Response of a PVC Lignin Blended Building Material to Fungi Attack

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ABSTRACT

Response of a PVC lignin blended building material to fungi attack

Poly vinyl chloride (PVC) products are susceptible to biological attack. To study this problem, lignin was considered as a material in blend with PVC. Lignin is a renewable, naturally occurring compound and is obtained as a by-product of pulping processes. At present, extensive research has been performed to explore the utilization of lignin. This research studies the feasibility of partially replacing a copolymer of vinyl chloride-vinyl acetate (VC-VAc) by lignin in flooring formulations and evaluates the effects of lignin on the resistance of these formulations to fungi attack. The lignin utilized in the study was an organosolv lignin-Alcell lignin.

To accomplish the objectives, a series of PVC controls and PVC-lignin blends with various plasticizers were inoculated with fungi. After a 28-day incubation, the changes in chemical structure were analyzed by Fourie Transform Infrared (ATR/FTIR) spectroscopy. Optical stereomicroscopy and optical microscopy were employed to observe the changes at the surface.

This research is the original assessment of the resistances of PVC-lignin blends to biodegradation. After a series of experiments, it was found that all fungi species grew on every composite and showed similar abilities of growth. PVC and PVC-lignin blends are susceptive to fungi attack and plasticizers are the main targets of fungi attack; the susceptibility of the plasticizers to biodegradation in descendent order is:

Di-ethylene glycol di-benzoate (Benzoflex 2-45) > Alchil sulphonate (Mesamoll) > Di-octyl-phthalate (DOP) > Tricresyl-phospate (Lindol);

Lindol is the most suitable plasticizer for the formulation of PVC-lignin flooring; Alcell lignin is biodegradable; the presence of Alcell lignin in the complex interactive system PVC-lignin-additives increases the biodegradability of polymeric materials.

ACKNOWLEDGEMENTS

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LIST OF SYMBOLS

AL	Alcell lignin
ACTT	American Type Culture Collection
ASTM	American Society of Testing Materials
ATR/FTIR	Attenuated Total Reflection Fourie Transform Infrared Spectroscopy
Ca	Calcium
Cl	Chloride
C=O	Carbonyl
DOP	Di-octyl phthalate
EHC	Environmental Health Criteria
ΔF	Difference of relative absorbance
$\Delta F'$	Difference of absorbance
H	Hydrogen
ICSCs	International Chemical Safety Cards
Lindol	Tricresyl phosphate
Mesamoll	Alchil sulphonate
MW	Molecular weight
Mw	Weight average molecular weight
Mn	Number average molecular weight
OH	Hydroxyl
OCH_3	Methoxyl
pH	Potential of hydrogen
phr	parts per hundred parts resin

PVC	Poly (Vinyl chloride) (In this thesis, PVC abbreviation will be used for VC-VAc copolymer.)
RH	Relative humidity
Ra	Relative absorbance
TCO_2	Total CO_2
Tg	Glass transition temperature
UV	Ultraviolet ray
VC-VAc	Vinyl chloride- vinyl acetate
VOCs	Volatile organic compounds
2-45	Di-ethylene glycol di-benzoate/ benzoflex 2-45
ν_{as}	Asymmetric stretching vibrations
ν_s	Symmetric stretching vibrations
δ_{as}	Asymmetric deformation vibration
δ_s	Symmetric deformation vibration
γ_r	Rocking vibration

CHAPTER 1 INTRODUCTION

1.1 Statement of the problem

Polyvinyl chloride (PVC) is one of the most important thermoplastics and has significantly influenced the industrial, domestic, and cultural aspects of modern life for several decades. Its advances in technology are illustrated by the huge range of information storage media, medical applications, transport uses, building and construction materials, toys, consumer goods, packaging (Titow, 1984). PVC has replaced the limited stocks of natural rubber and traditional construction materials such as clay and wood.

PVC is a hard and brittle polymer at room temperature. Commercial PVC is modified by various additives which include stabilizers, plasticizers, lubricants and pigments. The presence of additives improves the processing and utilization properties (Schwartz and Doogman, 1982). However, with the wide usage of PVC types, many problems have still arisen. One of these problems is that plasticized PVC may be degraded. The degradation of the vinyl polymer is characterized by discoloration, embrittlement, cracking, release of VOCs which cause indoor air pollution (Horsley, 1957). Biodegradation is an important aspect of plasticized PVC alteration. Micro-organisms may attack the entire PVC polymer or they may attack only one component of the composite. Occasionally, the physical properties do not change. Nevertheless, even a slight surface growth of fungi can make PVC look unattractive by the appearance of unwanted pigmentation. These factors have caused arguments about the potential application of polymeric materials (Mellan, 1996).

Fungi are very robust and under proper conditions, fungi can survive and reproduce, even in damp inorganic materials (e.g. glass, painted surfaces, and bare

concrete) if simple organic nutrients are available. Therefore, PVC components may be susceptible to fungal infestation. Therefore, the impact of fungi development in the presence of PVC material can not be ignored (Moore-Landecker, 1982).

While PVC is widely used by humans, people are paying more attention to lignin, a natural polymer which has only very few applications. Lignin is an essential component of plants. It occupies 20% to 30% of the weight of trees (Dean, 1989). It is well known that lignin is the most resistant natural polymer to microorganisms. It is found that white-rot fungi are the only organisms able to mineralize lignin efficiently to carbon dioxide and water through processes initially catalyzed by extracellular enzymes (Hatakka, 2001). Furthermore, lignin is an inexpensive by-product of The pulp and paper industry, usually used as fuel. Due to these characteristics, lignin is being considered as a composite to PVC or other polymers in order to increase the resistance of plasticized PVC to fungi or to decrease the cost.

Actually, a number of studies have been carried out in the past on the microbial degradation of PVC (Whitney, 1996) and lignin (Hatakka, 2001); however, no study has been carried out concerning the resistance of the PVC-lignin blends with various plasticizers. How lignin affect the resistance of plasticized PVC-lignin blends to fungi attack, what happens on the polymer after fungi attack, and which fungi play a more important role in the biodegradation of the PVC-lignin blends. All these problems need accurate answers. Clearly, studies on the resistance or biodegradability of the PVC-lignin blends are important in developing a new utilization of lignin.

1.2 Objectives of thesis

The present research aims to determine the resistance to fungi attack of plasticized PVC-lignin blends developed for flooring formulations. The final purpose

is to gain knowledge related to the effect of lignin on the resistance of the PVC-lignin blend. The main objectives of the research are to evaluate the effect of fungi growth on plasticized PVC-lignin blends, to determine the resistance of Alcell lignin, and to choose a suitable plasticizer for these particular formulations which has stronger resistance to fungi than the others. In response to the above problems, the present research was done based on observation of the visible effects, analysis of the changes of chemical structures, before and after the plasticized PVC and PVC-lignin blends are attacked by fungi. In order to reach the major objectives, several minor goals were set. These were:

1. to detect the rate of fungi growth on PVC formulations with or without lignin;
2. to observe the effects of different plasticizers on the resistance of materials to fungi attack;
3. to observe the changes in appearances of these materials;
4. to measure the effect of five mixed fungi on the mechanical properties of plasticized PVC without lignin and with lignin, and to compare the change of the mechanical properties of PVC controls and of PVC-lignin blends formulated with different plasticizers;
5. to analyze the effect of five mixed fungi on the chemical structures of plasticized PVC composites with and without lignin: comparing these two groups of data to confirm the influence of lignin on the resistance to biodegradation;
6. to observe the ability of bio-attack of individual fungus to plasticized PVC with and without lignin;

7. to detect the resistance of Alcell lignin to biodegradation used in this research.

The scope of the research is presented schematically in Figure 1.1.

