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Preliminary Study on Compost Production as Substrate for *Pleurotus sajor-caju* (Fr.) Singer Mushroom Cultivation in Mauritius

Yaswaree Mihilall • Ackmez Mudhoo • Romeela Mohee*

Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Mauritius, Mauritius

Corresponding author: * **rmh@uom.ac.mu** or **rkmohee@yahoo.com**

ABSTRACT

The in-vessel composting of a mixture of three potential substrates, namely banana leaves (B), mixed cardboard wastes (C) and green *Stenotaphrum dimidiatum* grass (G) was studied for three weeks. The first compost mix ratio monitored was 16 kg banana leaves, 8 kg cardboard wastes and 16 kg *Stenotaphrum dimidiatum* (wet basis). The initial wet moisture content, initial porosity and initial wet bulk density for the BCG mix were 69.5%, 86.9% and 152.7 kg/m^3, respectively. The peak temperature recorded for the BCG mix at day 10 was 55.1°C. The temperature then decreased to 35 to 45°C for a week. It was noted that there was an aggressive colonisation of an unidentified mushroom mycelium on the BCG mix. Mycelium and pinheads thrived mostly on the surfaces of the cardboard mix and throughout the feedstock, where temperatures averaged 35°C and less. The feedstock was removed from the reactor and mixed with 14 kg banana leaves, 14 kg grass (*Stenotaphrum dimidiatum*) and 1.5 L water (second BCG mix), and the composting experiment run afresh. A peak temperature with a maximum of 60.7°C was noted on day 6 and no mycelium growth was observed. It was hence inferred that temperatures should reach a minimum of 60°C to produce a sterile compost substrate for the growth of the oyster mushroom mycelium. The final parameters of the compost after 17 days were as follows: wet bulk density 271 kg/m^3, wet moisture content 54.3%, total dry solids 34.7%, net estimated volatile solids (VS) decrease of 40.5% (fixed ash basis), pH 7.5, electrical conductivity 1.57 mS/cm and respiration rate 2.52 mgCO_2.C/day.gVS.

Keywords: *Pleurotus sajor-caju* (Fr.) Singer, composting, substrate, mycelium

INTRODUCTION

Mauritius generates some 1218 tons of waste on a daily basis and this figure is expected to increase by the year 2020 under intensive development of various economic sectors (Mohee 1998). The composition of waste generated constitutes mainly of 45% yard wastes, 25% food wastes and 6–15% mixed paper wastes. Collection and treatment of the organic fraction of solid wastes can help meet with the reduction of the quantities of wastes reaching the landfill. Composting has been used for many years throughout the world for the stabilization of organic wastes (Hachicha *et al.* 2009; Niwagaba *et al.* 2009). This biological treatment provides a sound, cost-effective and efficient strategy at reducing the quantity of wastes reaching the landfill. Composting typically refers to the controlled decomposition and appropriate stabilisation of blended organic substrates under aerobic conditions that allow the development of thermophilic temperatures as a result of biologically produced heat to produce a final humus-like product with high organic matter content, that is stable, odour free, virtually free of pathogens and plant seeds, and that can have beneficial applications, namely revegetation, biofiltration and bioremediation (Das and Keener 1996; Leonard 2001; Lau *et al.* 2003; Benito *et al.* 2009; Shekdar 2009). In this process, the organic substances are reduced from large volumes of rapidly decomposable materials to small volumes that continue to decompose slowly. The process brings the ratio of carbon to other elements into a balance, thus providing adequate levels of nutrients to the crop in the absorbable state (Fabrizio *et al.* 2009; Fukushima *et al.* 2009). Examples of common waste materials that can be composted are yard and vegetable wastes, food garbage, straw, shredded paper and cardboard, cow, poultry and pig manure (Anastasi *et al.* 2005).

In-vessel composting systems are considered to be the best system for producing quality compost. The reason is because environmental conditions, including temperature, moisture, and aeration are closely controlled (Adhikari *et al.* 2009; Walker *et al.* 2009). This method offers much potential for detailed investigation into compost production and ecological and chemical factors in mushroom nutrition (Miller *et al*. 2006). Hence, the more selective the medium, the better it will support the desired mushroom strain (Oei 1996). The factor creating interest in the present study is the possibility of composting biowastes and lignocellulosic wastes in an in-vessel system and using the resultant compost product as substrate for the cultivation of the *Pleurotus sajor-caju* (Fr.) Singer mushroom. Other studies have been carried out to assess the growth of mushrooms on organic wastes, compost and compost-based substrates. Belewu and Belewu (2005) analysed the cultivation of the mushroom, *Volvariella volvacea*, using banana leaves and determine their effect on the chemical composition of the spent substrate (banana leaves), Pant *et al.* (2006) assessed the cultivation of oyster mushrooms on wheat straw and bagasse substrate amended with distillery effluent and Mandeel *et al.* (2005) evaluated the growth of three species of *Pleurotus*, namely *P. columbinus*, *P. sajor-caju* and *P. ostreatus* on untreated organic wastes including chopped office papers, cardboard, sawdust and plant fibres. Hernández *et al.* (2003) studied the use of wooden crates for composting a mixture of 70% grass (*Digitaria decumbens*) and 30% coffee pulp, combined with 2% calcium hydroxide as a method for preparing substrate for the cultivation of *Pleurotus ostreatus*, and concluded that it was possible to produce *P.*

Received: 21 April, 2009. Accepted: 21 September, 2009.

Original Research Paper

ostreatus on a lignocellulosic, non-composted, non-pasteurized substrate with an initial pH of 8.7, and that composting for two to three days improved the biological efficiency. Earlier, Derikx *et al.* (1990) identified and quantified several volatile sulphur compounds present in air emitted from stacks during the initial phase of the composting process performed to produce a substrate for mushroom cultivation.

More recently, Polat *et al.* (2009) determined the effects of spent mushroom compost (SMC), which is a waste product of mushroom processing through a year, on greenhouse cucumber growth as an organic matter source for the soil. Polat *et al.* (2009) investigated the effects of SMC on several yield related characteristics, such as total yield, fruit width, fruit length, total soluble solids, first quality fruit yield in total yield and nutrition content of the cucumber fruit. Polat *et al.* (2009) observed that the highest total fruit yield was obtained at 40 ton/ha and it was followed by 80 and 20 ton/ha SMC applications. The highest values of fruit width and the nitrogen contents in cucumber were found at 80 ton/ha SMC application. Based on their results, Polat *et al.* (2009) concluded that as an organic material source and amendment of greenhouse soil application of at least 6 months waited SMC was very effective and beneficiary for cucumber growth, productivity and recycling the spent mushroom compost. Adebayo *et al.* (2009) evaluated the yield of *Pleurotus pulmonarius* on different mixtures of cotton waste and cassava peel. *P. pulmonarius* demonstrated significantly higher colonization rate on cotton waste substrate (100 g cotton waste) 3 weeks after inoculation of spawn than any other substrate mixtures. The highest yield was observed on 100 g cotton waste with mushroom yield of 79.4 g followed by 80 g cotton waste + 20 g cassava peel with 72.0 g. Cassava peel (100 g) produced no mushroom fruit. The results of Adebayo *et al.* (2009) showed that cotton waste was a better substrate for cultivation of *P. pulmonarius* than cassava peel. However, with the high availability of cassava peel in Nigeria, the potential use of this waste as substrate adjunct (at 20% of substrate) can be suggested based on the findings of the study. Last but not the least, the amount of button mushroom (*Agaricus bisporus*) harvested from compost is largely affected by the microbial processes taking place during composting and the microbes inhabiting the mature compost. In their study, Székely *et al.* (2009) identified the microbial changes during the stages of the latter composting process. The dominant bacteria of the mature compost were also identified to reveal the microbiological background of the favorable properties of the heat-treated phase II mushroom compost. 16S ribosomal deoxyribonucleic acid (rDNA)-based denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) molecular fingerprinting methods were used to track the succession of microbial communities in summer and winter composting cycles. DNA from individual DGGE bands were reamplified and subjected to sequence analysis. The principal component analysis of fingerprints of the composting processes showed intensive changes in bacterial community during the 22-day procedure. Peak temperature samples grouped together and were dominated by *Thermus thermophilus*. Székely *et al.* (2009) observed that the mature compost patterns were almost identical by both methods (DGGE, T-RFLP). To get an in-depth analysis of the mature compost bacterial community, the sequence data from cultivation of the bacteria and cloning of environmental 16S rDNA were uniquely coupled by Székely *et al.* (2009) with the output of the environmental T-RFLP fingerprints (sequence-aided T-RFLP). Székely *et al.* (2009) deduced that the dominance of a supposedly cellulose-degrading consortium composed of phylotypes related to *Pseudoxanthomonas*, *Thermobifida*, and *Thermomonospora*.

