COMPOSTING AS AN ALTERNATIVE METHOD TO DISPOSE OF SLAUGHTERHOUSE WASTES IN PUERTO RICO

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ABSTRACT

The elaboration, and chemical, physical and biological properties of compost made of organic wastes from a slaughterhouse (SHW) plant were evaluated. Freshly cut (FYT) and semi-decomposed (SCYT) yard trimmings were evaluated as bulking agents (BA). Treatments consisted of combinations of BA (FYT and SCYT) with layers of SHW (none, single or double). All treatments combinations composted well and exhibited thermophillic temperatures after three days of composting. Chemical composition (pH, organic matter, OM; inorganic matter, IM; carbon, C; and nitrogen; N, content) varied depending BA and layers of SHW throughout the composting phases. However, the use of SCYT as BA resulted in greater N content and lower OM than FYT. The SHW composted in double layers resulted in a higher N content than none or single layers of SHW. However, even though it reaches higher temperatures, experimental analyses suggest that a longer period of time is needed to decompose it.

The physical and chemical characteristics were evaluated in finished compost in order to assess the stability and maturity, and compare them to three commercial composts (CC1, CC2, and CC3). All treatments evaluated produced mature and stable compost, which are a potential source of nutrients for plant growth. However, as the agronomic performance shows, the compost needs to be mixed with other nutrient sources (i.e. inorganic fertilizers) when added to soils in order to provide the optimum quantities of nutrients that *cv Mulato* needs in 42 days of growth. In a third study, the biological properties showed that an active heterogeneous population responsible for the breakdown of OM was identified at the first HC during the composting process of SHW and YT.

COMPOSTA COMO METODO ALTERNO PARA LA DISPOSICION DE RESIDUOS ORGANICOS DE MATADEROS COMERCIALES EN PUERTO RICO

RESUMEN

En el presente estudio se evaluó la elaboración de composta de residuos de mataderos comerciales y las características físicas, químicas y biológicas del producto final. El proceso de compostación fue evaluado utilizando como material absorbente (MA) grama fresca (GF) y semicompostada (GSC). Los tratamientos consistían de todas las combinaciones de MA (GF y GSC) y capas de RM (sin RM, sencillas o dobles). Todos los tratamientos de GF/RM y GSC/RM presentaron temperaturas termofílicas después de tres días de comenzar el proceso. La composición química (pH, materia orgánica, MO, materia inorgánica, MI, y nitrógeno, N) varió a través de las etapas de compostaje dependiendo de la combinación de materiales. La GSC presentaba mayor contenido de N y mejor descomposición de la MO que la GF. Doble capa de RM resultó en un mayor contenido de N al compararse con compostas sin RM o con capas sencillas. Aunque la doble capa de RM presentó las temperaturas más altas, se requirió mayor cantidad de tiempo para descomponer este tratamiento.

Se evaluaron las características físicas y químicas de las compostas experimentales con el objetivo de determinar su madurez y estabilidad al ser comparadas con tres compostas comerciales (CC1, CC2 y CC3). Todos los tratamientos evaluados produjeron una composta madura y estable que puede ser utilizada como una potencial fuente de nutrientes para las plantas. Sin embargo, según los resultados de la respuesta agronómica, la composta experimental necesita ser mezclada con otra fuente de nutrientes (fertilizantes inorgánicos) para proveer la cantidad óptima de nutrientes que el cv Mulato necesita en 42 días de crecimiento.

En un tercer experimento, se evaluaron las características químicas y biológicas de la composta en donde se encontró que una población activa y heterogénea de bacterias resultó ser la responsable del rompimiento de la MO durante el primer ciclo de calor.

A tod@s l@s "neci@s" porque de ell@s será el futuro.

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TABLE OF CONTENT

Contents	Pages

LIST OF TABLES

LIST OF FIGURES

Contents	Pages

Chapter 3 - Quality and agronomic performance of slaughterhouse wastes compost

Chapter 4 - Chemical and biological characterization of slaughterhouse wastes compost

CHAPTER 1

Literature review

Introduction

In Puerto Rico, as well as in many parts of the world, the generation and disposition of organic wastes are an increasing problem. Generally, the wastes from different kinds of anthropogenic activities are disposed of in landfills were they undergo an uncontrolled degradation which can ultimately become a source of pollution. To date, more than a half of the landfills, existing a decade ago, in Puerto Rico has been closed because they do not meet federal and state's regulations. Alternatives, other than disposing wastes into landfills, ought to be evaluated.

Many researchers have proposed rendering, incineration and open burning, burial, lactic acid fermentation, anaerobic digestion, alkaline hydrolysis, non-traditional technologies, and composting as methods to recycle different kind of wastes (Kasher and Phebus, 2004; Scudamore *et al.*, 2002; Blake and Donald, 2002; Kherrati *et al.,* 1998). However, out of all the possibilities, composting is the preferred choice since it can be implemented fairly rapidly in the farms of Puerto Rico.

Composting is a natural process that has occurred through millions of years and recently it has been used around the world, as a controlled process, for the disposal of animal wastes. This process entails a natural decomposition that takes place in the presence of oxygen, and generates a dark-brown to black soil like or humus product rich in plant nutrients and microorganisms. Presently in Puerto Rico, composting organic wastes from abattoirs hasn't been evaluated and considered by producers.

In the present study, composting as an alternative for the disposition of slaughterhouse organic wastes was evaluated. The objectives were:

1. To evaluate the efficacy of the process of compost production from slaughterhouse organic wastes using yard trimmings as a bulking agent.

2. To characterize the physical and chemical properties of the resulting product and its evaluation as a source of nutrients to agronomic crops.

3. To characterize the biological composition of the different composts.

Organic wastes production in Puerto Rico

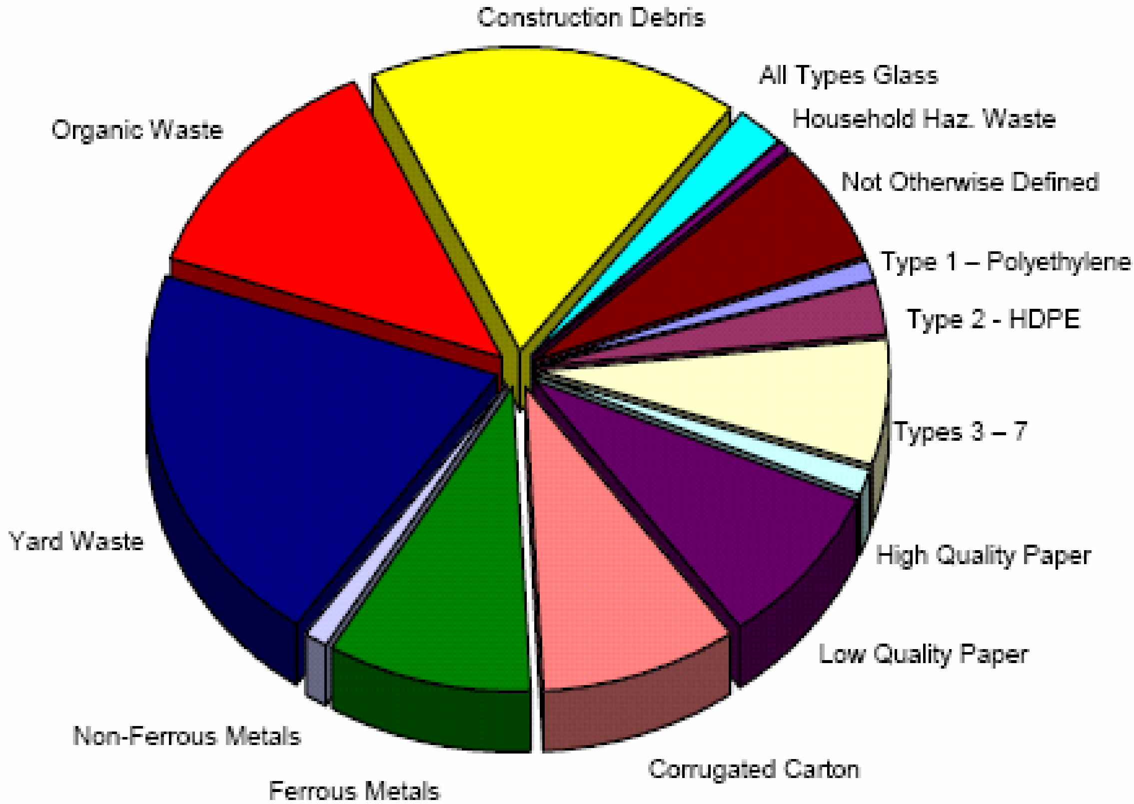
Waste generation is a direct result of anthropogenic activities. Pongrácz *et al.* (2004) defined waste as *"a human-made thing that is, in the given time and place, in its actual structure and state, not useful to its owner, or an output that has no owner, and no purpose"*. Enforcing proper disposition of waste has become a burden for governments all over the world, which requires regulatory agencies and environmental organizations to monitor the consequences of improper waste management. In order to accomplish an effective disposition it is necessary to know what a given waste is and its composition.

In Puerto Rico, several studies have been conducted to characterize the wastes that are commonly discarded. The Solid Waste Management Agency contracted with Wehran Puerto Rico, Inc. to conduct the 2003 Waste Characterization Study at landfills and transfer stations. In their study, Wehran (2003) classified wastes as: type1 (polyethylene); type 2 (high density polyethylene, HDPE); types 3 to 7 (polyvinyl chloride, PVC; low density polyethylene, LDPE; polypropylene, PP; polystyrene, PS, and mixed); paper (high and low quality); corrugated carton; metals (ferreous and non-ferreous); yard waste; organic waste; construction materials and debris; all types of glass; household hazardous wastes, and those not otherwise defined. Of all the wastes generated in Puerto Rico, yard and organic wastes comprised two of the most important (Figure 1.1).

The use of landfills to dispose of wastes was once considered an easy and economically viable method, however, its use have become problematic because of limited land availability and the environmental contamination it generates. The Strategic Plan for the Management of Solid Wastes in Puerto Rico prepared by the Solid Wastes Agency (SWA) states that the restrictions on the location and operation of landfills will result in their closure (ADS, 2004). From 1993 to 1994, there were 64 operating landfills in Puerto Rico. Currently, 52% of these are closed as a result of the Clean Air Act (1970), Resource Conservation and Recovery Act (1976), and Clean Water Act (1972). Nowadays, only 31 landfills (48% of the former 64) still operate in the Island and most of them have serious flaws that affect air and water quality (Figure 1.2). Furthermore, the quality of life of residents near these landfills is also affected.

Organic wastes from abattoirs

Slaughterhouses contribute to a high percentage of organic wastes received in these landfills. The disposal of organic wastes from commercial abattoirs represents a serious problem for the meat industry in Puerto Rico. The disposition of these types of wastes in landfills represents an increase in operational costs and a potential source of



Type of waste	%
Type 1 - Polyethylene	1.1
Type 2 - HDPE	2.9
Types 3 to 7	6.5
High quality paper	1.3
Low quality paper	8.7
Corrugated carton	9.3
Ferrous metals	9.4
Non-ferrous metals	1.1
Yard waste	20.4
Organic waste	12.9
Construction debris	17.1
All types glass	2.4
Household hazardous waste	0.5
Not otherwise defined	6.3

Figure 1.1. Average composition of solid waste discards in Puerto Rico (Source: Wehran PR Inc., 2003)

Figure 1.2. Landfills currently operating in Puerto Rico (Source: ADS, 2004)

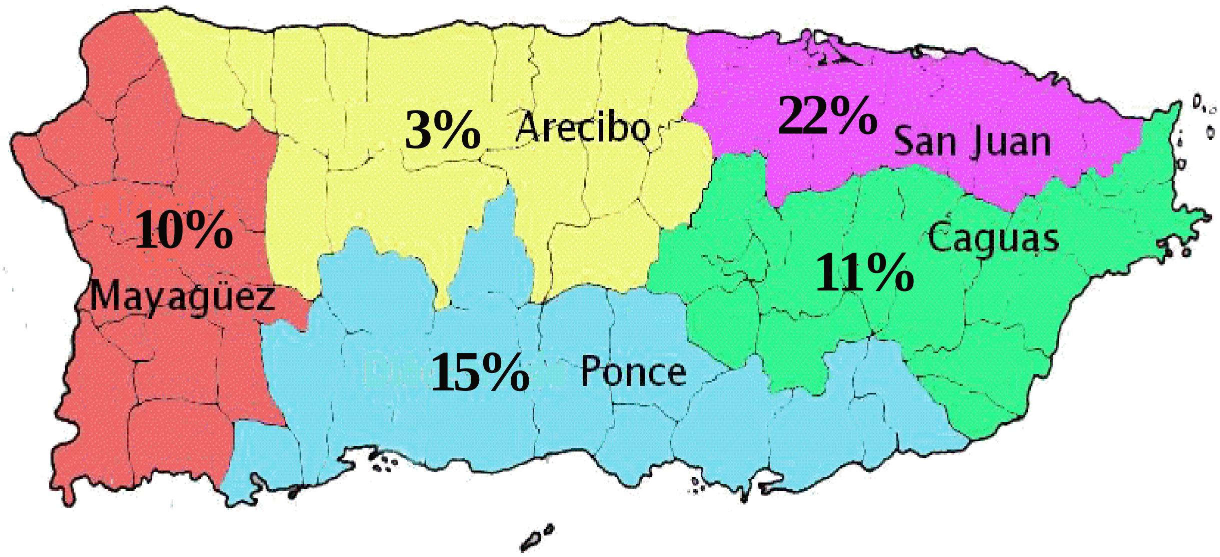


Figure 1.3. Comercial livestock production per region in Puerto Rico. This figure only applies to the comercial sector which represents 61% of the production. The remaining 38% left includes small farmers (data not shown)(DAPR, 2003b).

environmental pollution. The uncontrolled degradation of organic residues results in the generation of biogas and effluents that can cause important negative effects in the environment (Palma *et al.*, 1999). Biogas is a mixture of methane and carbon dioxide resulting from anaerobic decomposition of organic material (Oxford Dictionary of Biology, 2004). Methane and carbon dioxide emissions are significant with the global warming effect if they are not generated in a controlled environment. A major non-agricultural source of biogenic methane is refuse landfills; the contribution of methane from this source is still currently greater than that from agriculture (Merrington *et al.*, 2002).

In Puerto Rico, the slaughter and processing of livestock is performed in five regions (Figure 1.3). The Statistical Office of the Department of Agriculture of Puerto Rico reported in the annual slaughter summary for fiscal year 2001-2002, for animal type and location, that these five regions slaughtered 57,701,248 kg of meat. About 30% of the total of animal weight slaughtered represents organic residues from gastrointestinal and reproductive organs. This amounts to a production of approximately 57,350,348 kg of organic wastes per year (DAPR, 2003a). The evaluation of alternatives for the management and disposal of these types of wastes in Puerto Rico is urgently needed.

Disposal alternatives for organic wastes

Alternatives proposed to manage and dispose of SHW include rendering, incineration and open burning, burial, lactic acid fermentation, anaerobic digestion, alkaline hydrolysis, non-traditional technologies, and composting.

Rendering

Rendering involves heating ground carcasses in large boilers at a specific temperature, time, and pressure combination (Scudamore *et al.*, 2002). This process generates tallow, and meat and bone meals as by-products and is regulated by the United States Department of Agriculture (USDA). Alternatively, these by-products can be used as feedstuffs for livestock as permitted by the USDA. However, for years researchers have believed that the early cases of bovine spongiform encephalopathy (BSE) in Europe were caused by feeding to cattle rendered protein produced from the carcasses of scrapie-infected sheep or cattle with a previously unidentified transmissible spongiform encephalopathy. Changes in rendering operations in the late 1970's and early 1980's may have played a part in the appearance of the disease (APHIS-USDA, 2002).

Currently, the use of by-products generated from render carcasses posses a risk to the animal health because, if not done properly, this option can be a possible route to the spread of diseases. Puerto Rico does not have rendering facilities for ruminant carcasses.

Incineration and Open Burning

A mortality incinerator is a convection oven that burns a carcass under a controlled environment at a very high temperature, reducing the carcass to ashes (Henry, *et al.*, 2001). Open-air burning consists of burning carcasses in open fields, on combustible heaps called pyres, and with burning techniques that are unassisted by incineration equipment (Kasher and Phebus, 2004).

Pyres and incinerators for the disposal of organic wastes generated from livestock industries have been used for centuries. By the seventeenth century, European nation-states began to officially rely upon burning as a means of disposing of livestock infected with foot and mouth disease (FMD) and transmissible spongiform encephalopathy.

During the United Kindgdom's 2001 FMD outbreak, more than six million animals were slaughtered. Thirty percent of those were disposed of in burns (NAO, 2002). These pyres led to negative media images and public opposition.

Traditionally, open-air burning causes negative public perception because of the fact that this alternative can affect people's lifestyles (by posing health hazards) and are harmful to the environment. Open-air burning tends to be labor intensive, needs large quantities of fuel (since carcasses are mostly composed of water), depends on favorable weather conditions, causes environmental problems, and has poor public perception (Ellis, 2001, cited by Kasher and Phebus, 2004).

According to the regulations of the Environmental Quality Board (EQB) for the control of atmospheric contamination, open-air burning is prohibited in Puerto Rico (EQB, 1995). These regulations state that, effective January 11, 1981, no person shall dispose of any wastes by burning. In special cases, the EQB can make an exception to this rule for wastes generated from pineapple, sugarcane or rice production. These regulations were develop in compliance of the Environmental Public Policy Act (1970, amended July, 1995).

Incinerators are highly biosecure but expensive and difficult to operate and manage from a regulatory perspective (Kasher and Phebus, 2004). In Puerto Rico, the idea of establishing an incinerator to dispose of wastes has been subject of debate and is disconcerting to the community. In the past several incinerator projects have been proposed as an alternative for waste disposal. However, community leaders, and environmentalists opposed the project. The "Colegio de Médicos y Cirujanos de Puerto Rico" said that incineration of wastes could generate high quantities of toxic and carcinogenic materials with the potential to affect the residents of the town in question and towns nearby (Curbelo, 2004). In 2001, the Senate of Puerto Rico rejected incineration as a method to dispose of wastes (Bill R.C. of S. 9 of February 5th, 2001).

Burial

Burial involves excavating a grave or pit, filling the bulk of the excavation with dead animals, and then covering them with soil until the grave is filled. Blake and Donald (1992) state that the environment within the disposal pit allows for aerobic and anaerobic microorganisms to decompose organic materials. The majority of the degradation of the refuse is due to an array of anaerobic fermentations that create the objectionable odor normally associated with pits. Blake and Donald (1992) emphasize that the disposal pits should be located where ground water level is well below the pit bottom and where soil type permits good filtration of effluent. Major disadvantages for this method are the fact that residue remains for years at the spot and may affect ground water quality.

Lactic Acid Fermentation

Anaerobic fermentation is a process in which bacteria transform sugars into lactic acid. Carcasses are ground into small pieces (2.54 cm in diameter, or less), mixed with a carbohydrate source (sucrose, molasses, whey, or ground corn), inoculated with lactic acid bacteria, and stored in an oxygen free tank (Erickson *et al.*, 2004a). Lactic acid preserves and decontaminates carcass material.

Kherrati *et al.* (1998) studied lactic acid fermentation as a process of recycling slaughterhouses wastes. These wastes were mixed separately with 15% molasses and inoculated with a starter culture of *Lactobacillus plantarum*, and then incubated at 22-24 ˚C for 10 to 15 days. Results indicated that the product obtained from these types of waste had low pH, carbohydrate content decrease, and showed no significant change in dry matter, fat, and protein contents relative to the initial material.

Nevertheless, lactic acid fermentation is not a viable method for processing SHW since large quantities are produced; vast carbohydrate sources are needed, and the construction of an oxygen-free tank would involve high initial cost. In addition, there is limited information regarding the use of the end-product generated from this process.

