ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 4, pp: (199-207), Month: October - December 2018, Available at: www.researchpublish.com

Biodegradation of lignocellulosic waste using Cyanobacteria and development of organic manure

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Abstract: **This study presents degradation of lignocellulosic waste product from coir yarn industry – Coir pith using *Oscillatoria annae*. Degradation produced brown colored supernatant (cyanospray) and residue (cyanopith). The partially degraded coir pith (cyanopith) was converted into organic manure with the addition of jiwamrita. Morphological characteristics, functional group and the quality of cyanopith mixed with Jiwamrita in three different particle sizes were identified by Scanning Electron Microscopy (SEM), FTIR and HPLC analysis. Results indicated surface area modifications, presence of lignin derived compounds and the functional groups during the composting process. The minimum sized particle 0.01-0.1mm showed a significant variation than the other particle sizes on 30th day.**

Keywords**: Lignin, Degradation, Cyanopith, Cyanospray, Jiwamrita, Manure.**

I. INTRODUCTION

Coir pith is a highly lignocellulosic waste dumped in huge piles on roadside, because of its high lignin content and slow degradation in natural environment it creates environmental pollution problems (Bhat and Narayan, 2003). Disposal of solid waste is a major problem faced in most countries. As an alternative way of waste disposal composting can be done. Composting process will help to manage food and yard waste, also serve as a source of fertilizer for growing crops in the field (Sitavi *et al.*, 2018). Besides, various researches on degradation of coir pith by fungi and bacteria were already reported. Recent research shows that coir pith can be partially decomposed through the action of cyanobacteria and can be used as biofertilizer for all varieties of food crops (Anandharaj, 2007). Cyanobacteria are naturally occurring in most ecosystems and fix nitrogen gas from the atmosphere into forms which are useable by plants (Sterle *et al.*, 2015). Many studies have been conducted for improving the productivity of crops using cyanobacterial inoculants as a biofertilizer (Ali Salama, 2015).

Cyanopith is an organic biofertilizer produced by biodegradation of coir pith using freshwater cyanobacterium, *Oscillatoria annae* (Malliga *et al*, 2012). Jiwamrita is a plant growth promoting substance containing beneficial microorganisms (Vanaja *et al.*, 2009) besides improving the efficiency of applied manure (Manjunatha *et al.*, 2009). Manure contributes to soil fertility and tilts by the addition of organic matter and nutrients, such as nitrogen that is trapped by bacteria in the soil (Haynes *et al.*, 2003). Hence, the present study aims to analyse the degradation process of coir pith by fresh water cyanobacterium (*O. annae*) using SEM and to identify the lignin derived compounds and functional groups present in the coir pith based organic manure during composting of three different particle sizes using HPLC and FTIR analysis.

II. MATERIALS AND METHODS

A. Organism and culture conditions

Fresh water cyanobacterium (*Oscillatoria annae*) was obtained from the germ plasm of National Facility for Marine Cyanobacteria (NFMC), Bharathidasan University, Tiruchirappalli, Tamil nadu, India. The culture was maintained in BG-11 medium at 1500 lux at 25±2˚C with 30 days light / dark (10/14 hrs.) cycle (Anandharaj *et al*., 2012).

B. Cyanopith fertilizer

The coir pith based cyanobacterial product was known as cyanopith and this was used as a basal fertilizer (Malliga *et al.*, 2012).

C. Preparation of organic manure

The cyanopith fertilizer was ground and sieved into three different particle sizes (a. 1-10 mm; b. 0.1-1 mm; c. 0.01-0.1 mm). Then, the samples were mixed with Jiwamrita and incubated for 30 days. After incubation, the samples were collected w without contamination for further studies (Jenny and Malliga, 2014).

D. SEM analysis

Scanning electron microscope analysis was performed using SEM VEGA3 TESCAN at 1500 Hz vaccum frequency on three different particle sizes of organic manure to analyze the morphological changes induced during 30 days incubation. For the analysis, the samples of various sizes were coated with a thin gold layer by a sputter coater unit and scanned at 20µm range (Goodhew and Humphreys, 1988).

