Pathogen Die Off in Vermicomposting Process

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ABSTRACT

Vermicomposting has been utilised in waste management for a long time, as it is a good alternative to microbial composting. Vermicomposting toilets are an alternative to the common flush toilets to conserve water and reduce environment pollution due to disposal of sludge and wastewater from sewage treatment plants. The factors that are of main concern in using vermicomposted faecal matter in gardens are the health risks associated with the pathogens that are contained in faecal matter. Therefore this study investigated the pathogen die off in the vermicomposting process of human faecal matter. Faeces was collected in a Vermicom composting toilet using sawdust as a covering substrate. A portion of the samples was spiked with a known concentration of *Escherichia coli, Enterococcus faecalis* and *Salmonella typhimurium*. The faecal matter was then vermicomposted over a period of nine months. Sampling was undertaken monthly to determine the pathogen concentrations. The pathogen levels declined steadily over the composting period and were sufficiently reduced by the fourth month to the quality of class A or B composts according to the ARMCANZ (1995) guidelines for composts. A slight regrowth occurred in the sixth month. However, at the end of the composting period determined by the physical and chemical quality of compost, the pathogens declined to below detection levels.

Keywords: Vermicompost, pathogens, faecal matter, onsite treatment system

INTRODUCTION

Wastes generated by human activities can pose serious environmental problems if not handled appropriately. Industrialisation and overpopulation produce waste over the assimilative capacity of the environment. In large cities wastewater treatment plants are constructed to handle the liquid wastes generated by domestic and industrial activities. The treated effluent is disposed of in rivers and oceans. The resulting sludge from these plants is usually stabilised and sometimes reused as soil additives or simply ends up in landfill (Neuman & Bowden, 1989, Sidhu *et al*., 2000). These disposal methods will lead to land, water and air degradation causing health risks to plants and animals as the sludge contains high numbers of pathogens and heavy metals. Some waste treatment plants process this waste further by composting, which will reduce the pathogen levels.

The use of flush toilets wastes potable water and larger volume of wastewater are diverted to treatment plants. On-site treatment systems such as composting toilets are an alternative to these normal toilets. In isolated places such as mountains, drop toilets have been used safely for centuries. The Norwegian government conducted their first studies into composting toilets and their safety in 1973 and since then, the composting toilet and on-site waste treatment has gained more acceptance (Wynn, 2002).

Vermicomposting has been utilised in waste management for a long time, as it is a good alternative to microbial composting. Composting worms such as *Eisenia* fetida can eat each day around half of their body weight and the converted organic material is rich in plant nutrients and soil microbes. (Panikkar & Riley, 2003, DNR, 2003). Most studies conducted on vermicomposting focus on

green waste processing (Appelhof, 1997, Kale, 1998, Bansal & Kapoor, 2000). The advantages for using vermicomposting toilets are that dry vermicomposting does not use water for flushing and hence water is being saved. Furthermore, by using these toilets, no wastewater is generated that has to be dealt with in the wastewater treatment plants and the resulting compost from the toilets can be reused as soil amendments in the garden (Edwards & Steele, 1997). This will reduce the usage of chemical fertilisers in gardens and can be a sustainable way to handling one's own waste.

The factors that are of main concern in using vermicomposted faecal matter in gardens are the health risks associated with the pathogens contained in faecal matter. To date onsite vermicomposting of faecal matter and its quality has not been researched much. The period of composting that is required for faecal matter is not well understood. The microbial quality of the final compost for safe garden application is inconclusive. This investigation therefore focuses on the microbial quality of vermicomposted faecal matter and how safe it is for garden use.

MATERIALS AND METHODS

The procedure involved assessing the composting process of the faecal matter collected in a vermicomposting toilet and the microbial quality of the composted samples.

Vermicomposting Toilets (Vermicom)

A Vermicom toilet from Barefoot Engineering (Hobbs, 1999) was used for sample collections and maturation in this study. The toilet is made up of 113 litre polyethylene tub that measure 64 centimetres in diameter and 64.3 centimetres in height. Each toilet has four 30 millimetre snap in vents near the top edge of the tub, for aeration. The tub is fitted with a modified soak liner to ensure sufficient air circulation. It is covered with a plywood top that contains a standard toilet seat. The plywood top is fitted with a 12 Volt Direct Current fan and a 40 mm PVC pipe to expel odours and CO_2 that is produced by the worms and bacteria in the composting process. A drain coil is fitted for drainage of excess liquids at the bottom of the tub with a tap.

