

## Effect of Various Concentrations of Lignin Degrading Microbes for Efficient Coir Pith Treatment

Priya V\*, Sampath Kumar MC and Balasubramanya N

Department of Civil Engineering, Adhiyamaan College of Engineering, Tamil Nadu, India

\*Corresponding author: Priya V, Department of Civil Engineering, Adhiyamaan College of Engineering, Hosur 635109, Tamil Nadu, India, Tel: 04344260570; E-mail: vaishudurga@yahoo.com

Received: April 19, 2017; Accepted: April 29, 2017; Published: May 01, 2017

### Abstract

Coir pith as a by-product, remains in the soil for long time and results in the ground water pollution and erode the soil completely due to the leaching during Monsoon times. This implies the importance of lignin degradation in the coir pith using microbes at various concentration to increase the productivity of enzymes in a short duration of time. Enzymes are unstable proteins which promote chemical reaction in a process. Lignin is one of the major classes of compound that are present in the coir pith. Increased lignin content in the coir pith makes its natural degradation much slower due to the lignin-cellulose complex. It is very fortunate to imply the use of microbes in the degradation process. Lignin degrading enzymes released as a result of microbial action in the coir pith. The enzymes act as catalysts which are responsible for decomposition. This study helps in the detection of major enzymes as a result of lignin degradation ending in the usage of risk free, pollution free coir pith. This study aimed at the lignin degradation for the detection of lignin degrading enzymes using the fungi such as *Phanerochaete* and *Trichoderma viride*. The inoculum concentration of the microbe added in the coir pith was compared for the efficiency and the best treatment with the concentration of microbe was recorded. At the end of the research study, the higher concentration of the microbe produced higher enzyme release in shorter duration. *Clostridium* treated coir pith was better performing at all the concentrations than Rock phosphate.

**Keywords:** Coir pith; Degradation; Enzymes; Concentration; *Phanerochaete*; *Trichoderma viride*

### Introduction

#### Composting

Composting is the biological decomposition and stabilization of organic substrates to produce a final stable product under certain temperatures. Composting process consists of 3 major steps: pretreatment, hydrolysis, and fermentation [1]. The purpose of the pretreatment is to remove lignin and hemicelluloses content for the elimination of leaching process that leads to the soil pollution and ground water depletion. The pretreated material is followed by enzymatic hydrolysis by the use of microbes which boosts up the lignin degradation with the help of catalysts for the release of enzymes [2].

### **Coir pith deposition**

Coir pith as an end product of Coir fiber extraction is characterized as a light, spongy material which has higher water absorbing capacity. It will be deposited on the land usually which can degrade by itself but takes an ample of time, and always remains in the soil without properly getting degraded and causes the formation of leachate and further results in the land pollution [3]. Its accumulation during rainy seasons results in the leaching and higher amount of Polyphenols release that contaminates both the surface water and ground water. In order to eliminate the consequences, biological management practices should be adopted to avoid the pollution risks [4].

## **Materials and Methods**

### **Collection of coir pith**

The coir pith for the treatment was procured from an industrial area TANCI, Krishnagiri.

### **Inoculum preparation**

The microbes such as *Phanerochaete chrysosporium* and *Trichoderma viride* were purchased from MTCC and sub-cultured for its maintenance. The microbes were inoculated on potato dextrose agar (PDA) and incubated for their growth. They were sub-cultured every 15 days. The confirmation of the microbes was analyzed by microscopical analysis [5].

### **Pre-treatment trials**

The collected coir pith was treated with Rock phosphate and *Clostridium perfringens* earlier in order to accelerate the electrical conductivity reduction using organic method and biological method. For the faster degradation, the coir pith was subjected to delignification process using the fungal organisms such as *Phanerochaete chrysosporium* and *Trichoderma viride* at various concentrations such as 0.1%, 0.2%, 0.3%, 0.4%, 0.5% [6]. The organisms at the concentrated form perform well and speed up the degradation of coir pith easier and quicker. The parameters were observed after a period of 30 day [6].

### **Delignification enzymes detection**

The specific lignin degrading enzymes for the cause of delignification were measured. The efficiency of the treated coir pith and the individual ability of each fungal organism were recorded. The enzymes, namely Cellulose, Protease, lignin peroxidases, xylanases and Laccases [5] were detected and tabulated for a period of 30 days.

## **Results and Discussion**

### **Confirmation of fungal organisms**

Based on the individual characteristics and the microscopic analysis confirmation, the fungal organisms were classified and identified. *Trichoderma viride* has well defined conidiophore structure and has repetitive branches whereas *Phanerochaete chrysosporium* is thin walled and infrequently branched. It has hyphae leading to formation of conidia.

