs per dry matter gram as composting ingredient, and by the end of the process, removal efficiency for eggs ranged from 93 to 100 %. Fernandes et al. (1997) decreased the number of viable helminth eggs of sewage sludge by 86% after composting, and Soccol et al. (1997) decreased them by between 93 and 100% after composting sewage sludge with saw dust. Most of sludge sterilization occurs after the thermophilic phase of composting, which indicates that pathogen elimination occurs by combining high temperatures and disharmonious relationships between human pathogens and saprophytic organisms (Golueke, 1975).

Duarte et al. (2008) evaluated helminth eggs, protozoan cysts and oocysts in organic composts from domestic sewage and crop wastes. He found that helminth eggs may still be viable after composting and thermal treatment, a situation that poses contamination risks for humans and animals.

As shown by the parasitological analyses we performed, composting material from animal care wastes must not be used without previous treatment because they are potential disease carriers that may contaminate animals and even humans. Therefore, composting treatment is a viable and efficient procedure for animal care waste management that contributes positively to environmental preservation. However, even considering the pathogen removal index, the composts we prepared could not be used without restrictions on crops because helminth egg count was above 1.0 egg/4g total solids. Therefore, except for composting windrow number 5, the others can be classified as type B biosolids, which can be used with restrictions on coffee crops, silviculture and crops designed for fiber and oil production (Brasil, 2006).

Aerobic and facultative microorganisms, the most active agents involved in composting processes, prevail within temperature ranges of 20 to 45°C (mesophils) and 45 to 65°C (thermophils). These exothermic microorganisms release energy as heat, increasing compost temperature naturally (Reis et al., 2004). The results obtained indicate that all the composting piles underwent both the thermophilic and mesophilic phases. The maximum temperature, close to 60°C, was achieved on the 20th day of composting. Similar findings were reported by Thambirajah et al. (1995), Hanajima et al. (2001) and Georgacakis (1996).

Each microorganism group is specialized and develops within an optimal temperature range. The thermophilic phase is characterized by a fast decomposition rate, whereas the mesophilic phase exhibits slow decomposition of the organic material (Igue, 1984; Paul & Clark, 1989; Pereira Neto & Stentiford, 1992).

The high temperatures recorded and the time the composts remained within the thermophilic range were sufficient to significantly decrease microorganism content in the composted wastes. Microbiological activity increases the temperature in waste mixtures naturally, remaining at 60 to 65°C for several days, thereby eliminating pathogens (Hay 1996). An important point is that temperature did not exceed the limits found in other studies, a fact that could compromise the process.

Internal compost temperature was controlled by the aeration promoted by periodical manual turning. According to Pereira Neto (1996), the ideal temperature for composting is around 55ºC, an important variable to be controlled within the windrows because temperatures beyond 65ºC eliminate the mineralizing microorganisms that degrade organic residuals. Besides temperature control, Kiehl (1985) states that the passage of air,

through permeable ground and perforated pipes inserted in the windrows, accelerates decomposition and improves composting conduction by avoiding foul odors and the accumulation of flies.

All the composts were stable after nearly 100 days of composting. The composts can be considered stable when their mean temperature is close to room temperature (Kiehl, 1985).

Aerobic composting increases pH value. At the beginning of the process, pH becomes acid, from 5 to 6, due to organic acid and carbonic gas formation, but these composts react to the gases released by the organic matter, are neutralized and produce a mean between 8 and 8.5 that is maintained until the end of composting (Reis et al., 2004). However, in the present study pH oscillated from 6 to 8 throughout the entire experiment, except in windrows 2 and 4, which had pH value slightly above 8 and under 5, respectively at determinate measurement points. The low pH value in windrow number 4 was possibly caused by the accumulation of organic acids. Over the course of the experiment, these composts were metabolized and the pH value increased. An important point is that the pH was almost neutral by the end of the process. The use of acidic composts on crops can cause local and temporary soil acidification along with elevated temperatures due to microbial respiration, which compromises root development and plant production (Jahnel et al., 1999). Therefore, high pH fertilizers produced by composting are used more efficiently by the plants. In addition, their contribution to soil acidity and salinization is substantially decreased. Values of pH above 7 (neutrality) are typical in stable composts and are also reported in studies that composted other types of wastes (Georgacakis et al., 1996).

Although fecal material was used in the mixture subjected to composting, periodical pile turning and rigorous temperature and humidity control resulted in the production of a "Class B" biosolids (Brasil, 2006). Characteristics of the compost produced, such as humidity, nearly neutral pH, dark brown color, absence of foul odors, adequate macro and micronutrients, acceptable heavy metal content, and safe microorganism contamination from a sanitary standpoint (Magalhães, 2002) make it appropriate for agriculture purposes.

The findings we present also reveal that from an agroecological perspective, big companies are contributing to environmental preservation by developing studies that embrace economic, ecological and social principles.

CONCLUSIONS

Composting is an efficient treatment for solid wastes produced by animals held in animal care facilities. It eliminates or reduces microorganism content and produces "Class B" biosolids that can be used with restrictions on coffee crops, silviculture and crops designed for fiber and oil production.

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