Composting of a crop residue through treatment with microorganisms and subsequent vermicomposting

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Abstract

Preliminary studies were conducted on wheat straw to test the technical viability of an integrated system of composting, with bioinoculants and subsequent vermicomposting, to overcome the problem of lignocellulosic waste degradation, especially during the winter season. Wheat straw was pre-decomposed for 40 days by inoculating it with *Pleurotus sajor-caju*, *Trichoderma harzianum*, *Aspergillus niger* and *Azotobacter chroococcum* in different combinations. This was followed by vermicomposting for 30 days. Chemical analysis of the samples showed a significant decrease in cellulose, hemicellulose and lignin contents during pre-decomposition and vermicomposting. The N, P, K content increased significantly during pre-decomposition with bioinoculants. The best quality compost, based on chemical analysis, was prepared where the substrate was treated with all the four bioinoculants together followed by vermicomposting. Results indicated that the combination of both the systems reduced the overall time required for composting and accelerated the composting of ligno-cellulosic waste during the winter season besides producing a nutrient-enriched compost product.

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Keywords: Bioinoculants; Composting; Crop residue; *Pleurotus sajor-caju*; Vermicomposting

1. Introduction

Disposal of solid waste has become a major problem recently due to shortage of dumping sites and strict environmental laws. As a result emphasis is now on aerobic composting, defined as a microbiological process that converts waste into organic manure rich in plant nutrients and humus (Sharma et al., 1999). Even though recycling organic waste has been known since biblical times, there are many aspects that should be improved. One of these areas includes reduction in overall time required for composting, especially during the winter season.

Various studies have shown that vermicomposting of organic waste accelerates organic matter stabilization (Neuhauser et al., 1988; Frederickson et al., 1997) and gives a product rich in chelating and phytohormonal elements (Tomati et al., 1995) which has a high content of microbial agents and stabilized humic substances (Ferruzi, 1986). Furthermore, combining vermicomposting with composting also accelerated the composting process thus reducing the time required for composting (Frederickson et al., 1997; Nedgwa and Thompson, 2001). But since some epigeic earthworm species require pre-decomposed waste it would be desirable to decrease the pre-decomposition time period of the waste to be vermicomposted by these efficient selected earthworm species. This could be done by treating the waste initially with certain efficient microflora. Basidiomycetes are the best-known lignin degraders. *P. sajor-caju* has varying enzyme activities (Buswell and Chang, 1994). *Trichoderma* and *Aspergillus* degrade hemicellulose and cellulose, respectively. If these microflora were added during pre-decomposition of the waste, the time of composting might be reduced.

Keeping this in view, the main objectives of the present research work were to study the role of *Pleurotus sajor-caju*, *Trichoderma harzianum*, and *Aspergillus niger* in pre-decomposition of a crop residue; and the effect of these fungi, in addition to *Azotobacter chroococcum*, on the growth of earthworms and quality of compost based
on chemical analysis. Cellulose, hemicellulose and lignin content were estimated to evaluate the potential of the microflora.

The purpose of the study was to test the technical viability of this system, initially utilizing wheat straw, and later to be employed on other substrates, specifically municipal solid waste.

2. Methods

2.1. Microbial source

The fungal strains P. sajor-caju, A. niger and the bacterial strain A. chroococcum were procured from Indian Agricultural Research Institute (IARI), New Delhi. T. harzianum was obtained from G.B. Pant University, Pantnagar. The fungal cultures were maintained by subculturing them on Potato Dextrose Agar while bacteria was subcultured on Jensen’s Agar media.