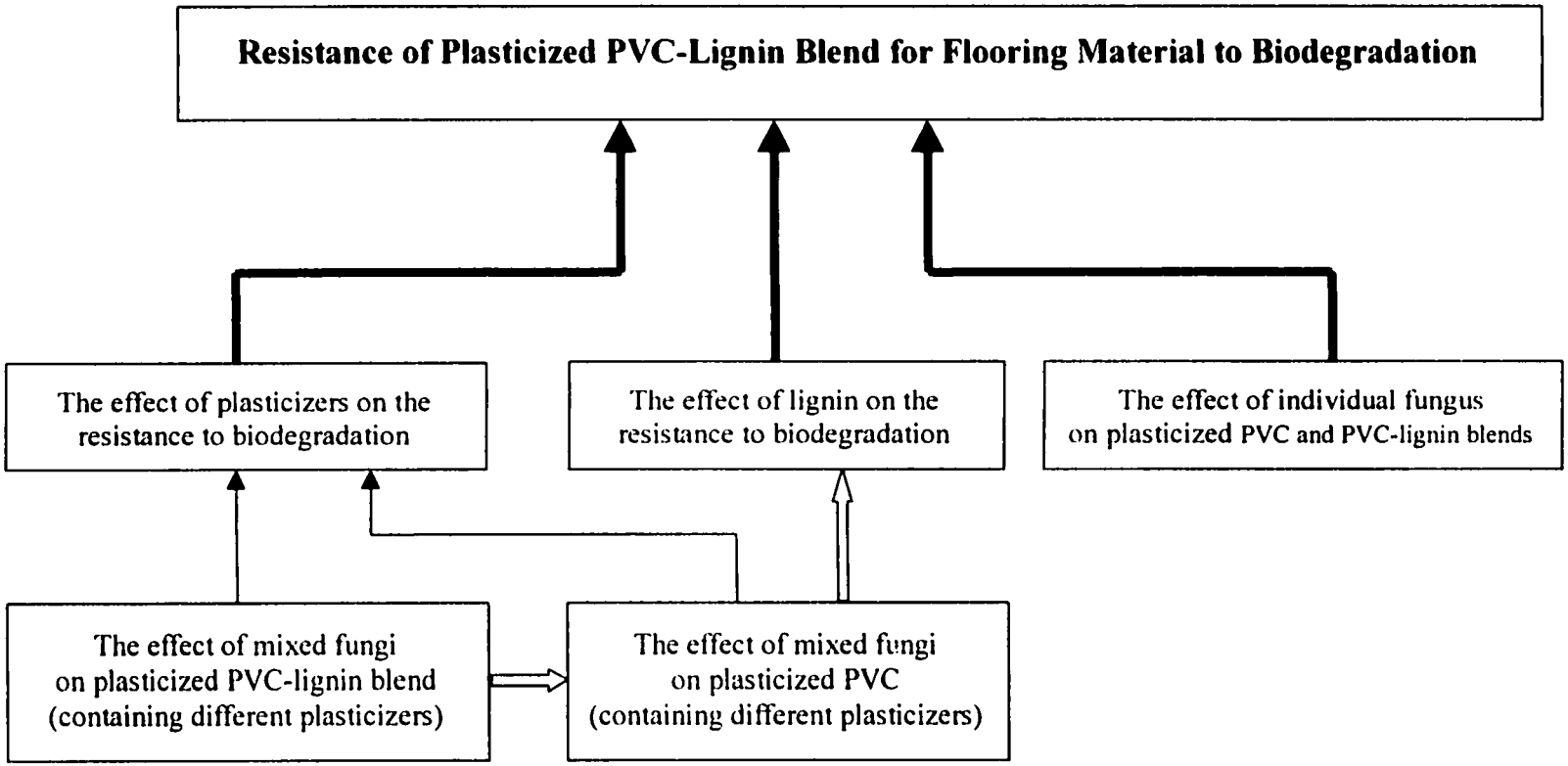


Figure 1.1 Scope of the research

1.3 Organization of thesis

This thesis is organized into five. Chapter 1 describes a series of problems that are related to this research and do not have yet accurate answers. The objectives of the thesis and the scope of the research are also described in this chapter. Chapter 2 covers a literature review of this research in which an introduction related to fungi, PVC, PVC additives, PVC flooring, lignin, and their biodegradation has been dealt with. In Chapter 3, the experimental methodology, experimental procedures, the preparation of test specimens, testing instruments, materials, and all detailed testing are described. This is followed by the experimental results and discussion in Chapter 4. Finally, the conclusions derived from the research are emphasized in Chapter 5. In addition, recommendations for further research are also presented.

1.4 Contribution

Lignin is one of the most abundant organic materials on the Earth making up a quarter to a third of the dry weight of wood. Lignin is removed from wood pulp in the manufacturing of paper. Vast amounts of lignin derivatives from the pulp and paper industry are created every year. Typically, lignin is used as an energy source. However, it is an ideal natural polymer that is renewable and much less expensive than PVC. For example, the price of organosolv lignin is $0.20 Cdn. per kg, while the price of Oxy 1810, a type of PVC used in this research, is $ 2.93 Cdn. Per kg (Zhu, 2000). Therefore, these characteristics make lignin a potential substitute of PVC. This experiment quantified the effect of lignin on the resistance of materials to biodegradation. Based on the results of the experiment, the feasibility of lignin used

as a PVC substitute can be estimated. This research provides a new utilization with experimental proof.

In addition to the contribution mentioned above, this study also quantifies the susceptibility of different plasticizers in PVC-lignin blends to fungi. It will help us to understand the characteristics of the plasticizers and it is important for determining the PVC-lignin blend formulations.

CHAPTER 2 LITERATURE REVIEW

2.1 Fungi

2.1.1 Introduction

Based on the available fossil records, fungi are assumed to have been present approximately one billion years ago. At present, about 70,000 species of fungi have been described; however, some estimates suggest that 1.5 million species may exist (Hawksworth et al., 1995). In fact, fungi are all around us and in some cases are the dominant organisms.

Fungi primarily fall into three groups according to appearance: yeast, moulds, and mushrooms. Most fungi appear to be terrestrial in origin; but all major groups have occupied marine and freshwater habitats. Most fungi are nonmotile.

Fungi constitute an independent group of natural organisms. They are carbon heterotrophs due to their lack chlorophyll. Therefore, they require external organic compounds as carbon sources. In order to digest the organic compounds, fungi produce hydrolytic enzymes to break down these nutrients. Fungi secrete digestive enzymes from their cells into the immediate environment where nutrients are simplified by these enzymes. Simplified nutrients pass into the fungal cell as a watery solution. Since they do not use light for their metabolic process, fungi can live in damp and dark places and some are anaerobic (Moore-Landecker, 1982).

2.1.2 Morphology

The majority of fungi are filamentous. As fungi grow, they produce an intertwined mass of threads that branch freely and often fuse together. The individual

tubular thread is called hyphae, while the body of the organism, called a mycelium, consists of a mass of threads. Fungi have a special hypha which is able to reproduce. The reproductive hypha is produced solely for the release of spores, and it is not the living and growing portion of the fungus. The surface-to-volume ratio of a fungus is very high thereby ensuring the excellent contact with its environment. Another specialized hyphae, known as rhizoids, fix fungus into the substrate (Esser and Kuenen, 1967).

2.1.3 Reproduction

Reproduction is the procreation of new individuals. A spore is a typical reproductive unit of fungi, which may be derived from either sexual or nonsexual reproduction. Spores are dispersed and reproduce under suitable conditions while they encapsulate a cell during unfavourable conditions. Fungi spores may be released actively or passively. There are several effective methods for dispersal of spores such as wind, rain or animals. Since many types of spores are air dispersed, fungal spores fill the air we breathe (Moore-Landecker, 1982).

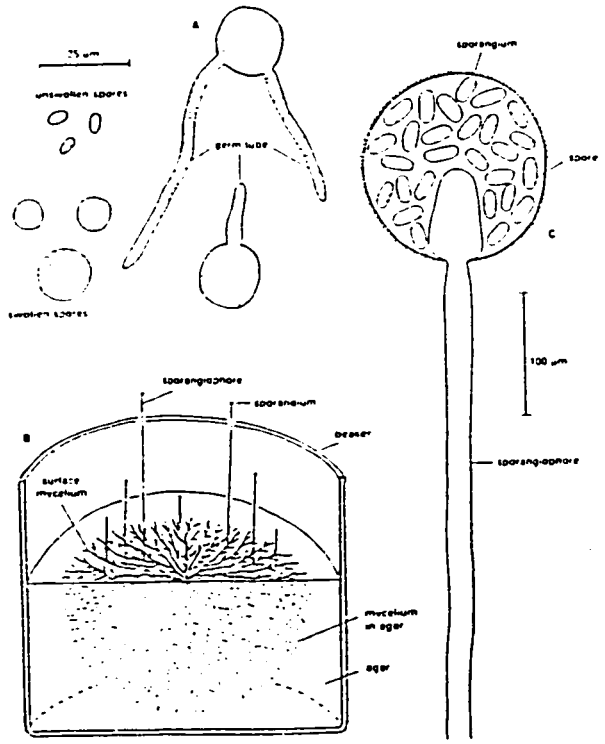
2.1.4 Fungi growth and requirements

2.1.4.1 Fungi growth

After a fungal spore is inoculated on a nutrient agar at approximately 20°C, the complete growth stages can be observed. During the first few hours, a spore swells considerably and one or more germ tubes grow out as short hyphae. Then these short hyphae soon branch and form the initial mycelium. After several days, a colony is produced. The colony of a fungus is hemispherical when the fungus grows in a deep

agar layer. Figure 2.1 shows a colony of a fungus and its structure (Ingold, 1984).

In nature, wherever adequate moisture, temperature, and organic substrates are available, fungi are present. Although fungi prefer to grow in warm and moist conditions, many species proliferate in cold, dry, or other inhospitable environments. It is important to recognize that optimum conditions for growth and reproduction vary widely with different species of fungi. Actually, many factors influence the growth of fungi such as: a) nutrition, b) oxygen, c) temperature, d) pH, and e) light. Many experiments demonstrate that nutritional and environmental requirements for sporulation usually are more restricted than those for vegetative growth. Moreover, the optimum conditions for sporulation and vegetative growth of different fungi are dissimilar (Ingold, 1984).



A: Spore germination B: A colony of fungi lives in a beaker
C: Single sporangiophore with spherical sporangium containing spores

Figure 2.1 A fungus colony structure (Ingold, 1984)

2.1.4.2 Requirements for fungi growth

The requirements for growth and sporulation of fungi are discussed below.

Nutrition:

- Water: Fungi can grow only in a watery solution or on aqueous agar or in high humidity air. Only nutrients in solution can be absorbed by a hypha (Ingold, 1984).
- Carbon sources
- Nitrogen
- Phosphorus
- Iron
- Potassium
- Magnesium
- Vitamins

Almost all fungi do not need calcium. Furthermore, some fungi have special requirements at certain stages or under certain conditions. For example, high concentration nutrients are usually favorable for their vegetative growth, while they are unfavorable for reproduction of fungi. Some experiments supported that fungi often sporulate after the nutrients have been exhausted by vegetative myceliums (Timnick, *et al.*, 1952, mentioned by Moore-Landecker, 1982).

