1. Methodology

1.1 Sample Collection

Coir pith was collected from the coir industries in Kattukada, Alappuzha district. Fungal species were procured from Central Coir Research Institute (CCRI), Kalavoor, Alappuzha. Bacteria (Azotobacter vinelandii, MTCC No. 124 and Azospirillum brasilense, MTCC No. 125) were procured from Microbial Type Culture Collection (MTCC), Chandigarh. The composting process conducted in Rajiv Gandhi Chair in Contemporary Studies, School of Environmental Studies, Cochin University of Science and Technology (CUSAT). The biochemical estimations were carried out at Central Coir Research Institute, Alappuzha and Rajiv Gandhi Chair.

1.2 Composting.

The experiments consisted of 4 lots mounted in 5 kg coir pith heaps in triplicate. The first lot consisted of raw coir pith, the second was supplemented with Pleurotus sajor caju alone, and the third lot with both fungus (Pleurotus sajor caju) and bacteria (Azotobacter vinelandii). The fourth lot was supplemented
with *Pleurotus sajor caju* and *Azospirillum brasilense*. The coir pith heaps were moistened daily and monitored regularly for 30 day. A control composting experiment using the conventional method was also carried out simultaneously.

1.3 Estimation of Chemical Properties.

1.3.1 Lignin.

The lignin in coir pith was determined by Klason lignin method (Stephen and Carlton, 1992).

1.3.2 Nitrogen.

Nitrogen in the coir pith samples were estimate by Alkaline permanganate method using Kjeldahl distillation Unit (Vogel, 1961).

1.3.3 Phosphorus.

Phosphorus was estimated by Vanado Molybdo phosphoric yellow colour method.

1.3.4 Potassium.

Potassium was estimated by Flame photometry.

1.3.5 Organic Carbon.

Organic carbon was estimated by Walkey and Black method

1.3.6 Ammonia.

Estimation of Ammonia in coir pith sample is done by Nesslerization method.

1.4 Estimation of Enzyme Activity.

1.4.1 Lignin Peroxidase.

Lignin peroxide activity was estimated by prepared reaction mixture containing 2 mM Veratryl alcohol (Km = 60 µM), 0.4 mM H₂O₂ (Km = 80 M), 50 mM tartaric acid and enough ligninase to give an absorbance change of 0.2/min.
One unit of enzyme activity is defined as the quality of enzyme required for the formation of 1 µM of Veratryl aldehyde per minute.

1.4.2 Manganese Peroxidase.

Manganese peroxidase activity estimated by prepared 50 mM sodium tartarate buffer (pH-4.5), added MnSO4, H2O2 and phenol red at 0.2%, 0.1 mM and 0.0025% concentration respectively to the final volume of 5 ml reaction mixture. Read the change in absorbance at 431 nm. Maintained a heat killed enzyme source as control. One unit of enzyme activity is defined as the amount of enzyme required for 0.1 OD change at 431 nm/min.

1.5 Particle Size Analysis of Coir Pith by Scanning Electron Microscope (SEM).

The surface particle size and pore size was studied using the Scanning Electron Microscope (SEM JEOL JSM 6380 LV).

1.6 Estimation of Phenolic Compounds Formation by High Performance Liquid Chromatography (HPLC).

HPLC analysis was carried out with Shimadzu LC-8A liquid Chromatograph. The LC-8A has been designed to offer simple scale-up from nanogram to gram quantities. The flow rate set to 0.6 mL/min. Photo Diode Array Detector (PDA) and Luna 5 micron C-18 column (Length 250×4.6 mm) were used for the analysis. Running time was for 30 minutes and the injection volume was 20 micro liters.

2 Result

2.1 Estimation of Chemical Properties.

The biodegraded coir pith using *Pleurotus sajor caju* in combination with *Azotobacter vinelandii* and *Azospirillum brasiliense* causes reduction in Lignin,
Organic carbon and enhancement in the Nitrogen, Phosphorous and Potassium (NPK) content. The details of the chemical parameters given below.