The substrate being used to date in Mauritius for the cultivation of mushrooms has been bagasse from sugarcane stems. However, bagasse is being increasingly used for energy production by factories. Therefore, an increase in price and a decrease in bagasse availability are inevitable. Growers of mushroom are increasingly having problems in obtaining bagasse as substrate. The challenge is therefore to find an alternative substrate using the local feedstocks available. The possibility of producing a substrate from controlled composting of biowaste and lignocellulosic waste has been envisaged in this study. In the same breath, a reasonable fraction of biodegradable wastes being generated can be diverted from landfill disposal, composted and then used as a higher valued product through mushroom production. Including this practice in the current agricultural systems can prove to be a profitable venture for local farmers and for the Mauritian agro-economy towards the production of fresh, protein-rich biomass. Given the actual critical economic situation, it is important for Mauritius to become self-sufficient in food production. Therefore, this preliminary study analyses the potential of producing a good quality compost substrate for cultivating *Pleurotus sajor-caju* (Fr.) Singer mushrooms using locally available waste materials, specifically biowaste and lignocellulosic wastes, through composting. Green wastes, namely, banana leaves and fresh *Stenotaphrum dimidiatum* grass were obtained from different home yards and the cardboard wastes was obtained from retail shops.

Pleurotus sajor-caju (Fr.) SINGER MUSHROOM

General characteristics

Pleurotus sajor-caju (Fr.) Singer can be classified according to its five main structures which are the cap, gills, stem, spores and mycelia. The cap is mmooth, narrowly convex becoming broadly convex with time, 50 to 200 mm in diameter, moist and hairless. The gills are hairless, decurrent and attached to the stem. The st*em i*s short, often horizontal and emerging from the substrate while the spores form a white to lilac-gray print on dark media. The lifecycle of the *Pleurotus sajor-caju* (Fr.) Singer involves two distinct stages, namely the vegetative stage and the fruiting stage. In the vegetative stage, substrate colonisation takes place by both mitotic and meiotic reproduction of haploid basidiospores which germinate to produce interconnected aggregates of mycelial hyphae and clamp connections (Oei 1996; Bessette *et al*. 1997). The mycelial network secretes enzymes and acids to break down the complex nutrients for absorption; heat and carbon dioxide are generated at the same time. The presence of a physical boundary or biological competitor will cause stoppage in substrate colonisation and the mycelium shifts to a transient stasis phase. In the fruiting stage induced by environmental stimuli, such as, temperature, relative humidity, light, carbon dioxide level and nutrient deficiency causes the mycelium to switch from colonisation to mushroom production (Oei 1996; Stamets 2000). This causes the tertiary dikaryotic mycelium to emerge as the primordia from the central mycelial plateau. Rhizomorphs providing nutrients to the growing primordia, cause the formation of multinucleate cells, which are separated afterwards by the septae. The resultant cells continue to expand until the universal veil breaks and the mushroom tissue differentiates into the cap, veil, stem and gills (Bessette *et al*. 1997).

Pleurotus sajor-caju (Fr.) singer cultivation

The world market for fresh, edible speciality mushrooms has experienced considerable growth over the past two decades with an annual production estimated to exceed 14 billion US$. Furthermore, Roach (2006) state that statistics from various groups around the European Union, United States and Australia as well as the Food and Agriculture Organisation have shown a sharp increase in the demand for mushrooms. The reason behind this surge is that modern consumers are progressively seeking health-added benefits to fresh foods compared to canned products (Schneider *et al.* 2005). Much desired mushrooms, such as Oyster and Shii-

Table 1 Growth parameters for *Pleurotus sajor-caju* (Fr.) Singer mushroom.

Lifecycle phase	Parameter				
	Incubation temperature (°C)	Relative humidity (%)	Carbon dioxide (mg/L)	Fresh air exchanges (per hour)	Light requirements (lux)
Spawn run 8-14 days	24-29	90-100	> 5000	1	n/a
Primordia formation 3-5 days	10-24	95-100	400-800	5-7	1000-1500
Fruitbody development 3-5 days	18-27	85-90	400-800	5-7	1000-1500

Cropping cycle: Every 7 to 10 days for two to three flushes

take make up nearly 60 per cent of all specialty mushrooms (Sivrikaya and Peker 1998; Roach 2006). The Phoenix Mushroom (*Pleurotus pulmonarius*) or Indian Oyster Mushroom has been misapplied by cultivators as *Pleurotus sajor-caju* (Moore and Chiu 2001). A common compromise to name this mushroom could be either *Pleurotus pulmonarius* var. *sajor caju* or *Pleurotus sajor-caju* (Fr.) Singer.

The local substrate formulation for *Pleurotus sajor-caju* (Fr.) Singer mushroom is bagasse as substrate, maize seeds as nutrient source for the spawn and calcium carbonate as supplement. Prior to mixing, the bagasse is allowed to ferment for 2-3 days. Afterwards, it is moistened until it reaches the desired water content. Polypropylene bags are filled with the resultant mixture to make a total weight of 750 g. The bags are then heat treated in an iron vessel (Oei 1996; Stamets 2000; Schneider *et al.* 2005) and allowed to cool to 48°C. A maximum of two days is allowed for conditioning. Afterwards, the bags are inoculated with mycelium spawn in a sterile area and then disposed on wooden or metal shelves to allow substrate colonisation. According to Oei (1996), the range at which the mycelium stays viable is between 5 and 35°C, while the optimal temperature range for spawn run ranges from 20 to 25°C. Conversely, Stamets (2000) states that the range should be between 24 and 29°C. Nevertheless, the scale for fruitbody development lies between 13 to 24°C with a maximum of 30°C (Stamets 2000; Obodai and Vowotor 2002). Spawn run takes approximately 2-3 weeks. The substrate is moistened by misting the opening of the bags 2-3 times a day depending on ambient temperature. The relative humidity of the incubation room should be kept at 80 to 90%. Primordia formation will occur in three to five days. Misting should be continued during the development of the fruit body, which takes another three to five days. Three flushes every 7-10 days can be envisaged. When growth parameters of *Pleurotus sajor-caju* (Fr.) Singer are considered for yield optimisation, the literature refers to Stamets (2000) as a benchmark to guide them in the production of this mushroom. The parameters are classified into four main groups, based on the mushroom lifecycle (**Table 1**).