Oxygen Supply

It has been widely recognized that nearly all fungi are strictly aerobic and require at least some free molecular oxygen. If there is completely devoid of oxygen in the atmosphere, growth of fungi will cease. Some fungi can grow and sporulate in the absence of measurable oxygen because these fungi can use combined oxygen instead

of free molecular oxygen (Smith and Onions, 1994).

Temperature

Temperature affects enzyme activity and the chemical activity of fungi. Therefore, temperature is a very important factor that influences the amount and growth rate. Generally, the rate for growth increases with an increase in temperature until the optimum is reached. Below the minimum temperature, fungi cannot grow. The optimum temperature range of most fungi is 22-27°C. However, this varies from species to species. Some fungi, for example *Mucor pusillus*, can still grow at 50°C (Ingold, 1984). Moreover, the optimum temperature for growth and reproduction are different.

pH

pH can affect the availability of certain ions which are absorbed as nutrients because some ions become insoluble at certain pH ranges. Another effect of pH is to alter the cell permeability. At low pH the protoplasmic membrane is saturated with hydrogen ions that limits the entrance of cations, while at high pH the protoplasmic membrane is saturated with hydroxide ions that limits the entrance of anions. In addition, pH also can affect the uptake of vitamins. Furthermore, external pH also affects the enzyme activity. For most fungi, the optimum pH is in the range of 5-6.5. Few fungi grow below pH 3 or above pH 9. The pH is rarely constant during growth because metabolism of fungi produces acidic or alkaline metabolites (Ingold, 1984).

Light

Light is also an important factor for fungi growth. Although the vegetative growth of most fungi is not sensitive to light, the reproduction of fungi is affected by light.

The effects are extremely complex. Different species or different isolates of the same fungus may show a different requirement of light. Also, each developmental stage may have a varying requirement of light. Some fungi can not sporulate in the dark and strong light may inhibit growth of fungi because it destroys required vitamins (Barnett, 1968; Robbins and Hervey, 1960, mentioned by Moore-Landecker, 1982).

Fungi show five different responses to light. Some fungi are evidently unconcerned to light. Some prefer less light at sporulation stage than growth stage. Some fungi repetitive require alternating light and darkness at sporulation stage while they have no special necessity of light at growth stage. Some fungi repetitive produce little spores in complete darkness while they produce more spores with enough light. Furthermore, some fungi could not sporulate without light (Moore-Landecker, 1982).

2.1.5 Common indoor fungi

Since this research focuses on PVC flooring, the characteristics of indoor fungi are of primary concern. It is well known that the most common mode of spore dispersal is air currents. By riding air, fungi can spray for a long distance. Therefore, indoor air contains plenty of fungi spores. In fact, fungal spores fill the air we breathe. The largest number of fungal spores ever sampled was over 5.5 million per cubic foot in Wales (Matthews, 1994). When conditions are proper, fungi will grow and multiply. Consequently, the indoor-air is contaminated. In the last 10 years, micro-organisms were the primary source of indoor air contamination in as many as 50% of indoor air quality (IAQ) cases (Lewis, 1994).

The species and concentrations of indoor fungi are strongly influenced by outdoor fungi and moisture. Usually, most indoor fungi originate from external sources which include plants, carbon-based products, building materials, and wood products. The

common locations in buildings for the majority of fungi growths include appliance drain pans, grain and meat food products, paper products, carpets, ceiling tile, air conditioning ductwork, plants, and soil used to pot plants.

Aspergillus and *Penicillium* can grow and reproduce effectively indoors and are commonly found in air samples of normal buildings. Likewise, *Chaetomium* and *Gliocladium* also are common indoor fungi (http://www.aehf.com/articles/moldlist.htm).

2.1.6 Fungi, humans, and nature

Fungi have a close relationship with humans. Humans, for a variety of purposes, have utilized fungi since ancient times. Some fungi are food sources, (e.g. mushrooms) and are good sources of nutrients. Besides being a direct source of food, fungi are also used in the processing of many foods. For example, during the fermentation of bread and cheese, various fungi are used. In addition, fungi are usually used in industrial production. Many metabolites of fungi have commercial value. These metabolites are separated from the substrates and used in many fields of our lives. These products involve organic acids, alcohol, antibiotics, pigments, vitamins, and enzymes. In spite of the fact that fungi have many benefits, they also cause many human, plant, and animal diseases.

Fungi play an important role in the natural cycle since they have to decompose organic compounds as their nutrients. One important method of taking nutrients is to live on dead organic matter and decay the dead organic matter to obtain nutritional supplements. In nature, the green plants convert inorganic chemicals of carbon to organic compounds. On the other hand, fungi or other organisms mineralize organic chemicals to fulfill the natural carbon cycle. Other elements, for example, nitrogen,

sulfur, phosphorus, are also cycled by the same approach. These cycles have special significances in the maintenance of the ecosystem's equilibrium. Fungi break down wastes from dead organic matter, thereby enabling the constituent materials to be available for reuse by other organisms within the ecosystem. Unfortunately, results of the breakdown can be both positive and negative, depending on the consequences. For example, saprophytic fungi cause food spoilage, ruin fabrics, destruct wood and wood products, etc. It is very clear that all of natural products encounter fungi. Furthermore, some synthetic materials including certain plastics and paints are also attacked by fungi (Moore-Landecker, 1982).

2.1.7 Fungi employed in present experiments

Aspergillus:

Aspergillus is the most abundant and common fungus that is found in soil and in air. The *Aspergilli* are capable of utilizing an enormous variety of substrates. The colonies of *Aspergillus* are white. Conidia are black and the reverse is colorless to pale yellow (Stevens and Ammirati, 1981).

Penicillium:

Like *Aspergillus*, *Penicillium* is also an abundant fungus species that is commonly found in soil, air and plant materials. It grows readily on many foods. The colonies are rapid growing, flat, cottony, initially white. Mature colonies become blue green in colour (Stevens and Ammirati, 1981).

Chaetomium:

Chaetomium is usually found in the soil, air, and plant debris. It grows well on damp paper and fabric. The colonies are rapidly growing, cottony and white in color initially. Mature colonies become gray in colour and the reverse is tan to red or brown

to black (Iarone, 1995).

Gliocladium:

Gliocladium is widespread and is commonly found in soil and plant debris. Colonies are usually rapid growing, spreading, cottony, and white to dark green with a colorless reserve (Stevens and Ammirati, 1981).

Aureobasidium:

Aureobasidiumgrows rapidly is most common mildew on painted surfaces and is frequently found in leaf surfaces and decayed fruits. It grows medium rapidly. The colonies are flat, smooth, moist, shine and leathery in appearance. Initially, the colonies are white or pale pink and then become black. The reserve is pale or black (Stevens and Ammirati, 1981; Larone, 1995).

2.2 Plasticized PVC

2.2.1 PVC resin

Polyvinyl chloride (PVC) is one of the most widely used polymers in the world. It is a chlorinated vinyl polymer that is obtained from the vinyl chloride monomer (VCM). The manufacturing process consists of two steps. Firstly, through a chemical reaction, ethylene and chlorine or ethyne and hydrogen chloride react to form vinyl chloride. Vinyl chloride is then transformed into a gas known as vinyl chloride monomer. The next step, called polymerization, converts the monomer into vinyl polymer, the number of repeat units in the molecular chain, n, ranges between 700 and 1500 (EHC 215, 1999); this corresponds to a theoretical molecular weight range of 40,000-90,000 (number average) or 70,000-500,000 (weight average) (Wilson, Alan S., 1995). The chemical structure of PVC is presented in Figure 2.2

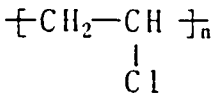


Figure 2.2 The chemical structure of poly (vinyl chloride)

PVC is a generic name. In practice, producers make a range of PVC polymers, which vary in morphology and in molecular mass. PVC molecular weight, particle size and particle size distribution are the parameters which influence the physical-mechanical properties of the finished product and its compatibility with plasticizers. Vinyl chloride polymer includes two types: homopolymer and copolymer. Homopolymer is the most abundant PVC polymer that consists of a single type of repeating unit. Copolymer, produced by the polymerization of two or more different monomers, shows a good processing property. The most important comonomer used with vinyl chloride is vinyl acetate. The chemical structure is illustrated in Figure 2.3. The presence of vinyl acetate reduces the processing temperature so that it avoids PVC thermal degradation (Miles and Briston, 1979).

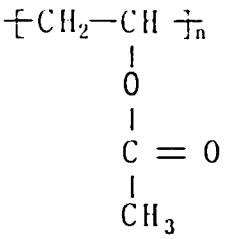


Figure 2.3 The chemical structure of poly (vinyl acetate)

Structurally, on every other carbon in the backbone chain, one of the hydrogen atoms is replaced with a chlorine atom and exhibits a head-to-tail model; namely, -CH_2-CHCl-CH_2-CHCl- (Titow, 1984). Chlorine gives vinyl two advantages. First, chlorine is an inexpensive chemical. Second, chlorine has excellent inherent flame

retardant properties. These properties make PVC an excellent choice for applications which require high resistance to ignition and flame spread. One of the most important uses is as a replacement for rubber wire insulation. Since 1950s, the PVC industry has grown steadily. Continuing development of new additives and novel compounding are driving the substitution of PVC for traditional materials such as metals and wood. Table 2.1 lists the categories of PVC utilization. Nearly 80 years have passed since the vinyl industry was invented (Fenichell, 1996) and PVC has been one of the most important synthetic materials. The total demand of PVC in North American was 15.278 billion tons in the year 2000. This demand is estimated to reach 17.8 billion tons by the year 2004. The annually growth rate, from 1995-2000, is 6.8 percent, and it will be 3.9 percent, from 2001-2004 (http://www.chemexpo.com/news/PROFILE010312.cfm).

