MICROBIAL DEGRADATION OF POLYVINYL CHLORIDE PLASTICS

By

Muhammad Ishtiaq Ali

Department Of Microbiology
Quaid- i- Azam University
Islamabad
2009

MICROBIAL DEGRADATION OF POLYVINYL CHLORIDE PLASTICS

A Thesis submitted in the partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

IN

MICROBIOLOGY

By

Muhammad Ishtiaq Ali

Department of Microbiology
Quaid- i- Azam University
Islamabad
2009

IN THE NAME OF ALLAH

THE MOST

COMPASSIONATE

AND

MERCIFUL

DEDICATED TO

AMMI AND ABBU JAN

MOST LOVING

CARING TO ME ON THIS HEAVEN

WHOM PRAYERS ALWAYS LEAD ME

TO SUCCESS

DECLARATION

The material contained in this thesis is my original work and I have not presented any part of this thesis/work elsewhere for any other degree.

Muhammad Ishtiaq Ali

CERTIFICATE

This thesis by Muhammad Ishtiaq Ali is accepted in its present form by the Department of Microbiology, Quaid-i-Azam University, Islamabad, as fulfilling the thesis requirement for the degree of Doctor of Philosophy in Microbiology

Internal examiner

Dr. Safia Ahmed

External examiner

Dr. Jahangir Arshad

External examiner

Dr. Ghazala Kaukab

Chairman

Prof. Dr. Abdul Hameed

Dated: 16th July, 2009.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AAC	Aliphatic-aromatic copolyester
ABI	Applied Biosystemic Inc.
AFM	Atomic Force Microscopy
APME	Association of Plastic Manufacturers Europe
ASTMD	American Society for Testing and Materials
BBP	Butyl benzyl phthalate
CFU	Colony forming unit
CSTEE	Scientific Committee on Toxicity, Ecotoxicity and the Environment
DBP	dibutyl phthalate
DDT	dichloro-diphenyl-trichloroethane
DEHA	bis-2ethylhexyl adipate
DEHP	di (2-ethyl hexyl) phthalte
DEP	diethyl phthalate
DIDP	diisodecyl phthalate
DINP	diisononyl phthalate
DMP	dimethyl phthalate
DO	dissolved oxygen
DOA	dioctyl adipate
DOP	dioctyl phtahalte
FTIR	Fourier-Transforms Infrared Spectroscopy
GOP	Govt. of Pakistan
HDPE	High density polyethylene
IARC	International Agency for Research on Cancer
ITS	Internal transcribed spacer
LLDPE	Linear low-density polyethylene
LPDE	Low density polyethylene
MDPE	Medium density polyethylene
MEA	Malt extract agar
MEP	Monoethyl phthalate
MSM	Mineral salt media

MSW	Muncipal Solid Waste
MW	Molecular weight
NCBI	National Center for Biotechnology Information
NMR	Nuclear Magnetic Resonance
OBPA	10, 10′ -Oxy bis-phenoxy arsine
ORP	oxidation-aromatic potential
OUR	oxygen uptake rate
PA	Phthalic acid
PAE	Polyarylene ether
PBT	Poly (butylene terephthalate)
PCL	poly (ε-caprolacton)
PCR	Polymerase chain reaction
PET	Polyethylene terephthalate
POP	Persistent organic pollutants
PP	Polypropylene
ppt	parts per trillion
PPVC	Plasticized polyvinyl chloride
PS	Polystyrene
PTFE	Poly (tetra-flouro ethylene)
PUR	Polyurethane
PVC	Polyvinyl chloride
rRNA	ribosomal RNA
SEM	Scanning Electron Microscopy
TAE	tri-acetate EDTA buffer
TCA	Tri-carboxylic acid cycle
TCDD	2,3,4,7,8-tetrachlorodibenzo dioxin
THF	Tetrahydrofuran
TOTM	tri-octyl tri-melliate
US EPA	United States Environmental Protection Agency
UV	ultraviolet.
VCM	monomer vinyl chloride

ACKNOWLEDGEMENTS

All praises to **Almighty Allah,** the most benevolent and merciful, the creator of the universe, who enabled me to complete this research work successfully. I offer Salaam to the **Holy Prophet Muhammad** (peace be upon Him) who is a blessing for all the worlds

I deem it my utmost pleasure to avail myself this opportunity in recording my immense gratitude and deep feelings of regards to my supervisor **Dr. Safia Ahmed**, Associate Professor, who inspite of his busiest tiring routine work, provide her guidance and valuable suggestion, enlightened supervision during the whole study period.

I would like to express my gratitude and profound regards to **Dr. Abdul Hameed** Professor and Chairman for his moral help and cooperation throughout the course of this research endeavor. I wish to express my warm and sincere thanks to **Dr. Farhia Hasan**, Assistant Professor, and **Dr. Amir Ali Shah**, Assistant Professor for their valuable advice and help.

I wish to express my warmest thanks to my all lab fellows especially, **Pir Bux Ghumro, Naima Atiq, Saadia Andleeb, Saadia Almas, Mayram shafique, Rehana, Arfat Yamin, Gulam Raza, Fazal, Masror, Khalid Mehmood, Dr. Habib, Mir Sadiq Shah, Nida** and **Hamza** who are the source of support and strength whenever I faced problems during my research work.

I am grateful to very carrying and sweet friends **Dr. Imran Javed, Bashir Ahmad** and **Zeeshan Haider** for their contribution and for practical help in the laboratory, and who were always with me at hard times with their maximum contribution in the completion of this work.

Special thanks for the wonderful and memorable company, care and support of my friends **Inayat ullah, Zeeshan Kashif, Shahzad Shah, Aurangzeb Iqbal,**

Isfahan Tauseef, Amir haider Shah, Zahid Azeem, Jahanzeb, Shafqat, Dr Tariq, Sajjad sb. and **Sajid Mehmood** and **Zeeshan Hyder.** I have no words to thank them for wonderful support in my research work.

I would also like to thank **Mr. Shabbir, Sharjel** office clerks, **Mr. Shehzad, Sohail. Shahid** and **Arslan** Lab. Attendant, for their cooperation throughout my research work

The generous support from Higher Education Commission, Pakistan is greatly appreciated. I have enjoyed considerable financial support from the HEC over the years in a whole range of ways.

I am also very much grateful to **Uzma Farooq**, who was always with me in all the hard times during my study and who always motivated me that just one step more. Thanks a lot.

Ordinary words for gratitude don't truly encompass the love & gratitude extended by all family especially, my brothers, **Dr Mumtaz Ali, Zulfiqar Ali, Iftikhar Hussain,** and sisters **Sajida Rehman, Saira Zaeed, Tahira Mumtaz, Saira Zulfiqar** and my Niece and Nephew **Iman , Maaz, Mateen** and **Salah-u-din** for their prayers, company, constant interest in my studies and encouragement, due to which I am here.

Finally, I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.

Muhammad Ishtiaq Ali.

ABSTRACT

Polyvinyl chloride (PVC) is the most widely used and is the most environmentally damaging plastic. The Polyvinyl chloride lifecycle (its production, use and disposal) results in the release of toxic, chlorine based chemicals. These toxins are building up in water, air and the food chain which results in severe health problems, including cancer, birth defects, genetic changes, chronic bronchitis, ulcers, skin diseases, deafness, vision failure, indigestion, and liver dysfunction immune system damage, and hormone disruption.

The Aims of the present study were to isolates polyvinyl chloride degrading microorganism from indigenous sources and to establish their biodegradation abilities. For the isolation of microorganism thin films of plastics (PVC, plasticized PVC with dioctyl phthalate (DOP) and dioctyl adipate (DOA); and PVC starch blends) were buried in soil in pots for 10 months in laboratory conditions. Microorganisms colonized and adhered to the surface of the plastic films were isolated and purified on agar media. They include bacteria, actinomycetes and different fungal strains. Four fungal strains having good ability to grow and adhere to the surface of the plastic film were selected by growing the isolates on mineral salt agar medium with plastic films as carbon source. The selected fungal strains were then identified on the basis of morphological and ribosomal RNA (5.8S, 18S, 28S) sequence analysis as *Phanerochaete chrysosporium* PV1, *Lentinus tigrinus* PV2, *Aspergillus niger* PV3 and *Aspergillus sydowii* PV4.

Biodegradability of the fungal strains for the four kinds of polyvinyl chloride films PVC, pPVC (DOP and DOA) and PVC starch blends was tested both in soil and liquid broth. Analysis of biodegradation was monitored by growth (biomass) on plastic films as sole carbon source in mineral salt broth under shaking condition. Changes in the structure of the polymers were recorded by scanning electron microscopic (SEM) analysis, Fourier transform infrared spectroscopy (FTIR) and Nuclear magnetic resonance (NMR) before and after treatment with fungal strains, both in soil burial (for 3-10 months) and shake

flask treatment (for 3-6 months). Further Gel permeation chromatography (GPC) analysis was used to measure the molecular weight of the polymer after treatment. Another parameter for biodegradability testing was gravimetric CO_2 measurement after treatment with respective fungal strains for four weeks in strum test.

Biomass quantification in case of pure PVC indicated that the strain *Phanerochaete chrysosporium* PV1 was the best grown having biomass of 0.25 mg/ml. The appearance of hexagonal rings in treated samples was observed in scanning electron microscopy due to fungal activity in PVC film buried in soil with *Phanerochaete chrysosporium* PV1 for 10 months. The measurement of CO_2 evolved during break down of polymer in Sturm test, showed that more amount of CO_2 was produced in test (8.31g/l) than in the control (4.90g/l) when PVC films were treated. A decrease in molecular weight of the PVC after fungal degradation was observed by gel permeation chromatography (178,292 Da) in test treated for six months as compared to the control (202,530 Da).

The changes in the structural properties of pure PVC film determined by FTIR by peaks shortening and almost disappearance at wave lengths of 2980-2910 cm^{-1} corresponding to alkyl group and appearance of new peaks at 2380-2340 cm^{-1} representing hydroxyl groups. Further the biodegradation of the PVC was confirmed with ^{13}CNMR which showed increase in signals of the treated sample in the range 29-47 ppm. The integration of the treated sample also decreased to 43.07 compared to control (43.81) in ^{1}H NMR spectra indicating degradation.

Similarly the fungal growth was also observed on the plasticized PVC films with DOP and DOA. The increase in the biomass was also observed in the treated sample than control as in case of the pPVC (DOP) the strains *Aspergillus niger* PV3 showed maximum biomass (0.2890 mg/ml) after seven week of incubation and the increase in the biomass was also observed in pPVC (DOA) about 0.2910 mg/ml by *Aspergillus niger* PV3. The scanning electron microscopy of the pPVC (DOP) films after microbial treatments showed pits, extensive spotting and changes in polymer surface. There was erosion and extensive

roughening of the surface of fungal degraded pPVC (DOA) film and also fungal adherence was observed by SEM. The results of the Sturm test showed more CO_2 production (29.79 g/l) than that in the control (9.07 g/l) in pPVC (DOP) and (32.79 g/l), in test than that in control (13.07 g/l) of pPVC (DOA). The significant decrease in molecular weights were observed in pPVC(DOP) (81,028 Da) as compared to control (84,300 Da), and in case of the pPVC (DOA) lower molecular weight in test (81,830 Da) was observed than in control (88,000 Da). The analyses of SEM and FTIR indicated preferred degradation with DOP as plasticizer than DOA.

The degradation of the PVC starch blends was also checked by isolated fungal strains. Good growth and adherence was observed on the PVC starch blended films. A maximum increase in the biomass (0.297 mg/ml) was observed in the starch blended film by *Phanerochaete chrysosporium* PV1. There was clear surface erosions and breakdown of the some parts of the film due to *Phanerochaete chrysosporium* PV1 degradative activity in scanning electron micrographs after shake flask experiments. The Sturm test showed amount of CO_2 evolved in test (33.79 g/l) was more as compared to the control without polymer (16.34 g/l). The decrease in the molecular weight of the polymer was observed by gel permeation chromatography in test (77,011Da) by *Phanerochaete chrysosporium PV1* than control (80,275 Da).

In case of fungal treated PVC starch blended film the FTIR results showed formation of some new peaks in the region 3077 cm^{-1} (corresponding to alkenes) and also decrease in the absorbance of the peaks at 2655-2529 cm^{-1} indicating degradation. In ^{13}CNMR analysis chemical shifting and appearance of the new peaks 29.85, 41.58, and 46.05 ppm and also significant increase in the intensity of resonances in ^{1}H NMR spectra confirmed degradation.

Overall it is concluded that polyvinyl chloride degrading fungal strains isolated from the soil had great potential to be used for treatment of solid waste containing plastics.

1. INTRODUCTION

1.1. History of Plastics

The development of synthetic polymeric materials began in the mid-nineteenth century as an effort to find alternatives to materials that were either very expensive or in short supply. Originally, plastics were mimicking and replacing natural products (lacquer, shellac, amber, horns, tusks, tortoise shell), and the early synthetic materials were chemical modifications of naturally occurring cellulosic polymers. The development of fully synthetic polymers did not occur until the early twentieth century, with fully cross-linked thermo set materials. Throughout the twentieth century, the pace of innovation developed along with the growth of the petrochemical industry, and scientists developed many new plastics (Table 1.1; Dylingowski and Hamel, 2005; Association of Plastic Manufacturers Europe (APME), 1999).

Table 1.1 Timeline for synthetic polymer development (Dylingowski and Hamel 2005)

Year	Plastic Type
1869	Cellulose Nitrate
1889	Rayon
1910	Phenol-formaldehyde
1927	Cellulose acetate
1931	Polyvinyl chloride
1931	Polyvinyl chloride, Polyvinyl acetate copolymer
1933	Polyethylene
1937	Polymethyl methacrylate
1937	Polystyrene
1938	Cellulose acetate butyrate
1938	Poly tetra fluoro ethylene ("Teflon")
1939	Polyamide ("Nylon")
1943	Silicone
1948	Acrylonitrile-butadiene-styrene
1956	Acetal
1957	Polypropylene
1958	Polycarbonates

Plastics are the product of the 20th century but today, they are largely synthetic materials made from an extremely inexpensive but non-renewable resource, crude oil.

1.2. Plastics and their Uses

Plastics are man-made long chain polymeric molecules (Scott, 1999).They are widely used economical materials, characterized by excellent all-round properties, easy molding and manufacturing. Traditionally plastics are very stable and not readily degraded in the ambient environment. As a result, environmental pollution from synthetic plastics has been recognized as a large problem. For instance, statistics published by the United States Environmental Protection Agency (US EPA) in 2003 indicated that before recycling approximately 236 million tons of municipal solid waste (MSW) was generated in the United States in that year of which 11.3% was composed of plastics. Only a small fraction of this plastic waste (mostly soft drink and other bottles) was recycled (US EPA, 2005).

Commodity plastics are used in packaging, disposable diaper backing, fishing nets, and agricultural film (Table. 1.2). They include polymers such as polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyurethane, poly (ethylene terephthalate), and nylon.

The most widely used plastics used in packaging are Polyethylene (LDPE, MDPE, HDPE and LLDPE), Polypropylene (PP), Polystyrene (PS), Polyvinyl chloride (PVC), Polyurethane (PUR), Poly (ethylene terephthalate) (PET), Polybutylene terephthalate (PBT), and nylons. The widespread applications of plastics are not only due to their favorable mechanical and thermal properties but also mainly due to the stability and durability (Rivard *et al,*. 1995).

Table 1.2 Uses of Synthetic Plastics (Vona *et al.*, 1965).

Plastic	Use
Polyethylene	Plastic bags, milk and water bottles, food packaging film, toys, irrigation and drainage pipes, motor oil bottles.
Polystyrene	Disposable cups, packaging materials, laboratory ware, certain electronic uses.
Polyurethane	Tires, gaskets, bumpers, in refrigerator insulation, sponges, furniture cushioning, and life jackets.
Polyvinyl chloride	Automobile seat covers, shower curtains, raincoats, bottles, visors, shoe soles, garden hoses, and electricity pipes.
Polypropylene	Bottle caps, drinking straws, medicine bottles, car seats, car batteries, bumpers, disposable syringes, carpet backings.
Polyethylene terephthalate (PET)	Used for carbonated soft drink bottles, processed meat packages peanut butter jars pillow and sleeping bag filling, textile fibers.
Nylon	Polyamides or Nylon are used in small bearings, speedometer gears, windshield wipers, water hose nozzles, football helmets, racehorse shoes, inks, clothing parachute fabrics, rainwear, and cellophane.
Polycarbonate	Used for making nozzles on paper making machinery, street lighting, safety visors, rear lights of cars, baby bottles and for house ware. It is also used in sky-lights and the roofs of greenhouses, sunrooms and verandahs. One important use is to make the lens in glasses.
Polytetraflouro-ethylene (PTFE)	PTFE is used in various industrial applications such specialized chemical plant, electronics and bearings. It is met with in the households as a coating on non-stick kitchen utensils, such as saucepans and frying pans.

1.3. Plastic Industry in Pakistan

The plastic industry in Pakistan is growing at an average annual growth rate of 15%. There are about 600-700 medium sized plastic processing units scattered all over Pakistan. About 60% of the units are located in and around Lahore and the remaining at Karachi, Hattar, Gadoon, Faisalabad, Multan and Quetta. Raw material is imported from Japan, Korea, Italy, Taiwan, Hong Kong, England, China, and Germany (Sabir, 2004).

1.3.1. Solid Waste in Pakistan

Solid waste in Pakistan is generally composed of plastic and rubber, metal, paper and cardboard, textile waste, glass, food waste, animal waste, leaves, grass, straws and fodder, bones, wood, stones and fines to various extents. The estimated figure of plastic waste generation across the country is 1.32 million tons per annum (Table. 1.3). This considerable content of plastic in the solid waste generated in Pakistan is of great concern. Plastic waste is released during all stages of production and post consumption every plastic product is a waste. Both the quantity and quality of plastic waste cause environmental problems.

Table 1.3 Plastic Waste Generations in Different Cities of Pakistan **[GOP, 1996]**

Sr. No.	*Cities*	*No. of Scavengers*	*Plastic Waste (Tons)*	
			Per Day	Per Year
1	Faisalabad	1500	44.4	13320
2	Gujranwala	1200	41.2	12360
3	Karachi	7000	412.8	123840
4	Hyderabad	1200	35.1	10530
5	Peshawar	800	29.9	8970
6	Quetta	600	31.0	9300

1.4. Plastic Waste Disposal/Management

The main difficulty of solid waste management is found in the variability of materials and sources. Synthetic polymeric packaging is the largest component followed closely by paper packaging. Used packaging is generally non-toxic but can have considerable impact as litter and is a significant contributor to landfill costs and a balanced approach to waste minimization and waste management has been suggested (Sturges, 2000). The hazard of discarding waste plastic, so-called "white pollution" is becoming more and more severe. The plastic waste stream emerges from domestic, industrial and municipal refuse (Jayasekara *et al.*, 2005). The plastic waste is disposed off through land filling, incineration and recycling.

1.4.1. Land filling

Millions of tons of plastic waste, including refuse sacks, carrier bags and packaging, are buried in landfill sites around the world each year. China generates about 16 million tons, India 4.5 million tons and the UK 1 million tons, of which more than 800,000 tons is waste polyethylene. Conventional polyethylene products can take longer than 100 years to degrade, taking up valuable landfill space (Sylvia, 1995).

In the United States, synthetic polymers are estimated to be approximately 20% of the volume of municipal solid waste, dumped as landfill. They are estimated to account for 8% of the total weight (Glover, 1993; Alexander, 1994), a similar figure to Germany. In Australia, most of waste from households and business ends up in municipal landfill sites and packaging is estimated to be 25% of the total waste by weight (common wealth of Australia, 1996). According to a study in Pakistan, the estimated figure of plastic waste generation across the country is 1.24 million tons per annum (GOP, 1996). There is no proper land filling of plastic waste in Pakistan, most of it is dumped as open garbage.

1.4.2. Incineration

Incineration or burning of plastics seems to be simple and straight forward but it is also not free of problems. Many polymers like polyvinyl chloride, poly (vinylidine chloride), poly (urethane) and other halogen and nitrogen-containing polymers can form corrosive and toxic substances upon burning and can cause health hazards or pollute the environment. Burning of PVC produces furans and dioxins which are carcinogenic and causes diseases of skin and respiratory tract. PVC also produces hydrochloric acid that causes problems in municipal incinerators. Thus, incineration has had a bad name in the environmental community as a result of the production of toxic fumes and ashes (Scott, 2000).

1.4.3. Recycling

Measures to deal with the growth of packaging waste also include reuse recycling. Recycling channels diverse material from waste streams to a reprocessing stream. Reuse is use of the product in its original form without extra processing other than cleaning. Recycling offers economic and environmental benefits if the costs of resources used, including collection, sorting, cleaning and reprocessing, are not greater than the resources saved. The quality of most materials deteriorates with processing. Recycling of household waste plastics is difficult when they are contaminated with biological residues or when they contain a mixture of different plastics. Direct recycling is applicable to wastes of the plastic production process. Indirect recycling has good future prospects if polymers can be separated and recovered. The cost of recovery is very high, it limits recycling activities. The other limiting factor is that the material to be reused must maintain the original quality and be no greater in cost than the virgin raw material. Recycling and reuse have limited value in solving waste management problems (Sturges, 2000).

Recycling inevitably leads to a reduction of desired physical properties. Plastic recycling is becoming more and more important in various industrial sectors. It

is topic of high priority due to large consumption of plastics. Many polymers possess excellent properties after their first use and can be utilized for many different applications after that use. Waste management strategy has stressed recycling of plastic wastes/packaging materials that have a small combustion value and which, at the same time, are feasible for recycling. Other options such as prevention reuse and recovery of energy can offer ecological and economic advantages over recycling according to the application area. Thus the growth of recycling industry is not a necessarily desirable policy target but it must perform at its optimum rate, both from an economic and an environmental point of view (Datta *et al.*, 1998).

1.5. Biodegradation of Plastics

Microorganisms such as bacteria, fungi and actinomycetes are involved in the degradation of both natural and synthetic plastics (Gu *et al.*, 2000a). The biodegradation of plastics proceeds actively under different soil conditions according to their properties, because the microorganisms responsible for the degradation differ from each other and they have their own optimal growth conditions in the soil (Glass and Swift, 1989).

Plastics (polymers) are potential substrates for heterotrophic microorganisms including bacteria and fungi. Polymer biodegradability depends on molecular weight, crystallinity and physical forms (Gu *et al.*, 2000b). Generally, an increase in molecular weight results in a decline of polymer degradability by microorganisms. In contrast, monomers, dimers and oligomers of a polymer's repeating units are much easily degraded and mineralized. High molecular weight result in a sharp decrease in solubility making them unfavorable for microbial attack because bacteria require the substrate be assimilated through the cellular membrane and then further degraded by cellular enzymes. However it should be pointed out that concurrent abiological and biological process may facilitate the degradation of polymers.

The high molecular weight and generally non-soluble polymers cannot be taken up into the microbial cell. Therefore, polymer-degrading hydrolases are excreted by the producing microbial cell into the surrounding milieu to allow the direct contact with the polymeric substrate. The polymer chains are then cleaved until short-chained water-soluble products are produced which can be transported through the cell membrane. Inside the cell, these degradation products are intracellularly metabolized into water, CO_2 and biomass and others (Gu *et al.*, 2000b).

Dominant groups of microorganisms and degradative pathways associated with polymer degradation are often determined by the environmental conditions. When O_2 is available, aerobic microorganisms are mostly responsible for destruction of complex materials, with microbial biomass, CO_2, and H_2O as final products. In contrast, under anoxic conditions, anaerobic consortia of microorganisms are responsible for polymer deterioration. The primary products will be microbial biomass, CO_2, CH_4 and H_2O under methanogenic conditions (Barlaz *et al.*, 1989a, b; Gu *et al.*, 2001; Gu and Mitchell, 2001) or H_2S, CO_2 and H_2O under sulfidogenic conditions. Both aerobic and strictly anaerobic microorganisms are involved in the degradation of polymers (Gu, 2003).

1.6. Polyvinyl Chloride (PVC)

Polyvinyl chloride (PVC) is arguably the most versatile of all the plastics (more correctly termed polymers). It is resistant to corrosion and weathering, a superb electrical insulator, impact and scratch resistant, tends not to crack, can be made rigid or flexible, and under many circumstances, it does not catch fire readily. PVC is also cheap to produce and can serve its designed function for decades. Due to its high chlorine content (57 wt% in virgin PVC) PVC is inherently more energy conservative (less fossil carbon is utilized) compared to its polymer alternatives. However, when energy consumption during manufacture is included PVC is on parity with the other major plastics (UBA 1999).

1.6.1. History

Polyvinyl chloride was accidentally discovered on at least two different occasions in the 19th century, first in 1835 by Henri Victor Regnault and in 1872 by Eugen Baumann. On both occasions, the polymer appeared as a white solid inside flasks of vinyl chloride that had been left exposed to sunlight. In 1926, Waldo Semon and the B.F. Goodrich Company developed a method to plasticize PVC by blending it with various additives. The result was a more flexible and more easily-processed material that soon achieved widespread commercial use (Scott, 1999).

Polyvinyl chloride is one of the three most important polymers currently used worldwide. This is because PVC is one of the cheapest polymers to make and has a large range of properties so can be used to make hundreds of products. PVC is formed by the polymerization of vinyl chloride (chloroethane) monomer units (Fig. 1.1). PVC consists of polar molecules which are attracted to each other by dipole-dipole interactions due to electrostatic attractions of a chlorine atom in one molecule to a hydrogen atom in another atom. These considerable intermolecular attractions between polymer chains make PVC a fairly strong material.

Polyvinyl chloride homopolymer is a semi-crystalline polymer with a relatively high melting temperature about 212 $^{\circ}$C with tensile strength 50-80 mpa, depending on formulation that can be lowered by plasticizing entities to produce semi-rigid and flexible items. The density is about 1380 kg/m^3 (Table: 1.4).

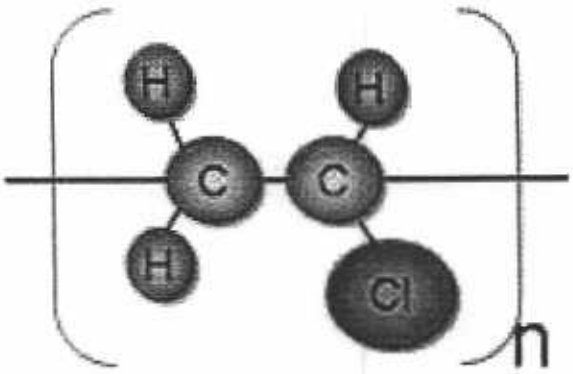


Figure 1.1 Structure of the Polyvinyl chloride

Table 1.4 Properties of PVC (http://en.wikipedia.org/wiki/pvc)

Polyvinyl chloride	
Density	1380 kg/m^3
Young's modulus (e)	2900-3300 mpa
Tensile strength(σ_t)	50-80 mpa
Elongation @ break	20-40%
Glass temperature	87 °C
Specific heat (c)	0.9 kj/(kg·k)
Melting point	212 °C
Water absorption (astm)	0.04-0.4

1.6.2. Applications of PVC

Polyvinyl chloride with a world-wide market second only to low-density polyethylene (Fauvarque, 1996), has been extensively used for a broad range of applications due to its safety, effectiveness, manufacturing technology and cost. The building products sector includes pipes (sewerage and potable water), cable and wiring covers, electrical switches and conduit, membranes, insulation, flooring, trim, and window frames. Consumer household uses include toys, blinds, wallpaper, furniture, shower curtains, electrical casings, imitation leather, and surface finishes (Fig. 1.2). Packaging and medical disposables are also minor but important applications (UBA, 1999).

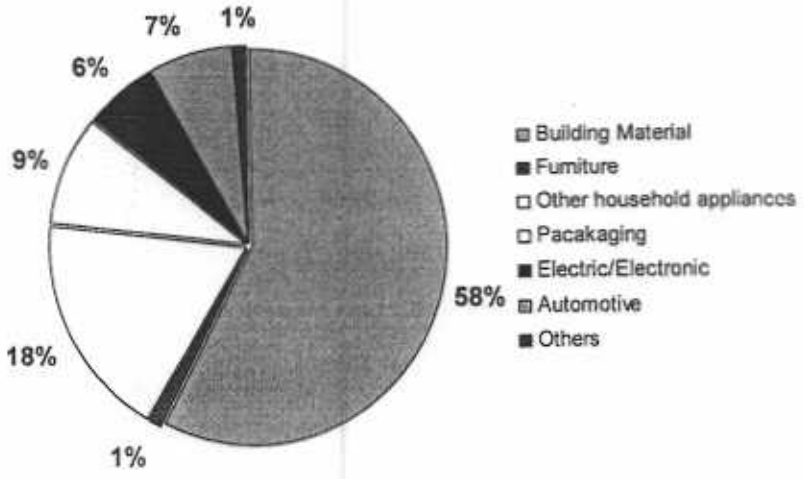


Figure 1. 2 Application of PVC (Source, Mechanical recycling of PVC wastes, Study for DG XI, January 2000).

In commercial terms, compounding PVC involves adding sufficient modifying components to the raw polymer to produce a homogeneous mixture suitable for processing and required performance at the lowest possible price. The

formulation is also determined by the processing technique to be employed. Modifiers for polymers may be classified into different groups (Table 1.5).

Table 1.5 Modifiers for PVC polymer

modifier	major function	how achieved	Example
plasticizer	soften PVC reduce Tg	separates PVC chains	phthalate esters aliphatic diesters epoxidzed oils phosphate esters
stabilizer	minimize or eliminate degrading effects of heat, light or oxygen on PVC	react with degradation product (HCl)	tin mercaptides barium-cadmium salts of fatty acids lead salts alkyl benzenes
lubricant	prevent adhesion of compound to processing	sweats out to form a film between PVC and equipment	calcium stearate normal and dibasic lead stearate
impact modifier	reduce brittleness	impedes crack development	acrylonitrile-butadiene-styrene ethylene-vinyl
processing aid	ensure uniform flow and good surface finish	affect melt viscosity of polymer	acrylates acrylonitrile-butadiene-styrene
filler	opacity compound increase hardness reduce cost	changes refractive index and reflective properties adds bulk to	calcium carbonate magnesium carbonate barium sulfate

Source of data (Titow, 1984 and Brydson, 1999).

1.6.3. Plasticizers

Plasticizers are the major modifier for PVC formulations in terms of percentage weight (between 15 and 50%) and, therefore, have the greatest

influence on the properties and behavior of the compounded PVC. A plasticizer is a material incorporated into a polymer or polymer mixture to increase its workability and its flexibility or elongation (Wilson, 1995). Plasticizers are essentially non-volatile solvents, with solubility parameters close to that of the polymer. Non-polymeric plasticizers are typically high boiling, oily, organic liquids, usually esters. Addition of plasticizer to a PVC polymer has two main functions; to assist in the processing stage by reducing the viscosity and melting temperature, and to modify the final product by softening it.

The majority of industrially produced plasticizers are used to manufacture plasticized PVC (pPVC). These plasticizers transform un-plasticized PVC (uPVC) into softer and more flexible form of the plastic. Primary plasticizers have the highest degree of compatibility with the PVC resin and are grouped into two classes- monomeric or polymeric. Modern monomeric plasticizers are synthetic organic chemicals with an ester base, such as adipate and phthalates.

The most common monomeric plasticizers include DOP (DEHP) bis-(2-ethylhexyl phthalate), DIDP (diisodecyl phthalate), DINP (diisononylphthalate), DOA (DEHA) bis-(2ethylhexyl adipate) and TOTM (tri-octyl tri-mellitate), (Roberts and Davidson, 1986; Whitney, 1996; Gumargalieva *et al.*, 1999). The polymeric plasticizers include various molecular weight plasticizers polymerized from adipic and glutaric acids in combination with various glycols. The end groups can also vary and include alcohols, esters and acids.

1.7. Hazards of PVC

The health and environmental effects of PVC and its additives have been the subject of intense debate (Hansen, 1999). The focus of this debate was initially concerned with the manufacture of PVC but has now shifted to the use and disposal of the material. The problems that are most often associated with the use of PVC in building and other product sectors arise from a number of factors, namely the release or extraction of the heavy metal based

stabilizers, uncertainty surrounding the health implications of the phthalate plasticizers and other additives, the formation of dioxins and hydrogen chloride gas and other substances during building fires and incineration, the long-term consequences of land filled PVC, and the poor recycling record of PVC waste (US EPA, 2000).

Dioxin has no commercial value and is extremely toxic, long-lived and ubiquitous in both the environment and our bodies. It is hormonally active in concentrations as low as 5 parts per trillion (ppt). The EPA has labeled dioxin a known carcinogen. (US EPA 2000) It is also unavoidable when PVC is incinerated or heated. Polyvinyl chloride is the largest contributor of the world's dioxin burden and it is highly persistent in the environment, traveling up the food chain, and accumulating in body fat.

The commonly employed phthalate plasticizers generally have low water solubility (although the values cited are variable). Phthalates are susceptible to a number of degradation pathways including hydrolysis, photochemical, and biodegradation (Staples *et al.*, 1997a). Acidic and alkaline hydrolysis of phthalates firstly affords the mono-ester and an alcohol moiety followed by formation of phthalic acid and a second alcohol moiety. This pathway is most relevant to higher animals where ingestion of phthalates results in rapid formation of the mono-ester in the stomach. Phthalates also undergo rapid aerobic and slow anaerobic biodegradation. Phthalates appear to persist in sediments where anaerobic conditions exist (VROM, 1998). According to the EPA, incineration of municipal and medical waste, which is heavily loaded with PVC, is the largest source of dioxinoid plasticizers (US EPA, 2000).

1.8. Degradation of Polyvinyl Chloride

Polyvinyl chloride is a known thermal and photo unstable polymer, degrades over 130°C (Pospisil *et al,*. 1999; Veronelli *et al,*. 1999).

Thermal decomposition of PVC results in an intense discoloration of the polymer, which is a result of the formation of long conjugated polyene

sequences that absorb in the visible region (Winkler 1959; Marks *et al,*. 1967 and Bacaloglu *et al,*. 1995). It is generally accepted that during degradation HCl molecules are eliminated in succession along the polymer chain yielding these conjugated polyenes.

