African Journal of Microbiology Research Vol. 4(15), pp. 1631-1634, 4 August, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2010 Academic Journals

Full Length Research Paper

Core sampling test in large-scale compost cells for microorganism isolation

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Accepted 5 July, 2010

Composting is a process by which organic wastes are transformed into fertilizer, preventing excess organic matter accumulation. Microbes that carry out this transformation have application in biotechnology. Composting cell assembling is a complex process, it can reach several m^3 of diverse materials. It is desirable a sampling methodology that allows the microbial analysis, however, this matter has not yet been approached by other researchers. In this work we tested soil auger to probe large-scale compost piles at the São Paulo Zoo, in São Paulo, Brazil. The criterion for auger selection was percentage loss of material and microbe isolation from samples.

Key words: Core sampling, compost, microorganism isolation, auger.

INTRODUCTION

Increasing population and consume has led to large amounts of organic waste accumulation, which has been a serious problem for the management in both urban and rural areas. Composting is an alternative technique to dispose organic waste, avoiding its accumulation. The end product generated is a natural fertilizer, which can be used in agriculture closing a self-sustainable cycle.

Composting is an ancient aerobic biological process carried out by microorganisms that can reach temperatures over 80°C favoring the activity of thermophilic species. The microbial metabolism changes the substrate composition over time, which in turn reflects on microbial population structure. The composting process involves chemical and biological reactions, release of carbon dioxide and heat due to microbial metabolic activity. Thus, composting is a rich source of microbial diversity studies suitable for prospection, aiming generation of biotechnological products.

The São Paulo Zoo located in São Paulo, Brazil, is one good example of self-sustainable conservation institution located in a large urban center that composts all its organic waste. The area of the park is a large patch of native and secondary vegetation of the Atlantic rainforest domain, among the richest resource for biodiversity on the planet (Figure 1). The organic compound production unit (UPCO) transforms about 2000 - 2500 kg/day of organic material into fertilizer within a 90 days period. The organic waste is generated mainly from food waste, droppings, beds and places of native and exotic wild animals and any carcasses of animals that died, mixed with a reasonable amount of vegetable matter, estimated at 1500 kg/day, from pruning of trees and plant debris like leaves, twigs and fallen trees native Atlantic rainforest in to the park.

The waste is placed in 8 m^3 chambers and 2 m deep (Figure 2A and B), where the organic matter is mixed in the proportion of about 30 parts of carbon to 1 nitrogen part in a stratified and aerated package of manure and organic matter (plant parts). The first stage of composting (active degradation) is marked by high bacterial activity and consumption of carbon sources of lower complexity, also, the volume of material decrease and the temperature can reach above 70°C. At this point which last about 45 days, the composting cells are overturned and the temperature falls to about 55°C (healing phase) avoiding anaerobic activity leading to putrefaction. During this process it is predicted that many mesophiles and thermophiles microbes propagate in this environment. (Ryckeboer et al., 2002)

Several composting analysis focusing on microorganism

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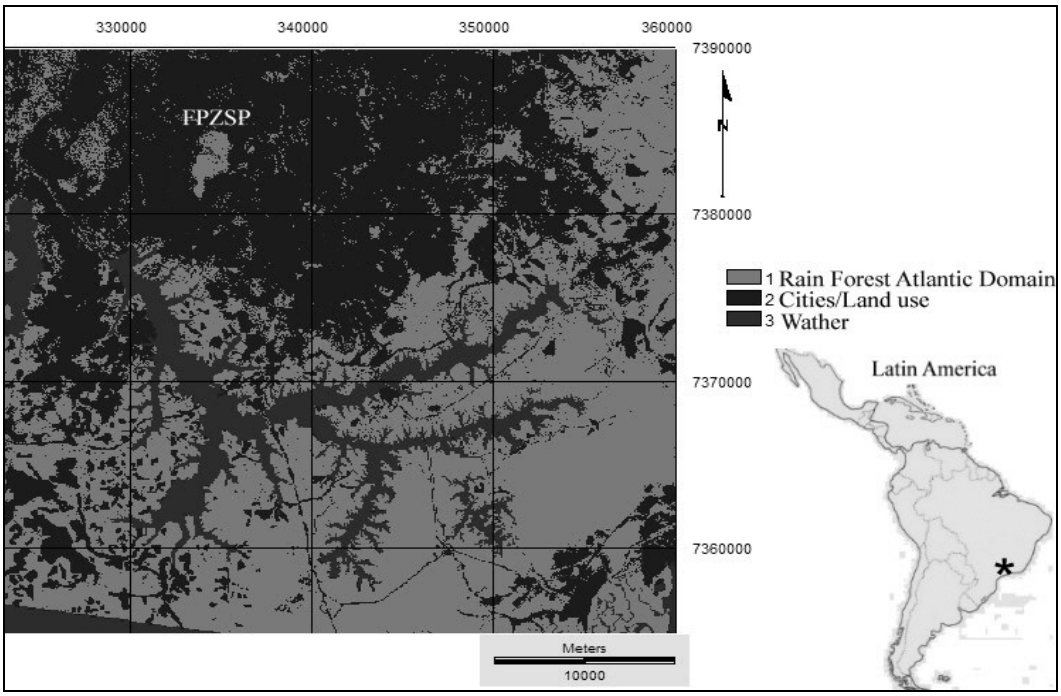