2.1.1 Lignin.

The investigations reveal that definite variations were observed in lignin content in the coir pith under various treatments with ligninolytic mushroom *Pleurotus sajor caju* in combination with *Azotobacter vinelandii* and *Azospirillum brasilense*. The periodical analysis of samples of coir pith drawn at regular intervals of 5 days from the experimental heaps shows the rate of decomposition on lignin in coir pith (20% to 18%) from the initial value of 32%, in the control (Raw coir pith). Biodegradation of coir pith using combination of *Pleurotus sajor caju* and *Azotobacter vinelandii* the maximum lignin reduction (17%) followed by the combination of *Pleurotus sajor caju* and *Azospirillum brasilense*. The details are given in table V c.

2.1.2 Nitrogen.

The investigations reveal that the treatment of coir pith with different mushroom species show compatible and novel changes in the case of Nitrogen. The percentage of nitrogen in the raw coir pith (control) showed no change during the course of composting. The value of Nitrogen for Raw coir pith is 0.73 and the others show variations. The treatments with both the combinations shows the enhancement of nitrogen to 0.78% and 0.79% for *Pleurotus sajor caju* & *Azotobacter vinelandii* and *Pleurotus sajor caju* & *Azospirillum brasilense* respectively. From the results, it is clear that all the treatments lead to the enhancement of Nitrogen.

2.1.3 Phosphorous.

The Phosphorous content in coir pith also show enhancement with the treatment of all the four mushroom species. The periodical analysis of coir pith samples under the treatment with mushroom and fungal species indicated an
increasing trend of phosphorous content during composting. The combinations used for composting of coir pith showed variation in phosphorous content ranging from 0.85% to 1.87% & 2.78% respectively for *Pleurotus sajor caju* & *Azotobacter vinelandii* and *Pleurotus sajor caju* & *Azospirillum brasilense*. The details of the amount of phosphorous given in table V a.

**2.1.4 Potassium.**

The values of Potassium in the biodegraded coir pith with the treatment with combination of mushroom and bacterial species were given in table V a. The potassium content in the coir pith maintained as control which shows no change throughout the composting process. However, the values under treatment with mushroom species showed variation in potassium content. The results obtained from the analysis of coir pith samples treated with all the mushrooms displayed the values of potassium in an increasing trend. The phosphorous content of the samples varied from 0.04% in raw coir pith to the maximum of 0.38% in Biodegraded Coir pith Compost (BCC) using *Pleurotus sajor caju* & *Azospirillum brasilense* and 0.36% with *Pleurotus sajor caju* and *Azotobacter vinelandii*. Coir pith samples drawn out from the experimental heaps at regular intervals indicated increase in the potassium content.

**2.1.5 Organic Carbon.**

The Organic Content (OC) value for the raw and Biodegraded coir pith Compost (BCC) is given in table V b. The use of mushroom species with nitrogen fixing bacteria for the decomposition was found to be effective for the reduction of OC content from 7.34% (raw coir pith) to 6.11% and 4.36% respectively for *Pleurotus sajor caju* with *Azotobacter vinelandii* and *Azospirillum brasilense*. The results indicated a decreasing trend in the carbon content in coir pith under the treatment with different combinations. The carbon content of the raw coir pith (untreated) kept as control and did not show any variation.
2.1.6 Ammonia.

Ammonia content of the compost was observed to be increased due to nitrogen fixation activity of Nitrogen fixing bacteria incorporated with \textit{Pleurotus sajor caju}. It is observed that the amount of ammonia increased at different intervals of decomposition. The raw coir pith accounts 1261.16 mg/kg which increased to 3016.27 mg/kg when treated with \textit{Pleurotus sajor caju} \& \textit{Azotobacter vinelandii} and 2991.12 mg/kg with a combination of \textit{Pleurotus sajor caju} \& \textit{Azospirillum brasilense}. The details have been given in table V b.

2.2 Enzyme Activity.

From the chemical analysis of Biodegraded coir pith it could be observed that coir pith contains three major constituents \textit{viz.} lignin, cellulose and hemicelluloses. Almost all white rot fungi produce enzymes \textit{viz.} Manganese Peroxidase (MnP) and laccase, but only some of them produce Lignin Peroxidase (LiP) (Hatakka, 1994; 2001). The enzymes produced by white rot fungi catalyze the initial polymerization of lignin are extracellular and unusually non specific (Cullen and Kersten, 2004). Lignin degradation by white rot fungi has been extensively studied and the results revealed that the three kinds of extracellular peroxidases \textit{viz.} lignin peroxidase (LiP), Manganese peroxidase (MnP) and Laccase are responsible for initiating the depolymerization of lignin in coir pith. In my study, the activity of both the enzymes \textit{viz.} MnP and LiP could be observed which leads to the degradation of lignin in the coir pith.