Photo degradation is considered to occur according to a radical mechanism. Initiation of degradation occurs by excitation of the polymer by irradiation of the material with ultraviolet (UV) light eliminating a chlorine atom, which can initiate dehydrochlorination at other polymer chains (Abbas and Sorvik 1973). The photo and thermal degradation releases hydrochloric gas (dehydrochlorination) that leads to the formation of conjugate polyene sequences (-CH=CH) in the polymer chains, giving the PVC a reddish brown color (Hollande and Laurent, 1997; Veronelli *et al,*. 1999).

1.8.1. Biodegradation of Polyvinyl chloride

Plastics are biodegraded aerobically in wild nature, anaerobically in sediments and landfills and partly aerobically and partly anaerobically in composts and soil. Carbon dioxide and water are produced during aerobic biodegradation and carbon dioxide, water and methane are produced during anaerobic biodegradation. Biodegradation is defined as any physical or chemical change in a material caused by biological activity. Microorganisms such as bacteria, fungi and actinomycetes are involved in the degradation of both natural and synthetic plastics (Gu *et al.*, 2000a).

Biodegradation of the PVC and pPVC would comprise a series of processes for decomposition of the polymer formula when exposed to conditions favorable for growth of microorganisms able to colonize PVC and pPVC. PVC is a strong plastic that resists abrasion and chemicals and has low moisture absorption. There are many studies about thermal and photo degradation of PVC (Braun and Bazdadea, 1986; Owen, 1984) but there only few reports available on PVC biodegradation. PVC having low molecular weight can be exposed to biodegradation by the use of white rot fungi (Kirbas *et al.*, 1999).

Plasticized PVC (pPVC) is highly susceptible to microbial attack in many different environmental situations. Biodeterioration of pPVC is now known to occur in a wide range of industrial, commercial, and structural applications (Flemming, 1998; Gaylarde *et al.*, 1999 and Griffin *et al.*, 1984). The susceptibility of pPVC results from the presence of plasticizers, commonly organic acid esters such as dioctyl phthalate (DOP) and dioctyl adipate (DOA), added to modify physical or mechanical properties of the polymer. Both bacteria (Booth *et al.*, 1968 and Eaton *et al.*, 1982) and fungi can degrade ester-based plasticizers. Loss of plasticizers from pPVC due to microbial degradation results in brittleness, shrinkage, and ultimately failure of the pPVC in its intended application (Berk *et al.*, 1957 and Roberts *et al.*, 1986).

Adhesion represent the first stage of the colonization process (Christensen *et al.*,1995). Several factors contribute towards successful binding among which the most important are the non-specific physiochemical forces, such as hydrophobic interactions and electrostatic attraction. Adhesion of *Aureobasidium pullulans* has been shown to be dependent on the chemical composition of the PVC and more precisely on the presence of plasticizers such as DOA and DOP (Webb *et al.*, 1999).

The majority of colonization studies have focused on polyurethane (Nakajima-Kambe *et al.*, 1999). By contrast there have been fewer studies on the degradation of pPVC *in situ*. 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). Despite the fact that the backbone structure of PVC is considered to be intrinsically resistant (Andrady *et al*,. 1994), there are studies reporting on partial degradation of PVC by white rot fungi under elevated oxygen levels (Kerbas *et al.*, 1999). However, the pPVC additives are more readily degraded and utilization of them as a carbon source causes brittleness, loss of plasticity and fragmentation (Roberts and Davidson, 1986).

The presence of different types of esters of adiphic and phthalic acids are considered as important factors for the increased susceptibility of pPVC to

fungal colonization (Whitney, 1996; Steinbuchel, 1996). The majority of reports on phthalates degradation have been by bacteria (Yu and Ward, 1995; Samsonova *et al.*, 1996; Valiente *et al.*, 1998; Zeng *et al.*, 2002; Murad *et al.*, 2007) and the mode of bacterial degradation of DOP is known, consisting of initial de-esterification, formation of protocatechuic acid, ortho-cleavage and entry into the tricarboxylic acid (TCA) cycle (Karpagam and lalithakumari, 1999). Only a few studies however have reported on phthalate degradation by yeasts (Gartshore *et al.*, 2003).

1.9. Aims and Objectives

Biodegradation of PVC by indigenous microorganisms was the specific aim which was fulfilled by the following specified objectives.

1. Isolation of microorganisms from plastic waste contaminated soil and sewage sludge with the ability to degrade synthetic plastics
2. Characterization of PVC degrading microorganisms
3. Degradation of PVC plastics in solid and liquid media using selected microorganisms
4. Analysis of biodegradation of synthetic polymer by measuring the evolution of CO_2 by the breakdown of polymers through Sturm test, by FTIR, SEM, NMR and GPC.

2. LITERATURE REVIEW

2.1. Production of Plastics

Since the discovery of man-made polymers continuous and systematic efforts have been made to make polymers more stable, mechanically stronger and chemically and environmentally durable. Rot-resistance and rust proof are the two important factors for the large-scale popularity and demand of synthetic polymers. Now the use of synthetic polymers, due to their low price, ready availability, wide spectrum colorability, and ease of fabrication in any desired shape has been accelerated to such that the disposal of the used products has become increasingly difficult (http://www.envis-icpe.com).

The industrialized countries of Western Europe, Austria, Germany, France and Denmark had about 90% share of plastic production in 1973. In 1993, the total world demand for plastics was over 107 million tones and it was estimated about 146 million tones in 2000. At present, the production growth of plastics is almost stand-still in the industrialized countries but for the developing nations the annual growth rates are still in order of 10-40% per annum.

2.2. Hazards of Plastics

Modernization and progress has had its share of disadvantages and one of the main aspects of concern is the pollution it is causing to the earth - be it land, air, and water. The disposal of plastic waste is a growing problem across the country. Most of today's plastics and synthetic polymers are produced from petrochemicals. As conventional plastics are persistent in the environment, improperly disposed plastic materials are a significant source of environmental pollution, potentially-harming life (Nir *et al.*, 1993). The plastic has both environmental and health hazards. There is a need of technical guidelines for protecting human health and the environment from the improper management and disposal of plastic wastes (Sturges, 2000).

2.2.1. Health hazards

Each year more than 140 million tones of plastics are produced worldwide. In many countries, plastics are disposed off through open, uncontrolled burning and land filling. Open burning releases pollutants into the air that could cause various health problems. In addition, the burning of PVC plastics produces persistent organic pollutants (pops) known as furans and dioxins. These pollutants circulate globally and have been associated with a number of adverse effects in humans, including immune and enzyme disorders and they are classified as possible human carcinogens. Health may be affected by the polymer itself, by chemicals added to the plastic to make it more flexible, stable or flame retardant, or by coloring agents. These substances may also be released to the air when the plastics are heated. When plastics are heated to form final products, monomers, additives and degradation products can be released. Small amounts of these may also be present in the resins before heating. They can affect the health of workers who use, clean or maintain the processing equipment (Jayasekara *et al.*, 2005).

2.2.2. Environmental hazards

Discarded, non-degradable polymers show several undesirable environmental problems. These polymers create a threat to diverse animal populations. They have a direct impact on marine ecosystems and are believed to be responsible for the death of a very large number of birds by ingestion or strangulation (Scott, 1990). Polymers found in the ocean have a considerable effect on marine life, and if ingested cause intestinal blockages in small fish or suffocation of other marine animals (dolphins and turtles). The amount of litter at sea seems to be increasing despite control measures. It is estimated that one million tones of plastics are dumped in the sea annually. Litter is also a danger to terrestrial wildlife (Whitney *et al.*, 1993) by tangling or by blocking digestion pathways. Entanglement can readily occur in materials with holes, or plastic bags. Non-biodegradable polymers also have the capacity to act as disease foci because they persist in the environment for a very long period of time enabling organisms to accumulate (Jayasekara *et al.*, 2005).

2.3. Hazards of Polyvinyl Chloride

Polyvinyl chloride production involves the creation of many toxic chemicals, as feed stocks, as additives or as by-products. Dioxins, including 2, 3, 4, 7, 8-tetrachlorodibenzo dioxin (TCDD), one of the most toxic synthetic chemicals known (National Toxicology Programme, 1991), and furans are inescapable by-products of the production of the basic feedstock of monomer of PVC (ICI Chemicals & Polymers Ltd, 1994). There is conclusive evidence that dioxin (TCDD) causes cancer in animals (NTP US, 1991). Epidemiology studies have also shown that exposure to dioxins is strongly associated with an increase in mortality of all cancers considered together (Zobe *et al.*, 1990; Manz *et al.*, 1991).

The epidemiology studies on dioxins have been reviewed by the US EPA, 1994. The review suggests that epidemiological evidence is consistent with experimental studies, and indicates that dioxins have the potential to cause many different types of cancer. Although the evidence was not considered sufficient to confirm that dioxin causes increased cancer incidence, the EPA concluded that dioxin (TCDD) probably increases cancer mortality of several types. In February 1997, 25 scientists from 11 countries met under the auspices of the international agency for research on cancer to review the evidence on the carcinogenicity of dioxins. They decided to re-classify TCDD as a known human carcinogen.

2, 3, 4, 7, 8-Tetrachlorodibenzo dioxin is also a known hormone disrupting chemical. TCDD dioxin and other dioxins have been shown to have adverse effects on the male reproductive system in animals and humans. Experiments on laboratory animals have shown that exposure to dioxin results in changes to the male reproductive systems which include reduced levels of testosterone, decreased sperm production, decreased fertility and reduced testicular weight (Peterson *et al.*, 1993). A single, very small dose of dioxin administered to rats on the fifteenth day of pregnancy (a critical time for the development of sexual differentiation in the rat fetus) caused male offspring to produce between forty

and fifty-six per cent less sperm than those whose mothers had not been exposed to dioxin (Mably *et al.*, 1991).

Concern over plasticizer migration from pPVC products has made phthalates the focus of considerable recent attention (Fiala *et al.*, 2000; Safe, 1999). The scientific committee on toxicity, ecotoxicity and the environment (CSTEE) has established standard protocols for the extraction of phthalates from plasticized PVC toys (CSTEE 2000). In 1999 the European commission banned the use of Di(2-ethylhexyl)phthalate (DEHP) in PVC toys and other easily mouthed items intended for children under 3 years of age as a precaution against the uncertain impact of phthalates on young children (EC 1999). Manufacturers of PVC toys have also voluntarily withdrawn the use of DEHP in many countries, including Australia.

The potential health effects of phthalates have been the subject of much debate in the scientific community. DEHP and some other phthalates cause liver cancer in rodents *via* a mechanism known as peroxisomal proliferation (Latruffe *et al.*, 1995; Parmar and Seth, 1997; Qi *et al.*, 2000). However, this mechanism is not considered relevant to humans because of the difference in the way that the human liver responds to phthalates compared to the rodent liver. Goll *et al.*, (1999) found no peroxisomal proliferation of human liver cells *in vitro* studies. In an *in vivo* study on marmosets (Kurata *et al.*, 1998) conducted by no peroxisome proliferation was detected after administering DEHP. Doull *et al.* (1999) concluded that "the hepatocarcinogenic response of rodents to DEHP is not relevant to human cancer risk at any anticipated exposure level". Based on studies of DEHP on cynomolgus monkeys Pugh *et al.*, (2000) concluded that "... phthalate esters do not appear to produce hepatic effects associated with peroxisome proliferation and hepatic carcinogenicity in humans". An expert panel convened by the American council of science and health (Koop *et al.*, 1999) concluded that "... there is no convincing evidence to date that early childhood cancers are caused by exogenous agents", such as phthalates. On the basis of mechanistic studies involving peroxisomal proliferation, the world health organization's

international agency for research on cancer has reclassified DEHP as "not classifiable as to carcinogenicity to humans" International Agency for Research on Cancer (IARC 2000).

Some phthalates have been shown to have effects on the testis of laboratory animals (Sjöeberg *et al.*, 1985; Parmar *et al.*, 1987; Mylchreest *et al.*, 1998) and are thus suspected of contributing to the indications of reduced male fertility observed in some populations (Li *et al.*, 1998; Sharpe, 1998).

An increasingly important area of concern regarding phthalates, and the major reason for the intense debate, is their implication as possible endocrine disrupting chemicals (Heinze and Adams 1997; Langer and Sang 1997). The effects of endocrine disrupting chemicals on wildlife and the extrapolation of these observations to possible human health problems have been debated extensively in the scientific literature. Much of the evidence for endocrine disruption of wildlife populations comes from sites with heavy environmental pollution (Tyler *et al.* 1998; Vos *et al.* 2000).

2.4. Degradation of Polyvinyl Chloride

Processes inducing changes in polymer properties (deterioration of functionality) due to chemical, physical or biological reactions resulting in bond scission and subsequent chemical transformations (formation of structural in homogeneities) have been categorized as polymer degradation. Degradation has been reflected in changes of material properties such as mechanical, optical or electrical characteristics, in crazing, cracking, erosion, discoloration, phase separation or de-amination the changes include bond scission, chemical transformation and formation of new functional groups (Pospisil and Nespurek, 1997).

Sensitivity of polymers to photo-degradation is related to the ability to absorb the harmful part of the tropospheric solar radiation. This includes the UV-b terrestrial radiation (~295-315nm) and UV-a radiation (~315-400nm) responsible for the direct photo-degradation (photolysis, initiated

photooxidation). Visible part of sunlight (400-760 nm) accelerates polymeric degradation by heating. Infra-red radiation (760-2500 nm) accelerates thermal oxidation (Gugumus, 1990; Pospisil and Nespurek, 1997).

2.4.1. Thermal degradation of PVC

Thermal decomposition (Bacaloglu and Fisch, 1995) of PVC results in an intense discoloration of the polymer, which is a result of the formation of long conjugated polyene sequences that absorb in the visible region. It is generally accepted that during degradation HCl molecules are eliminated in succession along the polymer chain yielding these conjugated polyenes. The dehydrochlorination process involves three successive steps. It starts with a relative slow initiation of HCl loss, which is followed by a rapid zipper-like elimination of HCl and thus the formation of polyenes, which is finally terminated. Dehydrochlorination is initiated mainly by structural defects (e.g. allylic and tertiary chlorine) at the polymer backbone. After the first elimination of HCl, allylic chlorine has been formed, which is a very active moiety, supporting the fast zipper-like elimination of HCl. The main issue is the type of mechanism by which the overall dehydrochlorination process takes place. Studies on some small aliphatic model compounds for PVC have been inconclusive. Various schemes have been proposed, which can be classified as involving uni-molecular eliminations, ionic, free radical chain, or polaron mechanisms.

2.4.2. Photo-degradation of PVC

Photo-degradation (Gibb *et al.*, 1974) is considered to occur according to a radical mechanism. Initiation of degradation occurs by excitation of the polymer by irradiation of the material with UV light eliminating a chlorine atom, which can initiate dehydrochlorination at other polymer chains. The extent of degradation depends primarily on the presence of photosensitive chromophores in the polymer chain as irregular structures and impurities, e.g. hydroperoxides, carbonyl groups, unsaturation and metal salts, often present in processed polymeric materials (Xu *et al.*, 1989). Whatever the nature of the

chromophores initially absorbing UV light in the original material, polyene structures, which rapidly accumulate in photolyzed PVC, become the predominant absorbing chromophores due to their large extinction coefficients.

In the presence of oxygen both thermal- and photodegradation are enhanced. There are various interpretations of the mechanism of the thermo-and photooxidative degradation of PVC. It is, however, almost generally accepted that in the presence of oxygen, radical chain reactions play an important role (Braun, 1971). The dehydrochlorination in oxygen is much faster than in nitrogen (Abbas and Sorvik 1973). This is probably due to the superposition of oxygen-initiated radical processes on the dehydrochlorination reactions, which is supported by the observation that the high rate of HCl elimination in thermo-oxidation is reduced by antioxidants. Also the degradation of PVC containing peroxide groups formed due to the presence of oxygen during polymerization (Braun and Wolf, 1978) was found to proceed faster compared with the degradation of PVC, prepared in an inert atmosphere.

2.4.3. Biodegradation of Polyvinyl chloride plastics

The polymer surface deterioration is an interfacial process. It can involve microorganisms that can colonize the polymer surfaces as biofilms. These biofilms consist of cells embedded in a polymer matrix of their own origin, containing polysaccharides and proteins (Flemming, 1998; Costerton *et al.*, 1987). Some of the major ways through which microorganisms deteriorate synthetic polymers are: fouling, which is an unwanted deposition and growth of microorganisms on surfaces; degradation of leaching components; corrosion including hydration, penetration, and color change due to biofilms which can contain organisms that produce pigments (Flemming, 1998).

Some fungi, such as *Phanerochaete chrysosporium* and *Aspergillus fumigatus* are known for their ability to degrade some recalcitrant pollutants such as synthetic polymers. The former is called white rot fungus and degrades lignin and synthetic polymers (Martins *et al.*, 2002; Thomas *et al.*,

1992; Lymar *et al.*, 1995) and the latter has been described as an effective degrader of plasticized PVC and dichloro-diphenyl-trichloroethane (DDT).

Plasticized polyvinyl chloride is highly susceptible to microbial degradation, especially by fungi. Plasticizers are added to PVC to improve its flexibility, process ability and extensibility, but they are also potential substrates for microbial growth (along with other additives), hence contributing to polymer degradation (Gumargalieva *et al.*, 1999; Webb *et al.*, 1999, 2000) and, potentially, failure in the plastics application.

Plasticized PVC is highly susceptible to microbial attack in many different environmental situations. The problem was first identified in U.S. government reports of the deterioration of military equipment (Brown, 1946; Wellman and McCallan 1945), and subsequent reports described defacement and deterioration of commercial pPVC products (Girard and Kods, 1959, Zabel and Terracina, 1979). Biodeterioration of pPVC is now known to occur in a wide range of industrial, commercial, and structural applications (Flemming, 1998; Gaylarde, 1999; Griffin and Uribe, 1984).

The biodegradation of pPVC was reported by Sabev *et al.*, (2006) in a recent study. After 10 months of soil burial experiments the physical properties of the pPVC were altered; changes in stiffness were the most significant for heavily colonized grassland-buried pPVC samples, whereas in forest soil, the extensibility of the pPVC was affected more than the stiffness. These results suggested that fungi are important colonizers of pPVC buried in soil and that enrichment of soil fungi capable of clearing DOA occurs during colonization of the plastic surface. The results also demonstrated that incorporated biocides have a marked impact on the richness of species colonizing the pPVC surface.

Complete mineralization of phthalate in the environment is restricted to microbiologically mediated processes with the biodegradation process reported to follow a series of stages common to all phthalate esters (Staples *et al.*, 1997 a, b and Ribbons *et al.*, 1984). Phthalate has the basic structure of

an esterified benzene dicarboxylic acid with two alkyl chains, and primary biodegradation involves the sequential hydrolysis of the ester linkage between each alkyl chain and the aromatic ring, forming the monoester and subsequently phthalic acid (PA) (Johnson *et al.*, 1984). This process is common to both aerobic and anaerobic degradation and has been reported in at least 10 bacterial genera (Eaton, 1982). Secondary biodegradation results in mineralization of the PA by a number of pathways (Pujar and Ribbons 1985, Elder and Kelly 1994). With diethyl phthalate (DEP), the formation of both the monoesters (monoethyl phthalate (MEP) and PA) has been observed following the biodegradation of DEP in aqueous solution as the sole carbon source by *Micrococcus sp.* and *Aureobacterium saperdae* NRRL B-14840 (Jackson *et al.*, 1996). The appearance and subsequent degradation of PA during the degradation of DEP, also in aqueous solution, has been reported Zhang and Reardon, (1990) although no monoester was detected. No other aromatic metabolites have been recorded during the primary degradation of a diester phthalate to PA.

The decomposition of phthalates by some bacteria has been reported (Zeng *et al.*, 2002). Murad *et al.*, (2007) reported the bacterial degradation of phthalic acid. A bacterial isolate, *Pseudomonas sp.* P1, was found to degrade phthalic acid. The highest percentage of degradation of phthalic acid was found at 37 °C and pH 8. i.e. 59% and 64% respectively, after 48 hours.

The fate and behavior of phthalic acid esters during anaerobic conditions in municipal solid waste landfills was investigated in laboratory scale bioreactors under conditions of anaerobic digestion by Bauer (1997). Dimethyl phthalate (DMP), being very water soluble, could be biologically transformed during the anaerobic degradation sequence of the municipal waste regardless of the length of incubation. A primary degradation of DEHP during the hydrolytic and acidogenic phases of anaerobic digestion could not be precluded. However, there was no evidence of a transformation of DBP and Butyl benzyl phthalate (BBP). During methanogenesis all the phthalic acid esters except DMP turned out to be persistent. Di (2-ethylhexyl) phthalate could not be decomposed abiotically at pH 9. It was concluded that in the biochemical environments of

municipal landfills, short chain polyarylene ethers (PAE) can be degraded by base catalyzed hydrolysis or by microorganisms which enzymically split the side chains. However, there was no cleavage of the aromatic ring long chain PAE like di-(2-ethylhexyl) phthalate.

In a recent study the five different bioslurry reactors were operated under different conditions to study the degradation of DEHP (1 mg g^{-1} soil) in soil. The process performance was assessed by monitoring DEHP concentration periodically using high performance liquid chromatography. More than 90% degradation was observed within 12 days of the cycle period in the augmented reactors. Metabolites formed during the degradation of DEHP in the slurry phase reactor were identified and the pathway was also established. The degradation process was found to follow zero-order kinetic model (Shailaja *et al.*, 2008).

Lee *et al.*, (2007) reported a white rot fungus, *Polyporus brumalis* for dibutyl phthalate (DBP) degradation. The degradation potential and resulting products were evaluated with HPLC and GC/MS. As DBP concentration increased to 250, 750, and 1,250 mM, the mycelial growth of *P. brumalis* was inhibited. However, growth was still observed in the 1,250 mM concentration. DBP was nearly eliminated from culture medium of *P. brumalis* within 12 days, with 50% of DBP adsorbed by the mycelium.

Polyesters are potentially biodegradable due to the hydrolysable ester bonds. Poly caprolactone (PCL) mixed with PVC alters its properties, such as high impact behavior, heat resistance temperature and technological processing. PCL was reported to be a very effective plasticizer for PVC (Karal *et al.*, 1997). In some cases, thermal and photo pre-treatment can facilitate the attack of microorganisms on the polymer surface (Veronelli et al., 1999). Martin-Franchetti *et al.*, (2007) studied the PVC blend with (PCL) degradation in soil using aerobic biodegradation. The morphology and structural changes of the blends were studied by FTIR, scanning electron microscopy, differential scanning calorimetry and contact angle measurements. The results showed that PCL films degrade faster than PVC/PCL and PVC films.

The biodegradation of the pPVC and its blends with cellulose was reported by Kaczmarek and Bajer (2006) the analysis was done by FTIR, SEM and for molecular weight estimation GPC was used. They found that biodegradation in soil occurs in pPVC and this process is accelerated in the composition of pPVC with cellulose. The bio decomposition yield of pPVC /cellulose blends (calculated as relative percentage weight loss) is several dozen times higher than that of pPVC. Studies on biodegradation of PVC/cellulose composites with cellulose part 25, 50 or 75 wt.% showed that biodegradation of PVC/cellulose composites depended on polysaccharide content and was more effective than PVC itself while dehydrochlorination, characteristic for PVC, was inhibited in the presence of natural polymer (Kaczmarek and Bajer, 2008).

In a recent study the blends of PVC and biodegradable aliphatic-aromatic copolyester (AAC) were prepared, and their physical, thermal, and mechanical properties were studied. Biodegradation in the presence of the lipases of *Rhisopus arrhisus* or *Candida cilindracea* was monitored as well. The physical properties of the blends, such as density and softening temperature, were between those of each component. Differential scanning calorimetry measurements showed that the blend components were completely amorphous and miscible. Obviously, PVC suppressed the crystallization of the partially crystalline copolyester. AAC was the thermally more stable component, and it seemed to improve the thermooxidative stability of PVC in the blends. The lipases of *R. arrhisus* and *C. cilindracea* no doubt catalyzed the ester hydrolysis of AAC, although the PVC matrix limited the rate and extent of the hydrolysis (Andricic *et al.*, 2008).

Loss of plasticizers from pPVC due to microbial degradation results in brittleness, shrinkage, and ultimately failure of the pPVC in its intended application Colonization processes occurring on pPVC in the environment have received comparatively little attention. Nothing is known about the temporal sequence of microbial colonization of pPVC in situ. Existing studies have examined fungal defacement of pPVC in tropical or subtropical climates

(Hamilton, 1983; Upsher and Roseblade, 1984). In both studies fungal growth was evaluated with a subjective, visual assessment of defacement of the pPVC. Neither study examined the role of bacteria in the colonization process.

Penicillium janthinellum was found to be an important colonizer of pPVC and was one of the few strains recovered from pPVC containing biocide 10, 10'-oxy bis-phenoxy arsine (OBPA). *Penicillium roseopurpureum* was also recovered frequently while *Penicillium canescens* was recovered only during the later stages. The high frequency of recovered *Penicillium* spp. probably reflects their widespread and abundant distribution in temperate soils (Domsh *et al.*, 1980a, b); they were amongst the most commonly isolated from the surface of a number of polymeric materials exposed to the environment and from soil-buried polyurethane (Barratt *et al.*, 2003). While many of the recovered fungi were shown to be Ascomycetes, a small number of Zygomycetes and basidiomycetes were also recovered, many of which were poorly sporulating or nonsporulating and may have been under-represented on the isolation plates compared to the more abundantly sporulating ascomycetes (Webb *et al.*, 2000) Moreover, it is well known that culture based recovery techniques do not necessarily reflect either true diversity or abundance (Borneman & Hartin, 2000) and that the majority of fungal species may be difficult to recover in the laboratory due to their fastidious nutrient requirements. However, despite these limitations, a total of 92 species were recovered, although only those capable of clearing plasticizer agar and recovered in high numbers or frequently during the course of the trial were subjected to rDNA sequencing for identification purposes. While many of the fungi recovered from the surface of the pPVC were capable of clearing DOA, few isolates were capable of clearing DOP (Webb *et al.*, 2000). Two Doratomyces isolates were the most abundant recovered DOP-clearing fungi. Phthalate plasticizers have previously been shown to be far less susceptible to microbial hydrolysis compared to adipate plasticizers (Berk et al., 1957; Eaton & Ribbons, 1982; Frankland et al., 1990; Nalli et al., 2002) and there have to our knowledge been no previous reports of phthalate ester clearing by fungi. As the majority of DOA-clearing fungi were unable to clear DOP, the two plasticizers must be hydrolysed by separate enzymic systems.

In order to delay microbial colonization and biodegradation of pPVC during a product's lifespan, a variety of biocides are often incorporated into the polymer blend (Jones et al., 1996) or immobilized on the material surface (James & Jayakrishnan, 2003). However, when the plastics are disposed of, these biocides could have a negative effect by reducing colonization and degradation of plasticizers. Soil is a rich microbial environment and natural habitat for fungi, where they play a major role in the decomposition of dead plant and animal materials (Thorn, 1997). Fungi play a major role in the biodegradation of organic materials due to their ability to secrete a variety of extracellular enzymes, and they actively invade and colonize substrates of different origins (Bennett & Faison, 1997).

Hjertberg and Grevert (1995) did not observe any sign of degradation of the PVC polymer in PVC samples after 25 years of application in soil. In a theoretical study on the degradation of PVC, they assumed that rigid PVC will not degrade at a practically relevant rate, and plasticized PVC will degrade slowly with the PVC chain remaining intact. This corresponds to observations on the PVC polymer reported by (Plate, 1997 ; Yabannavar 1993 and 1994.

Hjertberg and Grevert (1995) identified a loss of plasticizers from cables after 25 years of underground application. The measured content of plasticizer was 20% in the inner layer and 17% in the outer layer of the cable. A typical plasticizer content for cable insulation would mean 26- 28%. The obvious conclusion was that the plasticizer migrates out from the material. That was confirmed by the observation that the content of plasticizer is lower in the outer layer compared to the interior and the inner layers. The release of different plasticizers of PVC under soil buried test conditions and after long-term underground application was also reported in a number of studies, but without determination of the quantities of losses (Plate, 1997).

Richard *et al.*, (2007) reported the anaerobic landfill decomposition of plasticized polyvinyl chloride. By the addition of the combination of tin carboxylate heat stabilizer and a dimethylaminopropyl methacrylamide-

titanate adduct in PVC through a mechanism as yet not understood, the above combination directs decomposition under anaerobic conditions to chain scission, thus lowering molecular weight to the point of vulnerability to microorganisms.

2.5. Biodegradability Testing

When testing the degradation phenomena of plastics in the environment, there is a general problem concerning the type of tests to be applied, and the conclusions which can be drawn. In principle, tests can be subdivided into three categories:

1. Laboratory tests
2. Simulation tests
3. Field tests

2.5.1. Laboratory tests

The most reproducible biodegradation tests are the laboratory tests, where defined media are used (in most cases synthetic media) and inoculated with either a mixed microbial population (e.g., from waste water) or individual microbial strains, which may have been especially screened for a particular polymer. In such tests, which may be optimized for the activity of the particular microorganisms used, polymers often exhibit a much higher degradation rate than would be observed under natural conditions. This can be regarded as an advantage when studying the basic mechanisms of polymer biodegradation, but in laboratory tests it is only possible to derive limited conclusions on the absolute degradation rate of plastics in a natural environment. However, for many systematic investigations these tests are widely used (Marten, 2000).

A move towards more reproducible and controlled degradation tests involves the use of systems where only those extracellular enzymes known to depolymerase a particular group of polymers are used. This method cannot be used to prove biodegradation in terms of metabolism by a microorganism, but the system is valuable when carrying out systematic investigations, for

example on the correlation of polymer structure and biodegradability (Tokiwa and Suzuki, 1977; Vikman *et al.*, 1995; Walter *et al.*, 1995; Marten, 2000). Besides reproducibility, the shortening of test duration and minimization of the material needed are crucial points when performing extended systematic investigations, or when using biodegradation testing as a tool for industrial materials development. Although degradation experiments in compost or soil may take up to one year to complete, and tests with specially screened organisms may take only a few weeks, enzymatic degradation can be performed within hours to days. Recent approaches have been aimed at using very small polymer particles (nanoparticles) for enzymatic tests, in order to increase the surface area available to the degrading enzymes. Using this technique, enzymatic degradation tests with polyesters can be performed within seconds (Gan *et al.*, 1999; Welzel *et al.*, 2002).

The analytical tools used to monitor the degradation process depend on the aim of the investigation and the test environment used. Several different analytical methods have been used to test biodegradability which includes, visual observation, changes in mechanical properties and molar mass, weight loss measurement (determination of residual polymer), CO_2 evolution/O_2 consumption, determination of biogas, radio labeling and clear zone formation.

Visual observations

The evaluation of visible changes in plastics can be performed in almost all tests. Effects used to describe degradation include roughening of the surface, formation of holes or cracks, de-fragmentation, changes in color, or formation of bio-films on the surface. These changes do not prove the presence of a biodegradation process in terms of metabolism, but the parameter of visual changes can be used as a first indication of any microbial attack. To obtain information about the degradation mechanism, more sophisticated observations can be made using either SEM or atomic force microscopy (AFM) (Ikada, 1999). After an initial degradation, crystalline spherolites appear on the surface; that can be explained by a preferential degradation of

the amorphous polymer fraction, etching the slower-degrading crystalline parts out of the material. In another investigation, Kikkawa *et al.*, (2002) used AFM micrographs of enzymatically degraded PHB films to investigate the mechanism of surface erosion.

Changes in mechanical properties and molar mass

As with visual observations, changes in material properties cannot be proved directly due to metabolism of the polymer material. However, changes in mechanical properties are often used when only minor changes in the mass of the test specimen are observed. Properties such as tensile strength are very sensitive to changes in the molar mass of polymers, which is also often taken directly as an indicator of degradation (Erlandsson *et al.*, 1997). Whilst for an enzyme-induced de-polymerization the material properties only change if a significant loss of mass is observed, for abiotic degradation processes (which often take place in the entire material and include the hydrolysis of polyesters or oxidation of polyethylenes) the mechanical properties may change significantly, though almost no loss of mass due to solubilization of degradation intermediates occurs at this stage. As a consequence, this type of measurement is often used for materials where abiotic processes are responsible for the first degradation step, e.g., chemical hydrolysis for poly (lactic acid) of oxidation for modified polyethylenes (Breslin, 1993; Tsuji and Suzuyoshi, 2002).

Weight loss measurements: determination of residual polymer

The mass loss of test specimens such as films or test bars is widely applied in degradation tests (especially in field- and simulation tests), although again no direct proof of biodegradation is obtained. Problems can arise with correct cleaning of the specimen, or if the material disintegrates excessively. In the latter case, the samples can be placed into small nets to facilitate recovery; this method was used in the full-scale composting procedure of din vM54900. A sieving analysis of the matrix surrounding the plastics samples allows a better quantitative determination of the disintegration characteristics. For

finely distributed polymer samples (e.g., powders) the decrease in residual polymer can be determined by an adequate separation or extraction technique (polymer separated from biomass, or polymer extracted from soil or compost). By combining a structural analysis of the residual material and the low molecular weight intermediates, detailed information regarding the degradation process can be obtained, especially if a defined synthetic test medium is used (Witt *et al.*, 2001).