The variations in the lifecycle phase time and growth parameters will be normally determined depending by the type of substrate and local agro-climatic conditions. Various agricultural by-products can be used as substrates for the cultivation of the *Pleurotus sajor-caju* (Fr.) Singer mushroom. Some of these wastes include rice straw and wheat straw, mostly used in Asia and Europe respectively (Thomas *et al.* 1998). Additionally, banana leaves, peanut hull, coffee waste, corn leaves, mango fruits and seeds as well as sugarcane leaves and bagasse can be used (Martínez *et al.* 1984; Cangy and Peerally 1995; Moncaio Moda *et al.* 2005). In South East Asian countries sawdust is more commonly used as substrate (Moore and Chiu 2001). Furthermore, composted lignocellulosic wastes such as chopped office papers, cardboard, pulp mill sludge and sawdust as well as plant fibre, such as cotton waste can be used as substrate for *Pleurotus pulmonarius* production (Martínez *et al.* 1984).

Heat treatment, bagging, inoculation and daily care of the bags do not differ significantly among the substrate formulations. Furthermore, it should be emphasised that availability of the abovementioned feedstock should be taken into consideration before formulating the growth medium.

Composting typically refers to the controlled decomposition and appropriate stabilisation of blended organic substrates under aerobic conditions that allow the development of thermophilic temperatures as a result of biologically produced heat (Mudhoo and Mohee 2008). According to Miller *et al*. (2006), controlled composting is considered as the best system for producing substrate to be used in the cultivation of mushrooms. Reference is made to the in-vessel systems, which occupy the highest level in the composting technology hierarchy. The main reason is because the main parameters affecting composting (temperature, moisture, and aeration) are closely controlled thereby providing the opportunity for producing quality compost in a minimum amount of time.

PHASES OF CONTROLLED AEROBIC COMPOSTING

Compost quality will depend on the control of environmental factors that affect microbial life in composts. The most important parameters for the microorganisms (bacteria, fungi, and actinomycetes) are temperature, oxygen, moisture, pH and substrate composition (Kuo *et al.* 2005). Under optimal conditions, composting proceeds through three phases based on the temperature in the composting feedstock.

Phase 1: Initial Phase

In this phase, mesophilic bacteria utilise readily available organic matter, determining a rapid increase of temperature which can reach 40 to 45°C in a few days to several weeks (Kuo *et al.* 2005).

Phase II: High Rate Phase

Several species of thermophilic endospore-forming bacteria, fungi and actinomycetes are normally present in this phase. Thermophilic bacteria (mainly *Bacillus* spp.) are responsible for the degradation of proteins and carbohydrates, while actinomycetes and fungi contribute to the degradation of more complex compounds like cellulose and lignin. Hassen *et al.* (2001) found that high temperature (above 55°C with a maximum of 60 to 62°C) during thermophilic degradation phase caused a marked change in bacterial community of *E. coli* and faecal Streptococci, as well as yeasts and filamentous fungi, whereby populations decreased sharply. Therefore, pathogens are killed at this stage. Generally, fungi having a limited role in composting, are eliminated above 50°C, and their optimal temperatures are much lower (Kuo *et al.* 2005). During the composting process, microorganisms break down the organic matter and produce humus, carbon dioxide, water, ammonia, new cells and heat (Ghaly *et al.* 2006). In this phase, oxygen diffusion into the solid-liquid compost particles is the rate-limiting factor. Beffa *et al.* (1998) found that thermal hygienization can be achieved at temperatures between 65 to 75°C and not exceeding 80°C. The time-span of the high-rate phase ranges from a few days to several months (Haug 1993).

Phase III: Curing phase

In this phase, the amount of readily available nutrients becomes a limiting factor, allowing further stabilisation, reduction of pathogens and decomposition of cellulose and lignin (Kuo *et al.* 2005). The rate of carbon dioxide production declines, together with temperature. This period lasts from one to two months.

Table 2 Relative amounts and physical characteristics of composting substrates.

Physical parameter	Feedstock material		
	Banana leaves	Mixed cardboard waste	Green grass (*Stenotaphrum dimidiatum*)
Mass of each feedstock (kg)	16	8	16
Moisture content (%)	82.4 ± 2.3	7.1 ± 0.8	82.4 ± 1.7
Total Dry Solids (%)	15.3 ± 2.3	92.1 ± 0.8	15.9 ± 1.7
Total Volatile Solids content (%)	90.7 ± 0.1	93 ± 0.4	87.5 ± 0.1
Ash content (%)	9.3 ± 0.1	7.0 ± 0.4	12.5 ± 0.1
Carbon to nitrogen (C/N) ratio of substrates	41.2 ± 2.3	40.4 ± 2.2	39.4 ± 0.8

Table 3 Chemical characteristics of the BCG mix.

	Nutrient			
	Total nitrogen (Kjeldahl, N)	Total phosphorus	Total potassium	Total carbon
Method of analysis	Spectrocolorimetry (490 nm) (Bolleter *et al.* 1961)	Spectrocolorimetry (650 nm) (Gautheyrou and Gautheyrou 1989)	Flame photometry Modified Truog's method (Puchooa *et al.* 1999)	(100-%Ash)/1.8 (Haug 1993)
Level (ppm)	12883	1177	11910	450500

According to Oei (1996), conditioning is the process during which the mushroom substrate will promote the growth of thermophilic organisms (actinomycetes) to create a suitable environment for the growth of the desired mushroom, at the same time, discouraging the growth of nutrient competitors. The period of conditioning depends on the concentration and the type of nutrients to be transformed. However, the usual temperature for conditioning is 48°C. According to Psarianos *et al.* (1983), a temperature feedback control system can be implemented, where five temperature probes can be positioned across the pile and connected to a data logger. Consequently, temperature signals can be averaged and the centrifugal fan operated if the average temperature exceeds 65°C. Nevertheless, if the temperature remains below the trigger level, aeration can take place according to timer settings to maintain aerobic conditions. Controlled composting systems are relatively new in Mauritius. This field has not been explored much locally, and to a lesser extent when this system is associated with the production of the best quality substrate for oyster mushroom cultivation.

MATERIALS AND METHODS

Reactor design

The experimental reactor used was a horizontal plastic cylinder, 55 cm in diameter and 90 cm in length, with a volume of approximately 0.2 m^3. An opening (aperture) at the top of the drum allows the substrate to be loaded and unloaded. The same outlet was used for periodic grab sampling for different experimental analyses. There are 3 holes of aeration each 5.0 cm in diameter on top of the drum and 1 other hole (underdrain) at the bottom centre. At both ends of the cylinder, 5 cm near the bottom are two holes each 5 cm in diameter. Two polyvinyl chloride tubes having a length of 110 cm each, with a diameter of 5 cm, have been fitted alongside. Each tube has a number of perforations. Each perforation has a diameter of 0.2 mm and situated along the whole length of the pipes. More details of the experimental reactor may be obtained from Mohee and Mudhoo (2005) and Mudhoo and Mohee (2008).

Substrate preparation

The preparation of the feedstocks for the composting experiment was carried out in the composting facility on the University campus. The unit is a sheltered building made up of concrete. There is only a metal reinforced glass door which gives access inside. Once the materials reached the unit, they were shredded with a mechanical shredder to an average length of 5 cm to ensure uniformity during mixing. The compost mix ratio set was 16 kg green banana leaves, 8 kg dry cardboard wastes and 16 kg green *Stenotaphrum dimidiatum* grass (**Table 2**). The total nitrogen, total phosphorus and total potassium were also determined and the results are given in **Table 3**.