2.2.1 Lignin Peroxidase.

The enzyme assay was conducted for the enzyme produced by \textit{Pleurotus sajor caju}. Definite reduction of lignin is also observed by the action of these enzymes. A blank was also taken as control. Readings were taken using U.V-Visible spectrophotometer at different intervals. The details in the study are furnished in Table Vd.
2.2.2 Manganese Peroxidase.

Manganese peroxidase assay is based on the oxidation of phenol red during coir pith degradation (Tien et al., 1984). The principle function of manganese peroxidase (MnP) is to oxidize Mn2+ to Mn3+, using H2O2 as oxidant (Pudelski, 1987). Activity of the enzyme is stimulated by simple organic acids which stabilize the Mn3+, thus producing diffusible oxidizing chelates. Table Ve shows the variation in the enzyme activity by the organisms. The enzyme profile showed variation in activity with change in substrate, decomposition stage and method of composting.

2.3 Particle Size Analysis.

Size and surface characteristics of biodegraded coir pith was determined by Scanning Electron Microscope (SEM). All the treated and raw coir pith was subjected to SEM imaging and the images are given in Fig V 1 to V s. From the images it is clear that the raw coir pith has greater size than after biodegradation thereby confirming that while degradation the size of the coir pith is reduced.

2.4 Phenolic Compound Analysis.

Study on the presence of phenolic compounds in the coir pith was carried out using HPLC studies on their phenolic compounds such as Catechol, Gallic acid, Picric acid and Pyrogallic acid. From the peaks obtained from the HPLC analysis, it is clear that all the phenolic compounds present in different treatment samples and their presence could be confirmed. Details have been furnished in Fig. Vd to Vk.
### Table V a. Variation pattern of NPK (%) in Raw and Biodegraded coir pith

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Composting Days</th>
<th>Nitrogen CP</th>
<th>Nitrogen CP + PS</th>
<th>Nitrogen CP + PS + A.v</th>
<th>Nitrogen CP + PS + A.b</th>
<th>Phosphorus CP</th>
<th>Phosphorus CP + PS</th>
<th>Phosphorus CP + PS + A.v</th>
<th>Phosphorus CP + PS + A.b</th>
<th>Potassium CP</th>
<th>Potassium CP + PS</th>
<th>Potassium CP + PS + A.v</th>
<th>Potassium CP + PS + A.b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.14</td>
<td>0.14</td>
<td>0.14</td>
<td>0.19</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>0.11</td>
<td>0.21</td>
<td>0.24</td>
<td>0.28</td>
<td>0.85</td>
<td>0.86</td>
<td>1.20</td>
<td>1.06</td>
<td>0.04</td>
<td>0.11</td>
<td>0.11</td>
<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.14</td>
<td>0.2</td>
<td>0.21</td>
<td>0.21</td>
<td>0.85</td>
<td>0.90</td>
<td>1.48</td>
<td>1.40</td>
<td>0.04</td>
<td>0.12</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>0.19</td>
<td>0.21</td>
<td>0.44</td>
<td>0.46</td>
<td>0.85</td>
<td>1.10</td>
<td>1.56</td>
<td>1.86</td>
<td>0.04</td>
<td>0.16</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0.14</td>
<td>0.3</td>
<td>0.78</td>
<td>0.68</td>
<td>0.85</td>
<td>1.12</td>
<td>1.78</td>
<td>2.12</td>
<td>0.04</td>
<td>0.14</td>
<td>0.22</td>
<td>0.23</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>0.14</td>
<td>0.5</td>
<td>0.72</td>
<td>0.75</td>
<td>0.85</td>
<td>1.15</td>
<td>1.85</td>
<td>2.76</td>
<td>0.04</td>
<td>0.26</td>
<td>0.30</td>
<td>0.36</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>0.14</td>
<td>0.51</td>
<td>0.78</td>
<td>0.79</td>
<td>0.85</td>
<td>1.17</td>
<td>1.87</td>
<td>2.78</td>
<td>0.04</td>
<td>0.26</td>
<td>0.36</td>
<td>0.38</td>
</tr>
</tbody>
</table>

**CP** = Coir pith, **PS** = *Pleurotus sajor caju*, **A.v** = *Azotobacter vinelandii*, **A.b** = *Azospirillum brasiliense*
Table V b. Variation pattern of Organic carbon & Ammonia in Raw and Biodegraded coir pith