2.2. Experimental setup

Wheat straw was used as substrate for the pre-decomposition studies. Finely chopped substrate was pasteurized by dipping it overnight in 0.1% formalin. Pre-decomposition of the sterilized wheat straw was done in pits (1 m x 1 m x 1 m). The experiments were conducted with 5 kg of the substrate during October–December, 2000. Pure cultures of P. sajor-caju, T. harzianum and A. niger (all at 500 g mycelium per ton substrate) and A. chroococcum (at 50 ml/kg substrate having 10^6 cells per ml) were inoculated in different combinations:

P. sajor-caju (PS)
P. sajor-caju + T. harzianum (PST)
P. sajor-caju + T. harzianum + A. chroococcum (PSTA)
P. sajor-caju + T. harzianum + A. chroococcum + A. niger (PSTAA)
Fresh wheat straw—without any bioinoculants (Control)

For mesophilic aerobic digestion, turning was done manually every 4 days and the temperature was not allowed to exceed 26 °C by maintaining the moisture by adding water when necessary. The substrate with different treatments was pre-decomposed, in triplicates, for 40 days and then subjected to vermicomposting for 30 days.

2.3. Vermiculturing

For subsequent vermicomposting of the pre-decomposed waste, the earthworms Eisenia fetida were cultured in cow dung employing the windrow method.

2.4. Vermicomposting

The pre-decomposed substrates were vermicomposted, in the same pits as used for pre-decomposition, for a period of 30 days. Moisture was maintained to about 60% of the water holding capacity. 50 earthworms (Eisenia fetida) were introduced, in each pit, into the pre-decomposed wheat straw treated with different combinations of bioinoculants. Sampling was done at zero day and thirty-day. Composite samples (about 100 g) were collected from three sites in each pit. The earthworms and cocoons were removed manually and vermicompost was chemically analyzed.

2.5. Compost analysis

Total Kjeldahl nitrogen (TKN) and total organic carbon (TOC) of the pre-decomposed bioinoculated residue and the vermicompost were estimated by using a Micro-Kjeldahl method (Singh and Pradhan, 1981) and Walkey and Black’s Rapid Titration method (1934), respectively. Total phosphorus (TP) was determined spectrophotometrically while total potassium (TK) was detected by the flame emission technique. Cellulose, hemicellulose and lignin were fractionated sequentially by Dutta’s method (1981).

2.6. Statistical analysis

All the results reported are the means of three replicates. One way analysis of variance (ANOVA) was done using the INDOSTAT program. The objective of statistical analysis was to determine any significant differences among the parameters analyzed for different treatments during the composting process.

3. Results and discussion

The chemical analyses of fresh wheat straw are reported in Table 1.

Data in Table 2 reveal a significant decrease (p < 0.01) in the TOC content in all the treatments during

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC</td>
<td>30.10</td>
</tr>
<tr>
<td>TKN</td>
<td>0.69</td>
</tr>
<tr>
<td>C:N</td>
<td>43.62</td>
</tr>
<tr>
<td>TP</td>
<td>0.07</td>
</tr>
<tr>
<td>TK</td>
<td>0.50</td>
</tr>
<tr>
<td>Cellulose</td>
<td>55.59</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>23.07</td>
</tr>
<tr>
<td>Lignin</td>
<td>16.70</td>
</tr>
</tbody>
</table>

All values are mean of three replicates. All values are given in percentage except C:N ratio.
composting with bioinoculants. TOC decreased during vermicomposting (Table 3) indicating mineralization of organic matter. The best results were obtained when the wheat straw was treated with all the four bioinoculants together (PSTAA), where the TOC content decreased from 30.10% to 26.48% during composting and finally to 12.75% during vermicomposting. 20–43% loss of organic carbon as CO₂ was also observed during vermicomposting of paper mill and dairy sludges (Elvira et al., 1998).

