Enriching Vermicompost Using P-solubilizing and N-fixing Bacteria under Different Temperature Conditions

Hossein Ali Alikhani, Arash Hemati, Mehdi Rashtbari, Scott D. Tiegs, and Hassan Etesami

ABSTRACT
In the present study we evaluated changes in phosphorus (P), nitrogen (N), and humic acid (HA) contents of vermicompost (VC) in response to temperature increases, and inoculation with N$_2$-fixing and P-solubilizing microbes. Inoculants of Pseudomonas and Azotobacter were prepared and used to inoculate VC that was kept at 28 and 41 °C. Biological and chemical parameters of the VC were evaluated at 0, 20, 40, and 60 days. As incubation duration increased, bacterial population, N, available P, and HA content increased while organic carbon and pH decreased. These changes were most rapid during the initial 40 days of the experiment, and slowed subsequently. Increasing temperature from 28 to 41 °C reduced bacterial population and the efficiency of these bacteria in improving VC quality. Overall, our results indicate that inoculation of VC with microbes holds promise as a means of increasing the quality of VC, while our increased temperature treatment was less effective.

ARTICLE HISTORY
Received 28 July 2013
Accepted 17 May 2016

KEYWORDS
Azotobacter chroococcum; Eisenia fetida; humic acid; Pseudomonas fluorescens; vermicompost

Introduction
Organic-wastes recycling plays a vital role in sustainable agriculture, reducing pollutants in the environment, and nutrient enrichment of soils. Vermicomposting is a non-thermophilic process that involves using earthworms to alter physical, chemical, and biological reactions in organic wastes such that soil quality is improved. During the vermicomposting process organic materials are converted into stable compounds (humus) and, the final product, vermicompost (VC) is rich in humus and available phosphorous. The presence of earthworms in organic wastes increases rates of nutrient mineralization, phosphorus (P) availability, and by extension, nutrient uptake by plants. Additional consequences of the use of earthworms are increases in organic-matter conversion rate and microorganism’s diversity and activity (Fracchia et al. 2006; Le Bayon and Binet 2006). Eisenia fetida is an important and abundant species of surface-dwelling (epigeic) earthworms, used widely in composting and the treatment of organic wastes (Reinecke, Viljioen, and Saayman 1992; Elvira et al. 1996; 1998; Arancon et al. 2004; Greiner, Stonehouse, and Tiegs 2011).

VC quality depends on several factors including primary bed type (organic wastes), aeration, moisture, pH, temperature, and the species of earthworm used. So, in order to understand vermicomposting dynamic, it is essential to evaluate special conditions such as composition and abundance of microorganisms, organic carbon, total nitrogen, total P and potassium (K), humic acid (HA), and enzyme activity (Pramanik et al. 2007). VC is an efficient carrier of bacteria and an effective promoter of bacterial growth, and synergistic interactions between diazotroph bacteria and mycorrhiza increase plant growth (Gutiérrez-Miceli et al. 2008).
Recent research has revealed that changes in the microbial communities present in VC can improve its quality as an organic fertilizer (Padmavathiamma, Li, and Kumari 2008). For example, composting of organic wastes combined with rock phosphate and microbes caused dissolution of insoluble phosphates and resulted in an increase in available P for plants (Kumar and Narula 1999). Some nitrogen (N$_2$)-fixing bacteria can increase P availability through the production of organic acids (Kumar and Narula 1999). A number of diazotrophic bacteria, including species belonging to the genera *Pseudomonas, Burkholderia, Agrobacterium, Azotobacter, and Erwinia*, are able to solubilize phosphate in addition to carrying out biological N$_2$ fixation (Scervino et al. 2010). On the other hand, diazotrophs can mineralize organic forms of P by phosphatases that transform P from non-available, organically bound forms into bioavailable phosphate ions (Eivazi and Tabatabai 1977). For example, VC inoculated by *Thiobacillus* had positive effects on the transformation of rock phosphate into available P, as did *Burkholderia* and *Herbasprillum* (Busato et al. 2012; Mohammady Aria et al. 2010). Inoculation with N$_2$-fixing microorganisms increased N amounts in VC, and inoculation with phosphate-solubilizing microorganisms in the presence of rock phosphate increased P in VC, while direct application of rock phosphate, especially on natural soils, did not increase P availability (Kaushik and Garg 2004; Premono, Moawad, and Vlek 1996). Beauchamp et al. (2006) reported that the population of bacteria capable of nitrogen fixation which is used for processing of organic wastes in vermicomposting had not been reduced by the addition of earthworms (Hendrikson 1990). The microbial population increased in the end-product, however, suggesting that these microorganisms could be used in VC enrichment (Fischer et al. 1997).

