INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–175/AWB/2009/11–5–577–580
http://www.fspublishers.org

Full Length Article

Studies on Biodegradation of Cellulose Blended Polyvinyl Chloride Films

MUHAMMAD ISHTIAQ ALI[1], QAISER PERVEEN, BASHIR AHMAD, IMRAN JAVED, RAJA RAZI-UL-HUSSNAIN†, SAADIA ANDLEEB, NAIMA ATIQUE, PIR BUX GHUMRO, SAFIA AHMED AND ABDUL HAMEED
Department of Microbiology, Quaid-i-Azam University, Islamabad 45320, Pakistan
†Pakistan Scientific and Technological Center, Islamabad, Pakistan
[1]Corresponding author's e-mail: ishi_ali@hotmail.com

ABSTRACT

Wider applications of the plastics in packaging and agriculture, has raised serious issue of plastic waste disposal and its pollution. The synthetic plastics normally resist the microbial degradation and persist in the environment for longer times. The objective of this study was the isolation of the fungal strain having ability to adhere and degrade the cellulose blended polyvinyl chloride films. The films were buried in the soil mixed with municipal sewage sludge for six months. Two fungal strains PV1 and PV2 showed adherence on the surface of the Cellulose blended PVC film. The strain PV1 identified as *Phanerochaete chyrosporium*, was more efficient then the other in de-grading cellulos blended PVC films and was chosen for further studies. The biodegradability of plastics was determined by visual changes in the polymer, plate assay and Carbon dioxide production. The chemical changes like appearance and shortening of peaks using fourier transform infrared spectroscopy (FTIR) also confirmed the degradability of the polymer.

Key Words: PVC; Biodegradation; Strum test; FTIR

INTRODUCTION

Polyvinyl chloride has been extensively used for a broad range of applications due to its safety, effectiveness, manufacturing technology and cost second only to low-density polyethylene (Fauvarque, 1996). There is disadvantage of the use of the plastics as they resist to degradation and not undergoes degradation in natural condition. They not only increase the waste disposal and land filling but also release CO_2 and dioxins by burning, which cause pollution and global warming (Huang, 1995). There are many studies about thermal and photo degradation of PVC (Owen, 1984; Braun & Bazdadea, 1986) but there only few reports available on biodegradation of PVC (Kirbas *et al*., 1999).

The additives added in the polymer may enhance the physical and chemical degradation. The mixing of small amount of cellulose can bring some changes in the polymer properties and lead to its microbial degradation (Kazmarek & Bajer, 2007). Adhesion represents the first stage of the colonization process (Christensen *et al.,* 1995). Studies have been carried out on the microbial colonization and deterioration of pPVC by bacteria (Booth *et al.,* 1968) and fungi (Webb *et al.,* 2000). The biodegradation of the pPVC and its blends with cellulose was reported by (Kazmarek & Bajer, 2008). The aim of the present study was isolation and identification of the microbial strains having potential to degrade Cellulose blended PVC film.

MATERIALS AND METHODS

Sample preparation. Cellulose blended Polyvinyl chloride (PVC) films were prepared by casting in Petri plates. Polyvinyl chloride and cellulose (1:1) solution in chloroform was sonicated for 2 h in an ultrasonic water bath (35 KHz, 285W) and left overnight to dry in Petri plate. The films (6 × 2.5 cm) were sterilized by dipping it into 70% ethanol for 20 min (Calil *et al.,* 2006).

Soil burial treatment. The replicate pieces of cellulose blended PVC films (6 × 2.5 cm) were buried in the garden soil in pots for three months, inoculated with the sewage sludge for the isolation of microbial strains having ability to adhere and degrade the polymer film.