Table 2.1 Major categories of use of PVC

(http://www.chemexpo.com/news/PROFILE010312.cfm)

Usage	Percent	Usage	Percent
Construction: pipe and tubing windows and doors siding other construction, including flooring and pipe fittings	76 47 6 15 8	Consumer goods	6
Electrical fittings and wire and cable coatings	4	Transportation	2
Miscellaneous adhesives and coatings	4	Home furnishings	2
Packaging films and containers	6		

2.2.2 Compounding PVC

PVC resins are hard, brittle compounds due to the strong attraction bonds between hydrogen and chlorine atoms of adjacent polymer chains. Therefore,

unmodified PVC resins have limited commercial possibilities, and they are very sensitive to thermal and UV degradation. In order to obtain a required performance and make it easier to process, sufficient modifying components are added to the raw polymer to produce a homogeneous mixture. Depending on the modification of PVC microstructures, PVC may have a wide range of properties. The formulation is determined by the future application of the end product. Additives for polymers may be classified into the following groups:

- Plasticizers
- Stabilizers (thermal and light)
- Lubricants
- Impact modifiers
- Processing additives
- Fillers
- Pigments
- Biostabilizers

The functions and principals of each additive are listed in Table 2.2

Table 2.2 Additives for PVC polymer (Tadmor, Gogos, 1979)

Modifier	Major function	Basic principle
Plasticizers	Softening PVC, provide flexibility to the formula	Separating PVC chains
Stabilizers	Minimizing or eliminate degrading effects of heat, light or oxygen on PVC	Reacting with degradation Product (HCl)
Lubricants	Preventing adhesion of compound to processing equipment	Sweating out to form a film between PVC and equipment
Impact modifiers	Reducing brittleness	Inhibiting crack develop
Processing additives	Ensuring uniform flow and good surface finish	Affecting melt viscosity of polymer
Fillers	Opacity compound Increasing hardness Reducing cost	Changing refractive index and reflective properties Adding bulk to compound
Pigments	Giving vinyl a range of colors	Soluble in PVC and dispersed at a molecular level
Biostabilizers	Preventing or inhibit the growth of micro-organisms on the surface of the PVC	Giving toxicity to micro-organism

2.2.3 Plasticizers

A plasticizer is generally defined as a substance incorporated into a material that changes the physical and chemical properties of the material. Plasticizers of PVC are typically clear, high boiling organic liquids that make the plastic soft and flexible by maintaining a low glass transition temperature (Brydson, 1982). Usually, plasticizers are low or medium molecular weight materials. They are essentially non-volatile solvents, with solubility parameters close to that of the polymer.

Plasticizers are the major additives for PVC formulations. The useful proportion of plasticizer contained in PVC ranges in 20 p.h.r. ~ 100 p.h.r. (Wilson, 1995). Therefore, plasticizers have the greatest influence on the properties and behaviour of the compounded PVC.

Plasticizers fall into two categories based on their compatibility with the resin; namely, primary plasticizers and secondary plasticizers. Primary plasticizers are added for end use plasticization, and secondary plasticizers are used for processing. Secondary plasticizers are typically aromatics that are used to aid in the manufacturing of some PVC products. There are many different types of primary plasticizers used in PVC, such as phthalates esters, adipate esters, trimellitate esters, phosphate esters, polyester plasticizers, and sulfonate esters. Among them, phthalates are the most common plasticizers in PVC productions (Wilson, 1995).

2.2.4 Plasticization

2.2.4.1 Mechanism of plasticization

The process realized by plasticizers incorporated into PVC resins is called plasticization. The plasticizer is not bound chemically to polymers. It is considered as a weakening or selective breaking of some intermolecular bonds between the macromolecules of the PVC polymer. The purpose of the process is to increase this intermolecular space, known as a free volume that allows room for changes in shape and degree of flexing of the final material. Two basic parameters of plasticizers influence plasticization; namely, compatibility and efficiency. Specifically, they affect the stability of the plasticizer-polymer blend and the degree of modification achieved, respectively.

The process of plasticization usually is divided into four stages (Matthews, 1982; Brydson, 1982):

1. The plasticizer is mechanically mixed with the PVC resins and wets the surfaces of the PVC resins;
2. The plasticizer penetrates into the PVC resins and physically distribute itself

throughout the PVC macromolecule;

3. Plasticizer molecules attach themselves to the surfaces of the resin particles by physisorption. PVC resin chains are separated as the dipole-dipole interaction between the polymer chains is broken;
4. The structure of the PVC resin is reformed and the new structure contains the plasticizer.

The model of plasticization exhibits two types shown in Figure 2.4 (Wilson, 1995). As illustrated in Figure 2.4, for example, phthalates, which consist of a polar ester group and a linear group, ester groups and benzene group bond between the hydrogen-chlorine interacting atoms of the PVC adjacent macromolecules, while the linear group acts as a buffer between the polymer chains. As the linear group separates the polymer chains, the attraction between the hydrogen and chlorine bonds between adjacent chains is reduced. As a result, the flexibility of the overall material is increased.

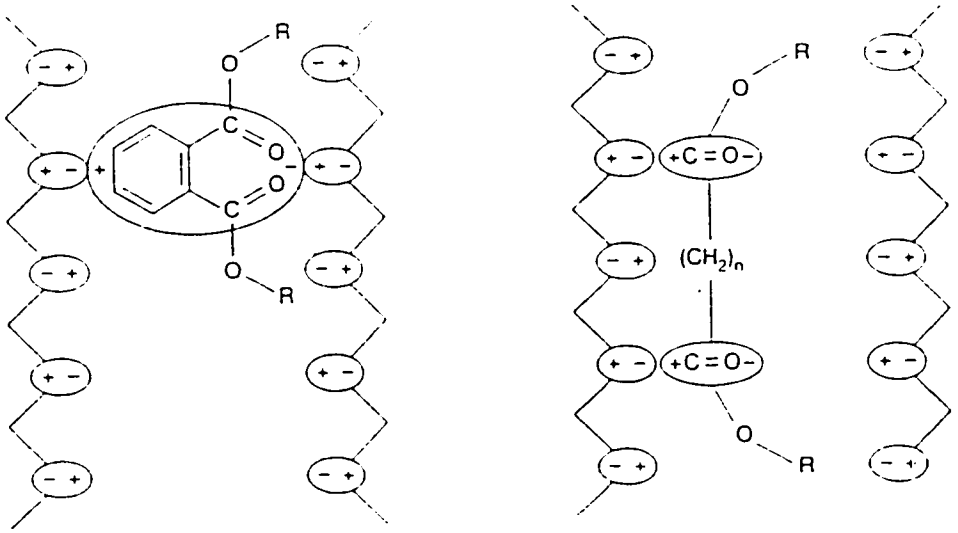


Figure 2.4 Leuchs model of plasticization (Wilson, 1995)

2.2.4.2 Compatibility of plasticizer with PVC

Compatibility, as Boyer viewed (mentioned by Mellan, 1961), is the governing quantity of plasticizer that can be added to a polymer before phase separation. Compatibility also identifies the stability of the plasticized polymer (Wilson, 1995). The compatibility of plasticizer depends on the properties of both plasticizers and polymer. The molecular mass, size, and shape of plasticizer greatly influences the compatibility. A plasticizer with molecular mass above 800 (except polyester or polymer) suggests a low compatibility. In addition, polarity of plasticizer is another important factor that influences the compatibility. As pointed out by Mead (reviewed by Mellan, 1961), any plasticizer chose to incorporate into PVC must contain a polar group or a polarizable group so that it can interact with the polar polymer molecules. Also, temperature is a concern for those plasticizers with distinct melting points. Furthermore, pressures added on the surface of plasticized PVC and ambient humidity also affect the compatibility of plasticized PVC. Generally, the compatibility of a plasticizer depends on the plasticization process, environment, characteristics of the polymer and plasticizer. It is not solely influenced by any individual factor but influenced by the result of their specific combination (Wilson, 1995).

2.2.5 Degradation of plasticized PVC

Degradation of a polymeric material is the decomposition of the chemical structure, resulting in an alteration of the physical properties. The ability to resist degradation is determined by the formulation, manufacture process, and the environment of use. Changes in any one of the components of the formulation, that is polymer, plasticizer or one of the other modifiers, will affect the whole composition

and may influence the lifetime as a whole. Degraded PVC manifests as discoloration, embrittlement, cracking, increased tackiness, and loss of physical properties. It is well known that degradation of PVC is a complex phenomenon resulting from a combination of chemical, physical, and microbiological causes. The polymer and its additives may all participate in the degradation. To some extent, they influence each other. For example, the oxidation of plasticizer makes it form a peroxy free radical which could initiate dehydrochlorination of PVC. Generally, degradation of plasticized PVC involves: volatilization, extraction, migration, thermo-oxidation, photodegradation, chemical degradation, and biodegradation. A detailed discussion about their approaches is introduced below.