### **Primitive tests**

The basic parameters such as pH, EC, TDS were analyzed prior the enzymes detection in order to prove that the microbes did not influence the coir pith's physical parameters (TABLES 1-27).

TABLE 1. Primitive tests.

Treatment	pH	EC ( $\mu$ S)	TDS (ppm)
<b>Rock Phosphate Treated</b>			
<i>Phanerochaete chrysosporium</i>	6.67	872	551
<i>Trichoderma viride</i>	6.89	841	520
<b><i>Clostridium perfringens</i> Treated</b>			
<i>Phanerochaete chrysosporium</i>	6.48	771	509
<i>Trichoderma viride</i>	6.40	750	521
Control	6.52	991	695

It was examined that the lignin degrading fungal organisms did not pose any serious threat to the physical characteristics of coir pith. Increase in EC or TDS coir pith leads to altering of chelating properties, polyphenol exudation, pH alteration that results in the chemical composition imbalance in coir pith.

TABLE 2. Cellulase activity in treated coir pith at 0.1%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.003	0.012	0.003	0.04	0.56
5	0.019	0.061	0.012	0.13	
10	0.095	0.16	0.065	0.19	
15	0.251	0.54	0.11	0.23	
20	0.271	0.76	0.147	0.31	
25	0.35	1.65	0.178	0.34	
30	0.391	2.78	0.189	0.387	
35	0.476	3.41	0.21	0.45	
40	0.61	4.21	0.23	0.51	

TABLE 3. Cellulase activity in treated coir pith at 0.2%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.008	0.07	0.006	0.09	0.56
5	0.19	0.67	0.072	0.19	
10	0.38	2.09	0.15	0.27	
15	0.51	2.87	0.22	0.33	
20	0.59	3.26	0.256	0.41	
25	0.65	3.69	0.28	0.58	
30	0.71	4.41	0.38	0.67	

TABLE 4. Cellulase activity in treated coir pith at 0.3%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.015	0.078	0.012	0.091	0.56
5	0.206	0.72	0.15	0.61	
10	0.391	2.11	0.23	1.38	
15	0.59	2.99	0.46	1.96	
20	0.781	3.45	0.59	2.12	
24	0.895	4.89	0.63	2.33	

TABLE 5. Cellulase activity in treated coir pith at 0.4%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.23	0.096	0.023	0.16	0.56
5	0.267	0.81	0.27	0.69	
10	0.403	2.43	0.37	1.97	
15	0.769	3.24	0.67	2.26	
20	1.09	5.26	0.87	2.54	

TABLE 6. Cellulase activity in treated coir pith at 0.5%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.33	0.12	0.078	0.25	0.56
5	0.41	1.33	0.55	1.49	
10	0.478	3.99	0.71	2.89	
14	1.78	6.22	1.13	3.09	

## Enzymatic Analysis

### Cellulase activity

The fungus *R. stolonifer* and *P. chrysosporium* produced near to the value of coculture at 28 days of fermentation [7]. Also, the coculture showed maximum cellulase enzyme activities on the 28 days of incubation while the activities of *R. stolonifer* and *P. chrysosporium* cellulase enzymes were observed maximally on the same day of fermentation. They also reported that the higher amount of cellulase and xylanase enzyme production by *Aspergillus terreus* during the course of solid state fermentation.

In this research study, cellulase enzyme was detected at the regular interval of time for a period of 40 days at various concentrations such as 0.1% to 0.5% to know the efficiency of the microbe degrading the coir pith when inoculated with *Phanerochaete* and *Trichoderma viride*. It was concluded that the cellulase enzyme measured was higher in *T. viride* than

*Phanerochaete* in the Rock phosphate treated coir compared to *Clostridium perfringens* treated. As the microbe concentration increased, the cellulose enzyme release also increased showing the capability of microbe acting with coir pith effectively.

