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Degradation of Lignocellulosic Coconut Waste by Selected Indigenous Microorganisms

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Abstract:- Lignin is regarded as the most plentiful aromatic polymer contains both non-phenolic and phenolic structures. It makes the integral part of secondary wall and plays a significant role in water conduction in vascular plants. The coconut waste were sun dried and made into pieces using mechanical shredder. The dried shredded coconut was subjected to 4 types of treatment namely T_1- Control, T_2- White rot fungi, T_3- *Trichoderma*, T_4- Consortium. Totally 6 different species of lignocelluloses compost bacteria were observed from the lignocelluloses samples in four pits. Among the bacterial species identified *Bacillus* and *Pseudomonas* were predominant in all treatments. The fungal species identified *P.chrysosporium* and *Trichoderma* were predominant in all treatments. Treated compost were tested for the enzyme activity namely lactase, polyphone oxidize, and lignin peroxides. The physicochemical parameters such as carbon, nitrogen source, pH, temperature and moisture were also analyzed. The physic chemical parameters were analyzed at 15 days intervals. Total organic carbon (31.01), organic nitrogen (1.07), C: N ratio was reduced (14.6:1) in T_4 – Consortium. Maximum degradation was recorded in the treatment T_4 followed by T_3. Finished compost obtained after 90 days.

Keywords:- *Lignin degradation; Fungi; Bacteria; Lignocellulolytic enzymes- lignin peroxidase; laccase; polyphenol oxidase; physicochemical parameters.*

I. INTRODUCTION

Lignin is the major no carbohydrate, 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 ($C_{10}H_{12}O_3$) 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, actinomycetes and lesser extent bacteria.

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^{2+} to Mn^{3+} which oxidizes phenol rings to phenoxy radicals leading to decomposition of compounds.

Lignin is the most structurally complex carbohydrate possessing a high molecular weight and the most recalcitrant, consisting of various biologically stable linkages (Perez *et al.*, 2001). The lignocellulose material of plant consists of three main compounds, namely cellulose, hemicellulose and lignin. After cellulose, lignin is the second most abundant renewable biopolymer in nature. It is most abundant aromatic polymer in the biosphere (Rahman *et al.*, 2013). Lignin causes a serious pollution and toxicity problem in aquatic ecosystem owing to its low biodegradability.Lignin is particularly difficult to biodegrade and reduces the bioavailability of the other cell wall constituents. Lignin is the well-known complex substance covalently bound to side chains of xylans of cell-walls. In biological treatment systems, a wide variety of microorganisms including fungi, actinomycetes and bacteria have been implicated in lignin biodegradation and decolourization of pulping effluent. Among them, white rot fungi have received extensive attention due to their powerful lignin-degrading enzymatic systems. The lignin degradation requires mono and dioxygenase enzyme to perform de-methylation cleavage and side chain oxidation. So that cross linkages collapse, complexity is reduced and enzyme accessibility impressed. The degradation products of lignin are vanillin, vanillic acid and vanilly alcohol etc. (William *et al.*, 1986). In the complex lignolytic enzyme system, peroxidases, laccases and H_2O_2 producing oxidases are the most studies enzymes peroxidases and laccase are defined as phenol oxidase (Kuhad, 1997).

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Collection of coconut leaf waste as the source of lignocellulosic substrates.Shredding of the substrate, (make them into process). Leaves are processed into shade for dry. Digging the pits of standard size.Filling the pits with known quantity of shredded lignocellulosic waste.Monitoring the pH, Moisture, Temperature and record the value.Analyzing the C, N, and C: N ratio.Isolating and identifying the efficiency of microbial population for the lignocellulosic waste inoculated pits. Analyzing the enzymes such as laccase, polyphenol oxidase, lignin peroxides.

II. MATERIALS AND METHODS

A. Collection And Preparation of Lignocellulosic Substrate

Coconut dried leaves were collected from the coconut growers around sundarakkottai village, Mannargudi Taluk, Thiruvarur district, Tamilnadu, India. The collected leaves were sun dried and made into pieces using mechanical shredder. The shredder was air dried under shade and weighed.

B. Collection of materials

White rot fungi- *P.chrysosporium*, *Trichoderma*, Consortium (cow dung + vermicompost + mushroom spent) was obtained from the Biominin Laboratories of the S.T.E.T Biofloral products development and Research centre, S.T.E.T women's College. Sundarakkottai, Mannargudi Taluk, Thiruvarur District.

C. Preparation of pits

Pits measuring about 3×2 were dug to a depth of feet.

30 kg of the air dried shredded coconut waste was filled in each pit. Totally four pits were prepared in this way three pits were used for each treatments and replicate.