<table>
<thead>
<tr>
<th>SI No</th>
<th>Composting Days</th>
<th>Organic Carbon (%)</th>
<th>Ammonia (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CP</td>
<td>CP + PS</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>7.34</td>
<td>7.34</td>
</tr>
<tr>
<td>2</td>
<td>05</td>
<td>7.34</td>
<td>7.30</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>7.34</td>
<td>7.25</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>7.34</td>
<td>7.11</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>7.34</td>
<td>6.86</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>7.34</td>
<td>6.84</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>7.34</td>
<td>6.80</td>
</tr>
</tbody>
</table>

CP = Coir pith, PS = Pleurotus sajor caju, A.v = Azotobacter vinelandii, A.b = Azospirillum brasilense
### Table V c. Variation pattern of Lignin (%) in Raw and BCC.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Composting Days</th>
<th>Lignin</th>
<th>CP</th>
<th>CP+PS</th>
<th>CP+PS+A.v</th>
<th>CP+PS+A.b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>32</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>32</td>
<td>30</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>32</td>
<td>28</td>
<td>22</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>32</td>
<td>26</td>
<td>21</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>32</td>
<td>25</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>32</td>
<td>23</td>
<td>17</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

**CP** = Coir pith, **PS** = Pleurotus sajor caju, **A.v** = Azotobacter vinelandii, **A.b** = Azospirillum brasilense.

### Table V d. Activity of Lignin Peroxidase (U ml⁻¹) in BCC

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Composting Days</th>
<th>CP+PS</th>
<th>CP+PS+A.v</th>
<th>CP+PS+A.b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>05</td>
<td>0.4</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>2.8</td>
<td>3.1</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>5.1</td>
<td>7.2</td>
<td>5.1</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>8.6</td>
<td>11.8</td>
<td>10.7</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>10.2</td>
<td>12.5</td>
<td>13.1</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>15.9</td>
<td>18.6</td>
<td>16.8</td>
</tr>
</tbody>
</table>

**CP** = Coir pith, **PS** = Pleurotus sajor caju, **A.v** = Azotobacter vinelandii, **A.b** = Azospirillum brasilense.

### Table V e. Activity of Manganese Peroxidase (U ml⁻¹) in BCC

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Composting Days</th>
<th>CP+PS</th>
<th>CP+PS+A.v</th>
<th>CP+PS+A.b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>05</td>
<td>0.2</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>2.5</td>
<td>4.1</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>4.1</td>
<td>6.8</td>
<td>7.1</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>6.8</td>
<td>10.2</td>
<td>12.4</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>11.2</td>
<td>15.9</td>
<td>15.8</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>14.4</td>
<td>18.8</td>
<td>18.1</td>
</tr>
</tbody>
</table>

**CP** = Coir pith, **PS** = Pleurotus sajor caju, **A.v** = Azotobacter vinelandii, **A.b** = Azospirillum brasilense.
Efficacy of combined action of Pleurotus sajor caju and Nitrogen fixing .......

**GRAPHS**

**Fig Vd.** HPLC Graph of Standards (Catechol).

**Fig Ve.** HPLC Graph of Standards (Gallic acid).
Efficacy of combined action of Pleurotus sajor-caju and Nitrogen fixing …….

Fig V f. HPLC Graph of Standards (Picric acid).

Fig V g. HPLC Graph of Standards (Pyrogallic acid).
Efficacy of combined action of Pleurotus sajor caju and Nitrogen fixing

Fig. V h. HPLC Graph of Standards (Resorcinol).

Fig. V i. HPLC Graph BCC with Pleurotus sajor caju
Efficacy of combined action of *Pleurotus sajor caju* and Nitrogen fixing…….

Fig. V j. HPLC Graph BCC with *Pleurotus sajor caju* and *A. vinelandii*

Fig. V k. HPLC Graph BCC with *Pleurotus sajor caju* and *A. brasilense*
Fig. V-I. HPLC Graph of Raw coir pith.

Fig. V-I. SEM images of Raw coir pith.
Efficacy of combined action of Pleurotus sajor caju and Nitrogen fixing.

Fig V m. SEM image of *Pleurotus sajor caju* BCC.

