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Full Length Research Paper

Biochemical aspects and formation of phenolic compounds by coir pith degraded by *Pleurotus sajor caju*

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Coir pith is a byproduct of fibre extraction from coconut husk. Coir pith has been produced in large quantities and is dumped as waste as its bulk at the production site itself and disposal efforts are in vain owing to the quantity. Several biological, biochemical and microbial methods have been tried and are underway to degrade the coir pith into useful product. A basidiomycete fungus *viz*. *Pleurotus sajor caju*, has the ability to slowly degrade the coir pith and is capable of detoxifying phenolic compounds by producing biopolymerizing enzymes. The present work is targeted to degrade the biochemical constituent present in coir pith includes lignin which is considered as recalcitrant under normal conditions and the production of phenolic compounds which are the break down products of lignin. The enhancement of Nitrogen, phosphorous and potassium (NPK) also shows the enrichment of the compost. Biodegraded product can be used as efficient organic manure and as hydroponic systems for growing roses and vegetables.

Key words: Coir pith, lignin, biodegradation, basidiomycete.

INTRODUCTION

Coconut (*Cocos nucifera*) is cultivated in tropical countries. The fibrous mesocarp of coir is used to make ropes. The waste of coir yarn industry (coir pith) gets accumulated in large quantities making their disposal difficult, though it is used as soil conditioner (Christopher et al., 2007). India is the leading producers of coconut. It is an important oil seed and cash crop grown in south Indian states especially in Kerala, Tamil Nadu and Karnataka. About 7.5× 10^5 tons of coir pith is produced annually in India (Pillai et al., 1952). It is available either from retted or unretted processing industries of coir fiber, where for every ton of fiber extracted, the coir dust is produced to the extent of 2 tons. Coir pith constitutes as much as 70% of the coconut husk. It is estimated that, at present there is an accumulated stock of $10×10^6$ metric tons of coir pith in the southern states of India (Ghosh et al., 2007). Fiber extracted from husk is used in production of mats, matting, rubberized coir mattresses, yarn, ropes etc. After extraction of coir fiber from husk, the, coir pith is unutilized (Photograph 1 and 2).

Coir pith is known as coir dust and is the major by-product of the coir fiber extraction industries. It is a ligocellulosic waste material consists of lignin 20 to 40%, cellulose 40 to 50%, hemicelulose 15 to 35% and protein 2.04% (Sjostrom, 1993). It has a high water holding capacity of 8 times its weight. It is a fluffy, light, spongy material with increased water-holding capacity and extremely compressive and has a sizable percentage of combustible matter along with low ash content. It is essentially a lignocellulosic material that decomposes very slowly in soil, because its pentosan/lignin ratio is 1:0.30; the minimum required for moderately fast decomposition in the soil is 1:0.50.(Ghosh et al., 2007). It should be noted that coir pith is resistant to

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Photograph 1. Accumulation of coir pith on coir fiber extraction industries.

Photograph 2. Collection of sample by the author.

biodegradation due to the presence of lignin (33 to 35%). Lignins constitute the second most abundant group of biopolymers in the biosphere. It is estimated that the planet currently contains 3×10^{11} metric tons of lignin with an annual biosynthetic rate of approximately 2×10^{10} tons (Argyropoulos and Menachem, 1997). It is an aromatic polymer composed of phenyl propane subunits including coumaryl, guaicyl and syringyl moeities that are covalently linked together by a variety of bonds, mainly β-aryl ether bonds. It is also present in the fiber and is responsible for the stiffness of coir. Oyster mushroom belonging to *Pleurotus* species has the ability to degrade lignin slowly under favorable conditions. This is reason for the selection of a suitable species of basidiomycetes fungus called *Pleurotus sajor caju*, which has the ability to slowly degrade. The cellulosic compounds present in the coir waste support the initial growth of this fungus and acts as co-substrate for lignin degradation.