Data pertaining to changes in TKN during pre-decomposition and subsequent vermicomposting are summarized in Tables 2 and 3, respectively. During pre-decomposition of the wheat straw the nitrogen content increased significantly (p < 0.01) in all the treatments (Table 2). The increment was maximum where all the bioinoculants were added together (PSTAA). The presence of A. chroococcum, a nitrogen-fixing bacterium, and the ability of P. sajor-caju to fix atmospheric nitrogen (Ginterova and Maxianova, 1975; Rangaswami et al., 1975) might have contributed to this increment. The process of degradation could have also been accelerated by synthesis of auxins like indole acetic acid and gibberellins; vitamins like thiamine, riboflavin, pyridoxin, cyanocobalamin, nicotinic and pantothenic acid; growth substances and antifungal antibiotics by A. chroococcum (Subba Rao, 1982), and this may have influenced the growth of other inoculated microflora. Further, it is inferred from Table 3 that the nitrogen content decreased during subsequent vermicomposting which may have been due to ammonification, NH₃ volatilization and denitrification (Martins and Dewes, 1992; Bernal et al., 1996). Our results are also supported by Benitez et al. (1999) who observed a 36% loss of total nitrogen during vermicomposting of sewage sludge.

Pre-decompostion and vermicomposting both resulted in a loss of carbon because of mineralization (Tables 2 and 3). The decomposition of the waste during vermicomposting was slow as compared to pre-decomposition process. That might have been due to higher initial N concentration, which might have increased the microbial activity in the beginning, thus decreasing the C/N ratio (Eiland et al., 2001). These results, however, contradict observations from the earlier work of Vincelas-Apka and Loquet (1997), who reported more

### Table 2
Composition of wheat straw after pre-decomposing with bioinoculants for 40 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TOC^a</th>
<th>TKN^b</th>
<th>C/N ratio</th>
<th>TP^b</th>
<th>TK^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>27.35 ± 0.52*</td>
<td>0.95 ± 0.10</td>
<td>28.79 ± 0.19*</td>
<td>0.070 ± 0.01</td>
<td>0.52 ± 0.01</td>
</tr>
<tr>
<td>PST</td>
<td>27.12 ± 0.12*</td>
<td>1.45 ± 0.06*</td>
<td>18.70 ± 0.02*</td>
<td>0.110 ± 0.01*</td>
<td>0.52 ± 0.01</td>
</tr>
<tr>
<td>PSTA</td>
<td>26.90 ± 0.10*</td>
<td>1.57 ± 0.02*</td>
<td>17.13 ± 0.01*</td>
<td>0.110 ± 0.02*</td>
<td>0.66 ± 0.02*</td>
</tr>
<tr>
<td>PSTAA</td>
<td>26.48 ± 0.11*</td>
<td>1.58 ± 0.02*</td>
<td>16.76 ± 0.13*</td>
<td>0.110 ± 0.01*</td>
<td>0.66 ± 0.01*</td>
</tr>
<tr>
<td>Control</td>
<td>29.86 ± 0.12</td>
<td>0.69 ± 0.04</td>
<td>43.28 ± 0.17</td>
<td>0.070 ± 0.00</td>
<td>0.50 ± 0.01</td>
</tr>
</tbody>
</table>

All values are mean and standard deviation of three replicates.

PS—P. sajor-caju.

PST—P. sajor-caju + T. harzianum.

PSTA—P. sajor-caju + T. harzianum + A. chroococcum.


Control—without any bioinoculant.

*Significant (p < 0.01).

^a Percentage decrease.

^b Percentage increase.

