The Pharma Innovation Journal 2019; 8(7): 141-146

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(7): 141-146
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www.thepharmajournal.com
Received: 10-05-2019
Accepted: 12-06-2019

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FTIR analysis: Biodegradation of complex hydrocarbons using *Coriolus versicolor*

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Abstract
Industrial Pollution has gross adverse effects on biotic and abiotic components of environment. Organopollutants arising from petrochemical industries mainly constitute polyaromatic hydrocarbons, which are toxic and mutagenic. Control and treatment strategies include Old-school physicochemical treatments, which are cost intensive and may produce harmfulbyproducts. Offering high efficiency and low cost, bioremediation represents a significant approach for environmental clean up. These strategies involve microorganisms and utilize their metabolic capability to degrade organic compounds. In this study, degradation of complex hydrocarbons was studied using fungus, *Coriolus versicolor* in Shake flask experiment, using methylene blue as indicator dye. Results of dye decolorization assay and FTIR analysis indicated that *Coriolus versicolor* was capable of effectively degrading or removing the test compounds (Petroleum ether, DPA, PVP, Picric acid and Pthallic acid) used in the present study. The extent of decolorization recorded at wavelength 510nm, was taken to indicate fungal growth and FTIR analysis of culture supernatants showing altered peak profile with test compounds indicated possible breakdown to simpler structures.

Keywords: Fourier transform infra red (FTIR), poly aromatic hydrocarbons, *Coriolus versicolor*, industrial pollutants

Introduction
Bioremediation is the use of living organisms, primarily microorganisms, fungi and plants, to degrade the environmental pollutants into less toxic forms. Living organisms through reactions that are a part of their normal metabolism transform complex compounds. Biodegradation of a compound is often a result of the actions of multiple organisms [1]. For bioremediation to be effective, conditions have to be manipulated to permit microbial growth and activity. Complex hydrocarbons like PAHs are most widespread organic pollutant owing to their physical and chemical properties and are associated with recalcitrance, bioaccumulation and toxicity. Phenolic compounds are ubiquitous in aqueous streams of industrial processes including: petroleum refining, coking and coal conversion, chemical plants, foundries and pulp and paper plants [2,3]. The presence of these compounds in drinking and irrigation water represents a health and environmental hazard. Although conventional methods of dephenolisation do exist, they are often expensive, deliver incomplete purification, can form hazardous byproducts and are often applicable to only to a limited concentration range.
White-rot fungus has the ability to degrade a wide variety of structurally diverse organic compounds, including a number of environmentally persistent organ pollutants [4]. The unique biodegradative abilities of fungus appear to be dependent upon its lignin-degrading system. White-rot fungi (*Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Coriolus versicolor*) have been demonstrated to oxidise PAH's rapidly with their extracellular ligninolytic enzyme systems and therefore have raised interest in the use of these organisms for bioremediation of contaminated soils [5].
In this context, the present study reports degradation of Test aromatic hydrocarbons by the fungus *Coriolus versicolor* in shake flask experiment using dye decolorization and FTIR analysis as parameters.

Material and Methods
Revival of culture: Lyophilized culture of the fungus (MTCC-138) was procured from Institute of Microbial Technology (IMTECH), Chandigarh. The fungus *Coriolous versicolor*was revived and maintained as a slant culture using standard protocol.

Preparation of Broth: Potato nutrient broth was used. Broth was inoculated with the fungus culture [6].

Preparation of fungal mat by spread plate method: Potato dextrose agar (PDA) media was used. Spreading was done using standard protocol [6].

Morphological Confirmation of Fungal Strain: A portion of mycelia mat from the plate was used for morphological conformation using Lactophenol cotton blue stain and observed under compound microscope at 10X and 40 X magnifications [7].

Experimental Design for Biodegradation Studies (shake flask experiment):

Test Group: In autoclaved nutrient broth (100ml), added 3-4 discs of fungal mat and respective amounts of organic compound (picric acid/petroleum ether-5mg/100 ml ;PVP/DPA/Pthallic acid-2mg/100ml), 1ml of methylene blue dye and 1ml of tween 80. Flasks were kept at 27 ^{0}C in orbital shaker at 120 r.p.m for 7 days. 2ml of media was taken out daily, centrifuged and O.D. of supernatant was measured at 510nm. Graphs were plotted between absorbance at λ max 510nm and time. Aliquots from the test groups were stored at -20^{0}C and then used for FTIR analysis [8].