Substrates

A layer of wood chip mulch was placed at the bottom of the toilets for good drainage. The toilet was provided with 500 g of mature worms (tigers *Eisenia fetida*) and 500 g of worm castings. The worm castings were added to provide a substrate for the worms to live in and to prevent the toilets from becoming extremely acidic at the start of collection. In addition, the worm eggs within the worm castings speed up the breeding up process during sample collection. Sawdust (SD) was chosen to cover the faecal matter after each use as it is a carbon source, easily available, cheap and easy to handle.

Sample Collection

The Vermicom toilet used for sample collection and maturation was supplied to a household that had 2 to 3 members utilising the facilities. The household was provided with sawdust and a scoop for taking uniform quantity of substrate. Approximately 80 faecal samples were collected in the toilet over a period of two months. After the collection period, the toilet was collected from the household and left for maturation at the Environmental Technology Centre (Murdoch University).

Bacterial seeding

Excess bacterial seeding was conducted in a fraction of the original sample to study the pathogen die off against the influence of a definite quantity of different pathogens during the composting process. A sample (2 kg) from the originally collected samples was transferred to a separate

Vermicom toilet bin and was seeded with bacterial cultures of *Salmonella typhimurium* (M3016), *Escherichia coli* (ATCC 4157) and *Enterococcus faecalis* (ATCC 29212), obtained from the Path Centre at Sir Charles Gardiner Hospital, Perth. The pathogens were cultured in broth for the spiking of the compost sample. *Escherichia* coli was grown in membrane lauryl sulphate (MELS) broth, *E. faecalis* was grown in tryptone soy broth (TSB) and *S. typhimurium* was grown in buffered peptone water (BPW). The bacterial concentrations were determined by dilution series and plate method.

Microbial analysis

Microbial analysis of the original sample and the spiked sample were conducted monthly over a period of nine months. One scoop each was collected from three different areas of the compost and then mixed together for analysis. For the microbial analysis 1 g of the collected sample was weighed out and added to 9 ml of distilled water and then mixed thoroughly for 10 minutes. One ml of the mixture was then added to 10 ml of distilled water and again shaken vigorously before testing. As the pathogen numbers decreased over time the Most Probable Numbers (MPN) test was utilised.

Total Bacterial Count

Total bacterial counts were carried out initially. However, as the pathogen numbers decreased, the total bacterial counts increased exponentially. By the third month the total bacterial count was over 10^8 CFU (Colony Forming Units) and it was too ambiguous to undertake total counts and was therefore not tested further.

Escherichia coli

Initially, while the *E.coli* concentrations were high, Standard Methods (1995a) was used for the determination. No vacuum filtration was undertaken due to the high pathogen concentrations and dilution series were made to determine bacterial numbers. As the bacterial numbers decreased over time Standards Australia (1995b) for MPN (Most Probable Number) method was used.

Enterococcus faecalis* (formerly *Streptococcus faecalis)

Standard Methods (1995c) was used for the determination of *E. faecalis*. No vacuum filtration was undertaken and dilution series were made to determine bacterial numbers. For *E. faecalis* the initial dilution had to be made up to 1:100. As the bacterial numbers decreased, the MPN Standard Methods (1995d) for *E. faecalis* was employed for the test series.

Salmonella typhimurium

For *S. typhimurium* the initial dilution was 1:100. Then, 0.01 ml aliquots were spread onto the SS (Salmonella-Shigella) agar plates with a spreader. Incubation was at 37°C for 24 and 48 hours. As the bacterial numbers decreased the MPN method as described by Sidhu *et al*. (2000) which has been specifically developed for compost was used. In this method 50 g each of compost is pre-enriched with buffered peptone water (BPW) to enable the detection of the pathogen. *S. typhimurium* was confirmed with biochemical tests as has been described in the Standard Methods (1995e).

Chemical and Physical analysis

The two composts were tested for phosphate, nitrate (water soluble) with a HACH kit and the pH with a Manutech two component soil test kit. Total kjeldahl nitrogen (N) was determined by the 2600-N, sulphuric acid digestion method and total carbon (C) was determined by the 6200-C, high temperature combustion method. The physical properties were determined by comparing the compost to potting mix that is commercially available.

RESULTS

Escherichia coli

The colony counts for both the compost (C) and the spiked compost (SC) was high ranging from 3.9 x 10^5 to 4.7 x 10^5. By the fourth month the pathogens were reduced to between 24 and 46 CFU/g of compost that almost stabilised during further composting. A slight regrowth occurred in the fifth month for C and in the sixth month for SC, as can be seen in figure 1.