### Table 3
Composition of subsequently vermicomposted (30 days) wheat straw

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TOC^a</th>
<th>TKN^b</th>
<th>C/N ratio</th>
<th>TP^b</th>
<th>TK^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>15.30 ± 0.18*</td>
<td>0.76 ± 0.16*</td>
<td>19.89 ± 0.31*</td>
<td>0.162 ± 0.003*</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>PST</td>
<td>14.91 ± 0.05*</td>
<td>0.87 ± 0.01*</td>
<td>16.98 ± 0.10*</td>
<td>0.165 ± 0.004*</td>
<td>0.52 ± 0.01</td>
</tr>
<tr>
<td>PSTA</td>
<td>14.85 ± 0.16*</td>
<td>0.89 ± 0.01*</td>
<td>16.54 ± 0.11*</td>
<td>0.170 ± 0.001*</td>
<td>0.52 ± 0.01</td>
</tr>
<tr>
<td>PSTAA</td>
<td>12.55 ± 0.05*</td>
<td>0.98 ± 0.003*</td>
<td>12.75 ± 0.27*</td>
<td>0.190 ± 0.001*</td>
<td>0.55 ± 0.04</td>
</tr>
<tr>
<td>Control</td>
<td>16.50 ± 0.05</td>
<td>0.70 ± 0.100</td>
<td>23.50 ± 0.66</td>
<td>0.145 ± 0.005</td>
<td>0.51 ± 0.02</td>
</tr>
</tbody>
</table>

All values are mean and standard deviation of three replicates.

PS—P. sajor-caju.

PST—P. sajor-caju + T. harzianum.

PSTA—P. sajor-caju + T. harzianum + A. chroococcum.


Control—without any bioinoculant.

*Significant (p < 0.01).

^a Percentage decrease.

^b Percentage increase.
rapid degradation of substrate during vermicomposting than composting.

Chemical analysis of the materials with different treatments showed a significant increase \((p < 0.01)\) in phosphorus and potassium concentrations (Tables 2 and 3) by the end of vermicomposting period, possibly because of mineralization of organic matter. However in the control, potassium concentration remained more or less the same as at the start of the process (Table 3), which could have been due to leaching of potassium during the vermicomposting process (Elvira et al., 1998; Benitez et al., 1999).

All the three organic components; cellulose, hemicellulose and lignin decreased significantly \((p < 0.001)\) both during pre-decomposition and subsequent vermicomposting (Tables 4 and 5) with maximum degradation with PSTAA treatment. The production of cellulose, hemicellulose and lignin degrading enzymes by the inoculated microbes during pre-decomposition might have accelerated the degradation process. Similar results were also reported by Rasal et al. (1988) who reported rapid decomposition of sugarcane trash with a mixture of cellulolytic fungi; Trichoderma viride.

### Table 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>33.89 ± 1.17*</td>
<td>19.35 ± 0.32*</td>
<td>11.56 ± 0.58*</td>
</tr>
<tr>
<td>PST</td>
<td>28.25 ± 0.21*</td>
<td>17.35 ± 0.10*</td>
<td>10.91 ± 0.07*</td>
</tr>
<tr>
<td>PSTA</td>
<td>28.10 ± 0.10*</td>
<td>17.21 ± 0.18*</td>
<td>10.53 ± 0.49*</td>
</tr>
<tr>
<td>PSTAA</td>
<td>27.88 ± 0.49*</td>
<td>16.79 ± 0.25*</td>
<td>10.48 ± 0.08*</td>
</tr>
<tr>
<td>Control</td>
<td>50.46 ± 1.48</td>
<td>22.45 ± 0.41</td>
<td>15.36 ± 0.15</td>
</tr>
</tbody>
</table>

All values are mean and standard deviation of three replicates.

**PS**—P. sajor-caju.

**PST**—P. sajor-caju + T. harzianum.

**PSTA**—P. sajor-caju + T. harzianum + A. chroococcum.

**PSTAA**—P. sajor-caju + T. harzianum + A. chroococcum + A. niger.

Control—without any bioinoculant.

* Significant \((p < 0.01)\).

### Table 5

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>28.49 ± 0.07*</td>
<td>19.13 ± 0.29*</td>
<td>5.13 ± 0.12*</td>
</tr>
<tr>
<td>PST</td>
<td>27.66 ± 0.05*</td>
<td>16.97 ± 0.40*</td>
<td>3.60 ± 0.20*</td>
</tr>
<tr>
<td>PSTA</td>
<td>25.79 ± 0.06*</td>
<td>16.54 ± 0.19*</td>
<td>3.20 ± 0.13*</td>
</tr>
<tr>
<td>PSTAA</td>
<td>16.73 ± 0.15*</td>
<td>15.91 ± 0.09*</td>
<td>3.00 ± 0.08*</td>
</tr>
<tr>
<td>Control</td>
<td>38.26 ± 0.25</td>
<td>20.56 ± 0.14</td>
<td>8.76 ± 0.16</td>
</tr>
</tbody>
</table>

All values are mean and standard deviation of three replicates.