E. HPLC analysis

HPLC is used to separate components of a mixture by using a variety of chemical interaction between the substance being analyzed and the column (Harzallah and Ben, 2005). The ability of cyanopith to degrade in the presence of jiwamrita at varying particle sizes was tested for the presence of compounds on 30th day of incubation. The sample was injected into C_{18} column for 10 min. retention time at 254 nm absorbance with UV detector.

F. FTIR analysis

The samples were mixed with spectroscopic grade KBr at a ratio of 1:100 and were made in the form of pellets at a pressure of about 1 MPa. The pellets were immediately put into the sample holder of Perkin Elmer spectrophotometer and operated in the range of 4000-400 cm^{-1} (Grube *et al*., 2006).

III. RESULTS AND DISCUSSION

G. SEM Analysis

SEM was performed to analyze the morphological changes (20 µm) of three different particle sizes of organic manure before and after degradation. Plate-1a indicated the rough surface and normal cell wall nature at 0th day which would have been slightly disintegrated on the 30th day by microorganisms. Plate-1b showed a moderate modification in the roughness and cell wall, whereas on the 30th day more demolished surface with slightly increased porosity was observed. Plate-1c indicated that the modification in the cell wall and decreased roughness was observed on 0th day. However, maximum smoothening of surface and increased porosity was observed in 0.01-0.1mm on the 30th day compared to other sizes of particles. Therefore, it indicates the delignification and massive degradation of cellulosic components by the activity of microbes during the composting process in smaller size (0.01-0.1mm) particle than bigger (0.1-1mm) and biggest (1-10mm) particles. This could be due to the degradation of lignin like substances has been enhanced by the activity of microorganisms during incubation because of increased surface area and aeration.

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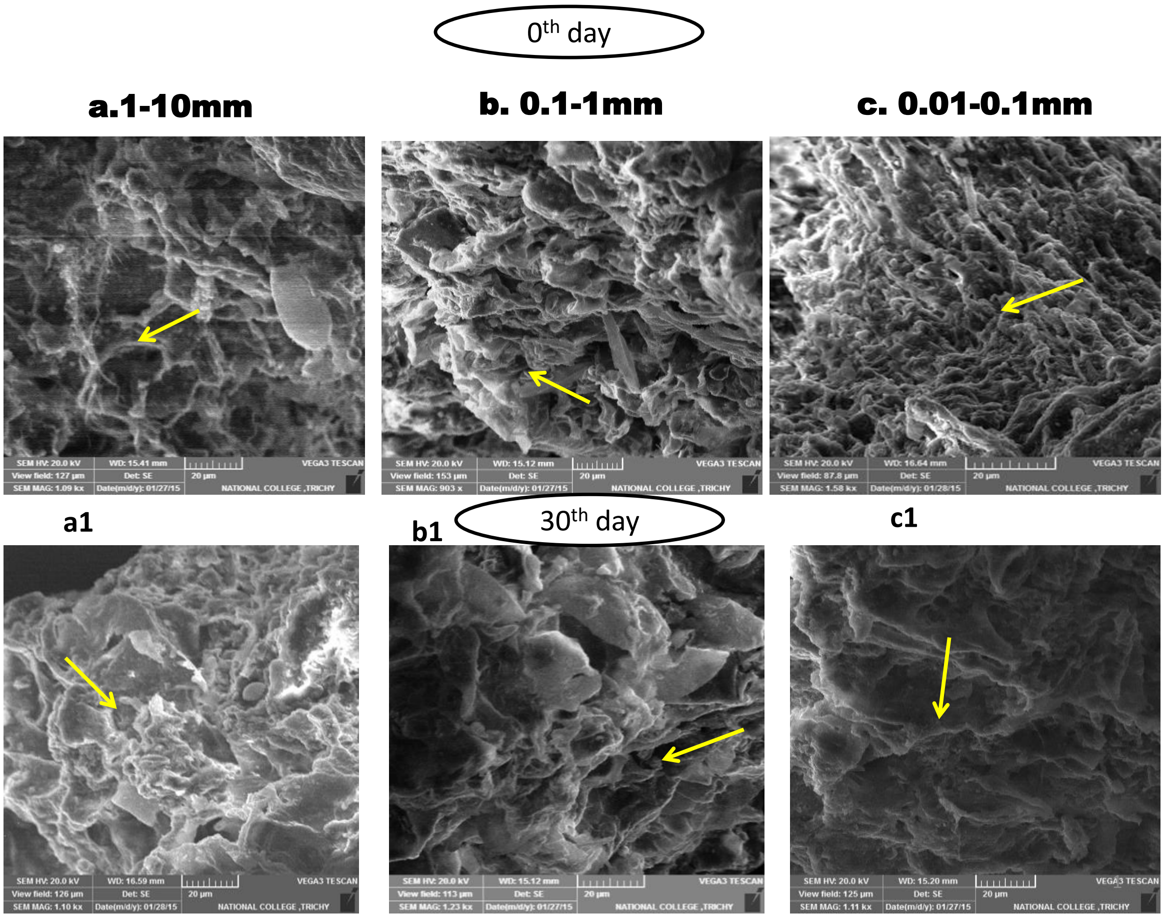
Plate1. Scanning electron microscopic view of different particle sizes of organic manure on 30th day



