



## Studies on degradation of lignocellulosic coconut waste by biological treatment methods

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### Abstract

Lignocellulosic biomass is a renewable and abundant resource with great potential for bioconversion to value added bioproducts. Lignin causes the serious pollution of toxins problem in aquatic ecosystem owing to its low biodegradability. In the present study was planned to degradate lingocellulosic material by biological treatment methods such as vermicompost, cow dung, mushroom spent waste. The experiment was designed as T<sub>1</sub> coconut shredder waste only, T<sub>2</sub> substrate + cow dung, T<sub>3</sub> substrate + vermicompost and T<sub>4</sub> substrate + mushroom spent waste. Lignocellulosic wastes was allowed for complete degradation nearly 90 days. During this degradation period, pH, temperature, moisture, total organic carbon, total organic nitrogen, C: N ratio, enzyme assay was estimated. Microbial population was also calculated. *Enterobacter sp*, *Bacillus sp*, *Klebsiella sp*, *Pseudomonas sp*, *Streptomyces sp* and *Azotobacter sp* and *Trichoderma sp*, *Pleurotus sp*, *Aspergillus sp*, *Rhizopus sp* and *P.chryso sporium* was identified by using standard manuals. In this result, physical chemical parameters was reduced than initial inoculation. Manganese peroxidase was absent in T<sub>1</sub> and T<sub>4</sub>. The lignin peroxidase was present in T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. Although final degradation was observed in 90% level in the T<sub>3</sub> using vermicompost followed by T<sub>2</sub>cowdung (75%) The findings has significant potent use in the agriculture for the treatment of lignin degradation and lignin related environment pollutants.

**Keywords:** lignolytic enzymes, lignin peroxidase, Manganese peroxidase, laccasse, bacteria, fungi, biodegradation

### Introduction

Lignocellulose, the major component of biomass, makes up about half of the matter produced by photosynthesis. It consists of three types of polymers – cellulose, hemicellulose, and lignin – that are strongly intermeshed and chemically bonded by non-covalent forces and by covalent cross-linkages. A great variety of fungi and bacteria can fragment these macromolecules by using a battery of hydrolytic or oxidative enzymes. In native substrates, binding of the polymers hinders their biodegradation. Molecular genetics of cellulose-, hemicellulose- and lignin-degrading systems advanced considerably during the 1990s. Most of the enzymes have been cloned, sequenced, and expressed both in homologous and in heterologous hosts. Much is known about the structure, genomic organization, and regulation of the genes encoding these proteins. It is beyond the scope of this review, however, summarize these findings. Our purpose is to provide an overview of the degradation of cellulose, hemicellulose, and lignin and the enzymatic systems involved. For a better understanding of enzymology and degradation, we will first briefly describe the structure of the cell wall and its components. Lignocelluloses in nature derive from wood, grass, agricultural residues, forestry wastes and municipal solid wastes. Several biological methods for lignocellulose recycling, based on the enzymology of cellulose-, hemicellulose- and lignin degradation, have been suggested. Among them, compostage and their use as raw material for the production of ethanol as an alternative combustible seem to be the most economically feasible. Moreover, the general use of alternative, environmentally friendly technologies that introduce lignocellulose enzymes at different stages of pulp

and paper manufacture as a pretreatment to pulping (biopulping), bleaching (biobleaching), or wastewater treatment has allowed considerable electrical power savings and a reduction of pollutants in the waste water from these industries. In addition, pretreatment of agricultural wastes with ligninolytic fungi enables their use as raw material for paper manufacturing. The use of microorganisms or their enzymes to enhance the de-inking of recycled fibers and the release of toners from once wastes is another promising field that is under research (Perez *et al.*, 2002) <sup>[11]</sup>.