Control group: Parallel control flasks of media containing organic compounds incubated without fungus were taken.

FTIR analysis: Aliquots of samples from shake flask experiments that were lyophilized to a fine powder were analyzed by FTIR spectroscopy (Perkin Elmer-spectrum RX-IFTIR) with range 4000cm^{-1} to 250cm^{-1} at Central Instrumentation Laboratory of Panjab University, Chandigarh [9].

Statistical analysis: All values were recorded and represented as mean ± standard deviation (S.D) of 5-6 independent observations. For comparison of data for two groups (test and negative control) of biodegradation studies, student's t- test was performed.

Result and Discussions

Morphological Confirmation of Fungal Strain after Revival of Culture

Coriolus versicolor belongs to the class Basidiomycetes with the top surface of the growth shows typical concentric zones of different colors, 1–3 mm thick and leathery texture. Cap like growth with rust-brown or dark brown and sometimes blackish zones could be observed. The fungal hyphae were stained with lactophenol cotton blue. Hyphae were observed under 100X magnification giving blue appearance (Figure 1). Nucleus of hyphae was darkly stained with long tail. Similar kinds of studies by Wilson *et al*, 1993 [10] and some others [11] have reported the use of *Coriolus versicolor* in biodegradation and confirmed the morphology.

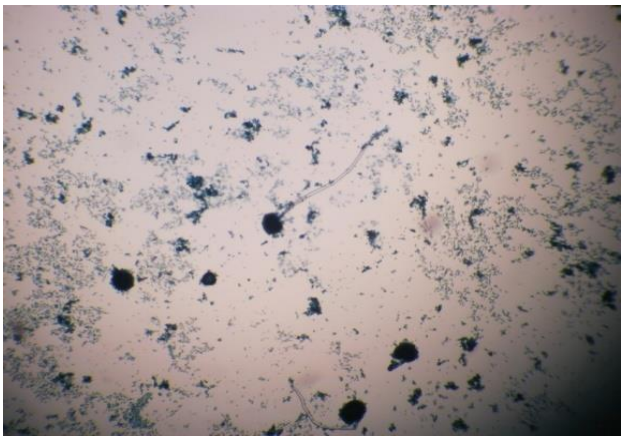
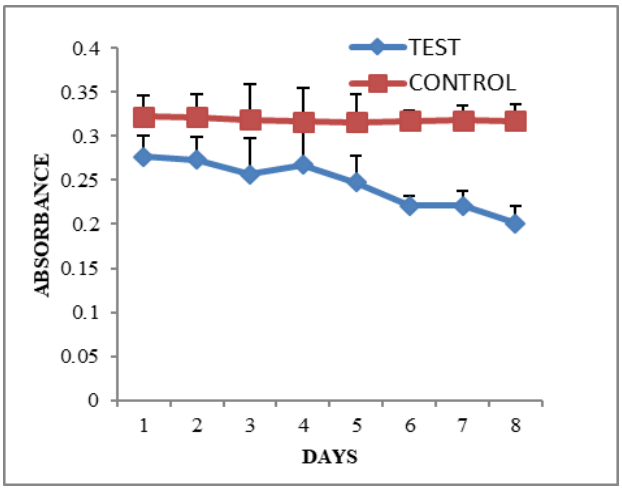


Fig 1: Fungal strain under 100X

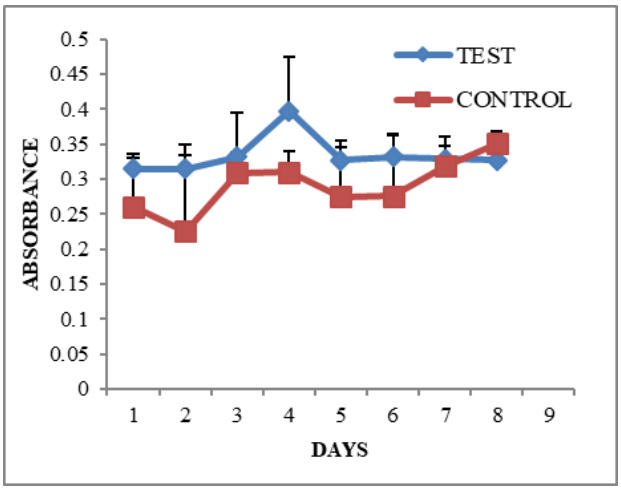
Degradation of organic compounds

Dye Decolorization in presence of test compounds (PVP, DPA, Picric acid, Pthallic acid and Petroleum ether):