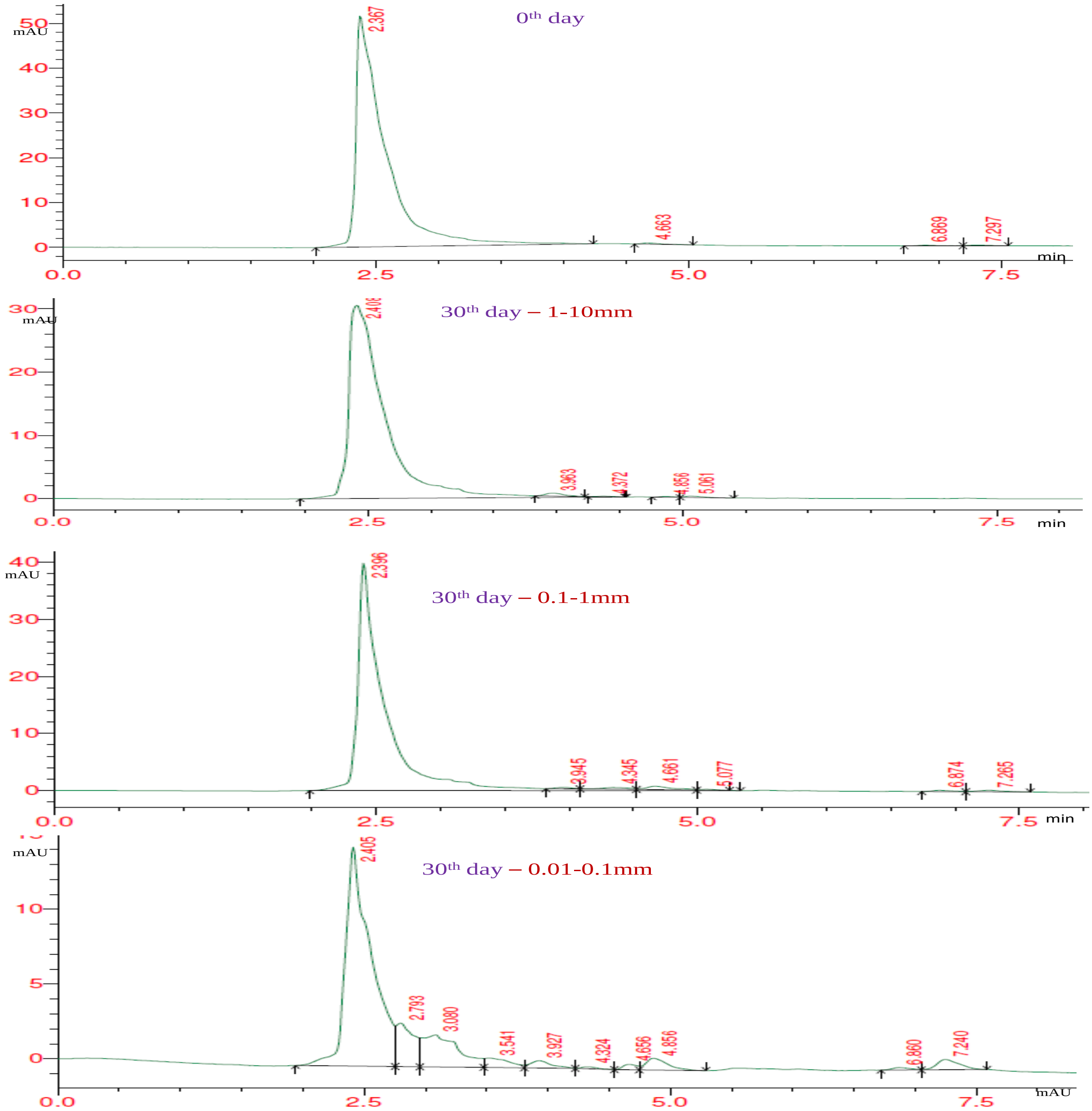
SEM analysis at 2 µm indicated smoothening of the surface and at 10 µm indicated a change of porous surface into pleet like structure. Fragmentation of the surface was observed in the SEM image taken at 50 µm. SEM analysis confirmed surface modifications induced due to the oxidative delignification treatment (Rojith and Bright, 2012). Baharuddin *et al*. (2009) exhibited that the surface structure of the untreated shredded empty fruit bunch consisted of firmly bound threads of lignin with the smooth surface of the structure, while the outer surface of the treated empty fruit bunch found altered with the presence of many holes indicating that the lignin has been disrupted. SEM micrographs revealed that the surface condition of the raw oil palm empty fruit bunches material was initially hard and rough and the pores were filled with silica bodies that acted to protect the plant structure (Wan *et al*., 2012). Due to the availability of silica bodies inside the pores the penetration of microbes into the cellulose layer into the internal tissue is restrained as reported by Wong *et al*. (2008).

