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CONFOCAL AND FTIR STUDIES OF COIR PITH DEGRADATION BY CYANOBACTERIUM (OSCILLATORIA ANNAE)

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ABSTRACT

Lignin degradation was done by several microorganisms such as bacteria, fungi and cyanobacteria. Cyanobacteria are a photosynthetic nitrogen fixing group that survive in wide variety of habitats (soil, rock and water) and abate various kinds of pollutants. Hence, the study was carried out to analyse the degradation process of coir pith by a fresh water cyanobacterium (Oscillatoria annae) and examine various parameters.

KEYWORDS : Lignin, degradation, Oscillatoria annae, coir pith, confocal, FTIR.

Introduction

Environmental pollution has been one of the largest concerns to science and the public. Bioremediation is a process by which living organisms degrade or transform hazardous organic contaminants or pollutants and wastes to less toxic compounds (Arun, et al., 2008). Large amounts of lignocellulosic "waste" (re generated through forestry and agricultural practices, paper-pulp industries, timber industries and many agro- industries) pose an environmental pollution problem. Deplorably, much of the lignocellulose waste is often disposed of by biomass burning, which is not restricted to developing countries alone, but is considered a global phenomenon (Levine, 1996). Lignocellulose consists of lignin, hemicellulose and cellulose (Betts et al., 1991); Sun and Cheng (2002) showed the typical compositions of the three components in various lignocellulosic materials. Lignin is a complex three dimensional, aromatic heteropolymer found predominantly in the xylem of plants (Donaldson, 2001). Hemicelluloses are typically found in the secondary wall and are linked with cellulose. However, they can also be found in the primary wall of the cell (Jeffries, 1994). The main structural component of lignocellulose is cellulose – a highly crystalline polymer composed of D-glucose, just like starch. These form long chains linked together through hydrogen bonds and Van der Waals interactions (Sanchez, 2009). Coir pith is similar to peat in appearance, light to dark brown and consists primarily of lignin and cellulose particles ranging from 0.2 to 2.0 mm (75% to 90%) in size (Cresswell, 1992). Coir pith is a major by-product of coir fibre extraction industries (Reghuvaran et al., 2009). It decomposes very slowly due to its high lignin content. Accumulation of coir pith every year leads to pollution of the environment. In recent years, these waste materials were converted to biofertilizer or compost using several microbes (Kamaraj, 1994). The waste products of coir yarn industry are coir dust and coir pith or coco peat which constitute about 70% of the husk. In spite of their limited use as soil conditioners, the quantity of coir dust produced is so enormous making its disposal difficult because of its lignocellulosic nature and slow degradation in the natural environment. Cyanobacteria, the oxygen evolving photosynthetic prokaryote originated 3.5 billion years ago, occupy a credential position between prokaryotes and eukaryotes. The resultant tandem operation of two photo systems is now known as oxygenic or plant- type photosynthesis (Atzenhofer et al., 2002). As these organisms have simple growth requirements, they could be attractive host for production of valuable organic products (Malliga and Viswajith, 2005) and it also showed coir pith degradation (Malliga et al., 1996; Anandhraj, 2008). Hence, the study was carried out to analyse the degradation process of coir pith by fresh water cyanobacterium (O. annae) and observed under microscopic view for 30 days and the degradation was also studied with confocal microscopy and FTIR.

2. Materials and methods

2. 1. Organism and culture conditions

Fresh water cyanobacterium (O. annae) was obtained from the germplasm 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 (Anandhraj et al., 2012).

2. 2. Coir pith

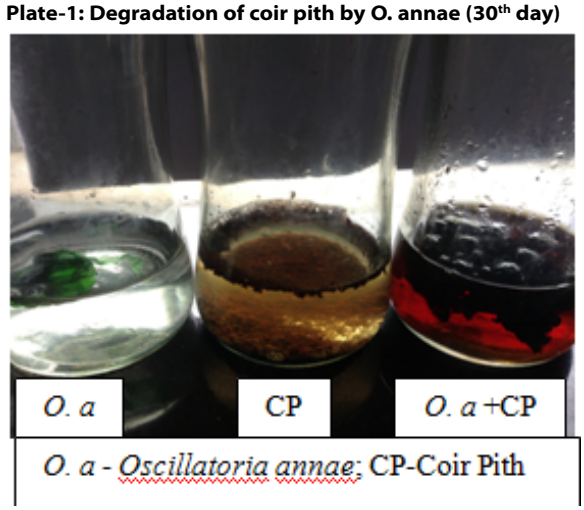
Coir pith was collected from coir industry, near Srirangam, Tiruchirappalli, Tamil nadu, India.