CO_2 evolution/O_2 consumption

Under aerobic conditions, microbes use oxygen to oxidize carbon and form carbon dioxide as one major metabolic end product. Consequently, the consumption of oxygen (Puchner *et al.*, 1995; Hoffmann *et al.*, 1997) or the formation of carbon dioxide (Sturm test) are good indicators for polymer degradation, and are the most often used methods to measure biodegradation in laboratory tests. Due to the normally low amount of other carbon sources present in addition to the polymer itself when using synthetic mineral media, only a relatively low background respiration must be identified, and the accuracy of the tests is usually good. Besides conventional trapping of CO_2 in Ba $(OH)_2$ solution followed by manual titration, infrared and paramagnetic O_2 detectors can also be used to monitor O_2 and CO_2 concentrations in the air stream. Although the automated and continuous measurements have advantages, they also have disadvantages. For example, the exact air flow must be measured, the signals of the detectors must be stable for long periods of time and, if slow degradation processes are to be determined, the CO_2 concentration or fall in O_2 concentration to be detected is very small, thereby increasing the likelihood of systematic errors. Under these circumstances, other concepts (e.g., trapping CO_2 in a basic solution, approx. pH 11.5) with continuous titration or detection of the dissolved inorganic carbon (Pagga *et al.*, 2001) may be useful alternatives. Other attempts to overcome problems with CO_2 detection are based on non-continuously aerated, closed systems. Here, either a sampling technique in combination with an infrared-gas analyzer (Calmon *et al.*, 2000) or a titration system (Mueller, 1999) was applied. Another closed system with a discontinuous

titration method has been described by Solaro *et al.*, (1998). Tests using small closed bottles as degradation reactors and analyzing the CO_2 in the headspace (Itavaara and Vikman, 1995) or by the decrease in dissolved oxygen (closed-bottle test) (Richterich *et al.*, 1998) are simple and relatively insensitive to leakages, but may cause problems due to the low amounts of material and inoculums used.

Although used originally in aqueous test systems for polymer degradation, CO_2 analysis was also adapted for tests in solid matrices such as compost (Pagga *et al.*, 1995), and this method has now been standardized under the name, controlled composting test (American Society for Testing and Materials ASTMD 5338 98e1; Din V 54900; ISO 14855; JIS k 6953). In fact, the controlled composting test was not a method simulating a composting process, because mature compost was used rather than biowaste as a matrix. Biowaste, which contains a large amount of easily degradable carbon, would cause too high a background CO_2 evolution for accurate measurement, and so pre-degraded biowaste (mature compost) is used. For polymer degradation in soil, CO_2 detection proved to be more complicated than in compost because of slower degradation rates that led not only to long test durations (up to 2 years) but also low CO_2 evolution compared with that from the carbon present in soil. One means of overcoming problems with background CO_2 evolution from the natural matrices compost or soil is to use an inert, carbon-free and porous matrix, wetted with a synthetic medium and inoculated with a mixed microbial population. This method proved practicable for simulating compost conditions (degradation at ~60°c) (Bellina *et al.*, 1999, 2000), but has not yet been optimized for soil conditions.

Determination of biogas

Compared with the formation of CO_2 in the presence of oxygen, anaerobic microorganisms produce predominantly a mixture of CO_2 and methane (called biogas) as one extracellular product of their metabolic reactions. The theoretical amount and composition of the biogas can be calculated from the chemical material composition with the so-called buswell equation (Buswell

and Muller, 1952). Degradation of plastics under anaerobic conditions is mainly monitored via biogas production (Gartiser *et al.*, 1998; Reischwitz *et al.*, 1998; Abou-zeid, 2001), and standards evaluating the anaerobic biodegradation are also based on such measurements (ISO/DIS 15985, ASTMD 5210, and ASTMD 5511). Measurement of gas volume can be performed using manometry or simple water displacement, and the composition of the gas produced can be analyzed (e.g., by gas chromatography) (Budwill *et al.*, 1996). As with CO_2 evolution, the basic problem is biogas evolution from anaerobic sludge, which are usually taken as environment. Attempts to reduce background biogas evolution were made by Abou-zeid (2001) by diluting the anaerobic sludge with a synthetic mineral medium.

Radio labeling

If the carbon of the polymer is radiolabeled (usually with ^{14}C), many of the above mentioned problems in testing biodegradation can be avoided. For instance, very low concentrations of $^{14}CO_2$ can be detected even if CO_2 from other carbon sources (e.g., biowaste) is evolved. Thus, radiolabeling is used especially when slowly degradable materials are to be investigated in a matrix containing carbon sources other than the plastics (Albertsson, 1978; Tuomela *et al.*, 2001). The disadvantages of this method are problems in producing the labeled material, and working with radioactive substances from an experimental point of view.

Clear-zone formation

A very simple semi-quantitative method is the so-called clear-zone test. This is an agar plate test in which the polymer is dispersed as very fine particles within the synthetic medium agar; this results in the agar having an opaque appearance. After inoculation with microorganisms, the formation of a clear halo around the colony indicates that the organisms are at least able to depolymerize the polymer, which is the first step of biodegradation. This method is usually applied to screen organisms that can degrade a certain

polymer (Nishida and Tokiwa, 1993; Abou-zeid, 2001), but it can also be used to obtain semi-quantitative results by analyzing the growth of the clear zones (Augusta *et al.*, 1993).

2.5.2. Simulation tests

As an alternative to field tests, various simulation tests in the laboratory have been used to measure the biodegradation of plastics. Here, the degradation might take place in compost, soil or seawater placed in a controlled reactor in a laboratory. Although the environment is still very close to the field-test situation, the external parameters (temperature, pH, humidity) can be controlled and adjusted, and the analytical tools available are better than would be used for field tests (e.g., for analysis of residues and intermediates, determination of CO_2 evolution or O_2 consumption). Examples of such tests include the soil burial test (Pantke and Seal, 1990), the so-called, controlled composting test (Pagga *et al.*, 1995; Tosin *et al.*, 1996; Degli-innocenti *et al.*, 1998; Ohtaki *et al.*, 1998; Tuominen *et al.*, 2002), test simulating landfills (Mccartin *et al.*, 1990; Smith *et al.*, 1990; Mccarthy *et al.*, 1992) or aqueous aquarium tests (Puchner *et al.*, 1995). On occasion, in order to reduce the time taken to conduct the tests, nutrients are added to increase the microbial activity and accelerate degradation.

2.5.3. Field tests

Although field tests, such as burying plastics samples in soil, placing it in a lake or river, or performing a full-scale composting process with the biodegradable plastic, represent the ideal practical environmental conditions, there are several serious disadvantages associated with these types of test. One problem is that environmental conditions such as temperature, pH, or humidity cannot be well controlled; secondly, the analytical opportunities to monitor the degradation process are limited. In most cases it is only possible to evaluate visible changes on the polymer specimen, or perhaps to determine disintegration by measuring weight loss. The latter approach is problematic however if the material breaks into small fragments that must be

quantitatively recovered from the soil, compost or water. The analysis of residues and intermediates is complicated by the complex and undefined environment. Since the pure physical disintegration of a plastic is not regarded as biodegradation in the sense of most definitions (as described earlier), these tests alone can never prove whether a material is biodegradable, or not (Tuominen *et al.*, 2002).

3. MATERIALS AND METHODS

3.1. Materials/Chemicals

The low molecular weight polyvinyl chloride (PVC), plasticizers dioctyl phthalate (DOP), dioctyl adipate (DOA), solvent tetrahydrofuran (THF) and all others compounds used were of analytical grade from Sigma-Aldrich, GmbH, Steinheim, Germany and Oxoid Ltd, Basingstoke, Hampshire, England.

3.2. Media for Cultivation and Degradation Experiments

Malt extract agar (MEA; Oxoid, Basingstoke, Hampshire, England) and Saboraud's agar (Sigma-Aldrich, GmbH, Steinheim, Germany) were used for the detection, isolation and maintenance of the fungal strains. Nutrient Agar was used for isolation of bacterial isolates and the basal mineral salt media (MSM Table 3.1) was used for determining the deteriogenic properties of the organisms.

Table 3.1 Composition of the Mineral Salts Media (MSM)

Ingredients	Amount (g/l)
K_2HPO_4	1.0
KH_2PO_4	0.2
NaCl	1.0
$CaCl_2.2H_2O$	0.002
Boric acid	0.005
$(NH_4)_2SO_4$	1.0
$MgSO_4.7H_2O$	0.5g
$CuSO_4$	0.001
$ZnSO_4.7H_2O$	0.01
$MnSO_4$	0.001
$FeSO_4.7H_2O$	0.01
Agar	20.0

Media sterilization was performed by autoclaving at 121°C and 15 lbs pressure for 20 minutes. The pH of media was adjusted (pH 4.5) prior to sterilization with 0.1 M sodium hydroxide or hydrochloric acid.

3.3. Sample Collection

Samples were collected from the plastics waste contaminated area near Quaid-i-Azam University, Islamabad, Pakistan and active sewage sludge was collected from a sewage treatment plant in Islamabad, Pakistan. This sewage sludge was mixed with garden soil collected from different places of Quaid-i-Azam University, Islamabad, Pakistan. This soil mixture was used for the isolation of polymers PVC and pPVC degrading microorganisms.

3.4. Polyvinyl Chloride Film Preparation

Polyvinyl chloride having low molecular weight was used for the preparation of film by dissolving the powdered PVC in, tetrahydrofuran (THF) (99% Merck) as a solvent and sonicating for one hour. The mixture was poured into petri plates and left for over night in oven to dry. Plasticized films were also prepared by mixing the blend of PVC and plasticizer (DOP and DOA) and starch in both 1:1 and 1:2 ratios of PVC and plastcizer. The PVC and pPVC films (6×2.5 cm) were sterilized by dipping it into 70% ethanol for 15min (Calil *et al.*, 2006).

3.5. Isolation of Plastic Degrading Microorganism

The plastic degrading microorganisms were isolated from soil by soil burial experiment.

3.5.1. Soil Burial Experiment

Pieces of PVC and pPVC films were washed and buried in soil (garden soil mixed with plastics waste contaminated sewage sludge) at room temperature (30 °C) for a period of 10 months. Soil was taken in a large pot amended with mineral solution to maintain the availability of mineral salts and moisture. After 10 months pieces of PVC and pPVC were taken from soil, rinsed with sterilized distilled water and placed on nutrient agar plates, at 30 °C for 2 weeks to observe the microbial growth.

3.5.2. Enrichment and screening of Plastic degrading microbes

The fungal isolates from soil were cultured in 250ml Pyrex flask containing 100mL mineral salt media (MSM), with PVC and pPVC films as a sole carbon source, in shaker at 155 rpm at 30 °C and pH 4.5. The biomass quantification was done on weekly base and the best growing strains were selected and further tested for biodegradation. The selected fungal strains were named as PV1, PV2, PV3 and PV4.

3.6. Identification of the Selected fungal Isolates

The fungal strains isolated from soil (garden soil mixed with plastic waste contaminated sewage sludge), attached on PVC and pPVC film pieces, and were purified on Saboraud agar plates. The fungal strains were identified by microscopic examinations at Microbiology Research lab, Quaid-i-Azam University Islamabad, Pakistan. Macroscopic identification was done by visualizing surface pigment and reverse pigment on Saboraud agar and microscopic characterization includes shape, color and structure of conidia, hyphae, conidiophores and conidial head.

3.6.1. Molecular Identification of fungal isolates

Fungal isolates were identified by PCR amplification and partial sequencing of the internally transcribed spacer (ITS) regions 5.8S rRNA and 28S rRNA using the fungal Universal primers ITS-1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS-4 5'-TCCTCCGCTTATTGATATGC-3' as previously described (Webb et al., 2000). Detailed methodology is as follows:

3.6.1.1. Isolation of genomic DNA

Fresh mycelia were harvested in deionised water from overnight plate cultures grown on MEA. The biomass was separated by centrifugation at 8,000 rpm using MSE Mistral centrifuge, UK for 10 min, and the supernatant was discarded. The pellet was frozen by placing the centrifuge tube into liquid

nitrogen, and tubes were then stored at −80 °C until the pellet was ground in a mortar under liquid N_2. DNA was extracted according to the method of Anderson *et al.*, (1996).

3.6.1.2. DNA Extraction Protocol

1. Filter recovered spore suspension through 2 x muslin or j-cloth, were added to liquid nitrogen in a cooled pestle and mortar and ground in to a fine powder ensuring constant freezing. They were then transferred to 12ml centrifuge tubes on ice.
2. A 5ml of 65 °C extraction buffer (0.7 M NaCl, 0.1 M Na_2SO_3, 0.005 M Tris/ Hcl (pH= 7.5), 0.005 M EDTA, 1%, SDS) for the isolation of the DNA was added (see appendix for constituents) and mixed well by shaking or vortexing.
3. Mixture was incubated at 65 °C for 20 min. A 5 ml of chloroform: isoamyl alcohol (24:1) was added vortexed and placed on ice for 30 min.
4. Centrifugation was done in MSE Mistral centrifuge for 30 min (4 °C at 3000 rpm). Upper phase was removed and kept and further transferred to a clean 12ml tube. Equal volume of isopropanol (propan-2-ol) was added and mixed gently. Mixture was left at room temperature (30 °C) for 10 min.
5. Centrifugation for 10 min was done at 4 °C at 3000 rpm and supernantant was discarded. To this 2 ml of sterile distilled water was added and left at room temperature to resuspend.
6. 1ml of 7.5M NH_4Ac was added. Mixed gently and placed on ice for 1h. Centifuged for 30 min (4 °C at 3000 rpm).
7. Supernatant was removed and transferred to another tube. And 0.54 volumes of isopropanol was added (approx. 1.62ml) and mixed gently. The DNA was visible as white strands. This was left at room temp for 10 min.
8. Centrifuged for 15min (4 °C at 3000 rpm and carefully removed and discarded the supernatant. Pellet was washed with 2ml 70% ethanol.

Centrifuged for 5 min (4 °C at 3000 rpm) and the supernatant was removed and allowed the pellet to air dry.

9. The pellet was resuspended in 500µl sterile Distilled water and 1µl RNase was added and stored at -20 °C.

3.6.1.3. PCR Amplification

Polymerase chain reaction (PCR) was used by using ITSI and ITS4 for the amplification of fungal 5.8S, 18s and 28s rRNA. The universal primers were used for the identification of the fungal strains. The V3 variable region at the 5' end of the 5.8 S, 18S and 28S rRNA were amplified with the fungal universal primers V3-1 (5' GCATATCAATAAGCGGAGGAAAAG) and V3-2 (5' GGTCCGTGTTTCAAGACGG) (Fell, 1993). PCR reagent concentrations were 0.2 µM of primers V3-1 and V3-2, 2.5 mM of $MgCl_2$, 200 µM for each of the four deoxynucleoside triphosphates, and 1.25 U of *Taq* DNA polymerase (Roche Diagnostics Ltd., Lewes, United Kingdom) per 50-µl reaction mixture. Amplification was performed for 30 cycles with denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min. ITS regions were amplified using fungal universal primers ITS-1 (5' TCCGTAGGTGAACCTGCGG) and ITS-4 (5' TCCTCCGCTTATTGATATGC) (White et al.,1990). PCR reagent concentrations were as for V3-1 and V3-2 with the exception of concentrations of 0.25 µM for primers ITS-1 and ITS-4 and 1.5 mM for $MgCl_2$. Amplification was performed for 35 cycles with denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min.

3.6.1.4. Agarose Gel Electrophoresis

DNA was observed following electrophoresis using 1% agarose in 1X TAE buffer) and staining with ethidium bromide (1 µg ml^{-1} in TAE buffer, Webb *et al.*, 1999).

3.6.1.5. Purification

Amplified products were purified using the QIAquick PCR purification kit (Qiagen Ltd., Crawley, United Kingdom).

3.6.1.6. Sequencing

PCR products (0.8 Kb fragments) were sequenced from the sequencing facilities of Faculty of Life Sciences, University of Manchester, UK.

Both strands of the amplified products were sequenced using the ABI BigDye Dideoxy Terminator Cycle sequencing kit (Applied Biosystems Inc., Warrington, United Kingdom). Cycle-sequencing conditions were denaturation at 96 °C for 10 s, annealing at 50 °C for 5 s, and extension at 60 °C for 4 min for 25 cycles, with a final extension at 60 °C for 4 min. The annealing temperature was increased to 55 °C for sequencing reactions using the V3-1 primer. Forward and reverse sequences were aligned using ABI Auto-assembler software (Applied Biosystems Inc.), and the overlapping consensus sequence was compared with sequences in the NCBI database using FASTA 3 sequence homology searches.

3.7. Biodegradation Studies

3.7.1. Soil burial

Low molecular weight polyvinyl chloride PVC films were buried in soil for 10 months, and pPVC (DOP, DOA) and blends with starch films were buried in soil for 6 months, at room temperature (30-35 °C), in a large pot amended with mineral salt media (MSM) to maintain the availability of mineral salts and moisture and inoculated with the selected fungal isolates. Structural change in the polymer was analyzed after soil burial.

3.7.2. Shake flask experiments

Mineral salt medium (100 ml) containing pieces of pure PVC, pPVC (DOP, DOA) and starch blends of PVC were used for biodegradability testing of

selected fungal isolates. Spore suspensions of fungal isolates were used as inoculum. Experiments were run for 6 months under shaking conditions at 30 °C except PVC starch blends which was treated for 3 months. Biomass quantification was done on weekly basis. The PVC films were analyzed for structural changes by SEM, Gel permeation chromatography (GPC), NMR and FTIR at the end of experiments.

3.7.2.1. Effect of UV pretreatment on biodegradation of PVC

The PVC film pieces were exposed to UV light (254 nm wavelength) for about 10 hours and then used in shake flask experiment with PV1 as inoculum. Plastics film was analyzed.

3.7.3. Sturm test

Carbon dioxide evolution as a result of PVC, pPVC (DOP, DOA) and starch PVC biodegradation was determined by Sturm Test (Muller *et al.*, 1992) (Fig. 3.1). The pieces of polymer films were added to the culture bottle (Test bottle) containing 300 ml of mineral salts medium without any other carbon source. Spore suspensions of fungal isolates were prepared in sterilized saline and were used to inoculate the test and the control bottles, containing mineral salt medium, to study the degradation of PVC and pPVC. Control bottle was without any plastic. Sterilized air was supplied to keep conditions aerobic. The test and control bottles were stirred continuously by placing them on the magnetic stirrer. The test was performed at room temperature (30 °C) for 4 weeks. After 4 weeks of culturing the change in biomass and the amount of carbon dioxide produced was calculated in the test and control bottles, gravimetrically. Evolution of CO_2, as a result of degradation of polymeric chain was trapped in the absorption bottles containing KOH (1M). Barium chloride solution (0.1M) was added to the CO_2 containing KOH bottles and as a result precipitates of barium carbonate (using CO_2 released from breakdown of polymer) were formed. CO_2 produced can be calculated gravimetrically by measuring amount (weight) of precipitates evolved by addition of $BaCl_2$. Change in both test and control was observed.

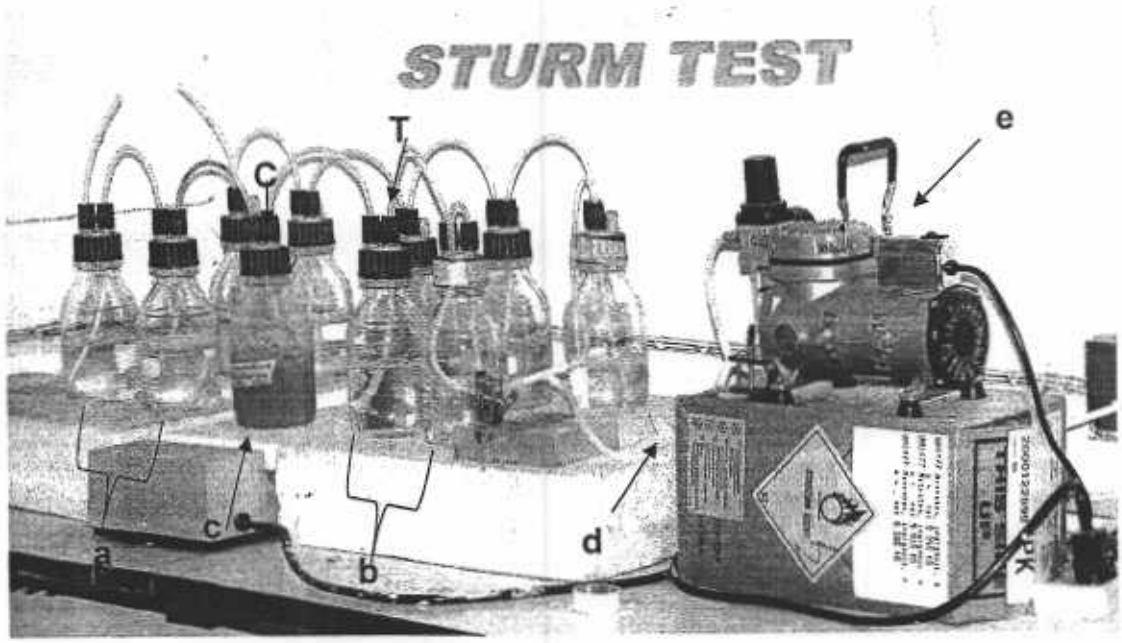


Figure 3.1 Experimental set up of sturm Test for measurement of carbon dioxide evolution during break down of plastic (PVC) material. a: CO_2 absorbing chambers, b: pretreatment chambers, c: culture vessel, d: 0.2 µm air filter, e: pressure air pump, T: Test, C: control

3.8. Analytical Methods

3.8.1. Light Microscopy

The pieces of PVC and pPVC buried in sewage sludge were taken. The plastic films were washed with sterilized distilled water and examined through microscope to observe the microbial adherence and attachment to PVC and pPVC surface.

3.8.2. Biomass quantification

This method is considered as a relatively simple and useful for directly quantifying the number of microorganisms attached to the polymer surface (Christensen *et al.*, 1995). It is used to evaluate the ability of chosen test

microorganisms to grow and utilize component from the polymer blend as sole carbon source. The quantification was done for the screening of isolates from degradation experiment by examining the proliferation and growth of microorganisms on the plastic material. The fungal isolates from soil were cultured in 250ml Pyrex flask containing 100mL mineral salt media (MSM), with PVC and pPVC films as a sole carbon source, in shaker at 155 rpm at 30 °C and pH 4.5, the fungal biomass was quantified on weekly basis. Separate flasks were set for every week sample. The whole contents of the flask containing fungal culture were filtered through Whattman #1 pre-weighed filter paper. Biomass on the filter paper was dried in oven at 50 °C to constant weight. The filter paper was weighed to get the dry biomass subtracting the weight of filter paper.

3.8.3. Scanning Electron Microscopy (SEM)

The surface morphology of the PVC and pPVC films was analyzed through scanning electron microscopy. Specimens were attached to stubs using Electrodag 915 (Acheson Industries, Reading, United Kingdom) and sputter coated (model S150 device; BOC Edwards) with gold before being examined using a Stereoscan 360 scanning electron microscope (Cambridge Instruments, Cambridge, United Kingdom).The images of the test samples were compared with those recorded on the original untreated samples (control).

3.8.4. Gel Permeation Chromatography (GPC)

The number- and weight-average molecular weights Mn, Mw of PVC and pPVC were determined using the Gel Permeation Chromatograph (GPC) made by Viscotek GPC max VE 2001(Texas) equipped with the refractometric (Shoedex RI-71) and viscometric (Viscotek) Model T50A) detectors. Two connected columns (GMHHR-MS type, TSK-GEL) were used.. Column flow rate was 1.mL/minute. Higher plate counts can be obtained at lower linear velocities. 100ul sample was injected at 35 °C detector temperature and 30 °C column temperature. The calibration was made using the polystyrene

standards (Aldrich). Tetrahydrofuran was used as solvent. The sample measurement time was 30 min/ sample.

3.8.5. Fourier Transform Infra Red (FTIR) Spectroscopy

Fourier Transform Infrared Spectrophotometer FTX 3000 MX Bio Rad Ex-Clibur^{Tm} FT-IR Series, USA was used for analysis of PVC and pPVC after degradation experiments. The polymer pieces were mixed with KBr and made into a tablet, which was fixed to the FTIR sample plate. Spectra were taken in triplicate at 400 to 4000 wave-numbers cm^{-1} for each sample.

3.8.6. Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the principal techniques used to obtain physical, chemical, electronic and structural information about molecules due to the chemical shift and Zeeman Effect on the resonant frequencies of the nuclei. It is a powerful technique that can provide detailed information on the topology, dynamics and three-dimensional structure of molecules in solution and the solid state. Both ^{13}Carbon NMR and Proton NMR spectroscopic analysis were used with Chloroform $^{13}CDCl_3$ as internal standard.

The films of PVC and pPVC were dissolved in the solvent (Chloroform $CDCl_3$) and then they were analyzed in the Bruker 400MHZ NMR spectrophotometer.

4. RESULTS

4.1. Isolation of PVC degrading microorganisms

Soil samples, collected from plastic waste contaminated sites with heap of solid waste debris from Quaid-i-Azam University, Islamabad, Pakistan and the sewage sludge collected from the Sewage Treatment Plant, Rawalpindi, Pakistan, were used as a source for isolating microorganisms having the ability to degrade PVC plastics.

A number of bacterial, actinomycetes and fungal strains were isolated. These microbes were tested for having the ability to adhere, grow and degrade PVC. Four fungal strains PV1, PV2, PV3 and PV4 were finally selected on the basis of their good growth, adherence and degradation potential of PVC plastics. The adherence and attachment of the fungal strains was observed in all polymer films, pure PVC film, pPVC film and PVC starch blends (Fig 4.1).

4.2. Identification of fungal isolates

For the identification of the fungal isolates, the morphology of the fungi on the malt extract media was studied. The colonies of PV1 were white color with serrated edges, the flat fruiting body appear 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. 4.2 a).

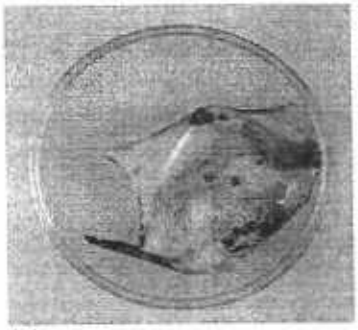
Colonies of the strain PV2 were light colored, smooth and flat on the surface of the agar plate, the colony surfaces have sporous appearance with smooth surface, whereas colonies of strain PV3 on Malt extract agar were initially light black, quickly becoming black with conidial production. The color of the mature sporulated mycelium was blackish. Reverse is pale yellow and growth may produce radial fissures in the agar (Fig. 4.2 b, c).

Initially the growth rate of the colonies of PV4 were slow, the color was off white but quickly turned to greenish shade having irregular white colored edges, on maturity of mycelium the appearance of light brown color observed in centre of colony, the growth texture was powdery (Fig. 4.2 d).

4.3. Molecular Identification of fungal isolates

The PCR product of 0.8 kb size of all of the isolated strains were visualized on 1% agarose gel with 1X TAE buffer (Fig. 4.3).

The four selected isolates were identified by 5.8S, 18S, 28S rRNA and internal transcribed spacer (ITS) region. The 0.8kb nucleotide fragment was observed in Agarose Gel Electrophoresis There nucleotide sequences (see Appendix) were compared with known sequences in National centre for biotechnology information (NCBI) using fast alignment sequences tool all (FASTA). The strains were identified on the basis of their homology as *Phanerochaete chrysosporium* PV1, *Lentinus tigrinus* PV2, *Aspergillus niger* PV3 and *Aspergillus sydowii* PV4 (Table 4.1). The sequences were deposited in NCBI Genbank and got the accession number as EU543990.1, EU543989.1, EU543987.1 , EU543988.1 for PV1, PV2, PV3 and PV4 respectively.



(a)

(b)

(C)

Figure 4.1. Fungal adherence and attachment on polymer films placed in MSM agar plates after two months incubation at 30 ^{0}C (a) pure PVC film (b) pPVC (DOP) film (c) PVC starch blends.

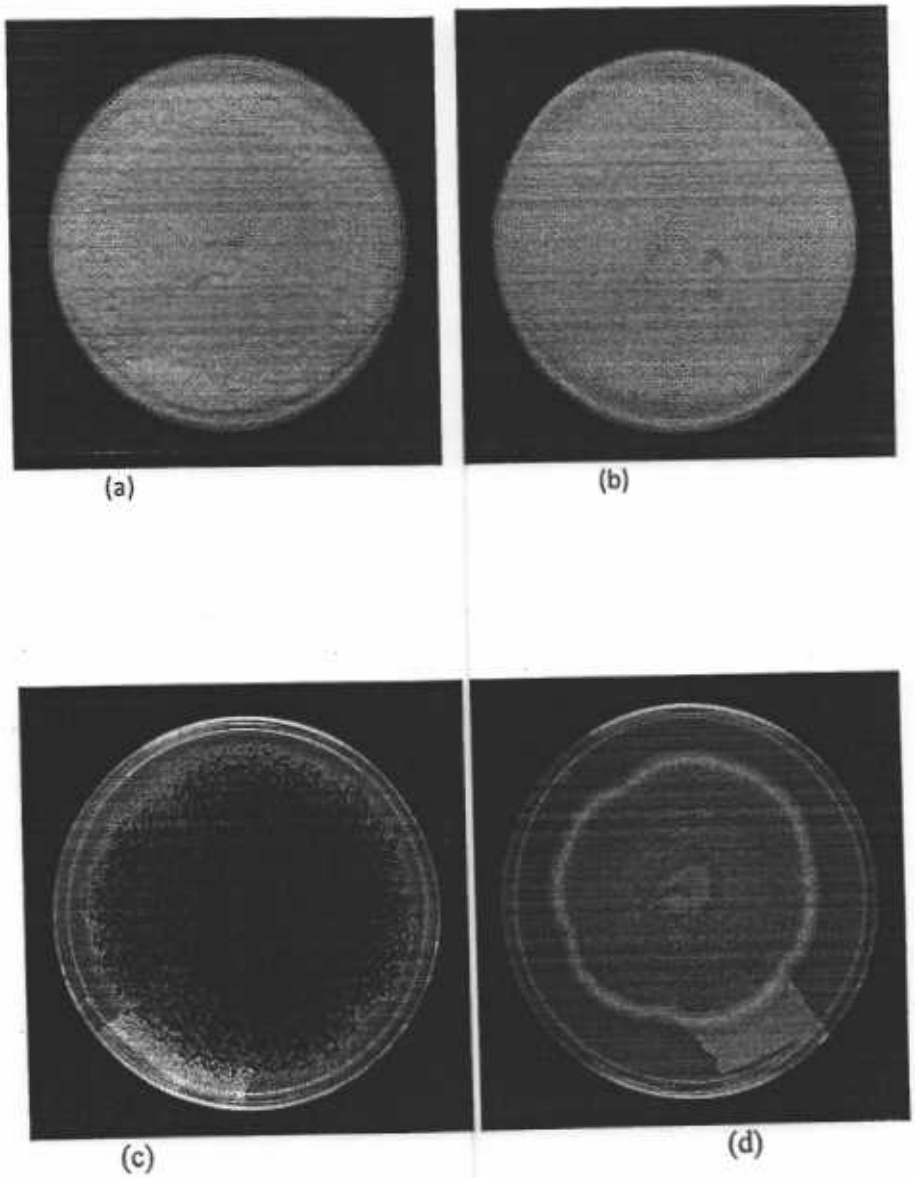


Figure 4.2. Morphology of colonies of fungal isolates on Malt extract agar plates. (a) PV1 (b) PV2 (c) PV3 and (d) PV4

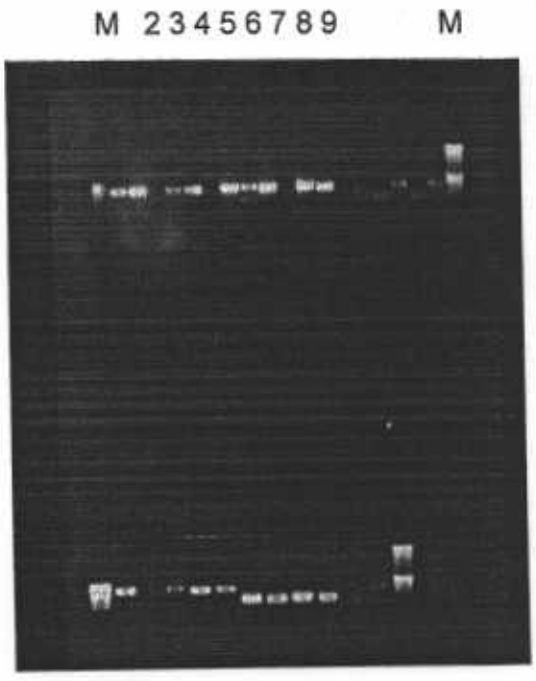


Figure. 4.3 Gel electrophoresis of isolated DNA on agarose gel electrophoresis
L1, Hyp4: 1kb DNA marker (Invitrogen)
L2: PV2, L4: PV1, L5: PV3,L5: PV4

Table 4.1. Molecular Identification of fungal isolates

Fungal isolates	Accession Nos. of the sequences giving homology with transcript	Score of homology	Query coverage	F value	% homology	identification
PV1	AF475147.1, AF475146.1, AY854086.1, AB361645.1, AB361644.1, AY219344.1	990-1086	95-99%	0.0	98-100%	*Phanerochaete chrysosporium*
PV2	AF516518.1, DQ056860.1, AF516520.1, AF516521.1	977-1173	94-99%	0.0	96-99%	*Lentinus tigrinus*
PV3	EU709774.1, EF634379.1, EF634376.1, EF634375.1, EF567979.1, EF175904.1, EU301660.1, EF151435.1,	992	100%	0.0	99%	*Aspergillus niger*
PV4	EF652451.1, AM883157.1, AM883154.1, AJ937748.1, AY373869.1, EF652473.1, EF652450.1, AM883163.1, AM883158.1	977-994	100%	0.0	99%	*Aspergillus sydowii*

4.4. Biodegradation of PVC

Biodegradability was first tested of plastic film kept with 10 months with microbes. Following burial for 10 months, the surface of the PVC was heavily colonised and discolored and some hyphal growth was visible to the naked eye at the surface of the films (Fig.4.4).

Further plastic films were incubated on mineral salt agar medium with inoculated fungi. Polyvinyl chloride film showed only the growth of *Phanerochaete chrysosporium* PV1 (Fig.4.5).

4.4.1. Growth of fungal Isolates on PVC plastics

The Biomass quantification was done for the degradability studies of isolates. The isolates were grown in MSM media having pure PVC under shaking condition. The biomass quantification was done on weekly basis. For the pure PVC the strain *Phanerochaete chrysosporium* PV1 showed maximum dry weight about 2.57mg/ml followed by *Aspergillus niger* PV3 after 7 weeks incubation in MSM media in shake flask experiment at 30^{0}C. There was gradual increase in dry cell mass from 3^{rd} week to 7^{th} week. However no or slow change in growth was observed in media inoculated with *Lentinus tigrinus* PV2 and *Aspergillus sydowii* PV4 (Fig.4.6).