D. Treatments

- Control without any treatment (lignin substrate)
- White rot Fungi + lignin substrate
- *Trichoderma* + lignin substrate
- Consortium (cow dung + vermicompost + mushroom spent) + lignin substrate

III. ISOLATION AND IDENTIFICATION OF MICROORGANISM FROM THE LIGNIN DEGRADING PITS

A. Serial Dilution (Lozano, 2009).

Microbiological analysis of the lignocellulosic compost was also carried out to analyze the diversity of microbial population. Serial dilution was performed by using the collected lignocellulosic compost to isolate the bacteria. 1gm of lignocellulosic compost was diluted in the tube containing 9 ml of sterile distilled water and mixed thoroughly to make 1:10 dilution (10^{-2}). 1ml of diluted sample was transferred to the next test tube and serially diluted in to the series of test tubes having 9ml of sterile pipettes up to 10^{-7} dilution. The isolated bacterial organisms were identified by Gram's staining, motility and biochemical characteristics. The fungal organisms were identified by Lactophenol cotton blue.

IV. ANALYSES OF PHYSICOCHEMICAL PARAMETERS OF LIGNOCELLULOSIC COMPOST

A. Determination Of Moisture Content (%) (Griffin, 1970)

The moisture content in a lignocellulosic compost 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.

B. Determination Of Ph (Booth Et Al., 1961)

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

C. Determination Of Temperature (Ahmed Et Al., 2003)

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

ORGANIC CONTENT

Organic carbon (%) (Walkey and Black, 1934)

Organic nitrogen (mg/kg) (Subbaiah and Asija, 1956)

C: N Ratio (Trojanowskii, 1980)

D. Microbial Load

A set of serial dilution was made a sample of each is placed into a liquified agar medium, and the medium poured into a Petridish. During solidified, with the fungi and bacteria cells locked inside the medium. Colonies grow with the agar, as well as on top of the agar and below the agar (between the agar and the lower dish). The procedure described above produces a set of pour plates from many dilutions, but spread plates (sample spread on top of solidified agar) can be used also. The agar plate allow accurate counting of the microorganisms.

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The standard formula

$$= \frac{\text{Colony count on an agar plate}}{\text{Total dilution of tube (used to make plate for colony count)} \times \text{amount plated.}}$$

E. Enzyme Assay

Lignin (500 ml) were analyzed for the enzyme activity, especially lignin peroxidase, laccase and polyphone oxidase.

Enzyme activity was measured by using culture supernatant of the microorganisms as enzyme source. Particularly supernatant was obtained by centrifugation of the culture fluid with 8000 rpm for 30 minutes.

F. Lignin Peroxidase (Crawford And Crawford, 1976)

Lignin peroxidase was assayed in an assay mixture of 1 ml phosphate buffer (pH 6.0) in a test tube and 1 ml of H_2O_2 and 0.8 ml of pyrogallol was added and the test tubes were equilibrated at 25°C, 0.2ml of enzyme source was added into the assay mixture and the mixture was incubated at 25°C for 3 min. Then, the optical density (OD) was measured at 460 nm. Control was also maintained with heat killed enzyme. One unit of enzyme activity was expressed as the amount of enzymes required for an increase of 1.0 absorbance unit/ml.

G. LACCASE (Kirk and Farell, 1987)

Laccases was assayed with in an assay mixture consist of 5 ml of sodium phosphate buffer (pH 6.0) containing 1 ml of 2 mm guaiacol into the test tube containing 0.1 ml of enzyme source and measured the absorbance at 470 nm. Unit of enzyme activity was expressed as the amount tetraguaiacol formed min-1 ml-1 of enzyme extract.

H. Polyphenol Oxidase (Mayer Et Al., 1980)

Polyphenol oxidase or catechol oxidase was measured with an assay mixture containing 3.5 ml phosphate buffer (pH 6.0) and 0.5 ml of 2 mm catechol into test tube and 0.5 ml of enzyme source was added into the mixture. An absorbance change was measured at 475 nm. 1minute of enzyme activity was calculated as change in absorbance by 0.001min-1 of culture filtered.

V. RESULT

➢ *Isolation of Microorganism From The Lignin Degrading Pits*

A. Serial Dilution Technique

Serial dilution technique was used to isolate the bacteria and fungi. Gram staining, motility test and biochemical tests were Indole, MR- VP, Citrate utilization test, Oxidase test, Catalase test. Lactophenol cotton blue technique was used to identify the fungi.