2. 3. Medium and growth conditions

2. 3. 1. Incubation

In a series of 250 ml conical flasks, 100 ml of sterile BG11 medium was poured and 1 g of cyanobacterial culture (O. annae) (Plate - 1) was inoculated, which was considered as positive control and to appropriate flask 1g of lignocellulosic material coir pith was added as negative control and medium was inoculated with the cyanobacterial culture and coir pith in ratio 1:10, which was considered as test.

Plate-1: Degradation of coir pith by O. annae (30th day)



O. a - Oscillatoria annae; CP-Coir Pith

2. 4. Estimation of derivative product

To estimate the degradation rate of all the three culture filtrates such as O. annae, CP (Coir Pith) and combination of these two were measured at 436 nm, from 0th to 30th day according to Dilara and Emily (2014).

2. 5. Estimation of pH

pH was determined during all the 30 days of incubation period for all the culture filtrates (O. annae, CP (coir pith), O. annae + CP).

2. 6. Confocal study

Initial day (0th day) and final day (30th day) of two cultures O. annae and O. annae + CP were viewed under LSM (Laser Scanning Microscopy) 710, HAL 100, ZEISS, Germany, 2011 equipped with HP ZR30W, in order to differentiate the structural modification of O. annae before and during degradation of coir pith.

2. 7. FTIR analysis

IR spectra of O. a, CP, pellet (cyanopith) and supernatant (cyanospray) (dried, powdered samples) were obtained by Perkin Elmer, version 2009, Fourier Transform Infrared Spectrometer (FTIR) equipped with detector, KBr as reference. IR spectra of the four samples were recorded in the ranges between 400 to 4000 cm^{-1} absorptions.

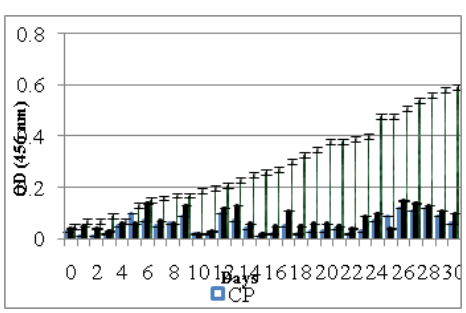
3. Results and Discussion

In the combined treatment of O. annae with coir pith showed luxuriant growth when compared to control culture (Plate - 1) on 30th day. This was due to the coir pith degradation by O. annae which releases intermediate compounds to the medium which would have enhanced the growth of O. annae in combined treatment. This clearly indicated that the cyanobacterial growth was not inhibited by the presence of the lignin content in coir pith (Abraham et al., 2008). O. annae degraded the lignocellulosic material coir pith containing lignin and holocellulose which was so far proved by other microorganisms such as bacteria (Perestelo et al., 1994), actinomycetes (Ferraz and Duran, 1995) and fungi (Bhat and Narayan, 2003). Palmer and Evans (1983) have reported that few actinomycetes such as Streptomycetes and Nocardia also degrade lignin.

3. 1. Determination of degradation rate

Rate of degradation of the samples was evidenced by OD values which were taken during 30 days incubation period. Coir pith cultured and O. annae individually cultured medium showed no significant difference in OD values, (Fig - 1) while, the combined treatment exhibited gradual increase started from 5th to 30th day. In nature, complete degradation of lignocellulose is a very complex process, usually involving a consortium of microbes (Distel et al., 2002a; Lynd et al., 2002). In particular, white rot fungi are the most effective degraders of lignocellulosic wastes (Lopez et al., 2002; Kirk and Farell, 1987; Hammel, 1997; Hatakka, 2001). Furthermore, bacterial strains were found to degrade and assimilate lignin (Ball, 1989; Chandra et al., 2007; Nishimura et al., 2006; Odier et al., 1981; Pometto and Crawford, 1986).

