



Short communication

Preliminary evaluation of pathogenic bacteria loading on organic Municipal Solid Waste compost and vermicompost

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ABSTRACT

The use of composts or vermicomposts derived from organic fraction of Municipal Solid Waste (OFMSW) brought about certain disagreement in terms of high level of bacterial pathogens, thereby surpassing the legal restrictions. This preliminary study was undertaken to compare the evolution of pathogenic bacteria on OFMSW compost against vermicompost (generated by *Eudrilus eugeniae*) with promises of achieving sanitation goals. Analysis to quality data showed that OFMSW vermicomposting caused a moderately higher reduction in total coliforms in contrast to composting. *E. coli* in OFMSW composts was found to be in the range of 4.72–4.96 log₁₀ CFU g⁻¹ whilst on a clear contrary, *E. coli* was undetectable in the final vermicomposts (6.01–6.14 logs of reduction) which might be explained by the involvement of the digestive processes in worms' guts. Both OFMSW composts and vermicomposts generated *Salmonella*-free products which were acceptable for agricultural usage and soil improvement. In comparison to compost, the analysis of this research indicated that earthworm activity can effectively destroy bacterial pathogenic load in OFMSW vermicomposts. But still, this study necessitates extra research in order to comprehend the factors that direct pathogenic bacteria in vermicomposting and earthworm-free decomposition systems.

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1. Introduction

World generation of solid waste is rising tremendously owing to the rise in population density and brisk urbanization throughout the past decades. The expanding rate at which organic residues from Municipal Solid Waste (MSW) are generated has become a serious problem that requires strategies for safe disposal and/or effective management. It is a normal practice in many rural areas of several developing countries to use partially degraded organic fraction of Municipal Solid Waste (OFMSW) as an alternative fertilizer (Edwards and Subler, 2011). Without any preliminary pre-treatment, the direct incorporation of OFMSW into agricultural soils may induce adverse effects from the release of some chemically decaying products which consequently can hinder root growth (Soobhany et al., 2017a). However, OFMSW frequently contain a variety of hazardous microbes, including pathogenic bacteria and the major sources of MSW contributing enteric pathogens were found to be from food waste, pet feces, absorbent products and biosolides (Gerba et al., 2011). In line with the

industrial ecology concept, challenges associated in tackling OFMSW need to be addressed because of its negative impact on the environment and the resulting damage to human health due to the presence of pathogenic compounds in certain cases. Biological treatments such as aerobic composting or anaerobic digestion are the most environmentally acceptable method to treat OFMSW. It has been shown from earlier studies that both technologies can make the most of recycling and recovery of waste components (Quiroga et al., 2014; Pandey et al., 2016). Yet, the most lucrative technique for management of OFMSW is through aerobic composting because of its low-tech nature than anaerobic digestion, high organic content (Mohee and Soobhany, 2014), high nutritional capacity (Soobhany et al., 2015a), production of a valuable end product at the same time and profitable utilization of the finish product (Soobhany et al., 2015b). Regardless of numerous reports which have been conducted on composting or vermicomposting of different organic wastes and review on bacterial pathogenic load (Hénault-Ethier et al., 2016; Soobhany et al., 2017b), various researchers have reported survivability of bacterial pathogen even when the specified condition in composting temperature exceeding 55 °C for at least 4 h was achieved (Hassen et al., 2001; Pourcher et al., 2005; Pandey et al., 2016). Moreover, the