TABLE 7. Laccase activity in treated coir pith at 0.1%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.004	0.004	0.004	0.008	0.054
5	0.017	0.012	0.016	0.012	
10	0.034	0.02	0.024	0.026	
15	0.042	0.029	0.036	0.031	
20	0.065	0.037	0.038	0.056	
25	0.079	0.046	0.041	0.076	
30	0.088	0.049	0.046	0.083	
35	0.099	0.053	0.048	0.091	
40	0.109	0.059	0.053	0.16	

TABLE 8. Laccase activity in treated coir pith at 0.2%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.008	0.007	0.006	0.011	0.054
5	0.028	0.018	0.023	0.017	
10	0.041	0.029	0.031	0.039	
15	0.065	0.032	0.037	0.087	
20	0.098	0.049	0.41	0.156	
25	0.17	0.053	0.049	0.184	
30	0.23	0.064	0.062	0.22	

TABLE 9. Laccase activity in treated coir pith at 0.3%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.015	0.016	0.012	0.018	0.054
5	0.038	0.029	0.023	0.037	
10	0.178	0.041	0.038	0.156	
15	0.254	0.061	0.057	0.243	
20	0.351	0.069	0.061	0.36	
24	0.39	0.078	0.075	0.374	

TABLE 10. Laccase activity in treated coir pith at 0.4%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.154	0.034	0.032	0.142	0.054
5	0.204	0.071	0.068	0.197	
10	0.278	0.079	0.074	0.254	
15	0.354	0.093	0.09	0.368	
20	0.476	0.12	0.113	0.451	

TABLE 11. Laccase activity in treated coir pith at 0.5%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.189	0.073	0.069	0.171	0.054
5	0.27	0.088	0.082	0.251	
10	0.39	0.152	0.123	0.387	
14	0.506	0.19	0.16	0.497	

### Laccase activity

Initiation of depolymerization of lignin is usually posed by the multinuclear enzyme called Laccases. In the study conducted by [7], the laccase activity increased gradually during fermentation and the maximum activity (5.1 IU/ml) was found to be in 28<sup>th</sup> days of fermentation carried out by co culture. Here, the laccases were maximum degraded by *T. viride* in *Clostridium perfringens* treated coir pith at 0.5% and showed higher rate of laccase enzyme release than the Rock phosphate treated coir that increased gradually from 0.1%. *Phanerochaete chrysosporium*, showed better performance with Rock phosphate as treatment than *Clostridium perfringens* treated coir pith.

TABLE 12. Lignin peroxidase activity in treated coir pith at 0.1%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	1.22	11.34	0.34	-0.002	2.6
5	1.67	12.72	1.65	-0.007	
10	2.89	14.29	2.09	-0.011	
15	3.11	14.76	3.18	-0.021	
20	4.21	15.33	4.25	-0.022	
25	4.86	18.34	4.77	-0.027	
30	5.17	22.7	5.19	-0.033	
35	6.36	25.11	6.88	-0.039	
40	7.1	27.5	7.41	-0.088	

TABLE 13. Lignin peroxidase activity in treated coir pith at 0.2%.

Day	Activity (mg/ml)				
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		Control
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	1.45	16.7	0.98	-0.005	2.6
5	3.06	24.98	3.78	-0.015	
10	5.32	26.87	5.12	-0.019	
15	6.94	27.12	5.96	-0.023	
20	7.87	29.82	7.04	-0.022	
25	8.39	31.45	8.76	-0.026	
30	8.8	33.4	9.2	0.002	

TABLE 14. Lignin peroxidase activity in treated coir pith at 0.3%.

Day	Activity (mg/ml)				
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		Control
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	1.61	18.11	1.44	-0.009	2.6
5	3.65	25.07	4.02	-0.028	
10	5.89	28.98	5.67	-0.099	
15	7.11	30.61	6.39	0.012	
20	9.33	33.65	8.88	0.045	
24	10.103	37.23	9.77	0.076	

TABLE 15. Lignin peroxidase activity in treated coir pith at 0.4%.

Day	Activity (mg/ml)				
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		Control
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	1.99	19.33	2.01	-0.012	2.6
5	3.78	26.09	4.77	0.024	
10	6.71	31.65	6.99	0.131	
15	8.65	34.89	7.09	0.198	
20	12.93	39.41	10.21	0.209	

TABLE 16. Lignin peroxidase activity in treated coir pith at 0.5%.

Day	Activity (mg/ml)				
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		Control
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	2.98	20.11	2.99	-0.023	2.6
5	4.55	25.72	5.44	0.045	
10	6.61	36.88	7.91	0.187	
14	13.45	41.90	11.34	0.226	

### Lignin peroxidase activity

Lignin peroxidase is an extracellular enzyme which plays a key role in breaking of lignin molecules by the process of lignin degradation. During the research study conducted by Kanmani et al. [7], almost all the selected fungi could produce significant level of lignin peroxidase (LiP) during the fermentation period which was comparatively higher than laccase activity.