B. Isolation of bacteria

Six different bacterial species were observed from four compost pits. The bacterial species were identified based on their morphological and biochemical characteristics Bergey's manual of determinative using bacteriology. The bacterial species were namely *Bacillus sp* and *Pseudomonas sp Enterobactersp, Azotobacter sp, Streptomyces sp, Klebsiella sp.* The bacterial species were *Bacillus sp* and *Pseudomonas sp* predominant in lignocellulosic compost. The fungal species were *Trichoderma, Pleurotus, Aspergillus, Rhizopus* and *P.chrysosporium* identified.

C. Physicochemical Parameters

The physical parameter includes pH, moisture content and temperature, and enzyme assay were also analyzed. The chemical parameters include carbon, nitrogen, C: N ratio.

D. Moisture

The present investigation revealed that there was high value of moisture contents were reported. The maximum moisture content was recorded in T_4 (49%) at 45-60 days interval. Then it was followed by T_3 and T_2 (42 % and 41 %) respectively. The minimum values were recorded in T_1 - 39 % (Table- 1).

E. pH

Among the pH ranges observed from the four pits, the pH values was recorded every 15 days interval. The maximum pH was recorded in T_4 (9.1) at 15-30 days interval. Then it is followed by T_2 and T_3 (8.0and 7.4) respectively. The minimum values were recorded in T_1-6.0 (Table-1).

F. Temperature

In present investigation the temperature were recorded in four pits. The temperature values were recorded every 15 days. The maximum temperature was recorded in T_4 (40.0° C) at 30-45 days interval. Then it is followed by T_3 and T_2 (37.0° C and 39.0° C) respectively. The minimum values were recorded in T_1 – 31.5°C (Table-1).

➢ *Chemical Parameters*

A. Estimation of Organic Carbon (%)

The availability of organic carbon was estimated by using Walkely and Black method. The total organic carbon was recorded in four pits. The values of raw carbon and composted values were 52.05 and 31.01 respectively. The reduced level of carbon content was observed in T_4- (31.0) (Table-2).

B. Estimation of Organic Nitrogen (Mg/Kg)

The availability of organic nitrogen was estimated by Subbaiah and Asija method. The total nitrogen was recorded

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in four pits. The values of raw nitrogen and composted values were 5.05 and 1.07 respectively. The reduced level of nitrogen content was present in T_4- (1.07) (Table-2).

C. Estimation of C: N ratio

In the investigation of C: N ratio was analyzed in compost pit. The C: N - raw 32.9:1 and C: N compost was ratio of 14.6:1. The reduced level of C: N ratio was observed in T_4 - 14.6:1 (Table-2).

D. Microbial Load

The samples were subjected to serial dilution and the viable count was made in the plate counting techniques. The microbial loads obtained were enumerated from four pits. Colonies were counted particularly in the dilution rate of 10^{-4}-10^{-7}. The number of colonies of (T_4) 22×10^{-4} CFU/ml, (T_2) 84×10^{-5} CFU/ml was recorded respectively (Figure 1 and 2).

E. Enzyme Assay

The present investigation revealed that there was high enzymatic activity were observed in four pits. The high concentration of lignin peroxidase enzymes (T_4 - 4.03±4.91IU ml), followed by laccase enzyme production observed in (T_3 - 3.64±3.01 IU/ml) and polyphenol oxidase (T_4 - 1.83±3.08IU/ml).

VI. DISCUSSION

Sasikumar*et al.*, 2014 reported that, totally 9 isolates of lignin degrading microbes were isolated using minimal salt media containing lignin (MSM-L) and lignolytic activities were preliminary screened by testing against methylene blue indicator dye containing LB medium. Eight microbial cultures showed positive results and the predominant isolates were identified as *Pseudomonas sp.* Lignin degrading bacteria, *Bacillus, Pseudomonas, Enterobacter, Azotobacter, Streptomyces, and Klebsiella,* were isolated in the lignin degrading pits.

Waksman et al., 1939, studied the degradation capacity of some microorganisms isolated from compost. Two thermophilic actinomycetes isolates degraded 0.7±2.5% of the lignin in 42 days at 50 ^oC, the thermophilic fungus , Thermomyces lanuginosus 4.2%, but neither of lignin degradation alone. In our study was correlated that T_2 and T_3 process by using Trichoderma sp and White rot fungi (Bacillus sp and Pseudomonas sp).However, the natural microbial population in manure degraded 11.5% of the lignin. The natural population also decomposed 62% of the total dry material as composed to 40% for the fungus (Waksman et al., 1939). In our study was reported that the Consortium was degraded in 90% of the total dry material as composed to white rot fungi and Trichoderma sp.