4.4.2. Analysis by Scanning Electron Microscopy (SEM)

Pure PVC film were buried in soil, inoculated with individual fungal strains, for 10 months were examined under SEM; There was a notable change on the surface of treated PVC film after microbial treatment with *Phanerochaete chrysosporium* PV1 showing initial pattern of degradation of the PVC film. The appearance of hexagonal rings in treated samples not present in control indicating the change in the surface of the polymer after soil burial due to fungal adherence *Phanerochaete chrysosporium* PV1 At higher magnification these hexagonal rings were more prominent and they were on the surface of

the PVC films (Fig.4.7). There were less/not significant changes in SEM analyses of films treated with other three fungal strains, PV2, PV3 and PV4.

4.4.3. Analysis by Strum test

Biodegradation of plastics was studies in liquid broth with fungal isolates *Phanerochaete chrysosporium* PV1, *Lentinus tigrinus* PV2. The breakdown of polymer results in the production of carbon dioxide. The carbon dioxide evolved was determined gravimetrically by Sturm test.

After incubation for 4 weeks, it was found that in case of Test (with PVC pieces) when treated with *Phanerochaete chrysosporium* PV1 the total amount of CO_2 produced was 8.31g/l, whereas, in control (no PVC pieces) it was 4.90g/l, however when treated with *Lentinus tigrinus* PV2 the total amount of CO_2 produced was 6.03g/l, whereas, in control (no PVC pieces) it was 3.05g/l). The dry cell mass of *Phanerochaete chrysosporium* PV1 in test was higher (0.099 mg/ml) than in control (0.053 mg/ml) also the increase in dry cell mass of *Lentinus tigrinus* PV2 was observed in test (0.087 mg/ml) (Table. 4.2).

The change in the carbon dioxide and dry cell mass produced by comparing with control confirmed that more activity of biodegradation was shown in the test having PVC pieces treated with fungal isolates than the control. However *Phanerochaete chrysosporium* PV1 showed better biodegradation ability than *Lentinus tigrinus* PV2.

4.4.4. Analysis by Gel Permeation chromatography

Gel permeation chromatography (GPC) was used to analyze the structural changes of pure polyvinyl chloride (PVC) after treatment with fungal isolates in shake flask. The GPC results showed that the weight-average molecular weight (*Mw*) of PVC decreased with longer exposure to the microorganism The Initial molecular weight of the pure PVC was 202,530 Da however after 6

months shake flask experiment decrease in molecular weight was observed, the *Phanerochaete chrysosporium* PV1 showed more change in the PVC molecular weight as decreased up to 178,292 than the rest of three fungal strains (Table 4.3).

Due to fungal degradation the molecular dispersity (Mn) was also changed. *Aspergillus niger* PV3 showed more change in the molecular dispersity (1.545) followed by *Phanerochaete chrysosporium* PV1 (1.572) as compared to the control 2.07 (Table. 4.3).

4.4.5. Analysis by Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) analysis of control of Polyvinyl chloride film piece and FTIR analysis of Polyvinyl chloride film piece, which were buried in soil showed the appearance of new peaks and shifting of the peaks at 2370-2350 cm $^{-1}$ (corresponding to O-H) region indicating the change in the structure of the PVC also decrease in peak height at 2277-2250 cm $^{-1}$ showing significant change due to microbial degradation (Fig.4.8).

The PVC film initially treated with UV and then exposed to *Phanerochaete chrysosporium* PV1 for four months in shake flask experiment showed more obvious degradation in PVC film, not only shifting of the peaks but also complete change in the structure of the polymer chains by peaks shortening and almost disappeared at 2980-2910cm^{-1} (corresponding to alkyl segment) also new peaks appeared at 2380-2340 cm $^{-1}$ (corresponding to O-H). The results showed that some of the double bonds of PVC were cleaved by microbial strain, indicating that UV treatment enhanced the biodegradation of the polymer (Fig. 4.9).

4.4.6. Analysis by Nuclear magnetic resonance (NMR)

In ^{13}CNMR spectra of the control sample the signal at 14.26 ppm was attributed to CH_2 while the signal at 32.03 ppm was assigned to CH-Cl carbon. While In addition to these signals some more signals appeared in the

range 29-47 ppm in treated film of PVC in shake flask experiment for six months with *Phanerochaete chrysosporium* PV1. The increase in number of signals indicated that some chemical change/shifting were taken place in the polymeric material. Further the appearance of new peaks in the fungal treated PVC film spectra indicated the chemical shift of peaks due to biodegradation activity of the microbes (Fig.4.10).

The ^{13}CNMR spectra of the PVC film when treated with the *Lentinus tigrinus* PV2 in shake flask also showed new peaks appearance in range of 20-40 ppm, the peaks at 23.16 ppm, 30.15ppm, 44.24ppm, 44.57ppm and 46. 41ppm (represent OC*H2CHClO) were the new peaks which were not present in the control PVC film (Fig.4.10). That showed there was notable change in the structure of the polymer film due to microbial attachment.

Proton magnetic resonance spectra of the PVC film showed the signal region for aliphatic protons (-CH_2-CH-Cl) was around 1.5 ppm and for (-CH2-CH-Cl) was 3.0-4.0 ppm. The total integration of the region for the control sample 0.3-3.1 ppm was 43.81 (Fig.4.11) while for the *Phanerochaete chrysosporium* PV1 treated sample the integration was decreased to 43.07 in the same region.

The ^{1}H spectra of the pure PVC treated with *Lentinus tigrinus* PV2 also showed decrease in integration to 43.52 in the same region. That decrease in integration clearly indicates that during the process, some of the protons have been exhausted (Fig.4.11).

Figure 4.4. Fungal adherence and attachment on the surface of PVC film (indicated by arrows) after 10 months soil burial experiment

Figure 4.4. Growth of fungal isolate *Phanerochaete chrysosporium* PV1 on PVC film

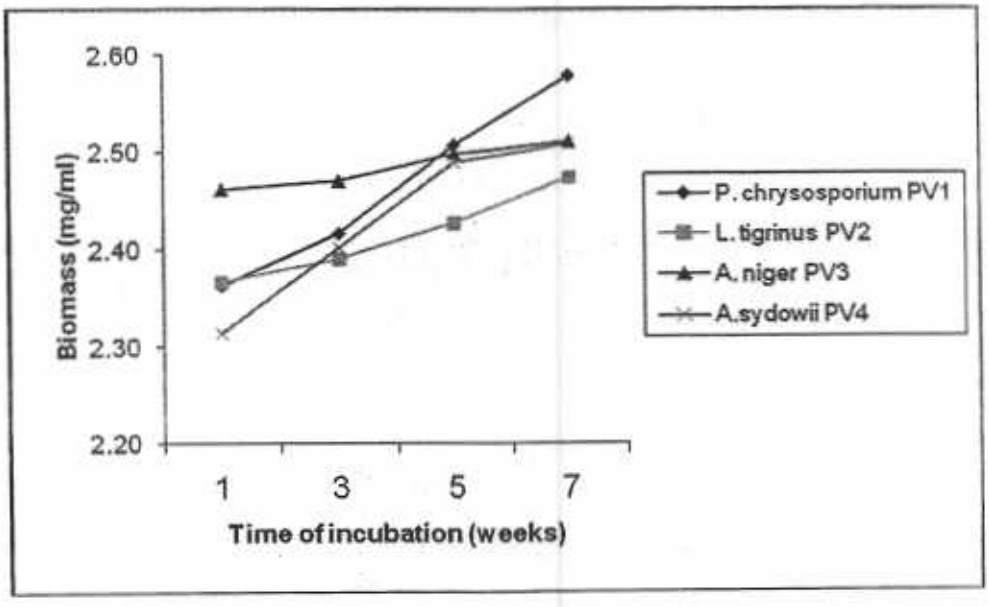


Figure 4.6. The growth of fungal isolates in mineral salt medium with pure PVC as sole carbon source.

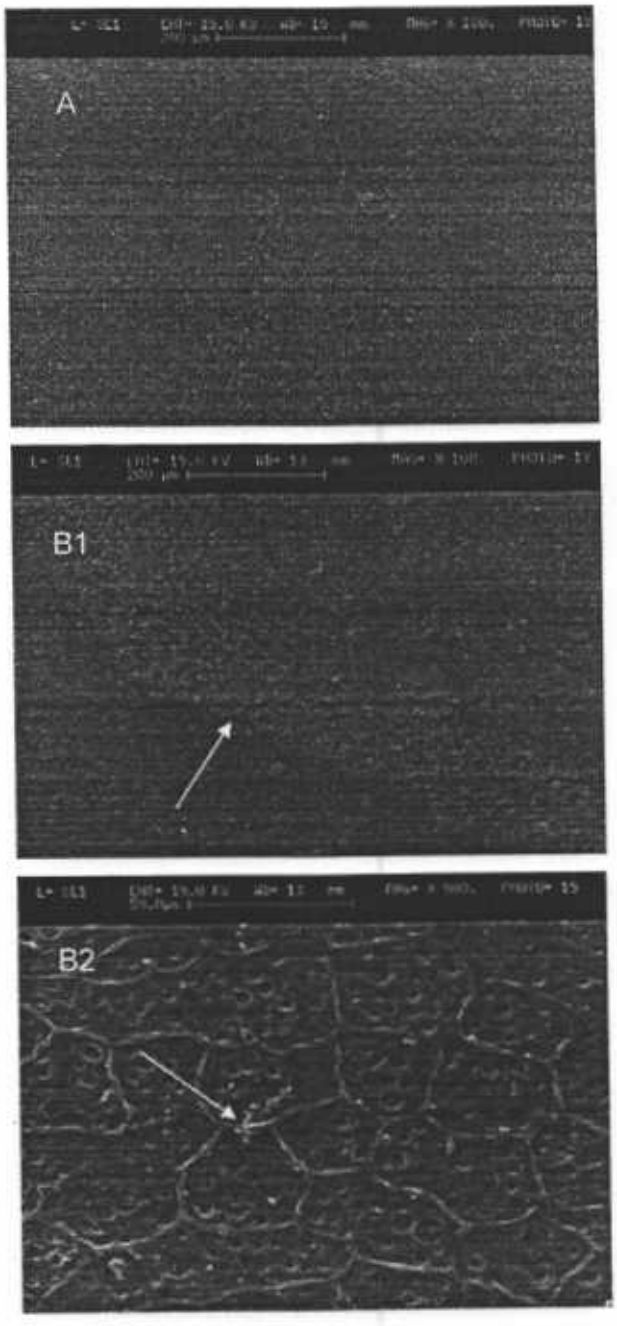


Figure 4.7. Scanning Electron Micrographs of the pure PVC films, Untreated film control (A); after 10 months soil burial experiment with *Phanerochaete chrysosporium* PV1 (B1, 100 x and B2 500x)

Table 4.2. Dry cell mass and gravimetric analysis of CO_2 evolution during breakdown of pure PVC by *Phanerochaete chrysosporium* PV1 and *Lentinus tigrinus* PV2 determined through Sturm test after 4 weeks of treatment (control; without PVC pieces)

Fungal isolates	CO_2 Produced (g/l)		Dry cell mass (mg/ml)	
	Control	Test	Control	Test
***P. chrysosporium* PV1**	4.90	8.31	0.053	0.099
***L. tigrinus* PV2**	3.05	6.03	0.063	0.087

Table 4.3. Gel permeation chromatography analysis of the pure PVC fungal degradation (after six months)

Strain	Molecular weight MW (Da)	Molecular dispersity (MW/Mn)
Control	202,530	2.077
P. chrysosporium PV1	178,292	1.572
L. tigrinus PV2	195,326	1.685
A. niger PV3	183,930	1.545
A. sydowii PV4	190,753	2.077

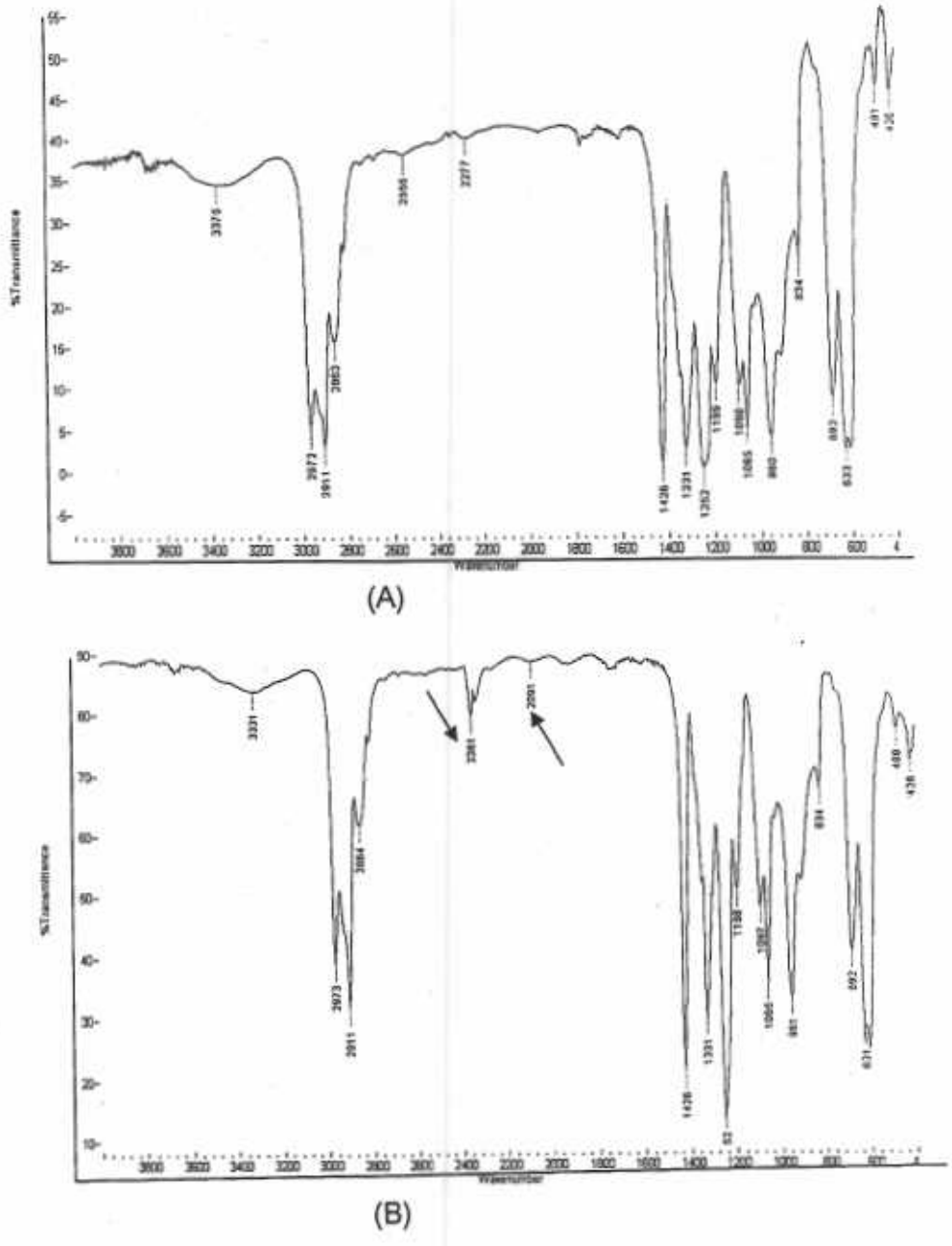
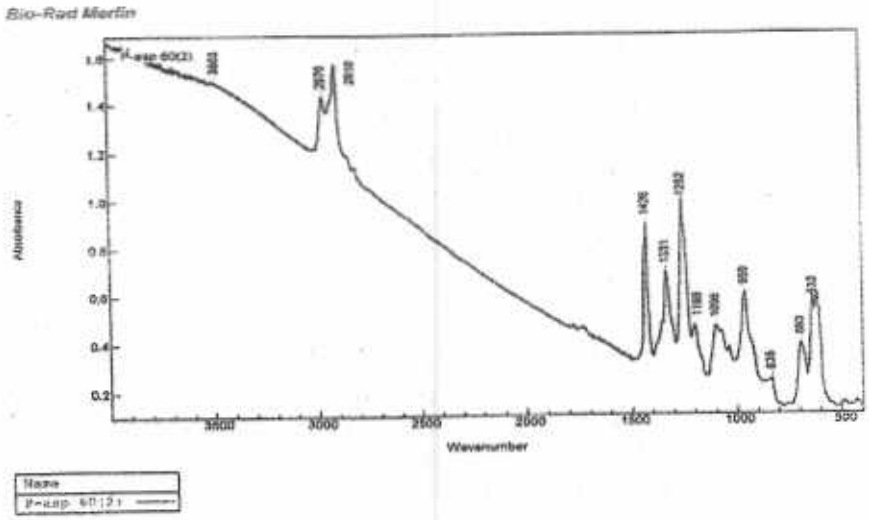
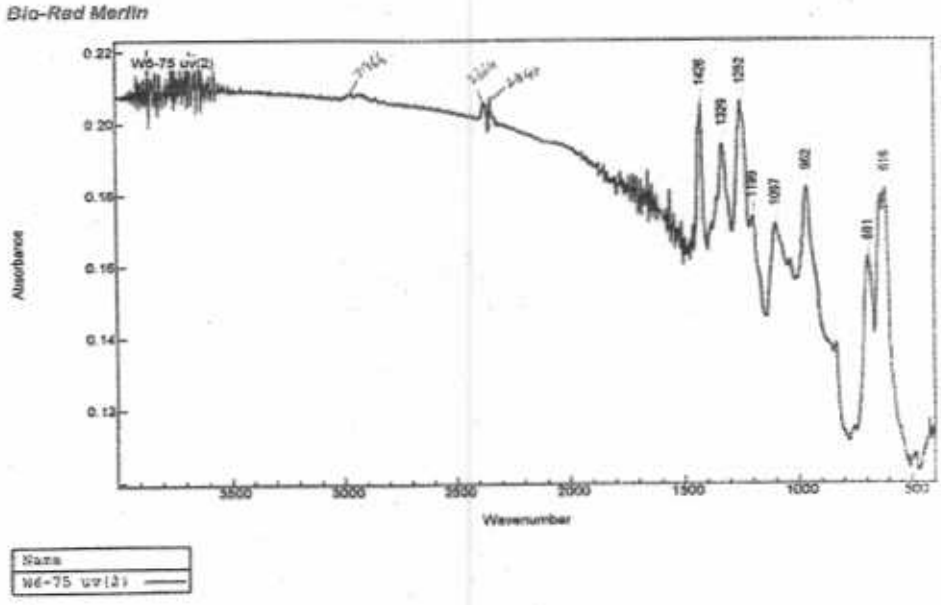


Figure 4.8. Fourier transform infra red spectra of polyvinyl chloride film pieces; control, untreated (A); After exposure to soil burial treatment for 10 months (B).



(A)



(B)

Figure 4.9. Fourier transform infrared spectra of polyvinylchloride Control (A); UV treated with films *P. chrysosporium* PV1 after four months (B)

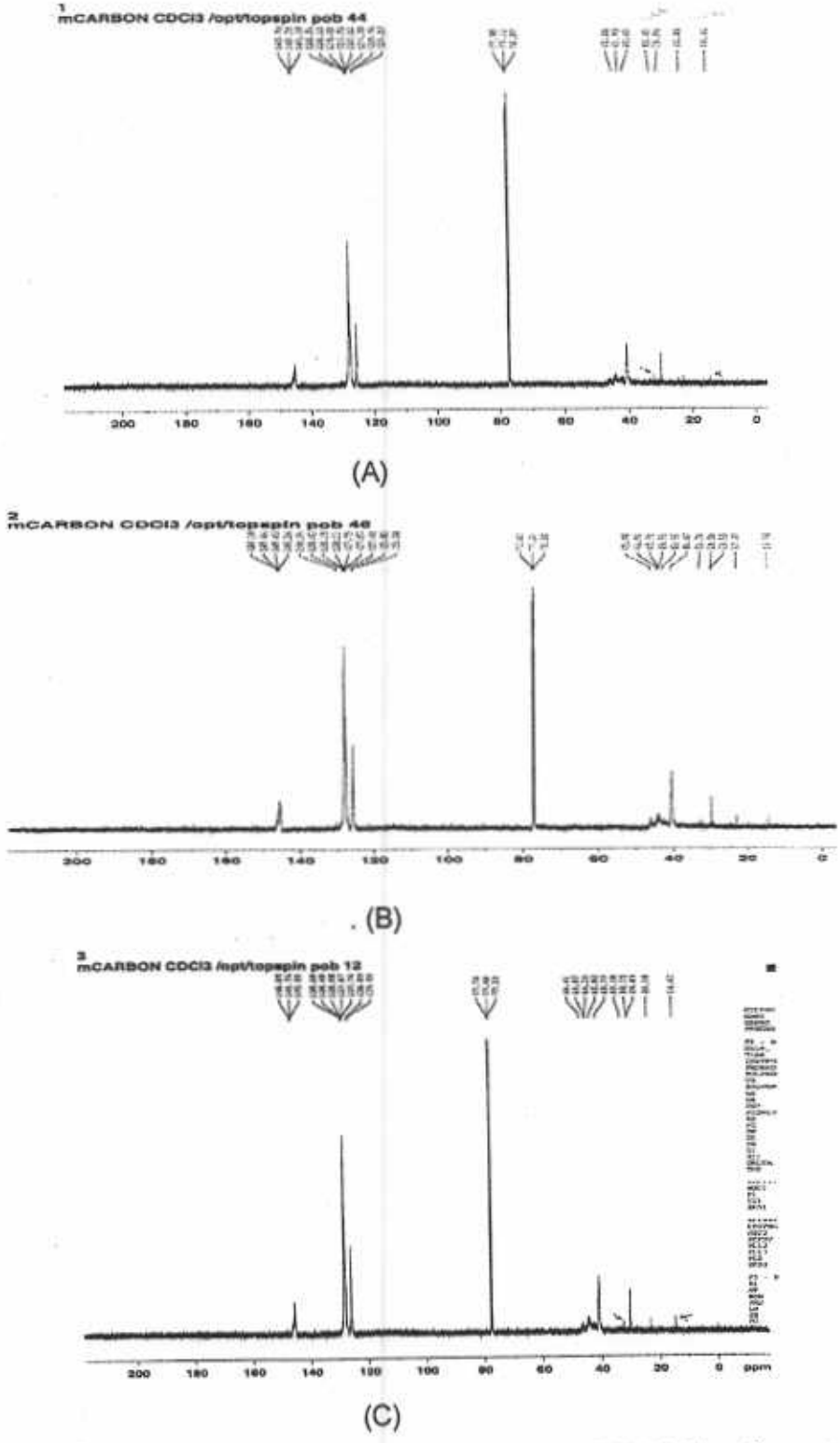
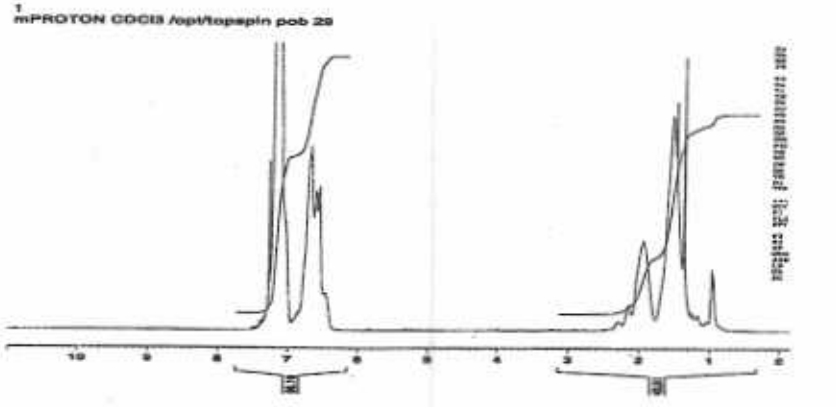
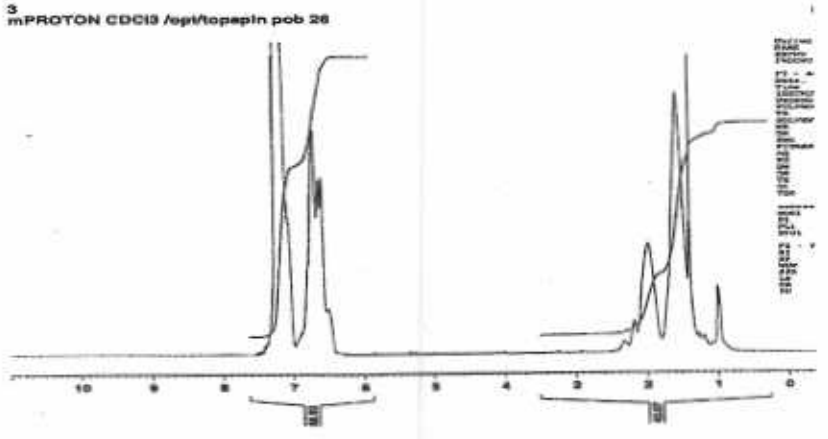


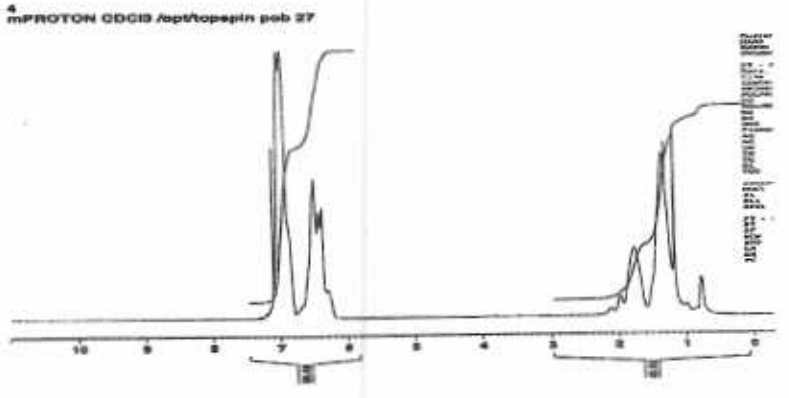
Figure 4.10. ^{13}C NMR spectra and peak assignments of PVC film, A) control, B) treated with *Phanerochaete chrysosporium* PV1, C) treated with *Lentinus tigrinus* PV2 after six months



(A)



(B)



(C)

Figure 4.11. ^{1}H NMR spectra of PVC film, A) control, B) treated with *Phanerochaete chrysosporium* PV1, C) treated with *Lentinus tigrinus* PV2 after six months.

4.5. Biodegradation of Plasticized PVC

Polyvinyl chloride films were made in the presence of plasticizers DOP and DOA. Both of the plastics were then tested for biodegradation.

The plasticized PVC films were incubated on mineral salt agar medium and inoculated with fungi. Both the plasticized (DOP and DOA) films showed only the growth of *Lentinus tigrinus* PV2 (Fig.4.12).

4.5.1. Growth of fungal Isolates on PVC plastics

The biomass quantification after growth in MSM with fungal isolates showed maximum dry weight 2.89 mg/ml after 7 weeks incubation by *Aspergillus niger* PV3 in shake flask experiments with pPVC (DOP). *Phanerochaete chrysosporium* PV1 showed initially increase in growth till 5th week there after no increase was observed. While *Lentinus tigrinus* PV2 and *Aspergillus sydowii* PV4 did not show significant growth (Fig.4.13).

A gradual increase in growth of *Aspergillus niger* PV3 was observed with maximum of (2.8 mg/ml) with pPVC (DOA) after 7 weeks incubation in shake flask experiment, however a increase in cell mass also observed in flasks inoculated with PV1 from 3rd to 5th week. The rest of the two strains *Lentinus tigrinus* PV2 and *Aspergillus sydowii* PV4 did not show significant increase in growth (Fig.4.14).

4.5.2. Analysis by Scanning Electron Microscopy (SEM)

Plasticized polyvinyl chloride (DOP and DOA) films were incubated with isolated fungal strains in mineral salt media in shake flask experiment for 6 months were examined under SEM; the results showed that there was not only clear adherence of fungus on the surface of pPVC (DOP and DOA) but also visible change in surface structure of the polymer film after microbial treatment.

The degradation pattern was more obvious in pPVC (DOP) after fungal (*Aspergillus niger* PV3) attack showing complete change in the surface structure of the treated film. The appearance of cracks and holes indicated that fungal strain utilizing pPVC as sole carbon source in shake flask experiment with mineral salt media after 6 months. The attachment and the adherence of the *Aspergillus niger* PV3 was also shown on the surface of the polymer leading to biodegradation potential of the *Aspergillus niger PV3* (Fig.4.15).

Surface analysis of pPVC (DOA) film after microbial treatment with *Aspergillus sydowii* PV3 in shake flask experiment for 6 months showed clear changes in the film structure. There was erosion and extensive roughening of the surface of fungal degraded pPVC (DOA) film .The appearance of fungal growth on the surface of pPVC (DOA) film was also indication of the adherence and degradation of the pPVC film (Fig.4.16).

Other 3 fungal strains PV1, PV2 and PV4 did not show significant structural changes in electron microscopic analyses.

4.5.3. Analysis by Strum test

The carbon dioxide evolved was determined gravimetrically by Sturm test, in reaction flasks containing PVC with DOP. After incubation for 4 weeks, it was found that in case of test pPVC(DOP) film pieces. when treated with *Phanerochaete chrysosporium* PV1 the total amount of CO_2 produced was 29.79g/l, whereas, in control it was 9.07g/l, however when treated with *Lentinus tigrinus* PV2 the total amount of CO_2 produced was 20.75g/l, whereas, in control it was 8.13g/l (Table 4.4). The increase in cell mass of *Phanerochaete chrysosporium* PV1 (0.116 mg/ml) and cell mass of *Aspergillus sydowii* PV4 (0.98 mg/ml) than in controls (Table. 4.4).

When biodegradability of plasticized PVC with DOA was tested by strum test using *Phanerochaete chrysosporium* PV1 the total amount of CO_2 produced

was 32.79g/l, whereas, in control it was 13.07 g/l, however when treated with *Aspergillus niger* PV3 the total amount of CO_2 produced was 27.67 g/l, as compared to control 11.37 g/l.The cell biomass was increased in *Phanerochaete chrysosporium* containing test flask (0.106 mg/ml) as compared to control (0.056 mg/ml) and same increase was observed in strain *Aspergillus niger* PV3 (0.089 mg/ml) (Table.4.5).

4.5.4. Analysis by Gel Permeation chromatography (GPC)

The change in the molecular weight was studied by analysis of pPVC films with gel permeation chromatography, the results showed that the molecular weight (*Mw*) of polymer was decreased due to biodegradation. There was significant decrease in the molecular weight of pPVC (DOP) with *Phanerochaete chrysosporium* PV1 (81,028) as compared to initial molecular weight of the 84,300 followed by *Lentinus tigrinus* PV2 (*81,928).*

An increase in the molecular dispersity was observed in the pPVC (DOP) by treating with fungal strains that confirming the change in the polymer. The more increase was observed in *Phanerochaete chrysosporium* PV1 (1.759) compared to rest of the fungal strains (Table.4.6).

A decrease in the molecular weight (*Mw*) of pPVC (DOA) was observed due to fungal degradation, all of the four fungal strains showed significant degradation results. The more change in molecular weight was observed by *Aspergillus sydowii* PV4 (81,830) *as* compared to other fungal strains (Table.4.7).

The increase in the molecular dispersity of the pPVC (DOA) was shown by all the fungal strains, *Aspergillus sydowii* PV4 showed maximum increase in the molecular dispersity (1.807) followed by *Lentinus tigrinus* PV2 (1.681) however *Phanerochaete chrysosporium* PV1 showed less increase as compared to other strains (1.591) (Table 4.7).

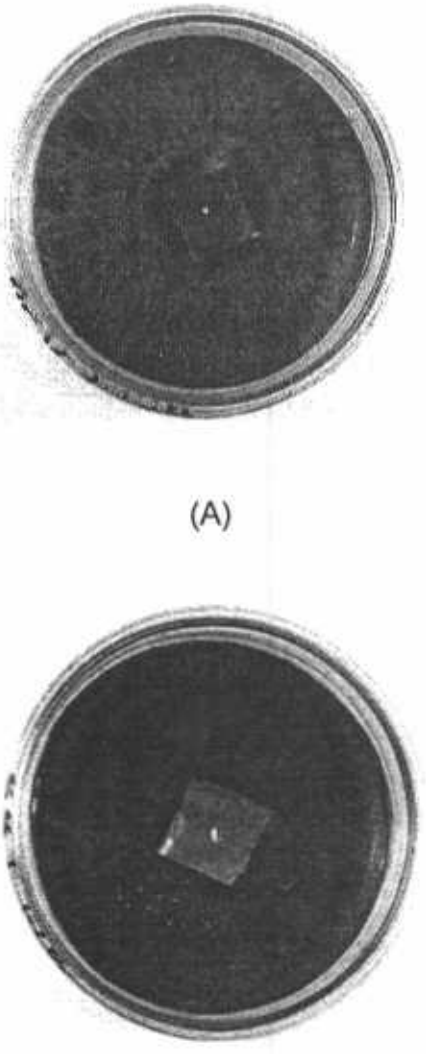
4.5.5. Analysis by Fourier Transform Infrared Spectroscopy (FTIR)

After treatment of pPVC (DOP) with *Lentinus tigrinus* PV2 and *Aspergillus sydowii* PV4 the structural changes in the polymer were determined by FTIR. The appearance of new peaks at 2400-2200 cm^{-1} and at 830-880 cm^{-1} (corresponding to –C=C–) and peaks shortening at 1710-1715 cm^{-1} (corresponding to carbonyl compound) confirmed the structural changes in the polymer. The results showed that some of the double bonds of pPVC (DOP) were cleaved by fungal strains (Fig.4.17).