In addition to enrichment of VC with microbes, researchers recently investigated the effects of the increase in temperature on vermicomposting and composting processes. Temperature is the most important factor for control of composting reaction rate as a result of its effects on microbial community and population structure (Palmisano and Barlaz 1996). Heat production beyond the thermophilic phase caused mortality of pathogens, and the final product had better usefulness as fertilizer (Larney et al. 2003). Frederickson and Howell (2002) reported that increasing the temperature during vermicomposting increased the reproduction and population size of earthworms in initial substrate.

Here we investigated whether the inoculation of VC with N$_2$-fixing and P-solubilizing bacteria could improve VC quality indices, and whether increased temperature altered VC properties. We hypothesized that inoculation of VC with bacterial strains could improve its quality and in combination with temperature treatments could significantly affect VC characteristics.

**Materials and methods**

**Vermicompost production**

VC was produced from *Eisenia fetida* grown in a mixture of cow manure and plant residues (ratio of 3:1 w/w) during a 5-month period at the VC research-educational station of College of Agriculture and Natural Resources of Tehran University (CANRTU), Iran. Cow manure and plant residues were initially decomposed in pits (0.7 m W, 0.5 cm H, and 2 m L) in sunlight for 1 month. After excess irrigation and leachate removal, *Eisenia fetida* were added to the pits at a density of about 500 per 100 kg of manure/plant residue material. The pit moisture was maintained at 50–60% by irrigating daily. Earthworms were removed from the VC after 4 months and physical and chemical properties of the VC analyzed (Table 1) (Gupta 2005).

<table>
<thead>
<tr>
<th>pH</th>
<th>EC (dSm$^{-1}$)</th>
<th>Total N (%)</th>
<th>OC (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Na (%)</th>
<th>Fe (%)</th>
<th>Ca (%)</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.63</td>
<td>2.14</td>
<td>1.20</td>
<td>24.37</td>
<td>0.82</td>
<td>6.52</td>
<td>1.10</td>
<td>0.57</td>
<td>8.50</td>
<td>20.3</td>
</tr>
</tbody>
</table>

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**Bacterial inoculation of VC**

Bacterial isolates were obtained from the Soil Science Department at Tehran University. The Ps 59 strain of *Pseudomonas* genus was selected due to its P-solubilizing ability, and strain Az 21 was selected from the *Azotobacter* genus, for its N\(_2\)-fixing ability, based on previous studies as MSc or PhD thesis (Rajaei 2010; Saghafi 2012). *Azotobacter* and *Pseudomonas* bacteria belonged to *Azotobacter chroococcum* and *Pseudomonas fluorescens* species, respectively. After renewing bacterial cultures, populations of fresh inoculant were adjusted to about \(4 \times 10^9\) cfu.mL\(^{-1}\), based on dilution factor and then 25 mL of each inoculant was applied per kg of VC (wet base) (Busato et al. 2012). Four different treatments (three replicates each) were applied to the VC:

- VCC: VC without inoculation (control)
- VC + Az: VC + *Azotobacter chroococcum* (Az 21)
- VC + Ps: VC + *Pseudomonas fluorescens* (Ps 59)
- VC + Az + Ps: VC + *Azotobacter chroococcum* (Az 21) + *Pseudomonas fluorescens* (Ps 59).

All treatments were kept at 60% water-holding capacity with addition of distilled water. VC samples (one kg per each replication) were incubated in a chamber with optimum aeration at 28 and 41 °C for 60 days and during incubation moisture of VC samples was maintained about 60% by distilled water. Enriched VC treatments were analyzed for physical, chemical, and biological characteristics at 0, 20, 40, and 60 days.

**Bacteria counts and measuring the biomass carbon**

Estimation of the cultivable bacterial population size was obtained by the colony formation unit (CFU) method (Page 1982). Biomass carbon was measured according to Wu et al. (1990).