Iniquez *et al*. (2011) showed significant changes in the structure of vascular bundle in agave bagasse sample after 126 days of composting. SEM images of cow dung manure, vermicompost and sludge and lake sediment showed crystal-like forms of humic acids aggregate along with various shapes (Rupiasih and Vidyasagar, 2009). Zulkarnain *et al*. (2015) reported that Microalga *Scenedermus dimorphus* cell surface exhibited morphological change with the larger pore size and shape was probably caused by the release of some ions which are bound to the biomass surface. The highly porous surface favours the diffusion of metal ions into the cell and leading to higher adsorption capacity (Dragana *et al*., 2011). The post-vermicomposted mixtures confirmed the presence of greater numbers of surface irregularities, which resulted in good vermicompost manure with high porosity and nutrient availability (Bhat *et al*., 2015).

H. HPLC analysis

HPLC methods were used for the extraction and separation of lignin-derived compounds present in three different particle sizes of organic manure with 2-10 retention times. The results revealed that more numbers of peaks were observed in the finest size among all the other sizes on the 30th day (Fig. 1).

Fig. 1 HPLC analysis for separation of compounds from organic manure (in filtrate) on 30th day



During incubation, the microbial activity was increased with the addition of jiwamrita thereby the lignin compounds could be degraded and the humic-like substances were formed and that improves the quality of organic manure. Supporting evidence showed that the HPLC analysis of water extract of the decomposing rice straw at different treatments in various intervals revealed the production of organic acids (citric acids, oxalic acid, maleic acid and formic acid) (Kumari *et al*., 2008).