The FTIR spectra of the pPVC (DOA) showed change in the structure of the polymer film by *Phanerochaete chrysosporium* PV1 as compared to the control. There was shifting of peaks appeared at 1000-900cm^{-1} indicating the change in the plastic film (corresponding to C-O) and appearance of new peak at 690cm^{-1} also confirmed the breakdown of the polymer due to fungal attack (Fig.4.18).

4.5.6. Analysis by Nuclear magnetic resonance (NMR)

The Proton NMR spectra of pPVC (DOA) showed the signal region for aliphatic protons (-CH_2-CH-Cl) and for (-CH2-CH-Cl) was 0-3.0 ppm. The total integration of the region for the control sample was 43.26ppm, while for the treated sample; the integration was increased to 43.39 in the same region. That increase in integration clearly indicated that during the process, some of the protons have been exhausted. Therefore, it is suggested that this decrease in integration in the polymer is due to fungal activity that bring changes in the polymer structure (Fig.4.19).



(A)

(B)

Figure 4.12. Growth of fungal isolate *Lentinus tigrinus* PV2 on pPVC(DOP) film (A) and pPVC (DOA) film (B).

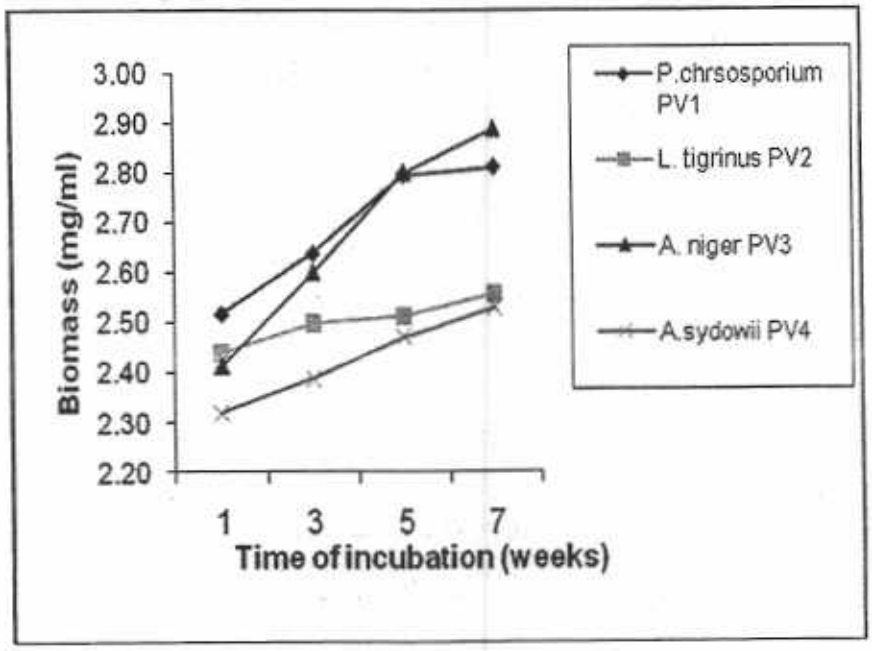


Figure 4.13. The growth of fungal isolates in mineral salt medium with p PVC (DOP) as sole carbon source

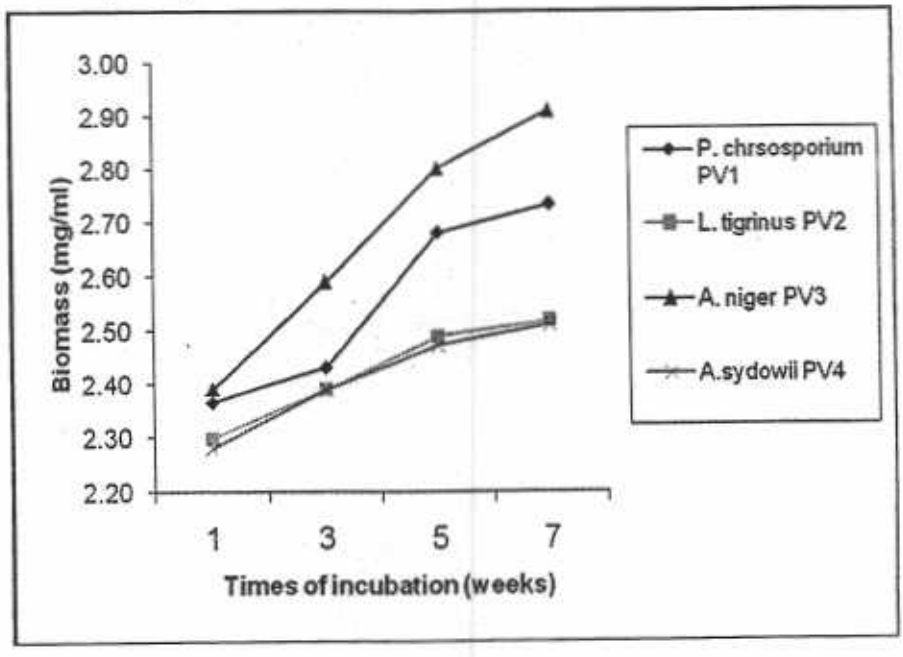


Figure 4.14. The growth of fungal isolates in mineral salt medium with p PVC (DOA) as sole carbon source

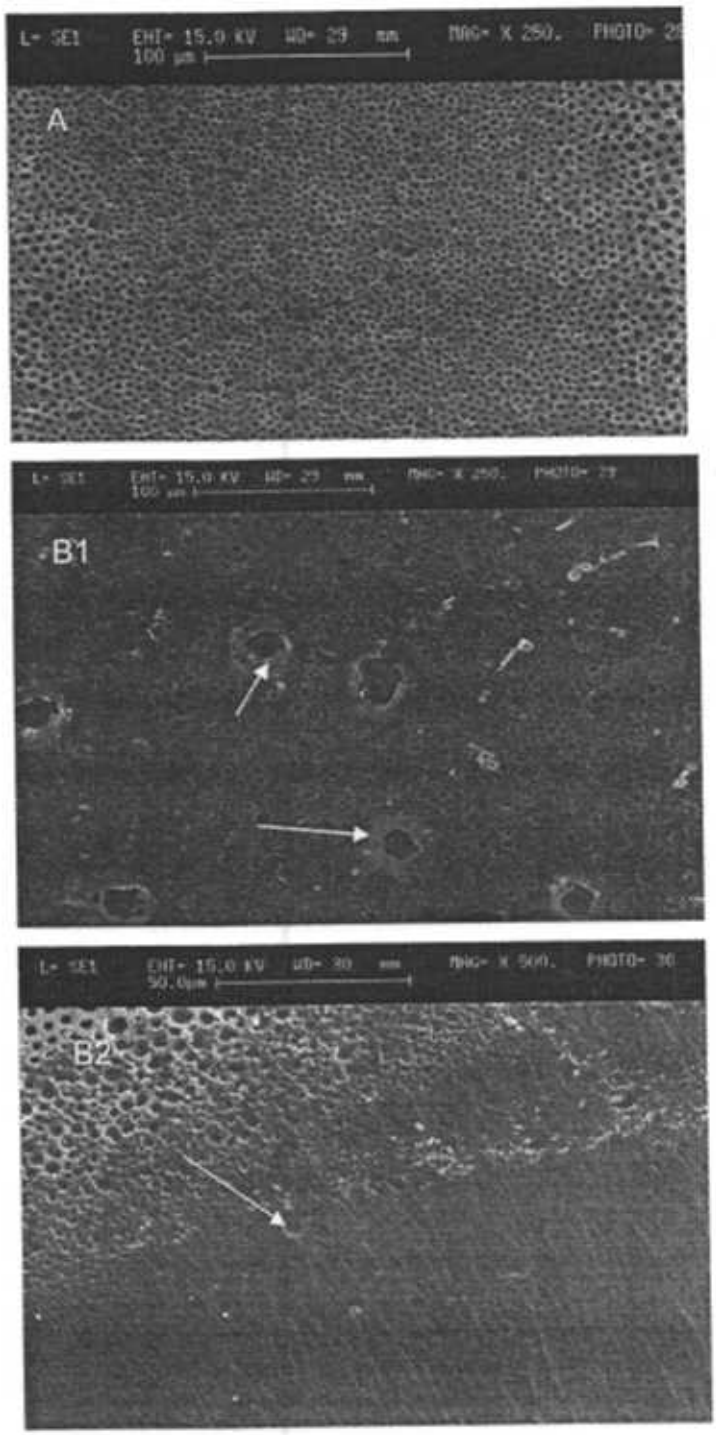


Figure 4.15. Scanning Electron Micrographs of the pPVC film (DOP) A) Untreated film; B1) after 6 months shake flask experiment with *Aspergillus niger* PV3 (B1) 250 x (B2) adherence of *Aspergillus niger* PV3 on film surface 500x

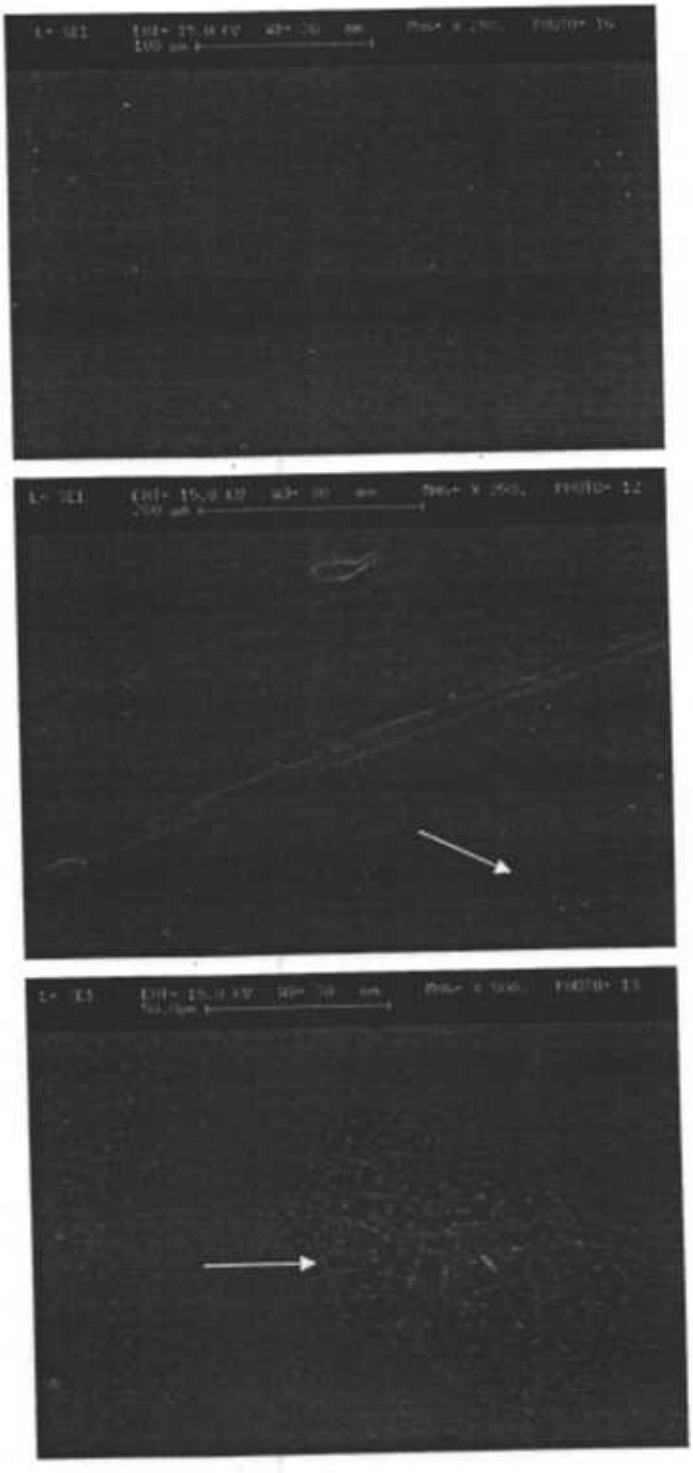


Figure 4.16. Scanning Electron Micrographs of the pPVC film (DOA), A) Untreated film; B1) after 6 months shake flask experiment with *Phanerochaete chrysosporium* PV1 250 x (B2) 500x

Table 4.4 Dry cell mass and gravimetric analysis of CO_2 evolution during breakdown of pPVC(DOP) by *Phanerochaete chyrososporium PV1* and *Lentinus tigrinus* PV2 determined through Sturm test after 4 weeks of treatment (Control; without pPVC pieces).

Fungal isolates	CO_2 Produced g/l		Dry cell mass mg/ml	
	Control	Test	Control	Test
***P. chrysosporium* PV1**	9.07	29.79	0.063	0.116
***L. tigrinus* PV2**	8.13	20.75	0.061	0.098

Table 4.5 Dry cell mass and gravimetric analysis of CO_2 evolution during breakdown of PVC +DOA by *Phanerochaete chrysosporium PV1* and *Aspergillus niger* PV3 determined through Sturm test after 4 weeks of treatment (Control; without pPVC pieces)

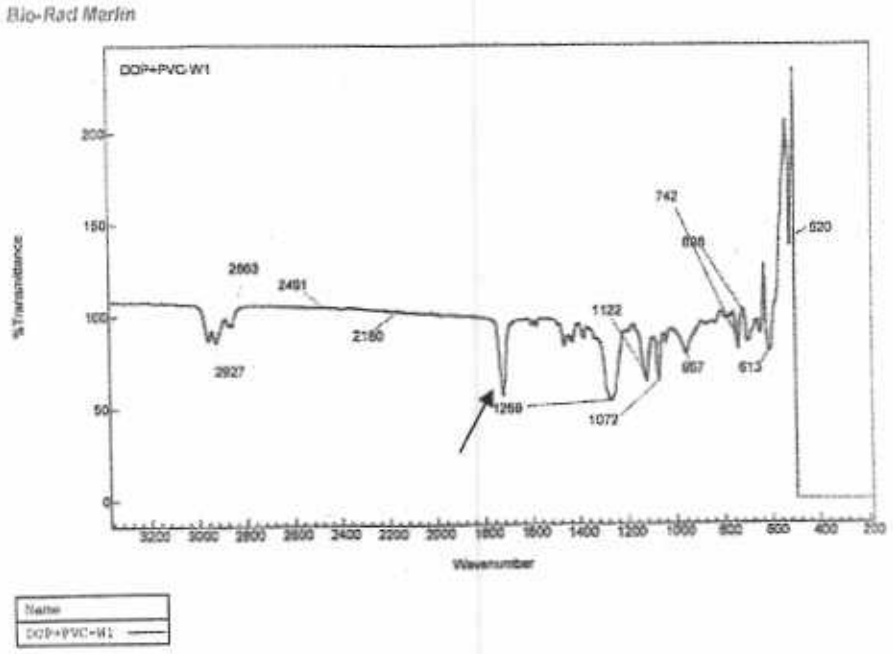
Fungal isolates	CO_2 Produced g/l		Dry cell mass mg/ml	
	Control	Test	Control	Test
***P. chrysosporium* PV1**	13.07	32.79	0.056	0.106
***A. niger* PV3**	11.37	27.67	0.058	0.089

Table 4.6 Gel permeation chromatography analysis of the pPVC (DOP) fungal degradation (after six months)

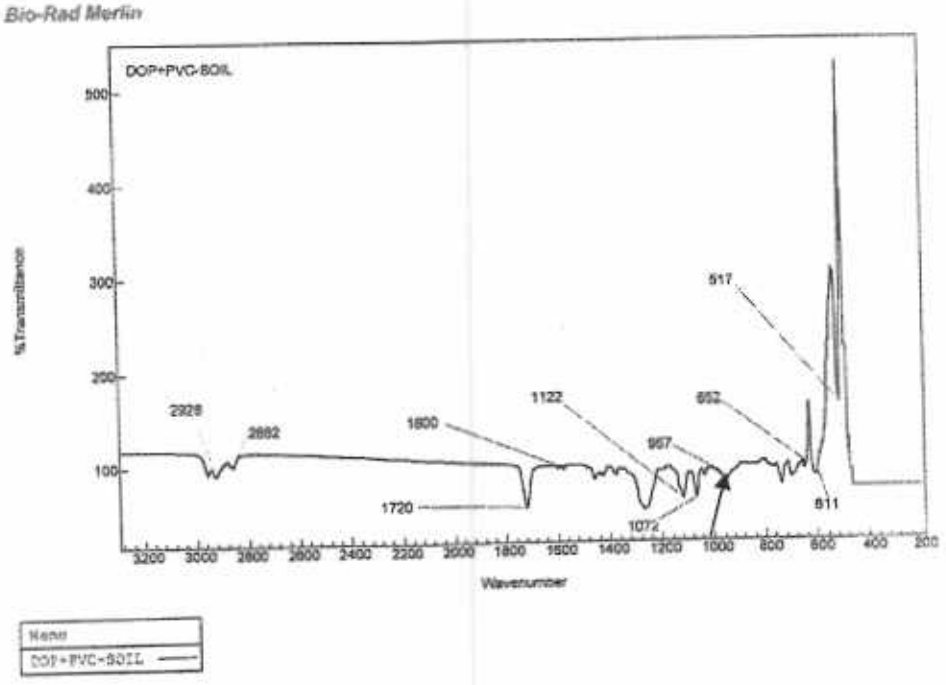
Strain	Molecular weight MW (D)	Molecular dispersity (MW/Mn)
Control	84,300	1.680
Lentinus tigrinus PV2	81,928	1.745
P. chrysosporium PV1	81,028	1.759
Aspergillus niger PV3	82,153	1.703
Aspergillus sydowii PV4	82,946	1.643

Table 4.7 Gel permeation chromatography analysis of the pPVC (DOA) fungal degradation (after six months)

Strain	Molecular weight MW (D)	Molecular dispersity (MW/Mn)
Control	88,000	1.400
L. tigrinus PV2	84,810	1.681
P. chyrososporium PV1	84,017	1.591
A. niger PV3	82,000	1.640
A. sydowii PV4	81,830	1.807

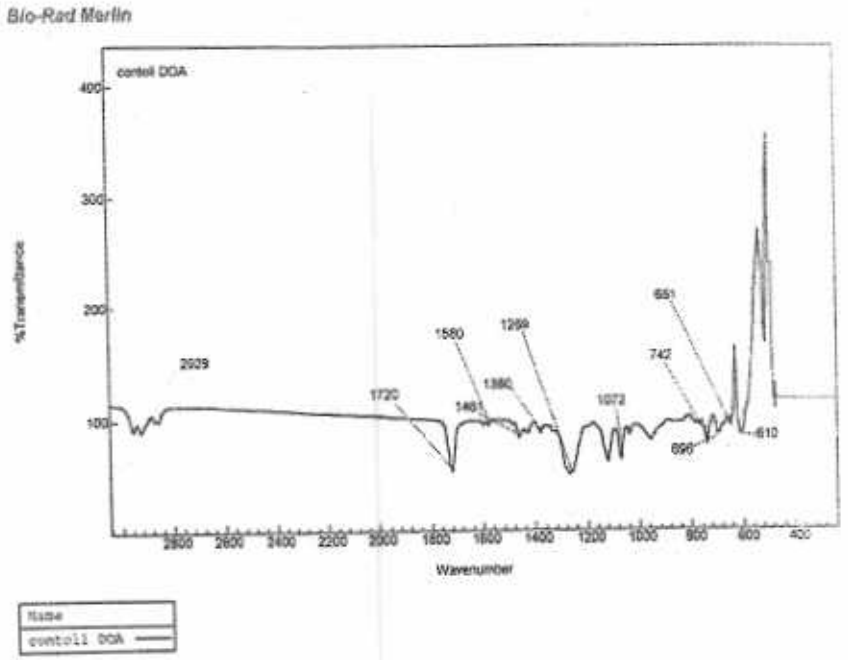


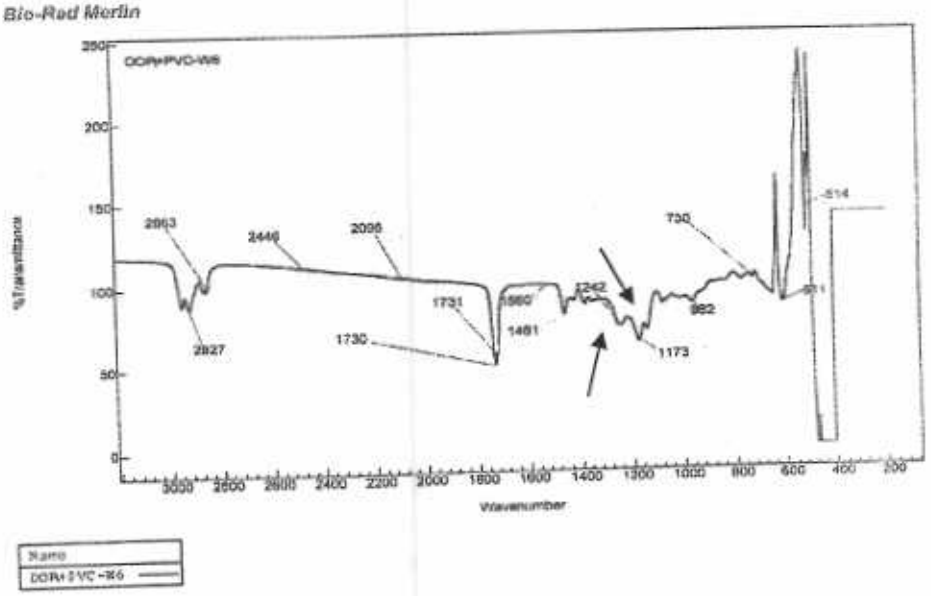
(A)



(B)

Figure 4.17 Fourier transform infrared spectra of PVC (DOP), A) after treated by *Lentinus tigrinus* PV2, B) after treated by *Aspergillus sydowii* PV4





(A)

(B)

Figure 4.18. Fourier transform infrared spectra of PVC (DOA), A) Control, B) after treated by *Phanerochaete chrysosporium* PV1

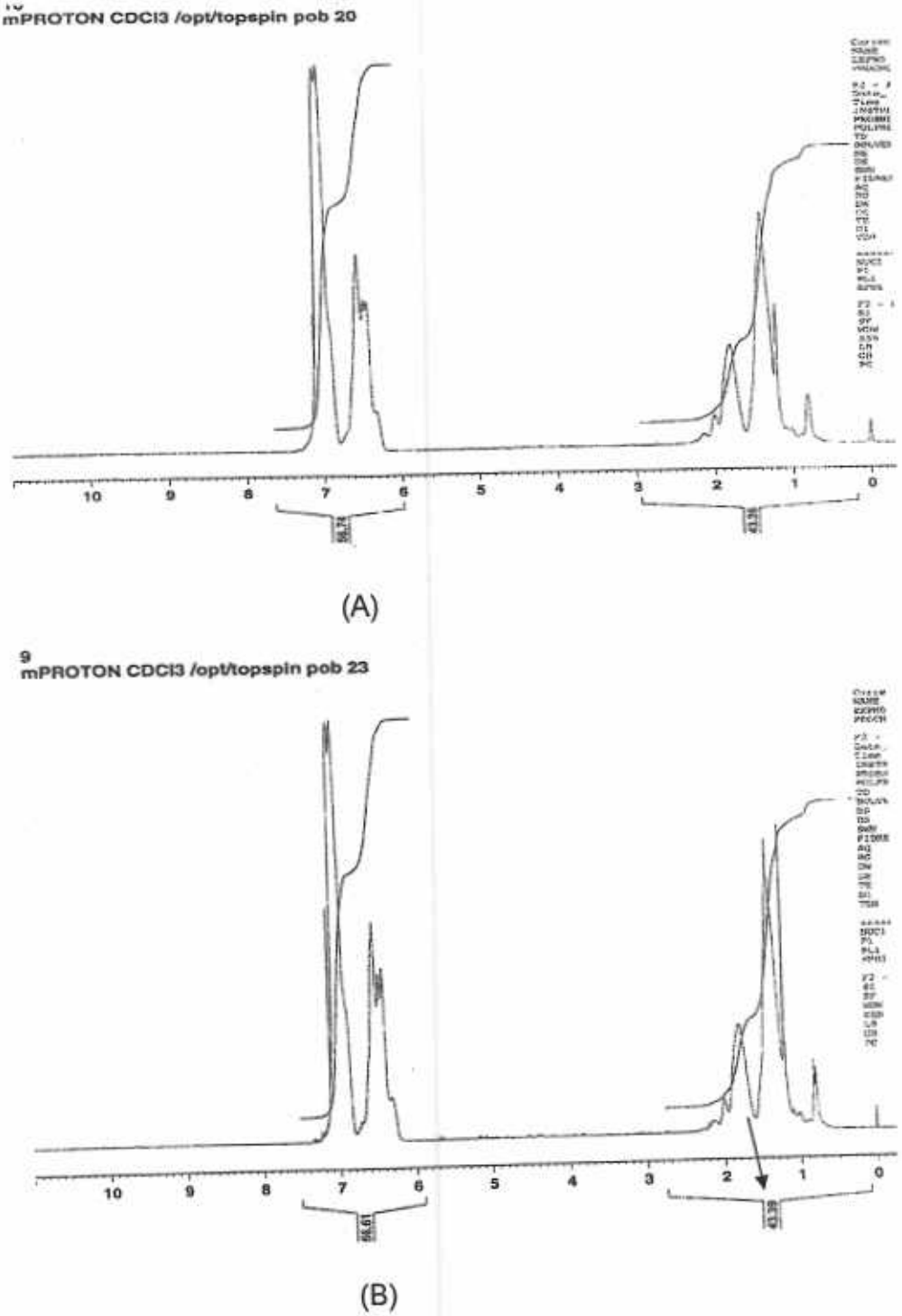


Figure 4.19. Proton Nuclear magnetic resonance ^{1}H NMR spectra, A) control, B) treated pPVC (DOA) film treated with *Phanerochaete chrysosporium* PV1 for six months in liquid media.

4.6. Biodegradation of Polyvinyl Chloride Starch Blends

Polyvinyl chloride starch blended films were incubated on mineral salt agar medium inoculated with fungal strains. Polyvinyl chloride film showed only the growth of *Aspergillus niger* PV3 (Fig.4.20).

4.6.1. Changes to the surface of PVC starch blends

The surface of the PVC starch blend film totally changed as compared to control after six months soil burial experiment. The samples were discoloured 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.4.21).

4.6.2. Growth of fungal Isolates on plastics

The Biomass quantification was done for the screening of isolates for degradation experiments. The isolates were grown in MSM media having PVC starch blends. The biomass quantification was done on weekly basis. During 7 weeks incubation in shake flask experiments the maximum growth was shown by *Phanerochaete chrysosporium* PV1 that was about 2.97mg/ml, followed by *Aspergillus niger* PV3, the *Lentinus tigrinus* and *Aspergillus sydowii* PV (Fig.4.22).

4.6.3. Analysis by Scanning Electron Microscopy (SEM)

Surface analysis of PVC starch blends after microbial treatment showed changes in the structure of the film. In the treated sample there was erosion and extensive roughening of the surface There was a notable change on the surface of treated PVC starch blend after microbial treatment showing appearance of holes on the polymer surface, there was clear surface erosion and breakdown of the some part of the film due to *Phanerochaete chrysosporium* PV1 activity in shake flask experiment after 3 months. The fungal growth was also observed on the polymer surface (Fig. 4.23). There

were also detoriation clearly observed in PVC starch films incubated with other fungal isolates (observed by light microscopy, results not shown) but electron micrographs were not taken.

4.6.4. Analysis by Strum test

Polyvinyl chloride with starch blends was treated with fungal isolates for 4 weeks in strum test. Carbon dioxide analysis showed that in case of *Phanerochaete chrysosporium* PV1 the total amount of CO_2 produced was 33.79g/l in test, whereas, in control it was 16.34g/l, however when treated with *Aspergillus sydowii* PV4 the total amount of CO_2 produced was 39.30g/l, whereas, in control (no PVC pieces) it was 21.07g/l (Table 12). When the biodegradation potential of the two strains tested was compared *Phanerochaete chrysosporium* PV1 than *Aspergillus sydowii* PV4 (Table 4.8). The dry cell mass of *Phanerochaete chrysosporium* PV1 in test was higher (0.136 mg/ml) than in control (0.056 mg/ml), also the increase in dry cell mass of *Aspergillus sydowii* PV4 was observed in test (0.118mg/ml) than control (0.058 mg/ml) (Table. 4.2).

4.6.5. Analysis of by Gel Permeation Chromatography (GPC)

To confirm the change in the PVC starch blends after fungal treatment the polymer films were analyzed by gel permeation chromatography. The results indicated that among the four fungal strains *Phanerochaete chrysosporium* PV1 showed maximum decrease in the molecular weight of PVC starch blends (77,011) than the control (80,275) followed by *Lentinus tigrinus* PV2 (79,369).The molecular dispersity was decreased as compared to control molecular dispersity. *Aspergillus niger* PV3 showed maximum decrease (1.572) of molecular dispersity (Table 4.9).

The decrease in the molecular weight (Mw) and decrease in molecular dispersity of PVC (PVC + starch) in shake flask experiment after treatment

with fungal strains confirms that activity of biodegradation and changes in the structure of the polymer film.

4.6.6. Analysis of by Fourier Transform Infrared Spectroscopy (FTIR)

Polyvinyl chloride starch blended film treated under shaking condition by fungal isolate for 3 months was analyzed through FTIR, the results showed that in case of treated samples new peaks were appeared at 3077 cm^{-1} (corresponding to alkenes), also the absorbance of peaks at 2655-2529 cm^{-1} decreased, a new sharp peak appeared at 1587 cm^{-1} .These changes confirmed the modification in the structure of the polymer films due to fungal degradation activity (Fig. 4.24).

4.6.7. Analysis by Nuclear Magnetic Resonance (NMR)

By comparing the C^{13}NMR spectra of the control sample of the PVC starch blend film and treated sample of the PVC starch blend with *Phanerochaete chrysosporium* PV1 clearly showed a chemical shifting and appearance of the new peaks 29.85, 41.58, and 46.05 ppm also significant increase in the intensity of resonances centered observed. That effect was more pronounced in the treated starch film (Fig.4. 25).

The Proton NMR spectra of PVC starch blends also showed the change in the polymer structure due to biodegradation. The signal region for aliphatic protons ($-CH_2-CH-Cl$) for (-CH2-CH-Cl) was 1.0-5.0 ppm. The total integration of the region for the control sample 0.3-3.1 ppm was 40.69 ppm while for the treated sample; integration was increased to 42.89 in the same region. That increase in integration also confirmed the change due to fungal degradation (Fig.4.26).