Dick, 1994, reported that these reductions indicate depressed microbial activity due partially to the scarce available C and N sources after the vermicomposting period as well as to the partial drying over this phase. It is well known that drying depresses microbial activity, particularly the metabolic activity of bacteria, also including the inactivation of extracellular hydrolytic enzymes.

A. Final Lignin Degradation Product of Lignocellulosic Waste From The Treatments

Factor	T_1	T_2	T_3	T_4
Moisture (%)	35	36	38	42
pH	6.8	6.0	6.9	7.0
Temperature (^oC)	31.5	35.5	28.5	36.5

Table 1. Physical properties of lignocellulosic compost in 90 days

T1- Control, T2 - White rot Fungi, T3 – *Trichoderma*, T4 – Consortium

Determination of C, N, C: N Ratio of raw and composted coconut waste

Determination	Percentage or Ratio			
	T_1	T_2	T_3	T_4
Carbon- raw	48.35	50.01	51.01	52.05
Carbon-composted	38.35	38.23	35.07	31.01
Nitrogen-raw	3.41	3.95	4.35	5.05
Nitrogen-Composted	3.21	2.48	2.08	1.07
	23.5 :	26.1 :	29.2 : 3	32.9 : 1

C : N –raw C : N - Composted	1 19.5 : 1	1 17.1: 1	16.5 : 5	14.6 : 1

Table 2. Chemical properties of lignocellulosic compost

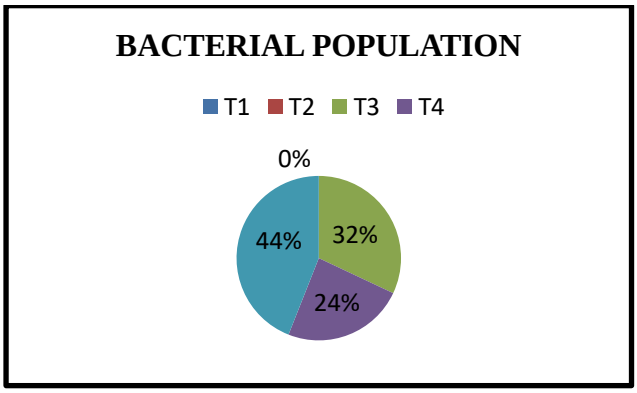

Fig 1:- Number of Bacterial Population in Lignocellulosic Compost

T1- Control, T2 - White rot Fungi, T3 – *Trichoderma*, T4 – Consortium

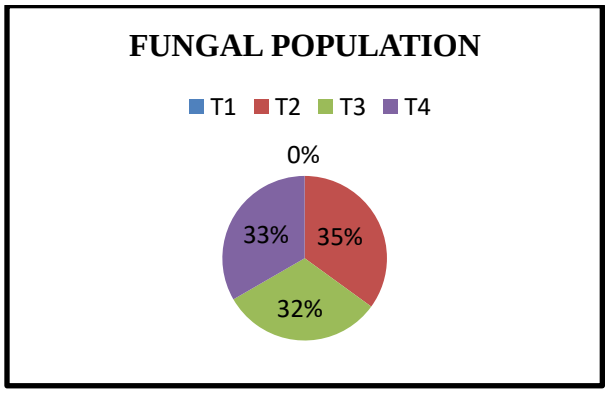

Fig 2:- Number of Fungal Population in Lignocellulosic Compost

T1- Control, T2 - White rot Fungi, T3 – *Trichoderma*, T4 – Consortium

VII. CONCLUSION

Totally 6 different species of lignocellulosic compost bacteria were observed from the lignocellulosic samples in four pits namely T_1- Control, T_2- White rot fungi, T_3- Trichoderma, T_4- Consortium. The identified bacteria namely Bacillus sp, Pseudomonas sp, Enterobactersp, Azotobactersp, Streptomyces sp, and Klebsiella sp.Among the bacterial species identified Bacillus and Pseudomonas were predominant in all treatments, fungi were observed from the lignocellulosic compost samples collected from the four pits. The fungi identified namely Trichodermasp, Pleurotussp, Aspergillussp, Rhizopussp, P.chrysosporium sp. Among the fungal species identified P.chrysosporiumand Trichodermawere predominant in all treatments.

Lignocelluloses materials including forestry, agricultural and agro industrial wastes contain several high value substances such as sugars, minerals and protein. Disposal of these wastes to the soil or landfill causes serious environmental problems, besides to constitute loss of these value added substances. Therefore, the development of processes for reuse of these wastes is of great interest. Since these wastes are rich in sugars, which are easily assimilated by microorganisms, they are very appropriate for used as degradation.

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