Fig - 1: Degradation rate of coir pith by O. annae

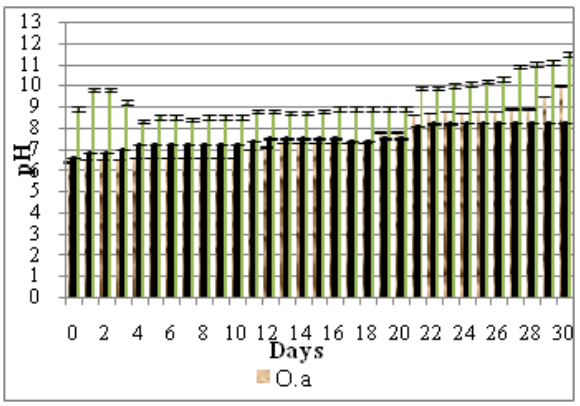


O. a - Oscillatoria annae; CP-Coir Pith

3. 2. Estimation of pH

pH of the medium (O. annae, coir pith and combined) was monitored during incubation period which was found to be the same in coir pith inoculated medium for all the 30 days. O. annae cultured medium showed moderate increase in pH, although, in combined (CB + CP) treatment pH was raised during 21-30 days period (Fig - 2). Maximum lignin degradation occurred at pH 6 which may be interpreted as increasing of pH during the growth of ligninolytic bacteria, which led to reach the alkaline range suitable for lignin biodegradation which was attained by Bacillus sp. (Chandra et al., 2007; Raj et al., 2007).

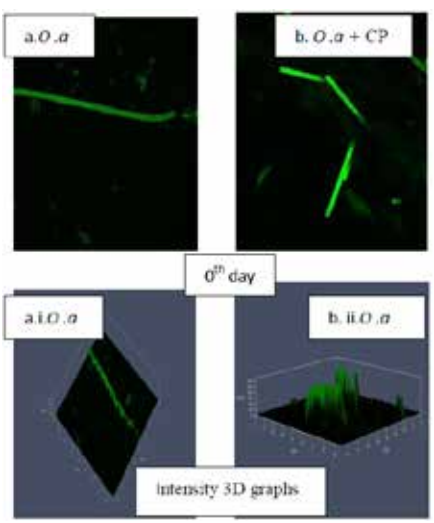
Fig - 2: Effect of pH during degradation of coir pith



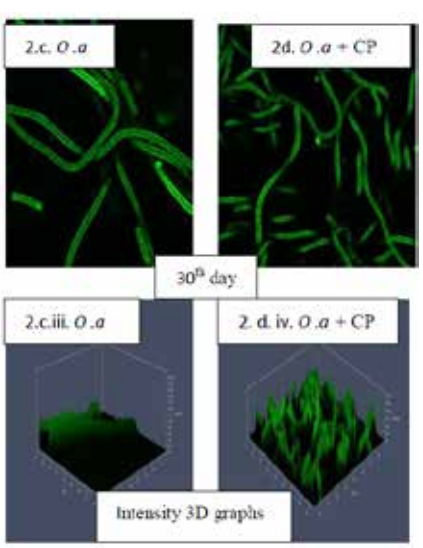
3. 3. Confocal study

At 0th day, O. annae (Plate – 2. a) and O. annae + coir pith (Plate – 2. b) showed no changes in the morphology and not much intensity was noticed (Plate a (i), b (ii)) under confocal microscope.

Plate -2: Confocal views of Oscillatoria annae and O. annae with coir pith (CP)



However, on 30th day O. annae (Plate – 2. c) and (Plate 2.d) showed enhanced growth which was supported by more visible intensity when compared to 0th day (Plate 2. c. iii and 2. d. iv) which might be due to the involvement in degradation process of coir pith by O. annae.

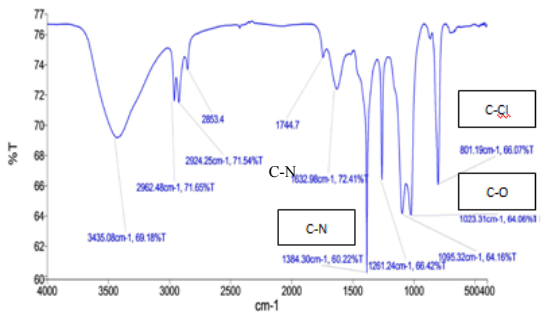


Sole et al. (2001, 2007) have denoted the manual method for obtaining images with CLSM (Confocal Laser Scanner Microscopy) for identifying and quantifying (biomass) the cyanobacteria in Ebro delta microbial mats and it was found to be time consuming method which produced useful results. The confocal microscope has allowed us to determine the biodiversity of the different samples with a degree of clarity greater than any other microscopic technique (Elia et al., 2004).