**Determination of the treatments characteristics**

Chemical properties of the VC were measured across all treatments (3 replicates each): total-N by Kjeldahl, available P by Olsen, water-soluble P according to Morphi and Rayli, soluble sodium and potassium by flame photometer, organic carbon by Walkley-Black, pH and electrical conductivity (EC) by pH-meter and EC-meter, respectively (Page 1982).

**Assessing the HA**

The HA was extracted with 0.5 M sodium hydroxide (NaOH) as described in Qi, Aldrich, and Lorenzen (2004); briefly, 20 g of air-dried samples of VC were mixed with 200 mL of 0.5 M NaOH under N\(_2\) at a ratio (VC/solution) of 1/10 (w/v). Before centrifuging, samples were shaked for 18 h at 150 rpm. The treated slurry was left to stand by 1, 7, and 9 days in dark at room temperature, then, the supernatant was separated by centrifugation at 10,000 rpm. The supernatant was acidified at a pH of 1.17–1.50 with 6 M hydrochloric acid (HCl) in order to separate the HAs from alkaline suspension containing HA and fulvic acid. Then the precipitated HAs were purified with HCl/hydrofluoric acid (HF) 0.1/0.3 M, and washed with distilled water until the pH of the hydrogen (H\(^+\))-exchanged soil was in the range 4–5, and finally dried at temperatures below 50 °C.

**Statistical analysis**

Two-way analysis of variance (ANOVA) was used to test the significant differences between treatments followed by Tukey’s post hoc tests. The probability levels used for statistical significance were \(P < 0.05\).
Results and discussion

Inoculation with microbes significantly increased bacterial populations relative to controls (Table 2), and cultivable bacterial populations increased through time. The greatest cultivable bacterial population (by CFU method) was observed on days 40 and 60. Lack of the growth of cultivable bacterial population probably had been caused by restriction of organic carbon in bed as well as nutrient limitation. Previous reports also indicated that after incubating stage cultivable bacterial population declined or remained constant due to nutrient limitation (Tiwari and Mishra 1993; Kaushik et al. 2008).

As temperature increased, cultivable bacterial population decreased, except in VCC (control). Highest temperature for Azotobacter growth is 55–60 °C and the lowest is about 0 °C, and the temperature at which this genus is most active is 20–30 °C (Greene 1932). Pseudomonas has most mobility at the range of 7.5–37 °C (Lynch 1980). Thus increasing the temperature limited the growth of Azotobacter and Pseudomonas and 28 °C was a suitable temperature for growth and reproduction. Unlike other treatments, microbial population increased in VCC, a result possibly related to the growth of thermophilic bacteria.

Carbon biomass is controlled by carbon availability, and addition of organic fertilizer provides available carbon for microbial survival (Hojjati and Nourbakhsh, 2006). VC with appropriate organic matter had the ability to supply available carbon for microorganisms and enriching of VC with bacterial treatments caused significant increase in microbial growth and carbon biomass.

The results of cultivable bacterial population counts suggest that the bacterial treatments encouraged cultivable bacterial growth and reproduction in VC until day 60. Results also show that nitrogen contents in all treatments increased as incubation time increased and Azotobacter treatment increased nitrogen content by 80% and 60% at 28 and 41 °C, respectively. The N content in treatments increased rapidly until day 40, and slowed subsequently. Nitrogen content in Pseudomonas treatment at 28 and 41 °C increased by 20% and 15%, respectively (Table 3). This reflects the N$_2$-fixing ability of this bacteria (Busato et al. 2012).

Results showed that available P in VC increased through time. Highest amounts of P were observed in Pseudomonas which caused a 40% and 36% increase in available P at 28 and 41 °C, respectively. Azotobacter treatment increased P availability by 25% and 19% at 28 and 41 °C, respectively, which had moderate ability to solubilize P. Similar to N, the P amount increased more rapidly in the first 40 days and the rate of increase slowed subsequently. Increasing the temperature had a negative effect on N and P availability, and 28 °C was better for overall nutrient availability, which could be due to increase in cultivable bacterial population (Table 3).

Table 2. Changes in cultivable bacterial population (CFU × 10$^4$) and C-biomass during (µg/g) the incubation of vermicompost with Pseudomonas and Azotobacter.