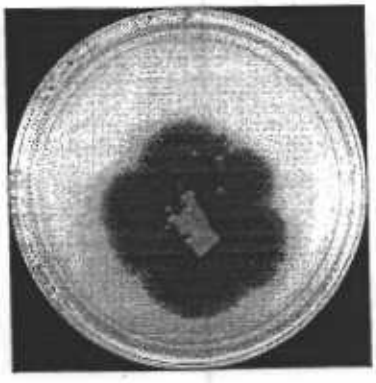


Figure 4.20. Growth of fungal isolate *Aspergillus niger* PV3 on PVC strach blended film

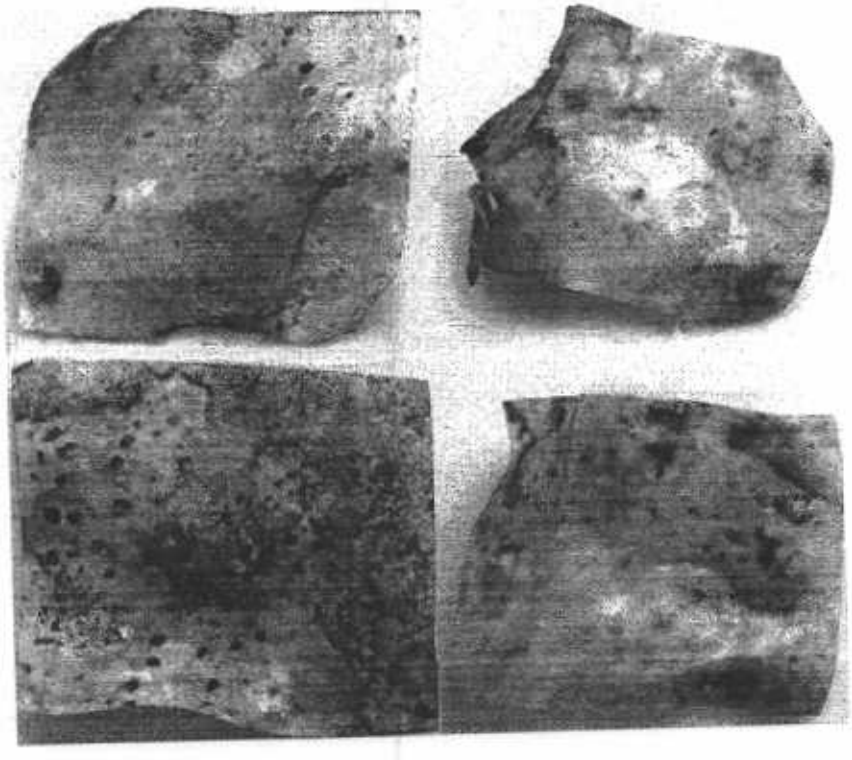


Figure 4.21. Fungal adherence and attachment on the surface of PVC starch blends film after six months soil burial experiment

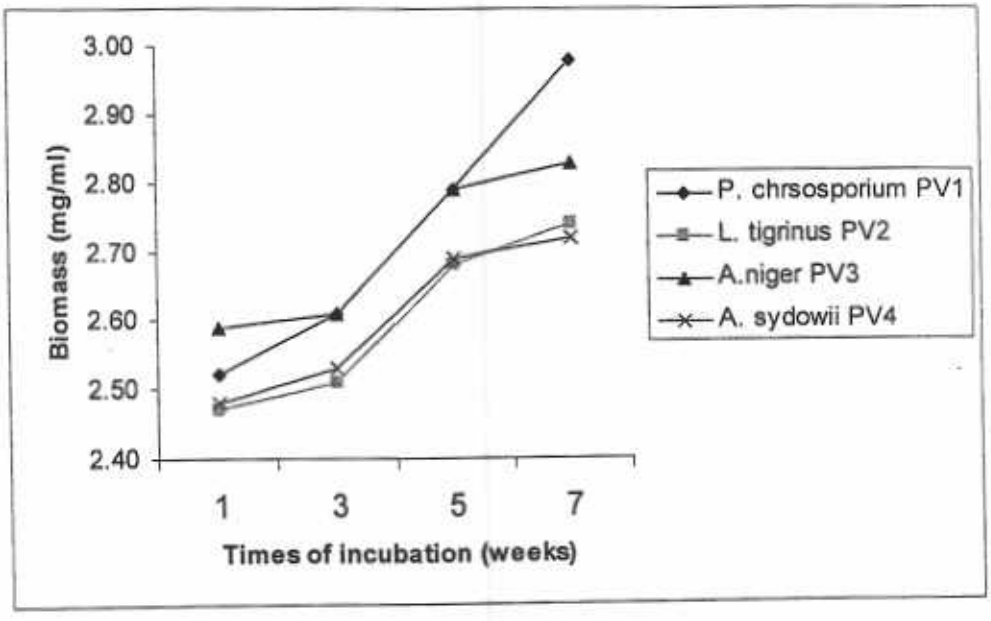


Figure 4.22. The growth of fungal isolates in mineral salt medium with PVC starch blends as sole carbon source

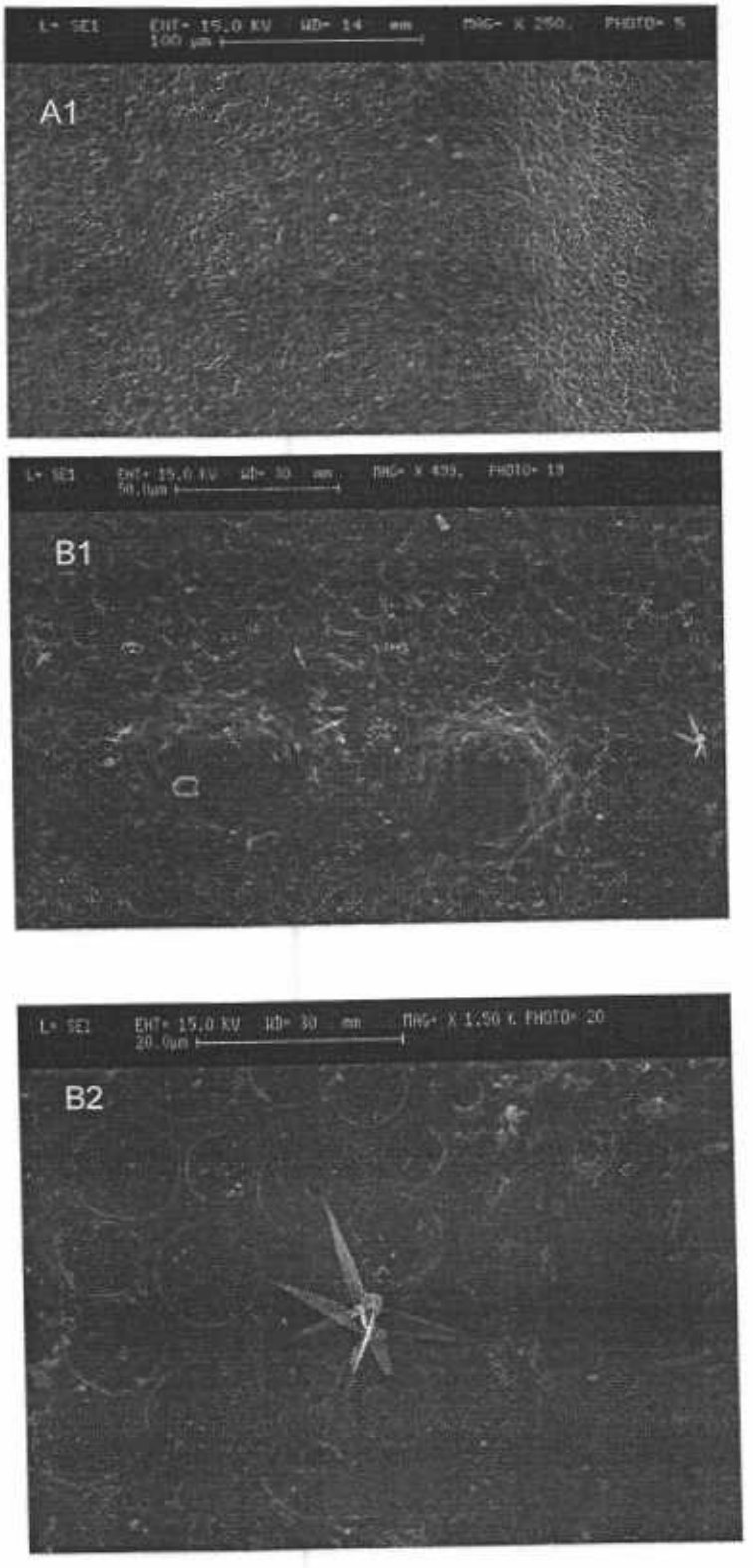


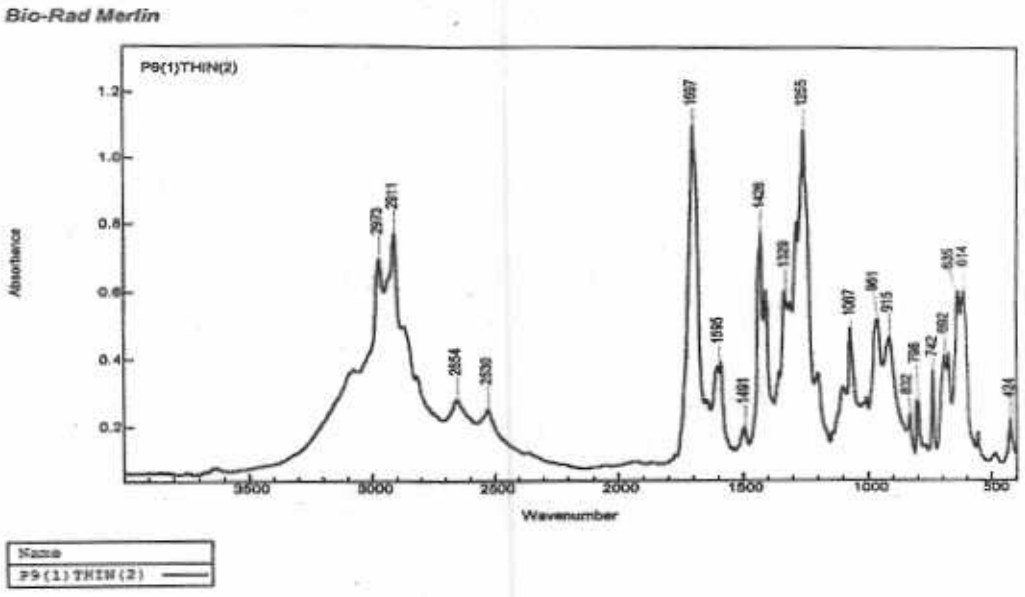
Figure 4.23. Scanning Electron Micrographs of the PVC starch blends film A) Untreated film; B1) after 3 months shake flask experiment with *Phanerochaete chrysosporium* PV1 250 x , (B2) 500x.

Table 4.8 Dry cell mass and gravimetric analysis of CO_2 evolution during breakdown of PVC starch blends by *Phanerochaete chrysosporium* PV1 and *Aspergillus sydowii* PV3 determined through Sturm test after 4 weeks of treatment (control; without PVC+ Starch pieces)

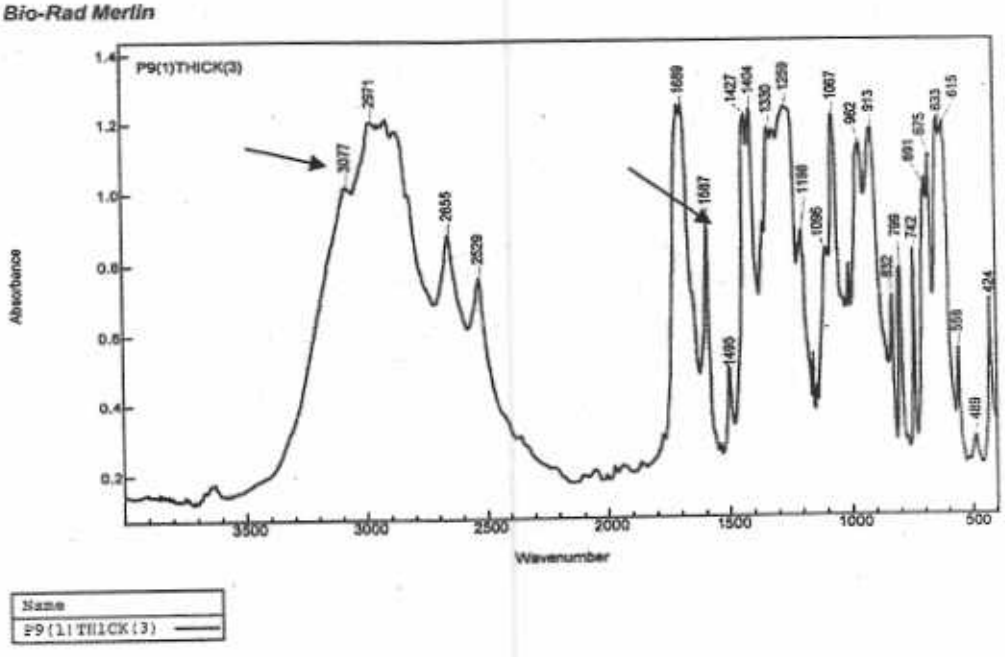
Fungal isolates	CO_2 Produced		Dry cell mass	
	Control	Test	Control	Test
***P. chrysosporium* PV1**	16.34	32.79	0.056	0.136
***A. sydowii* PV4**	21.07	39.30	0.058	0.118

Table 4.9 Gel permeation chromatography analysis of the PVC starch blends fungal degradation (after six months)

Strain	Molecular weight MW (D)	Molecular dispersity (MW/Mn)
Control	80,275	1.991
L. tigrinus PV2	79,369	1.902
P. chrysosporium PV1	77,011	1.731
A. niger PV3	78,866	1.572
A. sydowii PV4	79,233	1.759

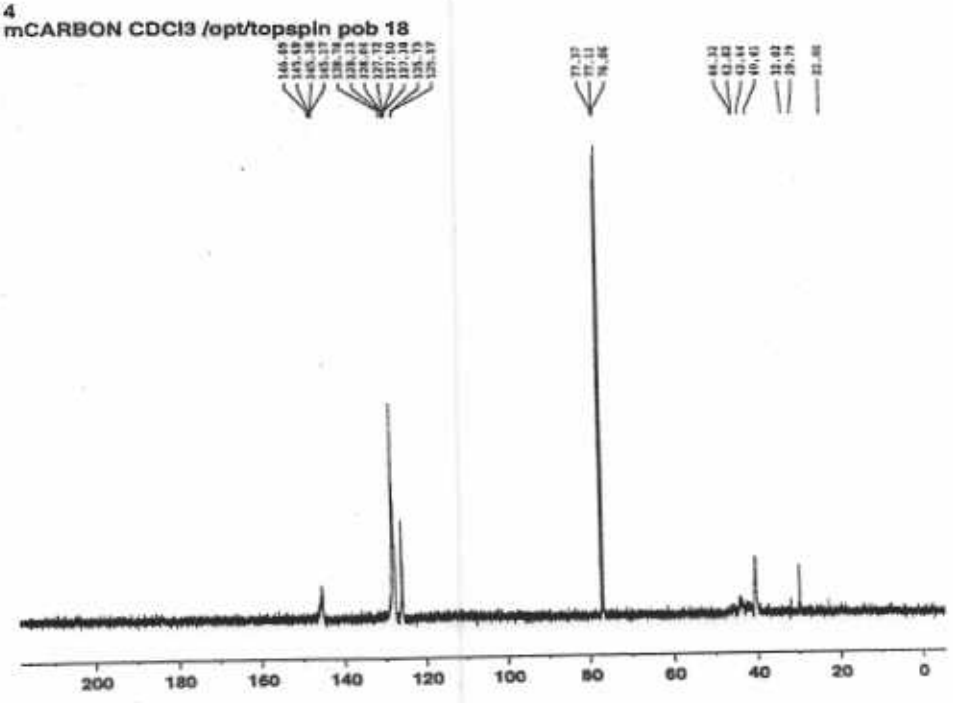


(A)

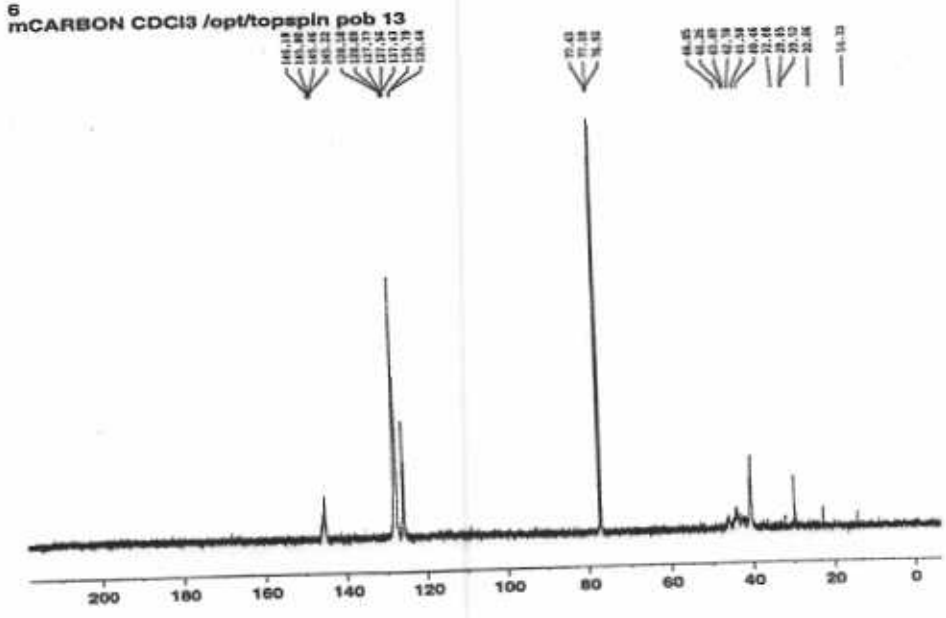


(B)

Figure 4.24. Fourier transform infrared spectra of Polyvinylchloride starch blends, A) control, B) after treatment with *Aspergillus sydowii* PV4 for 3 months.

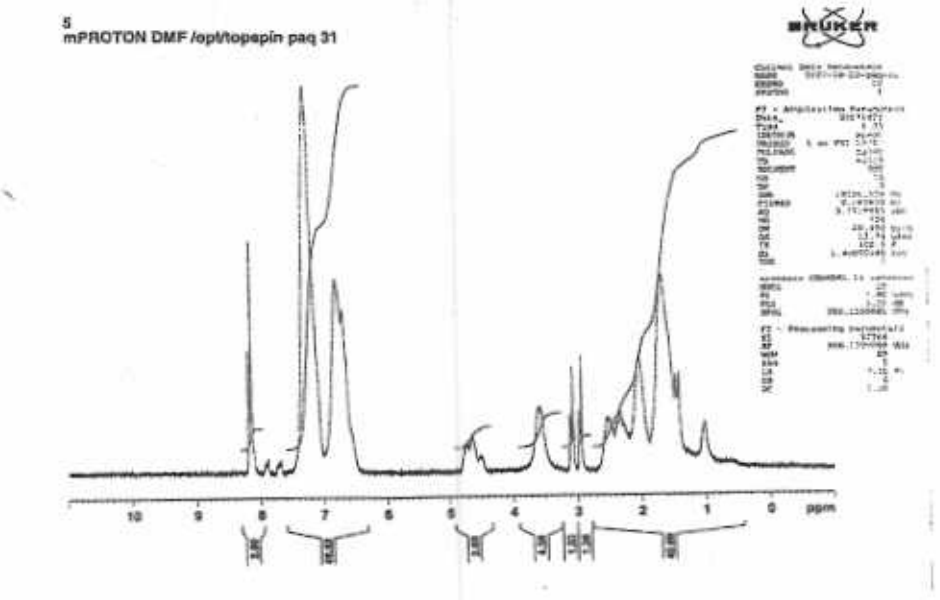


(A)

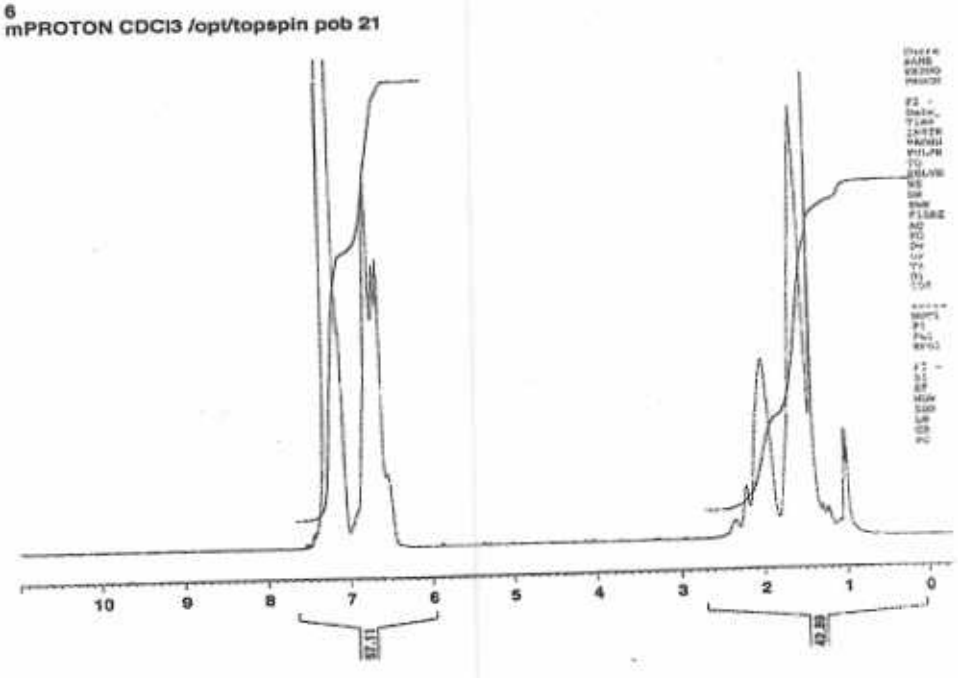


(B)

Figure 4.25. ^{13}CNMR spectra and peak assignments for PVC starch blends A) Control, B) treated with *Phanerochaete chrysosporium* PV1



(A)



(B)

Figure 4.26. Proton Nuclear magnetic resonance (^{1}H NMR) spectra of PVC starch blend, A) Control, B) treated with *Phanerochaete chrysosporium* PV1.

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, polyvinyl chloride, polystyrene, detergents like alkyl-benzene sulphonates and oils.

The objectives of the present study were the isolation of Polyvinyl chloride degrading microorganisms from the soil inoculated with sewage sludge, and to study the degradation potential of the microbes in laboratory. The detailed investigation of the microbial adherence and degradation of the PVC and pPVC containing DOP, DOA and starch blend was carried out.

Most biodegradation of plastics has been focused on polyurethanes (Bentham *et al.*, 1987; Kay *et al.*, 1991; Barrat *et al.*, 2003) bacterial polyesters (Mergaert *et al.*, 1996) and nylon (Deguchi *et al.*, 1997). By contrast there are only fewer studies reporting the pPVC degradation *in situ* (Upsher, 1984; Webb *et al.*,2000).There are some studies reporting on partial degradation of PVC by white rot fungus under elevated oxygen level (Kirbas *et al.*,1999).

The PVC and pPVC thin films were buried in sewage sludge soil for ten months for isolation of PVC and pPVC 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 by a number of researchers (Domb *et al.*, 1997; Hamid, 2000; Colin *et al.*, 1981; Seal & Pantke, 1988). The soil burial experiment were performed for isolation of microorganisms and to check their degradation potential also for other plastics (Pagga *et al.*,1995; Tosin *et al.*,1996; Degli-Innocenti *et al.*,1998; Ohtaki *et al.*,1998; Tuominen *et al.*, 2002). The isolated microorganisms were mostly fungi that showed maximum adherence and biodegradation of the PVC and pPVC. The results of present study are in accordance with Barratt *et al.*,

(2003). In our experiments after 10 months soil burial the surface of the PVC was heavily colonized and some hyphal growth was visible to the naked eye at the surface of the films (Fig.4.3). The physical properties of the pPVC were significantly altered due to plasticizer utilization (Sabev *et al.*, 2006).

Fungi play important role in biodegradation studies 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 (Semenov *et al.*, 2003). Degradation of plastic material did not related with colonization of films by microorganisms (Whitney, 1996).The plastic surface serve as a source of energy for adhering microorganisms, Griffin and Uribe, (1984) reported pPVC as a carbon source for the microorganisms. The fungal isolates were using the plastic as carbon and energy in the present study when the isolates were inoculated on mineral salts agar and liquid media containing plastic film as growth substrate.

The change in the color of the PVC film was observed in our soil burial experiment (Fig. 4.3). Visual observation showed that due to soil burial and shake flask experiment there was change in color and appearance of the PVC and pPVC films surface also the fungal hyphal growth was observed on the films after soil burial experiments. Changes in film and fungal growth on the PVC-p/cellulose sample were visible with the naked eye (Kaczmarek and Bajer 2007). It was found that an unaided visual observation of modified polyethylene film incubated with *A. niger* revealed irregular yellow and brown spots on the surface of the samples (Labuzek *et al.*, 2003). Coulthwaite *et al.*, (2005) reported that bacterial and fungal growth on the polymer surface can bring the discoloration pPVC samples due to microbial spoilage. The polyester discoloration during soil burial experiment (Dale and Squirrel 1990) was due to fungal activity (Pathirana and seal 1985). Fungal colonization and discoloration of rigid PVC/wood-flour composite was observed after 4 weeks of incubation. *Aureobasidium pullulans* was one of the predominant fungi causing the pPVC biodegradation (Benjamin *et al.*, 2004; Webb *et al.*, 2000).

Many microbes produce pigments that can diffuse into polymers and cause undesirable permanent staining (Kneale, 2002). However some bacterial spp. was isolated after soil burial experiment but they did not show good adhesion and degradation of PVC and pPVC. Bacterial growth might be inhibited by desiccation or *in-situ* acidification of pPVC (Hollande & Laurent, 1997; Martinez *et al.*, 1996).

In our study the four fungal strains were isolated on the basis of their adhesion and degradation potential from soil burial experiment and were further tested for their degradation potential. The isolated fungi belonged to white rot spp. and Aspergillus spp. and identified as *Phanerochaete chrysosporium* PV1, *Lentinus tigrinus* PV2, *Aspergillus niger* PV3 and *Aspergillus sydowii* PV4. Filamentous fungi are generally capable of attaching to the hydrophobic surfaces through the formation of hydrophobic proteins (Kershaw and Talbot, 1998). The white-rot fungi are capable of extensively degrading lignin (a heterogeneous polyphenolic polymer) within lignocellulosic substrates (Aghoury *et al.*, 2006; Eaton and Hale 1993). Significant depolymerization was observed in PVC under ligninolytic culture conditions when seven white rot fungal species were evaluated for their ability to depolymerize polyvinylchloride (Kirbas *et al.*, 1999).

Fungal growth was normally identified with basic morphological techniques only to genus level. However further identification was done by rDNA sequencing (Guarro *et al.*, 1999; Kurtzman and Robnett, 1998). Identification was done on the basis of 5.8S, 18S, 28S and Internal transcribed (ITS) regions (Kurtzman and Robnett, 1998) .

In the case of PVC, it is known that some of its plasticizers and stabilizers can be attacked by microorganisms; most plasticizers can be utilized by fungi and bacteria as a source of carbon, Some plasticizers such as phthalates, adipates, and sebacates were found to support growth of a large number of microorganisms (Wilhelm *et al.*, 2003; Juneson *et al.*, 2002; Murad *et al.*, 2007).

In our study pPVC (DOP, DOA) and starch blends of PVC showed better microbial adhesion and degradation than the pure PVC. Gumargalieva *et al.*, (1999) showed that the loss of a dialkyl phthalate (not specified) plasticizer for PVC under the influence of surface biodegradation by the microscopic fungus *Aspergillus niger* was much faster than the loss without fungal overgrowth. Both bacteria (Booth *et al.*,1968 and Eaton *et al.*,1982) and fungi (Lee *et al.*,2007; Berk *et al.*,1957 and Roberts *et al.*,1986) can degrade ester-based plasticizers and phthalic acid esters (Chen *et al.*,1997, Peciulyte , 1997; Wang *et al.*,1997; Ejlertsson *et al.*,1996; Inman *et al.*,1984; O'Connor *et al.*,1989). *Rhodococcus erthropolis* was reported for degradation of dibutyl phthalate and bis (2-ethylhexyl) phthalate (Anon, 1994; Cheng *et al.*, 1986). The fungus effectively removes plasticizer from the surface of the material by biodegradation (Gumargalieva *et al.*, 1999).

Microbial colonization of synthetic plastic films is normally slow, which affects the total period of biodegradation (Kaczmarek and Bajer 2008). In our study the pPVC (DOP) showed better degradation potential than (DOA). Loss of plasticizers from pPVC due to microbial degradation results in brittleness, shrinkage, and ultimately failure of the pPVC in its intended application. Adipate plasticizers were shown to be more susceptible to microbial hydrolysis than phthalate plasticizers (Kaczmarek and Bajer 2006; Yabannavar and Bartha, 1994; Berk, 1957; Nalli *et al.*, 2002).

In our study the pPVC and starch blends of PVC showed more adhesion of the microorganisms on the polymer surface than the pure PVC that was more rigid and consist of the carbon-carbon main chain that is hardly biodegradable (Chandra and Rustigi, Kawai, 1995; Tusji and Suzuyoshi, 2002). Plasticized PVC increases the susceptibility of the polymer for microbial degradation (Richard *et al.*, 2007; Booth *et al.*, 1968; Eaton & Ribbons, 1982).

In the present study Scanning Electron Microscopy demonstrated changes in physical structure of the PVC and pPVC (DOP) when they were exposed to the fungal strains in soil burial and shake flask experiments. There were

notable changes on the surface of treated PVC film after microbial treatment showing initial pattern of degradation of the PVC film (Fig.4.6). After biodegradation, the appearance of numerous holes and change in surface structure of p-PVC and PVC-starch blend were observed confirming, the biodegradation of the plasticizer and starch present in the PVC sample. A number of cracks and round holes were also reported on the eroded film surface through scanning electron microscopy by Kaczmarek and Bajer (2007), Martin *et al.*, (2007) and Zhao *et al.*, (2005).

The pPVC degradation was more obvious in pPVC (DOP). Phthalate plasticizers have far less susceptible to microbial hydrolysis compared to adipate plasticizers as previously reported (Shailaja *et al.*, 2008; Berk *et al.*, 1957; Eaton & Ribbons, 1982; Frankland *et al.*, 1990; Nalli *et al.*, 2002). Due to fungal (*Aspergillus niger*) adherence complete change in the surface structure of the treated (DOP) film was observed as the appearance of cracks and holes indicating that fungal strain was utilizing pPVC as sole carbon source in shake flask experiment with mineral salt media after 6 months. The results are in accordance with (Sabev *et al.*, 2006). Scanning Electron Microscopy studies demonstrated that *A. pullulans* colonized pPVC in the absence of other microorganisms and therefore acts as a primary colonizer of pPVC. *A. pullulans* was increasingly recognized as the major causative agent of defacement of various diverse materials, such as painted surfaces (Cooke 1959 and Winters *et al.*,1976) and wood (Kaarik, 1974), in addition to pPVC exposed to tropical conditions (Hamilton 1983 and Upsher *et al.*,1984).

In our study, in the treated sample of (PVC+ Starch) there was erosion and extensive roughening of the surface, due to *Phanerochaete chrysosporium* - PV1 treatment appearance of holes and cracks on the polymer surface were observed (Fig 7.30). Incorporation of starch into plastics, not only showed influence on its mechanical properties but also increased the susceptibility of plastics to microorganism (Fanta *et al.*, 1992; Imam *et al.*, 1993; Shogren *et al.*, 1992; Sung & Nikolov, 1992). Some changes on the surface of the starch were reported after 60 days biodegradation study (Arevalo *et al.*, 1996)

Vikman *et al.*, (1995) reported the enzymatic degradation of the starch base polymer. The growth of fungi was observed on both PVC-P and PVC-P/cellulose samples (Kaczmarek and Bajer, 2006).

Sturm test (ASTM D5209-91) was commonly employed for evaluation of the biodegradability of polymer materials (Muller *et al.*, 1992; Domb et al., 1997; Hamid, 2000; Calmon, 2000; Shah *et al.*, 2008). In the present study this technique was used to check the biodegradation of PVC and pPVC. The results showed a difference in the amount of carbon dioxide produced in test and control bottles indicating greater breakdown of the polymer in the test than the control. Increased amount of CO_2 produced during the biodegradation of pPVC was also reported by (Yabannavar and Bartha, 1994; Martin *et al.*, 2007).

It has been reported that the degradation of PVC by the chain scission reaction usually results in a decrease in weight-average molecular weight (*Mw*) with a possible increase in number average molecular weight (*Mn*) whereas the degradation by the cross linking of macromolecules results in an increase in *Mw* with a decrease in *Mn* (Torikai and Hasegawa, 1999). In our study change in weight-average molecular weight (Mw) and (Mn) number average molecular weight was observed. The average molecular weight (Mw) of the pure PVC was decreased but decrease in molecular weight was more obvious in pPVC (in case of DOA and DOP), however starch blended PVC also showed decrease in molecular weight and increase in molecular dispersity. Sombatsompop *et al.*, (2003) reported that addition of LDPE initially increased the Mw of PVC however the Mw decreased on latter stages, but no increase on Mn of the PVC by addition of LDPE. The GPC results confirmed the existence of a random-type degradation pathway of PVC as reported by Andrea *et al.*, (2002) for PVA. Fast decreases in Mn values of the poly butylene adipate were observed after 25 days of biodegradation (Zhao *et al.*, 2005). The number average molecular weight (Mn) was decreased and average molecular weight (Mw) increased, also the increase in molecular dispersity was observed during 3-6 months soil compositing of plasticized polyvinyl chloride (pPVC) (Kaczmarek and Bajer, 2006). Studies with the

same results of thermal or photochemical degradation of PVC were reported (Rabek, 1996 and Starnes, 2002).

The index of molecular motion measured by a dynamic mechanical test and by pulse NMR is applicable for evaluation of degradation (Mifune *et al.*, 1991; Fukumori *et al.*, 1992). In our study ^{13}CNMR spectra of the control sample the signal at 14.26 ppm was attributed to CH_2 while the signal at 32.03 ppm was assigned to CH-Cl carbon. While In addition to these signals some more signals have appeared in the range 29-47 ppm in treated film of PVC in shake flask experiment for six months with *Phanerochaete chrysosporium*. The increase in number of signals indicated that some chemical change/shifting were took place in the polymeric material (Fig. 4.9). Sombatsompop *et al.* (2003) revealed that the changed chemical structures of PVC in LDPE/PVC blends during melt blending by ^{13}C NMR (Sombatsompop *et al.*, The changes in the structure of the polymer during biodegradation of poly (vinyl alcohol), the chemical shifts were observed by ^{1}HNMR spectra (Andrea *et al.*,2002; Chiellini *et al.*,2002).

The C^{13}NMR spectra of treated PVC + starch film clearly showed a chemical shifting and appearance of the new peaks at 42.55, 44.19 46.05ppm and also significant increase in the intensity of resonances centered observed at 42.55 and 43.88ppm. This effect was more pronounced in the treated starch film (Fig.4.25). The loss of starch from polyethylene during biodegradation brought changes in the structure of the polymer indicated by chemical shifting of the peaks by ^{13}C NMR spectra (Dave *et al.*, 1997; Cheng *et al.*, 1986).

The increase in integration from 43.28 to 43.39 in ^{1}H- NMR spectra of pPVC(DOA) treated with *P. Chrysosporium* PV1 (Fig.4.19) clearly indicated that during the process, some of the protons have been exhausted. Therefore, it is suggested that decrease in integration in the peaks was due to fungal activity that bring changes in the polymer structure.^{1}H NMR analysis of plasticizers adipate and succinate showed that the relative content of adipate units in the polymer chain decreased after biodegradation, while that of

succinate units increased. Indicating that the adipate units were more easily attacked by *Aspergillus versicolor* than are succinate units (Zhao *et al.*, 2005).

In our study the FTIR spectroscopy was also used to observe changes in the molecular structure of the polyvinyl chloride and pPVC (DOP, DOA) and starch blended PVC as reported by others (Martin-Franchetti *et al.*, 2007; Santoro & Koestler, 1991). FTIR spectroscopy was used to examine the structural changes in the melt blends of PVC (Sombatsompop *et al.*, 2004). The clear change in the spectra was observed in our results, the appearance of new peaks at 2370-2350 cm $^{-1}$ (corresponding to O-H) region indicating the change in the structure of the PVC also peaks shorten at 2277-2250 cm $^{-1}$ showing significant change due to microbial degradation (Fig.4.8). Biodegradation of pPVC brought some structural changes in the FTIR spectra of the polymer (Kaczmarek and Bajer 2006). In the PVC spectrum, the bands typical for C-H stretching and deformation vibrations were present in 2800–3000 and 1500–1300 cm^{-1} ranges, respectively. The bands due to skeletal vibrations below 1300 cm^{-1} were also present. Three other bands (at 693, 636, and 610 cm^{-1}) were attributed to the carbon–chloride stretching frequencies, and they were useful in monitoring the process of PVC dehydrochlorination. The FTIR spectra of Poly (Vinyl alcohol) showed a sharp decrease in the bands and peaks of the polymer (Amanda *et al.*, 2001; Okaya and Ikari, 1992).