Fig V n. SEM image of *Pleurotus sajor caju* + *A. vBCC*. 
Efficacy of combined action of Pleurotus sajor caju and Nitrogen fixing …..

**Fig V o.** SEM image of Pleurotus sajor caju + A.b BCC

**Fig V t.** Enhancement of Nitrogen (%) during biodegradation of coir pith

**CP**: Coir Pith, **PS**: Pleurotus sajor caju, **A.v**: Azotobacter vinelandii, **A.b**: Azospirillum brasilense

**Graphs**

![Graph showing nitrogen percentage over composting days](image)

**Fig V t.** Enhancement of Nitrogen (%) during biodegradation of coir pith

**CP**: Coir Pith, **PS**: Pleurotus sajor caju, **A.v**: Azotobacter vinelandii, **A.b**: Azospirillum brasilense
Efficacy of combined action of *Pleurotus sajor caju* and Nitrogen fixing ….

**Fig V u.** Enhancement of Phosphorus during biodegradation of coir pith.

\[ \text{CP: Coir Pith, PS: Pleurotus sajor caju, A.v: Azotobacter vinelandii, A.b: Azospirillum brasilense} \]

**Fig V v.** Enhancement of Potassium during biodegradation of coir pith.

\[ \text{CP: Coir Pith, PS: Pleurotus sajor caju, A.v: Azotobacter vinelandii, A.b: Azospirillum brasilense} \]
Efficacy of combined action of Pleurotus sajor caju and Nitrogen fixing …….

**Fig. V w.** Reduction of Lignin during biodegradation of coir pith

CP - Coir Pith, PS - Pleurotus sajor caju, A.v - Azotobacter vinelandii, A.b - Azospirillum brasilense

**Graph V x.** Reduction of Organic Carbon during biodegradation of coir pith.

CP - Coir Pith, PS - Pleurotus sajor caju, A.v - Azotobacter vinelandii, A.b - Azospirillum brasilense
Graph V y. Enhancement of Ammonia during biodegradation of coir pith.
CP - Coir Pith, PS - Pleurotus sajor caju, A.v - Azotobacter vinelandii, A.b - Azospirillum brasilense

Graph V z. Enzyme Activity (Lignin Peroxidase)
CP - Coir Pith, PS - Pleurotus sajor caju, A.v - Azotobacter vinelandii, A.b - Azospirillum brasilense
Efficacy of combined action of Pleurotus sajor caju and Nitrogen fixing ……

Graph V a. Enzyme Activity (Manganese Peroxidase)

CP - Coir Pith, PS - Pleurotus sajor caju, A.v - Azotobacter vinelandii, A.b - Azospirillum brasiliense

3. Discussion

Coir pith has been reported to have a C: N ratio of 112:1 (Nagarajan et al., 1985). White rot fungi which degrade cellulose and hemi cellulose as well as lignin are widely used to increase the digestibility of agro-residues (Kurtzman, 1981; Neelakantan, 1987; Zabrazil, 1987). The studies of coir pith degradation with the combination of Pleurotus sajor caju and nitrogen fixing bacteria (Azotobacter vinelandii and Azospirillum brasiliense) shows a definite enhancement in nitrogen content after the coir pith biodegradation (Table V a). Biodegraded Coir pith Compost (BCC) using A.vinelandii and A.brasiliense has been observed to contain 0.78% and 0.79% nitrogen respectively. This is higher in comparison to that in raw coir pith and biodegradation with Pleurotus sajor caju alone. Barraquio et al., 2000; James et al., 2000 reported that, though a variety of nitrogen fixing bacteria like Acetobaccter, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Beijerinckia, Derxia, Enterobacter, Herbaspirillum, Klebsiella, Pseudomonas and
Zoogloea have been isolated from the rhizosphere of various crops, interest in the beneficial nitrogen fixing growth promoting rhizobacterial plant association has increased recently due to their potential use as biofertilizers (Vessey, 2003). As these bacterial species cannot act on the rigid chemical compounds present in coir pith, with the combination of fungal species Pleurotus sajor caju, the degradation has observed to be more effective. Phosphorous content has also been observed to increase significantly to an amount from 0.85% (raw coir pith) to 1.87% (A. vinelandii) and 2.78% (A. brasilense). A definite increase has also been observed in the potassium content in the different combinations from 0.04% in raw coir pith to 0.28% in BCC using Pleurotus sajor caju alone to 0.36% in combination with A. vinelandii and to 0.38% with A. brasilense. The use of these biological nitrogen fixing agents for the bio manure preparation is very important. It is reported that very significant reduction in the use of nitrogen fertilizer could be achieved if biological nitrogen fixation is made available to crop plants (Dawe, 2000).