<table>
<thead>
<tr>
<th>Incubation time (days)</th>
<th>Temperature °C</th>
<th>VCC</th>
<th>VCAz</th>
<th>VCPs</th>
<th>VCAzPs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CFU</td>
<td>C-biomass</td>
<td>CFU</td>
<td>C-biomass</td>
</tr>
<tr>
<td>0</td>
<td>28</td>
<td>1.2$^{Am}$</td>
<td>335.0$^{Ac}$</td>
<td>12.3$^{Ab}$</td>
<td>587.0$^{Ac}$</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>1.2$^{Am}$</td>
<td>335.0$^{Ac}$</td>
<td>12.3$^{Ab}$</td>
<td>586.6$^{Ab}$</td>
</tr>
<tr>
<td>20</td>
<td>28</td>
<td>2.3$^{Ab}$</td>
<td>381.3$^{Ac}$</td>
<td>29.3$^{Ac}$</td>
<td>895.6$^{Ac}$</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>2.4$^{Ab}$</td>
<td>403.3$^{Al}$</td>
<td>22.0$^{Rm}$</td>
<td>717.6$^{Rm}$</td>
</tr>
<tr>
<td>40</td>
<td>28</td>
<td>2.7$^{Ba}$</td>
<td>430.3$^{Bb}$</td>
<td>48.3$^{Ab}$</td>
<td>1234.6$^{Ba}$</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>2.9$^{Ba}$</td>
<td>465.3$^{Bk}$</td>
<td>38.0$^{Bl}$</td>
<td>1091.0$^{Bl}$</td>
</tr>
<tr>
<td>60</td>
<td>28</td>
<td>4.2$^{Ak}$</td>
<td>462.6$^{Ak}$</td>
<td>38.0$^{Bl}$</td>
<td>1091.0$^{Bl}$</td>
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<td>414.0$^{Ba}$</td>
<td>50.3$^{As}$</td>
<td>1241.3$^{As}$</td>
</tr>
<tr>
<td>C.V.</td>
<td></td>
<td>2.58</td>
<td>1.04</td>
<td>1.56</td>
<td>0.49</td>
</tr>
<tr>
<td>SEM±</td>
<td></td>
<td>0.039</td>
<td>0.44</td>
<td>0.28</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Means differ if they have a different letter at: lower case superscript (a, b, c...) for comparison of treatments at 28 °C, lower case superscript (k, l, m...) for comparison of treatments at 41 °C, and uppercase superscript for comparison of 28 °C and 41 °C (ANOVA; Tukey’s test, P < 0.05).
VC+Az+Ps (Azotobacter + Pseudomonas) had intermediate N and P availability suggesting no synergistic relation between bacteria types. VCC had the least amounts of P and N which indicate the positive effect of P-solubilizing and N$_2$-fixing bacteria in inoculated treatments (Table 3).

Frossard, Sinaj, and Dufour (1996) reported that water-soluble P was readily absorbed by plants. According to Figure 1, increasing incubation duration increased water-soluble P amounts, and the Pseudomonas treatment had the greatest amounts of water-soluble P. This represents the capacity of these bacteria to make available various forms of P in VC. Azotobacter treatment also increased water-soluble P and showed P-solubilizing ability as reported earlier by Kaushik et al. (2008). VCAzPs had intermediate value of water-soluble P relative to VCAz and VCPs, suggesting a neutral interaction between these bacteria. Also, lowest amounts of water-soluble P were observed in VCC. Figure 1 shows that water-soluble P increased rapidly until the 40th day and the rate of increase slowed subsequently. Darbyshire (1972) reported the N$_2$-fixing ability of Azotobacter at the range of 5–40 °C and with increase in the temperature more N$_2$ was fixed. A temperature of 41 °C was not optimum for bacterial growth and reproduction, in the study presented here, however. The results showed, however, that at this temperature Azotobacter has N$_2$-fixing ability and Pseudomonas has P-solubilizing capacity, but they were greater at 28 °C.