The photo-oxidative degradation of polymers did not always facilitate progressive attack by microorganisms, because the oligomer fractions produced during photo-oxidation may support microbial growth, but polymers with a high molecular weight resulted in little or no growth. In our study the changes in FTIR analysis were more obvious in PVC film initially treated with UV. UV treatment breakdown the backbone of the polymer and brought change in the polymer structure, the peaks shorten and almost disappear at 2980-2910 cm^{-1} (corresponding to Alkyl segment) also new peaks appeared at 2380-2340 cm $^{-1}$ (corresponding to O-H) indication the better biodegradation activity in UV treated films. The results showed that some of the double bonds of PVC were cleaved by microbial strain (Fig. 4.9). The results were in

accordance with the Volke-Sepulveda *et al.*, 2002 and Matsunaga and Whitney, 2000). Another study reported that the biodegradation of inert material such as PE, PVC takes more than 10 years and that of degradable material containing UV sensitizers takes 2 years or less (Albertsson and Karlsson, 1990).

Several attempts to introduce some naturally occurring polymers of microbial or plant origin, such as starch, Kiatkamjornwong *et al.*, (1999) and cellulose, Daneault and Kokta (1986) into a synthetic polymer structure have been reported. The resulting products have shown appreciable biodegradability of the naturally occurring fraction of the plastic mixture (Milstein *et al.*, 1992).

In our study PVC+ starch film after three months shake flask experiment with A*spergillus sydowii* showed clear change in the structure of the polymer films, the new peaks appeared at 3077 cm^{-1} (corresponding to alkenes), also the absorbance of peaks at 2655-2529 cm^{-1} decreased, a new sharp peak appeared at 1587 cm $^{-}$1. The spectra of biodegraded starch showed only a small difference from the spectrum of the original starch: a small increase in O-H stretching due to the enzymatic degradation products of starch was observed (Moreno-Chulim *et al.*, 2003; Suresh *et al.*, 1999). Dave *et al.*,(1997) reported FTIR spectra of the loss of starch from polyethylene during biodegradation bring changes in the back bone structure of the polymer by shortening of the peaks. Arevalo *et al.* (1996) reported change in the spectra of biodegraded starch, as the broad stretching of O-H and minor stretching band of C-H at 2921 cm^{-1}.

CONCLUSIONS

From the present study it was concluded that:

1- Soil and sewage sludge contain microorganisms fungi that are able to bring about some degradation of synthetic polymers.

2- The fungal isolates showing adherence and growth on the surface of Polyvinyl chloride films indicated their ability to utilize Polyvinyl chloride as a source of nutrient.

3- The increase is the biomass of the fungal strains incubated with the polymer (PVC, pPVC and starch blended PVC) in mineral salt media indicated the utilization of the plastics as a carbon source.

4- Production of carbon dioxide during the Sturm test indicated positive degradability test for the polyvinyl chloride film.

5- The four isolated fungal strains *Phanerochaete chrysosporium* PV1, *Lentinus tigrinus* PV2, *Aspergillus niger* PV3 and *Aspergillus sydowii* showed good degradation potential of polymer (PVC and pPVC starch blended PVC), however *Phanerochaete chrysosporium* PV1 showed better degradation potential then others.

6- The changes in the peaks of the FTIR spectra of the test samples as compared to control is an indication of breakdown of plastics (PVC, pPVC and starch blended PVC) as a result of fungal treatment.

7- Scanning Electron Microscopy indicated the changes in surface of the treated films of plastics (PVC, pPVC and starch blended PVC) after fungal degradation in soil burial and shake flask experiments.

8- Gel Permeation Chromatography indicated the decrease in the molecular weight of the treated films of the plastics (PVC, pPVC and starch blended PVC due to fungal degradation. The decrease in the molecular weight could be either degradation by cross linking or degradation by chain scission reaction that brings change in the molecular weight and molecular dispersity of the plastics.

9- The change in the integration and appearance of some new peaks in ^{13}CNMR and ^{1}HNMR spectra confirmed the change in polymer (PVC, pPVC and starch blended PVC after microbial degradation.

10- The pure PVC showed slow rate of degradation as compared to pPVC and starch blended PVC due to rigid nature of pure PVC.

11- It can also be concluded from our studies that extensive research and complete understanding is required to find the better solution of degrading plastics. The degradation potential of the isolated fungal strains can be enhanced to get better degradation results of the synthetic plastics.

FUTURE PROSPECTS

- The enzymatic degradation of the plastics (PVC, pPVC and starch blended PVC) could be done.

- The application of the enzyme in natural condition could be checked.

- The development of new processes for production of degradable synthetic plastics, bioplastics and natural plastics and methods to be used for their biodegradation require intensive research and large capital expenses and must be scaled-up to be economically competitive.

REFERENCES

Abbas. K. B., Sorvik. E. M (1973). On the thermal degradation of poly vinyl chloride. II. The effect of atmosphere. J. Appl. Polym. Sci. 17, 3577-3594.

Abou-Zeid. D (2001). Anaerobic biodegradation of natural and synthetic polyesters, Dissertation Technical University Braunschweig, Germany, Internet: http://opus.tu-bs.de/opus/volltexte/2001/246.

Ag. P. U (1999). Letter with information on existing recycling and recovery schemes for PVC in Germany to TNO-STB.

Albertsson. A. C (1978). Biodegradation of synthetic polymers II. A limited microbial conversion of 14C in polyethylene to 14CO2 by some soil fungi. J. Appl. Polym. Sci. 22, 419-3433.

Albertsson. A. C., Karlsson, S(1990). The influence of biotic and abiotic environments on the degradation of polyethylene. Prog. Polym. Sci. 15, 177-192.

Alexander. M (1994). Recalcitrant Molecules. Biodegradation and Bioremediation, Academic Press, San Diego. 272-286.

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

Anderson. M., Gull. J.K., Denning. D. W (1996). Molecular typing by random amplification of polymorphic DNA and M13 southern hybridization of related paired isolates of Aspergillus fumigatus. J. Clin. Microbiol. 34, 87- 93.

Andrady. A. L (1994). Assessment of environmental biodegradation of synthetic polymers. J. Macromol. Sci. Rev. Macromol. Chem Phys.C34, 25–76.

Andrea. C., Solaro. R., Chiellini. E (2002). Biodegradation of poly (vinyl alcohol) in selected mixed microbial culture and relevant culture filtrate. Polym. Degrad. Stab. 75 , 447–458.

Andricic. B., Kovacic, T., Perinovic, S., Klaric, I (2008) Polymer blends based on poly(vinyl chloride) and biodegradable aliphatic-aromatic copolyester. J. App. Polym. Sci. 109 (2), 1002-1008

Anon (1994). Degradation of dibutyl phthalate and bis(2-ethylhexyl)phthalate by *Rhodococcus Erythropolis*. Dokl. Akad. Nauk Belarusi. 38(6), 64-6

APME (Association of Plastic Manufacturers Europe) (1999). An analysis of Plastic Consumption and Recovery in Western Europe APME. Brussels (www.apme.org).

Arevalo-Niko. K., Sandoval. C.F., Galan. L.J., Imam. S.H., Gordon. S.H., Greene. R.V(1996). Starch-based extruded plastic films and evaluation of their biodegradable properties. Biodegradation. 73, 231–237.

Augusta. J., Muller .R. J., a Widdecke. H (1993). A rapid evaluation plate-test for the biodegradability of plastics. Appl. Microbiol. Biotechnol. 39, 673-678.

Bacaloglu. R., Fisch, M. H (1995). Degradation and stabilization of poly (vinyl chloride). V. Reaction mechanism of poly(vinyl chloride) degradation. Polym. Degrad. Stab. 47, 33-57.

Barlaz. M. A., Ham. R. K., Schaefer. D. M (1989a). Mass-balance analysis of anaerobically decomposed refuse. J. Environ. Engin. 115, 1088-1102.

Barlaz. M. A., Schaefer. D. M., Ham. R. K (1989b). Bacterial population development and chemical characterization of refuse decomposition in a simulated sanitary land6ll. Appl. Environ. Microbiol. 55, 55-65.

Barratt. S. R., Ennos, A. R., Greenhalgh. M., Robson, G. D., Handley, P. S (2003). Fungi are the predominant micro-organisms responsible for degradation of soil-buried polyester polyurethane over a range of soil water holding capacities. J. Appl. Microbiol. 95, 78–85.

Bauer. M. J (1997). Verhalten von Phthalsäureestern in anaeroben Hausmüllsickerwaessern aus Bioreaktoren und Deponien. University of Bayreuth, Dissertation.

Bellina. G., Tosin. M., Degli-Innocenti. F (2000). The test method of composting in vermiculite is unaffected by the priming effect. Polym. Degrad. Stab. 69, 113-120.

Bellina. G., Tosin. M., Floridi. G., Degli-Innocenti. F (1999). Activated vermiculite, a solid bed for testing biodegradability under composting conditions. Polym. Degrad. Stab. 66, 65-79.

Benjamin. D. A., Laurent. M. M., Harrison. J (2004). Mold susceptibility of rigid pvc/wood-flour composites J. Vinyl Add. Technol. 10, 4.

Bennett. J. W., Faison, B. D (1997).Use of fungi in biodegradation. In Man. Environ. Microb. 960–971. Edited by C. J. Hurst. Washington, DC: American Society for Microbiology.

Bentham. R. H., Morton. L. H. G., Allen, N. G (1987). Rapid assessment of the microbial deterioration of polyurethanes. Int. Biodet. Biodeg. 23, 377–386.

Berk. S., H., Ebert, and L. Teitell (1957). Utilization of plasticizers and related organic compounds by fungi. Ind. Eng. Chem. 49, 1115–1124.

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

Borneman. J., Hartin, R. J. (2000). PCR primers that amplify fungal rRNA genes from environmental samples. Appl. Environ. Microbiol. 66, 4356–4360.

Braun. D (1971). Thermal degradation of polyvinyl chloride. Pure Appl.Chem. 26, 173-192.

Braun. D., Bazdadea. E (1986). In L. I. Nass, C. A. Heiberger (eds.): Encyclopaedia of PVC, 2nd ed., vol. 1, Marcel Dekker, New York.

Breslin. V. T (1993). Degradation of starch-plastic composites in a municipal solid waste landfill. J. Environ. Polym. Degrad. 1,127-141.

Brown. A. E (1946). Problems of fungal growth on synthetic resins, plastics and plasticizers. U.S. Office of Scientific Research and Development, report no. 6067. U.S. Office of Scientific Research and Development, Washington, D.C.

Brydson. J. A (1999). Plastics Materials, Butterworth-Heinemann, Oxford, 6th edition.

Budwill. K., Fedorak. P. M., Page. W. J (1996). Anaerobic microbial degradation of poly (3-hydroxyalkanoates) with various terminal electron acceptors. J. Environ. Polym. Degrad. 4, 91-102.

Buswell. A. M., Muller. H. F(1952). Mechanism of methane fermentation, Ind. Eng. Chem. 44, 550-552.

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

Calmon. A., Dusserre-Bresson. L., Bellon-Maurel. V., Feuilloley. P., Silvestre. F (2000). An automated test for measuring polymer biodegradation. Chemosphere 41, 645-651

Cartwright. C.D., Owen. S.A., Thompson. I.P., Burns, R.G. (2000). Biodegradation of diethyl phthalate in soil by a novel pathway. FEMS Microbiol. Lett. 18, 27–34.

Chandra. R., Rustgi, R (1998). Biodegradable polymers. Prog. Polym. Sci. 23 (7): 1273-1335.

Chen. L., Imam S. H., Gordon S.H., Greene R.V (1997). Starch-polyvinyl alcohol crosslinked film-performance and biodegradation. J. Environ. Polym. Degrad. 5,118.

Cheng. G., Xiaoyang. L(1986). Microbiological degradation of dibutyl phthalate, a plasticizer. Huanjing Kexue. 7(6), 25-7.

Chiellini. E. Chiellini., F. Cinelli, P. (2002). Polymers from renewableressources in Geralds Scott, Degradable Poly mers Principles and Applications 2nd edn, Kluwer Academic Publishers. 163-233.

Christensen. G. D., Baldassarri. L., Simpson.W.A (1995). Mehtods for studying microbial colonization of plastics. Meth. Enzymol. 253, 477-500.

Colin. G., Cooney. J. D., Carlsson. D. J., Wiles. D. M (1981). Deterioration of plastic films under soil burial conditions. J. Appl. Polym. Sci. 26, 509-519.

Common wealth of Australia (1996). Packaging and Labelling, Report 49 Part An Introduction. Canberra, Australia: Industry Commission. Notes, 1–24.

Cooke. W.B (1959). An ecological life history of Aureobasidium pullulans (de Bary) Arnaud. Mycopathologia. Mycologia. Applicata. 12, 1–45.

Costerton. W. J., Cheng. K. J., Geesey. G. G., Ladd. T. J., Nickel. J. C.Dasgupta. M., Marrie. T. J (1987). Bacterial biofilms in nature and disease. Ann. Ver. Microbiol. 41, 435.

Coulthwaite. L., Bayley. K., Liauw. C., Craig. G., Verran. J (2005). The effect of free and encapsulated OIT on the biodeterioration of plasticised PVC during burial in soil for 20 months. Inter. Biodet. Biodeg. 56 , 86–93.

CSTEE (1999). Opinion on the toxicological characteristics and risks of certain citrates and adipates used as a substitute for phthalates as plasticisers in certain soft PVC products. Opinion adopted at the 11th CSTEE plenary meeting, 28 September.

Dale. R., Squirrell. D. J (1990). A rapid method for assessing the resistance of polyurethanes to biodeterioration. Int. Biodet. Biodeg. 26, 355–367.

Daneault. C., Kokta B. V (1986). The xanthate method of grafting. 107-114. In Carraher. C. E., Sperling. L. J (ed.), Renewable-resource materials: new polymer sources. Plenum Publishing Co., New York.

Dave. H., Rao. P.V.C., Desai. J..D (1997). Biodegradation of starch-polyethylene films in soil and by microbial cultures. World J. Microb. Biotech. 13, 655-658.

Degli-Innocenti. F., Tosin, M., Bastioli, C (1998). Evaluation of the biodegradation of starch and cellulose under controlled composting conditions. J. Environ. Polym. Degrad. 6(4), 197- 202.

Deguchi. T., Kakezawa. M., Nishida, T (1997). Nylon biodegradation by lignin-degrading fungi. Appl. Environ. Microbiol. 63, 329–331.

Domb. A.J., Kost, J., Wiseman, D.M (1997). Handbook of Biodegradable Polymers; Harwood Academic Publishers: Singapore.

Domsh. K. H., Gams. W., Anderson. T.-H (1980a). Compendium of Soil Fungi, vol. 1. London: Academic Press.

Domsh. K. H., Gams. W., Anderson, T.-H. (1980b). Compendium of Soil Fungi, vol. 2. London: Academic Press.

Dylingowski. P.J., Hamel.R.G (2005). Directory of Microbicides for the Protection of Materials A Handbook Paulus, Wilfried (Ed.)

Eaton. R. A., Hale. M.D.C (1993) Wood, decay, pests and prevention. Chapman and Hall, London.

Eaton. R. W., Ribbons. D. W (1982). Utilization of phthalate esters by micrococci. Arch. Microbiol. 132,185–188.

EC (1999). Commission Decision 1999/815/EC. European Commission, Brussels.

Ejlertsson. J., Meyerson. U., Svensson B.H (1996). Anaerobic degradation of phthalic acid esters during digestion of municipal solid waste under landfilling conditions. Biodegradation. 4,345-352.

El-Aghoury. R. K., Vasudeva D., Banun. M., Feldman. D (2006). Contribution to the Study of Fungal Attack on Some Plasticized Vinyl Formulations. J. Polym. Environ. 14,135–147.

Elder. D.J.E., Kelly. D.J (1994). The bacterial-degradation of benzoic-acid and benzenoid compounds under anaerobic conditions - unifying trends and new perspectives. FEMS Microbiol. Rev. 13, 441-468.

Erlandsson. B., S. Karlsson., Albertsson. A. C (1997). The mode of action of corn starch and a prooxidant system in LDPE: influence of thermooxidation and UV-irradiation on the molecular weight changes. Polym. Degrad. Stab. 55, 237-245.

Fanta. G.F., Swanson. C.L., Shorgren. R.L (1992). Starch –poly (ethylene-co-acrylic acid). Composite films. Effects of processing condition on morphology and properties. J. Appl. Polym. Sci. 44:,2037-2042.

Fauvarque. J (1996). The chlorine industry. Pure. Appl. Chem. 68, 1713-1720.

Fell. J. W (1993). Rapid identification of yeast species using three primers in a polymerase chain reaction. Mol. Mar. Biol. Biotechnol. 1,175–186.

Fiala. F., Steiner. I., kubesch. K (2000). Migration of di-(2-ethylhexyl)phthalate (DEHP) and diisononyl phthalate (DINP) from PVC articles. Dtsch. Lebensm. –Rundsch. 96, 51-57.

Flemming. H.-C (1998). Relevance of biofilms for the biodeterioration of surfaces of polymeric materials. Polym. Degrad. Stabil. 59,309–315.

Frankland. J. C., Dighton. J., Boddy. L (1990). Methods for studying fungi in soil and forest litter. Meth. Microbiol. 22, 343–404.

Fukumori. K., Sato. N., Kurauchi. T(1992). Mechanical properties and molecular mobility of NBR/PVC blends: stress relaxation behavior under constant strain. Nippon Gomu Kyoukaishi. 65(3),191-99.

Gan. Z., Fung. J. F., Jing. X., Wu. C., Kulicke.W. M (1999). A novel laser light-scattering study of enzymatic biodegradation of poly(ε-caprolactone) nanoparticles. Polymer. 40, 1961-1967.

Gartiser. S., Wallrabenstein. M., Stiene.G (1998). Assessment of several test methods for the determination of the anaerobic biodegradability of polymers. J. Environ. Polym. Degrad. 6, 159-173.

Gartshore. J., Cooper. D.G., Nicell. J.A (2003). Biodegradation of plasticizers by Rhodotorula rubra. Environ. Toxicol. Chem .22,1244–1251.

Gaylarde. C. C., Morton. L. H. G (1999). Deteriogenic biofilms on buildings and their control: a review. Biofouling. 14, 59–74.

Gibb. W. H., MacCallum. J. R (1974). The photodegradation of polyvinyl chloride V The effect of wavelength of irradiation on the dehydrochlorination reaction. Eur. Polym. J. 10, 529-532.

Girard. T. A., C. F. Koda (1959). Staining of PVC. Modern Plastics. 36:148.

Glass. J. E., G. Swift (1989). Agricultural and Synthetic Polymers, Biodegradation and Utilization, ACS Symposium Series 433, American Chemical Society, Washington DC. pp. 9-64.

Glover. R (1993). Markets for degradable plastics. Int. Biodeterior. Biodegrad. 31, 171–178.

Goll. V., Alexandre. E., Viollon-Abadie. C., Nicod. L., Jaeck. D., Richert. L. (1999). Comparison of the effects of various peroxisome proliferators on peroxisomal enzyme activities, DNA synthesis, and apoptosis in rat and human hepatocyte cultures. Toxicol. Appl. Pharmacol. 160, 21-32.

Government of Pakistan (1996). Data collection for preparation of national study on privatization of solid waste management in eight selected cities of Pakistan.

Griffin. G. J. L., Uribe. M (1984). Biodegradation of plasticized polyvinyl chloride. 648–657. In Biodeterioration 6: papers presented at the 6th

International Biodeterioration Symposium. C.A.B. International, Slough, United Kingdom.

Gu. J. D (2003). Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. Int. Biodet. Biodeg. 52, 69-91

Gu. J. D., Ford. T. E., Mitchell. R (2000a). Microbial corrosion of metals.In: Review. (Ed.), The Uhlig Corrosion Handbook, 2nd Edition.Wiley, New York. pp. 915–927.

Gu. J. D., Ford. T. E., Mitton. D. B., R. Mitchell (2000b). Microbial degradation and deterioration of polymeric materials. In. Revie. W. (Ed.), The Uhlig Corrosion Handbook, 2nd Edition. Wiley, New York. pp. 439–460.

Gu. J.-D., Mitchell. R (2001). Biodeterioration. In: Dworkin. M., Falkow. S., Rosenberg. E., Schleifer. K.-H., Stackebrandt. E. (Eds.), The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community. (3rd ed.) Springer-Verlag, New York.

Guarro. J., Gene. J., Stchigel. A. M (1999). Developments in fungal taxonomy. Clin. Microbiol. Rev. 12, 454–501.

Gugumus. F (1990). Photooxidation of polymers and its inhibition. In: J. Pospisil, P. P. Klemchuk (eds) Oxidation inhibition in organic materials, vol.II. CRC press. pp. 29-162.

Gumargalieva. K. Z., Zaikov. G. E., Semenov. S. A., Zhdanova. O. A (1999). The influence of biodegradation on the loss of a plasticiser from polyvinyl chloride). Polym. Degrad. Stabil. 63, 111–112.

Hamid, S. H (2000).Handbook of Polymer Degradation, 2nd ed.; Marcel Dekker: New York.

Hamilton. N. F (1983). Biodeterioration of flexible polyvinyl chloride films by fungal organisms. In Biodeterioration. 5, 663–678. Edited by Oxley. T. A., Barry. S. Chichester, UK: Wiley.

Hansen. O. G (1999). PVC in Scandinavia. Med. Device Technol.10, 31-33.

Heinze. J. E., Adams. A. J (1997) Endocrine disruptors: beyond the headlines. Can. Chem. News. 49, 17-18.

Hjertberg. T., Thomas. G (1995). Evaluation of possible degradation of pipes and cable insulation of PVC-Results obtained with samples collected after about 25 years use. Department of Polymer Technology, Chalmers Technical University of Gothenburg, Sweden.

Hoffmann. J., Reznicekova. I., Vanokova. S., Kupec. J (1997). Manometric determination of biological degradability of substances poorly soluble in aqueous environments. Int. Biodet. Biodeg. 39, 327-332.

Hollande. S., Laurent. J (1997). Study of discolouring change in PVC, plasticizer and plasticized PVC films. Polym. Degrad. Stab. 55, 141-145.

http:// www.envis-icpe.com. Production of plastics.

IARC (2000). IARC monographs on the evaluation of carcinogenic risks to humans. http://193.51.164.11/htdocs/announcements/vol77.htm

ICI Chemicals & Polymers Ltd (1994). Introduction to Report to the Chief Inspector. HMIP Authorisation AK6039. Formation of dioxins in oxychlorination, significance for human health and monitoring proposals. 27 Apr. 1994.

Ikada. E (1999). Electron microscope observation of biodegradation of polymers. J. Environ. Polym. Degrad. 7, 197-201.

Imam. S. H., Gordon. S. H., Thompson. A. R., Harry O'kuru. R. E., Greene. R. V (1993) Biotechnol. Tech. 7, 791–794.

Inman. J. C., Strachan. S. D., Sommers. L. E., Nelson. D. W (1984). The decomposition of phthalate esters in soil. J. Environ. Sci. Health. B19 (2), 245-57

Itavaara. M., Vikman. M (1995). A simple screening test for studying the biodegradability of insoluble polymers. Chemosphere. 31, 4359-4373.

Jackson. M.A., Labeda. D.P., Becker. L.A (1996). Isolation for bacteria and fungi for the hydrolysis of phthalate and terephthalate esters. J. Ind. Microbiol. 16, 301–304.

James. N. R., Jayakrishnan. J. A (2003). Surface thiocyanation of plasticized polyvinyl chloride and its effect on bacterial adhesion. Biomaterials. 24, 2205–2212.

Jayasekara. R., Harding. I., Bowater. I., Lornergan. G (2005). Biodegradability of selected range of polymers and polymer blends and standard methods for assessment of biodegradation. J. Polym. Environ. 13:,231-251.

John. D., Cattley. R., Elcombe. C., Brian. G., James. L., Swenberg, C.W.,Williams. G., Gemert. M. V (1999). A cancer risk assessment of di(2-ethylhexyl)phthalate: application of the new U.S. EPA risk assessment guidelines. Reg. Toxicol. Pharmacol. 29, 327-357.

Johnson. B.T., Heitkamp. M. A., Jones. J.R (1984). Environmental and chemical factors influencing the biodegradation of phthalic acid esters in fresh-water sediments. Environ. Poll. 8.101-118.

Jones. C. R., Handley, P. S., Robson. G. D., Eastwood. I. M., Greenhalgh. M (1996). Biocides incorporated into plasticized polyvinyl chloride reduce adhesion of *Pseudomonas fluorescens* BL146 and substratum hydrophobicity. J. Appl. Bacteriol. 81, 553–560.

Juneson. C., Ward. O.P., Singh. A (2002). Biodegradation of dimethyl phthalate with high removal rates in a packed reactor. World. J. Microbiol. Biotechnol. 18, 7–10.

Kaarik. A (1974). Sapwood staining fungi. The International Research Group on Wood Preservation. Document no. IRG/WP/125. Princes Risborough, England.

Kaczmarek. H., Bajer. K (2008). Study on the course of biodegradation of PVC/cellulose composites. Polimery. 53 (9), 631-638.

Kaczmarek. H., Bajer. K (2007). Biodegradation of Plasticized Poly (Vinyl Chloride) Containing Cellulose. Polym. Sci. 45, 903– 919.

Karal. O., Hamarcu. E., Baysal. B. M (1997), Blends of caprolactone – poly (dy\imetylsiloxane)–polycaprolactone triblock copolymer with poly (vinyl chloride): preparation and characterization. Polym. Degrad. Stabil. 38, 6071-6078.

Karpagam. S., Lalithakumari. D., (1999). Plasmid- mediated degradation of o- and p-phthalate by *Pseudomonas fluorescens.* World. J. Microbiol. Biotechnol.15. 565-9

Kawai. F (1995). Breakdown of plastics and polymers by microorganisms. Adv. Biochem. Eng. Biotechnol. 52,151-194.

Kay. M. J., Morton, L. H. G.,Prince, E. L (1991). Bacterial degradation of polyester polyurethane. Int. Biodet. Biodeg. 27, 205–222.

Kershaw. M. J., Talbot. N. J (1998). Hydrophobins and repellents: proteins with fundamental roles in fungal morphogenisis. Fungal. Genet. Biol. 23, 18–33.

Kiatkamjornwong. S., Sonsuk. S., Wittayapichet, S. and Prasassarakich, P, (1999). Degradation of Styrene-g-Cassava Starch Filled Polystyrene Plastics. Polym. Degrad. Stab. 66, 323-335

Kikkawa. Y., Abe. H., Iwata. T., Inoue. Y., Doi. Y (2002). Crystal morphologies and enzymatic degradation of melt-crystallized thin films of random copolyesters of (R)-3-hydroxybutyric acid with (R)-3-hydroxyalkanoic acids. Polym. Degrad. Stab. 76, 467-478.

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

Kneale. C (2002). Problems and Pitfalls in the Evaluation and Design of New Biocides for Plastic Applications. Avecia Protection and Hygiene UK, online at http://www.avecia.com/biocides/whats_new_archive/2002. .

Koop. C. Everett (1999) A scientific evaluation of health effects of two plasticisers used in medical devices and toys: a report from the American Council on Science and Health. Medscape, http://www.medscape.comMedscape/GeneralMedicine/journal/

Kurata. Y., Kidachi. F., Yokoyama. M., Toyota. N., Tsuchitani. M., Katoh. M (1998). Subchronic toxicity of di(2-ethylhexyl)phthalate in common marmosets: lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicol. Sci. 42, 49-56.

Kurtzman. C.P., Robnett. C.J (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwen. 73(4), 331-371.

Łabużek. S., Nowak. B., Pająk. J (2003). The Susceptibility of Polyethylene Modified with Bionolle to biodegradation by Filamentous Fungi. Pol. J. Environ. Stud. 13, 59-68

Langer, J.,Sang, S (1997). Endocrine disruption: cause for concern, cause for action. Can. Chem. News. 49, 16-17.

Latruffe. N., Pacot. C., Passilly. P., Petit. M., Bardot. O., Caira. F., Malki .M. C., Jannin. B., Clemencet. M. C., Deslex. P (1995). Peroxisomes and hepatotoxicity. Comp. Haematol. Int. 5, 189-195.

Li. L.-H., Jester. W. F., Orth. J. M (1998). Effects of relatively low levels of mono-(2-ethylhexyl) phthalate on cocultured Sertoli cells and gonocytes from neonatal rats. Toxicol. Appl. Pharmacol. 153, 258-265.

Lymar. E. S., Li. B., Renganathan. V (1995). Purification and characterization of a cellulose-binding glucosidase from cellulose-degrading cultures of *Phanerochaete chrysosporium*. App. Environ. Microbiol. 61 (8), 2976-2980.

Mably. T. A., Moore. R. W., Bjerke. D. L., Peterson. R. E (1991). The male reproductive system is highly sensitive to in utero and lactational TCDD exposure. Banbury report 35: Biological basis for Risk

assessment of Dioxins and related compounds. Cold Spring Harbour Laboratory Press. Cited in Allsopp, Costner & Johnston Body of Evidence p.27. Greenpeace

Manz. A., Berger. J., Dwyer. J.H., Flesch-janys. D., Nagel. S., Waltsgott. H (1991). Cancer mortality among workers in chemical plant contaminated with dioxin. Lancet. 338, 959-964.

Marks. G. C., Benton. J. L., Thomas. C. M (1967). Soc. Chem. Ind. (London) Monograph.26,204-235.

Marten. E (2000). Korrelation zwischen der Struktur und der enzymatischen Hydrolyse von Polyestern,Dissertation, Technical University Braunschweig,Germany.Internet:™http://opus.tubsde/opus/volltexte/2000/136∫.

Martin.F., S.M., Marconato. J. C., Agnelli. J. A., Monteiro. M. R (2007). Biodegradation of blend films in soil and soil with chorume. I.PVC/PCL. J. Biotech .2(4),20-25.

Martinez. J. G., Rodriguez. O. S., Sanchez. S., Ramirez. E., Allen.N. S. (1996). Prediction of photoageing stability of plasticized PVC films containing UV-stabilizers. Polym. Degrad. Stab. 54,49–55.

Martins. F. S.M., Domingos. R.N., Tombini. R.C (2002). Modifications on PVC films by ultrasound. Ultrason. sonochem. 9,139-141

Matsunaga. M., Whitney. P. J (2000). Surface changes brought about by corona discharge treatment of polyethylene film and the effect on subsequent microbial colonization. Polym. Degrad. Stab. 70, 325-32

McCarthy. S. P., Gada. M., Smith. G. P., Tolland. V., Press. B., Eberiel. D., Bruell. C., Gross. R. A1(992). The accelerated biodegradability of plastic materials in simulated compost and landfill environments. Annu. Tech. Conf. Soc. Plast. Eng. 50,816-818.

McCartin. S. M., Press. B., Eberiel. D., McCarthy. S. P (1990). Simulated landfill study on the accelerated biodegradability of plastics materials. Am. Chem. Soc. 31, 439-440.

Mergaert. J., Schirmer. A., Hauben. L., Mau. M., Hoste. B., Kersters. K., Jendrossek. D., Swings. J(1996). Isolation and identification of poly(3-hydroxyvalerate) degrading strains of *Pseudomonas lemoignei*. Int. J. Syst. Bact. 46, 769-773.

Mifune. N., Nagai. Y., Nishimoto. K., Ishimaru. S (1991). Study on change of material properties and dynamic properties of styrene-butadiene rubber vulcanizate by degradation. Kobunshi Ronbunshu. 48(7),437-442.

Milstein.O., Gersonde. R., Huttermann. A., Chen. M. J., Meister. J. J (1992). Fungal Biodegradation of Lignopolystyrene Graft Copolymers. Appl Environ. Microbiol, 58, 3225.

Moeller. S., Larsen. J., Jelnes. J.E., Faergemann. H., Ottosen. L.M., Knudsen. F.E (1996). Environmental aspects of PVC. Environmental project No. 313, Danish Environmental Protection Agency.

Moreno-Chulim. M. V., Barahona-Perez. F., Canche-Escamilla G (2002). Biodegradation of Starch and Acrylic-Grafted Starch by *Aspergillus niger*. J. App. Polym. Sci . 89, 2764–2770.

Muller. W. R (1999). Sauerstoff und Kohlendioxid gleichzeitig messen, LaborPraxis September. 94-98.

Muller.R. J., Augusta. J., Pantke. M (1992). An interlaboratory investigation into biodegradation of plastics. A modified Sturm-test. Mat. Organ. 27,179-189.

Murad. S., Hasan. F., Shah. A. A., Hameed. A,. Ahmed. Safia (2007). Isolation of phthalic acid degrading *Pseudomonas* sp P1 from soil. Pak. J. Bot. 39 (5) 1833-1841.

Mylchreest, E., Russell C., Cattley ., Paul. M., Foster. D (1998) Male reproductive tract malformations in rats following gestational and lactational exposure to di(n-butyl) phthalate: an antiandrogenic mechanism? Toxicol. Sci. 43, 47-60.

Nakajima-Kambe, T., Shigeno-Akutsu. Y., Nomura. N., Onuma. F., Nakahara. T. (1999). Microbial degradation of polyurethane, polyester polyurethanes and polyether polyurethanes. Appl. Microbiol. Biotechnol. 51,134– 140.

Nalli. S., Cooper. D. G., Nicell. J. A (2002). Biodegradation of plasticizers by *Rhodococcus rhodochrous*. Biodegradation. 13, 343–352.

National Toxicology Programme (1991). US Dept. of Health and Human services : NTP Chemical Repository (Radian Corporation).2,3,4,7,8-tetrachlorodibenzo-p-dioxin.

Nir. M. M., Miltz. J., Ram. A (1993). Update on Plastics and the Environment: Progress and Trends. Plast. Eng. 75-93.

Nishida. H., Tokiwa. Y (1993). Distribution of poly(β-hydroxybutyrate) and poly(ε-caprolactone) aerobic degrading microorganisms in different environments. J. Environ. Polym. Degrad. 1,227-233.

O'Connor. O. A., Rivera. M. D., Young. L. Y (1989). Toxicity and biodegradation of phthalic acid esters under methanogenic conditions. Environ. Toxicol. Chem. 8(7), 569-76.

Ohtaki. A., Sato. N., Nakasaki. K (1998). Biodegradation of poly (caprolactone) under controlled composting conditions, Polym. Degrad. Stab. 61(3), 499- 505.