Sunderland *et al*. (2003) developed HPLC method to detect CTC in pig faeces that also involved the SPE cleanup step, but the limit of quantitation (LOQ) of 3.5 mg/kg was too high for monitoring the lower level in pig manure. High performance liquid chromatography (HPLC) and gas chromatography-mass spectroscopy (GC-MS) analyses of aqueous extracts of cattle waste derived vermicompost showed the presence of significant amounts of indole-acetic-acid (IAA), gibberellins and cytokinins (Edwards *et al*., 2004). The nutrient status was enriched and the compounds were analyzed and separated by HPLC (Somasundari *et al*., 2014). The HPLC analysis confirmed the presence of fungicide residues in commercial compost (barks+ pruning residues+ urban and industrial sludge) and traces of some metabolites (Adriano *et al*., 2004).

I. FTIR analysis

FTIR is used to identify the functional groups and structural transformation of lignin, cellulosic and other components. Fig. 2 shows the FTIR spectra of three different particle sizes of organic manure on 0th and 30th days. Results revealed characteristic changes in the peaks in three different particle sizes of organic manure during incubation on 30th day. Nevertheless, the minimum particle size of organic manure has been degraded faster and exhibited a significant variation during incubation. This could be due to the degradation of lignin components by the activity of microorganism with the application of jiwamrita. It is observed that the lignin compound was completely disappeared in all the article sizes on the 30th day and exhibited new compounds on the 30th day in all the samples but observed maximum disintegrated compounds on minimum particle size (Table-1). The FTIR spectra clearly confirm the presence of amide, aromatic and aliphatic groups after degradation process and assessing compost maturity.