Shake flask experiment. Cellulose blended PVC films were incubated with the isolated microbes from soil burial experiments in shaking condition. Mineral salt media (MSM) used per 1000 mL contained in distilled water were; K_2HPO_4, 1 g; KH2PO4, 0.2 g; NaCl, 1 g; $CaCl_2.2H_2O$, 0.002 g; boric acid, 0.005 gm; $(NH_4)_2SO$, 1 g; $MgSO_4.7H_2O$, 0.5 g; $CuSO_4.5H_2O$, 0.001 g; $ZnSO_4.7H_2O$, 0.001 g; $MnSO_4.H_2O$, 0.001 g and $FeSO_4.7H_2O$, 0.01 g. Cellulose blended PVC film (3 pieces) in MSM (90 mL) were inoculated with 10 mL of spore suspension ($10 \pm 2.1 \times 10^6$ spores mL -1) and incubated at 30°C for 3 months. After every 4 weeks polymer samples were retrieved and evaluated visualy and with infrared spectroscopy measured on Bio-Rad Merlin FTIR (Excaliber Series FTS 3000 MX, USA).

To cite this paper: Ali, M.I., Q. Perveen, B. Ahmad, I. Javed, R. Razi-Ul-Hussnain, S. Andleeb, N. Atique, P.B Ghumro, S. Ahmed and A. Hameed, 2009. Biodegradation studies of cellulose blended polyvinyl chloride films. *Int. J. Agric. Biol.,* 11: 577–580

Sturm test. CO_2 evolution as a result of cellulose blended PVC biodegradation was determined by sturm test (Muller *et al.,* 1992). The pieces of polymer were added to culture bottles containing MSM (285 mL) without any carbon source. Spore suspension of *Phanerochaete chyrosporium* PV1 (2.9×10^6 spores mL^{-1}) was used as inoculum 5% (v/v) in test and control bottles (without plastic). Sterlized air was supplied to keep conditions aerobic and reaction bottles were stirred continuously by placing them on magnetic stirrer. After 30 days, gravimetric analysis of CO_2 production was done by trapping the gas in adsorption bottle containing KOH (1 M). The precipitates formed after titration with barium chloride solution (1 M) of test and control were filtered, weighed and calculated for CO_2 produced per liter.

RESULTS AND DISCUSSION

Soil burial experiment. The Cellulose blended Polyvinyl chloride films were buried in sewage sludge soil for six months for isolation of degrading microbe. (Otake *et al.,* 1995) examined several polymers that had been buried under soil for more than 32 years. It was found that a remarkable degradation was indicated for low-density polyethylene thin films, which were in direct contact with sewage sludge. Soil burial has been reported previously for PVC and pPVC biodegradation (Domb *et al.,* 1997; Hamid, 2000).

Plate assay. Cellulose blended PV films were incubated on mineral salt agar medium inoculated with isolated fungal strains. Only the growth of strain PV1 was observed on the film (Fig. 1). The formation of clear zones around the colonies is an indication that the polymer is hydrolyzed by the enzyme into water-soluble products (Nishida & Tokiwa, 1993). The surface of the PVC Cellulose blend film totally changed as compared to control after six months soil burial experiment. The samples were discolored and some hyphal growth was visible to the naked eye on the surface of the films however some cracks and film erosion was also visible (Fig. 2). Our results are in accordance with the (Labuzek *et al.,* 2003; Kazmarek & Bajer, 2007).

Identification of the microbes. The washed pieces of cellulose blended Polyvinyl chloride buried in soil were taken and immersed in mineral salt liquid medium. A fungal strain was found attached on the Cellulose blended Polyvinyl chloride film pieces after soil burial for 6 months. The fungal strain was identified as *Phanerochaete chyrosporium* PV1 on the basis of microscopic examination and morphologic characteristics.

Macroscopic features of white rot fungus. The colonies of PV1 were white color with serrated edges. The flat fruiting body appeared like a crust on the plate surface. Colonies were small initially relatively smooth surfaced but later developed a weft of aerial mycelium. The color of mature sporulated aerial mycelium was pinkish white (Fig. 3).

Microscopic examination. The spore staining of white rot fungus by malachite green showed that the spores, which are of round shaped and have hyphae, which are clearly visible. Microscopic examination of 2-weeks mycelial mats typically revealed simple septate hyphae ranging from 3-9 μm in diameter with sparse to moderate branching as well as the presence of thick-walled terminal. They have simple septate hyphe (Fig. 4).