The lignin degradation in nature has been considered to occur by the action of wood rot fungi mostly of the basidiomycete class (Odier et al., 1981). Most of the mushroom species have bee recognized for their property of degradation of the natural lignocellulosic wastes, in their original form or preformed (composted) form (Somasundaram Rajarathnam et al., 1998). White rot fungi, which have lignocelluloses degrading enzymes, play important roles in carbon recycling in nature, because lignin, next to cellulose is the second most abundant organic compound on earth (Kanmani et al., 2009). It is the most efficient lignin degraders, due to their ligninolytic system which is comprised of manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase (Hatakka, 1994; Higuchi, 2004). It is due to their powerful degrading capabilities towards varius recalcitrant chemicals, white rot fungi and their lignin degrading enzymes have long been studied for biotechnical applications such as bioleaching (Takano et al., 2001). The characteristic feature of *Pleurotus sajor caju* is that they contain higher protein content (28.03%), compared to common vegetables. It contains all the amino acids essential for human nutrition found in mushrooms and especially lycine, threonine and tryptophan which are present in appreciable amount. It degrades the phenolic group, as observed by the decrease in methoxy content, they also cause an oxidative shortening of side chain. Cleavage of the ring proceeds while still attached to the polymer. Enzymes such as laccase, phenol oxidases are also involved in the process of lignin degradation. It has drawn considerable attention as an appropriate host for the production of lignin degrading enzymes or direct application in lignocelluloses bioconversion processes (Ruggeri and Sassi, 2003; Bosco et al., 1999). White rot fungi in partilular, having great importances due to their potential use of their enzymes for bioremediation, industrial and biotechnological applications (Jordaan et al., 2004; Novotny et al., 2004).

Studies of Lignin biodegradation are also of great importance for possible biotechnological applications, since lignin polymers are a major obstacle to the efficient utilization of lignocellulosic material in a wide range of industrial processes (Eggert et al., 1996). Lignin in favorable conditions degraded to form phenolic components such as resorcinol, guaicol and catechol. Resorcinol otherwise called resorcinol, which is a chemical compound from the dihydroxy phenols. It is the 1, 3- isomer of benzenediol with the formula C6H4 $(OH)_2$. Resorcinol crystallizes from benzene as colorless needles which are readily soluble in water, alcohol and ether, but insoluble in chloroform and carbon disulfide. IUPAC name of Guaicol is 2-methoxy phenol having the molecular formula $C_7H_8O_2$, which is a naturally occurring organic compound with the formula C_6H_4 (OH)(OCH_3). This colourless aromatic oil is derived from guaiacum or wood creosote. Samples darken upon exposure to air and light. Guaiacol is present in wood smoke, resulting from the pyrolysis of lignin. Catechol**,** formerly known as pyrocatechol, or 1, 2-dihydroxybenzene, is an organic compound with the semi-empirical formula C_6H_4 $(OH)_2$. It is the *ortho* isomer, one of three isomeric benzenediols. This colourless compound occurs naturally in trace amounts. About 20 M kg are produced annually, mainly as a precursor to pesticides, flavors and fragrances.

MATERIALS AND METHODS

Coir pith collected from the fibre extracted units in areas of Cherthala, Alappuzha district in Kerala was used in the study. The *Pleurotus sajor caju* procured from the microbiology division of central coir research institute (CCRI), Alappuzha. The experimental protocol and biochemical analysis of coir pith was carried out at Rajiv Gandhi Chair in Contemporary Studies, Cochin University of Science and Technology (CUSAT) during September 2010 to November 2010.

Composting

One 1 Kg of washed coir pith in duplicates was laid on a shady area. Added 12 g of *Pleurotus sajor caju* spawn was thoroughly mixed with the sample of coir pith. Five grams of urea (M.W 60.06, SRL chemicals, Pvt Ltd) were added to the foregoing substrate. The heap was moistened by sprinkling water to maintain moisture to 200% and monitored for 30 days.

Chemicals

All chemicals (urea (extra pure), catechol, resorcinol and guaicol) and reagents were purchased from sisco research laboratories, Bombay, India.

Analysis

The composted coir pith was subjected to analysis for lignin content, organic cabon content, NPK, formation of phenolic compounds such as resorcinol, guaicol and catechol. Lignin was estimated by modefied Klason lignin assay method, Nitrogen was estimated by Kjel dahl method (Vogel, 1961) organic carbon by Walkey (1934) method, the estimation of phosphorous was done with spectrophotometry (CARY 50 Probe, UV-Visible spectrophotometer), potassium by flame photometry (ELICO flamephotometer CL 378, range 50-1000 ppm) and the estimation of phenolics by Thin Layer Chromatography (TLC). Thin layer chromatography was done with a flat plate of ordinary glass 20×20 cm^2 was washed with hot water and dried in oven. Prepare slurry of silica gel G in distilled water by mixing 30 g of silica gel G in 75 ml of distilled water. Stir the slurry evenly to the glass plate and allowed to dry. Apply the samples and the standards to one side of the plate with a micropipette or capillary tube as small spots. Pour the developing solvent system (benzene: ethyl acetate: acetic acid, 85: 15: 1) into a glass tank. Place the thin layer plates to this tank so that it stands in the solvents with the spotted end dipping in solvent. Once the solvent reaches three-fourth of the plate, remove It from the tank, dry and were exposed to iodine vapour. The

Table 1. Lignin Content,pH and organic carbon content of raw and treated Coir pith mushroom species.