Fig.2. FTIR analysis of different particle sizes of organic manure

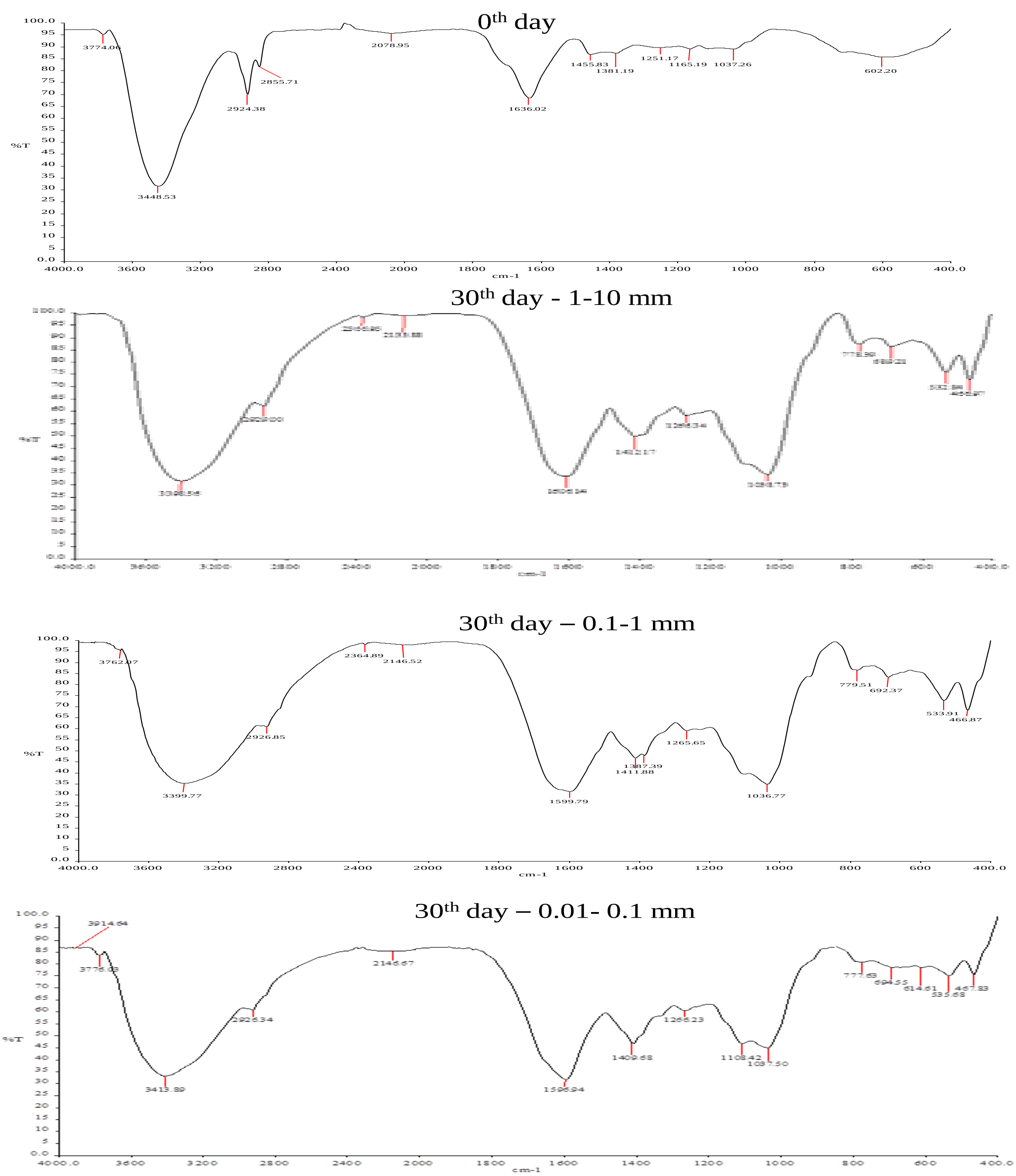


Table 1. FTIR analysis of three different particle sizes of organic manure on 30th day

S. No	Compounds	1-10mm		0.1-1mm	0.01-0.1mm
		0th day	30th day	30th day	30th day
1	OH bond stretching of alcohol & phenols	+	+	+	+
2	C-H stretching of alkanes	+	+	+	+
3	N-H bending of 1° amines	+	+	-	-
4	C-C stretch of aromatic compounds	+	+	+	+
5	C-N stretch of aromatic amines	+	+	+	+
6	C-O stretch of carboxylic acid, esters&ethers	+	+	+	+
7	C≡ C-H of alkanes	+	+	-	+
8	CH- lignin	+	-	-	-
9	1,2,Disubstitution (ortho)	-	+	-	+
10	C=CH (Aromatic –H)	-	+	+	+
11	C-O stretch –OH bending	-	-	-	-
12	Aromatic C-H	-	-	+	+
13	Aromaticcombination bands	-	+	+	+
14	N-O stretch of nitro compound	-	-	+	+
15	C-N stretch of aromatic amines	-	-	+	+
16	S-O stretching of sulphoxide	-	-	-	+
	Total	8	10	10	13

FTIR analysis of maximum particle size (1-10 mm) of organic manure resulted in peaks at 3448.53 cm^{-1}, 1636.02 cm^{-1} and 1455.83 cm^{-1} for OH and H bond stretching of alcohols and phenols, N-H bending of primary amines and C-C stretch of aromatic compounds were decreased into 3398.56 cm^{-1}, 1606.14 cm^{-1} and 1412.17 cm^{-1} respectively on the 30th day (Fig. 2a). However, the peaks at 2924.38 cm^{-1}, 2078.95 cm^{-1}, 1251.17 cm^{-1}, 1037.26 cm^{-1} and 602.20 cm^{-1} were increased to 2929 cm^{-1}, 2133.88 cm^{-1}, 1266.34 cm^{-1}, 1038.73 cm^{-1} and 689.21 cm^{-1} for C-H stretching of alkanes, aromatic combination bands, C-N stretch of aromatic amines, C-O stretch of carboxylic acid esters and ethers and C≡C-H of alkynes respectively. The peaks range of 1381.19 cm^{-1} for OH and –CO deformation from alcoholic phenolic OH and 1165.19 cm^{-1} for C-O stretch of polysaccharides were disappeared. Nevertheless, the new bands at 778.39 cm^{-1} for 1, 3, disubstitution (Meta) and 532.84 cm^{-1} for C=CH aromatic H were observed after incubation on 30th day.