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application of compost containing a high quantity of pathogens to agricultural systems could bring in food borne disease occurrences and through the food chain, could give rise to health issues to humans. Thus, there is concern about the efficacy of composting systems with regard to reducing or eliminating bacterial pathogens which could eventually help in preventing health problems, environment and sanitation hazards. Over the past few years, alternative biological methods for treating OFMSW have received much attention owing to its effectiveness in terms of sanitization and costing. One of the main biological approaches and environmentally sustainable technology to OFMSW management is vermicomposting, i.e. the treatment of organic wastes by earthworms acting in synergy with microbial populations (Soobhany et al., 2015c). A great deal of research revealed the capability of vermicomposting systems to effectively inactivate pathogens such as total coliform, *Salmonella* spp., *Escherichia coli* and *Shigella* spp. (Mainoo et al., 2009; Yadav et al., 2010; Aira et al., 2011). The mechanisms by which pathogens might be reduced or destroyed consist of the direct influences of mechanical interruption owing to ingestion and the grinding action of the gizzard of the earthworms (Edwards and Subler, 2011). To a certain point, it could be conjectured that the reduction or destruction of the pathogenic load largely depends on the earthworm species used for vermicomposting, that is, different earthworms have different capacity to inactivate pathogens and/or the pathogen considered (Soobhany et al., 2017b). Contradictory, Edwards and Subler (2011) reviewed on pathogen destruction through vermicomposting and it was reported in their review that Haimi and Huhta (1987) noted an increase in fecal *Streptococci* spp. after vermicomposting although given suitable conditions and time. Thus, the inability for entire bacterial pathogen destruction raised doubt with a high degree of disagreement concerning the viability of vermicomposting. In general, it is obvious that the effect of earthworms on pathogenic bacteria during vermicomposting process can be quite complex. Therefore for further comprehension, a more detailed preliminary consideration on comparing the evolution of pathogenic bacteria (total coliform, *E. coli*, *Salmonella* spp.) on OFMSW compost against vermicompost for quality evaluation is researched in this study. It should however be noted that this preliminary study was confined to the initial and final characteristics of the composting and vermicomposting products in terms of pathogenic bacteria only.

2. Materials and methods

2.1. Substrates collection

With respect to the appropriateness for vermicomposting, the OFMSW that was chosen were food waste, grass clippings, dry leaves and small branches, market waste, office shredded paper and newspaper, and cow dung. The organic waste was collected from the waste collecting trucks which consisted of mixed MSW such as kitchen waste, yard waste, paper waste, plastics, textiles, metal cans, glasses and others. To obtain the organic fraction of waste materials, the mixed MSW wastes were sorted manually. Cow dung was provided by the agricultural farm of the University of Mauritius and was homogeneously incorporated to the organic MSW in some scenarios to balance the C/N ratio and to boost up the composting process. Also, another purpose was that the cow dung could aid as a bedding material for the earthworms.

2.2. Experimental set-up

The mix calculation of the organic substrates and preparation of the mixtures from OFMSW was followed using the method explained by Soobhany et al. (2015b). Six scenarios were set up in

which three experiments were for composting denoted as S1 for food waste mix, S2 for paper waste mix and S3 for yard waste mix and the corresponding replicates for vermicomposting processes were S4, S5 and S6 for food, paper and yard waste respectively. The mix ratio of the OFMSW and cow dung used in this study was tabulated in Table 1.

Composting experiments (controls) were conducted in 244 L (effective size of $0.65 \times 0.60 \times 0.90$ m of L × W × D) wooden in-vessel composters for Scenarios 1, 2, 3 and vermicomposting experiments (thermophilic composting followed by vermicomposting processes) in 244 L wooden vermicbins for Scenarios 4, 5, 6 in a manner detailed earlier (Soobhany et al., 2015b). The composting experiments (S1, S2 and S3) started at the same time as the thermophilic composting for S4, S5 and S6. During the time of vermicomposting (after 3 weeks of thermophilic composting), the depth of the substrates in the vermicbin reduced to 0.25 m. Thus, vermicomposting experiments were carried out in vermicbins measuring $0.65 \times 0.60 \times 0.25$ m³ (Length × Width × Depth) and this provided an exposed top surface area of 0.39 m². An optimal of 1.60 kg worms/m² was used as worm stocking density in the setups for this experiment in order to obtain the maximum bioconversion of the feedstock into earthworm biomass as previously studied by Soobhany et al. (2015b). Thus, during the 3rd week of the composting process, a live-biomass loading of 0.624 kg of acclimated *Eudrilus eugeniae* earthworms were introduced into Scenarios 4, 5, and 6 when the temperature reached a mean value in a range of 25–30 °C. The composting and vermicomposting experiments (3 weeks composting followed by 7 weeks vermicomposting) lasted for a total period of 10 weeks.