The different substrates were then weighed with respect to the ratio to be mixed and then disposed on concrete floor. Using a rake and a shovel, the materials were spread and blended until a uniform mix was obtained. The resultant BCG mix was then filled in the reactor and the initial temperature was recorded using a thermocouple. Temperature changes were recorded over a period of three weeks. At the start of the second week, active aeration was carried out. The two aeration tubes found near the bottom of the drum were connected by means of two polyvinyl chloride shoulders and a polyvinyl chloride T-connection, both having a diameter of 5 cm, to a centrifugal fan operated at a rate of 0.009 m^3 air min^{-1}. As a trial, the blower was activated for 15 seconds every one and a half hour and when the temperature of the feedstock decreased in the reactor. On the third week, the feedstock was removed from the reactor and mixed with 14 kg banana leaves, 14 kg grass (*Stenotaphrum dimidiatum*) shredded to 5 cm length and 1.5 L water. The new BCG mix was then put back in the reactor for composting. Each week, random grab samples were taken directly from the outlet. Consequently, 2 L grab samples of composting material was taken from at least 15 points from the reactor. The sample was placed in a dry and clean plastic bag which was securely tied at both ends and kept for various analyses in the laboratory. Each test was carried out in triplicate. Hence, the values presented for the variation of the composting parameters in the graphs to follow are an average of a sample size 3 for each parameter. The composting parameters tested each week were as follows.

Temperature

The temperature of the compost pile was recorded everyday using a thermocouple (*CheckTemp1*, ± 0.1°C) with a probe of 1 m dipped at three different openings namely, T_1, T_2 and T_3 along the surface of the pile (Mudhoo and Mohee 2008). The probe was dipped slowly through a few centimeters and the temperature was allowed to stabilise on the monitor of thermocouple. The same procedure was carried out several times across the depths of the pile until the maximum temperature was recorded vertically down the initially point of temperature reading.

Bulk density

The initial wet bulk density of the samples was determined according to Agnew and Leonard (2003). A pre-weighed 1000 mL plastic container was filled with the compost sample material loosely up to the brim and the final weight was recorded. The bulk density was calculated by dividing the mass of the compost sample by the volume of the container. Masses were recorded to the nearest 0.1 g on a Mettler PM3000 top balance.

Particle size

The particle size of the feedstock materials was determined using a Ro-tap testing sieve shaker. 20-25 g of substrate was weighed and placed on the sieve having the largest mesh size. A total of 7 sieves having mesh sizes (26.67, 18.85, 13.33, 6.680, 3.327 and 1.651

mm) were used. A final metal vessel was placed at the bottom of the last sieve to collect any particle smaller than 1 cm.

Moisture content

100–200 g of sample was weighed in oven-dried aluminium plates and then placed in a preheated forced-convection oven at 105°C for 24 h and heated to constant mass. The samples were then equilibrated to room temperature in a dessicator for about an hour before recording the final weight. The moisture content was calculated by subtracting the final and initial masses of the plate with the compost sample and dividing it by the wet mass of the subsequent sample. Masses were recorded to the nearest 0.1 g on a Mettler PM3000 top balance.

Total dry solids

The percentage total dry solids content was calculated by subtracting the percentage moisture from 100%.

Volatile solids (VS)

1–2 g of oven-dried samples were weighed in pre-weighed dry porcelain crucibles and then placed into the muffle furnace and burnt to 550°C for about 2 hrs (BS1377 method). The volatile solids of each sample was determined by the equation

$$VS(\%) = \frac{M_{dry} - M_{burnt}}{M_{dry} - M_{crucible}} \times 100\% \quad (1)$$

where M_{dry} = mass of sample and crucible before burning (g), M_{burnt} = mass of sample and crucible after burning (g), $M_{crucible}$ = mass of empty crucible (g). The masses for *VS* determination were recorded to the nearest 0.001 g on the Mettler PM400 top balance.

Ash content

The percentage Ash was calculated by subtracting the percentage Volatile Solids from 100%.

pH

The pH was determined electrometrically in a slurry of the homogeneously ground oven-dried compost and calcium chloride solution. The original sample was sieved to <10 mm. 200 mL of 0.01 mol/L of calcium chloride solution was added to 20 g of the sample in a clean dry beaker. The mixture was stirred with a clean glass rod and the pH determined after one hour over the collected filtrate using a calibrated *EcoScan* pH (buffer 4, 7 and 10) meter. Excessive vigorous homogenisation was avoided in an effort to avoid gas exchanges which might alter slurry pH.

Electrical conductivity

20 g of fresh compost sample, sieved to less than 10 mm, was added to a beaker containing 200 mL of distilled water and stirred constantly for about 2 hrs. The mixture was then filtered. The filtrate was used to determine the electrical conductivity using a calibrated electrical conductivity meter (Model Lutron pH-201 Hand Held Digital pH meter).

Heavy metals

The heavy metals contents for lead and cadmium were determined using atomic absorption spectrophotometer. The experiment was carried out at the Heavy Metal Analysis Section of the Chemco Laboratory, Chemco Co. Ltd., Riche Terre, Mauritius. Reference for the experimental procedures was made in The *Standard Operating Procedure for Metal Analysis by AAS SOP MLD 005, California Air Resources Board, Inorganics Laboratory Section – October 2003, Revision 6.0*.

Respiration test

The respiration rate was measured as per the experimental method described by Mohee *et al.* (2008). If the level of carbon dioxide measured remained fairly constant, this would indicate that the compost has stabilised. The respiration test was performed in four jars. Three of the jars contained a known mass of fresh compost derived from the main compost pile and the fourth jar was a blank (no compost). The moisture content of the compost taken from the pile was determined prior to filling in the jars. 25.0 to 25.5 g of as-received compost was weighed, carefully deposited inside the jar and then thinly spread at the bottom. 20.0 mL of freshly prepared 1.0 mol/L potassium hydroxide was pipetted and transferred into a 100 mL beaker that fitted exactly through the neck of the jar. With the aid of a hook, the beaker was gently lowered into the jar and securely deposited alongside the compost. Care was taken not to cover any compost material under the beaker. The jar was then tightly sealed with a rubber bung. The date, time and mean air temperature were also recorded. This procedure was repeated for two other jars while a blank was prepared with the same volume of alkali.

The four jars were stored at room temperature (25.0 to 27.0°C). The amount of carbon dioxide released and absorbed by the potassium hydroxide trap was monitored over the next three days using simple titrimetric methods with standardised HCl acid. Standardisation was performed using aqueous sodium carbonate and methyl orange indicator following the standard procedures. The acid concentration used had a concentration of 0.966 mol/L.

Every 3 days, the potassium hydroxide containing beakers were carefully removed, an equal volume of fresh 1.0 mol/L potassium hydroxide replaced and the jars resealed. The titrations were carried out immediately. The burette was filled with the hydrochloric acid (HCl) and zeroed. Two to three drops of phenolphthalein indicator were added to the potassium hydroxide solutions. The potassium hydroxide was titrated with the acid until the endpoint was reached whereat colour of solution changed from pink (or purple) to colourless. The volume of acid required for each of the four potassium hydroxide samples was recorded to the nearest 0.2 mL. The date and time of the experiment were also recorded. Based on direct stoichiometric analysis, the higher the amount of carbon dioxide released from the compost sample and absorbed by the potassium hydroxide trap, the less acid will be needed for titration to endpoint. The mass of CO_2 generated by the compost sample may be calculated using the expression

$$CO_2.C(mg) = 12 \times (HCl_b - HCL_s) \times [HCl] \quad (2)$$

where HCl_b = mL HCl used in titration of blank, HCl_s = mL HCl used in titrating sample from jar containing compost, [HC*l*] = concentration of hydrochloric acid used (mol/L), and $CO_2.C$ = mass of CO_2 – Carbon generated (mg). The value for mg CO_2.C/g organic carbon/day was determined using the relationship mgCO_2.C/g organic carbon/day = mass of CO_2.C (mg/day)/organic carbon (g), where the organic content (g) was itself deduced from the equation Organic carbon (g) = (wet weight of sample) (100-%wet moisture) (%Carbon). The %Carbon was estimated from the equation %C = 0.55 (%VS) (Haug 1993).