Degradation of test compounds was studied in a culture flask experiment by inoculating the flask with 3-4discs of fully-grown fungal mat (72hours). Methylene blue dye (indicator) and Tween20 (surfactant) was also added to the culture flask.



A.



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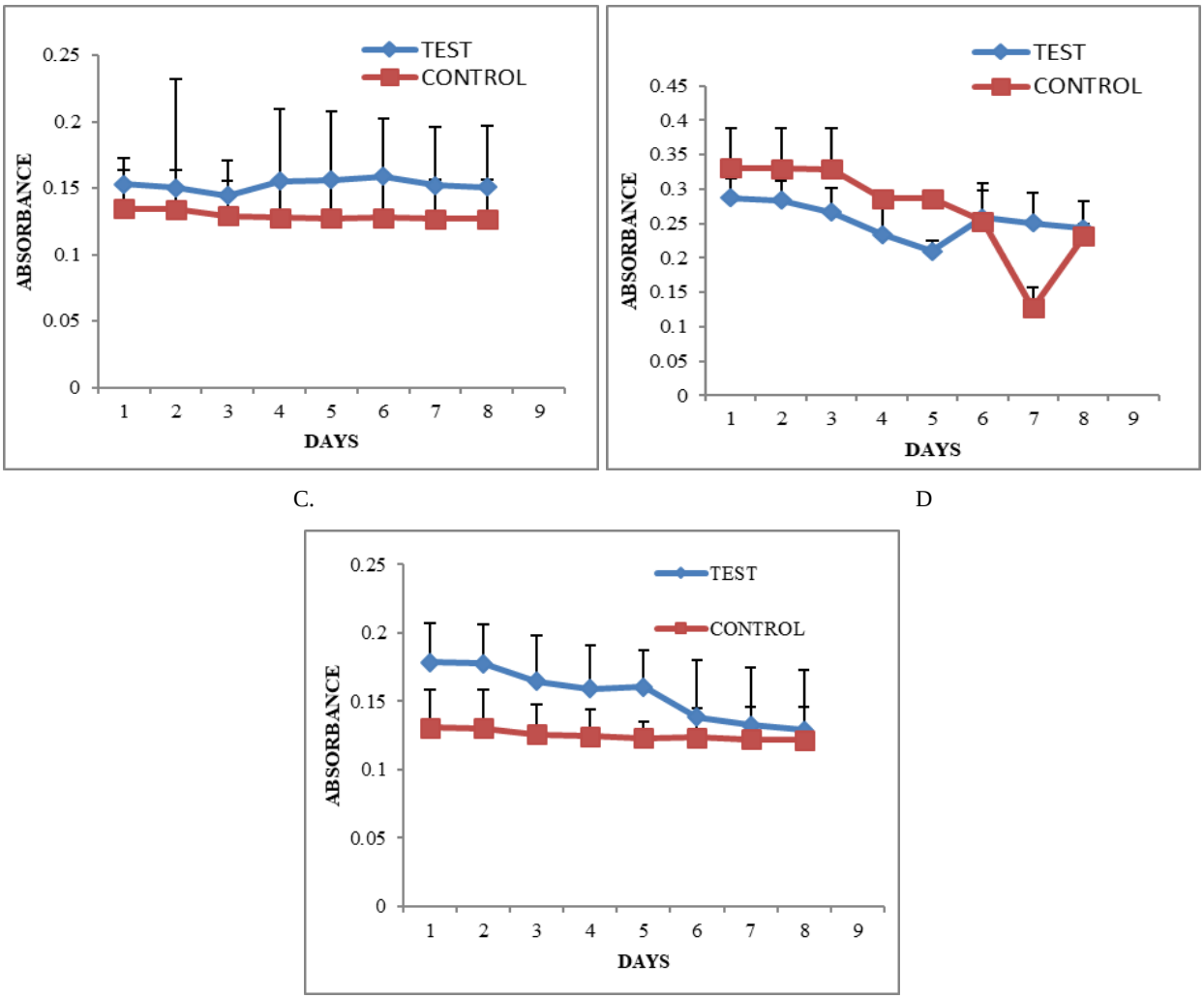
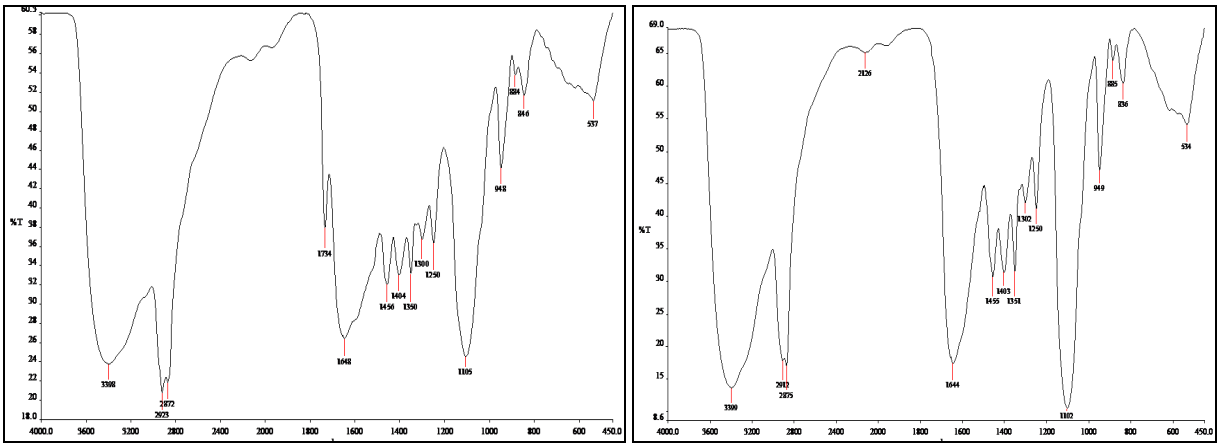