In terms of sanitation hazards thereby rendering threats to human health, three bacterial pathogens were assessed in this study to determine their diffusion in the end products as compared initially, following the legal requirements in Mauritius: Total coliform MPN (Most Probable Number) in 20 g samples, *E. coli* CFU (Colony Forming Units) in 20 g samples and *Salmonella* spp. CFU in 25 g samples. These bacterial pathogens were determined by analyzing samples of the initial and final OFMSW compost and vermicompost. The sample experimental determination procedure which was in line with Method TMECC 07.01 as per the TMECC (2001) was followed. The initial bacterial pathogens characterization of the fresh substrates mix from different scenarios is summarised in Table 2. Tukey's HSD test (IBM SPSS Package, Version 20) was used as a post hoc analysis to compare the means for the bacterial pathogen content.

2.3. Experimental analysis of pathogenic bacteria

2.3.1. Total coliform using the Most Probable Number (MPN) technique

Around 20 g sample was placed into a sterile stomach bag and 200 mL of buffered water peptone was added for a 1:10 dilution (10^{-1}) and homogenized for 1 min. Four 1:10 serial additional dilutions were prepared. Aseptically 1 mL of the dilutions 10^{-2} , 10^{-3} , 10^{-4} sample homogenate was transferred into each of three screw-top culture tubes containing 5 mL Brilliant Green Bile (BGB) 2% and an inverted Durham tube. The tubes were incubated for 24 h in a 37 °C ± 2 °C incubator. The number of tubes in each dilution set that was positive for gas formation was recorded. The MPN per g was computed using the MPN Index (Supporting information Table A-1) in a 3 tube dilution series.

2.3.2. *Escherichia coli* using the viable count method

Approximately 20 g sample was placed into a sterile stomach bag and 200 mL of buffered water peptone was added for a 1:10 dilution (10^{-1}) and homogenized for 1 min. Four 1:10 serial

Table 1

Substrates mix ratio for composting and vermicomposting scenarios.

Scenarios	Substrates mix	Composting technology employed	Mix ratio
S1	Food: Dry leaves: Paper	In-vessel composting (aerobic composting without earthworms)	5:0.5:1
S2	Market waste: Cow dung: Paper		4:5:1
S3	Grass: Cow dung: Dry leaves		2:2:1
S4	Food: Dry leaves: Paper	Vermicomposting (inoculation of <i>Eudrilus eugeniae</i>)	5:0.5:1
S5	Market waste: Cow dung: Paper		4:5:1
S6	Grass: Cow dung: Dry leaves		2:2:1

Table 2

Initial and final bacterial pathogens characterization of substrates mix.

	Total coliform (\log_{10} MPN g ⁻¹) ^a	<i>E. coli</i> (\log_{10} CFU g ⁻¹) ^a	<i>Salmonella</i> spp. (\log_{10} CFU g ⁻¹) ^a
Initial characterization of substrates mix			
S1	3.13 ± 2.33 a	6.15 ± 4.11 c	6.24 ± 3.51 b
S2	3.57 ± 3.08 a	6.09 ± 4.11 b	6.50 ± 4.59 c
S3	3.27 ± 2.55 a	6.00 ± 4.55 a	5.44 ± 3.81 a
S4	3.13 ± 2.33 a	6.14 ± 3.51 bc	6.24 ± 4.55 b
S5	3.54 ± 3.19 a	6.10 ± 3.81 bc	6.51 ± 3.51 c
S6	3.27 ± 2.55 a	6.01 ± 3.51 a	5.42 ± 4.29 a
Final characterization of substrates mix			
S1	2.77 ± 2.35 b	4.89 ± 4.62 b	Absent in 25 g
S2	2.84 ± 1.89 b	4.96 ± 4.46 b	Absent in 25 g
S3	2.89 ± 2.31 b	4.72 ± 4.74 ab	Absent in 25 g
S4	1.52 ± 0.63 a	n/d*	Absent in 25 g
S5	1.72 ± 1.49 a	n/d*	Absent in 25 g
S6	1.52 ± 0.63 a	n/d*	Absent in 25 g
Reduction of bacterial pathogens			
S1	2.88 log reduction	6.12 log reduction	6.24 log reduction
S2	3.49 log reduction	6.06 log reduction	6.50 log reduction
S3	3.03 log reduction	5.98 log reduction	5.44 log reduction
S4	3.12 log reduction	6.14 log reduction	6.24 log reduction
S5	3.54 log reduction	6.10 log reduction	6.51 log reduction
S6	3.26 log reduction	6.01 log reduction	5.42 log reduction

n/d*: Not detected in 20 g sample; below the detection limit (≤ 1000 CFU g⁻¹).