RESULTS AND DISCUSSION

Temperature

Heat generation results from microbial activity, so the composting process experiences an initial rise in temperature followed by declining and stabilized temperatures (Iyengar and Bhave 2006) as microbial activity decreases due to lower levels of available organic matter (Hagerty *et al.* 1973). The temperature was monitored daily from the start of the experiment and the three weeks onwards. The average temperature for the first three weeks was 41°C reaching a maximum of 51.5°C at day 9 as shown in **Fig. 1**. On day 7 and 8, a general decrease in temperature was recorded since active aeration was carried out on day 8. This resulted to a surge in temperature on day 9 and the resulting maximum temperature. The white mycelium of an unidentified mushroom was observed during that period. Aggressive colonisation was mainly found on the cardboard mix. Mycelium colonisation was allowed for further obser-

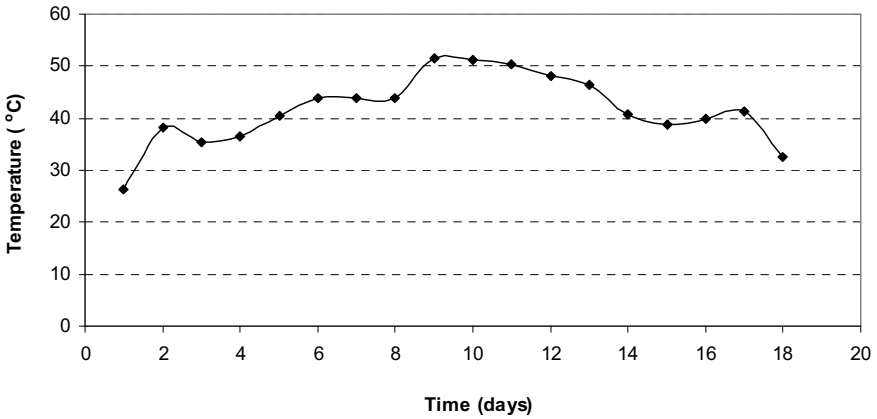

Fig. 1 Average temperature variations for BCG compost mix.

Fig. 2 Mushroom growth on the substrate being composted in the reactor.

vation because it was impracticable to identify the mushroom until pinhead formation and consequent pileus and stipe development. A pungent smell was detected due to mycelium spore production on the substrate. **Fig. 2** shows the mushroom growing on the substrate feedstock inside the reactor. The mushroom was supposed to belong to the Genus *Coprinus*. The growth of this mushroom on the compost substrate is normally undesirable since it will compete with the oyster mushroom for nutrients, specifically, cellulose.

Once the mushroom was identified, the blower was activated to raise the temperature so as to destroy the developing pinheads. This explained the increase in temperature on day 16 and 17. However, the temperature decreased on day 18 despite mechanical air supply. The compost was activated again by mixing the feedstock with 14 kg banana leaves, 14 kg grass (*Stenotaphrum dimidiatum*) and 1.5 L water. The second BCG mix was then put back again in the reactor and the composting experiment run afresh. **Fig. 3** shows the temperature variations of the second BCG mix.

The average temperature monitored was 44.6°C with a maximum of 60.7°C on day 6 for an airflow of 0.009 ± 0.0004 m^3 air min^{-1} (540 ± 24 L/h). The temperature remained above 55°C for three days (Finstein *et al.* 1986). The Environmental Protection Agency requirement of three days at 55°C or higher, to meet "Process to Further Reduce Pathogens" regulations (VanderGheynst 2007) was hence met with the second composting mix. Mechanical aeration was carried out on day 8 but the temperature rose by 1-2°C on day 9 and then decreased on day 10 and onwards. However, no mycelial growth was observed during the subsequent two weeks of monitoring. Similar trends in the evolution of compost matrix temperature have been reported by previous researchers. Mohee and Mudhoo (2005) recorded a peak temperature of 66.3°C on day 4 during the aerobic in-vessel co-composting of dry *Acacia* wood chips, mixed green vegetables and stabilized chicken manure whilst Mohee *et al*. (2008) reported a maximum temperature 67.2°C for the co-composting of waste white office shredded paper with boiler litter on day 5. De Guardia *et al.* (2008) equally observed that the temperature of the composting materials (sludge from effluents and wood chips mix) increased and decreased under forced aeration batch composting regime. The mean temperatures reached a maximum around 67°C when airflows were higher than 500 L/h. When airflows were 100 and 200 L/h, De Guardia *et al.* (2008) noted that the temperature increased up to 50 and 62°C, respectively, and remained constant for 2–5 days. These results hence indicate that the composting process in the present study had experienced sufficiently high thermophilic temperatures (Raviv *et al.* 1999; Venelampi *et al.* 2003; Hong and Park 2004) for reasonably long time periods.

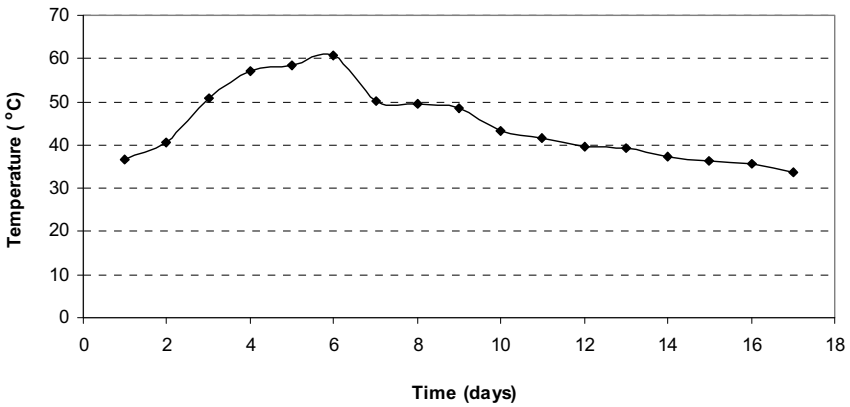

Fig. 3 Average temperature variations for second BCG compost mix.

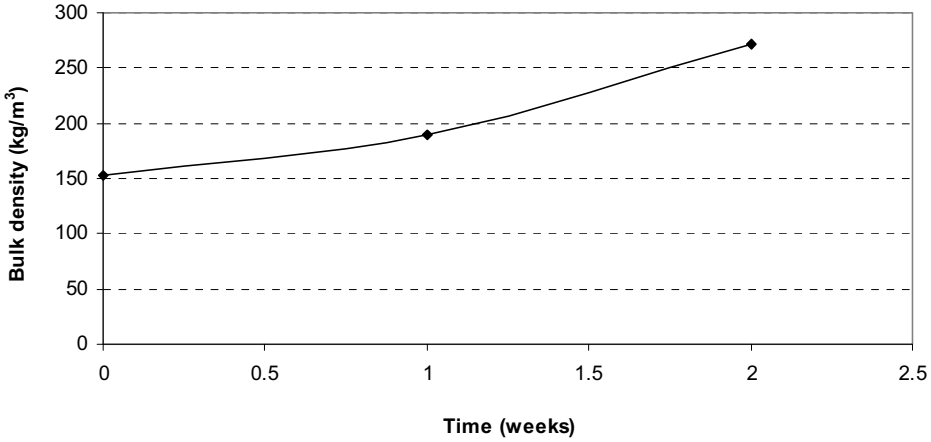

Fig. 4 Average wet bulk density for the second BCG mix.