Fig 2: Characteristic absorbance vs. time graph showing degradation of (a) polyvinyl pyrrolidone (b) diphenylamine (c) picric acid (d) phthalic acid (e) petroleum ether indicated by decolorization of dye.

The medium turned turbid with time indicating fungal growth and decolorization of dye was observed. There was a significant visual difference in the decolorization of the dye but it was not significantly reflected in the absorbance when observed spectrophotometrically at the specific absorbance maxima (max510nm) (Figure 2). On 4th day, the absorbance value increased probably indicating that the fungus secreted some compounds (enzymes and metabolites). The absorbance value was found to decline on day 7 indicating that the fungus was allowed growth in the presence of organopollutants left over in culture media as also shown by decolorization of dye. Hence probably on day 7 the organic compound was reduced to non toxic levels or was broken to simpler compounds further indicating that the levels was no longer inhibitory to the growth of fungi. Biodegradation of petroleum ether/PVP were reported in various studies [12, 13], where hyphae penetration was taken to indicate degradation.

FTIR analysis

The FTIR studies were performed to analyze proposed transformation or degradation of various organopollutants in the presence of *Coriolus versicolor*.

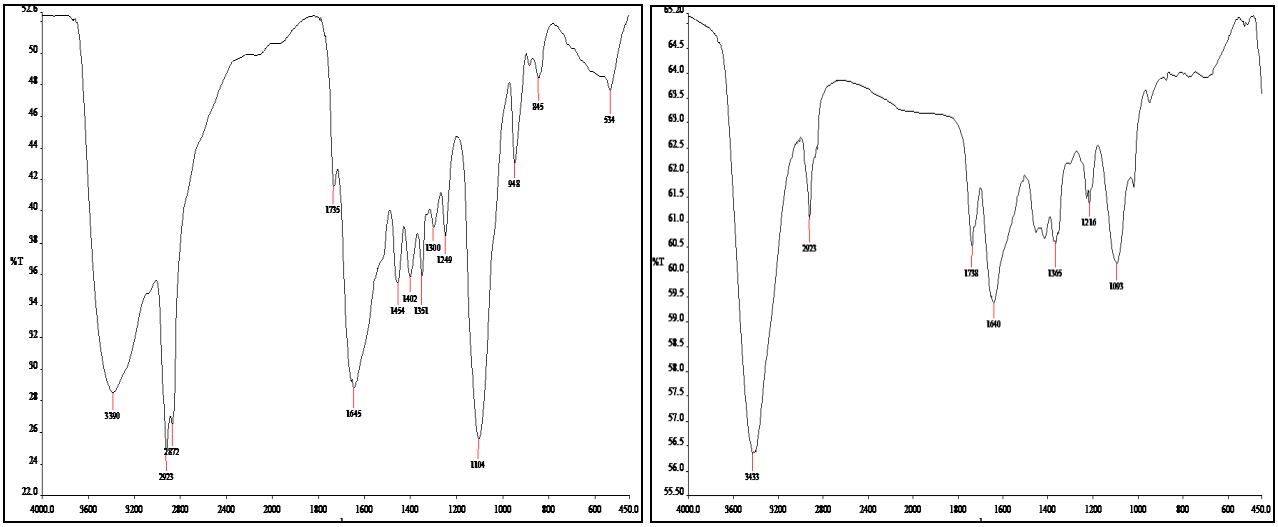


A. B.

Fig 3: FTIR spectrum of DPA (a) control (day1) and (b) test (day7)

FTIR spectrum of DPA control (day1) showed characteristic peaks at 3408cm^{-1} caused by heterocyclic aromatic amines and at 1644 cm^{-1} because of secondary amine N-H bond. A C-N stretch was observed at 1455 cm^{-1} representing primary amine whereas test group (Day 1) revealed peaks at 3398 cm^{-1},2921 cm^{-1},1633 cm^{-1}and 1101 cm^{-1}. Control group i.e. without fungus exhibited low % transmittance (high absorbance) values for some of the peaks indicative of compound (Figure 3). The % transmittance increased i.e. decreased absorbance was observed in test group (Day 1) where fungus was incorporated indicating physical removal of compound by fungal mycelia. DPA degradation studies analyzed by FTIR have been reported by Mecozzi, *et al* 2009 [9].

FTIR of un-degraded PVP control (Day 1) revealed prominent peak at 3390 cm^{-1} representing N-H stretch .While test group (Day 7) revealed peaks at 3343 cm^{-1}, 2493 cm^{-1},1738 cm^{-1},1640 cm^{-1},1365 cm^{-1},1216 cm^{-1},1093 cm^{-1} (Figure 4). Test group (Day 7) revealed peak at 3389 cm^{-1}which may be caused by normal polymeric –OH stretch. At 2872 cm^{-1} and 2921 cm^{-1} methylene C-H with symmetrical and asymmetrical stretch was observed. In test group (Day 7) C-O stretch was observed at 1640 cm^{-1}. Bhardwaj and Khan (2004) reported FTIR analysis of Polyvinyl chloride in which it was analyzed to be not broken down into simpler compounds [13].



A. B.

Fig 4: FTIR spectrum of PVP (a) test (day1) and (b) test (day7)