Okaya. T., Ikari. I (1992). Polyvinyl alcohol Developments. Wiley, New York.

Otake. Y., Kobayashi. T., Ashabe. H., Murakami. N., Ono. K (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 .

Pagga. U., Beimborn. D. B., Boelens J., Wilde. B. De (1995). Determination of the aerobic biodegradability of polymeric material in a laboratory controlled composting test. Chemosphere. 31, 4475–4487.

Pagga. U., Beimborn. D. B., Boelens. J., DeWilde. B (1995). Determination of the biodegradability of polymeric material in a laboratory controlled composting test. Chemosphere. 31, 4475-4487.

Pagga. U., Schefer. A., Muller. R. J., Pantke. M (2001). Determination of the aerobic biodegradability of polymeric material in aquatic batch tests. Chemosphere. 42, 319-331.

Pantke. M., Seal. K. J (1990). An interlaboratory investigation into the biodeterioration testing of plastics, with special reference to polyurethanes; Part 2: Soil burial experiments, Mat. und Organismen 25, 88-98.

Parmar. D., Seth. P. K (1997). Phthalic acid esters and liver. Environ. Xenobiot. 72-91.

Parmar. D., Srivastava. S.P., Singh. G.B., Seth. P.K (1987). Effect of testosterone on the testicular atrophy caused by di(2-ethylhexyl) phthalate (DEHP). Toxicol. Lett. 36, 297-308.

Pathirana. R. A., Seal. K. J (1985). Studies on polyurethane deteriorating fungi. Part 3. Physico-mechanical and weight changes during fungal deterioration. Int. Biodet. 21, 41-49.

Peciulyte. D (1997). Utilization of phthalate esters by microorganisms. Biologia. 2, 33-37.

Peterson. R. E., Theobald. H. M.,Kimmel G. L (1993). Developmental and reproductive toxicity of dioxins and related compounds: Cross species comparisons. Critical Reviews in Toxicology 23 (3)283:355. Cited in Allsopp, Costner & Johnston (1995) Body of Evidence p.23 Greenpeace.

Plate. D (1997). Schadstoff-Freisetzung aus Kunststoffen unter Deponie-Milieu Bedingungen Abfallwirtschafts journal. 10.

Pospisil. J., Horak. Z., Krulis. Z., Nespurek. S., Kuroda. S (1999). Degradation and aging of polymers blends. Thermomechanical and thermal degradation. Polym. Degrad. Stab. 65, 405-414.

Pospisil. J., Nespurek. S (1997). Highlights in chemistry and physics of polymer stabilization. Macromol. Symp. 115,143-163.

Puchner. P., Muller. W.R., Bartke. D (1995). Assessing the biodegradation potential of polymers in screening-and long-term test systems, J. Environ. Polym. Degrad. 3,133-143.

Pugh. G., Isenberg. J. S., Kamendulis. L. M., Ackley. D. C., Clare. L. J., Brown. R., Lington. A.W., Smith. J. H., Klaunig. J. E (2000). Effects of di-isononyl phthalate, di-2-ethylhexyl phthalate, and clofibrate in cynomolgus monkeys. Toxicol. Sci. 56, 181-188.

Pujar. B.G., Ribbons. D.W (1985). Phthalate-metabolism in Pseudomonas fluorescens PHK: Purification and properties of 4,5- dihydroxyphthalate decarboxylase. Appl. Environ. Microbiol. 49,374–376

Qi. C., Yijun. Z., Janardan. K. (2000). Peroxisome proliferator- activated receptors, coactivators, and downstream targets. Cell. Biochem. Biophys., 32, 187-204.

Rabek. J. (1996). Photodegradation of polymers – physical characteristics and application. Springer, Germany.

Reischwitz. A., Stoppok. E., and Buchholz. K. (1998). Anaerobic degradation of poly-3-hydroxybutyrate and poly-3-hydroxybutyrate-co-3-hydroxyvalerate, Biodegradation. 8, 313-319.

Richard. F., Grossman..J. E., Lorna S., Alessio. D (2007).Considerations affecting landfill biodegradability of PVC. J. Vinyl. Add. Tech. 13, 133–135

Richterich. K., Berger. H., Steber. J (1998). The two phase closed bottle test a suitable method for the determination of ready biodegradability of poorly soluble compounds. Chemosphere. 37, 319-326.

Rivard. C., Moens. L., Roberts. K., Brigham. J., Kelley. S (1995). Starch esters as biodegradable plastics: Effects of ester group chain length and degree of substitution on anaerobic biodegradation. Enz. Microb. Tech. 17, 848-852.

Roberts. W. T., Davidson. P. M (1986). Growth characteristics of selected fungi on polyvinyl chloride film. Appl. Environ. Microbiol. 51, 673–676.

Sabev. H. A., Handley. P. S., Robson G. D (2006). Fungal colonization of soil-buried plasticized polyvinyl chloride (pPVC) and the impact of incorporated biocides. Microbiology. 152, 1731–1739.

Sabir. I. (2004). plastic industry in Pakistan. http://www.jang.com.pk/thenews/investors/nov2004/index.html.

Safe. S (1999). Endocrine disruptors and human health an update. Organohalogen Comp. 42, 15-18.

Samsonowa. A.S., Aleshchenkowa. Z.M., Syomochkina. N.F., Baikova. S.V (1996). Microbial decontamination of effluents from phthalate esters. DECHEMA Monographs. 133, 607–610.

Santoro. E. D., Koestler. R. J (1991). A methodology for biodeterioration testing of polymers and resins. Int. Biodet. Biodeg. 28, 81–90.

Scott. G (1990). Photo-biodegradable plastics: Their role in the protection of the environment. Polym. Degrad. Stab. 29, 135–154.

Scott. G (1999). Polymers in modern life. In: Polymers and the Environment. The Royal Society of Chemistry, Cambridge, UK.

Scott. G (2000). Biodegradable Polymers [Web Page]. Accessed 2000 March 30. http://www.denison.edu/chem/DCS/journal/scottv1n1.shtml.

Seal. K. J., Pantke. M (1988). Microbiological testing of plastics: ongoing activities of IBRG plastics project group to improve standard test procedures. Int. Biodegrad. 24, 313–320.

Semenov. S.A., Gumargalieva. K.Z., Zaikov. G.E (2003). Biodegradation and durability of materials under effect of microorganisms. VSP, Boston.

Shah. A. A,. Hasan. F., Akhtar. J. I., Hameed. A., Ahmed. S (2008). Degradation of polyurethane by a novel bacterium consortium isolated from soil. Ann. Microbiol. 58(3) 381-386.

Shailaja. S., Mohan. Venkat. S., Krishna. M. R., Sarma. P. N (2008). Degradation of di-ethylhexyl phthalate (DEHP) in bioslurry phase reactor and identification of metabolites by HPLC and Ms. Int. Biodet. Biodeg. 62 (2), 143-152.

Sharpe. R. M (1998). Natural and anthropogenic environmental estrogens: the scientific basis for risk assessment. Environmental oestrogens and male fertility. Pure. Appl. Chem.70, 1685-1701.

Shogren. R. L., Swanson. C. L., Thompson. A. R (1992). Extrudates of cornstarch with urea and glycols: structure/mechanical property relations Starch. 44 (9), 335-338.

Sjoeberg. P., Lindquist. N. G., Montin. G., Plöen. L (1985). Effects of repeated intravenous infusions of the plasticizer di(2-ethylhexyl) phthalate in young male rats. Arch. Toxicol.58, 78-83.

Smith. G. P., Press. B., Eberiel. D., McCarthy. S. P., Gross. R. A., Kaplan. D. L (1990). An accelerated in laboratory test to evaluate the degradation of plastics in landfill environments. Polym. Mat. Sci. Eng. 63, 862-866.

Solaro. R., Corti. A., Chiellini. E (1998). A new respirometric test simulating soil burial conditions for the evaluation of polymer biodegradation. J. Environ. Polym. Degrad. 5, 203-208.

Sombatsompop. N., Sungsanit. K., Thongpin. C (2003). Analysis of Low-Density Polyethylene-g-Poly(vinyl chloride) Copolymers Formed in

Polyvinyl chloride /Low- Density Polyethylene Melt Blends with Gel Permeation Chromatography and Solid-State 13C-NMR. App. Polym. Sci. 92, 3167–3172.

Soo-Min. L., Lee. J-W., Koo. B-W., Kim. M-K., Choi. D-H., Choi. In-G (2007). Dibutyl Phthalate Biodegradation by the White Rot Fungus, *Polyporus brumalis*. Biotechnol. Bioeng. 97, 6.

Staples. A., Adams. W. J., Parkerton. T. F., Gorsuch. J. W., Biddinger. G. R., Reinert. K. H (1997a). Aquatic toxicity of eighteen phthalate esters. Environ. Toxicol. Chem. 16, 875–891.

Staples. C. A., Peterson. D. R., Parkerton. T. F., Adams. W. J (1997b). The environmental fate of phthalate esters: a literature review. Chemosphere 35, 667–749.

Starnes. W.H (2002). Structural and mechanistic aspects of the thermal degradation of polyvinyl chloride. Prog. Polym. Sci. 27, 2133.

Steinbuchel, A. (1996) Biodegradability of polymers and mechanisms of biodegradation. In: Biodeterioration & Biodegradation. Papers of the 10th International Biodeterioration & Biodegradation symposium-Hamburg, ed. W. Sand, DECHEMA Monograph, p. 245-254 Frankfurt: Schon & Wetzler.

Sturges. M (2000). Packaging Technology. Science.13, 37–42.

Sung. W . Nikolov, ZL. (1992) Accelerated degradation studies of starch- filled polyethylene films.Ind. Eng.Chem Res. 31: 2332-2339

Suresh. C., Dobey. A. K., Srikanta. S., Umeshkumar. S., Karanth. N. G (1999). Appl. Microbiol. Biotechnol. 51, 673.

Sylvia. K (1995). Materials. World. J. Indust. Microbiol. 17, 463-469.

Thomas. D.R., Carswell. K.S., Georgiou. G (1992). Mineralization of biphenyl and PCBs by the white rot fungus *Phanerochaete chrysosporium*. Biotech. Bioeng. 40, 1395-1402.

Thorn. G (1997). The fungi in soil. In Modern Soil Microbiology,pp. 63–127. Edited by J. D. Van Elsas, E. M. H. Wellington & S. T. Trevors. New York: Marcel Dekker.

Titow. W.V (1984). PVC Technology to 2, 3, 7, 8-TCDD after the 1953 accident. Fourth edition, Elsevier Applied Science, London. Int. Arch. Occ. Environ. Health. 62, 139-157.

Tokiwa. Y., Suzuki. T (1977). Hydrolysis of polyesters by lipases. Nature 270, 76–78.

Torikai. A.; Hasegawa. H(1999). Accelerated photodegradation of poly(vinyl chloride). Polym. Degrad. Stab. 63, 441.

Tosin. M., Degli-Innocenti. F., Bastioli. C (1996). Effect of the composting substrate on biodegradation of solid materials under controlled composting conditions,.J. Environ. Polym. Degrad. 4, 55-63.

Tsuji. H., Suzuyoshi. K (2002). Environmental degradation of biodegradable polyesters 1. Poly(ε-caprolactone), poly[(R)-3-hydroxybutyrate], and poly(l-lactide) films in controlled static seawater. Polym. Degrad. Stab. 75, 347-355.

Tuomela. M., Hatakka. A., Raiskila. S., Vikman. M., Itavaara. M (2001). Biodegradation of radiolabelled synthetic lignin (14C-DHP) and mechanical pulp in a compost environment. Appl. Microbiol. Biotechnol. 55, 492-499.

Tuominen. J., Kylma. J., Kapanen. A., Venelampi. O., Itavaara. M., Seppala J (2002). Biodegradation of lactic acid based polymers under controlled composting conditions and evaluation of the ecotoxicological impact. Biomacromolecules. 3, 445-455.

Tyler. C., Jobling. S., Sumpter. JP (1998). Endocrine disruption in wildlife: a critical review of the evidence. Crit. Rev. Toxicol. 28, 319- 361.

US EPA (2005). Municipal solid waste. United States EPA. (www.epa.gov/epaoswer/nonhw/uncpl/facts.htm).Toxicol. 28, 319-361.

U.S. EPA (2000). Toxicological Review of Vinyl Chloride, Available: http://www.epa.gov/iris/toxreviews/ [accessed 2 September 2005].

U.S. E.P.A. Environmental Protection Agency (1994). Health assessment document for 2,3,7,4,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. (EPA/600/BP-92/001a-c).

UBA (1999). Areas of action and criteria for a preventative sustainable substance control policy using PVC as an example. English translation. The German Federal Environmental Agency.

Upsher. F. J (1984). Fungal colonization of some materials in a hot wet tropical environment. Int. Biodet. 20, 73–78.

Upsher. F. J., Roseblade. R. J (1984). Assessment by tropical exposure of some fungicides in plasticized PVC. Int. Biodet. 20, 243- 254

Valiente. N., Lalot. T., Brigodiot. M., Marechal. E (1998). Enzymic hydrolysis of phthalic unit containing copolyesters as a potential tool for block length determination. Polym. Degrad. Stab. 61, 409-15.

Veronelli. M., Mauro. M., Bresadola. S (1999). Influence of thermal dehydrochlorination on the photoxidation kinetics of PVC samples. Polym. Degrad. Stab. 66, 349-357.

Vikman. M., Itavaara. M., Poutanen. K (1995). Measurement of the biodegradation of starchbased materials by enzymatic methods and composting. J. Environ. Polym. Degrad. 3, 23 - 29.

Volke-Sepulveda. T., Saucedo-Castaneda G., Gutierrez-Rojas M., Manzur A., Favela-Torres. E. (2002) Thermally treated low density polyethylene biodegradation by Penicillium pinophilum and *Aspergillus niger*. J. Appl. Polym. Sci. 83. 305–314.

Vona. I. A., Costanza. J. R., Cantor. H. A., Roberts. W. J (1965). In W. M. Smith (ed.): Manufacture of Plastics. 66, 141-142.

Vos. J. G., Dybing. E., Greim. HA., Ladefoged. O., Lambré. C., Tarazona. JV., Brandt. I., Vethaak. A.D (2000). Health effects of endocrine-disrupting

chemicals on wildlife, with special reference to the European situation. Crit. Rev. Toxicol. 30, 71-133.

VROM (1998). Position paper of the Netherlands on PVC. The Dutch Ministry of Housing, Spatial Planning, and the Environment.VSP, Boston.

Walter. T., Augusta. J., Muller. R. J., Widdecke. H., Klein. J (1995). Enzymatic degradation of a model polyester by lipase. Enzy. Microb. Tech. 17, 218- 224.

Wang .J., Liu. P., Shi. H., Yi. Q (1997). Chemosphere. 8, 1747.

Wang. M.Q., Saricks. C. L., Wu M (1997). Fuel-Cycle Fossil Energy Use and GreenhouseGas Emissions of Fuel Ethanol Produced from U.S. Midwest Corn, prepared for Illinois. Department of Commerce and Community Affairs, Center for Transportation Research, ArgonneNational Laboratory, Argonne, Ill.,

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

Webb. J.S., van der Mei. H.C., Nixon. M., Eastwood. I.M., Greenhalgh. M.,Read.S.J, Robson. G.D. Handley. P.S(1999). Plasticisers increase adhesion of the deteriogenic fungus Aureobasidium pullulans to polyvinylchloride. Appl. Environ. Microbiol. 65, 3575- 3581

Wellman. R. H., McCallan. S. E. A (1945). Fungus resistance of pesticides. U.S. Office of Scientific Research and Development, report no. 5683.

Welzel. K., Belal. E., Muller. R. J., Deckwer. W. D (2002). Enzymatischer Abbau von Polyester-Nanopartikeln, Chem. Ing. Tech. 5, 574

White. T. J., Bruns. T., Lee. S., Taylor. J (1990). Amplification and direct sequencing of fungal rRNA genes for phylogenetics. 315–322. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols: aguide to methods and applications. Academic Press, San Diego, Calif .

Whitney. P.J (1999). A comparison of two methods for testing defined formulations of PVC for resistance to fungal colonization with two methods for the assessment of their biodegradation, Int. Biodet. Biodeg. 37 (1996), 205–213.

Whitney. P. J (1996). A comparison of two methods for testing defined formulations of PVC for resistance to fungal colonization with two methods for the assessment of their biodegradation. Int. Biodet. Biodeg. 37, 205– 213.

Whitney. J., Swaffield. C. H., Graffham. A. J (1993). The environmental degradation of thin plastic films. Int. Biodet. Biodeg. 31, 179–198.

Wilhelm. H.M., Sierakowski. M.R., Souza, G.P. Wypych, F (2003). Starch films reinforced with mineral clay. Carbohy. Polym. 52(2), 101-110.

Wilson. A. S (1995). Plasticisers: Principles and Practice, The Institute of Materials,London,.

Winters. H., Guidetti. G (1976). Extracellular enzymes from fungal isolates involved in the biodeterioration of paint films. Dev. Ind. Microbiol. 17, 173–176.

Witt. U., Einig. T., Yamamoto. M., Kleeberg. I., Deckwer. W. D., Muller. R. J (2001). Biodegradation of aliphatic-aromatic copolyesters: evaluation of the final biodegradability and ecotoxicological impact of degradation intermediates. Chemosphere. 44, 289-299.

Xu. P., Zhou, D., Zhao, D (1989). Studies of the thermal stability of poly vinyl chloride. Influence of light on the thermal stability of PVC. Eur. Polym. J. 25, 575-579.

Yabannavar. A. V., Bartha. R (1994). Methods for assessment of biodegradability of plastic films in soil. Appl. Environ. Microbiol. 60, 3608–3614.

Yabannavar. A., Bartha. R (1993). Biodegradability of some food-packaging materials in soil. Soil. Biol. Biochem. 25, 1469-1475.

Yu. J. J., Ward. O. P (1995). Bioremediation of di(2-ethylhexyl) phthalate in contaminated soil. In Bioremediation of Recalcitrant Organics, ed. R Hinchee, D. B Anderson and R. E. Hoeppel, 323-330.

Zabel. R. A., Terracina. F (1979). The role of Aureobasidium pullulans in the disfigurement of latex paint films. Dev. Ind. Microbiol. 21,179-190.

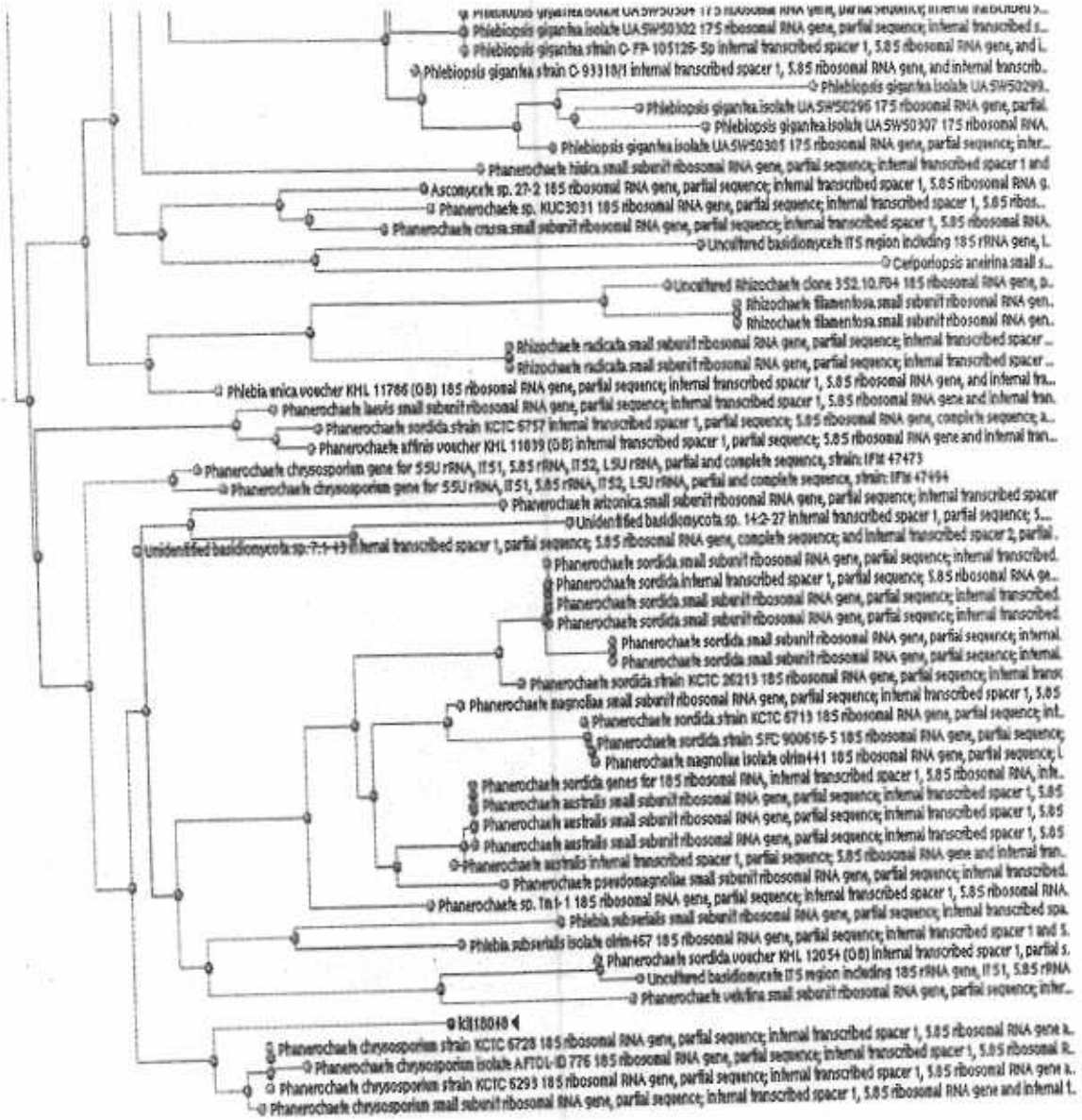
Zeng. F., Cui. K. Y., Fu. J. M., Sheng. G. Y., Yang, H.F (2002). Biodegradability of di(2-ethylhexyl) phthalate by Pseudomonas fluorescens FSI. Water. Air. Soil. Pollut. 140, 297-305.

Zhang. G., Reardon., K. F (1990). Parametric study of diethyl phthalate biodegradation. Biotechnol. Lett.12(9), 669-704.

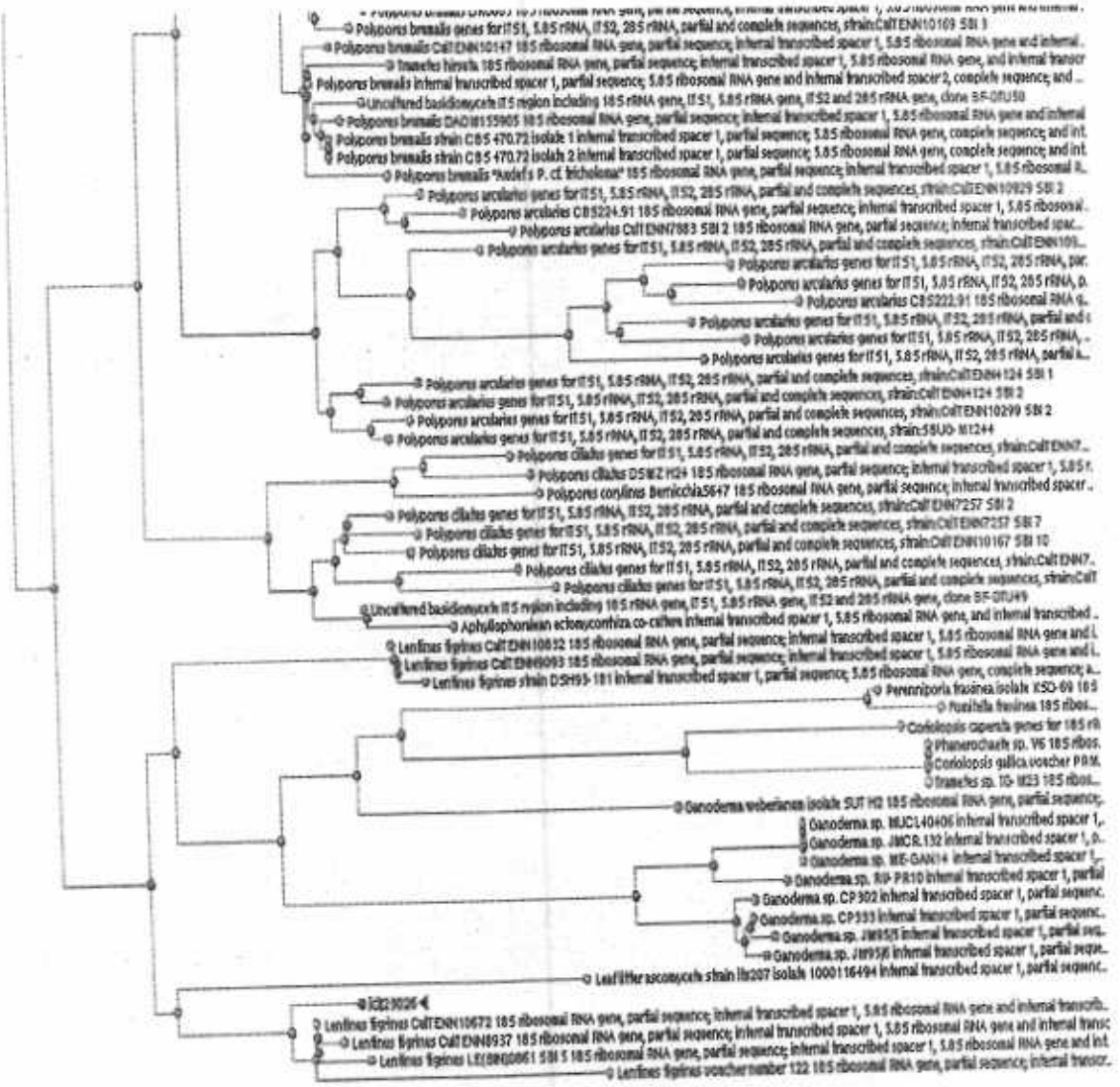
Zhao. J. H., Xiao.Q. W., Jun. Z., Guang. Y., Feng. H. S., Qing. Y (2005). Biodegradation of poly(butylene succinate-co-butylene adipate) by *Aspergillus versicolor*. Polym. Degrad. Stab. 90,173-179.

Zober. A., Messerer P., Huber P (1990). Thirty-four year follow-up of BASF employees exposed to 2,3,7,8-TCDD after the 1953 accident. Int. Arch. Occ. Environ. Health. 62, 139-157.

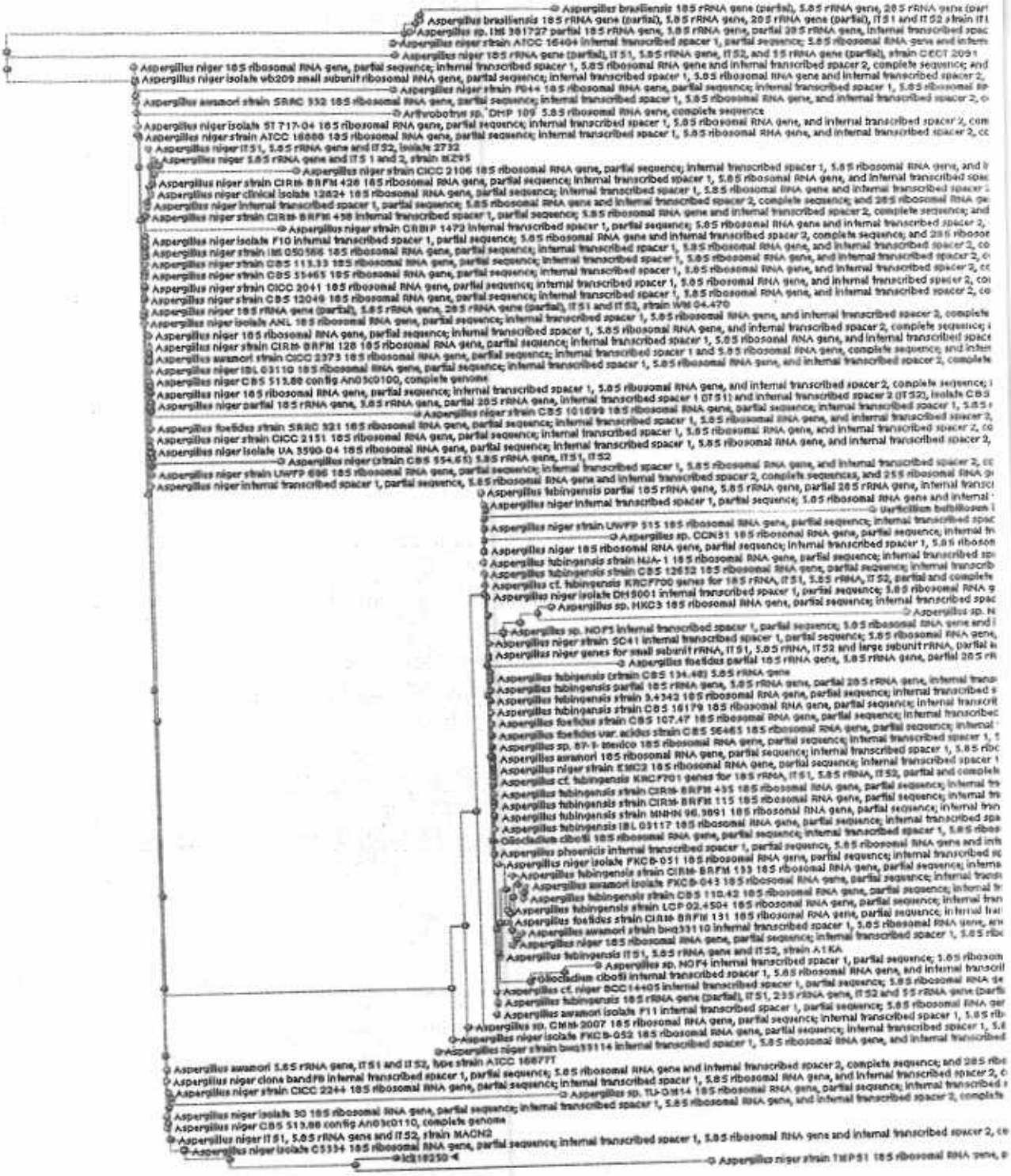
Phylogenetic tree of *Phanerochaete chrysosporium* PV1



Phylogenetic tree of *Lentinus tigrinus* PV2



Phylogenetic tree of *Aspergillus niger*



Phylogenetic tree of *Aspergillus sydowii*

Nucleotide Sequence of isolated fungal strains

Phanerochaete chrysosporium PV1

ITS1 partial, 5.8S, ITS2 and 28S partial 589

TTAACGAGTAACTGAACAGGTTGTAGCTGGCCTCTCGGGGCATGTGCACGCCTGGCT
CATCCACTCTTCAACCTCTGTGCACTTGTTGTAGGTCGGTAGAAGAGCGAGCATCCT
CTGATGCTTTGCTTGGAAGCCTTCCTATGTTTTACTACAAACGCTTCAGTTTAAGAA
TGTCTACCTGCGTATAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTC
TCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAG
TGAATCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATTCCGGGGAGCATGCC
TGTTTGAGTGTCATGGTATCCTCAACCTTCATAACTTTTTGTTATCGAAGGCTTGGA
CTTGGAGGTTGTGCTGGCTTCTAGTCGAGTCGGCTCCTCTTAAATGTATTAGCGTGA
GTGTAACGGATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTAACATA
AGCTTGCGCTTCTAACCGTCCTTCAGTTGGACAACTTACTTTGACATCTGACCTCAA
ATCAGGTAGGACTACCCGG

Aspergillus sydowii PV2

ITS1, 5.8S, ITS2 AND 28S partial

CTGAGTGCGGGCTGCCTCCGGGCGCCCAACCTCCCACCCGTGAATACCTAACACTGT
TGCTTCGGCGGGGAGCTCCCTCGGGGGCGAGCCGCCGGGGACTACTGAACTTCATGC
CTGAGAGTGATGCAGTCTGAGTCTGAATATAAAATCAGTCAAAACTTTCAACAATGG
ATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAACTGCGATAAGTAATGTGAAT
TGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGCATTCC
GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCCCGGCTTGTGTGTTGG
GTCGTCGTCCCCCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGTGTCCGGTC
CTCGAGCGTATGGGGCTTTATCACCCGCTCGACTAGGGCCGGCCGGGCGCCAGCCGA
CGTCTCCAACCATTTTTCTTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAA
CTTAAGCATATCAATAAGCGGAGGA

Aspergillus niger PV3,

5.8S, ITS2 and 28S partial

CCGATGCGGGTCCTTTGGGCC**CAACCTCCCATCCGT**GTCTATTGTACCCTGTTGCTT
CGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCCCGTG
CCCGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGA
ATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAA
CGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTT
TGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTG
CTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGACGGG
CCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACA
TGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTG
ACCTCGGATCAGGTAGGGATACCCGCT

Lentinus tigrinus PV4

Final 18S partial, ITS1, 5.8S, ITS2 and 28S partial

TTCCGTAGGTGAACCTGCGGAAGGATCATTATCGAGTTTTGAAACGGGTTGTAGCTG
GCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTGT
GGGTTTCAGGAGCTTCAAGGGCGTTTCTTACGCCGGAGTTGTGACTGGGCCTACGTT
TACTACAAACTCTTACAAGTATCAGAATGTGTATTGCGATGTAACGCATCTCTATAC
AACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGC
GCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATTCTCAACCTAA
CGGGTTCTTAACGGGACTTGCTTAGGCTTGGACTTGGAGGCTCTTGTCGGCTTGCTT
TCGTCAAGTCGGCTCCTCTCAAATGCATTAGCTTGGTTCTTTGCGGATCCGGCTCAC
GGTGTGATAATTGTCTACGCCGCGACCGTTGAAGCGTTTTAATGGGACTAGCTTCTA
ACCGTCTCCTCGCGAGACAGCATTCATCGAACTCTGACCTCAAATCAGGTAGGACTA
CCCGCGTA