Figure 1: Reduction in *E. coli* during the vermicomposting process

Enterococcus faecalis

The counts of *E. faecalis* for the compost samples are shown in Figure 2. The bacterial numbers initially ranged from 3.55 x 10^5 to 5.95 x 10^5 in the first month. The bacterial numbers declined rapidly in both C+ SD and SC+SD and were found to be between 0.1 and 0.4 CFU/g by the fourth month of composting. In the fifth month, levels were low in both composts ranging between 0.3 to 0.9 CFU/g of compost. There was a slight regrowth at the sixth month in both composts. Confirmatory test (Catalase test) conducted to verify the *E. faecalis* colonies for the final samples indicated that no true *E. faecalis* colonies were present after nine month of composting.

Figure 2: Reduction in *E. faecalis* during the vermicomposting process

Salmonella typhimurium

The initial counts for *S. typhimurium* were relatively low in both composts ranging between 4.85 x 10^3 to 7.75 x 10^3 CFU/g of compost. There was a rapid decline of bacterial numbers in both samples. By the fourth month the die off had reached the level of 0.3 CFU/50g of compost in both samples. By the sixth month only below 0.3 CFU/g of compost could be detected both samples (Figure. 3).

Figure 3: Reduction in *S.typhimurium* during the vermicomposting process

Chemical and physical quality

The chemical quality of the final sample after 9 months of composting have been represented in table1. Compost C+SD was found to be low in nitrate at 0.75 g/kg of compost at the end of the composting period, whereas in SC+SD nitrate was relatively high at 3.25 g/kg. The phosphate levels ranged from 3.15 g/kg in C to 3.28 g/kg in SC. The pH of both composts were slightly acidic at pH 5.5. The total carbon and kjeldahl nitrogen were determined to find out if C had reached stability. The final carbon to nitrogen level was good at 21:1.

Table 1 Chemical quality of the composted samples

	Nitrate(mg/kg)	Phosphate (mg/kg)	pH	Nitrogen (mg/g)	Carbon	C : N ratio
C+SD	0.75	3.15	5.5	13	27%	21:1

SC+SD	3.25	3.275	5.5			

The physical appearance changed over time such that at the end of nine month the compost was of crumbly consistency like potting mix and had an earthy smell. Though there was a pathogen reduction by the fourth month, the physical nature in terms of odour, consistency and colour only reached the compost quality requirements after nine months of composting.

DISCUSSION

For composting to be successful as a waste treatment option, the emphasis should be on producing a good quality product for which a demand exists rather than as an alternative waste disposal option. Compost quality can be evaluated by two main criteria: 1. Quality of compost in relation to its use as a soil conditioner and, 2. Level of contamination as a legacy of the source of the raw material (Hofstede, 2003). In the present study the quality of the composts was assessed on the microbial, chemical and physical quality, to determine if the compost is safe to handle with regard to pathogens levels and the fertiliser quality.

The presence and the abundance of indicator bacteria such as *E.coli, E. faecalis* and pathogen such as *Salmonella* sp. in the compost indicate the microbial quality of the product and its safety for disposal or garden use. *Escherichia coli* are bacteria that are found to naturally occur in the intestines of human beings and can cause diarrhoea and a severe shigella-like illness (VanDemark & Batzing, 1987) if ingested. *Enterococcus faecalis* occurs naturally in faecal matter and is an important indicator bacterium for faecal contamination (VanDemark & Batzig, 1987). *Salmonella typhimurium* is commonly found in food that has gone bad, especially meat products and eggs and can cause typhoid fever and food poisoning. It can also be found in live birds that can often be the pathogen vectors. However, this pathogen is usually not found in human faeces unless the faeces originate from a carrier (VanDemark & Batzig, 1987). The presence of these pathogens in the compost indicates that the faecal matter has not been composted to completion.