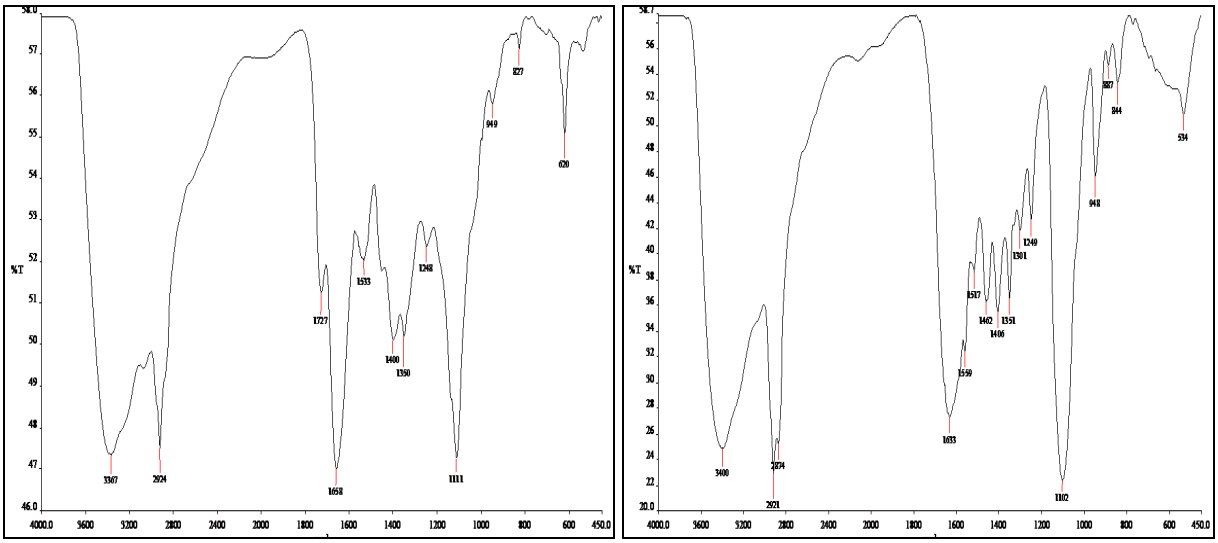
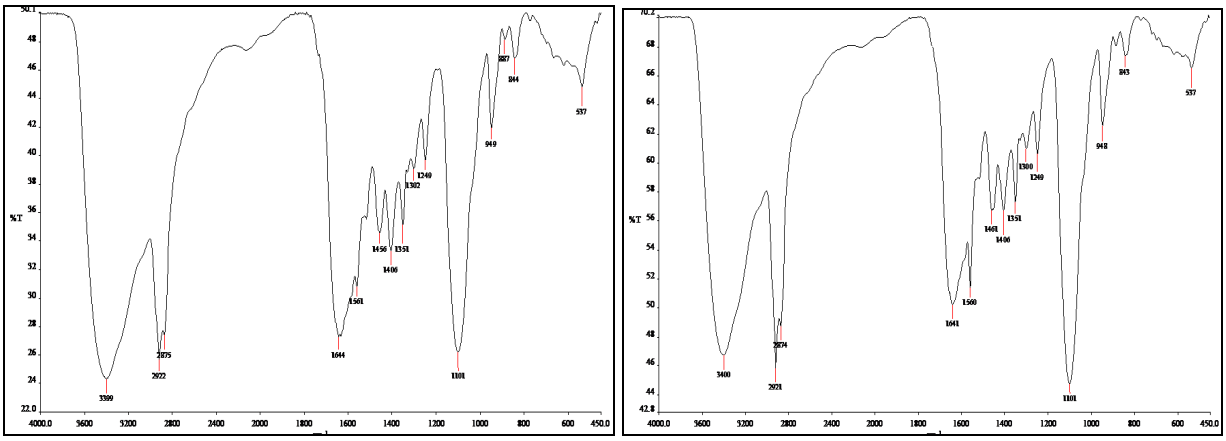


Fig 5: FTIR spectrum of Picric acid (a) control (day1) and (b) test (day7)

FTIR of undegraded Picric acid control (Day1) revealed prominent peak at 3367 cm^{-1} representing primary amines. N-H stretch with strong appearance. A –CH stretch (asymmetric/symmetric) was observed at~2830 cm^{-1} and 2305 cm^{-1} aromatic hydrocarbon-H in plane ring stretch was observed (Figure 5). The test group (Day1) revealed prominent peaks at 3408 cm^{-1}, 2923 cm^{-1},1732 cm^{-1},1660 cm^{-1} and 1535 cm^{-1} with low % transmittance i.e. high absorbance compared to peaks of Day 7 test group. The absorption at Day 7 are found to be~ 1480 cm^{-1} and 1180 cm^{-1} which are due to aromatic C=C absorption stretching vibrations compared with Day 1 having values ~1580 cm^{-1} and 1500 cm^{-1}.