![Figure 1. Amounts of water-soluble P in VC during the incubation of vermicompost with Pseudomonas and Azotobacter (VCC: VC, VCAz: VC+ Azotobacter, VCPs: VC+ Pseudomonas and VCAzPs: VC + Azotobacter + Pseudomonas).](image)

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**Table 3. Changes in nitrogen and available phosphorous content (%) of VC during the incubation of vermicompost with Pseudomonas and Azotobacter.**

<table>
<thead>
<tr>
<th>Incubation time (days)</th>
<th>Temperature</th>
<th>P</th>
<th>N</th>
<th>P</th>
<th>N</th>
<th>P</th>
<th>N</th>
<th>P</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VCAz</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VCPs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VCAzPs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>28</td>
<td>8.2$^{Ab}$</td>
<td>12.0$^{Ab}$</td>
<td>8.2$^{Ab}$</td>
<td>12.0$^{Ab}$</td>
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<td>12.0$^{Ab}$</td>
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<td>41</td>
<td>8.2$^{Bk}$</td>
<td>12.0$^{Bk}$</td>
<td>8.2$^{Bk}$</td>
<td>12.0$^{Bk}$</td>
<td>8.2$^{Bk}$</td>
<td>12.0$^{Bk}$</td>
<td>8.2$^{Bk}$</td>
<td>12.0$^{Bk}$</td>
</tr>
<tr>
<td>20</td>
<td>28</td>
<td>8.2$^{Ab}$</td>
<td>12.2$^{Ab}$</td>
<td>9.5$^{Ac}$</td>
<td>15.1$^{Ac}$</td>
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<td>8.6$^{Ac}$</td>
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</tr>
<tr>
<td></td>
<td>41</td>
<td>8.2$^{Bk}$</td>
<td>12.0$^{Bk}$</td>
<td>8.4$^{Bk}$</td>
<td>14.4$^{Bk}$</td>
<td>8.7$^{Bk}$</td>
<td>12.6$^{Bk}$</td>
<td>8.4$^{Bk}$</td>
<td>13.1$^{Bk}$</td>
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<td>40</td>
<td>28</td>
<td>8.1$^{Bk}$</td>
<td>12.2$^{Bk}$</td>
<td>9.7$^{Bk}$</td>
<td>20.7$^{Bk}$</td>
<td>11.1$^{Bk}$</td>
<td>13.8$^{Bk}$</td>
<td>10.4$^{Bk}$</td>
<td>16.2$^{Bk}$</td>
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<td>11.5$^{Bk}$</td>
<td>9.5$^{Bk}$</td>
<td>19.3$^{Bk}$</td>
<td>10.9$^{Bk}$</td>
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<td>15.9$^{Bk}$</td>
</tr>
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<td>28</td>
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<td>12.4$^{Bk}$</td>
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<td>21.7$^{Bk}$</td>
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<tr>
<td>C.V.</td>
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<td>0.27</td>
<td>1.84</td>
<td>0.57</td>
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</tr>
<tr>
<td>SEM±</td>
<td></td>
<td>0.01</td>
<td>0.12</td>
<td>0.03</td>
<td>0.04</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
<td>0.018</td>
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</table>

Means differ if they have a different letter at: lower case superscript (a, b, c…) for comparison of treatments at 28 °C, lower case superscript (k, l, m…) for comparison of treatments at 41 °C, and uppercase superscript for comparison of 28 °C and 41 °C (ANOVA; Tukey’s test, P < 0.05).
Total amounts of organic carbon decreased during incubation process during the first 40 days, and the rate of decrease slowed after 40 days. VCAzPs had the greatest decrease in organic carbon, representing the humification and stabilization of organic matter in VC with increasing incubation duration (Figure 2) (Veeken et al. 2000). By increasing the temperature organic carbon decreased at a slower rate, reflecting the smaller bacterial population.

Table 4 shows the results of measuring sodium and potassium. Sodium and K changed little during incubation, but generally the amounts of both elements decreased through time. This decrease is most probably due to the incorporation of Na and K into humic matters complexes. Decrease in the amount of these elements at 28 °C was greater than at 40 °C.