Anaerobic Digestion

Anaerobic digestion decomposes organic matter and produces biogas. This controlled process is often referred to as biomethanization and biodigestion and it can eliminate carcasses and produce energy at the same time. In some cases, it is necessary to reduce the size of the carcasses and sterilize them on-site before proceeding with the anaerobic digestion (Erickson *et al.*, 2004b).

Complex organic matter (lipids, nucleic acid, polysaccharides, carbohydrates, proteins and fats) is hydrolyzed into soluble organic molecules (fatty acids, monosacharides, amino acids, purines and pyrimidines). The soluble organic molecules are fermented by acetogenic bacteria to organic acids (volatile fatty acids) in order to produce carbon dioxide and hydrogen. The final step is methanogenesis, in which methane and carbon dioxide are produced from intermediate products (Erickson *et al.*, 2004b). A disadvantage of this process is the use of large quantities of acids to regulate the pH, which may damage equipment in a short time.

Alkaline Hydrolisis

Thacker (2004) described alkaline hydrolysis as an alternative for the reduction and sterilization of animal wastes. This process uses sodium hydroxide or potassium hydroxide to catalyze the hydrolysis of biological material into a sterile aqueous solution consisting of small peptides, amino acids, sugars, and soaps. To accelerate the process, high temperatures are applied. The thermal conditions and alkali concentrations destroy the protein coats of viruses and the peptide bonds of prions (Taylor, 2001 cited by Thacker, 2004). A mayor set back of this process is that the effluent produced has high pH, temperature, and biological oxygen demand.

Non-Traditional Technologies

Non-traditional methods that might be used to deal with large scale animal mortalities and wastes are thermal depolymerization, plasma arc process, refeeding, napalm, ocean disposal, non-traditional rendering, and novel pyrolisis technology (Jones *et al.*¸2004).

Composting

Composting is a method widely used around the world as an alternative to dumping different types of wastes and is one of the SWA's preferred methods to dispose of waste disposal. Mukhtar *et al*. (2004) described carcass composting as a natural biological decomposition process that takes place in the presence of oxygen. The end product of this process is a dark-brown or black soil like material or humus with a musty odor rich in plant nutrients and microorganisms. This method "buries" animals above ground with a carbon rich material (bulking agents) which gives microorganisms enough nutrients to decompose the wastes.

Composting envolves the same process that decays leaves and other organic debris in nature but conditions are controlled so that materials decompose faster (Rynk *et al.*, 1992). Carcass composting offers several benefits, including reduced environmental pollution, generation of a valuable by-product (soil amendment), and destruction of many pathogens (Mukhtar *et al.*, 2004). Compost is a method utilized for the reduction, recycling, and reutilization of crop residues (Bernal *et al.*, 1998), fruit processing wastes (Van Heerden *et al.* 2002), household wastes (Das *et al.*, 2003; Sullivan *et al.*, 2002; Michel *et al.*, 1995), animal mortalities (Fonstad *et al.*, 2003; Keener *et al.*, 1997; Carter *et al.*, 1996), and manure (Fukumoto *et al.*, 2003).

This method entails the degradation of organic matter in a controlled and aerated environment. Microorganisms decompose refuse generating a stable by-product composed of the biomass of both dead and living microorganisms, and the undegradable parts of the raw materials (USDA-NRCS, 2000) (Figure 1.4). This by-product is known as the compost. Decomposition occurs best when there is an adequate supply of the essential nutrients carbon, nitrogen, oxygen, phosphorus, potassium, hydrogen, sulfur, iron, and magnesium plus 10 to 20 trace elements (Blake and Donald, 1992). The characteristics of organic wastes, their moisture and their ability to biodegrade, affect the composting process (de Guardia *et al.*, 1998).

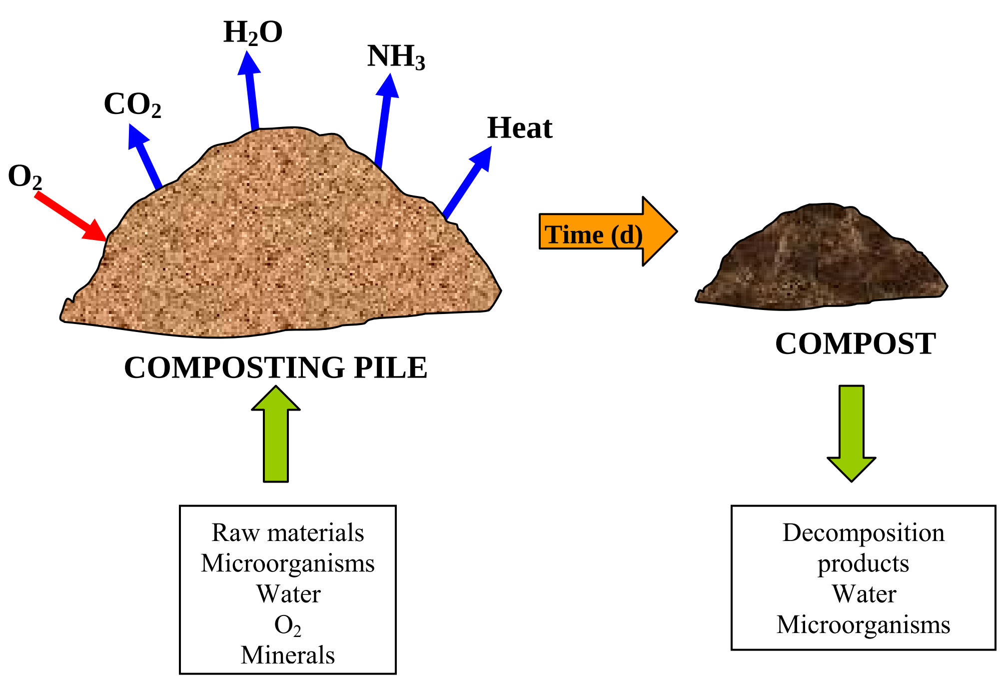


Figure 1.4. Composting process.

Biochemistry of the process

The principal purpose to start a composting pile is to degrade raw organic materials to produce a simpler and stable product. These raw materials are composed mainly by organic matter that is composed of high-molecular weight compounds, and as simpler and humic substances (McCarthy *et al.,* 1990, cited by Epstein, 1997). However, in soil organic matter is classified as the non-mineral fraction of soil and any vegetable or animal matter (Coleman *et al.,* 1989).

The biological degradation of organic matter in the composting process is governed by a heterogeneous population of microorganisms. Microorganisms are classified as psycrophiles, mesophiles, and thermophiles according to the optimum temperature at which they grow and reproduce. Psycrophiles thrive at temperatures ranging from 0 to 25˚C; mesophiles and termophiles grow best at temperatures ranging from 26 to 45˚C, and >45˚C, respectively.

The microbial communities within the pile are mainly composed of bacteria, fungi, and actinomycetes. Bacteria are responsible for the initial decomposition of raw materials and the stabilization of most readily available nutrients, such as simple sugars and products of fungal decomposition. Fungi and actinomycetes tend to be present in the degradation of organic acids, sugars, starches, hemicelluloses, celluloses, proteins, and polypeptides, during later stages of the process when temperatures are mesophillic. Many fungi can degrade woody materials, waxes, proteins, hemicelluloses, lignin, and pectin. Actinomycetes degrade amino acids and lignin (USDA, 2000). During composting, microorganisms break down organic materials and transform them into new complex compounds (Rynk *et al.*, 1992). As microbes degrade the materials, their metabolic activities allow the variation in the population.

Microbial growth is further enhanced when in addition to these nutrients (especially C and N), an aerobic environment and adequate moisture levels are present. When these conditions are optimized, microbial activity generates thermophillic and mesophillic temperatures which allow the rapid degradation of materials and the production of water, carbon dioxide, ammonia, and other volatile compounds (Epstein, 1997).

Optimization of the composting process can be achieved if nutrients, moisture, oxygen, temperature, and pH are combined in adequate proportions.

Nutrients

Raw organic materials contain the C, N, P, K, and other nutrients needed by microorganisms to grow and reproduce. Blended raw materials should provide sufficient quantities and proportions of nutrients so that microorganisms can decompose them. Of all the nutrients needs of the microorganisms, the proportion of carbon to nitrogen is the most important factor in constructing a compost pile. Carbon and nitrogen have the greatest effect on the composting process and therefore are used as the primary indicators of nutrient adequacy (USDA, 2000). Microorganisms use carbon for both energy and growth while nitrogen is essential for protein synthesis and reproduction (Rynk *et al.*, 1992).

The quantities of these two nutrients are not as important as their proportion. In the composting process the relative amounts of carbon and nitrogen are compared. This is called the C:N ratio of the pile. The initial C:N ratio is one of the most important characteristics determining the success of the process. If the mixture of materials used to make a compost has a low C:N ratio (not enough C), the surplus N supply is converted to NH_3 and NH_4^+, resulting in offensive odors. Conversely, if there is too much C (high

C:N ratio) the low N supply can limit microbial activity resulting in slow carcass decomposition and cool temperatures (Morse, 2001). The composting process slows after initial rapid cell growth and depletion of available N, resulting in a subsequent reduction of cellular growth (Epstein, 1997). As microorganisms die and their N becomes available, the C:N ratio decreases, and the process continues.

It has been reported that an ideal C:N ratio ranges from 25:1 to 30:1 or 20:1 to 35:1 (Fonstad *et al.*, 2003; Carter *et al.*, 1996). However, Laos *et al.* (2002) reported that a C:N ratio in the range from 10:1 to 40:1 can result in a successful composting process. Michel *et al.* (2004) suggests that in order to minimize N loss during dairy manure composting with sawdust and straw as amendments, the C:N ratio should be between 40:1 and 50:1.

As the composting process progresses, the C:N ratio decreases because a large part of the C is continuously released as CO_2 while the majority of the N is recycled through the cellular material of the microorganisms (USDA, 2000). While degrading organic compounds, microbes waste from 60 to 70% of the C as CO_2, and incorporate (immobilize) only 30 to 40% of the C into their body as cellular components (Henis, 1986 cited by Barrington *et al.*, 2002). Richard *et al.* (2002b) reported that at the end of the process, the compost should have a C:N ratio of 10:1 to 15:1.

Wiley and Pierce (1955; cited by Epstein, 1997) described the composting process using the following chemical equation:

$$C_pH_qO_rN_s \cong aH_2O + bO_2 \rightarrow C_tH_uO_vN_w \cong cH2O + dH_2O + eH_2O + CO_2$$

where $C_pH_qO_rN_s \cong aH_2O$ is the hydrated organic matter, bO_2 is the consumed oxygen, $C_tH_uO_vN_w$ is the compost, cH2O and dH_2O are the evaporated water, and CO_2 is the carbon dioxide produced. The small letters represent constants for different conditions.

Organic wastes composed of animal mortalities tend to have a high N content and thus for composting, large amounts of C must be added in the form of cover materials or bulking agents (Morse, 2001; Keener *et al.*, 1997). Cover materials affect the moisture content, free air space, decomposition rate, temperature, C:N ratio, and O_2 concentrations during composting (Michel *et al.*, 2004).

Several on-farm and off-farm wastes can be used as bulking agents. Straw, bark, sawdust, sand, hay, paper, grass clippings, leaves, and rice hulls, among others, can be blended at different proportions with organic residues from animals in order to generate ideal conditions for the composting process to take place. Availability, cost, and, physical characteristics are the most common characteristics used in choosing a cover material.

Michel *et al*. (2004) compared the effects of hardwood sawdust, and wheat straw, versus sand bedding on the decomposition rate, overall mass, volume, and C and N losses during full scale windrow composting of dairy manure. Dairy manure composting using sawdust and straw as bulking agents resulted in extensive reductions in manure volume and weight. Sand bedded manure composting had more modest weight and volume reductions. None of the straw amended or sand bedded compost reached temperatures exceeding 55˚C in conjunction with extensive volume and volatile solid losses.

Minkara *et al.* (1998) characterized and compared the degradation rates and nutrient values of rice hull and crawfish by-products. They also examined the efficacy of adding raw crawfish by-products to partially composted rice hulls. This experiment was divided into three phases: phase I corresponded to the composting of rice hulls and ground crawfish by-products; phases II and III were initiated by recycling the product of phase I and II, respectively, with a batch of crawfish by-products. In general, treatments with the lowest percentage of initial volatile solids had the highest loss of dry mass and organic matter (treatments of the third phase). Biodegradability analysis conducted in this experiment showed that rice hulls and crawfish by-products can be composted when initial C:N ratio is within the range of 10 to 42:1. However, these researchers stated that mixtures containing a higher percentage of crawfish by-products to rice hulls or recycled product tend to degrade at a faster rate. In addition, nutrient analysis of compost samples showed that repetitive additions of crawfish by-products increases the concentration of important nutrients like P, K, Mg, Mn, S, and Ca.

Laos *et al.* (2002) evaluated the composting of fish processing wastes and biosolids with wood by-products and yard trimmings as bulking agents. They used fish offal and a mixture of sawdust and wood shavings in a 3:1 proportion. Biosolids were mixed with wood shavings and yard trimmings at a 1:1 ratio by volume. In the fish offal composting, results indicated that a thermophillic period persisted for approximately 60 days. Thermophillic temperatures over 55˚C were sustained long enough to satisfy the USEPA requirements for processes to further reduce pathogens. Fish offal composting with wood shavings by the in-vessel system was adequate to ensure organic matter stability, although the process was retarded during winter. Furthermore, the biosolids compost's seasonal patterns were related to the bulking agent used. In summer both agents exhibited optimal behavior, while in winter yard trimmings were more adequate to achieve temperature requirements for pathogen reduction.

Moisture and Aeration

An ideal moisture level should be maintained so that microorganism can perform their metabolic activities, and O_2 can flow through the pile. Water provides the medium for chemical reactions, transports nutrients, and allows the microorganisms to move

(Rynk, *et al.*, 1992). According to Richard *et al.* (2002a), the optimum moisture content for the biodegration of materials in compost varies from 50 to 70% in the raw materials used to make the pile and the composting phases. On the contrary, Dougherty (1999) suggested that moisture content should be kept between 40 to 60% on a mass basis.

Below a level of 40% moisture content, degradation slows and below 15% microbial activity ceases completely. At a level above 70%, water begins to inhibit the flow of oxygen by lowering the free air pore space within the pile affecting the balance of elements needed by microorganisms to thrive (Fonstad *et al.*, 2003). Throughout the process, moisture content fluctuates as water is lost to evaporation (USDA, 2000), leaching, or precipitation. Monitoring moisture content several times is essential to guarantee the effectiveness of the process.

Water affects O_2 availability. Oxygen is needed because the microorganisms that degrade the organic matter are aerobic. Through aeration, O_2 is inserted into pile but the amount of air that is being supplied does not necessarily reflect the amount of O_2 used by the microorganisms. Microbes function in the aqueous environment of the pile and oxygen diffusion in water is slower than in air (USDA, 2000). Ideally, an O_2 concentration of no less than 5% should be available for the microbes to survive (USDA, 2000; Dougherty, 1999). If these conditions are not met anaerobic microorganisms will govern the degradation of the material, generate offensive odors, and slow the rate of decomposition.

During the process, as microorganisms degrade the organic materials pile porosity decreases as materials settle under their own weight (Dougherty, 1999). This reduction in porosity results in a decrease in air flow and a subsequent lower microbial activity (Richard, 2000). Furthermore, phytotoxic compounds can be produced as a result of anaerobic conditions (Epstein, 1997). Necessary aerobic conditions can be maintained by physically turning the mass, injecting air into the pile, or by passive aeration.

Temperature

Temperature is an indicator of microbial activity during the composting process, thus it should be monitored regularly. The composting process is characterized by mesophillic (25 to 45˚C) and thermophillic (>45˚C) temperatures (Epstein, 1997). When raw materials are blended and ideal conditions are present, microbes start to degrade the organic matter and as a result of their metabolic activities energy is released in the form of heat. The rising temperature in the pile allows the degradation to be more effective and enhances the probability of pathogen inactivation (Morse, 2001). The temperature rise is affected not only by the type of microorganisms present and the co-composting materials used, but also by moisture content, as well as the size and depth of carcasses in the composting materials (Mukhtar *et al.*, 2004).

Heat produced by microbial activity degrades carcasses, killing pathogens such as New Castle's Disease Virus, Infectious Bursal Disease Virus, salmonella, and coliform bacteria (Cummings *et al.*, 1993). Senne *et al.* (1994) evaluated a two-stage composting procedure to determine the survival of highly pathogenic avian influenza (HPAI) virus and the adenovirus that causes the egg drop syndrome-76 (EDS-76). In this experiment they found that two-stage composting was effective in destroying the adenovirus of EDS-76 and HPAI viruses in tissues from infected birds. Adenovirus of EDS-76 was isolated from only one of the 20 tissue composite bags recovered at the end of the first 10 days of the compost cycle. The bag of tissues from which the isolation was made had been positioned within the lower layer of birds near the outside of the compost bins, where recorded temperatures were lower. The virus HPAI could not be isolated at the end of the first 10 days of the compost cycle. No virus was isolated for either disease following 20 days of composting.

Furthermore, the high temperature allows hot air to leave the pile taking with it water and CO_2 which maintains an aerobic environment. As compost reaches maturity and stabilization, mesophillic temperatures predominate in the pile.

Stability and Maturity

Cooperband *et al.* (2003, cited by Gigliotti *et al.* 2005) defined stability as a function of the content of labile compounds in the composting mixture as well as the O_2 demand required for aerobic decomposition. They defined maturity as the capacity of the compost to support plant growth and related it to the presence of phytotoxic compounds, the oxygen demand and the evolution of organic matter. Wu *et al.* (2000) defined stability as the rate or degree of organic matter decomposition; maturity was defined as the degree of decomposition of phytotoxic organic substances produced during the active composting stage. Stability of the composts occurs when its temperature remains constant, is biologically less active, has a neutral to slightly alkaline pH, resembles humus, has an earthy smell and is dark brown to dark in color.

Specific guidelines to determine compost stability and maturity are not available in the composting industry. Researchers have made much effort to asses which characteristics of the compost can be utilized as specific indicators to accurately predict stability and maturity. The United States Composting Council (Thompson *et al.*, 2002) suggests some parameters to evaluate both of these properties with respect to the end use of the product. These include pH, salinity, nutrients, organic matter, moisture, particle size, inert material, pathogens, and presence of weed seeds.

Wu *et al.* (2000) established pH, electrical conductivity, O_2 production, evolution rate, seed germination rate, and dissolved organic carbon as criteria to monitor the stabilization and maturation processes. In addition, compost quality includes contents of N, P, K, and S which are essential nutrients for plants. Ammonium, nitrates, nitrites, pH, heavy metals, and germination index are criteria used to determine the quality of the compost (Thompson *et al.*, 2002).

Germination index is a phytotoxicity measurement that can be affected by the salinity of the compost. Das *et al.* (2003) suggest germination indexes greater than 70% for mature compost. On the other hand, Woods End Research Laboratory (2000) suggests a germination index greater than 90% for compost of high quality.

Animal Compost

Recently, animal mortality composting has been reexamined from a scientific perspective, as methods such as incineration or burial come under increased environmental scrutiny (Standford *et al.*, 2000). Poultry mortalities composting has led the research in this area. Research has also been conducted using this method to dispose of mortality wastes in different types of animal industries.

Fonstad *et al.* (2003) investigated the possibility of using compost as a viable method of dead animal disposal for hog producers in Saskatchewan, Canada. This management option was demonstrated and analyzed on a commercial hog operation over a two-year period. For the study, pile construction was made on a continuous basis as mortalities became available. The pile was built by constructing a manure/straw mix base 0.6 m thick with an area of 4 x 6 m. Groups of dead animals (120 to 150 kg per group) were placed on the base and covered with a 0.6 m thick mix of manure/straw.

Additional piles were constructed as each pile reached a limit of approximately 2000 – 2500 kg of carcasses. A pile constructed with only the straw/manure mix was used as a control treatment.

