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Assessment of biodegradability of PVC containing cellulose by white rot fungus

Fakhrul Hassan, Fazal Akbar, Farooq Rashid, Sohaib Roomi, Abdul Ghafoor, Muhammad Suleman*

Center for Biotechnology and Microbiology, University of Swat, Pakistan.
Email: suleman@uswat.edu.pk; sulemanspk@yahoo.com

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ABSTRACT

Aims: Polyvinyl chloride (PVC) is the most widely used and environmentally damaging plastic. Processing, production and disposal of PVC cause release of toxic chlorine based compounds into environment. The objective of the present study was to assess the biodegradability of cellulose blended PVC by white rot fungi i.e. *Phanerochaete chrysosporium*.
Methodology and results: Biodegradability of the strain for the polymer was tested on plate assay, sturm test, soil burial and shake flask experiments. The biodegradability of the polymer was determined by visual changes, plate assay and carbon dioxide production. Morphological changes in the polymer such as pits, extensive spotting, clear surface erosion, fungal attachment, roughening and deterioration of some parts were observed using scanning electron microscopy. Chemical changes like appearance and shortening of peaks using fourier transform infrared spectroscopy also confirmed the biodegradability of the polymer.
Conclusion, significance and impact of study: The present study confirmed that mixing of small amount of cellulose increases the hydrophilicity of the polymer and lead to its microbial degradation and *Phanerochaete chrysosporium* has great potential for the treatment of solid waste containing plastics.

Keywords: Persistent organic pollutants (POPs), Polyvinyl chloride (PVC), Tetrahydrofuran (THF), white rot fungi (*Phanerochaete chrysosporium*).

INTRODUCTION

Pure Polyvinyl chloride (PVC) is usually unstable to heat and light, but mechanically tough and fairly good weather, water and chemicals resistant (Gartiser *et al*., 1998). PVC is very necessary for construction of market, the largest volume of PVC markets are insulated wire and cable; and packaging, including film, sheet, and bottles (Molinaro, 1999). The use of plastic has many disadvantages as they resist degradation and do not undergo degradation in natural conditions. However, in the process of incineration of wasted PVC, they not only release carbon dioxide (CO_2) and dioxins that cause pollution and global warming but also increase waste disposal and land filling (Huang, 1995). PVC plastics produces persistent organic pollutants (POPs) known as furans and dioxins (Jayasekara *et al.*, 2005). Dioxins are highly toxic and bio-accumulates which make them very dangerous for human health (Birnbaum *et al.*, 2003). Many studies have reported thermal and photo degradation of PVC but reports regarding biodegradation of PVC is very few in number (Kirbas *et al.*, 1999). White-rot fungi are the member of Basidiomycotina, that are able to degrade a wide variety of environmentally persistent xenobiotics and organopollutans to CO_2. To degrade these compounds (xenobiotics and organopollutans) a nonspecific and nonstereo-selective lignin degrading system is required. The lignin degrading system consists of a group of peroxidases commonly known as ligninases, which catalyze the initial oxidative depolymerization of lignin polymer (Shah *et al.*, 1992). Low molecular weight PVC can be exposed to biodegradation by the use of white rot fungi. The polymer surface deterioration is an interfacial process and microorganisms have the ability to colonize the polymer surfaces as biofilms. These biofilms consist of cells embedded in a polymer matrix of their own origin, containing polysaccharides and proteins (Costerton *et al.*, 1987; Flemming, 1998; Kirbas *et al.*, 1999). The physical and chemical degradation may be enhanced by the additives added in the polymer. The mixing of small amount of cellulose can bring some changes in the polymer properties and lead to its microbial degradation (Kaczmarek and Bajer, 2007). The aim of this study is to determine the adherence and degradation capability of *Phanerochaete chrysosporium* to cellulose blended PVC films in soil burial experiment, liquid and solid media. Besides, observation of the morphological changes in cellulose blended PVC biodegradation is also carried out using plate test and scanning electron microscopy (SEM). The changes in the functional groups during

***Corresponding author**

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biodegradation of blended polymer by fourier transform infrared (FTIR) spectroscopy are also observed in this study.

MATERIALS AND METHODS

Sample preparation

Cellulose blended PVC films were prepared by standard method as described by Calil *et al.* (2006) and the films were sterilized by 70 % ethanol for 20 min.