Lignin is the major noncarbohydrate, polyphenolic structural constituent of wood and other plant material that encrusts the cell walls and cements the cells together, A highly polymorphic substance, with a complex cross - linked, highly aromatic structure of molecular weight about 10,000 derived principally from coniferyl alcohol (C<sub>10</sub>H<sub>12</sub>O<sub>3</sub>) by extensive condensation polymerization. The aromatic polymer lignin is well – known for resistance to microbial degradation because of its high molecular weight and presence of various biologically stable carbon-to-carbon and ether linkages. Microorganisms that degrade plant lignin via an oxidative process are fungi (Tien and Kirk, 1983) <sup>[16]</sup>, *Actinomycetes* (Hernandez, *et al.*, 2001) <sup>[10]</sup> and a lesser extent bacteria (Trojanowski *et al.*, 1977) <sup>[15]</sup>.

The structure of lignin mainly composed of phenolic and nonphenolic components. Lignin forms an integral part of secondary walls in plants and it plays an important role in enhancing the efficiency of water conduction in vascular plants. In the lignin, digestibly many various fungi and bacteria are proficient for genes production. These enzymes comprising of lignin peroxidases (e.g. lignin peroxidase, and

manganese peroxidase and laccases as well. These are heme containing glycoprotein which requires hydrogen peroxide as oxidant. Lignin peroxidase degrades non-phenolic lignin units. Manganese peroxidase acts on phenolic and non-phenolic lignin units through lipid peroxidation reactions (Sindhu *et al.*, 2011). It oxidizes Mn<sup>2+</sup> to Mn<sup>3+</sup> which oxidizes phenol rings to phenoxy radicals leading to decomposition of compounds. *P. chrysosporium*, *Ceriporia lacerata*, *Cyathus stercoleris*, *C. Subvermispora*, *Pycnoporus cinnabarinus*, and *Pleurotus ostreatus* produce enzymes which are involved in lignin degradation (Periyasamy *et al.*, 2015) [12].

In this study, an investigation was attempted to degrade the lignocellulosic waste using biological treatments.

## Materials and Methods

### Collection and Preparation of Lignocellulosic Substrate

Coconut dried leaves were collected from the coconut grooves in and around Sundarakkottai village, Mannargudi Taluk, Thiruvarur District, Tamil Nadu, India. The collected leaves were sun dried and made into pieces using mechanical shredder. The shredder was air dried under shade and weighed.

### Collection of Cow Dung, Vermicompost, Mushroom Spent Waste Material

Cowdung was obtained from the local cowshed near the STET women's college, Sundarakkottai, Mannargudi Taluk, Thiruvarur District.

Vermicompost and Mushroom spent waste was obtained from the Biominin Laboratory of the STET Biofloral products Development and Research centre, STET Women's College, Sundarakkottai, Mannargudi Taluk, Thiruvarur District.

### Preparation of Pits

Pits measuring about 3×2ft were dug to a depth of 1ft. Weight quantity (30 kg) of the air dried shreds was filled in each pit. Totally four pits were prepared in this way three pits were used for other treatments and replicate.

### Treatment-1

#### T<sub>1</sub>-control

In T<sub>1</sub> pit containing the Lignocellulosic substrate (Shredded leaf material of coconut) without adding any additive was treated as control. The substrate containing pits was spread with water and 60 % moisture content was maintained. The substrate were well mixed and allowed to decompose. The substrate was well mixed periodically. So as to enhance the process of decomposition.

### Treatment-2

#### T<sub>2</sub>-Cow dung

Substrate augmented with cow dung

The substrate was added with 5kg of cow dung and mixed thoroughly and allowed to decompose. Suitable moisture content was maintained and monitored.

### Treatment -3

#### T<sub>3</sub>-Vermicompost

Substrate augmented with Vermicompost

The substrate was added with 5kg of Vermicompost and mixed

thoroughly, and allowed to decompose. Suitable moisture content was maintained and monitored.

### Treatment – 4

#### T<sub>4</sub> – Mushroom spent waste

Substrate augmented with mushroom spent waste

The substrate was added with 5kg of mushroom spent waste mixed thoroughly and allowed to decompose suitable moisture content was maintained and monitored.

## Isolation of Microorganism from the Lignin Degradation Pits

### Serial Dilution (Aneja, 1996)

Microbiological analysis of the soil was also carried out to analyze the soil quality. Serial dilution was performed by using standard methods.

