Enteric Pathogen modification by Anaecic Earthworm, Lampito Mauritii

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ABSTRACT: The biosolids from municipal wastewater treatment plant contains several enteric microbial pathogens, predominantly Salmonella and Escherichia species in the range of 15-18 × 10^4 CFU/g and 11-12 × 10^4 CFU/g respectively. The present study investigates the influence of earthworm, Lampito mauritii on enteric pathogen during vermicomposting of municipal sewage sludge. The Biosolids (B), Rice straw (R) and Cow dung(C) were used in this study. The BR (3:1) and BRC (3:1:1) combination proved to be best in the removal of enteric pathogens. Both these combinations reduced the bacterial pathogens like Salmonella and Escherichia species to nil during 60 days stabilization period of vermicomposting. The gut analysis also proved that Salmonella species ranging 15-17 × 10^3 CFU/g and Escherichia species ranging 10- 14 × 10^2 CFU/g were completely eliminated in the gut after 70 days of vermicomposting period.

The methodology of biosolid waste management has shifted from conventional disposal strategies to conversion into value added products (Liang et al 2003). The technique for proper management of biosolids was vermicomposting, which uses earthworms for breakdown of the organic wastes. However, a broad spectrum of pathogenic microorganisms has been reported to be present in biosolids (Abdennaceur Hassen et al 2001). Many researchers on solid waste management suggested bio composting of solid wastes before applying to soil in order to achieve biological transformation of the organic matter and to avoid potential risks of pathogens (Gliotti et al 1997 and Masciandaro et al 2000). Biocomposting of solid wastes bring about stabilization of the organic matter and effectively reduces pathogen concentrations in sludges to very low levels (Burge et al 1987). However, absolute removal of pathogens becomes difficult to achieve and many survives the composting process (Sidhu et al 2001). Various researchers have established the viability of vermi-technology for treatment of different wastes (Ndegwa et al 2000; Elvira et al 1997).

In the vermireactors the earthworms maintain aerobic conditions in the organic wastes through proper mixing, moreover the biochemical process is enhanced by microbial decomposition of the substrate in the intestines. The earthworms convert a portion of the organic present in the wastes into worm biomass and excrete undigested/partially-digested matter as worm cast, which are rich in nutrient source (Benitez et al 1999). The earthworms also enhance the biological activity in the soil by improving the environment suitable for microbes (Mulongoy et al 1989). However, the information on the fate of enteric pathogenic microorganisms during vermicomposting was less. This paper aims to study the fate of major enteric pathogens like Salmonella sp. and Escherichia sp. in the biosolids during vermicomposting process.

MATERIALS AND METHODS
The sewage sludge was obtained from municipal sewage treatment plant. The adult Anaecic earthworms, Lampito mauritii were used in this study. The worms were collected by hand sorting and maintained in soil amended with cattle manure.

Different mixture of substrates used in treatments were a. Mixture of Biosolids and Rice straw, 3:1 (BR) b. Mixture of Biosolids, Rice straw and Cow dung, 3:1:1 (BRC) c. Control sample containing biosolids alone (B)

The vermicomposted sample was extracted in buffer solution and appropriate dilutions were made and plated using pour plate technique and incubated at 37°C. Salmonella was enumerated using Salmonella Shigella Agar (SS agar) and Escherichia using Eosin Methylen Blue agar (EMB agar). The results were calculated after 24 hours of retention time and were expressed as CFU g⁻¹ (Colony Forming Units per gram of biosolids). In midgut analysis whole body was dissected under sterile environment and analysis were carried out.

RESULTS AND DISCUSSION
The Salmonella sp. and Escherichia sp. decreased gradually from 7th day itself in both BR and BRC, and decreased to nil level after 50th day but in control experiments these populations were not reduced (Fig 1 and 2).

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In the initial stages of the vermicomposting pathogens like Salmonella sp. and Escherichia sp. were found to be dominant in the mid gut. The Salmonella sp. from 15-18 x10^3 CFU g^-1 decreased in the treatments of BR and BRC up to 1x10^1 CFU g^-1 in the midgut. The Escherichia sp. was found in gut of earthworm grown in control but it was completely removed in 35th day of both BR and BRC grown earthworm (Table 1).

**Table 1** Bacteriological analysis of Gut

<table>
<thead>
<tr>
<th></th>
<th>No. of Days</th>
<th>0</th>
<th>35</th>
<th>70</th>
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<tbody>
<tr>
<td><strong>a. Salmonella in gut</strong></td>
<td></td>
<td></td>
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<tr>
<td>Biosolids</td>
<td></td>
<td>16-17x10^3</td>
<td>5-8x10^3</td>
<td>2-3x10^3</td>
</tr>
<tr>
<td>BRC</td>
<td></td>
<td>15-18x10^3</td>
<td>1x10^3</td>
<td>Nil</td>
</tr>
<tr>
<td>BR</td>
<td></td>
<td>15-18x10^3</td>
<td>1x10^3</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>b. Escherichia in gut</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biosolids</td>
<td></td>
<td>10-11x10^2</td>
<td>7-8x10^1</td>
<td>1-3x10^1</td>
</tr>
<tr>
<td>Biosolids + Rice straw + Cow dung (BRC)</td>
<td></td>
<td>11-14x10^2</td>
<td>1-2x10^1</td>
<td>Nil</td>
</tr>
<tr>
<td>Biosolids + Rice straw (BR)</td>
<td></td>
<td>10-12x10^2</td>
<td>1-3x10^1</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Although earthworms enhances the soil microbial activity by improving the environment favorable and much more suitable for its growth (Syers et al 1979) the fate of microorganisms during the gut transit through earthworms is still controversial (Wolter et al 1999). The experimental results of gut analysis proved the removal of Salmonella and Escherichia in 35 days under suitable substrate. Moreover, these enteric bacterial populations were decreased at the end of vermicomposting period, indicating the nature of earthworms in the removal of microorganisms. These results correlates with the findings of various researchers proving earthworms include microorganisms in their substrates as a food source and can digest them selectively (Edwards et al 1996; Bohlen et al 1995). Many researchers also proved that the indigenous microflora play a significant role in the suppression of pathogenic bacterial population regrowth in composted sludges (Sidhu et al 2001). These results suggest that vermicomposting can be used for safe management of solid waste generated from wastewater treatment plant and its bioconversion into valuable compost free from enteric bacterial populations.

Acknowledgement: The author A. Ganesh Kumar is thankful to Council of Scientific and Industrial Research (CSIR) India, for awarding Research Fellowship and providing all facilities to carryout this work.
REFERENCES