Soil burial experiment

The cellulose blended PVC films were washed and buried in the garden soil in pots for four months, inoculated with 15 mL of spore suspension of *P. chrysosporium* which were taken from the culture having ability to adhere and degrade the polymer film. Spore suspension was prepared in mixture that consists of 50 uL Tween 20 and 100 mL of distilled water. Morphological and chemical changes in the film were analyzed after four months of soil burial by scanning electron microscopy (SEM) (samples were coated for 30 sec in sputter coater SP1-Module TM. Then images were taken using JEOL JSM-5910 and FTIR spectroscopy (The polymer pieces were mixed with KBr and pellets were prepared and fixed to the FTIR sample plate. Spectra were taken at 400 to 4000 wave-numbers cm^{-1} for each sample.

Shake flask experiment

Three pieces of cellulose blended PVC films (1 cm^2) were incubated in each flask with the spore suspension of *P. chrysosporium* in shaking condition. Mineral salt media (MSM) was used per 1000 mL contained in distilled water, K_2HPO_4, 1 g; KH_2PO_4, 0.2 g; NaCl, 1 g; $CaCl_2{\cdot}2H_2O$, 0.002 g; boric acid, 0.005 g; $(NH_4)_2SO_4$, 1 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; $CuSO_4{\cdot}5H_2O$, 0.001 g; $ZnSO_4{\cdot}7H_2O$, 0.001 g; $MnSO_4{\cdot}H_2O$, 0.001 g and $FeSO_4{\cdot}7H_2O$, 0.01 g, inoculated with spore suspension of *P. chrysosporium* and incubated at 30 °C for one to three months. After completion of experiment polymer samples were isolated and analyzed by SEM.

Sturm test

Cellulose blended PVC biodegradation to evolve CO_2 was determined by sturm test (Muller, 1992). The culture bottles containing 285 mL of MSM lacking carbon source were inoculated by three pieces of PVC film. The fungi (*P. chrysosporium)* were used as inoculum in both test and control bottles (without plastic). The sterilized air was supplied continuously and reaction bottles were stirred continuously to provide the aerobic condition. After 30 days, the gravimetric analysis of CO_2 production was carried out by trapping the gas in adsorption bottle containing (1 M) KOH. After titration with (1 M) barium chloride solution of both test and control the precipitates formed were filtered, weighed and calculated for CO_2 produced/liter.

Plate assay

About 100 mL of MSM was prepared in 250 mL flask and then 2 g of technical agar was added to the media and autoclaved. The media was poured into Petri plates inside the hood. After solidification of media, three pieces of cellulose blended PVC were put on the film on each petri plate. The spores of *P. chrysosporium* were inoculated on the test plates with the help of loop and left the control plates without inoculation. Then the plates were incubated at 30 °C for two months. After two months polymer samples were analyzed by SEM.

RESULTS

Analysis by scanning electron microscopy (SEM)

Soil burial experiment

The surface of cellulose blended PVC film totally changed as compared to control after four months of soil burial experiment. The test film was discoloured and some hyphal growth were visible to the naked eye on the surface of the films, however, some cracks, pits, erosion and a clear breakdown was also visible when analyzed by SEM (Figure 1a and 1b).

Shake flask experiment

The surface features of cellulose blended PVC films in shake flask experiment of one and three months samples were analyzed by SEM. In the treated samples there was erosion, extensive roughening, holes and breakdown of some parts when compared to the surface of control (Figure 2a, 2b and 2c). The SEM analysis of the cellulose blended PVC films in plate assay also showed changes in the structure of the film after treatment with *P. chrysosporium*. There were erosion, roughening, holes, breakdown of the some parts and clear hyphal growth. The formation of clear zones around the colonies is an indication that the polymer is hydrolyzed by the enzyme into water-soluble products (Figure 3a and 3b).

Analysis by strum test

The total amount of CO_2 produced in test and control was 19.74 g/L and 10.86 g/L respectively. The CO_2 produced after mineralization of polymer for 30 days was found to be 8.88 g, which showed remarkable degradation of the polymer in test.