2.2.5.1 Volatilization, extraction and migration

One important indication of degradation is the loss of plasticizer which is manifested as a migration of the plasticizer from the bulk phase to the surface layer. The effects of plasticizer loss on the physical properties of PVC are a loss in total weight, a slight reduction in sheet thickness, an increase of elastic modulus, an increase in tensile strength, and a reduction in ultimate strain and flexibility. The plasticizer loss is influenced by plasticizer type, temperature, sheet thickness, environmental conditions and exposure time (Mellan, 1961). The loss of plasticizer develops quickly when there is a large concentration gradient of the plasticizer between the PVC bulk phase and the PVC surface layer. Here the gradient must have sufficient energy to overcome the bonding of the ester group of the plasticizer, allowing the linear group to separate the chains of PVC and provide migration paths between the chains. The most common processes that a cause concentration gradient of plasticizer are volatilization, extraction, and migration.

Volatility

Volatility is the evaporation of a plasticizer from the PVC into the surrounding air. Since molecules of plasticizer are not chemically bound to the polymer, they move from their original position quite readily even at low. Two mechanisms controlling the rate of volatility are diffusion to the surface and evaporation from the surface. Under different conditions, either one of these can be the rate-determining step. If the rate of diffusion in the bulk is faster than that of evaporation, the total rate is dominated by the rate of evaporation. On the contrary, if the rate of evaporation is faster than that of diffusion, the total rate will be governed by the rate of diffusion (Gumargalieva, *et al.*, 1998). For most normal uses of plasticized PVC, the rate of evaporation determines the speed and the extent of plasticizer loss.

Extraction

Extraction is a process where a plasticizer is transferred from the PVC bulk phase into a liquid/polymer interface. The liquid includes organic liquid and water (or water and surfactants). Plasticizers are deemed to be completely miscible with most organic liquids, so the factor governing the extraction is the rate of migration to the interface.

The loss of Plasticizers can also occur when they are exposed to high levels of atmospheric moisture and water. Most plasticizers for PVC are hydrophobic and have low solubility in water (between 0.1-1.0 percent). However, water molecules can displace plasticizer molecules from the polymer. The displaced plasticizer forms an oily film on the surface. Usually, this kind of extraction is controlled by the rate of dispersion from interface to water. However, if surfactants are present in the water, they disperse and remove the plasticizer film, encouraging further extraction. With very high concentrations of surfactant, removal from the interface may become faster than diffusion through the PVC. In this case, the process becomes diffusion controlled

(Mellan, 1961).

Migration

When a polymer contacts any absorbent material, including surface coatings, structural components, and adhesives, plasticizers are removed from the PVC. It is called migration. The process is considered to be dependent upon the rate of diffusion of the plasticizer from the polymer into adjacent materials (Mellan, 1961).

2.2.5.2 Thermo-oxidation and photodegradation

When plasticized PVC encounters heat with oxygen present, thermo-oxidation occurs. It manifests as colour development, hydrogen chloride release, and deterioration in physical properties. Polymers may or may not be involved in the chemical reaction. The thermo-oxidation of plasticizer may initiate the change of the polymer microstructure. The favourite site of oxygen attack is on alkyl groups in the plasticizer molecule, especially in the branching point of the carbon chain because of the relative ease of removal of the attached tertiary hydrogen. When the hydrogen is removed, at that point a peroxy free radical is formed. The peroxy free radical can then take away chlorine atoms from the PVC chain thereby initiating the dehydrochlorination of PVC. Under a normal service condition, thermo-oxidation will not influence the stability of the PVC composition (Miller, 1981).

Photodegradation is similar to the thermo-oxidative degradation, but the initial energy is light. Plasticizers absorb the shortest wavelength of radiation; as a result, the bonds of plasticizers break and free radicals are formed. The result is the same as that of thermo-oxidation, namely the dehydrochlorination of PVC. Consequently, the physical properties of PVC are deteriorated and the compatibility of the plasticizer with PVC is decreased (Wilson, 1995).

2.2.5.3 Chemical degradation

Chemical instabilities of plasticizers manifest mainly as hydrolysis and oxidation. Plasticizers usually are esters which are susceptible to hydrolysis under strongly acidic or alkaline environments. The susceptibility of different esters to hydrolysis typically follows the order shown below:

adipates> linear phthalates > branched phthalates > triaryl phosphates (Wilson, 1995).

2.2.5.4 Biodegradation

Microbiological attack is an important mode that causes the degradation of PVC. PVC resin is usually highly resistant to microbial growth (Summer, at al., mentioned by Pankhurst *et al.*, 1968). Early work done by Potts (reviewed by Swift, 1990) indicated that high molecular weight polymers did not support fungal growth. The first reason may be the hydrophobic nature of the polymer. In addition, synthetic polymers contain chemical bonds that do not occur or are uncommon in nature, and so perhaps micro-organisms have not yet evolved the appropriate enzymes to initiate the breakdown of synthetic polymers. However, plasticized PVC is susceptible to biodegradation because of the presence of additives which are organic compounds with relatively low molecular weights. The susceptibility of PVC formulations to microbial attack is related to the amount and types of these additives. The presence of a plasticizer is the main cause of biodegradation of PVC. Moreover, Mellan (1961) stated that the combination of plasticizers with a PVC resin is more susceptible than either individually.

It is difficult to determine the chemical processes involved in the decomposition of plasticized PVC in nature. It is considered that these procedures involve digestive enzymes. The basic principle of biodegradation is that micro-organisms utilize a

plasticizer as a substratum to grow on the surface and decompose the plasticizers and PVC. The first step being that the micro-organisms exist at the surface of PVC and they produce enzymes that diffuse into the pores. The enzymes destroy specific groups of plasticizers, and the digested products diffuse back to the surface where they are metabolized (Cole, 1990). Structurally, for biodegradation, the ester groups of plasticizers normally provide the weak link for initiation of breakdown (Wilson, 1995).

Many micro-organisms such as fungi, bacteria, and alga can attack plasticized PVC. Fungi are the most effective because they can produce esterase, which is capable of breaking ester linkages. Consequently, both fungi and bacteria are able to utilize the fragment molecules of plasticizers as substrate. Fungi attack can raise the rate of plasticizer loss because they effectively remove plasticizer from the surface of the material. In this situation, the rate of plasticizer loss is controlled by the rate of diffusion (Zaikov, 1998). The susceptibility of different plasticizers to biodegradation is the same as that of chemical degradation.

Besides the effect of plasticizer on the biodegradation of PVC, the biodegradability of PVC copolymer is influenced by the comonomer vinyl acetate. Experiments have proved that poly vinyl acetate is a soluble biodegradable polymer (http://www.age.psu.edu/extension/factsheets/c/C17.pdf). A soil experiment showed that the poly vinyl acetate degrades slowly over 12 to 18 months. The high humidity and high organic content can accelerate the rate of degradation of poly vinyl acetate (http://www.kiwipower.com/QEI_Atlas_article.pdf). However, because the amount of vinyl acetate in the copolymer is low and the molecular weight of PVC copolymer is very high, the VC-VAc compound still has a high resistance to biodegradation.

2.3 PVC flooring

PVC flooring has long been the most popular surface flooring in the United States. In general, there are two types of vinyl flooring: sheet flooring and tile. They are available in any styles and colours. PVC flooring is so popular because it has some outstanding characteristics, for example, resiliency, durability, easy of installation and maintain, low cost, and a high moisture-resistance. The other advantages of PVC flooring include noise reduction and a high degree of comfort when. Moreover, most PVC flooring has fire-resistant characteristics. It can resist burning and typically does not continue burning when an external flame is removed. Recently, new technologies have improved PVC's performance, especially in the areas of durability against rips, tears and gouges. In general, every flooring formulation is different. Typically, PVC flooring contains PVC resin, fillers, plasticizers, stabilizers, and pigments. In this study, lignin was added to replace part of the polymer.

2.4 Lignin

2.4.1 Introduction

The lignin molecule is a highly complex polymer that forms an extensive network within the cell walls of plants. As an essential component of plants, lignin gives them rigidity, water-impermeability, and resistance against microbial decay (Moore-Landecker, 1982). There are many types of lignins, with different types occurring in different species of plants or even coexisting in the same plant. However, almost all lignin found in nature is made from three monomers, which are phenyl propane units substituted with one or two methoxyl groups. Their structures are shown in Figure 2.5 (Feldman and Banu, 1997). These basic units are naturally

bonded together by random combination reactions. Approximately, 40-60% of all inter-unit linkages in lignin are by ether bond, and the rest bonded by carbon-carbon bonds. It is very difficult to determine the exact chemical structures of lignin; moreover, some new structures are detected continually (Hatakka, 2001)

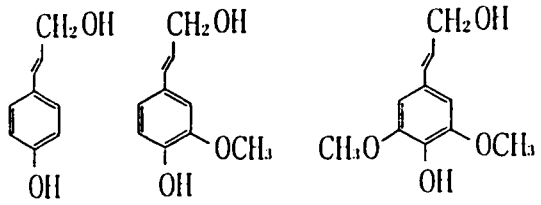


Figure 2.5 The structures of the lignin monomers

Usually, lignin is stable below 200°C. When temperature is a little above 200 °C, carbonization begins, and when the temperature reaches 300 °C, the structure of lignin has changes slightly. When temperature is up to 460 °C, lignin macromolecules encounter depolymerization, decarboxylation, dehydration. As a result, low molecular weight compounds are formed (Zhu, 2000). Usually, lignin has a strong resistance to biodegradation (Hatakka, 2001).

Lignin can be used as a corrosion inhibitor, filler in certain plastics, adhesives, coatings, or fertilizers. Intensive efforts have been underway for twenty years to incorporate lignin into plastics. However, lignin is still used as fuel. Recently, data show that of the 50 million tons of lignin produced annually in the world, only 3 million tons of lignosulfonates and 100,000 tons of alkali lignin are used for purposes other than fuel (Faix, 1992). Therefore, it is necessary to develop new methods to utilize lignin.