Results show an increase in temperature and oxygen consumption after the addition of carcasses to the pile. However, when addition of carcasses was completed and as compost reached stability, the volume of the pile was reduced by 50 to 60%. Incorporation of mortalities into the compost enhanced the nutrient content of the final product with increases over the control of 108, 130, and 51% for nitrogen, phosphorus, and potassium, respectively. In the mortalities compost, the mass of objectionable materials constituted 0.04% of the total material (dry-basis) and consisted of recognizable remains. Observations of the compost pile during pile turning revealed that the carcasses decomposed rapidly.

Standford *et al.* (2000) determined the suitability of year-round composting of lamb and mature sheep mortalities within the arid prairie of Canada. Four studies with varied depth of the mortality layer within the bin, number of layers of mortalities per bin, age of animal (lamb vs. mature sheep) and time of year (summer or winter) were performed using 2:1:1 (manure: mortalities: chopped straw) proportions. Results reveal that the larger the carcass, the greater the length of time required to complete composting. Composting neo-natal lambs was faster than composting mature sheep, although ambient temperatures may also had a minor influence on the speed of the process. Lambs were composted over the spring/summer period and mature sheep over the fall/winter period. Excluding the mature sheep composting, all flesh and wool was converted to a gray ash layer at the completion of the primary composting.

These researchers report that the majority of heavily ossified bones were intact at the end of the secondary composting of mature sheep. Composting two layers of neonatal lambs was as effective as composting a single layer, though double layers sustained higher temperatures. Based on these results, composting up to 1 ton of mortalities in a single layer may be successful, provided that the length of time to complete the process is not critical and that the compost is properly aerated.

Looper *et al.* (2002) utilized animal composting procedures and determined manure characteristics, composting temperatures, and length of time needed to compost dairy cows and calves. A compost pile was established by spreading a sand/manure mixture on a level site to a depth of approximately 25 to 30 cm. A single mortality was then placed on the mixture and covered with the compost mixture (50:50 manure: old feedstuff) to a depth of 0.45 to 0.61 m.

Results showed that internal temperatures on the compost piles reached 61.3˚C within three days of pile establishment. Temperatures gradually declined thereafter and averaged 43.5 ± 0.4˚C during the last three weeks of the experiment. At six weeks, carcasses were 70 to 75% decomposed. Carcasses uncovered at four months were more difficult to find. Approximately, 7 to 10 bones per carcass were found and 100% of the flesh was decomposed. Post-composting percentages of C and N were reduced by approximately one-half the pre-composting value. In this study, the estimated cost of composting a mature dairy cow was $2.00 per mortality.

Compost utilization

Compost can be utilized as a source of nutrients for agronomic crops and horticulture ((Kideer, 2001; Stofella *et al.* 1996), erosion control (Risse and Faucette, 2001), bioremediation (Kiikkilä *et al.*, 2001), or as cover material for the production of a new compost. For the utilization of compost as a source of nutrients, the chemical (i.e. organic matter, nitrogen, macro and micro nutrients) and physical characteristics (i.e. water holding capacity and particle density) are pertinent. Effects upon soil and plant characteristics are also important.

Epstein (1997) states that the application of compost to soil favorably affects water holding capacity, erosion control, cation exchange capacity, pH, macroelements availability, and soil structure, porosity and aeration. Large quantities of compost applied during several years are needed to observe changes in soil characteristics.

Sullivan *et al.* (2002) reported that the application of food waste compost did not affect the performance of the grass *Festuca arundinacea* during the first year of application, but it gave a higher yield during the second and third year. This forage grass extracted more nitrogen from the soil when organic fertilizer was applied with the compost than without, even though the compost did not affect nitrogen extraction from the inorganic fertilizer. These results suggest that the mineralizable nitrogen from the compost and the nitrogen from the inorganic fertilizer can be considered as additives for plant growth. Yermiyahu *et al.* (2001) reported that compost application regulates boron uptake from *Capsicum frutescens* (pepper) by controlling the concentrations of this micromineral in the soil which has a beneficial effect because an excess of boron is phytotoxic.

The origin of compost will determine its potential use as a source of nutrients in agriculture, the eventual decrease in costs for waste disposal, and the contribution to reduce environmental pollution. Research is needed in Puerto Rico to determine the effectiveness of composting animal mortalities in farms and slaughterhouses.

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CHAPTER 2

Evaluating yard trimmings as a bulking agent during the composting of organic slaughterhouse wastes

Abstract

The objective of this experiment was to evaluate the efficacy of fresh (FYT) and semi-decomposed (SCYT) yard trimmings as bulking agents (BA) on the composting process of organic wastes from a commercial slaughter house (SHW). Treatments consisted of combinations of BA (FYT and SCYT) with layers of SHW (none, single or double), and seven composting phases. Mixtures were weighted in a 2:1 (BA:SHW) proportion and placed in composting bins of identical dimensions (0.91 m^2). Moisture level was adjusted to 60% on a weight basis at the start of each composting phase. Temperature was recorded daily to determine the time (d) needed to reach the first and second heat cycles. Composting characteristics such as: pH, carbon to nitrogen (C:N) ratio, organic (OM) and inorganic matter (IM), and nitrogen (N) concentration were measured at 0 d, at the peak of the first and second heat cycles, and 0, 20, 40 and 60 d of maturation in the composting process. Data were analyzed using a 3 (layers of SHW) x 2 (BA) x 7 (composting phases) factorial design and ANOVA procedures of SAS. All treatments of FYT and SCYT exhibited thermophillic temperatures after three days of composting. Regardless of treatment combination, pH ranged from 7 to 8. Contents of OM were higher ($P<0.05$), but IM was lower ($P<0.05$) in FYT than SCYT at all composting phases regardless of SHW layers. Nitrogen content was ($P<0.05$) higher for treatments using SCYT as BA. At 60 d of maturation N and C:N values were similar for

all treatments. At the end of the maturation phase, the C:N ratio was similar for none and single layers of SHW, but lower ($P<0.05$) in double layers of SHW. In summary, FYT and SCYT resulted in excellent BA for the composting of SHW. Treatments with SCYT composted better than FYT as judge by the OM decomposition and N content. Double layers of SHW presented higher temperatures and N content. Further studies are needed to evaluate the use of this compost as an organic nutrient source.

Introduction

The disposal of slaughterhouse wastes (SHW) represents a major concern for meat producers in Puerto Rico. Presently, SHW are disposed in landfills therefore causing environmental pollution. The application of biological procedures and technologies for the reduction and stabilization of such wastes has been extensively studied. Composting is a biotechnological procedure that has been evaluated with regard to the rate of decomposition of animal tissue wastes with an excellent outcome (Morse 2001; Eldridge, 1996). This procedure also offers the advantage of reutilization of the composted product as an organic nutrient source. A preliminary study conducted in our laboratory demonstrated that the success of SHW composting is influenced by the characteristics of the bulking agent (Sanabria *et al.,* 2003). Reports by López *et al.* (2003) and Sanabria *et al.* (2003) indicated that dead animal tissue (SHW and discarded fish) mixed with wood shavings or hay compost more slowly than SHW mixed with yard trimmings. However, evaluation of other bulking agents to determine appropriate conditions for composting of SHW is needed.

The objective of this study was to evaluate fresh (FYT) and semi-decomposed (SCYT) yard trimmings as bulking agents (BA) over the composting of SHW.

Methodology

Slaughterhouse wastes (small ruminant digestive and reproductive tracts) were obtained from a commercial abattoir located at the Lajas Agricultural Experimental Sub-Station of the University of Puerto Rico at Mayagüez (UPRM). Freshly-cut (FCYT) and semi-decomposed yard trimmings (SCYT) were obtained from the composting facilities located at the Alzamora Farm of UPRM and used as bulking agents (BA) for single or double layers of animal tissue wastes (SHW) placed in composting bins of identical dimensions (0.91 m^2). Both BA (without SHW) were used as a control. In treatments containing animal tissue, SHW were placed between 45.72 cm layers of the bulking agent in a 2:1 proportion (w/w). The compost piles were hydrated to obtain approximately an initial 60 to 70% moisture content on a weight basis and were maintained constant over the entire composting process. Triplicate samples from the SHW and BA (composting phase 0) were obtained to determine initial dry matter (DM), organic matter (OM) and inorganic matter (IM), C and N content, and C:N ratio, (AOAC, 1991; Rynk, 1992).

The DM of a sample was determined using a 65° C oven for 48 hours. The IM content was determined by igniting an oven dried sample in a muffle furnace at 600°C for six hours. The remaining sample represented the IM fraction and the incinerated material estimated assuming 56% of the material was OM. The C content was established by knowing the OM content. Total N determination was performed using the micro-Kjeldahl procedure (AOAC, 1991).

In each treatment, temperature was monitored daily with thermometers (Sudbury Compost Thermometer, NASCO, Fort Atkinson, WI) situated at the center of each pile. Four core samples from each treatment were obtained in the seven composting phases (Figure 2.1). Composting phase 1 and 2 started once the temperature reached its

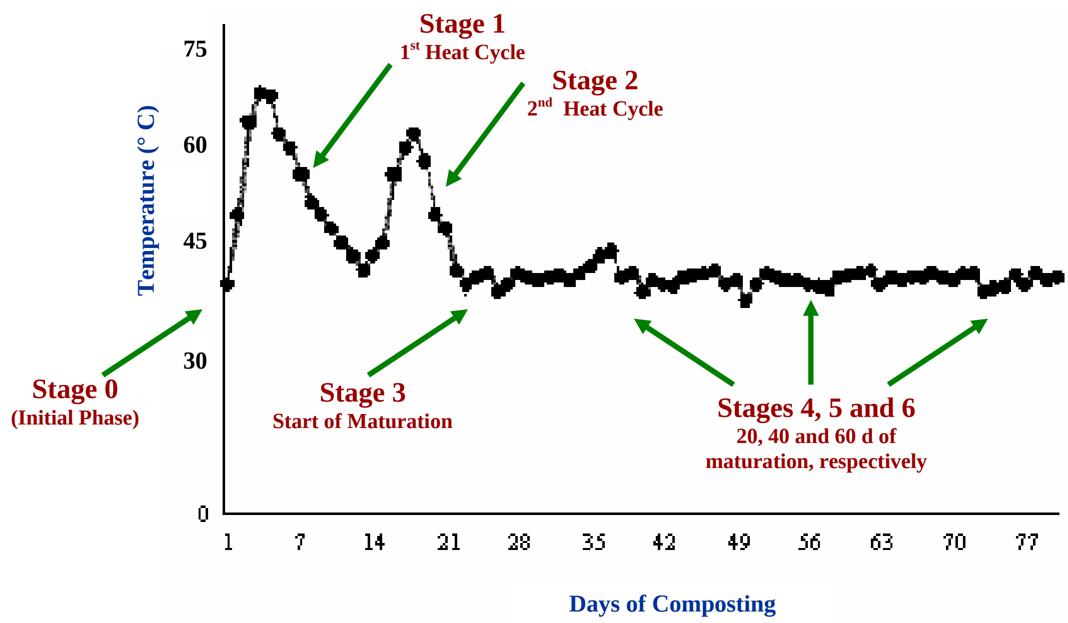


Figure 2.1. Sampling times during the composting process of slaughterhouse wastes using freshly cut or semi-composted yard trimmings as a bulking agent.

maximum value and then dropped 5.56° C after the first and second heat cycles, respectively. Triplicate samples from each treatment were collected at 0, 20, 40, and 60 d of maturation, composting phases 3 – 6, respectively. Samples for each treatment and composting phase were collected after turning the compost piles, composite, and analyzed to determine pH and chemical composition as described for the fresh material. Data were analyzed according to a completely randomized design with a 3 (layers of SHW; none, single or double) by 2 (bulking agents; FYT or SCYT) by 7 (composting phases) factorial arrangement (Steel and Torrie, 1990) using the General Linear Model subroutine of SAS (1990). Treatment means showing significant difference in the ANOVA were ranked using the Tukey test option of SAS (1990). All statements of significance were based on the probability level of 0.05 unless otherwise noted.

Results and Discussion

As expected, initial chemical composition of the mixtures evaluated in this experiment reflected the individual characteristics of materials utilized in each composting treatment (SHW, FYT, and SCYT). All treatments evaluated contained an initial C:N ratio between 13:1 and 35:1. Theoretically, a proper mix of composting materials should have a value within the range from 25:1 to 30:1. (Looper 2001; Thompson *et al.*, 2002).

Changes in nutrient concentration within mixtures evaluated over the full composting process were also related to the original characteristics of the compost piles. The types of BA evaluated differ in composting characteristics through the process. Nitrogen content was higher ($P<0.05$) in treatments containing SCYT than in mixtures with the FYT during the whole process (Figure 2.2). Piles made with FYT as BA had higher ($P<0.05$) OM content and a lower IM content than compost elaborated using SCYT regardless of levels of SHW (Figures 2.3 and 2.4). The C:N ratio, an indicator of N availability for the process of biological degradation (Thompson *et al.* 2002) was down-trending throughout the composting phases; but was variable, and reached a final value between 12:1 and 18:1. The BA and layers of SHW influenced the changes of C:N in the composting piles through the composting phases. Piles made using SCYT as BA had lower ($P<0.05$) C:N ratio all the way through the process but values were similar at the end (Figure 2.5). Freshly cut yard trimmings had lower ($P<0.05$) pH than SCYT during the second and third composting phases, however, pH was similar for both BA during the maturation period (Figure 2.6). The pH value in compost depends on the types of materials used. Typical compost pH ranges between 6.8 and 7.3 (Watson, 2003).

In this experiment, the layers of SHW influenced the composting process.

Nitrogen content was higher ($P<0.05$) in treatments containing single or double layers of SHW than in BA composted alone (Figure 2.7). The C:N ratio was greater ($P<0.05$) in treatments without SHW than one or two layers of SHW throughout the composting phases but values were similar at the end (Figure 2.8). In this experiment, for all layers of SHW, pH varied but remained nearly neutral over the composting process regardless of layers of SHW (Figure 2.9). These changes in chemical composition observed during the composting process, suggests that microbial ecology was changing and different substrates were being use, which would imply that a very heterogeneous population of microbes that can use C and N as a source of energy existed in the composting process of SHW (Epstein, 1997).

All treatments evaluated in this experiment reached thermophillic temperatures (higher than 45°C) during the first and second heat cycles (Figure 2.10). Changes in temperature have been identified as the first sign of microbial activity in the composting process. Heat production is the fermentation end-product of an active microbial population utilizing organic wastes as energy source (Dziejowski and Kazanowska, 2002). In this experiment, treatments composed of BA alone and single layers of SHW reached the beginning of the maturation phase faster than treatments with double layers of SHW. However, higher temperatures ($P<0.05$) were observed in piles containing double layers of SHW than in treatments with a single layer or BA composted alone. The duration (d) of thermophillic temperature within each heat cycle was also longer for treatments containing double layers of SHW. These results suggest that piles containing greater proportions of animal tissue compost slower than vegetative material composted alone, but reach higher temperature during longer periods of time as seen in both heat cycles. This greater heat production may contribute to the elimination of undesirable and pathogen microorganism during the composting process.

For all treatments evaluated in this experiment, neither rodent infestation nor offensive odors were a problem during the composting process. Final appearance of the composted products was similar to typical organic fertilizers (Figure 2.11).

Conclusion

These results suggest that FYT and SCYT can be effectively used as BA in the composting of organic wastes from slaughterhouses. Using SCYT can result in a better BA as shown by its chemical characteristics (lower OM and C:N, and higher N content). Composting can be effectively performed with single and double layers of SHW. Experimental analyses suggest that a longer period of time is needed to decompose double layers of SHW, even though it reaches higher temperatures. Further studies are needed in order to evaluate the potential use of composted SHW for this purpose.

Implications

This experiment offer meat processing plants an alternate method to dispose SHW, therefore it can potentially reduce organic contaminants in landfills. The data presented suggests that composting single and double layers of SHW with FYT and SCYT resulted in a successful method to reuse this type of waste. Consequently, these results can aid governmental agencies to prove the effectiveness of this method and implement it as recycle technique to reduce wastes.

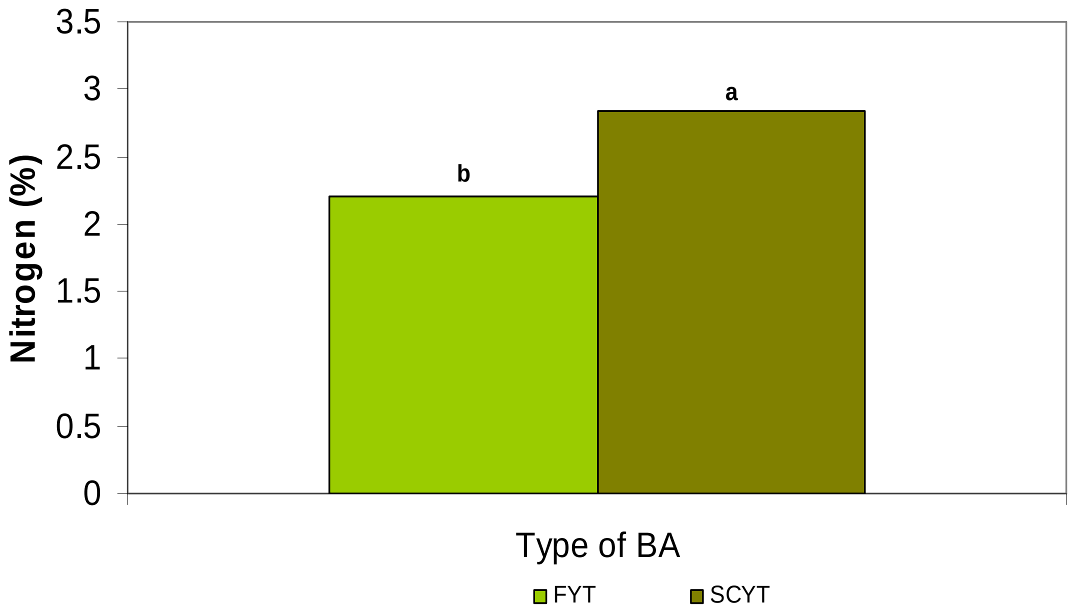


Figure 2.2. Effect of bulking agent on nitrogen changes. Different letters shows significant difference between the treatments.

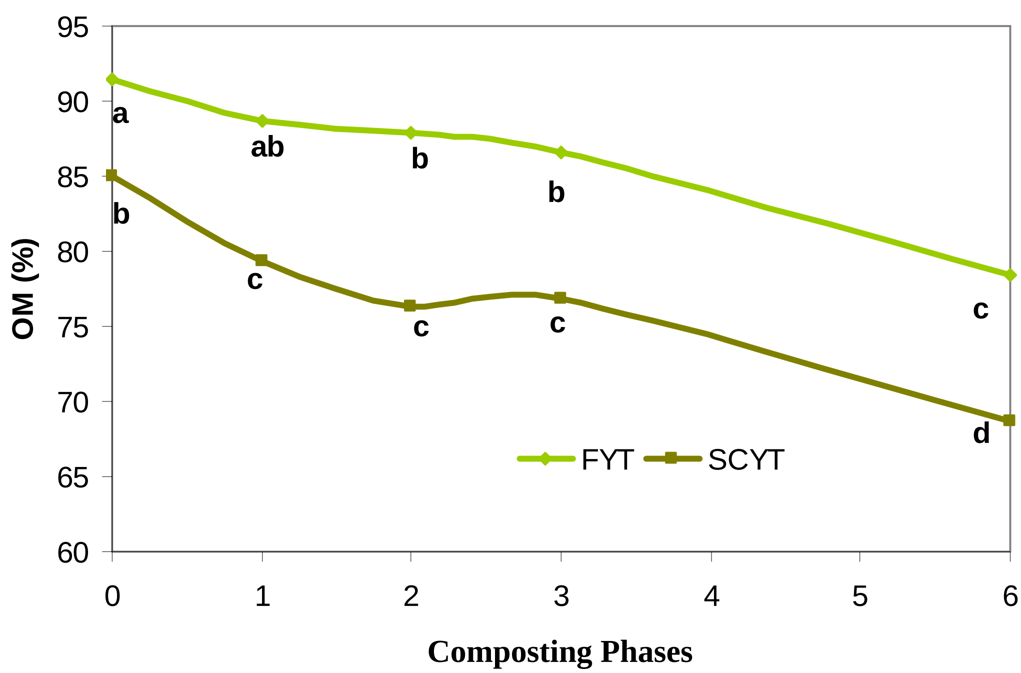


Figure 2.3. Effect of bulking agent on organic matter changes through the composting phases. Different letters shows significant difference between the treatments.