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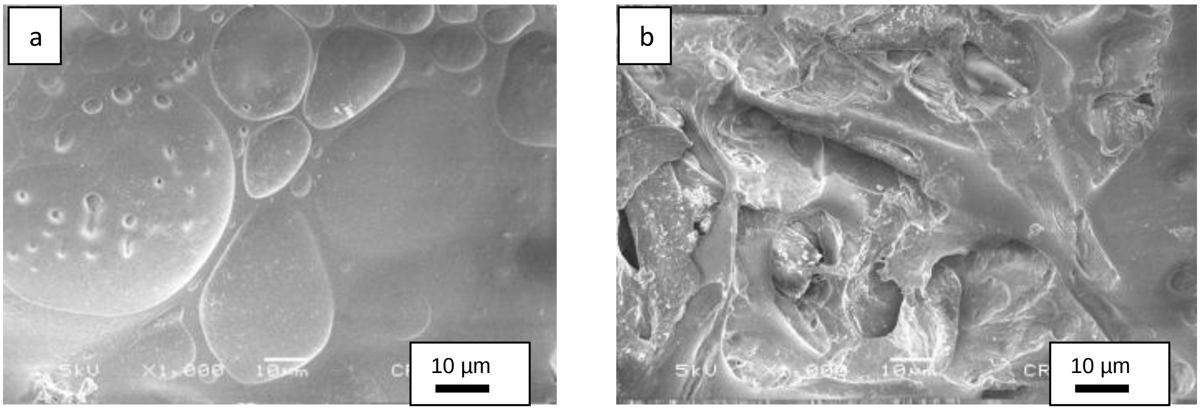

Figure 1: (a) Scanning electron micrographs of the untreated (control) cellulose blended PVC films after 4 months soil burial experiments at 1000X; **(b)** Scanning electron micrographs of the cellulose blended PVC films, treated with *P. chrysosporium* after 4 months soil burial experiment at 1000X.

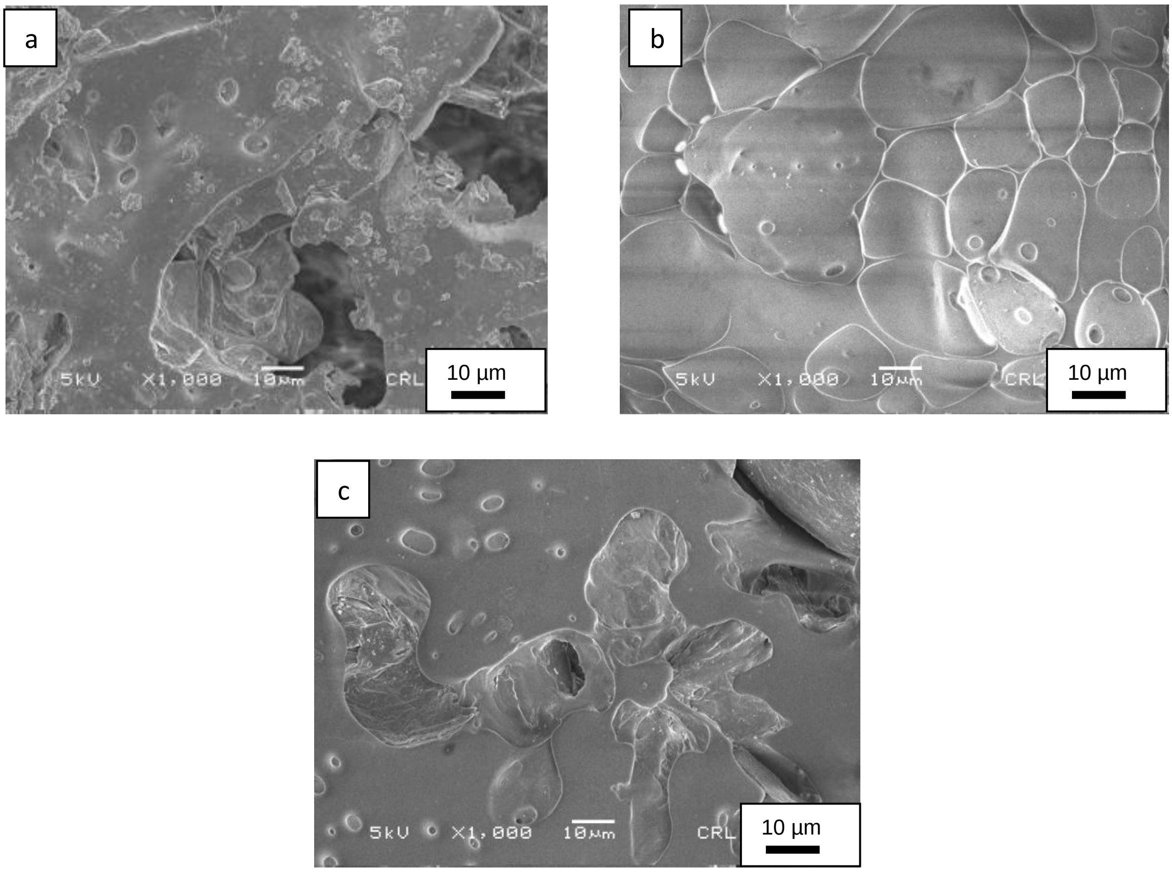

Figure 2: (a) Scanning electron micrographs of the cellulose blended PVC films treated with *P. chrysosporium* after 1 month shake flask experiment at 1000X; (b) Scanning electron micrographs of the untreated (control) cellulose blended PVC films after 3 months shake flask experiment at 1000X; (c) Scanning electron micrographs of the cellulose blended PVC films treated with *P. chrysosporium* after 3 months shake flask experiment at 1000X.