2.4.2 Delignification

In plants, lignin is bonded together with cellulose and hemicellulose. It can't be obtained without isolation from cellulose and the other polysacharides. Lignin is a sticky, dark, water-resistant, brittle sap that causes many difficulties in paper

processing. In order to gain paper with high quality, most of the lignin must be removed from the pulp. Therefore, a vast amount of industrial lignin derivatives from pulp and paper industries result as a by-product. Various delignification processes can be used to remove and isolate lignin from pulp. The properties of these industrial lignin and their functions, for example, molecular weight, functional groups, and molecular linkages are different. There are four important factors which influence the properties of lignin: the source (woody plant) of lignin, the processes used to remove lignin from the plant, processes used to purify the lignin and nature of the chemical modification of the lignin after isolation. The characteristics of industrial lignins are different from the native lignin. At present, the main delignification processes include (Zhu, 2000):

- Kraft Pulping (alkaline process)
- Sulphite or acid pulping
- Organosolv pulping and steaming

These processes produce three main types of industrial lignin which are kraft lignin, lignosulfonates, and organosolv lignin. Among these processes, organosolv pulping is a relative new technology. Organosolv pulping is the common name for approaches utilizing organic solvents to isolate lignin. There are two types of organosolv pulping processes that have been used commercially, and they are: Organocell and Alcell. In this research, Alcell lignin was used, which derives from the Alcell process. In the Alcell process, hardwood chips are cooked with an ethanol and caustic soda. Lignin, hemicelluloses and other various components of wood are extracted from the chips into the ethanol forming black liquor. The black liquor is

then flashed, and the lignin is recovered by precipitation, settling, centrifugation, and drying (Lora, *et al*, 1988). Almost all the ethanol is recovered (Stockburger, 1993).

The properties of Alcell lignin are different from the Kraft lignin and lignosulfonates. Alcell lignin is highly hydrophobic and insoluble in neutral or acidic aqueous media, but soluble in moderate to strong alkaline solutions and certain organic solvents. Alcell lignin has a low molecular weight; the number average molecular weight is lower than 1000 (Lora, *et al*, 1988).

2.4.3 Biodegradation of lignin

Lignin is the most resistant natural polymer to bio-organism attack. However, it is biodegradable since wood decay fungi can secret the necessary enzymes for the lignin degradation. These enzymes are results of centuries of biological adaptations by the micro-organisms to lignin. At present, some bacteria have been identified to be capable of attacking lignin by erosion, tunnelling, and cavity formation (Hatakka, 2001). Compared to the bacteria, fungi are more efficient to attack lignin. Wood decay fungi are usually separate into three groups: white-rot fungi, brown rot fungi, and soft rot fungi. Among them, white-rot fungi are the only organisms able to degrade lignin efficiently to carbon dioxide and water. However, it has not been verified that lignin could be used as a sole carbon and an energy source.

The processes caused by white-rot fungi are of two types. The first type is that white-rot fungi occupy cell lamina of plants and erode cell walls, and as a result, large voids are formed. Another type of damage is that fungi decay plants, and the plants form white-pocket or white-mottled type of rots rather than large voids. The reason for forming this second type of damage is because some white-rot fungi prefer to remove lignin rather than cellulose. Therefore cellulose is maintained in plants

although lignin is decayed. In addition, some white-rot fungi could cause two types of damages. After white-rot fungi were found, some research established that brown-rot fungi could modify lignin to a limited extent (Eriksson *et al.*, reviewed by Hatakka, 2001). The hyphae of brown-rot fungi penetrate from one cell to another through existing pores in wood cells and causes the wood to break into fragments. In contrast to white-rot fungi and brown-root fungi, soft-rot fungi attack wood and cause a brown soft appearance. Recently, more research is ongoing and many fungi species have been discovered to have a lignin-decay capability. For example, some fungi usually thought to degrade carbohydrate in soil, forest litter, and compost also show the ability of lignin degradation in their environment (Tuomela *et al.*, 2000, reviewed by Hatakka, 2001).

The degradation of lignin is an enzyme-catalytic reaction. The initial reactions of lignin degradation are mediated by extracellular enzymes which are primarily produced by white-rot fungi or other wood-decay fungi (Kirk and Farrell, 1987, mention by Hatakka, 2001). The extracellular enzymes produce low molecular weight radicals, for example the hydroxyl radical, which penetrates into the cells of lignin and decomposes lignin into low molecular weight residuals. These low molecular weight residuals are further hydrolyzed and oxidized by various intracellular enzymes (Bailey, *et al.*, 1990). After the initial lignin degradation is undertaken, further decomposition of lignin can be performed by a wide range of fungi.

Before the beginning of the 1980s, only laccase was found in the degradation of lignin. The discovery of lignin peroxidases and Manganese peroxidases, which were found in 1983 and 1984 respectively (Hatakka, 2001), starts a new study stage of lignin degradation. Experiments have verified that the catalytic mechanism of lignin peroxidase is that the enzyme initiates the one-electron oxidation and form a cation

radical that causes a breakdown reaction. As more fungi have been researched, it has been discovered that enzymes secreted by each fungi are different. In addition, the mechanisms of each enzyme-catalyzed lignin degradation vary.

In generally, lignin degradation is primarily an aerobic process, and in an anaerobic environment lignin can persist for very long periods and the aromatic rings of lignin are not decomposed by micro-organisms (Hatakka, 2001).

2.4.4 PVC-lignin blend

Due to the fact that is an abundant and inexpensive polymer with unique properties, many studies have been performed to incorporate lignin into PVC. Studies done at Concordia University revealed an interaction occurred between the OH groups of lignin and the hydrogen of PVC. Experiments demonstrated that the weathering stability and the impact strength of the PVC-lignin blends are lower than the PVC controls. The losses of PVC-lignin properties were explained as the effect of a slight decomposition of lignin caused by the high temperature imposed by rigid PVC processing (Feldman and Banu, 1997). Subsequently, another experiment also completed by a Concordia University team compared the plasticized PVC-lignin blends with plasticized PVC controls. The result demonstrated that PVC-lignin blends exhibited a slight increase in stiffness accompanied by moderate losses in strength and elongation at break. Moreover, the mechanical properties were also affected strongly by the morphology of the polymer, especially the lignin particle size and distribution although the experiment indicated that the PVC-lignin blend had a homogenous structure (Feldman, *et al.*, 2001). Based on these experiments, it is well known that the presence of lignin influences the characteristics of PVC materials.

CHAPTER 3 METHODLOGY AND MATERIALS

3.1 Overview of experimental methods

The study is based on the fundamental consideration that fungi are heterotrophic organisms that must use preformed organic matter. In this research, vinyl polymer, additives, and Alcell lignin are the potential carbon sources. Fungi decompose and use these carbon sources and may cause biodegradation, which reveals the chemical breakdown. Consequently, the chemical structures and physical properties are changed. Comparing the changes of PVC controls and PVC-lignin blends before and after fungi growth, the effects of lignin on the resistance of PVC materials to biodegradation would be identified. The experiments fell into three stages. In different stages, specimens were made in various shapes and formulations. In addition, the fungi used in eacht stage were different.

Stage 1 consisted of two series of tests. The micro-organism used in Series 1 of Stage 1 was a five-fungus- species-mixed spore suspension. In Series 2 of Stage 1, each individual spore suspension of five fungi was inoculated on the surfaces of specimens respectively. In this stage, the specimens used were sheets of flooring material, and they contained: VC/VAc copolymer, different plasticizers, stabilizer, lubricant, filler, with or without Alcell lignin.

In Stage 2, only one fungus spore suspension was employed, and the specimens were films that contained: VC/VAc copolymer, different plasticizers, stabilizer, and lubricant, with Alcell lignin or without it. In this stage, filler was excluded.

The experiment of Stage 3 was conducted in order to confirm the biodegradability of the Alcell lignin, so the specimens used in this stage were Alcell lignin alone and lignin with different plasticizers.

The detailed experimental procedures are highlighted in Figure 3.1. In these experiments, observation for visible effects and growth rates of fungi were performed. The optical microscopy, optical stereoscopic microscopy, and ATR/FTIR spectroscopy were employed. The weight loss gravimetric method was also used.