Maximum amount of Lip activity (8.1 IU/ml) was observed on the 28<sup>th</sup> day of fermentation by using co culture method. But *R. stolonifer* produced very low level of activity (3.5 IU/ml) on the same fermentation period. In this study, the LiP activity was produced in higher amount than the other lignin degrading enzymes.

The best LiP activity was in the Rock phosphate treated coir pith with 0.5% *T. viride* which almost shown complete degradation of coir pith by the release of enzyme whereas in the *Clostridium* treated coir pith, slower degradation activity was observed gradually from 0.1% to 0.5%.

TABLE 17. Protease activity in treated coir pith at 0.1%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.76	1.11	1.12	1.54	0.56
5	1.13	2.33	2.45	2.97	
10	1.78	2.87	3.1	3.66	
15	2.11	3.41	3.75	3.98	
20	2.65	3.76	4.11	4.14	
25	3.07	4.12	4.53	4.51	
30	3.42	4.51	4.87	4.77	
35	3.78	4.89	5.08	5.02	
40	4.34	5.44	5.27	5.48	

TABLE 18. Protease activity in treated coir pith at 0.2%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	1.04	1.63	1.36	2.31	0.56
5	3.22	3.05	3.03	4.02	
10	3.76	3.65	3.98	4.49	
15	4.09	4.17	4.56	5.27	
20	4.73	5.89	5.2	5.34	
25	4.95	6.12	5.78	5.94	
30	5.1	6.3	6	6.35	



TABLE 19. Protease activity in treated coir pith at 0.3%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	1.16	2.02	1.45	2.76	0.56
5	2.49	2.9	3.11	3.67	
10	3.92	3.37	3.96	4.85	
15	4.35	4.02	4.19	5.23	
20	4.90	5.43	5.08	5.79	
24	5.33	6.66	6.22	6.71	

TABLE 20. Protease activity in treated coir pith at 0.4%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	1.31	2.33	1.88	2.77	0.56
5	2.53	2.81	2.94	3.81	
10	4.02	4.92	4.03	4.76	
15	4.78	6.22	5.55	6.05	
20	5.87	7.03	6.93	7.12	

TABLE 21. Protease activity in treated coir pith at 0.5%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	1.56	2.45	2.03	3.01	0.56
5	3.71	3.71	4.12	4.18	
10	5.01	6.03	6.09	6.12	
14	6.03	7.55	7.21	7.42	

### Protease activity

Several hypotheses in the role of proteases in wood rotting fungi. They indicated their possible implication in the release of lignolytic enzymes from the fungal cell wall [6]. One of the functions of the proteases produced by white rot fungi is to recycle nitrogen by break down of proteins released into the medium for cell autolysis [4]. Proteases enzyme at the end of lignin degradation are responsible for breaking down of peptide bonds during hydrolysis process. The efficiency of protease individually at all the concentrations was very well proven in all the treatments. Highest protease activity was measured in both the fungal degrading organisms which helped in lignin degradation in 14 days at 0.5% concentration and 40 days at 0.1%.

TABLE 22. Xylanase activity in treated coir pith at 0.1%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.043	1.06	0.47	2.76	0.56
5	0.126	1.79	1.65	3.01	
10	0.398	2.32	2.03	3.43	
15	0.92	2.8	2.65	3.91	
20	1.2	3.09	2.89	4.12	
25	1.51	3.54	3.22	4.87	
30	1.62	3.98	3.74	5.33	
35	1.76	4.43	4.03	6.01	
40	1.84	4.76	4.29	6.83	

TABLE 23. Xylanase activity in treated coir pith at 0.2%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.086	1.32	0.89	3.22	0.56
5	0.553	2.21	1.16	4.95	
10	1.34	2.71	1.92	5.24	
15	1.7	3.86	2.45	6.54	
20	1.89	4.09	3.28	6.78	
25	1.99	5.02	3.95	7.11	
30	2.12	5.32	4.61	7.21	

TABLE 24. Xylanase activity in treated coir pith at 0.3%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.276	1.91	1.91	3.89	0.56
5	0.561	3.49	3.14	4.21	
10	0.791	4.1	3.87	6.19	
15	1.32	4.56	4.07	6.81	
20	1.87	5.31	4.4	7.21	
24	2.43	5.89	4.98	7.78	

TABLE 25. Xylanase activity in treated coir pith at 0.4%.

Day	Activity (mg/ml)				Control
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	0.769	2.75	2.65	5.31	0.56
5	1.31	4.97	4.23	7.22	
10	1.56	5.59	4.76	7.77	

15	2.05	6.01	5.13	8.21	
20	2.9	6.17	5.78	8.45	

TABLE 26. Xylanase activity in treated coir pith at 0.5%.

