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Short communication

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

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A R T I C L E I N F O

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A B S T R A C T

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 $\log_{10}$ 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.

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 pretreatment, 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 homogenously 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 × 0.60 × 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 vermibins 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 vermibin reduced to 0.25 m. Thus, vermicomposting experiments were carried out in vermibins measuring $0.65 \times 0.60 \times 0.25\ m^3$ (Length × Width × Depth) and this provided an exposed top surface area of $0.39\ m^2$. An optimal of 1.60 kg worms/m^2 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 stomacher 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 stomacher 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