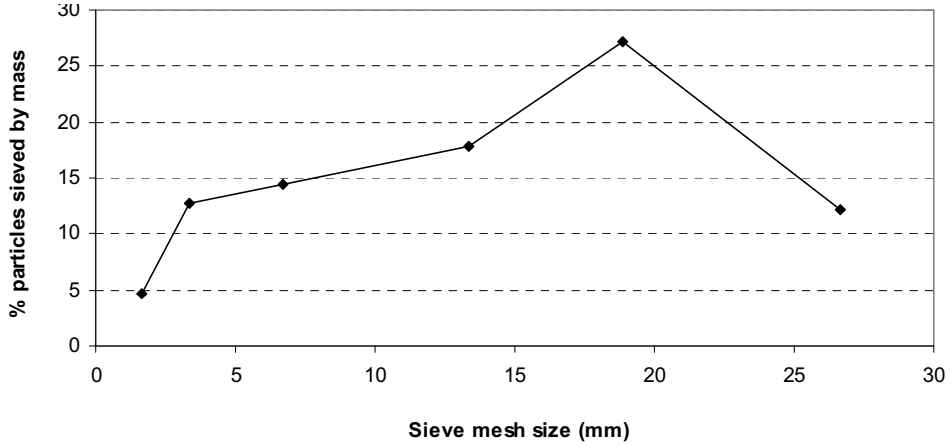

Fig. 5 Percentage particle size sieved by mass for the second BCG mix.

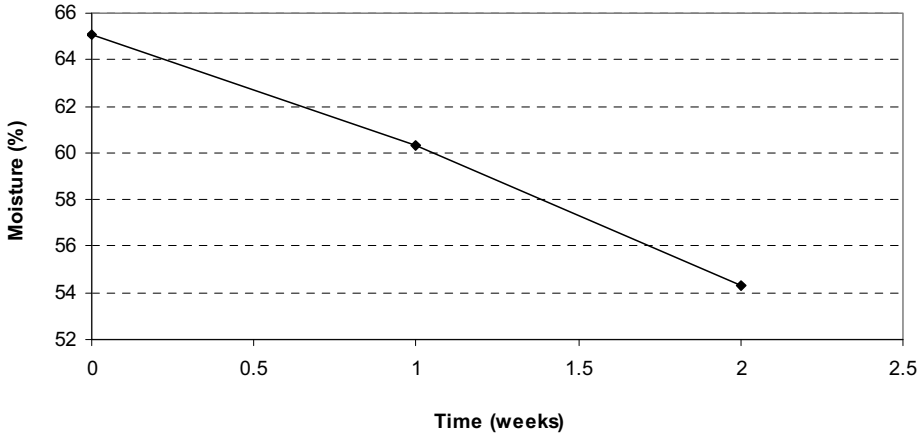

Fig. 6 Average moisture content for the second BCG mix.

Wet bulk density

The starting wet bulk density for the second BCG mix was 152.7 kg/m^3. On weeks 1 and 2, the bulk density increased to 190 and 271 kg/m^3 as shown in **Fig. 4**. This may be explained by the fact that during the decomposition process, physical structure decreases and bulk density increases. Larney *et al.* (2000) stated that bulk density should normally increase with composting time, based on a mass decrease usually accompanied by more pronounced volume reductions (Iyengar and Bhave 2006). As supported by the results in **Fig. 4**, the initial feedstock materials, their physical preparation and the mix ratios used may have synergistically influenced the changes in bulk density (Agnew and Leonard 2003).

Particle size

To ensure a constant uniform average air flow across every horizontal cross-section of the waste material bed, feedstock particle size should equivalently range between 12 to 50 mm (Mathsen 2004). As a result, the surface area for microorganisms to consume and accelerate decomposition is increased. **Fig. 5** shows the percentage particle size of the new substrate mix at the start of the composting experiment was within the acceptable range.

Moisture content

The initial moisture content of the first BCG mix was 70% and was not adjusted because the mixed cardboard waste had a relatively low moisture content of approximately 7%.

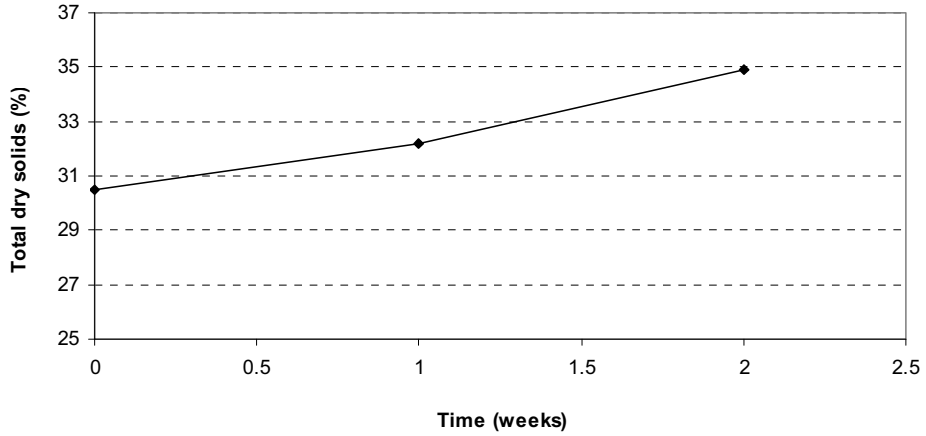

Fig. 7 Percentage dry matter for the second BCG mix.

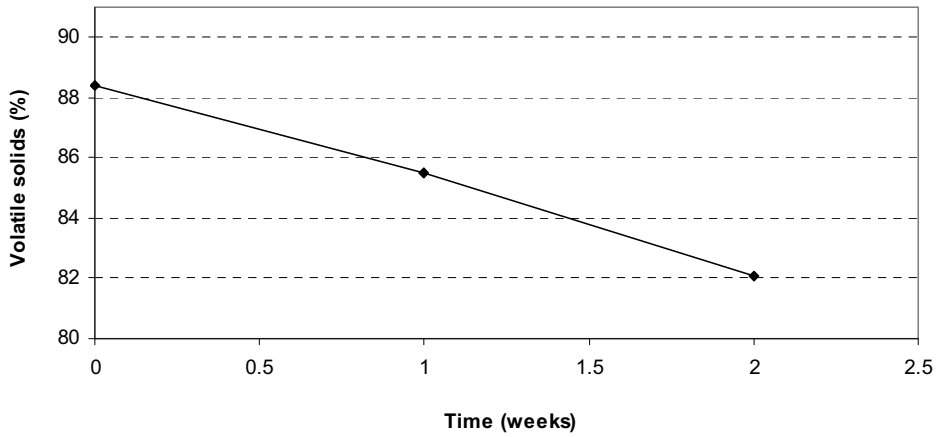

Fig. 8 Volatile Solids for the second BCG mix.