Fig. 1. Growth of fungal isolate PV1 on cellulose blended PVC film

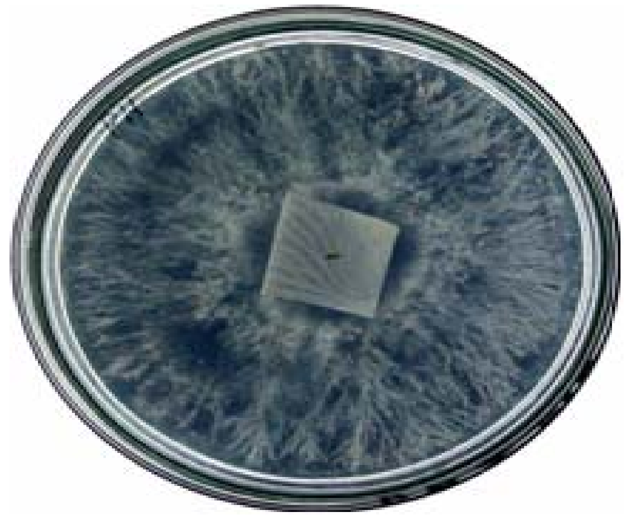


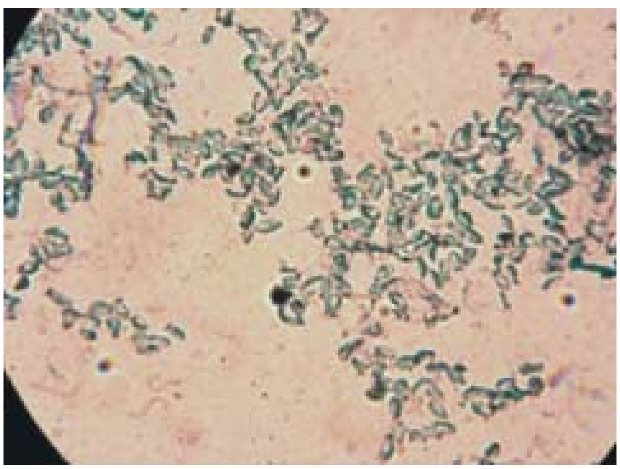
Fig. 2. Fungal adherence and attachment on the surface of cellulose blended PVC film after six months soil burial experiment

Analysis by strum test. Gravimetric analysis of CO_2 produced in test and control was 21.28 and 11.07 g L^{-1}, respectively. The CO_2 produced after mineralization of polymer for 30 days was found to be 10.21 g, which showed significant degradation of the polymer. The result is in agreement with the studies done by Sielicki *et al.* (1978). There was also a significant change in the dry cell mass of *Phanerochaete chyrosporium* PV1 in test higher (0.136 mL^{-1}) than in control (0.056 mL^{-1}).

Analysis of by fourier transform infrared spectroscopy (FTIR). When Cellulose blended PVC films treated with fungal isolates when studied under FTIR the area of peak at wavelength 3361 cm^{-1} was broad and greater in control as

Fig. 3. Morphology of colonies of fungal isolates on malt extract agar plates *Phanerochaete chyrosporium* PV1

Fig. 4. Microscopic examination of *Phanerochaete chyrosporium* PV1 (100 x)



compared to the sample spectrum; similarly a small peak appeared near this peak in sample, which was not present in control. The peak at wavelength 1647 cm^{-1} (C=C stretching vibration of aromatic ring) in sample was sharp and larger as compared to the control. Similarly the small peak between wavelength 1956 cm^{-1} and 2358 cm^{-1} (corresponding to O-H) disappeared in the sample spectrum, which was present in control (Fig. 4). FTIR spectroscopy was used to examine the structural changes in the melt blends of PVC (Sombatsompop *et al.,* 2004). Biodegradation of pPVC brought some structural changes in the FTIR spectra of the polymer (Kazmarek & Bajer, 2007).