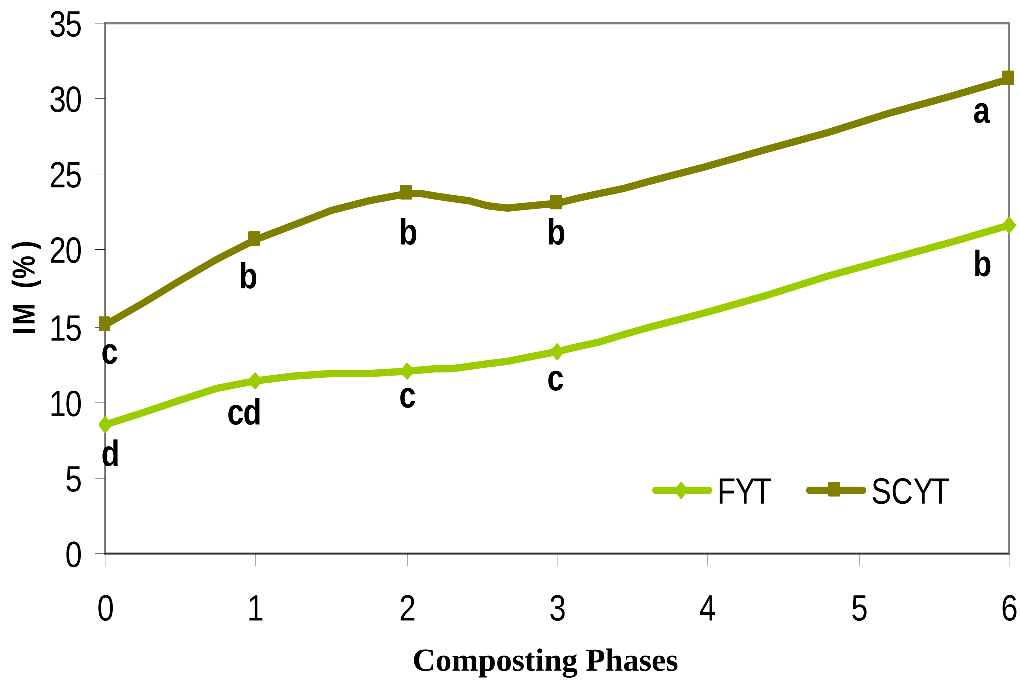


Figure 2.4. Effect of bulking agent on inorganic matter changes through the composting phases. Different letters shows significant difference between the treatments.

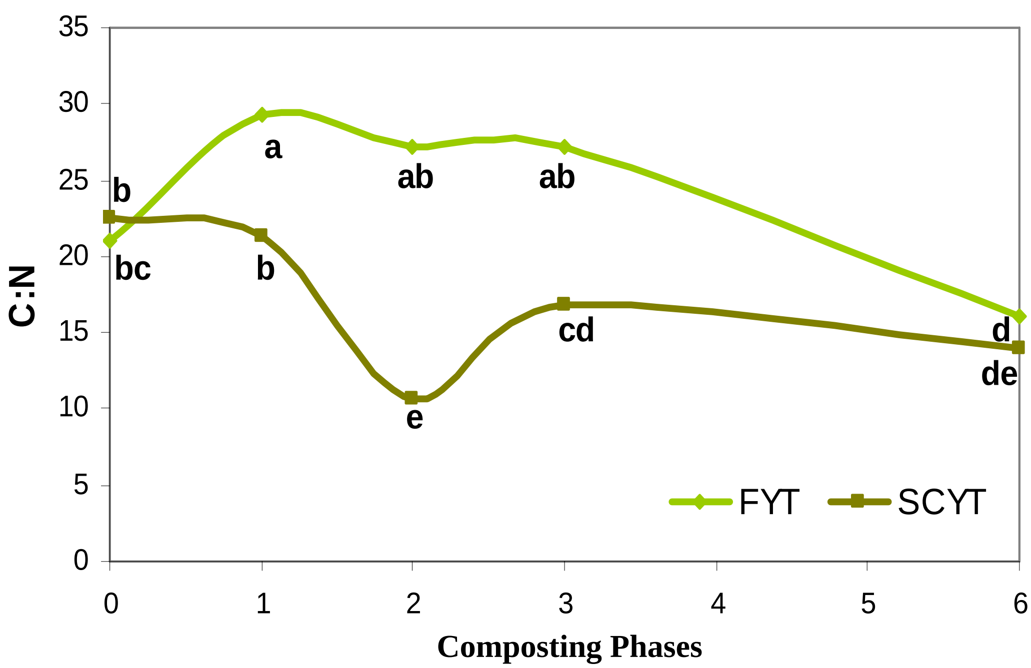


Figure 2.5. Effect of bulking agent on C:N changes through the composting phases. Different letters shows significant difference between the treatments.

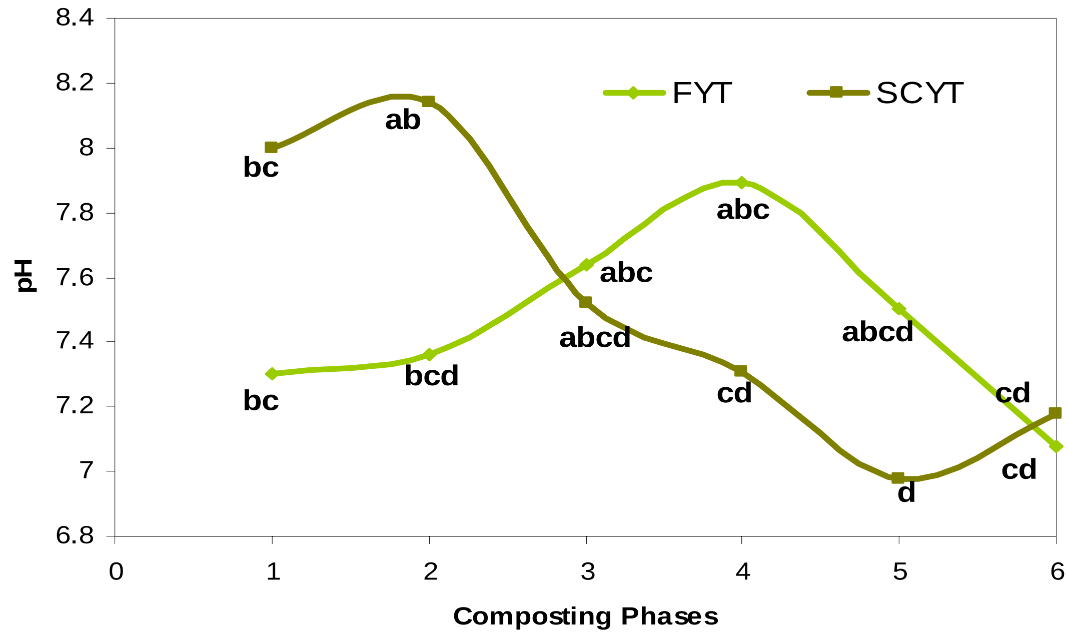


Figure 2.6. Effect of bulking agent on pH changes through composting phases. Different letters shows significant difference between the treatments.

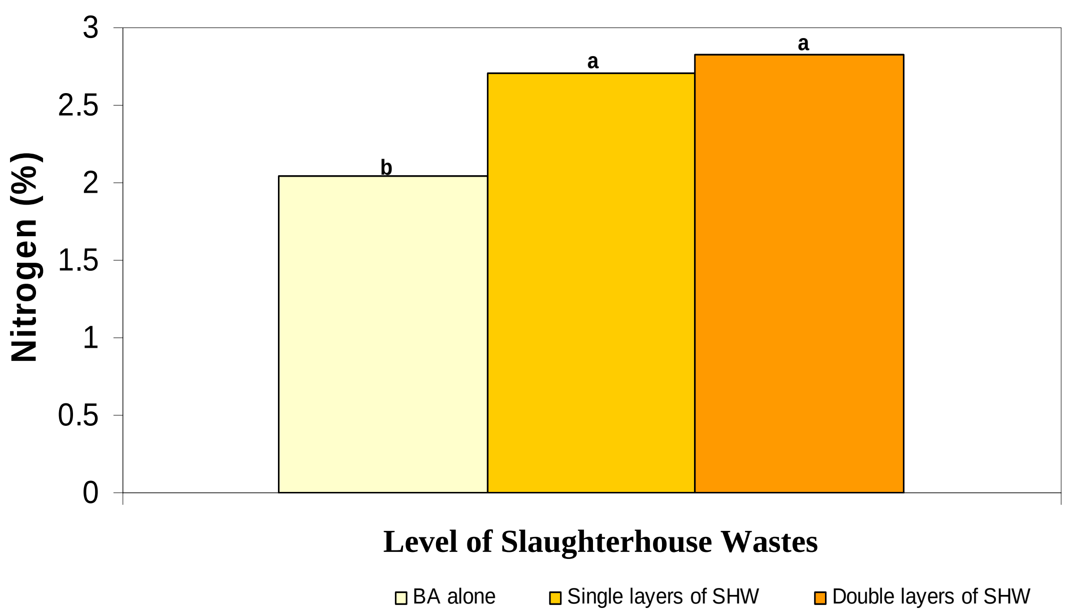


Figure 2.7. Effect of levels of slaughterhouse wastes on nitrogen changes. Different letters shows significant difference between the treatments.

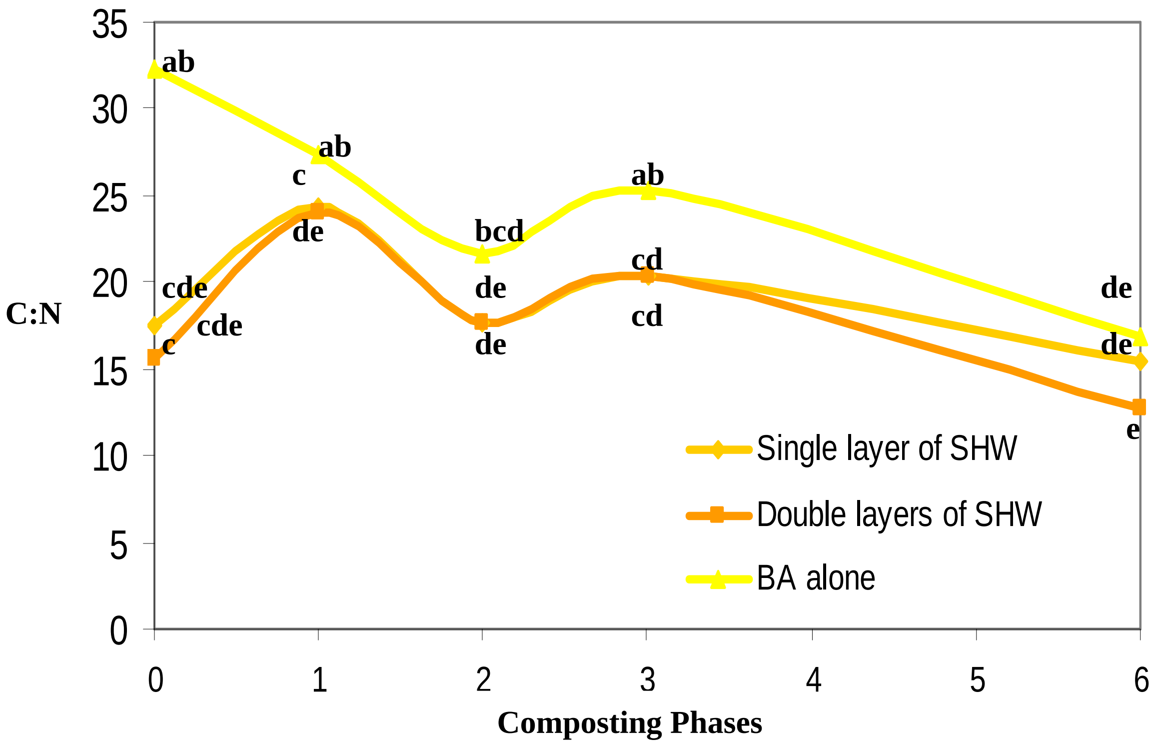


Figure 2.8. Effect of levels of slaughterhouse wastes on C:N changes through the composting phases. . Different letters shows significant difference between the treatments.

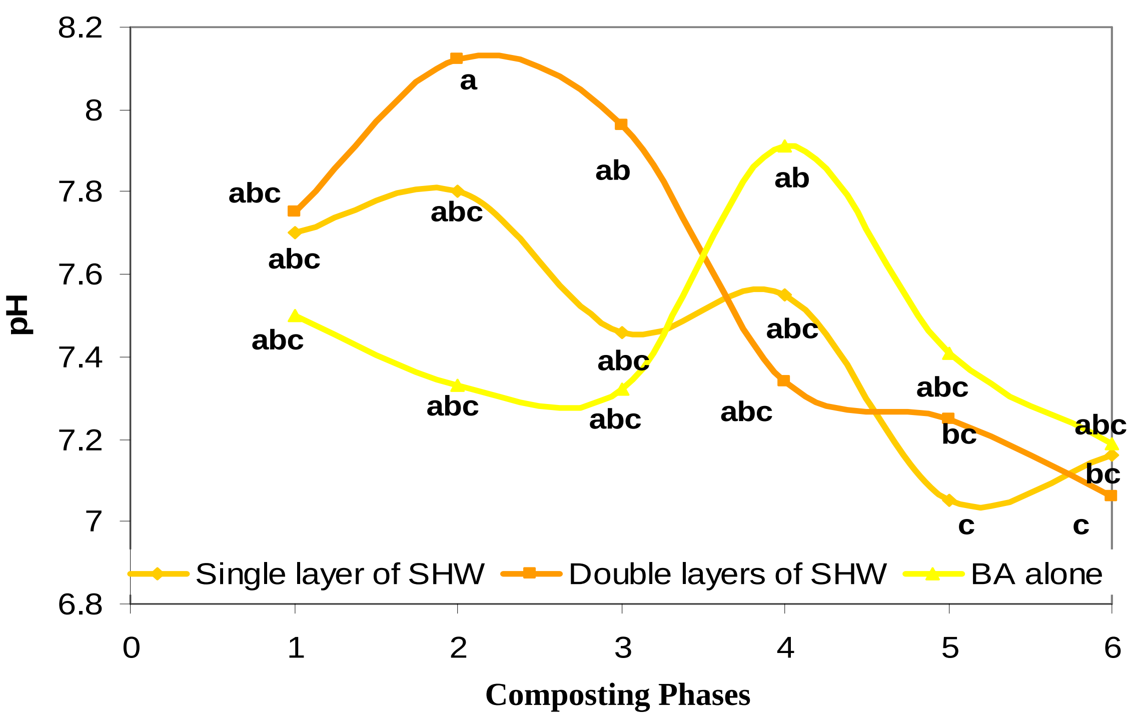


Figure 2.9. Effect of levels of slaughterhouse wastes on pH changes through composting phases. . Different letters shows significant difference between the treatments.

Table 2.1. Standard error and significance levels of the chemical composition of experimental composts.

		P>|t|[1]						
Component	SEM[2]	BA[3]	LSHW[4]	CS[5]	BA x LSHW	BA x CS	LSHW x CS	BA x C x CS
OM	1.1381	0.0001	0.1209	0.0001	0.3528	0.0443	0.2942	0.7558
IM	1.1381	0.0001	0.1209	0.0001	0.4104	0.0443	0.2942	0.7558
C	0.6323	0.0001	0.1209	0.0001	0.3528	0.0443	0.2942	0.7749
N	0.3635	0.0001	0.0001	0.0001	0.8113	0.5379	0.5379	0.6298
C:N	1.684	0.0001	0.0001	0.0001	0.9271	0.0001	0.0005	0.1734
pH	0.2351	0.4677	0.2800	0.0001	0.8856	0.0001	0.0073	0.4473

[1]Significance levels
[2] Standard error of the mean
[3] Bulking agent
[4] Layers of slaughterhouse wastes
[5]Composting stage

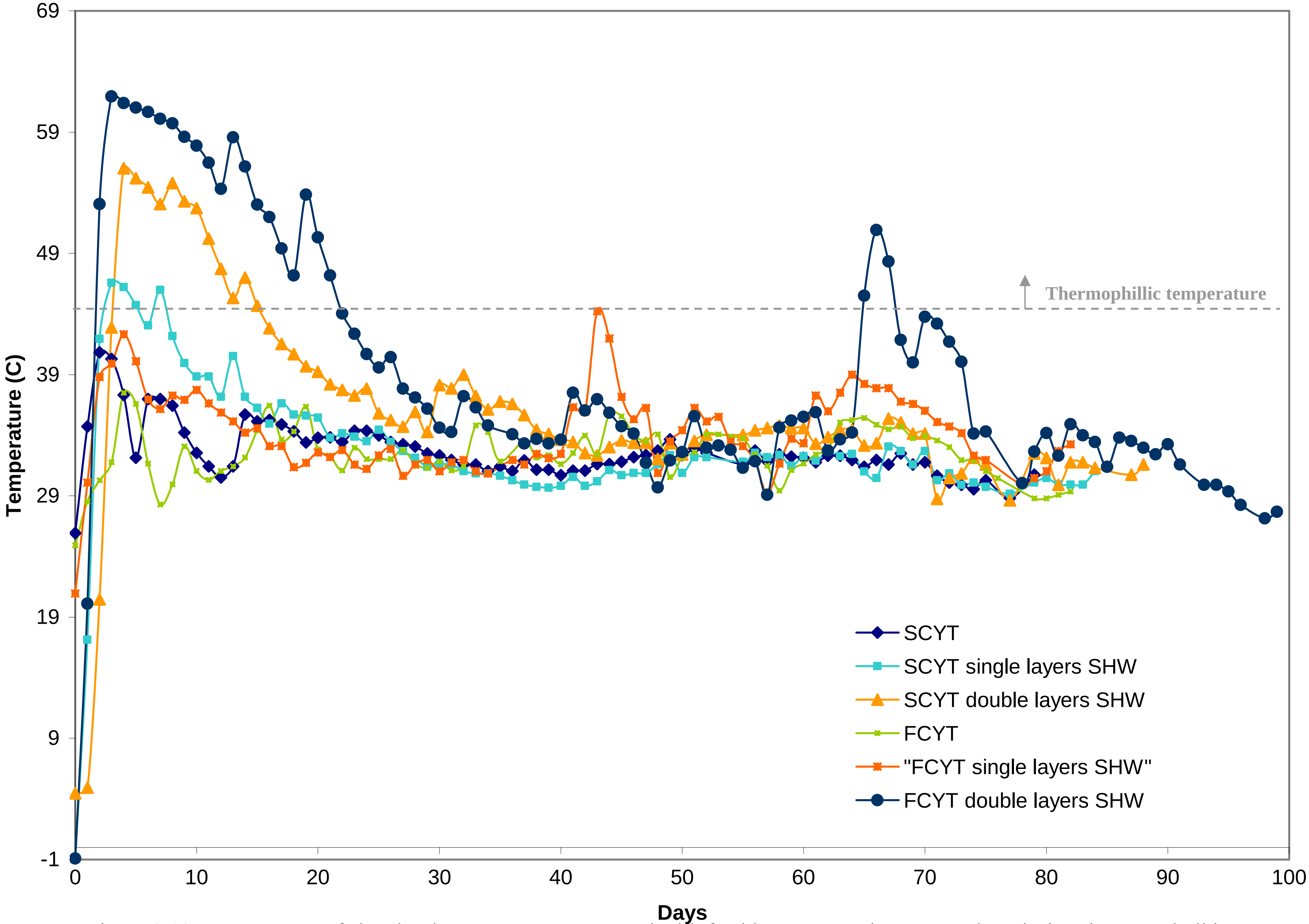
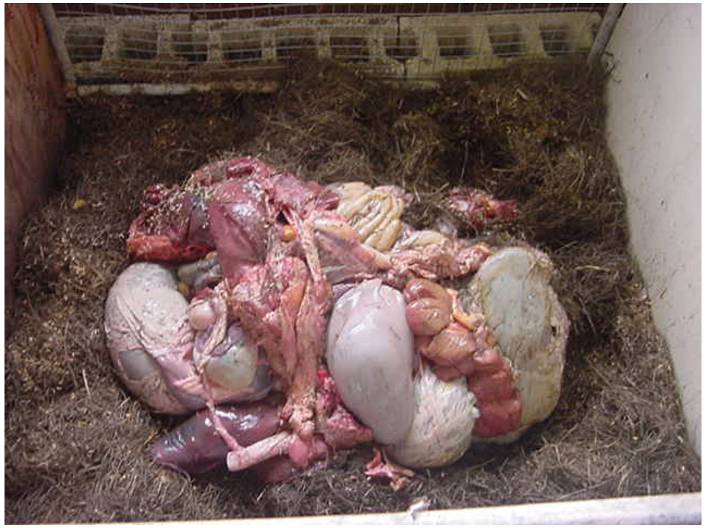


Figure 2.10. Temperature of slaughterhouse wastes composted with freshly cut or semi-composted yard trimmings as a bulking agent.



d 0

d 0

d 91

d 99

Figure 2.11. Initial and final appearance of SHW composted with or semi-composted fresh yard trimmings as bulking agents.