Figure 1. Location of FPZSP, sector south of the city of São Paulo. Landsat image with patches of urban occupation and land use (black), Atlantic Rain Forest domain (grey) and water course (dark grey).

Figure 2. Large-scale cell composting at UPCO. (A) empty composting cell; (B) front view of two composting cells; (C) auger containing compact compost; (D) manual kit of Eldeman`s auger (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands): Type 1, riverside auger - for the coarse sandy soils and little mixed cohesive (between 35 to 40 cm in length); Type 2, Edelman auger for clay and cohesive soils (between 25 to 30 cm in length); Type 3, Edelman auger for sandy soils and non-cohesive (25 to 30 cm in length) and Type 4, Edelman auger for very dry sandy soils with little or no cohesion (between 25 to 30 cm in length).

Table 1. Auger tested and criteria sampling.

	1) **Penetration in the substrate**	**2) Catching sample**	**3) Retention of the sampled volume**
Type 1	Difficult and tough, with three attempts to achieve penetration at 1.5 m.	Difficult to recover the material, setting core on the walls of the substrate.	Losses from 30 to 40% of the sample.
Type 2	Difficult, with three attempts to achieve penetration at 1.5 m.	Easy removal.	Losses from 50% of the sample due to the large opening of the auger.
Type 3	Reasonable, advancing 1.5 m in an attempt penetration.	Easy removal	Losses from 50% of the sample.
Type 4	Easy, attain at 1.5 m in an attempt to penetrate.	Easy removal	Retention of 100% of the sample.

Sampling procedure: Each composting cell was divided into 4 equal quadrants, which were sampled for each type auger. Stalks were adjusted to reach up to 2 meters high. We sampled five different regions (four at the corners and one at the center of the cell) at approximately 1.5 m depth. Before introducing the auger to remove the material the temperature of each spot was measured. The samples were homogenized in buckets with metal spatula, stored in clean plastic bags and put in thermo container for transportation to the laboratory where microorganism isolation took place.

community structure have been carried out in the past years, however, most of these studies used small-scale reactors. The work of Schloss et al. (2005) and Hansgate et al. (2005) present data from sequencing of the small subunit of the ribosome from feed pellets and wood chips, which according to these authors offers no obstacles for homogeneous sampling. Also, soil-sampling methods using augers have been documented before, in order to evaluate diverse aspects regarding sample quantity and quality (van Galen-van Beers et al., 2002; Anikwe and Nwobodo, 2002). On the other hand, large-scale composting cells represent a challenge for homogeneous representative sampling, due to its large size and compactation (Figure 2C).

Different types of augers are used to sample different kinds of soil, size and shape of the instrument directly impact on sampling (Van Galen and Van Beers et al., 2002). Compost is different from soil regarding its mineral constituents, processes formation, consistence, particle size and density. Considering the need for standardization of sampling procedure, 2 large-scale piles of compost (45 days old, revolved for aeration and 37 days not revolved), containing various substrates were probed with different soil augers at UPCO in Brazil. The main criterion for auger selection was the penetration of the auger in the substrate and retention of sample volume and microorganism isolation from the compost samples removed with different augers.