Lignin is the most abundant aromatic polymer on earth, being produced by plants. The first step in lignin degradation is depolymerization, catalysed by lignolytic enzymes. Degradation of lignin is carried out by a group of basidiomycetes categorized as white rot fungi (Kamitsuji et al., 2004) Lignocellulosic wastes (LCW) refer to plant biomass wastes that are composed of cellulose, hemicelluloses and lignin (Mtui, 2009). Out of these three, lignin is the toughest materials which degrade very slowly in nature. In the present study, a reduction of lignin was observed by composting with combination of fungi and bacteria. Raw coir pith contains 32% lignin and which is reduced to 17% and 18% after biodegradation (Table V c). It should be noted that coir pith is resistant to biodegradation due to the presence of lignin. Normally coir pith is dumped as agricultural waste and accumulates as a waste product as heaps of course and fine dust (Ghosh et al., 2007). The coir pith decomposes very slowly in soil as its pentosan-lignin ratio is below 0.5, and because of chemical and structural complexity of lignin-cellulose complex (Ramalingam et al, 2004). High content of
Efficacy of combined action of *Pleurotus sajor caju* and Nitrogen fixing……

Lignin in coir pith causes very slow decomposition following which it is used as raw organic manure for crops (Vinodhini *et al.*, 2005). The degrading capacity of the combined consortium is more than that by the individual organisms. Even, white rot fungi which are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials (Sun and Cheng, 2002), when added with appropriate bacterial species, interesting results could be obtained.

Ammonia is the indicator of nitrogen fixation, and attempts have been made in the present study to show the enhancement of ammonia in coir pith after biodegradation. Table V b shows the value of ammonia in raw and biodegraded coir pith. Reghuvaran *et al.*, 2011 reported a definite amount of increase in ammonia with the biodegradation of coir pith could be observed (Reghuvaran *et al.*, 2011). Organic carbon was also observed to reduce considerably during the biodegradation (Table V b). There are reports stating that coir pith contains 87% organic matter and 13% ash content (Thampan, 1987).

Extracellular enzymes play an important role in the degradation of lignocelluloses. In our study the activity of Lignin peroxidase (LiP) and Manganese peroxidase (MnP) were estimated and the results have been tabulated in Table Vd & Ve. White rot fungi have been observed to secrete these lignin degrading enzymes. White rot fungi, including *P. ostreatus*, produce a wide range of enzymes (laccase, peroxidases, cellulases and xylanases) that degrade lignocelluloses (Kues and Liu., 2000). Several studies on *Pleurotus* species have been reported to grow on a wide spectrum of lignocellulosic waste materials due to their ability to secrete a range of degrading enzymes (cellulases, hemicellulases, xylanases, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccases) and to produce protein rich biomass of fruiting bodies (Madan and Bisaria., 1983; Buswell and Chang., 1993; Rajarathnam *et al.*, 1998). Among the ligninases produced from LCW (Lignocellulosic Waste), laccases have been studied the most (Nazareth and Sampy, 2003; Moldes *et al.*, 2003, 2004; Couto *et al.*, 2003; Couto and Sanroman,
Efficacy of combined action of *Pleurotus sajor caju* and Nitrogen fixing ……

2006; Mishra and Kumar, 2007; Alcantara *et al.*, 2007; Minussi *et al.*, 2007), followed by Manganese peroxidase and Lignin peroxidase (Couto *et al.*, 2001, 2003; Wuyep *et al.*, 2003; Velazquez-Cedeno *et al.*, 2004; Couto and Sanroman, 2005; Alam *et al.*, 2005; Asgher *et al.*, 2006; Songulashvili *et al.*, 2007; Elisashvili *et al.*, 2008). Combined consortium of microorganisms produce more enzymes when compared to biodegradation by *Pleurotus sajor caju* alone and thereby action of the consortium accelerates the lignin degradation. As the combined action of these organisms yields more extracellular enzymes it can be concluded that, the presence of these nitrogen fixing bacteria could attribute to accelerating the enzyme production of *Pleurotus sajor caju* and thereby speed up the biodegradation of coir pith. These results are in concordance with many works, the main enzymes involved are lignin peroxidase, manganese peroxidase and laccase (Hao *et al.*, 2006; Mtui and Nakamura, 2007, 2008; Mtui and Masalu, 2008).