Analysis of soil burial experiment. The FTIR analysis of Cellulose blended PVC film showed that the peak at wavelength 3245 cm^{-1} was present in control, which was absent in the sample. The length of the peak at wavelength

Fig. 5. Fourier transform infrared spectra of cellulose blended PVC films after shake flask experiment with *Phanerochaete chyrosporium* PV1

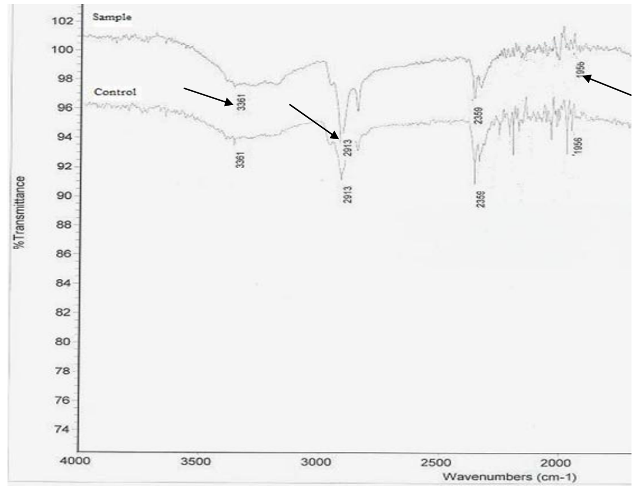
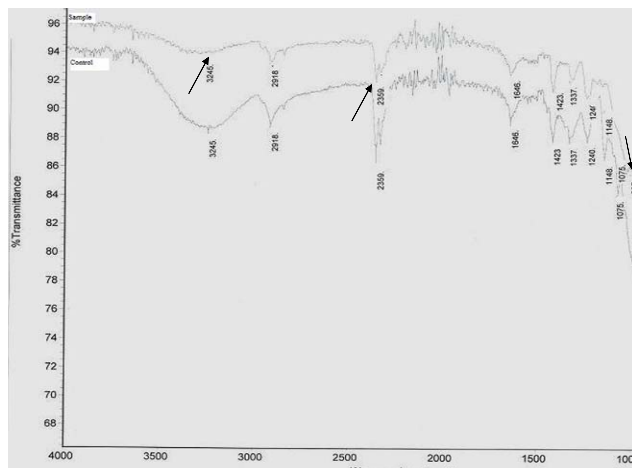


Fig. 6. Fourier transform infrared spectra of cellulose blended PVC films, after soil burial treatment with *Phanerochaete chyrosporium* PV1



2359 cm^{-1} (corresponding to O-H) was sharp in control, which was broad in sample, which attribute to degradation. Similarly, the sharp peak at wavelength at 987.4 cm^{-1} (corresponding to C-O) in control was broad in sample spectrum showed C-O-C bond stretches (Fig. 5). Our results are in accordance with the studies of Amanda *et al*. (2001). The FTIR spectra of poly (vinyl alcohol) showed a sharp decrease in the bands and peaks of the polymer (Okaya & Ikari, 1992).

CONCLUSION

Soil and sewage sludge contain microorganisms (fungi) that are able to bring about some degradation of synthetic polymers. The fungal isolate *Phanaerochaete chrsosporium* PV1 showing adherence and growth on the surface of starch blended PVC films indicated their ability to utilize PVC as a source of nutrient. Production of carbon dioxide during the Sturm test indicated positive degradability test for the starch blended PVC films. The changes in the peaks of the FTIR spectra of the test samples as compared to control is an indication of breakdown of plastics starch blended PVC as a result of fungal treatment.