FTIR of medium particle size of organic manure (0.1-1mm) displayed peaks at 3404.73 cm^{-1}, 2930.64 cm^{-1}, 1266.92 cm^{-1}, 1042.00 cm^{-1} and 536.58 cm^{-1} for OH and H bond stretching of alcohols and phenols, C-H stretching of alkanes, C-N stretch of aromatic amines, C-O stretch of carboxylic acid esters and ethers and C=C-H aromatic H respectively. But, on 30th day these functional groups were decreased into 3399.77cm^{-1}, 2926.84 cm^{-1}, 1265.65 cm^{-1}, 1036.77 cm^{-1} and 533.91 cm^{-1} (Fig. 2b). Moreover, the peaks at 2140.95 cm^{-1}, 1597.44 cm^{-1} and 1410.18 cm^{-1} were slightly increased into 2146.52 cm^{-1}, 1599.79 cm^{-1} and 1411.18 cm^{-1} for aromatic combination bands, N-O stretch of nitro compounds and C-C stretch of aromatic compounds respectively whereas, the new peaks at 1387.39 cm^{-1}, 779.51 cm^{-1} and 692.37 cm^{-1} for OH and –CO deformation from alcoholic phenolic OH, 1,3, disubstitution (meta) compounds and C≡C-H of alkynes were also formed, the peaks at 1106.46 cm^{-1} for C-O stretch of polysaccharide was disappeared after 30th day incubation.

FTIR analysis of minimum particle size (0.01-0.1mm) of organic manure exhibited more number of peaks than the other particle sizes of organic manure. The peaks range at 3395.45 cm^{-1}, 2925.16 cm^{-1}, 1106.66 cm^{-1}, 1036.49 cm^{-1}, 775.69 cm^{-1} and 534.79 cm^{-1} were slightly increased into 3413.89 cm^{-1}, 2926.34 cm^{-1}, 1108.42 cm^{-1}, 1037.50 cm^{-1}, 777.63 cm^{-1} and 535.68 cm^{-1} for OH and H bond stretching of alcohols and phenols, C-H stretching of alkanes, C-O stretch of polysaccharide, C-O stretch of carboxylic acid esters and ethers, 1,3, disubstitution (meta) and C=CH aromatic H respectively on 30th day (Fig. 2c). However, the peaks at 1412.16 cm^{-1} for C-C stretch of aromatic compounds and 1267.53 cm^{-1} for C-N stretch of aromatic amines were slightly decreased into 1409.68 cm^{-1}, 1266.23 cm^{-1} respectively. Moreover, the peak at 1330.90 cm^{-1} for OH and –CO deformation from alcoholic phenolic OH and 1601.93 cm^{-1} for N-H bending of primary (1°) amines were disappearing and the new peaks were formed at 2146.67 cm^{-1}, 1596.94 cm^{-1}, 694.55 cm^{-1}, 614.61 cm^{-1} for aromatic combination bands, N-O stretch of nitro compounds and C≡C-H of alkynes respectively after incubation on 30th day.