Day	Activity (mg/ml)				
	Trial 1 (Rock Phosphate Treated)		Trial 2 ( <i>Clostridium perfringens</i> Treated)		Control
	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	<i>Phanerochaete</i>	<i>Trichoderma viride</i>	
1	1.63	2.75	2.65	5.31	0.56
5	2.05	5.59	4.76	7.77	
10	2.88		5.13	8.21	
14	3.26	6.89	6.15	8.9	

### Xylanase activity

The coir waste was used as lignocellulose materials for bioconversion. During the bioconversion, hemicellulose was effectively degraded by producing the enzyme xylanase [7]. The increased amount of xylanase (16.4 IU/ml) was recorded after 28 days of incubation period. Xylanases are involved in the breakdown of lignin cell wall for the release of the enzymes. In this study, highest activity was recorded in *Clostridium* treated coir pith showed quick degradation using *T. viride* in 14 days at 0.5% concentration when compared to *Phanerochaete* and rock phosphate treated coir pith. Overall, the activity was gradually increased from 0.1% to 0.5%.

TABLE 27. Lignin content in treated and untreated coir pith.

Treatment	Total Lignin (mg/g)					
Rock Phosphate Treated Initial	0.1% - 41 <sup>st</sup> day	0.2% - 31 <sup>st</sup> day	0.3% - 25 <sup>th</sup> day	0.4% - 21 <sup>st</sup> day	0.5% - 15 <sup>th</sup> day	
<i>Phanerochaete chrysosporium</i>	1506.3	146.3	100.3	95.1	91.2	81.2
<i>Trichoderma viride</i>		144.3	99.6	90.2	88.4	85.6
<i>Clostridium perfringens</i> Treated						
<i>Phanerochaete chrysosporium</i>	1506.3	130.2	81.3	75.1	68.2	50.1
<i>Trichoderma viride</i>		137.9	85.1	79.6	72.1	53.2

### Estimation of lignin in the degraded coir pith

By the action of *Pleurotus sajor* Caju the amount of lignin could be reduced from 32% to 20% [2]. It is also seen that the action of *Pleurotus sajor* Caju on the washed sample of coir pith is more than that on the unwashed sample. This can be explained by the fact that washing exposes the cell walls thus increasing the surface area for decomposition. In this study, the lignin content was degraded using the lignin degrading microbes at various concentrations and recorded that the higher concentration of the lignin degrading microbes such as *Phanerochaete* and *Trichoderma viride* helped in the faster reduction of lignin content in the collected coir pith. On higher concentration, the microbe tends to act faster in the coir by using up all the lignin and produces enzymes.

## Conclusion

It is evident that the coir pith can be converted to effective manure for agriculture in a shorter period of time when higher concentration of microbial inoculum was used. The action of microbe in the treated coir pith was very active at all the concentrations that showed a remarkable reduction in the lignin content of coir pith after treatment with lignin degrading microbes. This is an alternative method for solving the serious problem of coir pith from causing pollution.

## REFERENCES

1. Keay L, Wildi BS. Proteases of the genus *Bacillus*. I. Neutralproteases. *Biotechnol Bioeng*. 1970;12(2):179-212.
2. Reghuvaran A, Ravindranath AD. Biochemical aspects and formation of phenolic compounds by coir pith degraded by *Pleurotus sajor caju*. *J Toxicol Env Heal Sci*. 2012;4(1):29-36.
3. TAPPI T 222 OM-02. Acid-insoluble lignin in wood and pulp, In: 2002-2003 TAPPI Test Methods. Atlanta: Tappi Press, USA; 2002.
4. Dosoretz CG, Dass SB, Reddy CA, et al. Protease mediated degradation of lignin peroxidase in liquid cultures of phanerochaete chrysosporium. *Appl Environ Microbial*. 1990;56(2):3429-34.
5. Nidadavolu H, Ram PM SVSSSL, Maringanti SC, et al. Bioactive compounds from discarded mushroom beds. *Turk J Biol*. 2012;36(3):275-82.
6. Eriksson KE, Petterson B. Purification and partial characterization of two acidic proteases from the white rot fungus *Sporotrichum pulverulentum*. *Eur J Biochem*. 1982;124(3):635-42.
7. Kanmani P, Karuppasamy P, Pothiraj C, et al. Studies on lignocellulose biodegradation of coir waste in solid state fermentation using *Phanerocheate chrysosporium* and *Rhizopus stolonifer*. *Afr J Biotechnol*. 2009;8(24):6880-7.