This material was used to provide the main carbon source and act as bulking agent. The high moisture content of the feedstock at the beginning of the composting thus favoured mushroom growth. According to Stamets (2000), any substrate is colonised by a mushroom mycelium if its moisture content is between 60-75%. Nevertheless, the moisture content of the new mix was brought to 65.1%). The moisture content then decreased continuously during the first two weeks, as shown in **Fig. 6**, reaching 54.3% in week 2. The decrease in moisture content may be attributed to loss of water due to evaporation during the thermophilic phase (Ghaly *et al.* 2006; Mohee *et al.* 2008).

Total dry solids

The content remaining after a compost sample has been dried is known as total dry solids (or dry matter). The initial percentage of dry matter of the second BCG mix was 30.5% as shown in **Fig. 7**. During the second week, this percentage increased to 34.9%. As the moisture content of the compost decreased, the total dry solids increased.

Volatile solids

Volatile solids (VS) is the measure of carbon-based materials in the compost and therefore quantifies the amount of organic constituents converted to carbon dioxide. It gives a good indication on how and to what extent biological degradation has occurred. Values of volatile solids present in different feedstocks were between 65 and 99%. **Fig. 8** shows the change in VS during the first two weeks of the composting process for the second mix. The initial volatile solids content was 88.4%. However, in the following two weeks, this value decreased to 85.5 and 82.1%, respectively (Lemus and Lau 2002). These corresponded to a net estimated VS decrease of 40.5% (fixed ash basis) resulting from the microbial decomposition of the organic matter. This net decease in VS hence indicates that microbial decomposition and hence degradation of biodegradable organic matter in the feedstock materials had taken place (Huang *et al.* 2004; Joo *et al.* 2007; Mohee *et al.* 2008).

Ash content

Ash is the inorganic part of compost. It is mainly composed of minerals such as calcium, magnesium, phosphorus, and potassium. The initial ash content was 11.6%. This percentage increased to 14.5 and 17.9%, respectively, during the following two weeks (**Fig. 9**). The ash component increases due to the loss of volatile solids (Mohee and Mudhoo 2005; Alburquerque *et al.* 2006) during the composting process. As the decomposition proceeds, the weight loss and increase in ash content causes the wet bulk density to increase as supported by **Fig. 4**. Similar trends for an increase in ash contents were recorded by Wang *et al.* (2004) during the co-composting of dairy manure and straw and dairy manure and sawdust for the initial 20 days of both composting experiments.

pH

The average acceptable pH for microbial activity ranges between 5.5 and 9.0 (Rynk 1992). The initial pH for the new BCG mix was 7.72 as shown in **Fig. 10**. This value then decreased to 6.4 during the first week and then increased again to 7.53 in the second week. The decrease in pH can be attributed to the formation of organic acids as a result of biological degradation. However, the increase in pH after the first week is due to the decomposition of the organic matter, proteins and amines that produce ammonia gas. The pH of the composting material dropped during the initial days of composting due to the formation of organic

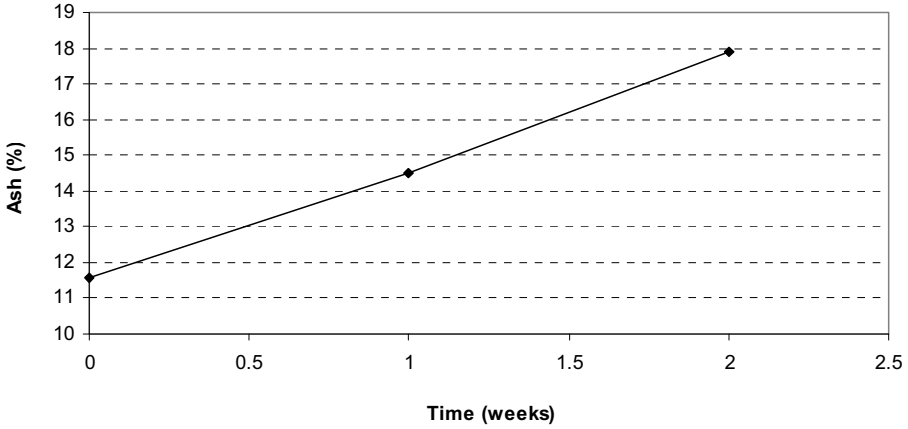

Fig. 9 Ash content for the second BCG mix.

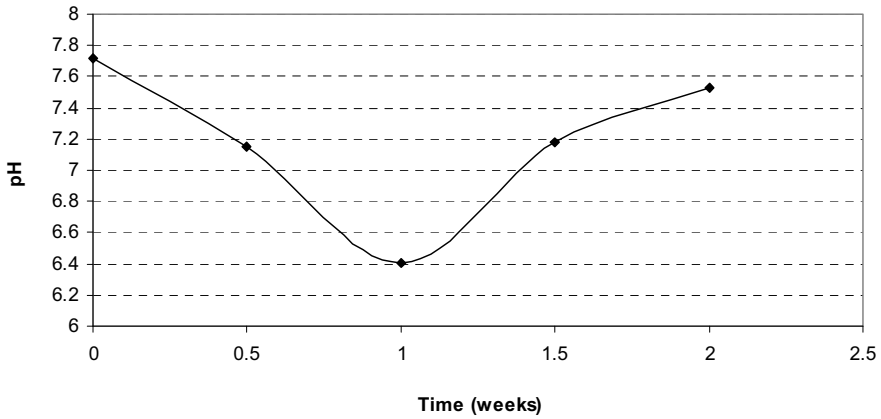

Fig. 10 pH for the second BCG mix.

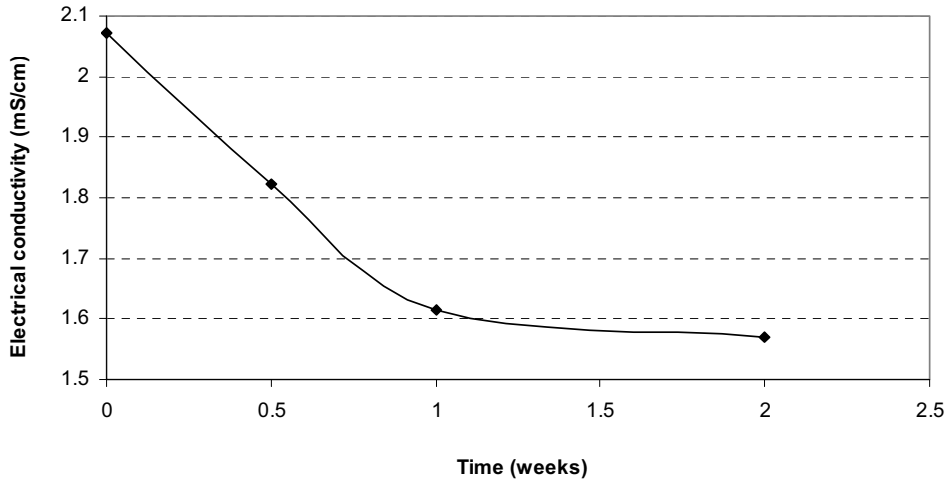

Fig. 11 Variation of electrical conductivity in the new BCG mix.

acids, i.e., amino acids and other volatile fatty acids (Hagerty *et al.* 1973). After this period, the pH tends to move towards neutral again when these acids have been converted to carbon dioxide by the microbial action (Iyengar and Bhave 2006). This drop in pH values as observed in the present study for the initial days of decomposition of biodegradable organic matter is in agreement with the observations made by Seo *et al.* (2004). These results, therefore, show that the average pH (varying from 6.4 to 7.7) of the composting materials was acceptably within the normal range (6.5–8.5) for optimum microbial activity. pH was, thus, not a limiting factor for the compost set-up studied.