S/ No	Treatment sample	Lignin content		pH		Organic carbon	
		30th day (%)	30th day (%)	15th day (%)	30th day (%)	15th day (%)	30th day (%)
1	Raw coir pith	32	32	6.3	6.3	6.28	6.28
2	Composting with *Pleurotus sajor caju*	32	20	6.7	6.8	6.17	6.16

Table 2. Nitrogen, phosphorous and potassium contents of raw and treated coir pith mushroom species.

S/No	Treatment sample	Nitrogen		Phosphorous		Potassium	
		15th day (%)	30th day (%)	15th day (%)	30th day (%)	15th day (%)	30th day (%)
1	Raw coir pith	0.73	0.73	0.24	0.24	0.28	0.28
2	Composting with *Pleurotus sajor caju*	0.84	0.86	0.29	0.48	0.42	0.41

samples then identified by comparing the Rf value of the standards.

RESULTS AND DISCUSSION

The lignin content, pH and organic carbon of coir pith before and after decomposition with the mushroom species was estimated and the results obtained are presented in Table 1.

Composting is the most suitable technique for transforming organic waste into usable agricultural amendments (Vargas et al., 2007). Although the waste composition is very diverse, lignocelluloses is the most abundant component which is responsible for limiting degradation (Dixon and Langer, 2006). The lignin content of raw coir pith has observed as 32% lignin. Where as the coir pith when treated with *Pleurotus sajor caju*, the lignin content showed varying levels. The decomposition is to 30.4%. It is observed that the amount of lignin in the sample first increased from 32 to 35% in the first five days. Then the amount of lignin started decreasing and in a span of thirty days the lignin content decreased to about 20%. The decrease in lignin content can be explained by the hypothesis that may be the fungi first extracts all the lignin from the cells and then acts on it. Our experiment proved that by the action of *Pleurotus sajor caju* the amount of lignin could be reduced from 32 to 20%. 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.

The pH of the raw coir pith on 15th day and 30th day of composting did not show any variation and it remained at 6.3. The pH was showed acidic and at no point of time it was either neutral or towards alkaline, however without the difference, it was not significant.

The organic carbon content of coir pith on 15th and 30th day did not show any variation and it was 26.28%. Where as the values under treatment at time intervals showed variation. The organic carbon content of coir pith after 15 days of treatment with *Pleurotus sajor caju* showed that a decrease in the carbon content from 6.28 to 6.17 (15 days) and the 30th day sample shows the reduction of carbon 6.16%. This shows the organic carbon in the pith is utilized by the organism and break down to form its products.

Nitrogen, phosphorous and potassium (NPK) content of raw and treated coir pith with mushroom species (*Pleurotus sajor caju*) on 15th and 30th day of composting are presented in Table 2 and Figure 1. The results of the study revealed some interesting information on different variables on account of decomposition. The nitrogen content of the raw coir pith was 0.73%, but after the treatment of 15 days of treatment, the amount of the same is increased to 0.84% and which is again increased to 0.86%. In the case of Phosphorous, the raw coir pith holds 0.24% and which in reach to 0.29% in 15th day and which again increased to 0.48% after the time of

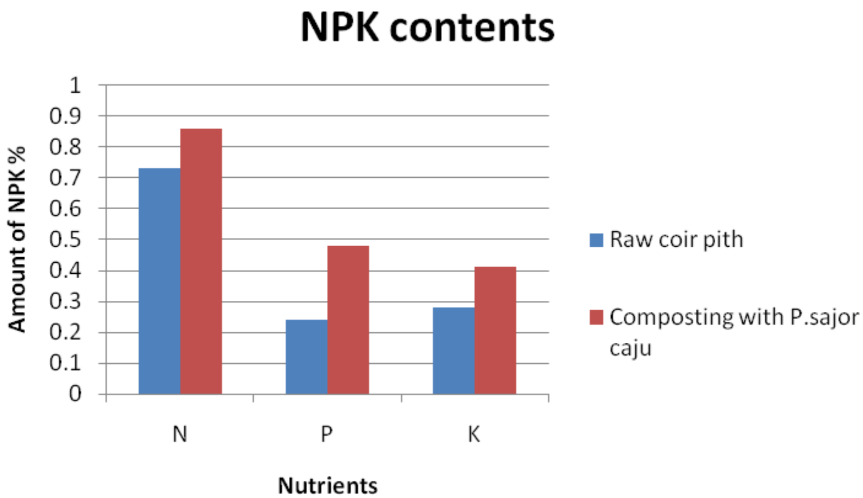

Figure 1. Nitrogen, phosphorous and potassium content of raw and biodegraded coir pith.