^a Given in \log_{10} concentration. To get the concentration in general number form, the value displayed in the table (e.g. 6.14) is taken to the power of 10 (i.e. $10^{6.14} = 1\ 380\ 384$). Values designate mean ± standard deviation based on 2 samples. Different letters in the same columns for each substrate mix are statistically different (Tukey's HSD test, $p < 0.05$).

additional dilutions were prepared. 0.1 mL of samples 10^{-3} and 10^{-4} from dilution tubes were transferred onto the surface of the petri dishes containing MUG Sorbital Agar. The petri dishes were incubated for 24 h in a $36\ ^\circ\text{C} \pm 1\ ^\circ\text{C}$ incubator. Using the colony counter, the number of cfus was counted on the plates. The general formula for the viable count on plates is given in Eq (1):

$$N (\text{CFUs per mL or g}) = \sum C / [V \times \{n_1 + (0.1 \times n_2)\} \times d \times d] \quad (1)$$

where, N: Number of CFUs per mL or g of sample, $\sum C$: Sum of cfus counted on all selected plates of two successive dilutions, V: Volume of inoculum added to each plate (mL), n_1 : Number of plates selected at the 1st dilution, n_2 : Number of plates selected at the 2nd dilution, d: Dilution factor of the first dilution.

2.3.3. *Salmonella* spp. using the viable count method

About 225 mL of buffered water peptone was added to 25 g sample in a stomacher bag for a 1:10 dilution (10^{-1}), homogenized and incubated. One serial additional dilution (10^{-2}) was prepared in Rappaport Vassiliadis Enrichment Broth (RVEB). The 10^{-2} dilution was then incubated and three serial additional dilutions were prepared in RVEB. 0.1 mL of sample from dilutions 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} was transferred onto the surface of the petri dishes containing XLD Agar. The general formula which was used for viable count on plates was the same as described in Eq (1).

3. Results

The initial and final bacterial pathogenic loading (Total coliform, *E. coli* and *Salmonella* spp.) of waste material in the different scenarios was summarised in Table 2.

4. Discussion

4.1. Total coliform

The total coliform in the final composts and vermicomposts is given in Table 2. Of all the initial characterization of the different feedstocks, total coliform was high in the paper waste mix of S2 and S5 with a value of $3.57 \pm 3.08 \log_{10}$ MPN g⁻¹ and $3.54 \pm 3.19 \log_{10}$ MPN g⁻¹ respectively, which might be due to the presence of market wastes and cow dung and these organic materials are the main source for total coliform (Mainoo et al., 2009; Monroy et al., 2009; Lalander et al., 2013). The log reduction (\log_{10} MPN g⁻¹) in total coliform as compared to initial level for composting scenarios S1, S2 and S3 was 2.88, 3.49 and 3.03 respectively. The reduction in total coliforms might be due in part to the relatively high temperatures reached during the thermophilic phase as shown in Fig. A-1 (Supplementary material). This decrease in total coliform during the composting processes corresponded with earlier findings made by Bustamante et al. (2008) during the co-composting of winery and distillery wastes. As compared to the composting processes, vermicomposting scenarios S4, S5 and S6 demonstrated a