**PS**—P. sajor-caju.

**PST**—P. sajor-caju + T. harzianum.

**PSTA**—P. sajor-caju + T. harzianum + A. chroococcum.

**PSTAA**—P. sajor-caju + T. harzianum + A. chroococcum + A. niger.

Control—without any bioinoculant.

* Significant \((p < 0.01)\).

### Table 6

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of earthworms</th>
<th>Number of cocoons</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>115 ± 2.00*</td>
<td>35 ± 4.58</td>
</tr>
<tr>
<td>PST</td>
<td>121 ± 12.12*</td>
<td>39 ± 3.00*</td>
</tr>
<tr>
<td>PSTA</td>
<td>126 ± 5.56*</td>
<td>42 ± 6.55*</td>
</tr>
<tr>
<td>PSTAA</td>
<td>127 ± 5.56*</td>
<td>47 ± 8.00*</td>
</tr>
<tr>
<td>Control</td>
<td>89.16 ± 3.81</td>
<td>20 ± 4.00</td>
</tr>
</tbody>
</table>

Initial number of earthworms = 50.

All values are mean and standard deviation of three replicates.

**PS**—P. sajor-caju.

**PST**—P. sajor-caju + T. harzianum.

**PSTA**—P. sajor-caju + T. harzianum + A. chroococcum.

**PSTAA**—P. sajor-caju + T. harzianum + A. chroococcum + A. niger.

Control—without any bioinoculant.

* Significant \((p < 0.01)\).

Trichurus spiralis, Pachemlyces fusisorus and Aspergillus sp. along with nitrogen fixing bacteria Azotobacter sp. Treatment with Trichoderma reesei reduced the degradation time period of mixed plant residues (Sharma et al., 1999). The simultaneous activity of microflora present in the gut of earthworms and in the waste might have intensified cellulolysis and lignolysis (Loquet et al., 1984). The earthworms digest long chains of polysaccharides, enhancing microbial colonization. Simultaneously the structure of lignin changes, probably due to microbial oxidation and demethylation. The microbial cleavage of the aromatic rings of lignin leads to new polysaccharide and humins in the organic matter (Beyer et al., 1993).

A pronounced increase in the number of earthworms as well as the cocoons was observed during vermicomposting (Table 6). The maximum growth of earthworms with Azotobacter concludes the dual role of bacteria, i.e., in having been utilized as food material and enriching the substrate with nitrogen through nitrogen fixation process. Flack and Hartenstein (1984) obtained similar results in regard to Azotobacter on earthworms. Various studies have shown that earthworms utilize microorganisms in their substrates as a food source and can digest them selectively (Edwards, 1988; Edwards and Bohlen, 1996). The increase in earthworms’ growth may also be attributed to a low C:N ratio (Nedgwa and Thompson, 2000) of the pre-decomposed substrate and positive role of bioinoculants used in the present study.

### 4. Conclusion

The chemical analyses of the compost produced by pre-decomposing the waste with efficient microbes followed by vermicomposting point towards the feasibility of this process. The compost produced by all the treatments and especially the one that used all the four bioinoculants (PSTAA) was rich in total nitrogen and
phosphorus and had a low content of lignin. From the lignin degradation point of view, the results presented above suggest that this system would be the best for lignocellulosic waste treatment, specifically during the winter season. It would be helpful to further reduce the pre-decomposition time (i.e. from 40 to 10 days) which would enable us to potentially convert the waste into value-added products in a short time.

References