### Identification of Bacteria

The bacterial culture was identified by using Bergey's manual of determinative bacteriology.

### Identification of Fungi

The fungal culture was identified by using manual such as manual of soil fungi (Gill man, 1957) [8], Dematiaceous Hypomycetes (Ellis, 1971) [6], More Dematiaceous Hypomycetes (Ellis and Ellis, 1976) [7], Hypomyceta (Subramaniam, 1971) [14].

### Optimization

#### Analysis of Physico Chemical Parameters

This lignin degradation was subjected to analysis for pH, C: N ratio, N, P, K, weight loss, and degradation rate. Samples were taken for day 0 (starting day) and the 60 day (when the experiment partially degraded) for determining the trends with time. In addition, physical parameters, namely, conductivity, salinity, and color change, were observed.

#### Determination of Soil Moisture Content (Griffin, 1970) [9].

The moisture content in a soil sample was determined by drying a known quantity of sample in a hot air oven at 105°C ±2°C and estimating the loss of weight.

#### Determination of Soil pH (Booth *et al.*, 1961) [5].

20 grams of soil sample was air dried and taken in a beaker of 50 ml of distilled water and the pH of the soil sample was recreated by using pH meter with electrode.

#### Determination of Temperature (Ahmed *et al.*, 2003)

Temperature was an important physical factor in limnological studies. The sample temperature was measured by using standard centigrade thermometer graduated from 0° to 100°C.

#### Total Organic Carbon, Nitrogen and CN (Walkey and Black, 1934) [17].

Total organic carbon, total organic nitrogen and total organic carbon nitrogen ratio was done by Walkey and Black, 1934 [17].

#### Enzyme Assay (Adrika Raybaren *et al.*, 2014) [1].

Enzyme assay was performed by the reference of Adrika Raybaren *et al.*, 2014 [1]. Lignolytic enzymes were estimated

namely -laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP).

**Enzyme Separation**

The 21-day fungal cultures were first filtered using filter paper. The filtrate was centrifuged at 5000 rpm for 12min. Potassium phosphate buffer (pH 5.8) was the running buffer for the DEAE Sephadex anion exchange column (HIMEDIA DEAE Sephadex A-50). The samples were eluted with a linear gradient of 0.3-0.9M NaCl in the same buffer with a flow rate of 0.6 ml min<sup>-1</sup>. The fractions containing the proteins were collected and the effluents were assayed to determine enzymatic activity.

**Percentage of Lignin Degradation (Adrika Raybaren et al., 2014) [1].**

Lignin degradation was measured in terms of percentage of degradation by means of dry weight measures. Initial weight was taken, after the period of completion of lignin degradation weight was measured in dry weight basis in all the inoculated pits.

$$\text{Weight loss} = \frac{\text{Initial weight} \times \text{Final weight}}{\text{Initial weight}} \times 100$$

**Physico-Chemical Properties**

pH, Temperature and Moisture content was reported on the table (1-4).

**Table 1:** T<sub>1</sub>-Control Lignocellulosic Waste Only

S.NO	Days (composting)	Ph	Temperature (°c)	Moisture Content (%)
1.	1-15	9.0	37.0	40.0
2.	15-30	7.5	37.1	39.8
3.	30-45	7.1	35.0	39.0
4.	45-60	6.8	33.0	38.0
5.	60-75	6.4	33.0	37.0
6.	75-90	6.9	32.5	36.5

**Table 2:** T<sub>2</sub>-Lignocellulosic Waste + Cowdung

S. NO	Days (Composting)	pH	Temperature (°C)	Moisture Content (%)
1.	1-15	7.5	39.0	41.0
2.	15-30	7.2	38.7	40.5
3.	30-45	7.5	38.0	39.7
4.	45-60	7.3	37.4	38.5
5.	60-75	7.0	37.0	37.5
6.	75-90	6.7	34.0	35.0