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CHAPTER 3

Quality and agronomic performance of slaughterhouse wastes compost

Abstract

Determining the quality of compost is important in order to assign a suitable end use for the product. The objectives of this study were to evaluate physical and chemical characteristics of composted slaughterhouse wastes (Chapter 2), and to determine its stability and maturity. The agronomic response of the gramineous forage *Brachiaria brizantha* cv Mulato to application of 0 and 400 kg N/ha as SHW compost was also evaluated. The treatments evaluated (described in Chapter 2) were none, single, and double layers of SHW with semicomposted (SCYT) or fresh yard trimmings (FYT) as bulking agents (BA) and three commercial composts (CC1, CC2, and CC3). Physical, chemical, and biological measurements such as pH, OM, total N, N-NH_4, N-NO_3, C:N ratio, mineral profile, cation exchange capacity, particle density, electrical conductivity, relative germination, root elongation, and germination index were used to asses the SHW compost quality. Among treatments, FYT composted alone had higher ($P<0.05$) OM (663 to 687 g/kg) and C (368 to 382 g/kg) concentration; CC1 and CC2 presented higher IM concentration (779 and 743 g/kg, respectively), but C:N ratio did not differ (10 to 15). The mineral profile, cation exchange capacity, particle density, and electrical conductivity of the experimental treatments showed typical values for composts. No significant differences were observed for relative germination (>90%), but root elongation (>79%), and

germination index (>87%) differed ($P<0.05$). At six weeks of grass growth, chlorophyll, N (%), and biomass (g/pot) exhibited low values in all treatments. The treatments evaluated resulted in a mature and stable compost, which can be a potential source of nutrients for plant growth. However, as indicated by the agronomic performance, compost alone cannot provide the optimum quantities of nutrients that cv Mulato needs for rapid development even at equivalent N rate of 400 kg/ha.

Introduction

Compost quality is defined by its stability and maturity. Mature and stable compost releases nutrients to the soil gradually during a crop growth cycle. In contrary, immature and unstable compost can cause problems during its storage and subsequent use due to anaerobic conditions and the generation of phytotoxic compounds (i.e. volatile organic acids).

Stability refers to the organic matter's degree of decomposition. Moreover, a definition provided by Thompson (2002) refers as the stage in composting at which microbial activity diminishes with a corresponding decrease of available organic carbon and other energy sources.

During the composting process microorganisms utilize carbon as their source of energy. At first, microbes decompose the readily available carbon (i.e. starches, sugars, amino acids) but as it becomes less available in the pile, they start to utilize other sources that are harder to degrade (i.e. cellulose, hemicellulose, lignin), thus affecting the rate of decomposition. Epstein (1997) reported that when these changes occur the rate of decomposition becomes slower and the compost starts to stabilize. Changes in temperature are the primary indicator of microbial activity and consequently of stability. Continuous mesophillic temperatures, following the thermophillic phase of composting, indicate a stable product, since they result from decreased microbial activities.

Maturity is an organo-chemical state that refers to the degree of decomposition of phytotoxic compounds produced in the active phases of compost and indicates the presence or absence of these compounds in stable to very stable compost (Thompson, 2002; Wu et al., 2000). Phytotoxicity can be caused by excessive concentrations of soluble salts, heavy metals, ammonia, carbon dioxide, and fatty acids or a high degree of compactation (Wu *et al.*, 2000; Epstein, 1997). As a result of phytotoxicity, germination and plant growth can be adversely affected.

The maturity of compost can be determined using different parameters. Physically, the change in color and odor that occurs in the pile can be indicative of degradation activities. The process of color change is initiated when soluble organic compounds are oxidized during the thermophillic phase. Color will be darker as more complex polymeric organic constituents are oxidized and humic compounds accumulate (Thompson, 2002). In addition, mature compost should have a distinctly different odor from that of the raw materials.

The humus like smell of the mature compost is caused by actinomycetes and fungi. Becker (1995; cited by Epstein, 1997) reported that the characteristic odor of soil is primarily the result of the gasses, geosmin and 2-methylisoborneol, which are by-products of fungi and actinomycetes. However, other characteristics should be used concomitantly to establish maturity and stability, as temperature, odor and color are too subjective to be used as sole criteria.

Presently, there is neither an accepted methodology nor a specific parameter to determine the maturity and stability of the compost. However, the use of a combination of different physical, chemical, and biological criteria such as pH, OM, total N, N-NH_4, N-NO_3, C:N ratio, mineral analyses (Al, B, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Si, Zn), cation exchange capacity (CEC), particle density, electrical conductivity, and germination index can be used to asses the compost's quality.

The objective of this study was to evaluate the physical and chemical parameters following the composting of none, single, and double layers SHW with FYT and SCYT and then assess the stability and maturity of the resulting product. The agronomic response of a grass crop fertilized with the compost was also evaluated. Experimental composts were also compared, in terms of stability and maturity, with three commercial composts.

Methodology

Stability and maturity tests

Finished composts, obtained from previous experiments (Chapter 2) were compared with three commercial composts produced in Puerto Rico and tested to determine their stability and maturity. Two of the commercial composts were made from vegetable residues, yard trimmings and goat manure with or without alluvial residues (CC1 and CC2, respectively). These were obtained from the composting facilities located at the Alzamora Farm, UPRM. The third commercial compost (CC3) used was made from sewage sludge and wood chips and was obtained from Caribbean Composting at Arecibo, PR.

To determine compost stability and maturity samples from each compost were subject to analysis for dry matter, OM, IM, N, and C content, and C:N ratio, as described in Chapter 2. The pH was measured using deionized water and compost at a rate of 2:1 (v/w). Soluble salts content was measured using the electrical conductivity test described by SMEWW (1999). Cation exchange capacity was measured using the method of ammonium saturation (Thompson *et al.*, 2002; Black *et al.*, 1965). The concentration of N-NH_4 and N-NO_3 were determined from a solution of a saturated paste extraction (Thompson *et al.,* 2002; Keeny and Nelson, 1982). Particle density was measured using the method described by Blake and Hartge (1986) but instead of water, ethanol was used as the solvent. Samples from each compost were digested in a microwave system (10 min.) and analyzed for total nutrient concentration (P, K, Ca, Mg, Fe, Mn, Zn, Cu, B, Mo, Cd, and Na) in a commercial laboratory (Soil, Plant and Water Laboratory, University of Georgia).

Data obtained from chemical and physical assays were analyzed according to a completely randomized designed with 9 treatments (FCYT and SCYT with none, single and double layers of SHW; CC1, CC2, and CC3) replicated three times using the GLM procedure of SAS (Steel and Torrie, 1990). Treatment means showing significant difference in the ANOVA were ranked using the Tukey multiple comparison tests option (SAS, 1990). All statements of significance were based on the probability level of 0.05 unless otherwise noted.

The finished and commercial composts maturity was evaluated using biological assays. Compost extracts (2:1 water to compost, v/w) were filtered using Whatman No.1 paper and placed in petri dishes with five corn (*Zea mays*) seeds. Petri dishes containing the extracts and seeds were placed in a germination incubator in a dark for seven days at 27°C. Distilled water was used as a control. Germination percent and root elongation relative to water were determined at the end of the incubation period. The germination index was the product of the germination percent of each treatment relative to water (Das *et al.* 2003; Thompson *et al.*, 2002).

Data from biological assays were analyzed according to a completely randomized designed with 10 treatments (FCYT and SCYT with none, single and double layers of SHW; CC1, CC2, CC3, and water) replicated five times using the GLM procedure of SAS (Steel and Torrie, 1990). Treatment means showing significant difference in the ANOVA were ranked using the LSD procedure.

Agronomic value of the compost

Experimental and commercial composts were compared to determine their ability to provide nutrients to crops (agronomic value). The experiment was performed using 50 pots (10 treatments with 5 replications) with a 15.24 cm diameter located in a greenhouse. The type of soil used was a Fraternidad series (Fine, smectitic, isohypothermic Typic Hapluderts). Two application rates (0 and 400 kg N/ha) were evaluated for a six week period using *Brachiaria brizanta cv Mulato* as vegetative material. Compost were applied in the first two centimeters of soil in the pot and blended uniformingly. Plant seeds were germinated and seedlings transplanted to the pots when they reached 10 cm height.

At 42 d, post planting biomass, leaf color, and N concentration were determined. Biomass was determined as the weight of dry vegetative material (g/pot). Leaf color was determined from the concentration of chlorophyll (Minolta SPAD-502 Chlorophyll Meter, Spectrum Technologies, Inc, Minolta Co., LTD Japan). Five leaves per pot per treatment were selected to measure chlorophyll concentration. Three measurements were taken from the middle of each leaf and averaged to obtain a final value per leaf. The N concentration was measured by distillation following the micro-Kjeldahl digest (AOAC, 1991).

Data were analyzed according to a completely randomized design with 10 treatments replicated five times using the GLM procedure of SAS (Steel and Torrie, 1990). Treatment means showing significant difference in the ANOVA were ranked using the LSD procedure.

Results and Discussion

Stability and maturity tests

The pH and chemical composition of the experimental and commercial compost differed among treatments (Table 3.1). The pH values ranged from acid to neutral. Commercial composts (CC3 and CC1) presented higher ($P<0.05$) pH than the experimental composts. The highest numerical pH was found in CC3 (7.05). All pH values observed are consider appropriate for stable compost. Dry matter content was highest ($P<0.05$) for CC3, than experimental composts, while experimental treatments and CC1 did not differ. Organic matter in CC2 was similar to treatments containing SCYT, but higher in FYT. Among treatments, the three compost made with FYT had higher ($P<0.05$) OM (663 to 687 g/kg) and C (368 to 382 g/kg) concentration than the SCYT composts. The composts CC1 and CC2 presented markedly higher ($P<0.05$) IM concentration (779 and 743 g/kg, respectively). Treatments with FYT had higher ($P<0.05$) N concentration than SCYT and CC composts. CC1 and CC2 presented lower ($P<0.05$) N content than experimental composts with SCYT. All three CC presented lower ($P<0.05$) N concentration especially CC1 and CC2. Carbon to nitrogen ratio ranged from 10.99 to 15.01 being relatively similar among treatments.

Previous reports are in agreement with these results in terms of chemical compositions for their composts. Madrid *et al.* (2000) evaluated compost elaborated with different combinations of coffee husks, guinea-grass stem, fruits and vegetables wastes, and rabbit manure. The pH values varied from 6.9 to 7.3, and total Kjeldahl N and organic carbon concentration ranged from 12.5 to 23.3 g/kg and 259.1 to 152.4 g/kg, respectively. The C:N ratio and electrical conductivity values did not differ significantly among treatments and varied from 11 to 19, and 4.06 to 5.89 dS/m, respectively. Overall, the compost elaborated from coffee husk and rabbit manure were the highest quality.

Standford *et al*. (2000) evaluated the feasibility of year-around composting of lamb and mature sheep mortalities and determined the value of these composts as fertilizer. Finished compost of both lamb and sheep had a 7.1 pH value. Chemical analysis showed that the lamb compost had a 473 g/kg of moisture, 231 g/kg of C, 18 g/kg of N, 12.7 C:N ratio, and 12.3 dS/m electrical conductivity. Corresponding figures for the sheep compost were 502 g/kg, 283 g/kg, 23 g/kg, 12.2, and a 14.2 dS/m. Cummings *et al.* (1993) evaluated the chemical characteristics of co-composted poultry mortalities and poultry litter. Such compost results in a composition of 362 g/kg moisture; 309 g/kg ash; 38 g/kg of total N; 363 g/kg of total C, and a C:N ratio of 9.8.

Compost is a source of nutrients for plant growth. Evaluating the quantities of nutrients in the compost may help determine if this product meets the requirements of agronomic crops. In general, and because of the decomposition that organic wastes undergo in the composting process, there is a limited quantity of macro and micronutrients in the compost.

The CEC is the capacity of compost, mineral, and organic soils to hold exchangeable cations to counter balance the fixed negative charges in the material (Thompson, 2002). These characteristics influence nutrient availability and retention (Havlin *et al.*, 2005). Harada and Inoko (1980; cited by de Guardia *et al.,* 1998) reported that values greater than 100 meq/100g OM for animal wastes constitute a stability indicator. Table 3.2 shows the CEC values obtained for the evaluated treatments.

Treatments of SCYT and FYT with all combinations of SHW had statistically similar CEC. Treatments with SCYT had also similar CEC than commercial composts, but FYT composted alone or in combination with single layers of SHW had higher ($P<0.05$) CEC than commercial composts. In related experiments, Bernal *et al.* (1998) presented similar values (124.4 meq/100g) for compost made of sewage sludge and cotton waste, whereas García and Valencia (2004) found low CEC values (46.3 meq/100g) for poultry litter compost.

For a compost to be stable and mature N-NH_4^+ and N-NO_3^- must be present in an adequate proportion. In theory, stable composts must have lower N-NH_4^+ than N-NO_3^- concentration. Furthermore, these two forms of N are important because plants absorbs N as both N-NH_4^+ and N-NO_3^-. In the present study, N-NH_4^+ values were higher ($P<0.05$) than those of N-NO_3^- except for FYT with none and double layers of SHW, and CC2 (Table 3.2). Of all the treatments, CC3 exhibited by far the highest N-NH_4^+ value (366.82 μ/ml). Nitrate concentration showed similar values amongst all composts. Bernal *et al.* (1998) reported values of 437 and 4884 ppm of N-NH_4^+ and N-NO_3^-, respectively. Nitrate values in the present study were markedly lower than that presented by Bernal *et al.* (1998).

Other important parameters in the evaluation of compost quality are particle density and salinity. In this experiment, particle density showed significant difference among treatments (Table 3.2). Salinity in compost, measured as electrical conductivity, did show a significant difference ($P<0.05$) among treatments. However, not a tendency or a clear response of the effect of SHW composted with FYT or SCYT on EC or PD was observed in the experiment. Woods End Research Laboratory (2005) presents standards for the evaluation of salinity in compost testing. This institution classifies the salinity concentration as very low (<1.0 dS/m), medium low (1-2 dS/m), medium (2-5 dS/m), medium high (5-10 dS/m), and very high (>10 dS/m). When using these standards it can be concluded that salinity was low for all treatments. Ionic components contributing most to salinity are sodium, potassium, chloride, nitrate, sulfate, ammonium, and volatile organic acids (Woods End Research Laboratory, 2005).

In this experiment, mineral analyses of the treatments showed a great deal of variability (Table 3.3). Phosphorus values were higher ($P<0.05$) for CC3 (19028.39 ppm), followed by CC2 (1017 ppm) and FYT with all combinations of SHW. Potassium content ranged from 6,700 ppm to 1,300 ppm among treatments. Magnesium and Pb concentration were also similar for all composts. Aluminum, Cr, Cu, Na, Ni, S, and Zn concentration were similar for the SCYT and FYT composts with all combinations of SHW, and for CC1 and CC2. However, these treatments differed from CC3 which presented the highest values ($P<0.05$). Silicon concentration was similar among composts with the exception CC3 which presented the lowest (392.05 ppm) values, respectively. The SCYT and FYT composts with all combinations of SHW presented similar values of Mn, while commercial composts, especially CC2, had the higher values (899.53 ppm). Calcium values was highest ($P<0.05$) for CC3; CC1 had similar values to all SCYT and FYT composts, while CC2 was lower than FYT with a single layer of SHW. Finally, B values were lower ($P<0.05$) for SCYT with double layers of SHW than for several of the other treatments.

In general, CC3 presented the highest ($P<0.05$) values for most of the minerals evaluated with the exception of Fe, K, Mg, Mn, Pb, and Si. Overall, commercial composts tended to show numerically higher values relative to the experimental composts.

Environmental regulatory agencies in several countries have established standards for maximum concentration of heavy metal in composts. The United States Environmental Protection Agency (EPA) limits Cr, Cu, Pb, Ni, and Zn to 1,200, 1,500, 300, 420, and 2,800 mg/kg, respectively. The Canadian government, under the Canadian Environmental Protection Act, limits Cr, Cu, Pb, Ni, and Zn concentrations more restrictively to 210, 128, 83, 32, and 315 mg/kg, respectively (Epstein, 1997). The experimental composts in the present study comply with those requirements.

Cummins *et al*. (1993) reported mineral concentration data for poultry mortalities compost in Alabama. Compared with the experimental composts in the present study, these researchers produced material with higher Cu, Mn, Cr, Pb, and B contents (615, 556, 57, 30, and 38 mg/kg, respectively) and lower Fe, and Zn content (4,705 and 499 mg/kg, respectively).

As for relative germination (Figure 3.1) of corn seeds exposed to the composts, no significant differences ($P>0.05$) were observed (Table 3.5). Although relative root elongation (>79%) and germination index (>87%) differ it did not showed a clear response between treatments (Figure 3.2). It seems to be that piles with FYT were higher than SCYT, and similar to CC1 and CC2. These results shows that the experimental composts were very mature and have a low probability of containing phytotoxic compounds that could consequently affect plant germination and growth. Das *et al*. (2003) evaluated the stability and maturity of synthetic and natural bulking agents in food waste composting. In their study the germination index for all treatments exceeded 70% which they considered indicative of stability in the compost.

Agronomic value

For the evaluation of the agronomic value of the composts a tropical grass *Brachiaria brizantha cv Mulato* was utilized. After six weeks of growth, chlorophyll, N percentage, and biomass were determined. All nine composts (experimental and commercial) showed low agronomic potential as evidenced by chlorophyll, N, and biomass production values of the grass at that stage of growth (Table 3.6).

Chlorophyll concentration in the grass was evaluated using a chlorophyll meter. This meter estimates the amount of the green pigment present by measuring the amount of light that is transmitted through a leaf. Chlorophyll values, expressed as SPAD units, were higher ($P<0.05$) for the SCYT with double layers of SHW but this treatment was statistically similar to FYT with all single or double layers of SHW, and to CC1 and CC3.