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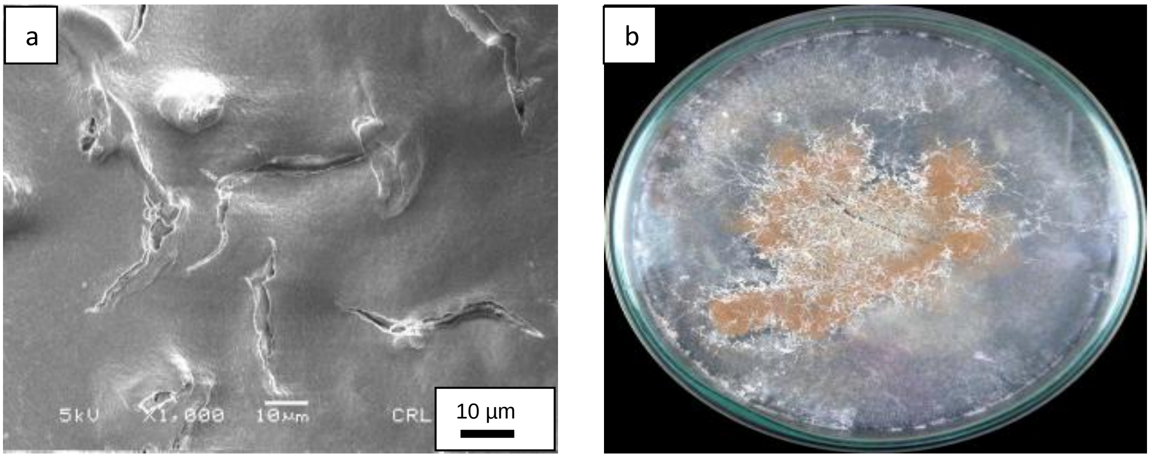

Figure 3: (a) Scanning electron micrographs of cellulose blended PVC films treated with *P. chrysosporium* after 2 months MSM agar plate assay at 1000X; (b) Fungal adherence and attachment on cellulose blended PVC films in plate assay.

Analyses by Fourier Transform Infrared spectroscopy shake flask experiment

FTIR analysis of the treated PVC film showed few changes in sample spectra as compared to control spectrum. The peak at wavelength 3449 cm^{-1} was present in control which was absent in test sample and contribute to NH stretches. A sharp decrease in intensity of characteristic peak at wavelength 2911 cm^{-1} was observed in the test sample, which attributed to change on the surface. The peak at wavelength 1195 cm^{-1} was totally disappeared in the test sample which was present in the control spectrum that attribute to C=O methane hydrogen bonding confirming the degradation pattern in the PVC film (Figure 4 a and b).

DISCUSSION

The list of pollutants which pose environmental and health hazard and are tough for biodegradation, is long one and includes solvents, wood preservative chemicals, plasticizers, refrigerants, coal tar wastes, pesticides, biphenyls, polychlorinated and polybrominated biphenyls, synthetic fibers, plastics, PVC, polystyrene, detergents like alkyl-benzene sulphonates and oils.

The objectives of the present study were to assess the degradation potential of the white rot fungus i.e. *P. chrysosporium* in the laboratory. The detailed investigation of the fungus adherence and degradation of the cellulose blended PVC was carried out.