**Table 3:** T<sub>3</sub>- Lignocellulosic Waste + Vermicompost

S. No	Days (composting)	Ph	Temperature (°c)	Moisture content (%)
1.	1-15	7.0	37.0	40.5
2.	15-30	7.5	36.5	39.5
3.	30-45	7.3	34.3	38.0
4.	45-60	7.1	31.0	37.3
5.	60-75	7.0	30.0	34.8
6.	75-90	6.5	28.5	32.5

**Table 4:** T<sub>4</sub>-Lignocellulosic Waste +Mushroom Spent Waste

S. No	Days (composting)	Ph	Temperature (°c)	Moisture content (%)
1.	1-15	8.0	38.0	41.0
2.	15-30	8.3	37.3	40.9
3.	30-45	7.8	36.5	41.3
4.	45-60	7.0	34.0	40.5
5.	60-75	6	33.3	39.5
6.	75-90	6.9	32.8	35.0

**Chemical Parameters**

**Effect of Total Organic Carbon (%)**

In all the treatments, total organic carbon was measured

**Results**

Lignocellulosic wastes was extracted from the natural sources and our study is planned to degrade lignin by the treatments are designed as T<sub>1</sub>-coconut shredder waste alone, T<sub>2</sub>-cow dung, T<sub>3</sub>- vermicompost and T<sub>4</sub>-mushroom spent waste. Totally six bacterial species and five fungal species are identified in the pits. The crude lignin was extracted after degradation period of 90 days, extraction was done by alkaline delignification method, using 4% sodium hydroxide, a dark colour alkali lignin was obtained and stored in air tight bottle and used for further experiment.

Enzyme assay, total organic carbon, total organic nitrogen, C: N ratio, pH, temperature, moisture were recorded.

**Experiment Design**

As it is most convenient to use natural lignocellulosic waste as substrates biological pretreatment in the present study four wastes are easily available namely coconut shredder waste (substrate), substrate + cow dung, substrate + vermicompost, substrate + mushroom spent waste were selected for enzyme production. The design for treatment of lignocellulosic waste into T<sub>1</sub>- control without any treatment (lignin substrate only), T<sub>2</sub>-cow dung slurry +lignin substrate, T<sub>3</sub>- vermicompost + lignin substrate, T<sub>4</sub>- mushroom spent waste + lignin substrate.

before and after treatments (raw and composting material). The following organic carbon values are measured raw and composted material.

Among the treatments, maximum level of organic carbon was estimated in the T<sub>3</sub> using vermicompost (45.35, 40.27).

**Effect of Total Organic Nitrogen (mg/kg)**

In all the four treatments, organic nitrogen was recorded before and after treatments (raw and composting). The following organic nitrogen values are measured raw and composted material.

Among the treatments, maximum level of nitrogen was estimated in the T<sub>3</sub>-using vermicompost (1.17, 0.68).

**Effect of C: N Ratio**

In all treatments, C: N ratio was recorded before and after treatments (raw and composting). The following C: N ratio values are measured raw and composted material.

The reduction of C/N ratio in all types of coconut waste due to reduction of total organic carbon (TOC) via the respiration process in the form of carbon-di-oxide (CO<sub>2</sub>).

Among the treatments, maximum level of C:N ratio was estimated in the T<sub>3</sub>-vermicompost (21.7:1,12.5:1).

**Effect of Enzymes (IU/ML)**

The enzymes were present in all the treatments namely laccase, manganese peroxidase and lignin peroxidase. The following enzyme assay values are measured (IU/ML). Lignin peroxidase, Laccase was present in all the four treatments of lignin degradation. In that enzyme assay, Lignin peroxidase was higher in the treatment T<sub>3</sub> using vermicompost 0.97 IU/ML followed by T<sub>2</sub> 0.83 IU/ML. Manganese peroxidase was present in T<sub>2</sub>, T<sub>3</sub> 1.03 IU/ML and 1.25 IU/ML, and absent in T<sub>1</sub>, T<sub>4</sub>.