3. 4. FTIR analysis

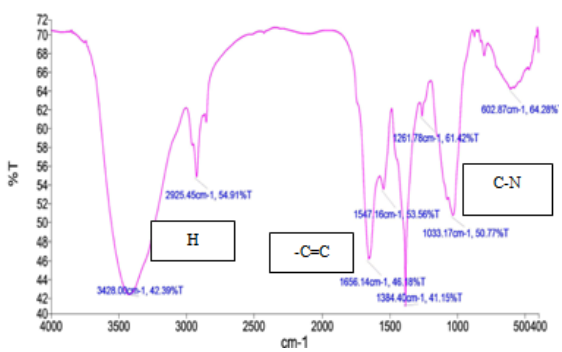
In case of coir pith sample, 3 bond stretches were prominent, (Fig – 3. a) CN- aliphatic amines, C-Cl- Alkyl halides and C-O-Carboxylic acids, esters, ethers, alcohols

Fig – 3. a: FTIR analysis on 30th day coir pith



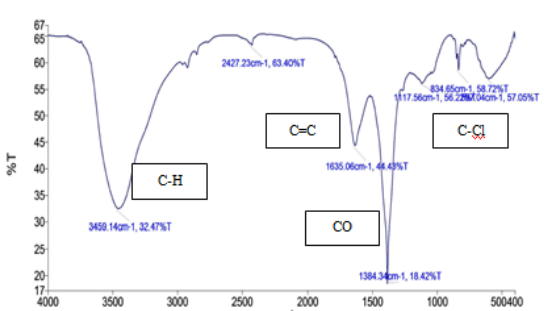
Whereas, in O. annae stretch bonds (Fig – 3. b) of H-Phenols, C=C – Alkenes and C-N- Aliphatic amines were evident

Fig – 3. b: FTIR analysis on 30th day O. annae



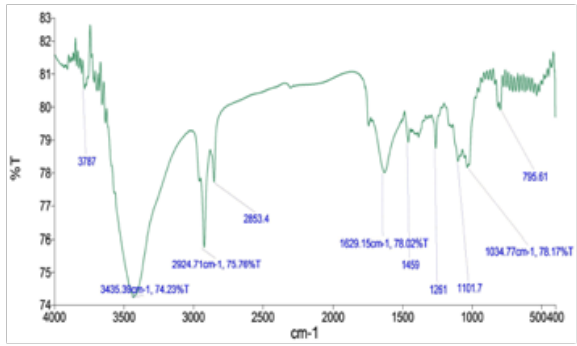
Additional one number of stretch bonds (Fig – 3. c) was demonstrated in supernatant (cyanospray) which are CH- Alkanes, -C=C- Alkynes, CO-Alcohols, and C-Cl-Alkyl halides

Fig – 3. c: FTIR analysis on 30th day supernatant (cyanospray)



Indeed, more number of bonds were observed (Fig – 3. d) in pellet (cyanopith) to all other samples i.e. O-H –Alcohols, H-Phenols, CH-Alkanes, C-Cl- Alkyl halides and C-N-Aliphatic amines respectively

Fig – 3. d: FTIR analysis on 30th day pellet (cyanopith)



This phenomenon could possibly be explained as a result of degradation process of coir pith by O. annae, more number of stretch bonds were visible in cyanopith than the individual samples. To the best of our knowledge, no reports are available on the above mentioned work done with coir pith. However, Xin Dong et al. (2011) analysed lignin extracts at 1740 cm^{-1} has similar amount of phenolic hydroxyl groups. According to Gosselink et al. (2004), the hydroxyl, methoxyl, carbonyl, and carboxyl groups are the most important chemical functional groups in lignin, and can be used for identification of lignin (Shamsuri and Abdullah, 2010).

4. Conclusion

O. annae was proficient in degrading coir pith with release of compounds which was examined by OD reading. Confocal microscopic view was implemented to observe intensity of the filaments in terms of growth and also FTIR studies confirmed degradation by exhibiting more functional groups in combined treatment when compared to control.

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