Due to a lag phase in the bacterial growth the pathogen numbers in the spiked compost are at the beginning almost identical to the non spiked sample. This lag phase occurs, when bacteria are introduced to a new growth medium and need to adjust. This phase only lasts a few hours generally (VanDemark & Batzig, 1987). The samples were only tested monthly, and in the meantime in both the samples C + SD and SC + SD the vermicomposting process took place and the worms were able to ingest the microbes (Doube & Brown, 1998). Therefore, the spiked sample appears only slightly higher in the second month of testing. Both samples contained less then the ARMCANZ (Agriculture and Resource Management Council of Australia and New Zealand Water Technology Committee, 1995) requirement (MPN method) for *E. coli* levels for grade A compost. ARMCANZ (1995) requirements for grade A compost are, that the thermotolerant coliforms has to be less than 100 per gram of compost (Table 2). However *E.faecalis* was entirely eliminated in both samples by the end of the composting period in nine months.

For *Salmonella sp*. ARMCANZ (1995) recommend <1 CFU/50 g of compost for grade A1 compost for safe use. The spiked compost had initially a low number of *S. typhimurium*. However, a sudden increase in bacterial numbers occurred in the second month, from whereon the bacterial numbers decline rapidly. This sudden increase from the initial to the second month could have been due to the conditions being favourable for this pathogens growth and the different preferences of food sources of the composting worms. This is also supported by Sidhu *et al*.

(2000) who found that regrowth can occur in composts if conditions are favourable. After six months of composting the salmonella levels met the ARMCANZ (1995) requirements (MPN method) for grade A1 compost for both samples (Table, 2).

Table 2: Pathogen Levels in the Final Compost Compared to ARMCANZ requirements

Pathogen	Grade 1A	Grade 1B	C + SD	SC+ SD
Salmonella (per 50g)	<1	<10	0.3	0.3
Thermo-tolerant coliforms (per gram)	<100	<1000	110	46

C = Composted Faecal Matter; SC = Spiked Compost; SD = Sawdust

From Table 2 it is clear that both samples show sufficient evidence of pathogen reduction for it to be safe to handle. Both samples had a significant pathogen die off which correlates with the findings of Lotzof (2002) who found that *Escherichia coli* could be sufficiently reduced by vermicomposting to render the compost safe to be used in the garden. The studies conducted by Eastman *et al*. (2001) and Eastman (2000) also support these findings. They found that within 144 hours the pathogens were significantly reduced compared with the control plot, which had only a slight reduction of pathogens.

Both composts were slightly acidic at pH 5.5, which is beneficial for the plant nutrient uptake as plants are not able to take up nutrients such phosphorus and potassium at a high pH (Jones *et al*., 1996). The critical pH values of composts necessary for positive results, when applied to vegetable crops should be 5 to 7.5 (O'Malley *et al*., 2003). Nitrate is the best form of nitrogen for plants, as it is readily taken up through the roots. It is therefore essential for a good fertiliser to contain this form of nitrogen. There are to date no Australian Standards (1999) regulating the levels of phosphate and nitrate in compost. O' Malley *et al*. (2003) measured the level of nitrate in Karakatta sands as being 1.2 mg/kg. The composts were all found to have much higher levels of nitrate ranging from 0.75 g/kg in C+SD to 3.75 g/kg in SC+SD and can therefore be a good additive to soils low in nitrate.

The phosphate concentration was good in both samples being between 3.15 g/kg in C+SD and 3.28g/kg in SC+SD. There are to date no Australian Standards (1999) regulating the levels of phosphate and nitrate in compost

The carbon (C) to nitrogen (N) ratio measured at the end of the experiment was reasonably good at a ratio of 21:1, indicating that composting process had gone to completion as most of the C source had been consumed by the composting worms and bacteria. The Integrated Waste Management Board (2003) found that ratios of less than 20:1 indicate that the compost is stable. The compost contained a relatively high level of total Kjeldahl N measuring 13mg.N/g. The final C:N ratio was found to be and 22:1, which indicates that some of these composts could be used as fertilizers in a diluted form.

The faecal matter composted with sawdust as a substrate was found to be of good quality compared to potting mix. No faecal matter could be distinguished in the compost. As the Standards Australia (2001) for waterless toilets states, there should be no recognisable faecal matter in the compost, and the there should not be an offensive odour given off by the compost.

CONCLUSION

Both composts achieved the standards set for *S. typhimurium* to grade A composts after a composting period of six to nine month (ARMCANZ 1995). For *E. coli* C was found to be slightly above the grade A requirements (ARMCANZ, 1995) but well below the upper limit grade B compost. SC achieved grade A compost requirements for both pathogens. Due to the high levels of Phosphate the composts reached soil conditioner quality. It can therefore be concluded that vermicomposting of faecal matter is an effective method of pathogen reduction and the resulting compost is of good consistency and texture and can be safely used in the garden as soil amendment.

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