**Identification of Microorganisms**

**Identification of Bacteria**

Identification of bacteria from the lignocellulosic waste treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> using substrate, cow dung, vermicompost and mushroom spent waste present in *Enterobacter sp*, *Klebsiella sp*, *Bacillus sp*, *Streptomyces sp*, *Pseudomonas sp* and *Azotobacter sp*.

**Identification of Fungi**

Identification of fungus from the lignocellulosic waste treatments such as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> using by cow dung, vermicompost and mushroom spent waste, such as In the treatments nearly 5 fungal species were isolated and identified namely *Aspergillus sp*, *Rhizopus sp*, *Pleurotus sp*, *Trichoderma sp*, *P. chrysosporium*.

**Percentage of Lignin Degradation**

The complete lignin degradation was observed in T<sub>3</sub> using vermicompost for 90 days of duration. Initial weight of lignocellulosic substrate (30kg lignocellulosic waste +5kg of substrate) was reduced into 15kg of organic manure. Next better degradation of lignin was noticed in T<sub>2</sub> (18 kg). This degraded organic manure is one of the most value added humic products of agriculture, A product or mass is produced which does not decompose or produce offensive odors as readily as the original material (Table-5,). In our study clearly highlighted that maximum level of lignocellulosic coconut waste was effectively degraded nearly 90% by using

vermicompost. This process was also mediated by the microbial population. Degradation of lignin could be beneficial for the sustainable production of organic manure. (Table-5, plate-1).

**Table 5:** Dryweight Measurement of Complete Lignocellulase Degraded Products

Treatment	Initial weight (kg)	Final weight (kg)
T <sub>1</sub>	30	26
T <sub>2</sub>	30+5	18
T <sub>3</sub>	30+5	15
T <sub>4</sub>	30+5	20

T<sub>1</sub> – (30 Kg Lignocellulosic Waste)

T<sub>2</sub> – (30 Kg Lignocellulosic Waste + 5 kg Cow Dung)

T<sub>3</sub> – (30 kg Lignocellulosic Waste + 5 kg Vermicompost)

T<sub>4</sub> – (30 kg Lignocellulosic Waste + 5 kg Mushroom Waste)

**Plate-1 Final Lignin Degradation Product of Lignocellulosic waste from the treatments**



T<sub>1</sub> Lignocellulosic Waste Only



T<sub>2</sub> Lignocellulosic waste + cow dung



T<sub>3</sub> Lignocellulosic waste + Vermicompost



T<sub>4</sub> Lignocellulosic waste + Mushroom spent waste

### Summary and Conclusion

Lignocellulolytic microorganisms have attracted a great deal of interest as potential biomass degraders for large-scale applications due to their ability to produce vast amounts of extracellular lignocellulolytic enzymes. Lignin, the most recalcitrant component of lignocellulosic material, acts as a barrier for any solutions or enzymes by linking to both hemicelluloses and celluloses and prevents penetration of lignocellulolytic enzymes to the interior lignocellulosic structure. The present study was planned to investigate the degradation of lignocellulosic waste materials using cow dung, vermicompost, mushroom spent waste.

The substrates namely cow dung, vermicompost, mushroom spent waste was used for lignin degradation. Lignocellulosic wastes was allowed for complete degradation nearly 90 days. During this degradation period, pH, temperature, moisture, total organic carbon, total organic nitrogen, C: N ratio, enzyme assay was estimated. Microbial population was also calculated. Although final degradation was observed in 90% level in the T<sub>3</sub> using vermicompost followed by T<sub>2</sub> cowdung (75%). Lignin degradation from decayed plants more effectively which in bioconversion of lignocellulosic biomass into other value added end products such as biofuel, sugar productions and management of waste to wealth. The study of lignin degradations using cow dung, vermicompost, and mushroom spent waste could be beneficial for the sustainable production of chemical, materials, fuels for renewable plant sources.

Further investigation is required to identify the novel mechanism involved in degradation treatments and their action, to identify new lignin degrading microbes and through study of their genomic, biochemistry, amino acid, proteomics and reducing the duration of the process, will uncover the role of lignolytic enzymes in the coming year.

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