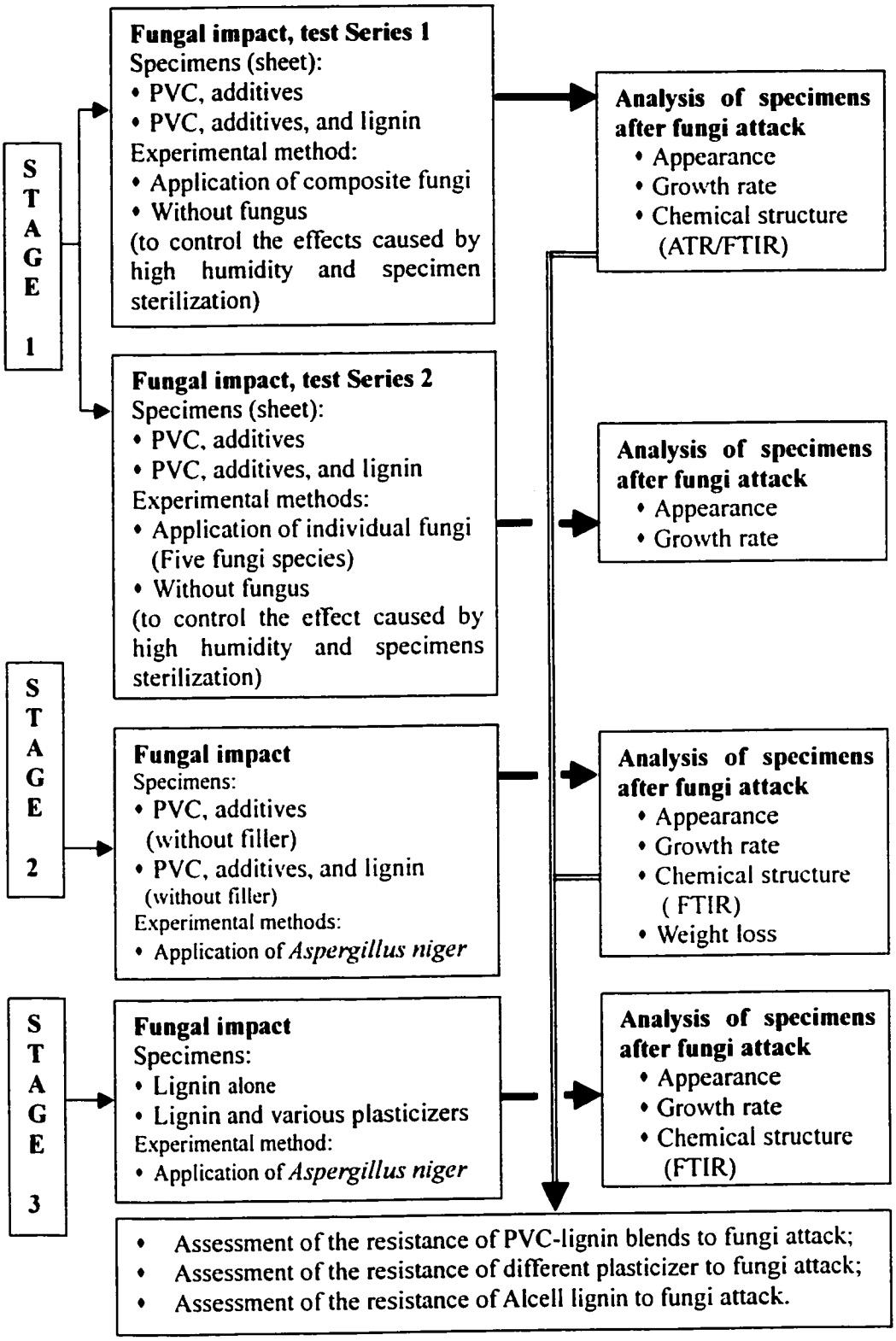


Figure 3.1 Scope of experiments

3.2 Preparation of mixed fungi spore suspension

In the present research, the five fungi employed are listed in Table 3.1. They were obtained from American Type Culture Collection (ATCC) in the form of frozen-dried powder and were stored in double vials.

Table 3.1 Types of fungi and optimum media (ATCC, 2002)

Fungus	ATCC No.	Optimum Medium
Aspergillus niger	9642	336 potato dextrose agar
Penicillum pinopnilum	11797	336 potato dextrose agar
Chaetomium globosum	6205	329 mineral salt agar
Gliocladium virens	9645	336 potato dextrose agar
Aureobasidium pulluians	15233	28 Emmon's modification of Sabouraud's agar

The procedure of preparing the fungi spore suspensions followed the Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi (ASTM G21-96) In order to revive frozen-dried cultures, 0.5 mL sterile water was added to the inner vial of each double vial, respectively, and then transferred the entire contents to separate test tubes which contained 5 mL sterile water. After over night rehydration, these cultures were transferred to optimum media described in Table 3.2. These cultures were stored in a refrigerator kept at approximately 5°C. In order to gain spore suspensions, subcultures of each fungus were incubated at the optimum growth conditions, namely a temperature of 25°C and a relative humidity of 100 %.

Table 3.2 Composition of culture media (ATCC, 2002)

Media	Description
336 Potato dextrose agar (PDA)	Diced potatoes, 300.0 g Glucose, 20.0 g Agar, 15.0 g Distilled water, 1.0 L Autoclave at 121°C for 15 minutes
28 Emmons' modification of Sabouraud's agar	Sabouraud's Glucose Agar 65g Distilled water, 1.0 L Adjustment pH to 6.8-7.0 Autoclave at 121°C for 15 minutes
329 Mineral salts agar	$NaNO_3$, 2.0 g $MgSO_4$, 0.5 g KCl, 0.5 g $Fe_2(SO_4)_3.H_2O$, 0.01 g KH_2PO_4, 0.14 g K_2HPO_4, 1.2 g Agar, 15.0 g Yeast extract, 0.02 g Distilled water, 1.0 L Autoclave at 121°C for 15 minutes Adjustment pH to 7.2. Placement strip of sterile filter paper on cooled agar slant and inoculation on filter strip.

After incubating for 30 days, these subcultures had sporulated. A solution of 10 mL sterile 0.05 g/l sodium dioctyl sulfosuccinate was poured into one subculture, and the surface growth of the subculture was gently scraped. Subsequently, the spore charges were poured into a sterile glass vial containing 45 ml sterile water and 12 solid glass beads, 5 mm in diameter. After separating the spores from fruiting bodies and breaking the spore clumps by shaking the glass vial, the shaken suspension was filtered through a thin layer of sterile glass wool in a glass funnel, and the filtrate was held in a 50 mL sterile centrifuge tube. The filtered spore suspension was centrifuged for 20 minutes at 4,000 rpm. The residue was maintained and resuspended in 50 ml of sterile water and centrifuged again. This step was repeated three times and the final residue was resuspended with a nutrient-salt solution (Table 3.3). Next, a 5mL spore suspension was removed with a sterile pipette to a test tube respectively. The amount

of spores was counted using a bright line counting chamber with a bright line hemacytometer (Micromaster LR33310). Based on the concentration of the spore suspension, a sterile nutrient-salt solution was added to dilute the original spore suspension to gain the required spore concentration. Following the procedure mentioned above, five different spore suspensions were obtained. The numbers of spores per ml in final suspensions were listed in Table 3.4. Finally, a 5mL spore suspension of each of five fungi was withdrawn with a pipette to a glass vial and the final mixed spore suspension was gained. This fungi spore suspension was prepared for the Stage 1.

The procedures for preparing the fungi spore suspensions used for Stage 2 and Stage 3 were the same as that of Stage 1. Instead of the five used in Stage 1, Stage 2 employed one kind of fungus: *Aspergillus niger*. The number of spores per ml in spore suspension of Stage 2 was 1,070,000. Likewise, Stage 3 employed *Aspergillus niger* as an experimental micro-organism and the number per mL was 1,160,000.

Table 3.3 Compositions of nutrient-salts solution and nutrient-salts agar (ASTM, 1996)

Compound	**Weight (g)**
Potassium dihydrogen orthophosphate (KH_2PO_4)	0.7
Magnesium sulfate ($MgSO_4.7H_2O$)	0.7
Ammonium nitrate (NH_4NO_3)	1.0
Sodium chloride (NaCl)	0.005
Ferrous Sulfate ($FeSO_4.H_2O$)	0.002
Zinc Sulphate ($ZnSO_4.7H_2O$)	0.002
Manganous Sulfate ($MnSO_4.H_2O$)	0.001
Potassium monohydrogen orthophosphate(K_2HPO_4)	0.7
Distilled water	1 liter
Note: Nutrient-salts agar is of nutrient-salts solution with 15.0g agar. Adjust the pH of the media between 6.0 and 6.5 by adding 0.01N NaOH	

Table 3.4 Numbers of spores/ml in final spore suspension

Aspergillus niger (spores per ml)	*Penicillum pinopnilum* (spores per ml)	*Chaetomium globosum* (spores per ml)	*Gliocladium virens* (spores per ml)	*Aureobasidium pulluians* (spores per ml)
1,000,200	980,600	890,000	1,050,000	1,120,000

3.3 Preparation of experimental specimens

3.3.1 Raw material for Vinyl flooring

3.3.1.1 PVC

The polymer used in this study is a vinyl chloride-vinyl acetate (OXY 1810) copolymer, supplied by Occidental Chemical Corporation, TX, U.S. In this research, it was referred to as PVC. The properties of the copolymer are showed in Table 3.5. The chemical structures of PVC and poly vinyl acetate are illustrated in Figure 2.2 and Figure 2.3. The biodegradability of this copolymer is mentioned in Chapter 2.

Table 3.5 Properties of OXY 1810 PVC copolymer

(PVC data sheet, Occidental Chemical Corporation, 2001)

Relative molecular mass	54,000
Physical state	solid powder
Color	white
Specific gravity	1.37
Bulk density, g/cm^3	0.63
Bound vinyl acetate, wt %	9.7
Particle size % retained, 40 mesh (in μm) % through, 200 mesh h (in μm)	 0 10
Volatiles, %	1.0

3.3.1.2 Plasticizer

In this research, four plasticizers were used, and they are listed in Table 3.6. Their

physical and chemical properties are presented below.