A. B.

Fig 6: FTIR spectrum of pthalic acid (a) control (day1) and (b) test (day7)

FTIR spectrum of Phthalic acid as present in culture media at the start of degradation experiment showed prominent peaks at 3434 cm^{-1},2926 cm^{-1},2100 cm^{-1},1642 cm^{-1},1101 cm^{-1} in case of Test group (Day1) while Test group (Day7) showed characteristic peaks at 3400 cm^{-1}, 2921 cm^{-1},1641 cm^{-1}, 1101 cm^{-1}. Test group (Day 1) showed a peak at 1457 cm^{-1} representing C-C stretch. A bending vibration of Carboxylic acid was observed at 1300 cm^{-1} on Day 7 of Test group (Figure 6). Bending mode of phenolic group was observed from 1304 cm^{-1} - 1352 cm^{-1} in case of Test group (Day1). Similar studies were also reported by Bhat *et al*(2011) who studied the analysis at different concentrations and pH [14].

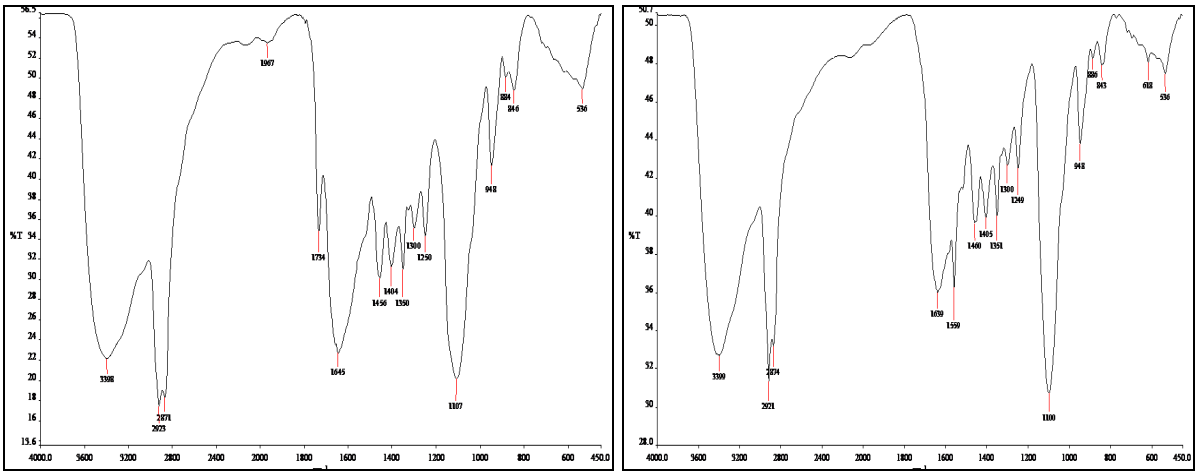


Fig 7: FTIR spectrum of petroleum ether (a) control (day1) and (b) test (day7)

FTIR of Petroleum ether control (Day1) revealed prominent peak at 2923 cm^{-1} representing primary amine, N-H with strong appearance. A C-H stretch (symmetric/ asymmetric) was observed at 2871 cm^{-1} and 1645 cm^{-1} aromatic hydrocarbon, C-H in a plane ring stretch was observed (Figure 7). The test group (Day1) revealed prominent peaks at 3399 cm^{-1}, 2921cm^{-1}, 1645 cm^{-1}, 1104cm-1 with high % transmittance i.e. low absorbance as compared to peaks of test group (Day7) which appeared at low % Transmittance .Similar studies have also been reported by Olfat & Karimi (2005) in which degradation of crude oil was observed in presence of *Coriolus versicolor* [12]. The vibrational spectrum of a molecule is considered a unique physical property and is characteristics of the molecules as such; the IR spectrum can be used as a fingerprint for identification, by comparison of spectrum of unknown with previously recorded reference spectra [15].

The present study indicated that fungus *Coriolus versicolor* has the potential to degrade organic compounds as indicated by dye decolorization using spectrophotometric analysis. The fungal growth turned the media turbid with time and decolorization of dye was observed. Decrease in absorbance value with the growth of fungi indicated reduction of organic compound to non –toxic levels or break down to simpler compounds. Supporting evidence for biodegradation of compounds came from FTIR analysis of various groups, where a change in the peak intensities and patterns was reflective of bioconversion/removal. Moreover, FTIR spectroscopy can be a very useful tool in performing preliminary tests in order to predict remediation performance to select an appropriate approach for clean- up technologies.

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