Fungi play an important role in biodegradation due to its ability to secrete extracellular enzymes (Bennett and Faison, 1997). The fungal growth on polymer surface is not necessarily in accordance with other assessment methods for quantitative analysis of polymer biodeterioration, because fungal growth on polymer surface can be even or invisible. Degradation of plastic material did not related with colonization of films by microorganisms (Whitney, 1976). In our study the visual observations showed changes in the colour and appearance of the cellulose blended PVC film surface in soil burial, shake flask experiment and plate assay. The fungal hyphal growth was also observed on the surface of cellulose blended PVC films after soil burial experiment. Our results are similar to the study of (Kaczmarek and Bajer, 2007). Changes in the colour, appearance and fungal hyphal growth were also observed in PVC/cellulose sample with naked eye. Coulthwaite *et al*. (2005) also reported that bacterial and fungal growth on the polymer surface could bring the discoloration of PVC samples due to microbial spoilage. Dale and Squirrel (1990) also showed the polyester discolouration during soil burial experiment. All these studies correlate with our results and showed that the attachment of microbes to the polymer surface cause change in their colour and appearance.

In the present study SEM micrograph demonstrated changes in physical structure of the cellulose blended PVC when they were exposed to the *P. chrysosporium* strain in soil burial, shake flask and plate assay experiment. There were notable changes on the surface of treated cellulose blended PVC film after treatment showing initial pattern of degradation of the cellulose blended PVC film. After biodegradation, the appearance of numerous holes, cracks, pits, erosion and changes in the surface structure of treated samples of cellulose blended PVC was observed by comparing to untreated samples. Our results are similar to the work of Kaczmarek and Bajer (2007), Martin- Franchetti *et al*. (2007) and Zhao *et al.* (2005) they also reported cracks, pits, erosion and holes on the eroded film surface through scanning electron microscopy.

Sturm test was commonly employed for evaluation of the biodegradability of polymer materials (Muller *et al.*, 1992; Domb *et al*., 1997; Calmon, 2000; Hamid, 2000). In the present study sturm test or CO_2evolution test was used to check the biodegradability of cellulose blended PVC by *P. chrysosporium*. In this test different

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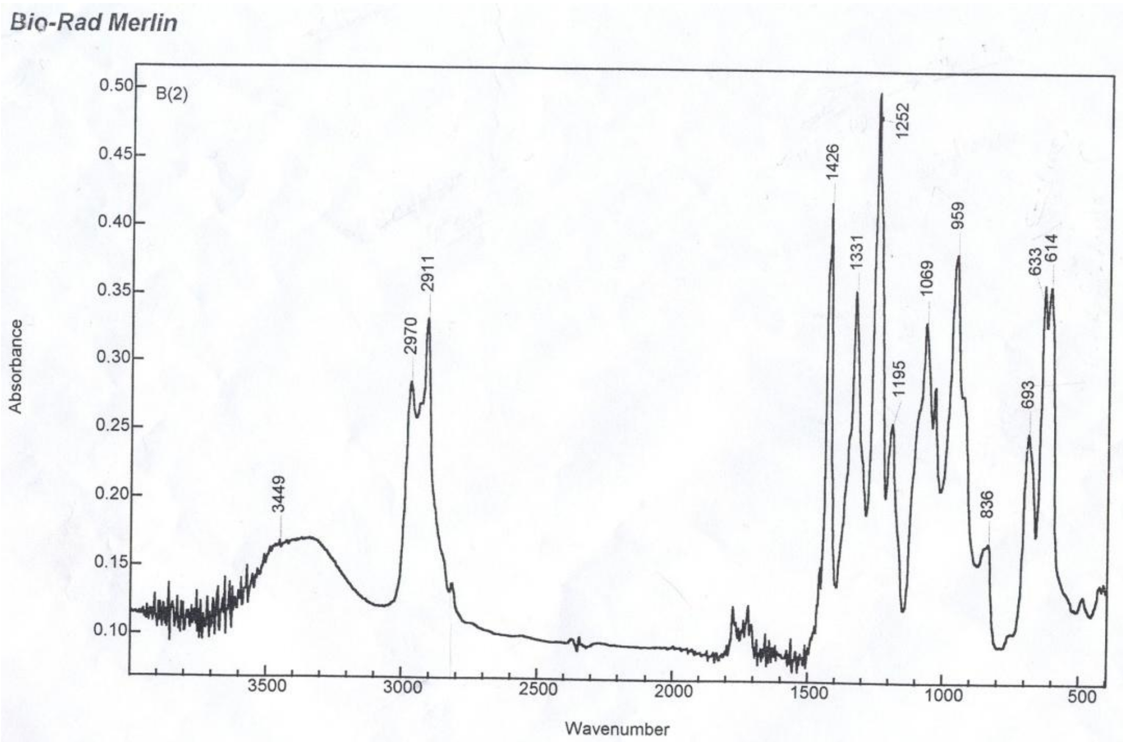

Figure 4 (a): Fourier transform infra red spectra (FTIR) of control PVC.