moderately higher decrease in total coliform of $3.12 \log_{10} \text{MPN g}^{-1}$, $3.54 \log_{10} \text{MPN g}^{-1}$ and $3.26 \log_{10} \text{MPN g}^{-1}$ respectively. The moderately high reduction in total coliform in vermicomposts might be due to competitive interactions between coliforms and microorganisms that were specific to the bacterial activity of earthworm gut enzymes which was similarly justified by Monroy et al. (2009). As can be observed from Table 2, the presence of total coliforms was slightly greater in all the three different controls (food, paper and yard) as compared to its respective vermicomposts which might be explained, probably by means of an antagonism mechanism during vermicomposting or by the involvement of the digestive processes in worms' guts (Soobhany et al., 2017b). The difference in total coliforms from each pair of composting and vermicomposting processes indicated the ability of *Eudrilus eugeniae* to reliably reduce the levels of total coliforms during stabilization of OFMSW. On the contrary, the effect of earthworm *Eisenia andrei* on total coliform was insignificant during vermicomposting of cow manure (Aira et al., 2011). While not carrying the same weight of evidence as reported from controlled scientific studies, the experience from this research study was definitely with the concept that it is possible to achieve a more effective reduction in pathogenic total coliform during vermicomposting compared to composting. However, there is no respective limit set out by MS 164 (2010) for total coliform content.

4.2. Escherichia coli

E. coli is mostly considered as an indication to fecal pollution and as an indicator of the fate of fecal pathogenic microorganisms. At the start of the experiment, *E. coli* was present in the food waste mix, paper waste mix and yard waste mix since *E. coli* is generally found in animal waste (Aira et al., 2011; Hill and Baldwin, 2012; Lalande et al., 2013) and food waste (Mainoo et al., 2009). The *E. coli* content for the six sets is shown in Table 2. The reduction in *E. coli* in the food, paper and yard wastes mixes as compared to initial level for S1, S2 and S3 was $6.12 \log_{10} \text{CFU g}^{-1}$, $6.06 \log_{10} \text{CFU g}^{-1}$, and $5.98 \log_{10} \text{CFU g}^{-1}$, respectively. According to the US EPA standards (1999), effective pathogen inactivation is achieved only if the composting system is subjected to a minimum operating temperature of 40°C for a period of 5 days with temperatures exceeding 55°C for at least 4 h of this period. Thus, it could be deduced that thermo-composting alone, as shown in the temperature profile of the composting processes from Fig. A-1 (Supplementary material) promoted a decrease in *E. coli* but still, did not inactivate completely this microorganism. Similarly, Pourcher et al. (2005) showed even temperature as high as 66°C did not inactive completely this pathogenic bacteria. The decrease in *E. coli* in composts corresponded with previous studies during co-composting of other organic wastes (Bustamante et al., 2008; Carthy et al., 2011). But, however, these studies showed that *E. coli* were reduced to undetectable levels ($<1.77 \log_{10} \text{MPN g}^{-1}$) during composting. Whilst on a clear contrary, vermicomposts from S4, S5 and S6 did not show the presence of *E. coli* analyzed in the 20 g sample which was therefore below the detection limit ($\leq 1000 \text{ CFU/g}$). Thus, the log reduction in *E. coli* from the OFMSW vermicomposts was computed to be 6.14 for S4, 6.10 for S5 and 6.01 for S6. To some extent, from the results of this study, it could be deduced that the earthworms *Eudrilus eugeniae* have the capacity to inactivate *E. coli* effectively in all the three sets of OFMSW vermicomposting. It has been suggested that killing of *E. coli* is prominently achieved through earthworm actions, secretion of coelomic fluids, selective grazing and alteration of microbial community composition during vermicomposting (Domínguez and Edwards, 2004). Brown and Mitchell (1981) provided solid evidence that the stimulation of endemic bacteria by earthworm

activity may lead to pathogen destruction through competitive or antagonistic interactions. In comparison to its respective equivalent vermicomposts, S1, S2 and S3 were not free of *E. coli* and therefore, could not substantially apply to land prior to pre-treatment. Hénault-Ethier et al. (2016) observed that *E. fetida* negatively influenced *E. coli* survival and detected an earthworm density independent relationship (survival of *E. coli* did not decrease with increasing earthworm density). In addition, previous studies reported a decrease in *E. coli* after the vermicomposting process of other organic wastes with other earthworm species (Aira et al., 2011; Hill and Baldwin, 2012; Cao et al., 2016). Yet, the laboratory analysis of this research provided a fairly consistent and conclusive indication that *Eudrilus eugeniae* activity can effectively destroy *E. coli* in OFMSW. According to Mauritius Standards (MS 164, 2010) for compost quality, pathogens test for *E. coli* should be $\leq 1000 \text{ CFU/g}$ and it could be deduced that the three types of composts (S1, S2 and S3) generated from OFMSW did not pass the Mauritius Standards but its respective vermicomposts (S4, S5 and S6) were well within the limits.