Electrical conductivity

Electrical conductivity is an indirect measure of the soluble ions in a solution of compost and water (U.S. Composting Council 2002). Excess soluble salts can cause phytotoxicity to plants. To avoid damage to plants when using compost, total soluble salts in the final blend should be 1.5 - 2.0 mS/cm (Kuo *et al.* 2005). However, an electrical conductivity of 6 mS/cm is acceptable for fruits and vegetables (Cooperband 2002). **Fig. 11** shows the variation of electrical conductivity. At the start, the electrical conductivity was 2.07 mS/cm. The value then decreased to 1.57 mS/cm in week 2. The final electrical conductivity varies significantly with many factors among which the type and relatively amounts of materials composted, the soluble salt content in the compost (Krogmann 1999), the time of composting and the amounts of leachate drained off (Khoshgoftarmanesh and Kalbasi 2002) are the most important. A total volume of 3.1 L of leachate had been collected from the underdrain. Several final electrical conductivity values (**Table 4**) have been reported for different composts but it remains difficult

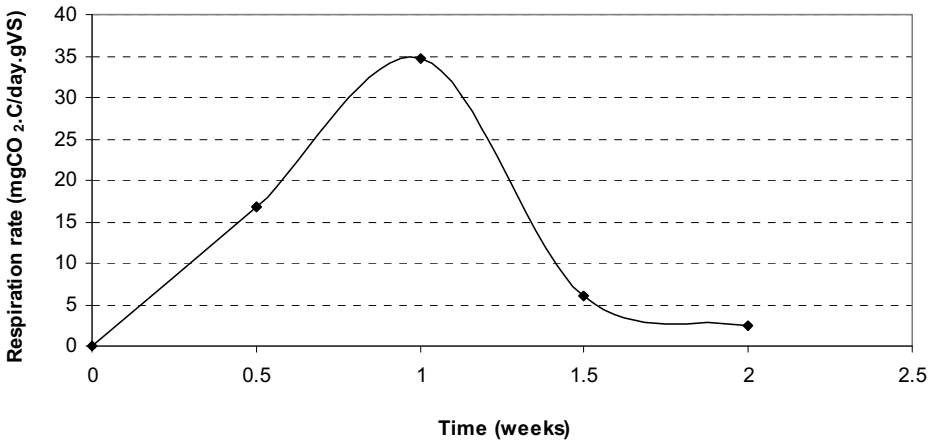

Fig. 12 Evolution of respiration rate with time for the second BCG mix.

Table 4 Final electrical conductivity values for finished composts.

Reference	Electrical conductivity (mS/cm)	Material
Campbell and Tripepi (1991) [a]	1.1-1.9	Yard waste compost
Garcia *et al.* (1991) [a]	7.8-9.8	Mixed organic city refuse
Grebus *et al.* (1994) [a]	1.5-3.3	Yard trimmings compost
Wang *et al.* (2004)	8.7-9	Dairy manure-sawdust compost
Wang *et al.* (2004)	21-21.4	Dairy manure-Straw compost
Hao et al. (2005)	15.0-16.3	Cattle manure compost amended Phosphogypsum
Mohee and Mudhoo (2005)	8.22-8.58	Mixed green vegetables and chicken manure compost with woodchips
Gea *et al.* (2005)	1.31	De-inking sludge from the recycled paper manufacturing industry
Mohee *et al.* (2008)	3.44-5.43	Shredded Paper, Broiler litter
Gómez-Brandón *et al.* (2008)	2.57-3.03	Cattle manure compost
Hachicha *et al.* (2009)	9.21	Olive mill wastewater sludge issued from the evaporation ponds and poultry manure compost
In this study	1.57	Mixed banana leaves, mixed cardboard and *Stenotaphrum dimidiatum* grass compost

[a] Data Source: Agnew and Leonard (2003)

to confirm compost stability of the compost mix studied with reference to these data.

Heavy metals

The heavy metal contents of the fresh second BCG mix in terms of lead and cadmium were found to be below the detection limits, 0.01 and 0.002 mg/L, respectively. These values were well below the levels specified by the Mauritius Standards (Mauritius Standards, 2008, pers. comm., Department of Environment, Ministry of Environment, Mauritius), European Standards (2005) and Brinton (2005).

Respiration rate

Active composting generates significant carbon dioxide due to the biological activity in the feedstock. For the second BCG mix, on day 0 (**Fig. 12**), the quantity of carbon dioxide generated was quasi nil. However, there was a sharp increase in gas evolution (34.3 $mgCO_2$.C/day.gVS) on day 6. This corresponded to the first week of active thermophilic composting supported by the maximum temperature rise of 60.7°C and the three-day temperature retention of 55°C (**Fig. 3**). On day 7, there was a sharp decrease in the amount of carbon dioxide generated (Wang *et al.* 2004) falling to 20.3 $mgCO_2$.C/day.gVS, 5.1 $mgCO_2$.C/day.gVS and 2.48 $mgCO_2$.C/day.gVS on day 10, 14 and 18, respectively. This rapid decline may be explained by a reduction of thermophilic activity due to the depletion of substrates in the feedstock. According to the California Compost Quality Council Standards and Brinton (2005), 2-8 $mgCO_2$.C/day.gVS correspond to 'stable compost' and is usually suitable for seeds. Hence, the compost produced after 20 days from the second mix was stable. Similarly, Kalamdhad *et al.* (2008) assessed the stability of compost using respiration techniques for different C/N ratio waste combinations of grass cutting mix vegetable waste, cattle manure, food waste and sawdust in a rotary drum composter. The CO_2 evolution rates for C/N ratio 16, 22 and 30 decreased from initial values of 7.88, 7.24, and 7.01 $mgCO_2$.C/day.gVS to 0.76, 0.84 and 1.24 $mgCO_2$.C/day.gVS, respectively, after 20 days of composting (corresponding to 90.3, 88.8 and 81.9% decrease in CO_2 evolution during the three composting experiments, respectively). In the present study, an 85.7% decrease from the peak CO_2 evolution rate after 9 days of composting has been recorded for an initial mix of C/N ratio 34.9. These similar results strongly reflect the viability of the rotary drum composting reactor for various types of organic wastes waste with varying C/N ratios and in producing a final stable compost.

CONCLUSION

In this study, the possibility of composting a defined substrate mix composed of banana leaves, cardboard wastes and *Stenotaphrum dimidiatum* grass was assessed. The temperature of the second BCG mix run during the composting experiment peaked to 60.7°C and remained above 55°C for three days compared to the first BCG mix which reached a temperature of 55.1°C for only one day. This explained the absence of mycelial growth in the second run. The various parameters monitored and their respective variations and end values collectively support that the second composting experiment ran as a typical composting process and the final compost product was stable. The above findings reveal an opportunity for the commercial implication of *Pleurotus sajor-caju* (Fr.) Singer mushrooms cultivation for utilization of different feasible and cheap recyclable organic residues.

FUTURE WORK

As sequels to the present preliminary study, the compost thus produced will be used and assesses as a substrate for growing *Pleurotus sajor-caju* (Fr.) Singer mushroom. The

growth of the mushrooms and the yield will be compared with four other local substrates, namely, bagasse, composted market waste, composted yard waste and wood chips. Furthermore, pileus size and stipe length will be measured in each case. Substrates suitable for mushroom production require a balance between readily available nutrients and nutrient sources that require more enzymatic breakdown. Hence, the cellulose content of the determined feedstocks will be monitored during the different composting phases during the subsequent composting runs. The best substrate for *Pleurotus sajor-caju* (Fr.) Singer mushroom will be then determined according to the subsequent yields and nutritional composition of each yield obtained on the different locally available substrates.

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