RESULTS AND DISCUSSION

Sampling

Among the various types of equipment for sampling of soils, augers represent a practical and easy tool. It has different types of end probes, which allows collection of several material types according to particle size, weight, humidity and consistency. In this paper 4 types of augers were tested to probe 2 different stages of large compost cells. These types of augers are usually applied for soil sampling, which is similar but not equal to compost (Catani et al., 1954; Silva et al., 2003; Souza et al., 2006). Therefore, several auger types seeking for the best fit to sample the compost cell were tested.

The auger type 1 presented difficulties for the penetration of the substrate, with 3 attempts to reach 1.5 m deep. The material was trapped inside the tube and its removal was not complete with about 30 to 40% loss of compost. The auger type 2 also presented difficulties in penetrating the substrate, requiring three attempts to reach the depth of 1.5 m. The probe´s opening appropriate for clay soils facilitated the withdrawal of the probe, but with 50% loss of the material. The auger type 3 penetrated easily in the substrate, reaching the depth of 1.5 m on the first attempt. It was easily removed, but the sample loss was of about 50%. The auger type 4 showed good penetration in the substrate (1.5 m deep) and it was easily removed with 100% retention of the material (Table 1).

Regardless of the sample retention by the different kinds of augers, all the recovered material was subjected to microorganism isolation on selective media (data not shown), with no significant difference on yields (Table 2). Since auger number 4 showed no loss during removal it was decided to further test it in 6 more composting piles to reassure the choice. Microbe isolation number was very similar considering the same dilution factor (data not shown) indicating the auger choice did not impact the ultimate goal of the sampling which is microbe prospecting. These tests were important to define the appropriate type of auger to collect compost for microbiological test. Therefore, the choice of probe 4 was due to the easy penetration in the substrate and

Table 2. Microorganism yields after sampling procedure with different augers.

Probe	Depth (m)	Total microbe isolated	(ºC)	Bacteria	Filamentous fungi	Yeast
1	1.2	39	44.5	29	10	0
2	1.4	33	52.2	29	4	0
3	1.0	32	49	13	12	7
4	1.0	37	48	12	13	12
Total	-	141	-	-	-	-

Isolation procedure: samples were diluted in 5 ml of sterile saline (0.9% NaCl), agitated in vortex for 5 min and decanted for 2 h for particles precipitation at the bottom of the test tube at room temperature. The samples were serial diluted in saline and 100 µl aliquots from 10^{-6} dilution were platted in Nutrient Agar and Sabouraud Agar (HiMedia), plates were incubated at 30˚C for 16 h. Isolated colonies were counted and further purified to obtain single colonies in Nutrient Agar or Sabouraud Agar. Microscopic observation was conducted for all isolates for preliminary classification (bacteria, fungi and yeast).

extraction without loss of material for the isolation of microorganisms.

Even though the cells were at different stages of the composting processes, the test showed that both presented similar results with the auger type 4 (Table 1). This means that the consistency and granulation of the composting material are close to sandy soils with little or no cohesion. Despite the different stages of the composting cells regarding compactation and cohesion, it was shown that the auger type 4 gave a better chance for sampling collection, easily penetrating the substrate at the desired depth, also it had a better sample retention, ensuring a significant and representative sample. This test provided important and interesting information for procedure standardization during large-scale compost sampling for microorganism isolation. Therefore, it is suggested the use of the Eldeman auger number 4 for sampling composting materials under similar conditions and nature of composting cells described here.

ACKNOWLEDGMENTS

The authors would like to thank Solotest LTDA for providing augers kit for test, Tortuga Cia Zootécnica Agrária for funding this work and Fundação Parque Zoológico de São Paulo´s staff for their help during sampling procedures, Dr. Luiz Juliano Neto and Dr. João Batista da Cruz for suggestions and critical reading of the manuscript. Conselho Nacional de Pesquisa e Desenvolvimento (CNPq), Fundação de Amparo a Pesquisa do Estado de São Paulo (Grants 2009/52030-5 to LJN, ALB, RCP, MAV and 2007/50536-3 to MAV).

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