During the incubation period, pH significantly decreased and VCPs had the greatest reduction. Decrease in pH could be attributed to organic acid production and increase in HA (Busato et al. 2012), which may have also increased soluble P. In fact, Pseudomonas increased P availability by producing organic acids and increasing HAs. During the first 40 days, pH decrease was rapid and then slowed. VCAz had less change in pH than VCPs, which could be due to fixed N and less organic acid production. VCC had the least pH change (Figure 3). During incubation EC reduced with the reduction in VCPs being greater than for other treatments (VCAz and VCAzPs) (Figure 4). As temperature increased changes in pH and EC showed less reduction (excluding VCC).

We observed that as incubation duration increased, the amount of HA increased in all treatments. VCAz had the greatest extraction, then VCPs, compared to other treatments which had fewer HAs.

### Table 4. Changes in sodium and potassium content (%) of VC during the incubation of vermicompost with Pseudomonas and Azotobacter.

<table>
<thead>
<tr>
<th>Incubation time (days)</th>
<th>Temperature</th>
<th>K</th>
<th>Na</th>
<th>K</th>
<th>Na</th>
<th>K</th>
<th>Na</th>
<th>K</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VCC</td>
<td>VCAz</td>
<td>VCPs</td>
<td>VCAzPs</td>
<td></td>
<td>VCC</td>
<td>VCAz</td>
<td>VCPs</td>
<td>VCAzPs</td>
</tr>
<tr>
<td>0</td>
<td>28</td>
<td>65.20^Aa</td>
<td>11.02^Aa</td>
<td>65.20^Aa</td>
<td>11.02^Aa</td>
<td>65.20^Aa</td>
<td>11.02^Aa</td>
<td>65.20^Aa</td>
<td>11.02^Aa</td>
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<td>11.02^Aa</td>
<td>65.20^Aa</td>
<td>11.02^Aa</td>
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<td>11.02^Aa</td>
<td>65.20^Aa</td>
<td>11.02^Aa</td>
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<td>10.95^Ab</td>
<td>64.40^Ab</td>
<td>10.90^Ab</td>
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<td>10.91^Al</td>
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<td>10.95^Ak</td>
<td>64.34^Al</td>
<td>10.76^Al</td>
<td>64.07^Al</td>
<td>10.91^Ak</td>
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<td>0.47</td>
<td>0.12</td>
<td>0.48</td>
<td>0.12</td>
<td>0.45</td>
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<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
<td>0.02</td>
<td>0.05</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Means differ if they have a different letter at: lower case superscript (a, b, c...) for comparison of treatments at 28 °C, lower case superscript (k, l, m...) for comparison of treatments at 41 °C, and uppercase superscript for comparison of 28 °C and 41 °C (ANOVA; Tukey’s test, P < 0.05).

![Figure 2](image.png) Changes in organic carbon content of VC during the incubation of vermicompost with Pseudomonas and Azotobacter (VCC: VC, VCAz: VC+ Azotobacter, VCPs: VC+ Pseudomonas and VCAzPs: VC+ Azotobacter + Pseudomonas).
During incubation VCPs lost a greater proportion of its organic matter. According to Veeken et al. (2000) these materials probably converted to more stable materials, thus it is expected that VCPs had more HA. Surprisingly, lower amounts of HA were observed in this treatment. Considering that condition and extractant type were the same, low pH in VCPs most probably caused reduction in extractant power (NaOH) and lower amounts of HA were extracted in this treatment. On the other hand, VCAz with higher pH compared to VCPs extracted more HA by increasing extractant power. With respect to greater pH reduction in treatments VCAz, VCPs, and VCAzPs compared to VCC, bacterial treatments extracted more HA which indicated the efficiency of these bacteria genera in stabilizing organic matter in VC (Figure 5). Increasing the temperature has little effect on HA extraction in bacterial treatments.

Unlike the previous measured indices, which had the most rapid increase between 20 and 40 days, the most rapid increase in HA occurred between 40 and 60 days. This could be due to the fact that HA is the final and stable product of organic matter, therefore are the latest products of microorganisms. As processing duration was increased, more HA was produced (Veeken et al. 2000).
Conclusions

Incubation duration is one of the important criteria in VC enrichment and according to results of our study and those of others, 45–60 days at 28 °C is sufficient to induce beneficial physical and chemical properties of VC. Incubation for more than 60 days would continue to provide improvements to soil properties, but at a decreasing rate of increase. Finally, future work should be carried out on various soil–useful microbial communities in incubation process and at various temperatures.

References


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