The peak range at 1620 cm^{-1} and 2939 cm^{-1} indicates a lignin change, 3407 cm^{-1} and 3531 cm^{-1} vibration infers structural changes in hydroxyl groups in the phenolic and aliphatic structure (Rojith and Bright, 2012). A strong hydrogen bond was observed at 3931 cm^{-1} due to OH stretch. Also, the band at peak range of 2925 cm^{-1} is present which represents the C-H stretch. The band at 1433 cm^{-1} confirms the presence of amide group and 1641 cm^{-1} peaks indicates C=C aromatic group. The peak range of 3404 cm^{-1}, 2858 cm^{-1}, 1723 cm^{-1}, 1380 cm^{-1} and 1033 cm^{-1} indicate the change in –OH stretch, C-H stretch, aliphatic CH_3 stretch and amide group respectively (Karthika *et al*., 2014). The abundance of N containing functional group changes during digestion and decomposition of dairy manure (Calderon *et al*., 2006).

The bands located at 2916 cm^{-1} and 2845 cm^{-1} for the hydrogen vibrations of the C-H group of aliphatic methylene group from the degradation of hemicellulose (Bernabe *et al.*, 2011). Jouaiphy *et al*. (2005) reported that microorganisms utilized aliphatics and carbohydrates such as polysaccharides, cellulose and hemicelluloses for their energy requirement during the composting process and more easily disappear than cellulose or lignin. The absorption band at 1730 cm^{-1} indicates that the metabolic products such as aldehydes, ketones and esters (Tandy *et al*., 2010). Bands at 1260 cm^{-1} to 1240 cm^{-1} were referred to carboxylic acid groups and tertiary amides, which were visible in the raw oil palm empty fruit bunches and disappeared in 40 days of composting process (Wan *et al*., 2012). According to Wong *et al.* (2008), lignin is the most difficult component to decompose and the bands at 1157 cm^{-1}, 1120 cm^{-1} and 1053 cm^{-1} are attributed to S-O polysaccharide compounds. The decrease of the C-H stretching bands corresponding to aliphatic methylene bands at 2920 cm^{-1} and 2850 cm^{-1} can be an indicator of the degradation process and these aliphatic methylene groups are part of many organic molecules and their degradability varies in a wide range (Iniquez *et al*., 2011).

Xin *et al*. (2010) suggest an increase in aromatic structures, nitrogenous compounds and alkenes and a decrease in carbonyl groups (COOH, ketone and aldehydes) as well as aliphatic groups in swine manure. The FT-IR spectra of all solid residue samples were similar and difference in the intensity of some peaks indicated that some reactions such as dehydration, decomposition of protein, decarboxylation and aromatization happened (Rui *et al*., 2015). FTIR analysis indicated the presence of the hydroxyl and carboxyl groups on the biomass surface which play an important role in the biosorption process, suggesting that the process takes place mainly by ionic exchange (Zulkarnain *et al*., 2015). FTIR analysis of treated coir concludes changes in functional groups and IR bands converted from strong to weak represented degradation (Arun Kumar *et al*., 2015).

IV. CONCLUSION

Coir pith which is one of the lignocellulosic compound and pollution causing agent can be degraded by cyanobacterium *Oscillatoria annae* and degraded coir pith can be used as biofertilizer. The present study deals with the three different particle sizes of organic manure (cyanopith mixed with jiwamrita) incubated for 30 days. SEM results exhibited that the minimum particle size (0.01-0.1mm) of organic manure showed more surface modifications and smoothening of the surface than the other particle sizes and control. FTIR results proved that there is a characteristic variation in the peaks in three different particle sizes of organic manure during incubation on the 30^{th} day. Nevertheless, the minimum particle size of organic manure has been degraded faster and exhibited a significant variation during incubation. HPLC results exhibited that the more no. of peaks were observed in the finest size among all three particle sizes on the 30^{th} day. Hence, this study concluded that the minimum particle size (0.01-0.1mm) of organic manure has high nutrient status as compared to that of other particle sizes (1-10 mm; 0.1-1 mm) during incubation period. Further, cyanobacterial based organic manure can be act as good source for plant growth.

ACKNOWLEDGEMENT

The author is grateful to Model Organic Farm (MOF), Bharathidasan University, Tiruchirappalli, Tamilnadu for the facility and completion of this work.

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