4.3. Salmonella spp.

Salmonella spp. is considered as the major and specific problem of the hygienic quality of compost. At the start of the experiments, *Salmonella* spp. was found in all the three pairs of composting and vermicomposting processes owing to the presence of cow dung (Létourneau et al., 2010) and food wastes (Hassen et al., 2001). At the start of the experiment, *Salmonella* spp. was found to be much higher in the paper waste mix of S2 and S5 which might be due to the presence of both market wastes and cow dung, whereby they are main source of these pathogenic bacteria. As presented from Table 2, *Salmonella* spp. was absent in all the three types of OFMSW composts (S1, S2 and S3) and OFMSW vermicomposts (S4, S5 and S6) at the end of the experiments which might be due in part to the relatively high temperatures reached during the thermophilic phase in Fig. A-1 (Supplementary material). The absence of *Salmonella* spp. in the vermicomposts might be due to the stimulation of an endemic microflora, which when grown with *Salmonella* in liquid cultures caused nearly total elimination of the pathogen as comparably justified by Brown and Mitchell (1981). Still, Domínguez and Edwards (2004) indicated that temperatures above 30°C may promote chemical and microbiological activities in the substratum. A *Salmonella*-free was reported from the composting of separated solid fraction of pig manure (Carthy et al., 2011) which was in agreement with the composting end products of this study. Mainoo et al. (2009) reported a decline in population of pathogenic *Salmonella* spp. during vermicomposting of pineapple wastes with *Eudrilus eugeniae* while on the contrary, presence of *Salmonella* spp. was obtained in urine diverting vermicompost toilet using *Eisenia fetida* (Lalande et al., 2013). Also, it was found that earthworm *Eisenia fetida* decreased *Salmonella* spp. by 97.8%–99.9% compared to cultures with no earthworms (Brown and Mitchell, 1981) which somewhat corresponded to the findings of this study, wherein different species of earthworm and feedstocks material have been used. The destruction in *Salmonella* spp. after vermicomposting processes of the food waste, paper waste and yard waste from OFMSW was in accord with previous research on sewage sludge by *Eisenia fetida* (Rodríguez-Canché et al., 2010) which suggested a consistent anti-microbial response on gram-negative bacteria from the gizzard through the intestinal tract of the earthworms. Interestingly, the OFMSW composts and vermicomposts were in acceptable level as specified by MS 164 (2010) which recommend *Salmonella* spp. should be absent in 25 g of fresh sample.

5. Conclusions

Analysis to quality data showed that *Eudrilus eugeniae* caused considerable reduction in total coliform bacteria in OFMSW vermicomposts (3.12–3.56 log reduction) compared to its respective composts (2.88–3.49 log reduction). The laboratory analysis of this research provided a fairly conclusive indication that *Eudrilus eugeniae* activity can effectively reduce *E. coli* in OFMSW vermicomposts to below detection limits. In contrast, composting caused a decline in *E. coli* in which 5.98–6.12 logs of reduction were obtained but did not pass the limit of ≤ 1000 CFU/g as set out by MS 164 (2010) and would restrict its application as an organic fertilizer. Both OFMSW treatments generated *Salmonella*-free composts and vermicomposts which were substantially acceptable for agricultural land practice and soil enhancement. Vermicomposts derived from OFMSW were of better quality in terms of bacterial pathogens than its respective composts thereby preventing environment and sanitation hazards. Yet, this preliminary study necessitates extra research in order to comprehend the factors that direct pathogenic bacteria in vermicomposting and earthworm-free decomposition systems.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jenvman.2017.11.029>.

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