Nitrogen percentage was higher ($P>0.05$) for FYT with single layer of SHW (0.94%) and, as expected, lower ($P>0.05$) for SCYT with double layers of SHW, SCYT alone, and water (0.74, 0.73, and 0.73%, respectively). In this experiment, no correlation ($r^2=0$) was found between chlorophyll content and nitrogen percent (Figure 3.3). It was expected that as chlorophyll values increased, nitrogen percent would follow the same trend. A possible explanation for this is that N wasn't the only nutrient limiting the growth of the plant. As for biomass, CC3 application resulted in a markedly higher value (4.01g DM/pot) than the other treatments evaluated, which gave similar weights of biomass (Figure 3.4). This result differs from the findings of García and Valencia (2004). These authors studied the effect of poultry litter compost on the growth of *Brachiaria brizantha cv Mulato*. They evaluated compost inclusion rates of 0, 16, 31, and 62 kg N/ha at three harvesting intervals (30, 60, and 90 d). Their results showed that plants receiving applications of 62 kg N/ha from poultry litter compost and harvested at 90 d exhibited the highest biomass yields (31, 56, and 81g at first, second, and third harvest, respectively). Plants treated with 62 kg N/ha had the highest N concentration (1.46%) which was higher than that observed in the present study.

Stratton and Recheigl (1998) evaluated municipal solid waste compost, yard waste compost, and food plus yard waste compost applications to ryegrass (*Lolium multiflorum L.*). The inclusion rates of 168, 336, and 672 kg N/ha were chosen by estimating that half of the total N in the compost might be available in the first growing season. A difference between that experiment and the present study and the study of García and Valencia (2004) was that P and K were supplemented to meet the plant's requirements. These researchers found that supplemental N was needed in addition to all of the composts to achieve an acceptable stand. Over the two-year experimental period, amendments of compost doubled the yields of ryegrass compared to unamended plots grown with chemical fertilizers alone.

Conclusions

The physical and chemical parameters evaluated in this study for the SCYT or FYT with and without SHW showed these to be stable and mature product comparable to several commercial composts. Both the experimental and commercial composts exhibited an adequate chemical composition and germination index. However, commercial composts tend to have a higher mineral content and a lower

CEC. As the agronomic performance shows, experimental compost need to be mixed with other nutrient source (i.e. inorganic fertilizers) in order to provide the optimum quantities of nutrients that *cv Mulato* needs in 42 days of growth.

Implications

The quality of compost is important in determining the end use for the product. In this experiment, the quality was determined using different parameters that correspond to the maturity and stability of the compost. The data presented suggest that the composts evaluated were mature and stable and could be used as a source of nutrients for plant growth. However, as the agronomic performance shows, compost alone does not provide the optimum quantities of nutrients that *Brachiaria brizanta cv Mulato* needs in 42 days of growth. Therefore, it is suggested that inorganic fertilizer and compost be used concomitantly to provide the essential nutrients for plant nutrition.

Table 3.1. Chemical composition of the experimental and commercial compost.

Treatment	pH	Dry matter	OM	IM	N	C	C:N ratio
		g/kg					
SCYT	5.94^{bc}	443.5^{bc}	591.5^{b}	408.5^{b}	22.1^{bc}	328.6^{b}	14.85^{ab}
SCYT- single SHW	5.82^{bde}	469.6^{bc}	600.1^{b}	399.9^{b}	22.1^{bc}	333.4^{b}	15.61^{a}
SCYT- double SHW	5.80^{bde}	470.9^{bc}	572.9^{b}	427.1^{b}	23.8^{bc}	318.3^{b}	13.43^{ab}
FYT	5.56^{cde}	330.7^{c}	671.0^{a}	329.0^{c}	27.6^{ab}	372.8^{a}	13.50^{ab}
FYT- single SHW	5.98^{bd}	372.6^{c}	687.6^{a}	312.4^{c}	31.5^{a}	382.0^{a}	12.11^{ab}
FYT- double SHW	5.12^{e}	315.8^{c}	663.2^{a}	336.8^{c}	33.6^{a}	368.5^{a}	10.99^{b}
CC1	6.78^{a}	494.3^{bc}	256.9^{c}	743.1^{a}	11.8^{d}	142.7^{c}	12.19^{ab}
CC2	6.37^{ab}	625.3^{ab}	220.2^{c}	779.8^{a}	11.6^{d}	122.3^{c}	10.65^{b}
CC3	7.05^{a}	789.3^{a}	553.2^{c}	446.8^{b}	19.8^{c}	307.3^{c}	15.61^{a}
SEM[1]	0.142	3.74	0.946	0.946	0.123	0.526	0.866
P>|t|[2]	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0036

[1] Standard error of the mean
[2] Significance level
Different letters in each column represents significant differences ($P<0.05$).

Table 3.2. Chemical evaluations of experimental and commercial composts.

Parameter	Treatments: SCYT	SCYT- single SHW	SCYT- double SHW	FYT	FYT- single SHW	FYT- double SHW	CC1	CC2	CC3	SEM[5]	P>|t|[6]
CEC[1] (meq/100g)	90.36^{abc*}	107.40^{abc}	108.06^{abc}	134.18^{a}	123.87^{a}	109.12^{ab}	64.74^{bc}	55.42^{c}	56.40^{bc}	10.94	0.0005
$N\text{-}NH_4$[2] (µg/mL)	99.86^{bc}	78.71^{bc}	195.13^{b}	6.49^{c}	7.13^{c}	5.09^{c}	15.28^{bc}	6.62^{bc}	366.82^{a}	33.9	0.0001
$N\text{-}NO_3$[3] (µg/mL)	23.44	14.01	7.39	8.92	6.11	17.58	19.11	6.62	17.07	6.773	0.4024
PD[4] (g/cm^3)	1.43^{c}	1.30^{c}	1.71^{bc}	2.64^{abc}	2.37^{bc}	3.49^{ab}	3.94^{a}	1.81^{bc}	2.04^{bc}	0.246	0.0001
EC (dS/m)	1.37^{b}	1.32^{bc}	1.09^{e}	1.30^{bcd}	1.34^{b}	1.31^{bc}	1.22^{cd}	1.20^{d}	1.62^{a}	0.0208	0.0001

[1] Cation exchange capacity; [2] Ammonium nitrogen; [3] Nitrate nitrogen; [4] Particle density
[5] Standard error of the mean
[6] Significance level
*Different letters in each row represent significant differences (P<0.05).

Table 3.3. Mineral analysis of experimental and commercial composts.

	Treatment								
Parameter (ppm)	SCYT	SCYT- single SHW	SCYT- double SHW	FYT	FYT- single SHW	FYT- double SHW	CC1	CC2	CC3
Al	1539.80^{b*}	1702.47^{b}	1745.80^{b}	1325.13^{b}	1053.27^{b}	1388.13^{b}	2510.13^{b}	5812.80^{b}	29219.47^{a}
B	5.66^{bcd}	7.19^{abdc}	2.33^{e}	12.24^{b}	12.01^{ab}	11.17^{abcd}	9.33^{abd}	6.05^{abc}	15.85^{ab}
Ca	18780.55^{a}	18889.21^{a}	18452.55^{a}	25252.55^{bd}	25602.55^{d}	23622.55^{a}	23209.21^{abd}	19559.21^{ab}	40962.55^{c}
Cr	9.31^{b}	13.25^{b}	11.79^{b}	10.13^{b}	5.99^{b}	5.81^{b}	10.55^{b}	23.34^{b}	58.59^{b}
Cu	35.55^{b}	42.05^{b}	34.21^{b}	29.73^{b}	44.22^{b}	31.80^{b}	48.00^{b}	59.31^{b}	598.67^{a}
K	3250.33^{abc}	6716.00^{a}	1309.40^{c}	3228.00^{abc}	2831.67^{abc}	1336.86^{c}	5982.00^{ab}	3673.33^{abc}	2122.00^{bc}
Mg	5177.42	5640.75	3922.75	8469.75	8316.09	6952.75	5242.09	8904.75	3263.42
Mn	311.67^{cd}	327.47^{bc}	332.73^{bc}	226.80^{bc}	247.40^{c}	286.00^{c}	502.80^{b}	899.53^{a}	422.87^{b}
Na	371.61^{b}	536.94^{b}	201.14^{b}	259.17^{b}	373.27^{b}	320.27^{b}	284.47^{b}	1019.74^{a}	1119.67^{a}
Ni	17.23^{b}	14.80^{b}	18.02^{b}	20.54^{b}	18.43^{b}	11.27^{b}	31.75^{ab}	31.54^{ab}	58.14^{a}
P	2996.39^{d}	3079.06^{d}	2181.39^{d}	3533.06^{cd}	6009.73^{bc}	6094.39^{bc}	2765.06^{d}	7017.06^{b}	19028.39^{a}
Pb	19.85	22.16	13.15	1.73	3.23	6.87	4.85	35.17	34.85
S	1406.31^{b}	1680.97^{b}	2372.02^{b}	2831.69^{b}	2648.02^{b}	2778.02^{b}	1403.69^{b}	2805.35^{b}	11670.69^{a}
Si	1664.92^{ab}	1571.25^{ab}	1452.25^{bd}	1195.75^{ab}	1143.19^{b}	1535.59^{ab}	2108.92^{a}	1846.92^{ad}	392.05^{c}
Zn	211.76^{b}	287.02^{b}	324.62^{b}	403.42^{b}	286.36^{b}	255.52^{b}	178.62^{b}	252.06^{b}	904.89^{a}

*Means in rows with unlike superscripts differ ($P<0.05$).

Table 3.4. Standard error and significance levels of mineral analysis.

Parameter (ppm)	SEM[1]	P>|t|[2]
Al	995.33	0.0001
B	1.17	0.0001
Ca	1064.28	0.0001
Cr	6.09	0.0003
Cu	12.697	0.0001
K	822.57	0.0037
Mg	1242.85	0.0481
Mn	26.109	0.0001
Na	90.51	0.0001
Ni	6.32	0.0022
P	488.92	0.0001
Pb	12.597	0.4609
S	265.04	0.0001
Si	132.8	0.0001
Zn	62.41	0.0001

[1] Standard error of the mean.
[2] Significance level

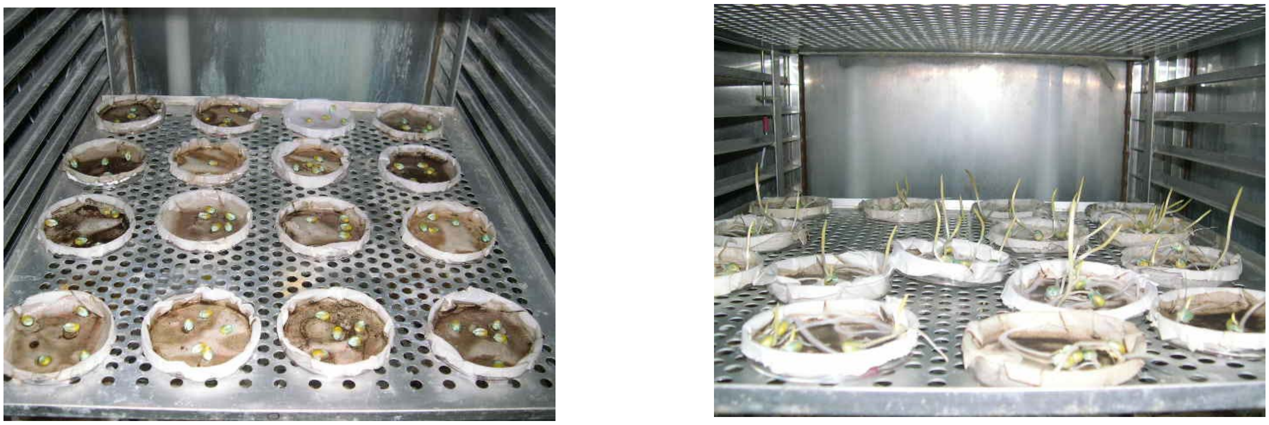

Figure 3.1. Germination chamber with evaluated treatments before (left) and after (right) the incubation period.

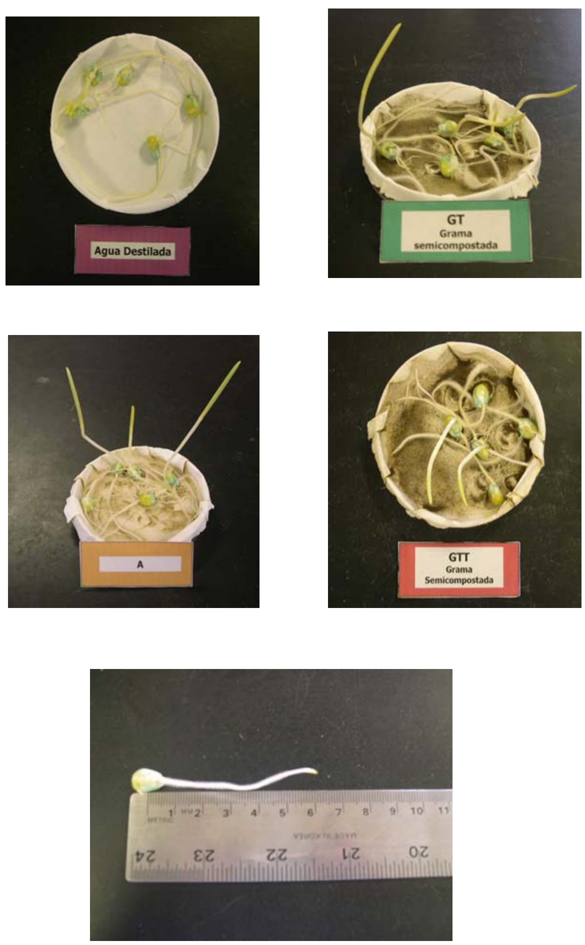

Figure 3.2. Germination and root elongation of the experimental and commercial composts.

Table 3.5. Germination index, germination percent and root length of corn seeds exposed to the experimental and commercial composts.

Treatments	Relative Germination	Relative Root Length	Germination index
SCYT	100	79.36[c]	87.32[c]
SCYT- single SHW	108.96	114.32[ab]	127.25[ab]
SCYT- double SHW	104.48	86.52[bc]	91.76[bc]
FYT	108.96	129.63[a]	144.8[a]
FYT- single SHW	111.94	124.96[a]	139.88[a]
FYT- double SHW	111.94	118.32[a]	132.46[a]
CC1	105.54	124.82[a]	136.47[a]
CC2	104.48	109.98[abc]	121.87[abc]
CC3	104.48	120.64[a]	132.21[a]
Water	99.15	100.02[abc]	109.65[abc]
SEM[1]	4.90	11.42	13.08
P<|t|[2]	0.6103	0.0251	0.0202

[1] Standard error of the mean.
[2] Significance level
*Means in columns with unlike superscripts differ (P<0.05).

Table 3.6. Agronomic evaluations of the experimental and commercial composts used as a growing media for *Brachiaria brizantha*.

Parameter	Treatment: SCYT	SCYT- single SHW	SCYT- double SHW	FYT	FYT- single SHW	FYT- double SHW	CC1	CC2	CC3	Water	SEM	P<|t|
Chlorophyll (SPAD units)	18.42^{ab*}	19.11^{ab}	21.74^{c}	17.33^{b}	19.77^{abc}	20.13^{abc}	20.85^{ac}	17.36^{b}	20.70^{ac}	18.52^{ab}	0.568	0.0001
N (%)	0.73^{bc}	0.91^{ab}	0.74^{bc}	0.82^{abc}	0.94^{a}	0.92^{ab}	0.78^{bc}	0.8^{abc}	0.8^{abc}	0.73^{bc}	0.0538	0.0563
Biomass (g DM/pot)	2.00^{a}	2.10^{a}	1.95^{a}	1.40^{a}	1.78^{a}	1.28^{a}	1.70^{a}	1.28^{a}	4.01^{b}	1.29^{a}	0.1781	0.0001

*Means in rows with unlike superscripts differ (P<0.05).

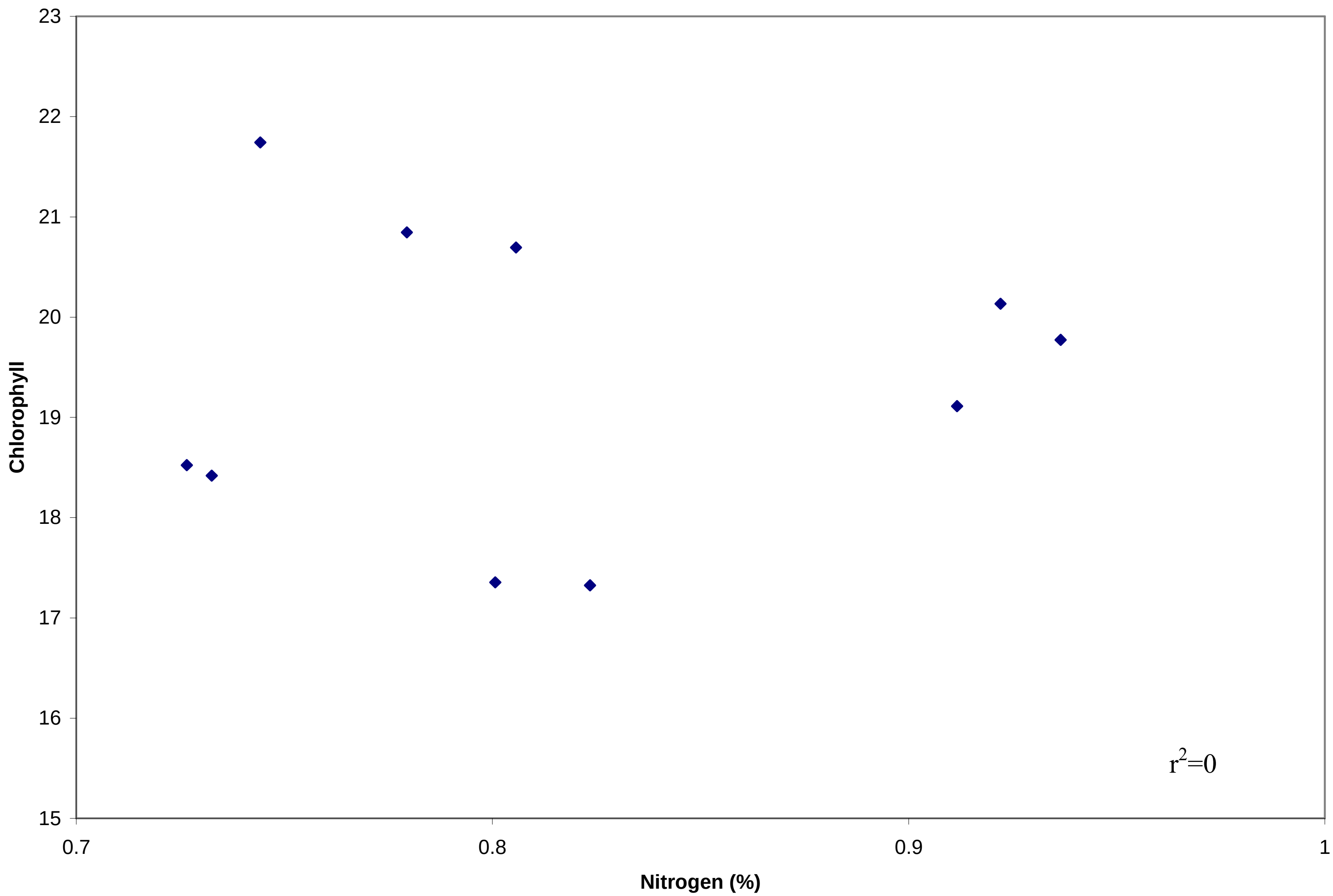

Figure 3.3. Chlorophyll concentration and nitrogen content in the leaves of *Brachiaria brizantha*.

Figure 3.4. *Brachiaria brizantha* six weeks after the application of 400 kg N/ compost/ pot.

*1= Semicomposted yard trimmings alone (SCYT), 2= SCYT and single layer of SHW, 3= SCYT and double layer of SHW, 4= fresh yard trimmings alone (FYT), 5= FYT and single layer of SHW, 6= FYT and double layer of SHW, 7=Commercial Compost 1, 8=Commercial Compost 2, 9=Commercial Compost 3.