composting (30th day). The same enrichment of the nutrient amount is also observed in the case of Potassium also the, it accounts 0.28% potassium in the case of raw coir pith. After the degradation of 15 days it was increased to 0.42%.But there was a bit reduced amount of Potassium was observed when it reached to the 30th day (0.41%) (Figure 1).

It is observed from the available reports, the mushroom species with its enzymatic action degrade only the lignin content and leaving the other components intact (Arora et al., 2002). But the present study confirms that when the coir pith was treated with the mushroom *Pleurotus sajor caju*, the nutrient content of coir pith such as Nitrogen, Phosphorous and Potassium showed the variation and it get increased. In addition to the fungi, several reports brought strong evidence of some bacteria can degrade lignin effectively (Crawford, 1978; Haider et al., 1978; Kawakami., 1976; Trojanowski et al., 1977).

Cullen and Kersten (2004) reported that the enzymes from white rot fungi that catalyse the initial depolymerization of lignin are extra cellular and unusually non specific. The lignin degradation of white rot fungi is extensively studied, and their results revealed that the three kinds of extra cellular phenol oxidases, namely lignin peroxidases (LiP) Manganese peroxidase (MnP) and laccase (Lac) are responsible for initiating the depolymerization of lignin(Moriya et al., 2001). The present work is in line with these findings that, the depolymerization of the lignin causes the reduction in their percentage from 32 to 20% with the treatment of coir pith with the mushroom. From this it may be described that these enzymes also produced by Mushroom and are involved in the lignin polymerization.

This work is in harmony with the findings of Reghuvaran et al. (2009) that they indicate the degradation of coir pith different mushroom species in combination with the bacterial species. Instead of nitrogen fixing bacteria, here urea was used as the main nitrogen supplier and the action of the mushroom itself showed a definite capability to degrade the lignin and thereby exhibited a drastic reduction in lignin percentage. It is reported that the maximum yields of coconuts when coir pith compost was added with trace amounts of chemical fertilizers (Venkitaswamy, 2003). But the present work of composting with the mushroom species causes the enhancement of MPK content with in the coir pith compost itself by the action, and can cause the improved activity of organic manure.

The thin chromatographic results of the phenolic standards and the coir pith samples are given in Table 3. Spotted samples of coir pith along with phenolic standards such as resorcinol, guaicol and catechol. On comparing the values retention factor (Rf) of raw and coir pith samples, with these standards we can interpret the presence of phenolic compounds. It is very evident from the results that up to the fifteenth day sample, only onespot was obtained whose value corresponded to the value of catechol and guaicol. On the twenty-fifth and thirtieth day sample, three spots were obtained corresponding to catechol, guaicol and resorcinol. Thus it can be suggested that these phenolic compounds are formed during the degradation of coir pith. It can also state that these compounds are the breakdown products of lignin thus decreasing the total lignin content (Figure 2 to 4).

Thus the present study confirms that the mushroom species, *Pleurotus sajor caju* influences lignin degradation effectively. Coir pith is highly non-degradable mainly due to the presence of high percentage of lignin. It is one of the major pollutants of land and water in south Indian states. Our works find application in such places. From our work we can conclude that by the action of

Table 3. Thin layer chromatographic results of phenolic standards and coir pith samples.

No of days	Solvant front(cm)	Solute front(cm)		Rf value	Colour
		Resorcinol	3.5	0.2554	Yellow
		Guaicol	10.0	0.7299	Brown
0th Day		Catechol	4.9	0.3576	Brown
		Raw coir pith	5.5	0.4014	White
		Sample i	----	--------	-------
		Resorcinol	6.0	0.4545	Yellow
5th Day		Guaicol	11.4	0.8636	Brown
		Catechol	6.8	0.5151	Brown
		Sample i	5.4	0.4091	White
		Resorcinol	6.2	0.4460	Yellow
		Guaicol	12.1	0.8705	Brown
10th Day		Catechol	7.5	0.5395	Brown
		Sample i	7.4	0.5323	Brown
		Sample ii	7.7	0.5539	Brown