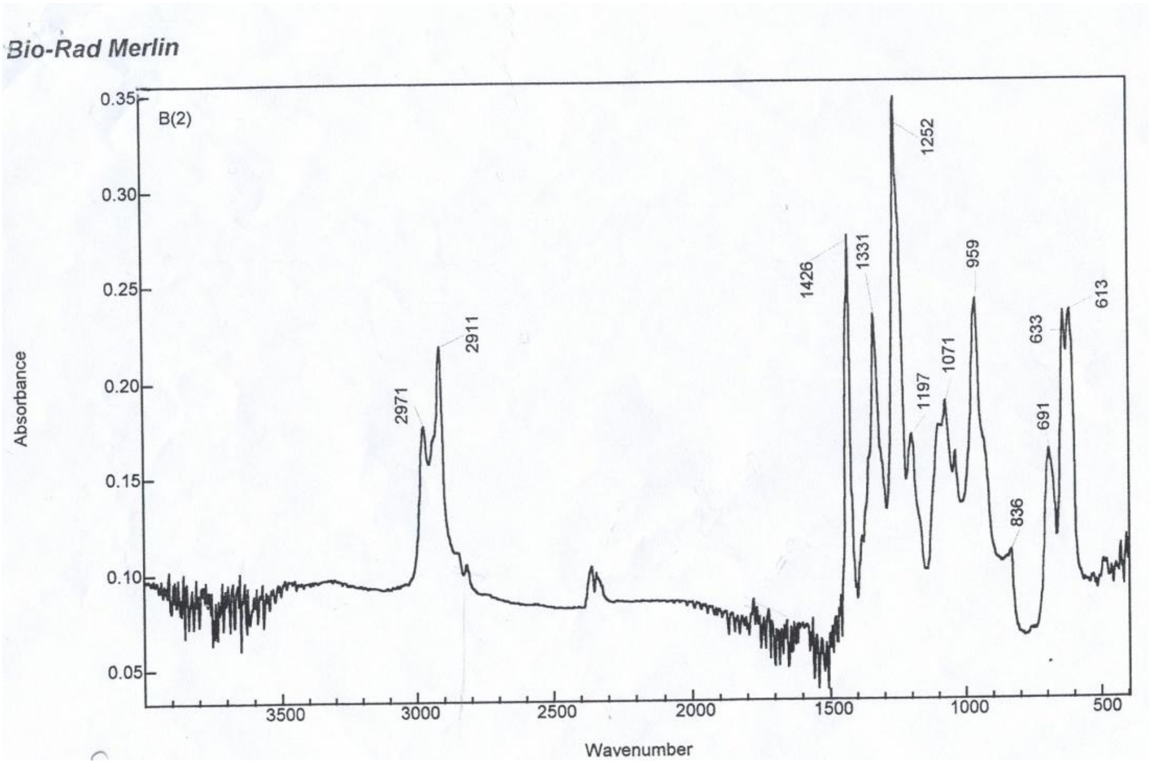

Figure 4 (b): Fourier transform infra red (FTIR) spectra of PVC after treatment.

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amount of $CO_2$was produced in test and control bottles due to biodegradation of the polymer. The amount of CO_2 produced in test and control bottles was 19.74 g/L and 10.86 g/L respectively which is in agreement to the previous study done by (Ali *et al*., 2009) who recorded that amount of CO_2 produced in test and control bottles was 21.28 g/L and 11.07 g/L respectively.

In the present study FTIR results showed an agreement to the work done by Sombatsompop *et al.* (2004), who examined the structural changes in the melt blends of PVC, while similar results were also found by Kaczmarek and Bajer, 2007, who reported that biodegradation of PVC brought some structural changes in the FTIR spectra of the polymer. Our results also show similarities with the data of Amanda *et al.* (2001). Okaya & Ikari, 1992, also reported that the FTIR spectra of poly (vinyl alcohol) showed a sharp decrease in the bands and peaks of the polymer. Our results are also in agreement with Ali*et al.* (2009).

CONCLUSION

The fungal strain *P. chrysosporium* showing adherence and growth on the surface of cellulose blended PVC films indicated their ability to utilize the polymer as a carbon source. Production of CO_2 in sturm test indicated positive degradability test. The changes in the peaks of the FTIR spectra of the test samples as compared to control is an indication of breakdown of polymer. SEM indicated the changes in surface of the treated films. It is concluded from the results that *P. chrysosporium* has great potential to be used for treatment of solid waste containing plastics.

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