The structure of coir pith has also been observed to vary during composting. Scanning Electron Microscope images of raw and biodegraded coir pith show variation in coir pith structures (Fig V 1 to V 6). It has been observed that the average size of coir pith reduces after degradation which could be attributed to the breakdown of components. The raw coir pith particle size has been observed to be 816×480μm. *Pleurotus sajor caju* mediated BCC displayed size of 708×376 μm, *Pleurotus* and *A. vinelandii* BCC showed a size of 569×337 μm and the BCC using *Pleurotus* and *A. brasilense* BCC displayed size of 365×231 μm. The big sized particles show high aeration porosity and those containing small sized particle size show high water holding porosity *i.e.* smaller particles do not hold much aeration and bigger particles do not hold much water (Jeyeseeli and Poul Raj, 2010).

Large amounts of coir pith accumulate in the vicinity of coir processing units (approximately 7.5 million tons annually in India), causing severe disposal problems, fire hazards and ground water contamination due to release of phenolic compounds (Namasivayam *et al.*, 2001). This also causes pollution of ground water
Efficacy of combined action of *Pleurotus sajor caju* and Nitrogen fixing ……

due to the percolation of leachates containing residual phenol from these dumps (Gopal and Gupta, 2001) especially during rainy season. The environment also serves as an ideal breeding ground for rodents and insect pests (Grimwood, 1975). Polyphenols are present /produced during coir pith biodegradation. Lignin, a main contributor of the total carbon of agro-industrial wastes, produces polycyclic aromatic hydrocarbon components such as benzopyrine, catechol, hydroquinone, phenanthrine and naphthalene when degraded by heat (Kjallstrand et al., 1998). Here the phenolic substances like catechol, gallic acid, picric acid, pyrogallic acid and resorcinol were taken as standards for HPLC analysis. The Rf (Retension Factor) values of the samples were almost similar for these standards. Gallic acid has the Rf value 4.358, which is almost similar to that observed in *PS+A.v BCC* (4.312) and *PS+A.b BCC* (4.326). The Rf values were studied for Picric acid (4.388), Pyrogallic acid (4.257) and Resorcinol (4.204). Catechol (3.280; 5.616). The similar Rf values were observed in the *CP+PS* (5.861), *PS+A.v BCC* (3.608; 5.868) and *PS+A.b BCC* (3.507; 5.635). Presence of resorcinol (3.460; 5.371) was also observed in the samples of biodegraded coir pith. Presence of phenolic compounds *viz.* Picric acid and Pyrogallic acid could also be confirmed (Fig V d to V k). In contrast, only one definite peak was observed in the HPLC graph of raw coir pith which shows the phenolics were absent in coir pith before biodegradation (Fig. V l). These results are in concordance with reports which state that resorcinol, pyrogallic acid and catechol were present in leachate samples drawn from coir retting areas. (Ravindranath and Sarma, 1998).

The potential of bioconversion of lignocellulosic wastes into value added products is emphasized in recent studies (Philippoussis & Zervakis 2000a; Poppe 2000). In our studies, we can conclude that coir pith can be converted to effective organic manure with biodegradation with *Pleurotus sajor caju* and Nitrogen fixing bacteria like *Azotobacter vinelandii* and *Azospirillum brasilense*. The combined action of these organisms with the white rot fungi, there was a marked reduction in
the lignocelluloses content and particle size, enhancement of NPK content and the resultant product can be used as effective manure for plants.

4. Conclusion

Bioremediation has been reported to be more effective through consortium action than single culture. In the present study, Pleurotus sajor caju has been experimented in combination with nitrogen fixing microorganisms viz. *Azotobacter vinelandii* and *Azospirillum brasilense*. This was aimed at replacing urea in the composting process. The study could yield results viz. increase in nitrogen content from 0.19% to 0.79%. The increase in the nitrogen content could be attributed to the inoculation of *Azotobacter vinelandii* and *Azospirillum brasilense*. Thus biodegradation of coir pith using a consortium of cultures consisting of lignin degrading fungi and nitrogen fixing bacteria could yield a value added fertilizer from biological waste.