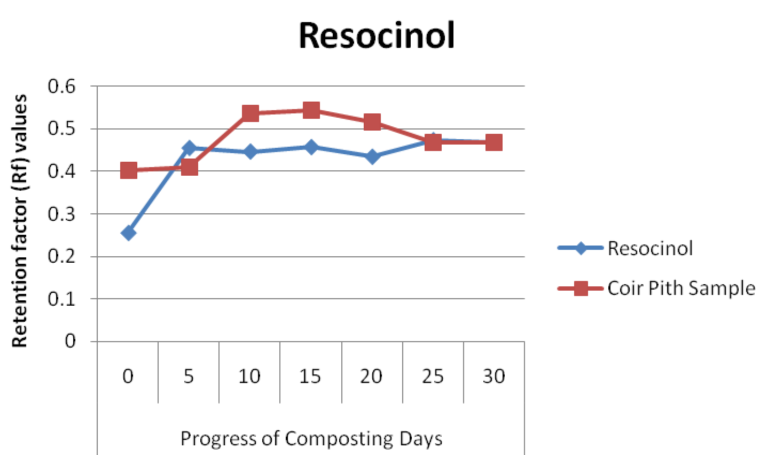

Figure 2. Enhancement of resorcinol on biodegraded coir pith sample.

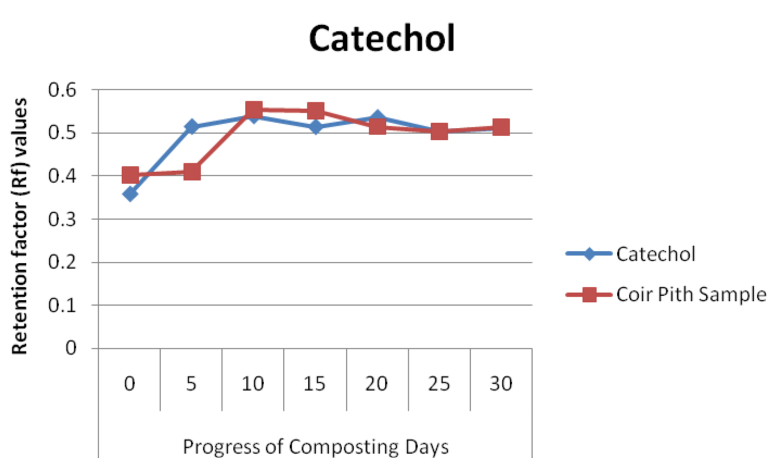

Figure 3. Enhancement of catechol on biodegraded coir pith sample.

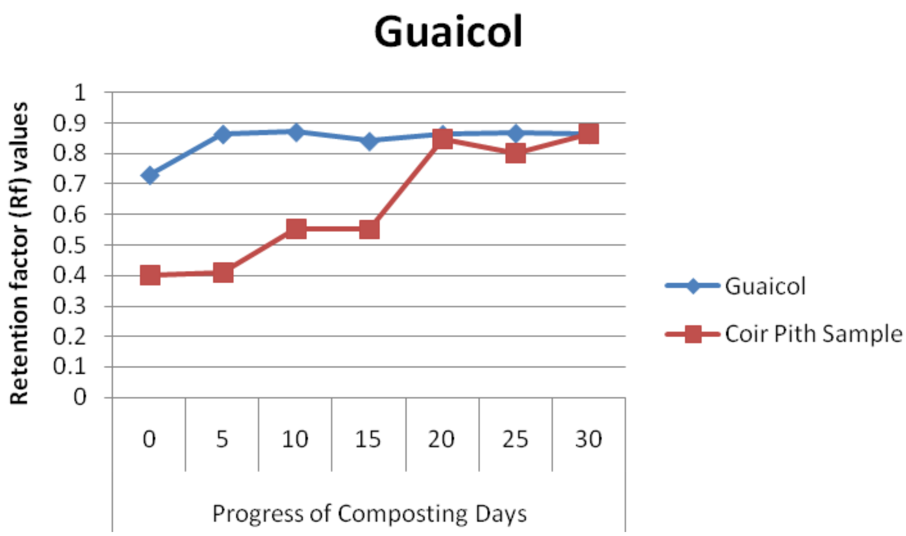

Figure 4. Enhancement of catechol on biodegraded coir pith sample.

Pleurotus sajor caju, the amount of lignin in the coir pith can be reduced considerably thus converting the waste pith into a useful product in an eco-friendly manner. The product obtained after bio-degradation can be used as manure and as hydr Coir pith oponic systems for growing roses and vegetables.

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