REFERENCES

Amanda, I.M.G., B.L.O. López and L. Sierra, 2001. Biodegradation of poly (vinyl alcohol-co-ethylene) with the fungus *Phanerochaete chrysosporium*. *Mat. Res. Innovat*., 4: 148–154

Booth, G.H., A.W. Cooper and G.A. Robb, 1968. Bacterial degradation of plasticized PVC. *J. Appl. Bacteriol.*, 31: 305–310

Braun, D. and E. Bazdadea, 1986. *In*: Nass, L.I. and C.A. Heiberger (eds.), *Encyclopaedia of PVC. 1*. Marcel Dekker, New York

Calil, M.R., F. Gaboardi, C.G.F. Guedes and D.S. Rosa, 2006. Comparison of the biodegradation of poly (ε-caprolactone), cellulose acetate and their blends by the Sturm test and selected cultured fungi. *Polym. Test,* 25: 597–604

Christensen, G.D., L. Baldassarri and W.A. Simpson, 1995. Methods for studying microbial colonization of plastics. *Meth. Enzymol*., 253: 477–500

Domb, A.J., J. Kost and D.M. Wiseman, 1997. *Handbook of Biodegradable Polymers*. Harwood Academic Publishers: Singapore

Fauvarque, J., 1996. "The chlorine industry."*Pure. Appl. Chem.,* 68: 1713–1720

Kazmarek, H. and K. Bajer, 2007. Biodegradation of Plasticized Poly (Vinyl chloride) containing Cellulose. *Polym. Sci*., 45: 903–919

Kazmarek, H. and K. Bajer, 2008. Study on the course of biodegradation of PVC/cellulose composites. *Polym. Composites*, 53: 631–638

Hamid, S.H., 2000. *Handbook of Polymer Degradation*, 2nd edition. Marcel Dekker: New York

Huang, S.J., 1995. Polymer waste management-biodegradation, incineration and recycling. *J. Macromol. Sci. Pure*., 32: 593–597

Kirbas, Z., N. Keskin and A. Guner, 1999. Biodegradation of polyvinylchloride (PVC) by white-rot fungi. *Bull. Environ. Contam. Toxicol.*, 63: 335–342

Labuzek, S., B. Nowak and J. Pająk, 2003. The Susceptibility of Polyethylene Modified with Bionolle to Biodegradation by Filamentous Fungi. *Environ. Stud.,* 13: 59–68

Muller, R.J., J. Augusta and M. Pantke, 1992. An interlaboratory investigation into the biodeterioration of plastics, with special reference to polyurethanes; Part 1: A modified sturm test. *Material und Organismen,* 27: 179–189

Nishida, H. and Y. Tokiwa, 1993. Distribution of poly (β-hydroxybutyrate) and poly (ε-caprolactone) aerobic degrading microorganisms in different environment. *J. Envion. Polym. Degrad.*, 1: 222–233

Okaya, T. and I. Ikari, 1992. *Polyvinyl Alcohol "Developments*". Wiley, New York

Otake, Y. and T. Kobayashi, H. Ashabe, N. Murakami and K. Ono, 1995. Biodegradation of low-density polyethylene, polystyrene, polyvinyl-chloride and urea-formaldehyde resin buried under soil for over 32 years. *J. Appl. Polym. Sci.*, 56: 1789–1796

Owen, E.D., 1984. *Degradation and Stabilization of PVC*. Elsevier Applied Science Publishers, Barking

Sielicki, M., D.D. Focht and J.P. Martin, 1978. Microbial degradation of [C14C] polystyrene and 1, 3-diphenylbutane. *Canadian J. Microbiol.*, 24: 798–803

Sombatsompop, N., K. Sungsanit and C. Thongpin, 2003. Analysis of Low-Density Polyethylene-*g*-Poly (vinyl chloride) Copolymers Formed in Poly (vinyl chloride)/Low-Density Polyethylene Melt Blends with Gel Permeation Chromatography and Solid-State 13C-NMR. *J. Appl. Polym. Sci.,* 92: 3167–3172

Webb, J.S., M. Nixon, I.M. Eastwood, M. Greenhalgh, G.D. Robson and P.S. Handley, 2000. Fungal colonization and biodeterioration of plasticized polyvinyl chloride. *Appl. Environ. Microbiol*., 66: 3194–3200

(Received 21 May 2009; Accepted 26 May 2009)