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CHAPTER 4

Chemical and biological characterization of slaughterhouse wastes compost

Abstract

The chemical and microbiological properties of compost made from semicomposted yard trimmings (SCYT) alone or SCYT mixed with SHW were evaluated in seven phases. Mixtures of YT:SHW were weigh in a 2:1 proportion and placed in composting bins (0.91 m^2). Temperature was recorded to determine the time (d) needed to reach the first (1HC) and second heat cycles (2HC). Composting characteristics were measured at 0 d, at the peak of 1HC and 2HC, and at maturation after reading the 2HC (0, 20, 50 and 70 d). During 1HC, bacterial isolates were cultivated in both treatments and characterized using the Biolog® System. The chemical composition data was analyzed using a 2 (layers of SHW) x 7 (composting phases) factorial arrangement of treatments. The pH was neutral for YT alone and YT:SHW mixture and ranged from 7.41 to 6.82 throughout the process. There was a progressive decrease in OM and C, and a relative increase in IM and N in both treatments. At 70 d of maturation C:N values were similar between treatments, but were lower ($P<0.05$) than the initial values. Only the YT:SHW exhibited thermophillic temperatures. However, in both treatments at the 1HC a diverse bacterial community was isolated and identified.

Introduction

The disposal of organic wastes from slaughterhouses (SHW) in Puerto Rico has become an environmental problem. Currently, SHW are disposed of in landfills causing land and water pollution. The uncontrolled degradation of organic wastes generates leachate and biogas that cause a negative impact on the environment (Palma *et al.*, 1999). Compost is a stable product that results from recycling organic wastes (i.e. manure, yard trimmings), which is used as a soil conditioner and as a source of nutrients for plants. The active component mediating biodegradation of the organic wastes is the resident microbial community present during the different stages of decomposition (Peters *et al*., 2000). In general, microorganisms present in compost include bacteria, actinomycetes, and fungi (Trautmann and Olynciw, 2003; Epstein, 1997). During the process microbes transforms the organic residues into stable organic products and produce carbon dioxide, water, heat, and humus, amongst other compounds (Epstein, 1997). The heat produced can destroy pathogenic organisms if they are exposed to high temperatures for a sufficient period of time.

In order to enhance compost quality and reduce the time of organic matter breakdown it is desirable to identify the microorganisms predominate the different stages of degradation. Researchers have used several protocols based on methods such as random amplification polymorphic DNA (Zhang et al., 2002), the polymerase chain reaction (Peters et al., 2000), phospholipid fatty acids profiling and carbon source utilization studies (Carpenter-Boggs et al., 1998) to identify the presence or absence of pathogens and microbial populations responsible for the organic matter degradation in the compost.

The Biolog® System (Hayward, CA) is a carbon source utilization technology that can identify different types of aerobic and anaerobic bacteria, yeast and fungi. After isolation and purification, microorganisms are inoculated in a 96 well plate in which they utilize or oxidize compounds from a preselected panel of different carbon sources. The objectives of this study were to evaluate the chemical and biological characteristics of compost and identify the bacterial population associated with the thermophillic stage of slaughterhouse organic wastes composted with yard trimmings.

Methodology

Slaughterhouse organic wastes (SHW), small ruminant digestive and reproductive tracts) were obtained from a commercial abattoir located at the Lajas Agricultural Experimental Station, UPRM. Semicomposted yard trimmings (SCYT) were obtained from the composting facilities located at the Alzamora Farm, University of Puerto Rico Mayagüez Campus. Two compost treatments were evaluated: SCYT (used as control treatment) and the mixture of SHW and SCYT. Single layers of animal tissue wastes were placed in composting bins of identical dimensions (0.91 m^2) using SCYT as a bulking agent (BA). Animal tissue was placed between 45.72 cm layers of SCYT in a 2:1 proportion (w/w). The compost piles were hydrated to obtain approximately an initial 60% moisture content on a weight basis, which was maintained over the composting process.

Chemical characterization

Triplicate samples from each original material (composting phase 0) were obtained to determine initial C and N content, C:N ratio, organic matter (OM) and inorganic matter (IM) (Rynk 1992; AOAC 1991). Temperature was monitored daily with compost thermometers (Sudbury Compost Thermometer, NASCO, Fort Atkinson, WI) situated at the center of each pile SCYT and SCYT:SHW. Compost treatments were sampled during seven phases, as described in Chapter 2. Composting phases 1 and 2 were considered to begin once the temperature reached its maximum value and then dropped 5.56° C after the 1HC and 2HC, respectively. Four core samples from each treatment were collected at day 0, the first (1HC) and second heat cycle (2HC) and at 0, 20, 50, and 70 d of the maturation process (composting phases 3 – 6, respectively) after turning the piles. The composite samples were analyzed to determine pH and chemical composition as described for the original material. Data were analyzed according to a completely randomized design with a 2 (layers of SHW; none or single) by 7 (composting phases) factorial arrangement (Steel and Torrie 1990) using the General Linear Model subroutine of SAS (1990). Treatment means showing significant difference in the ANOVA were ranked using the Tukey test of SAS (1990). All statements of significance were based on the probability level of 0.05 unless otherwise noted.

Biological characterization

Samples for microbial identification were taken from each treatment replicate at 1HC (composting stage 1). Colonies were isolated on Triptic Soy Agar (TSA) after the samples were serially diluted in distilled and sterilized water (10^{-1} – 10^{-10}). Thirty bacterial isolates for both treatments were chosen randomly and tested for C-source utilization pattern and identified using the Biolog® System (Hayward, CA). Bacterial isolates were cultured on TSA and Biolog Universal Growth (BUG) media for 24-hours. Cultures were then suspended in GN/GP inoculating fluid, adjusted to required optical density and inoculated on the 96 well Biolog plates. Plates were incubated at 30°C for 24 hours. Color development pattern was compared to the database and isolates were identified at species level.

Results and Discussion

Chemical characterization

For both treatments, the final pH value was similar, however the pH in SCYT compost alone remained neutral during the entire composting process, while pH in compost SHW differed ($P<0.05$) between phases, varying from neutral (phases 1, 3 and 4), to alkaline (phase 2) and slightly acid (phases 5 and 6) (Figure 4.1).

The C and OM concentration decreased ($P<0.05$), but IM increased ($P<0.05$) over the composting process for SCYT and YT:SHW (Figures, 4.2, 4.3, and 4.4 respectively). In this experiment the C:N ratio was initially higher (45.5:1 for SCYT and 37.4:1 for SHW) than the recommended value (25:1 to 30:1; Rynk et al., 1992). Although, in both treatments it was down-trending, reaching a final value of 21.28 and 14.81 for the SCYT and SHW compost, respectively (Figure 4.5).

In both treatments, the N content was variable over the composting phases, but was higher ($P<0.05$) in SHW than in SCYT compost (Figure 4.6). These changes in chemical composition observed during the composting process, suggest that microbial ecology was changing and different substrates were being used; this would imply that a very heterogeneous microbial population existed in the composting of

SHW, consisting of microbes that can use C and N as a source of energy (Epstein, 1997).

These results are in agreement with previous studies that evaluated SHW composted with fresh and semicomposted yard trimmings (Sanabria *et al.*, 2004). In this experiment, the observed changes in pH and organic composition show similar trends at 60 days of the start of maturation.

In SCYT:SHW combination, thermophillic temperatures were reached only in the first heat cycle. The maximum value reported for that compost was 50° C on the day six. During the whole composting process, temperature in SCYT alone remained mesophillic reaching a maximum value of 37°C. However, temperature in SCYT was higher than the environmental therefore indicating that an active microbial population was present (Figure 4.7).

As expected, little or no trace of the original material was recognizable at the end of the process. The finished composted material exhibited dark brown to black color (Figure 4.8) and the dry matter weight of the compost was reduced from 36.89 and 35.91 kg at the beginning of the process, to 13.71 and 18.40 kg DM at the end of maturation for the SCYT and SCYT:SHW, respectively (Figure 4.9). This corresponded to a significant reduction of 37% and 51% of the original material, respectively.

Microbiological characteristics

Compost quality optimization is directly related to the population of microorganisms present (Peters et al., 2000). In this experiment microbial characterization associated with the decomposition of SCYT and SHW compost was conducted using the Biolog® System. Under optimum conditions, the microorganisms transform organic raw materials (i.e. lining) into a stable organic material termed compost by breaking them down into simple compounds (i.e polyphenolics) and reforming them into new complex compounds (i.e. fulvic acids) (Epstein, 1997; Rynk et al., 1992). It is important to know and identify the microorganisms that are capable of biodegrading specific compounds and nutrients in order to determine what use the compost will have once finished. In this experiment, the bacteria that could be identified included 7 and 8 different genus for SCYT and SHW composts, respectively (Table 4.1).

In both treatments, most of the bacteria identified were gram negative (*Pantoea stewarti* and *Providencia stuarti* for SCYT and SHW compost, respectively), enteric or non enteric, and positive to the oxidize test. However, gram positive bacteria were also identified. The gram-positive groups consisted of rods like *Bacillus cereus/thuringensis*, and cocci like *Staphylococcus sciuri,* and *Macrococcus equipercicus*. *Bacillus cereus/thuringensis* and *Vibrio diazotrophicus* were identified in both treatments indicating that active mesophillic and termophillic bacteria were present in the degradation process. *B. thuringensis* grows well in mesophillic and thermophillic temperatures (40-45° C). *Bacillus cereus* and *Staphylococcus sciuri* have a wide range of compatible temperature (35-45° F and 6.5-46° C, respectively) being their optimum temperature below 40° C (Cowan et al., 1974).

Some bacteria isolated in this experiment are classified as weak pathogens for humans. *Bacillus cereus* is a ubiquitous soil bacterium that produces toxins, which damage vital organs even though it is considered a weak pathogen because only inmunocompromised patients present signs of infection. The genus *Pasteurella* spp. is well known for causing parasitic infections in mammals and birds. *Pasteurella trehalosi* is a widely occurring pathogen of sheep that under stressful conditions produces an acute systemic infection affecting the upper alimentary tract and lungs of young adults (Davies et al., 1997). *Providencia stuartii* is an enteric bacterium usually isolated from human clinical specimens but its infection symptoms and habitat are not known. *Staphilococci* are human and animal pathogens but the clinical significance of *S. sciurii* and *Macrococcus equipercicus (*closely related to genus *Staphilococci*) remains controversial. *M. equipercicus* has been isolated only from equines where it seems to form part of the cutaneous flora. *Burkholderia glumae* is a bacterial grain/ seedling rot pathogen (Maeda et al., 2004). *Pantoea stewartii* causes a vascular wilt of corn and some related plants. *Aquaspirillum metamorphum, V. tubiashi* and *V. diazotrophicus* are water pathogens for fish, fresh water shellfish and bivalve mollusks. All isolated strains, with the exception of *Burkholderia glumae*, *V. diazotrophicus,* and, *P. stewartii* reduce nitrates to nitrites. *Burkholderia glumae* and *V. diazotrophicus* fixes atmospheric and marine nitrogen, respectively. *Providencia stuartii* is part of the *Proteus* genus in which all species are capable of producing bacteriocins (Cowan, 1974).

Ghazifard et al. (2001) identified termophillic and mesophillic microorganisms in municipal solid waste compost between 0 to 28 days of composting. They found that at the initial stages of composting, *Escherichia, Klebsiella, Aeromonas*, *Alcaligenes, Enterococcus* and *Bacillus* were the dominant microorganisms. After 20 days of composting they could only identify one mesophillic bacterium (*Bacillus spp*) because temperatures were termophillic.

Previous reports have shown that the endogenous microorganisms found in compost can reduce the predisposal of soil borne pests. Muhammad and Amusa (2003) reported that compost-inhabiting microbes had inhibitory effects on the seedling blight inducing pathogens of maize and cowpea in northwestern Nigeria. They reported that *B. cereus* reduced the mycelia growth of *Sclerotium rolfsii, Fusarium oxysporum, Phytium aphanidermatum, Helminthosporium maydis, and Rhizoctonia solani*, with inhibitory scores ranging from 35.5% to 53.3%.

Variable bacteria isolated in this experiment indicate that a heterogeneous population of organisms is involved in the decomposition of SHW and SCYT. Further studies are needed to establish the relationship between fungi, actinomycetes, and bacteria and how their populations are altered with the changes of nutrients and temperature, and their specific role in the composting process of SHW.

Conclusion

The composting process represents an alternative for the disposal of SHW using SCYT as BA. Composting of SHW and SCYT is characterized by typical chemical changes that occur during the aerobic degradation process of organic wastes. Even though SHW compost was the only treatment to exhibit thermophillic temperatures, an active and heterogeneous microbial population was evidently involved in this process for both treatments. However, in order to determine how the microbiological evolution of the process affects the degradation of organic residues further studies should characterize the microbial population during the initial and final phases of composting.

Implications

Determining the chemical and microbiological properties of a compost pile is important because it can aid to understand the decomposition process that organic residues undergo. However, as this study demonstrated, certain opportunistic and weak pathogens may be present if the compost does not reach sufficiently high temperatures during the active stages of decomposition. The evolution of microbial population during the entire process should be further evaluated to determine how physical and chemical properties affect the metabolism of the active microflora and consequently the presence of undesirable and pathogenic microorganisms.

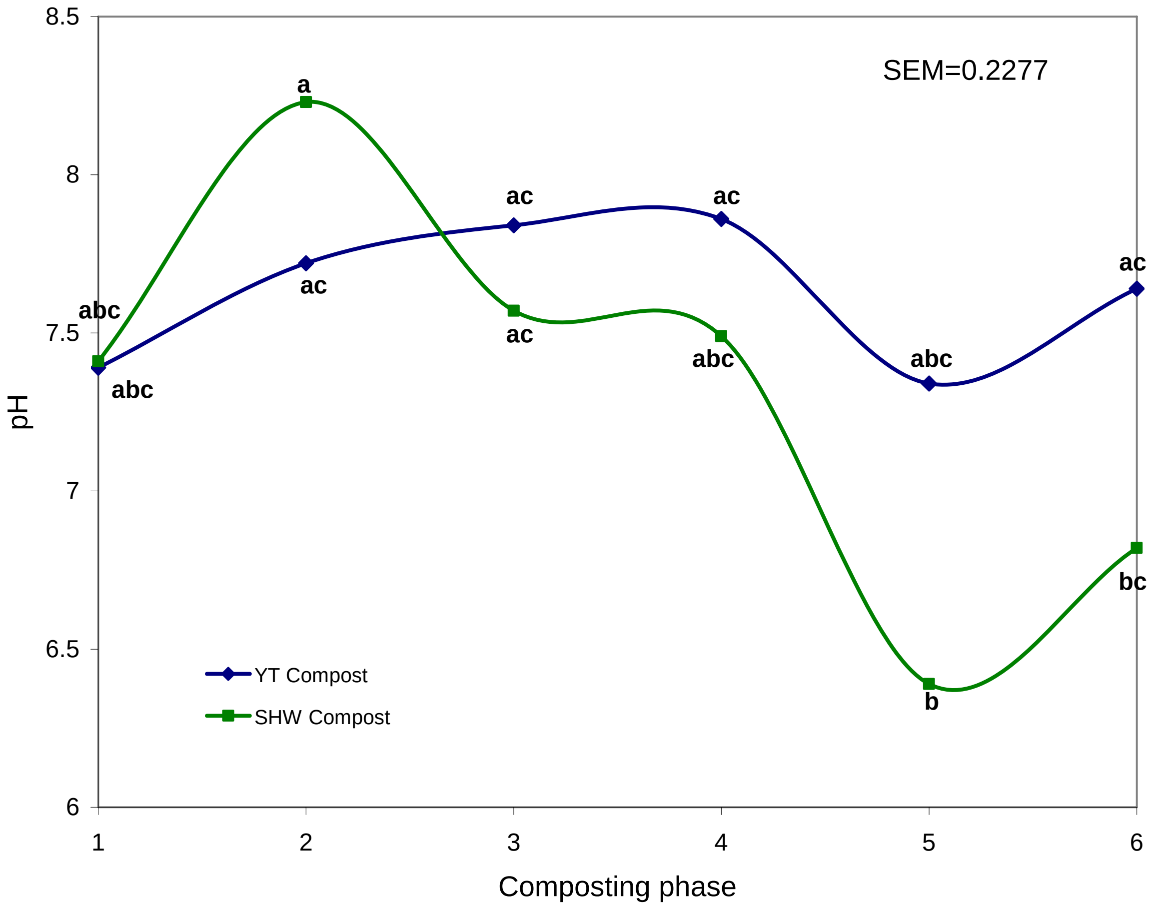

Figure 4.1. pH value for the SCYT and SHW compost in the seven composting stages. Different letters in the same component shows significant difference between the treatments.

*Standard error of the mean

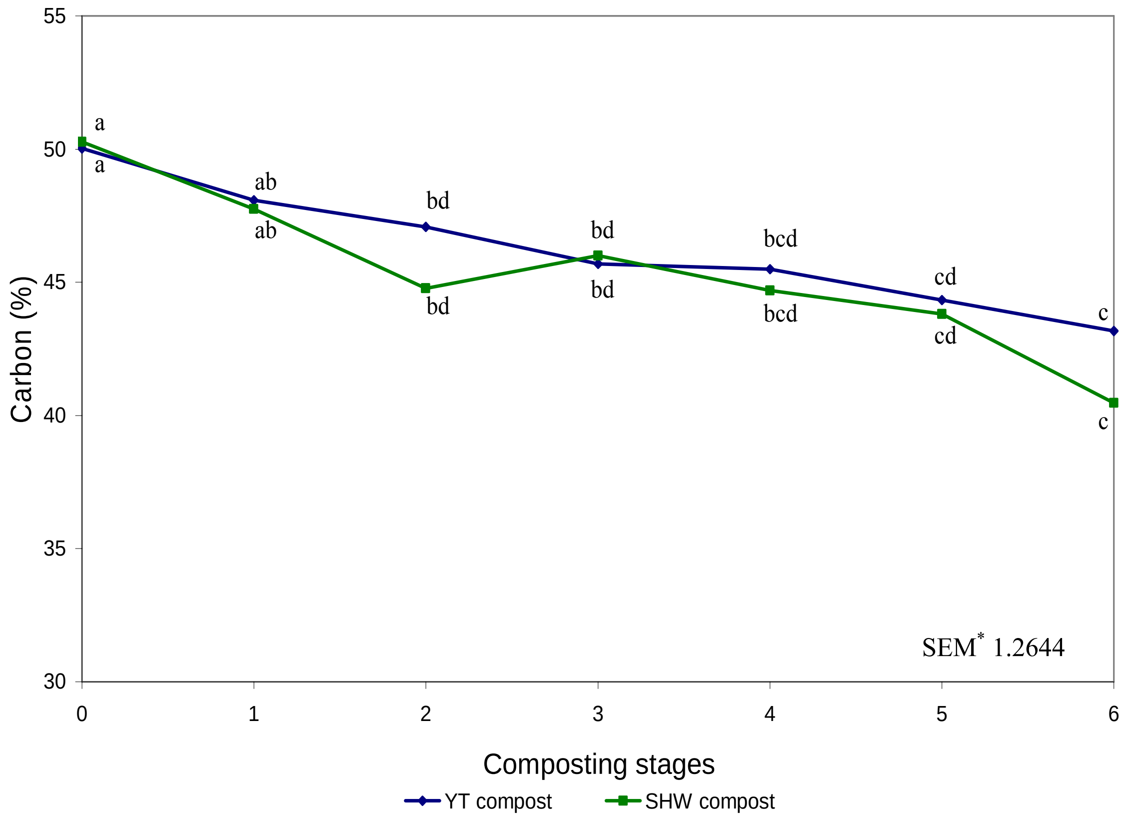

Figure 4.2. Carbon content of the SCYT and SHW compost during the seven composting phases. Different letters represents significant differences between treatments and composting phases.

*Standard error of the mean

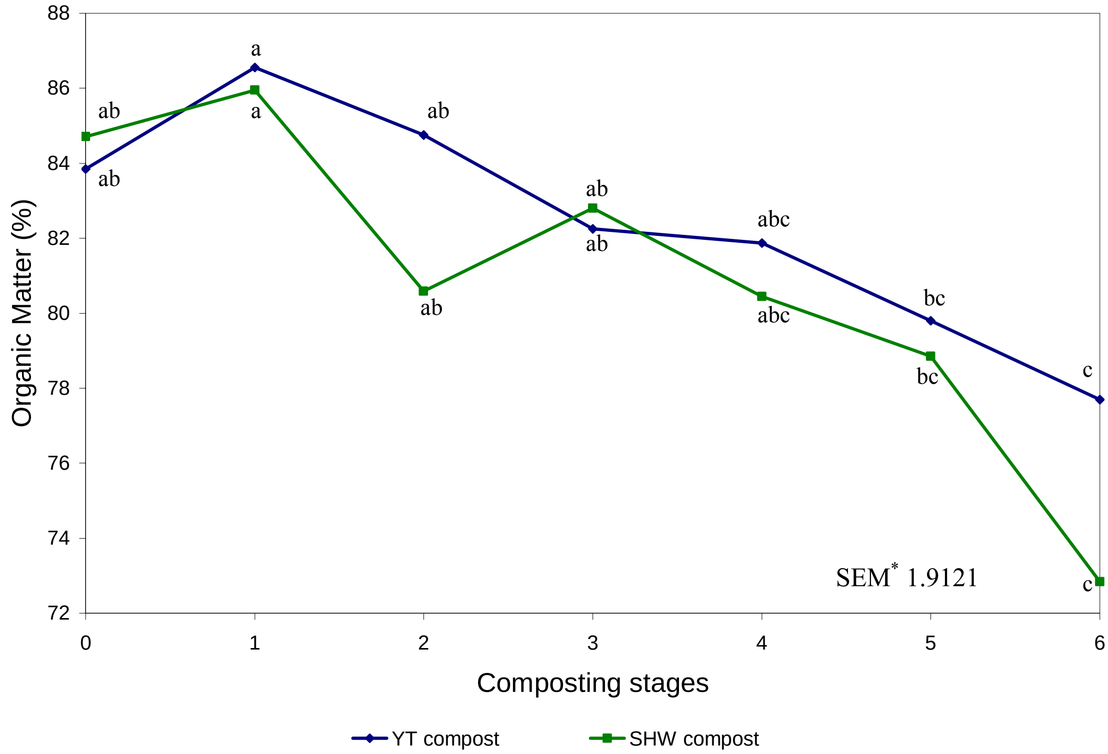

Figure 4.3. Organic matter content of the SCYT and SHW compost during the seven composting phases. Different letters represents significant differences between treatments and composting phases.

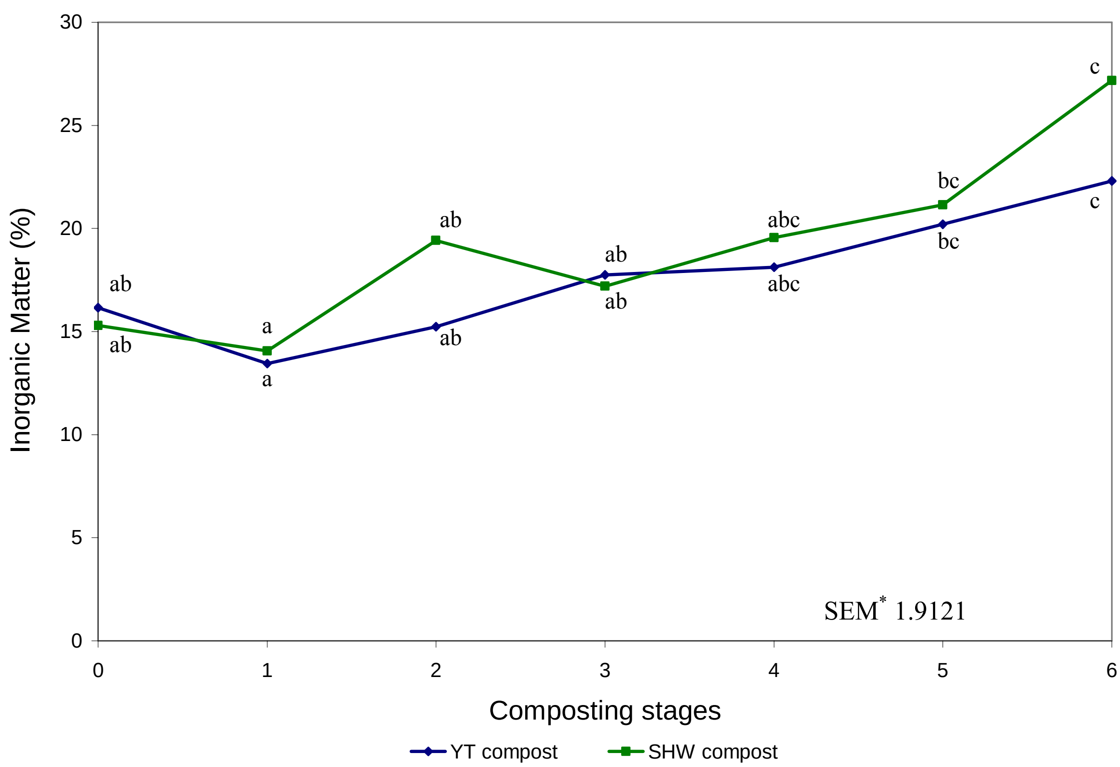

Figure 4.4. Inorganic matter content of the SCYT and SHW compost during the seven composting phases. Different letters represents significant differences between treatments and composting phases.

*Standard error of the mean

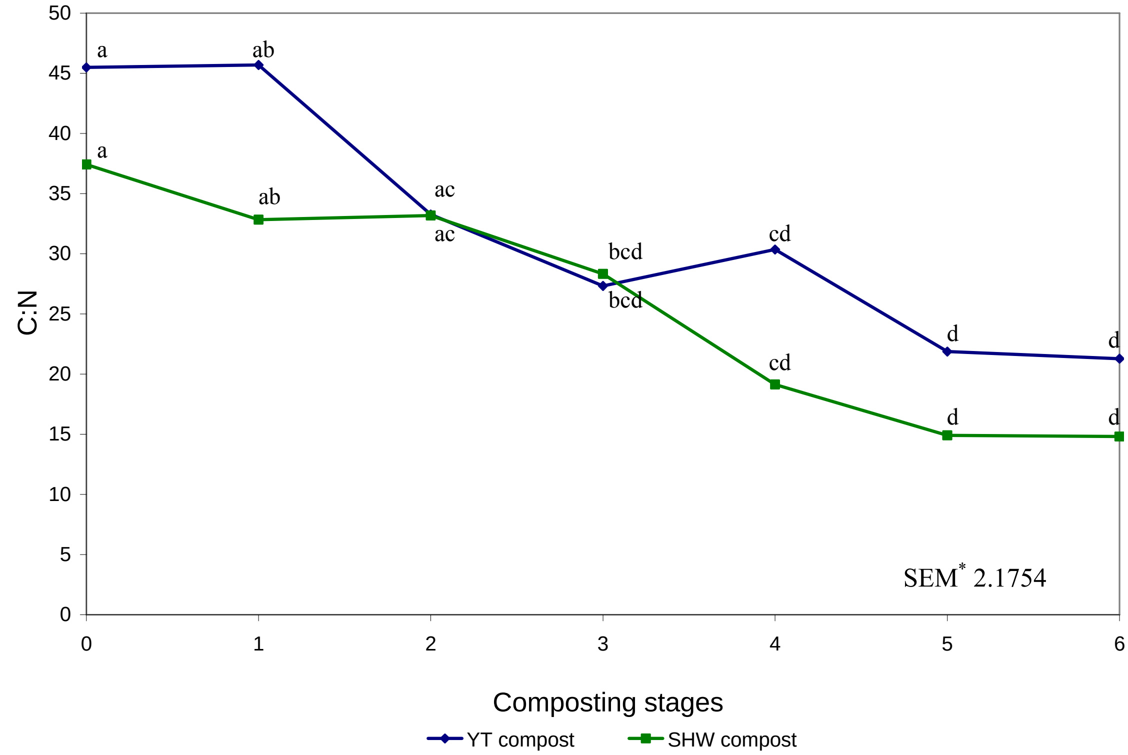

Figure 4.5. C:N ratio of the SCYT and SHW compost during the seven composting phases. Different letters represents significant differences between treatments and composting phases.

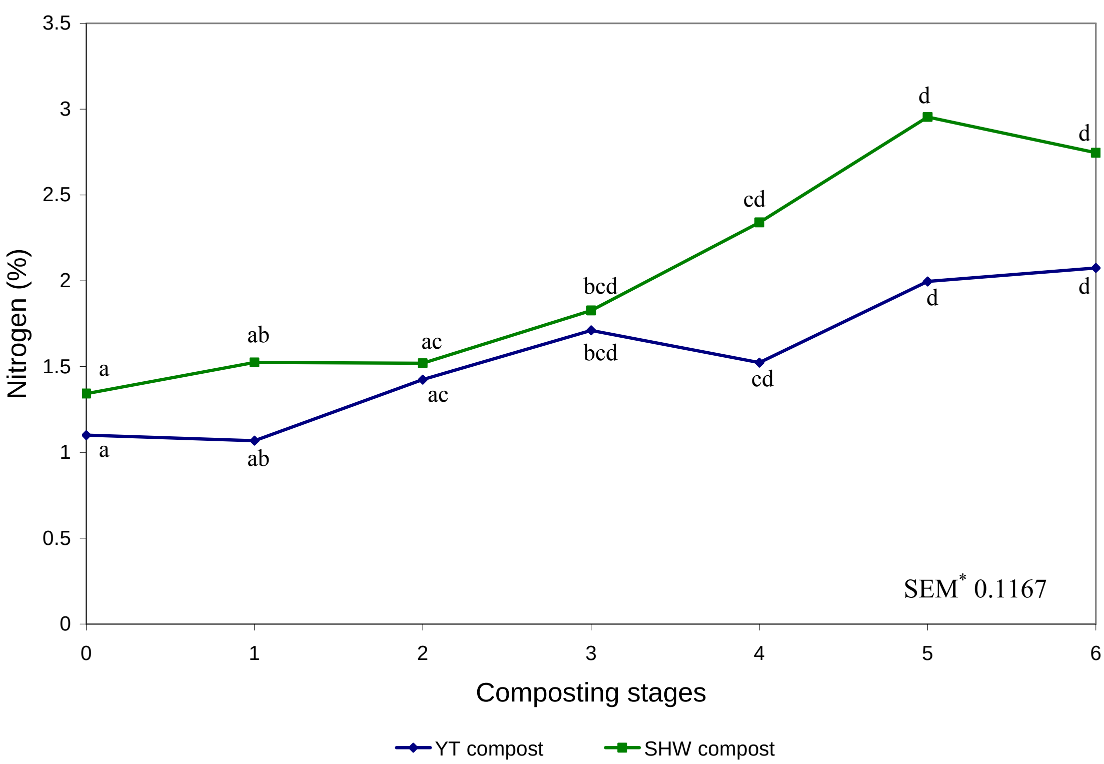

Figure 4.6. Nitrogen of the SYT and SHW content during the seven composting phases. Different letters represents significant differences between treatments and composting phases.

*Standard error of the mean

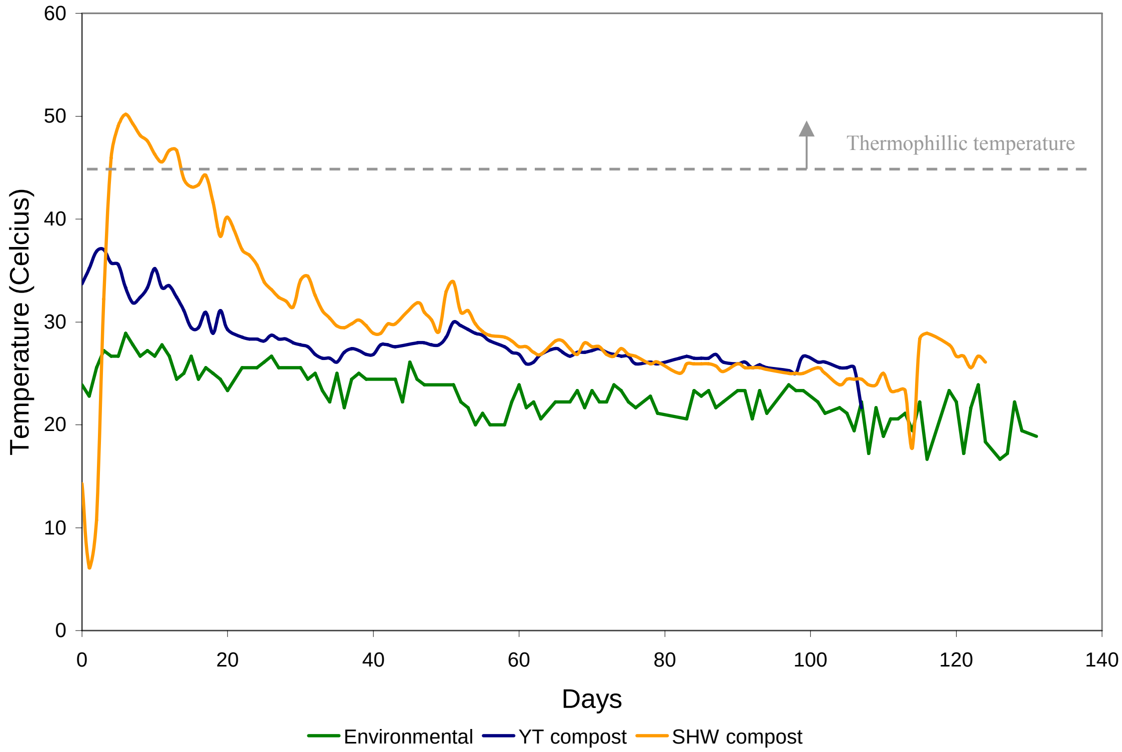

Figure 4.7. Temperature of the SCYT and SHW compost during the seven composting phases.

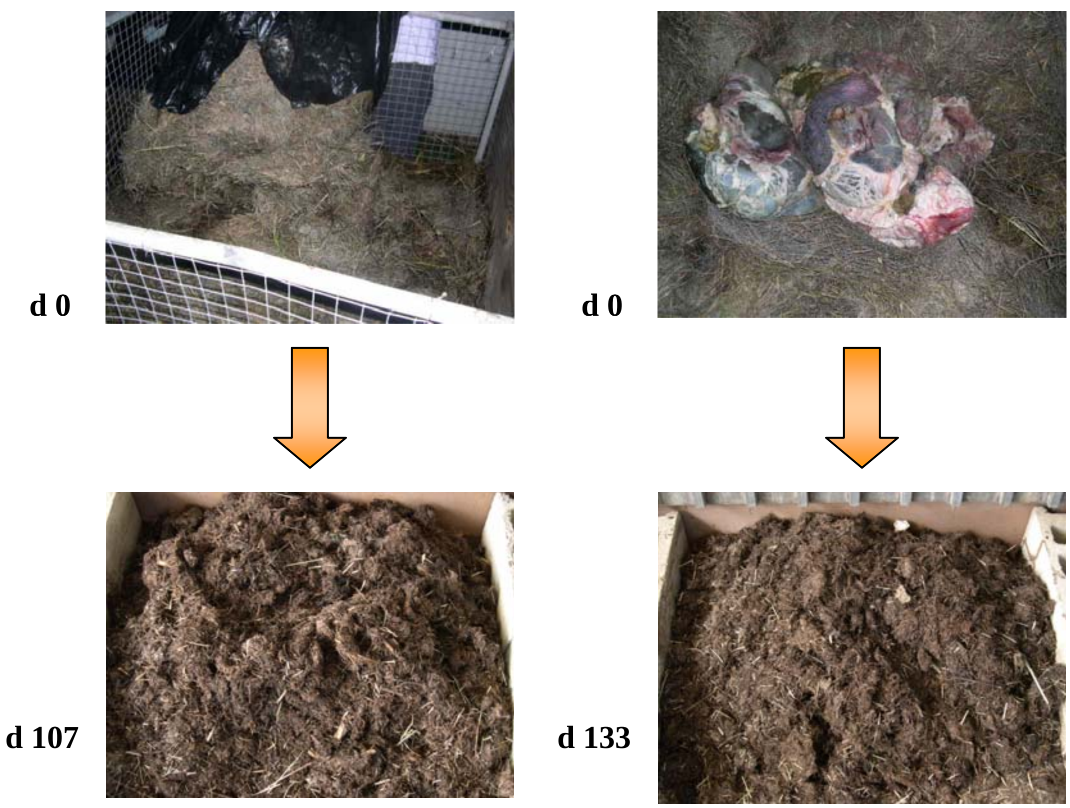

Figure 4.8. Initial and final appearance of SCYT (left) and SHW (right) compost at phase 0 and 6th of the process.

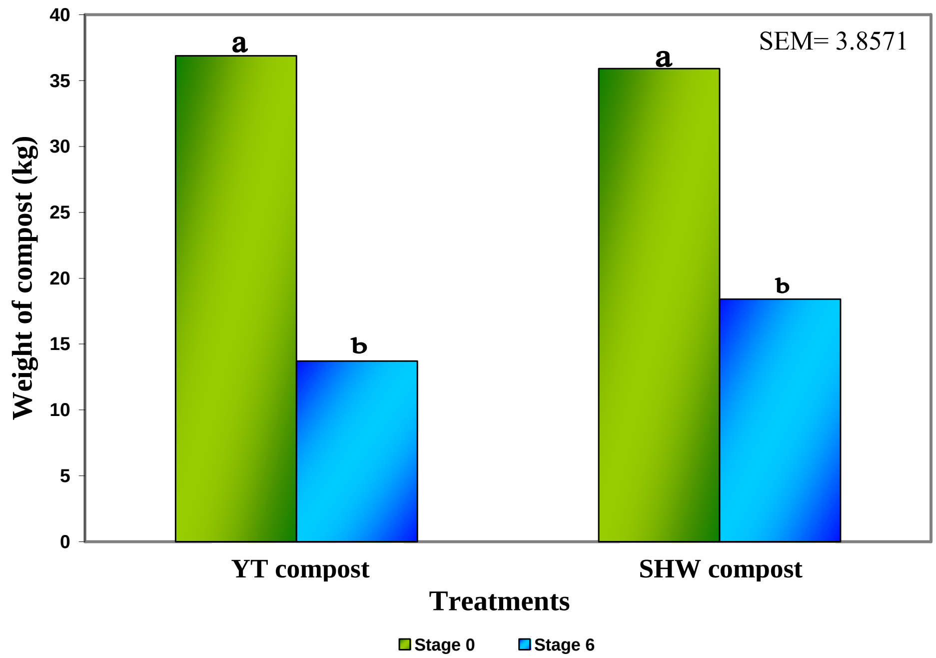

Figure 4.9. Weight of SCYT and SHW compost at phase 0 and 6th of the process.

Table 4.1. Bacteria isolated from SCYT and SHW composts during the first heat cycle using the Biolog® System.

Treatment	Temperature (°C)	Bacteria	Probability (%)
YT compost	35.55°	*Macrococcus equipercicus*	98
	28.33°	*Vibrio tubiashii*	79
	28.33°, 33.33°[1]	*Vibrio diazotrophicus*	99
	28.33°	*Burkholderia glumae*	99
	28.33°	*Aeromomas encheleta*	100
	35.55°	*Bacillus cereus/thuringensis*	100
	28.33°	*Pantoea stewartii ss stewartii*	99
	35.55°	*Staphylococcus sciuri*	99
SHW compost	45.55°	*Pasteurella trehalosi*	81
	45.55°	*Aquaspirillum metamorphum*	99
	45.55°	*Alcaligenes faecalis faecalis*	99
	45.55°	*Pasteurella canis/stomatis*	74
	45.55°	*Bacillus cereus/thuringensis*	100
	43.33°	*Vibrio diazotrophicus*	96
	43.33°	*Providencia stuartii*	99

1= Bacteria isolated at two different temperatures in the first heat cycle.

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