Table 3.6 Types of Plasticizers

Plasticizer/ Trade name	Abbreviation	Supplier
Di-octyl-phthalate	DOP	Fisher Scientific
Di-ethylene glycol di-benzoate/ Benzoflex 2-45	2-45	Velsicol
Tricresyl-phospate/Lindol	Lindol	Akzo Nobel
Alchil sulphonate/Mesamoll	Mesamoll	Bayer AG

- **Di-octyl-phthalate (DOP)**

Chemical structure of DOP (see Figure 3.2):

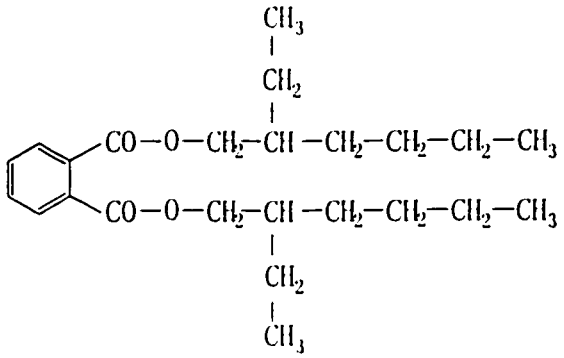


Figure 3.2 Chemical structure of DOP

Physical and chemical properties of DOP (see Table 3.7):

Table 3.7 Physical and chemical properties of DOP (Guidelines for drinking water quality. Environmental Health Criteria (EHC) 131, 1992)

Molecular formula	$C_{24}H_{38}O_4$
Relative molecular mass	390.57
Physical state	liquid
Colour	colourless to yellow oily liquid
Odour	very slightly aromatic
Melting or freezing point(°C)	-46°
Boiling point or range (°C)	370 °C at 101.3 kPa
Specific gravity (density)	0.98
Viscosity (mPa.s at 25°c)	82
Flash point (°C)(open cup)	210 [a]
Vapour pressure	0.056×10^{-7} kPa at 20 °C
Henry's Law constant	$1.1\text{-}2.8 \times 10^{-6}$ atm-m^3/mol
Solubility in water	0.23-0.34 (mg/litre)
Octanol-water partition coefficient (log P_{ow})	4.88

a: Mellan, Ibert (1961).

Biodegradability of DOP:

In the presence of several microorganisms in soil, sludge, sediment, and water, aerobic degradation of DOP has been found to occure. However, it is only 40-90% degraded after 10-35 days (ECETOC, 1985; mentioned by EHC 131, 1992). Anaerobic degradation is much slower, or possibly even non-existent. Aerobic degradation of DOP depends on temperature. According to Mathur's (1974b) experiment (mentioned by EHC 131, 1992), the quick rate degradation occurs at relative high temperature. At 4 and 10 °C, only marginal degradation was taking place. Another experiment onducted by Shanker et al. (1985) (mentioned by EHC131, 1992), who incubated garden soil containing DOP at a concentration of 500 mg/kg, indicated that 75% and 90% of the DOP had been degraded after 20 days and 30 daysrespectively. Meanwhile, his experiment showed negligible degradation was detectable when sterilized soil was used. The fungus *Penicillium lilacinum* can degrade approximately half of the initial amount of DOP within 30 days, which was found by Engelhardt et al. (1977) (mentioned by EHC 131, 1992).

Kurane et al. (mentioned by EHC 131, 1992) found the first step of the biodegradation of DOP was the hydrolysis of the diester to the monoester by esterase with low substrate specificity. The conversion of monoester into phathalic acid was detected by Engelhardt et al. in 1975 (referred by EHC 131, 1992). Finally, the ring was opened and degraded to pyruvate and succinate and then to CO_2 and H_2O.

- **Di-ethylene glycol di-benzoate (2-45)**

Chemical structure of 2-45 (see Figure 3.3):

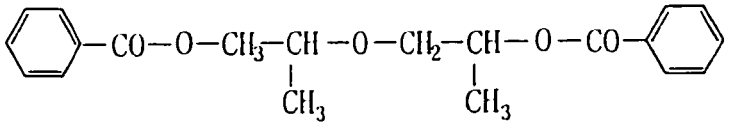


Figure 3.3 Chemical structure of 2-45

Physical and chemical properties of 2-45 (shown in Table 3.8):

Table 3.8 Physical and chemical properties of 2-45 (Product information bulletin from Velsicol Chemical Corporation, 2001)

Molecular formula	$(C_6H_5CO_2CH_2)_2O$
Relative molecular mass	314.4
Physical state	liquid
Colour	clear colourless
Odour	mild ester odor
Melting or freezing point(°C)	28
Boiling point or range (°C)	236 at 0.7 kPa
Specific gravity (density)	1.20
Viscosity (mPa.s at 25°c)	65-66
Flash point (°C)(open cup)	232
Vapour pressure	1.73×10^{-5} Pa at 25°C
Henry's Law constant	7.0×10^{-10} atm-m^3/mol
Solubility in water	38.3 mg/l at 30°C
Octanol-water partition coefficient (log P_{ow})	3.0

Biodegradability of 2-45:

According to the test performed by Huntingdon Life Sciences (http://www.epa.gov/chemrtk/diglydib/c13271tp.pdf), it was found that 2-45 was degraded readily at aerobic condition connected with activated sludge. The test indicated that 17% of TCO_2 was released after 2 days of bio-organism attack, 71% of TCO_2 is released after 10 days.

- **Lindol** (commercial product: mixture of isomers)

Chemical structure of Lindol (see Figure 3.4):

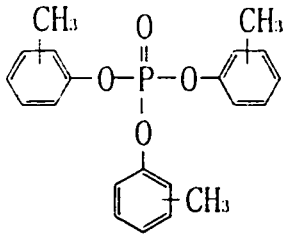


Figure 3.4 Chemical structure of Lindol

Physical and chemical properties of Lindol (introduced in Table 3.9):

Table 3.9 Physical and chemical properties of Lindol (Environmental Health Criteria (EHC) 110, 1990)

Molecular formula	$C_{21}H_{21}O_4P$
Relative molecular mass	368.4
Physical state	liquid
Colour	colourless
Odour	very slightly aromatic
Melting or freezing point (°C)	-33
Boiling point or range (°C)	241-255 (4 mmHg) 190-200 (0.5-10 mmHg)
Specific gravity	1.165
Viscosity (cSt)	60 (25 °C), 4.0 (100 °C)
Flash point (°C)	257
Vapour pressure (mmHg)	1×10^{-4} (20°C), 10 (265°C)
Henry's Law constant	$1.1\text{-}2.8 \times 10^{-6}$ atm-m^3/mol
Solubility in water	0.36(mg/litre)
Octanol-water partition coefficient (log P_{ow})	5.11

Biodegradability of Lindol:

Many studies have demonstrated that Lindol exposed to natural micro-organisms undergo a primary biodegradation. Its rates in both die-away and activated sludge studies show the similar results. A study conducted by Hattori *et al* (mentioned by EHC 110, 1990) showed Lindol (1 mg per litre), sampled from the Neya and Oh River water (Osaka, Japan), was almost completely degraded within 5 days under non-sterilized conditions.

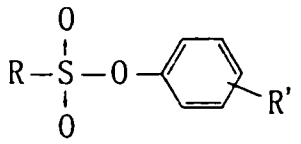
The ultimate biodegradation of Lindol was measured by Saeger *et al* (mentioned by EHC 110, 1990). At 26.4 mg Lindol/litre, the carbon dioxide evolution reached 82 % of its theoretical value.

The degradation pathway for Lindol most probably involves stepwise enzymatic hydrolysis to orthophosphate and phenolic moieties (Barrett *et al.*, 1969; Pickard *et al*, 1975). The phenol would then be expected to undergo further degradation. Analysis using thin-layer chromatography and gas chromatography-mass spectrometry demonstrates that the major metabolite of tri-cresyl phosphate hydrolysis is

hydroxybenzoic acid and two unidentified components, with a half-life of 7.5 h (Ku & Alvarez, mentioned by EHC 110, 1990).

- **Mesamoll**

Chemical structure of Mesamoll (see Figure 3.5) (Wilson, Alan S., 1995):



R= $C_{15}H_{31}$ average in Measamoll R'= H and CH_3

Figure3.5 Chemical structure of Mesamoll

Physical and chemical properties of Mesamoll:

Table 3.10 lists the physical and chemical properties of Mesamoll.

Table 3.10 Physical and chemical properties of Mesamoll (Technical sheet from Bayer, 2001)

Molecular formula	$C_{15}H_{31}SO_3C_6H_5$ or $C_{15}H_{31}SO_3C_6H_4CH_3$
Physical state	liquid
Colour	clear, slightly yellowish
Odour	very slightly aromatic
Specific gravity (density)	1.03-1.07
Viscosity (mPa.s) at 20°C	95-125
Flash point (°C)(open cup)	210-240
Solubility in water	not miscible with water
Octanol-water partition coefficient (log P_{ow})	soluble in all common organic solvents

Biodegradability of Mesamoll:

Due to its chemical structure, a phenyl sulphonate ester group exists which has a low susceptibility to hydrolysis; Mesamoll has a high resistance to degradation during

exposure to weather, micro-organisms or alkaline media (Wilson, 1995).

3.3.1.3 Heat stabilizer

Dibutyltin dilaurate (DBTL) is employed as a heat stabilizer, which was provided by Sigma Aldrich Canada Ltd (2001). Its physical and chemical properties are showed in Table 3.11.

Table 3.11 Physical and chemical properties of DBTL (International Chemical Safety Cards (ICSCs): 1171, 1995)

Molecular formula	$(C_4H_9)_2Sn(OOC(CH_2)_{10}CH_3)_2$
Relative molecular mass	631.6
Physical state	oily liquid or waxy crystals
Colour	yellow
Melting or freezing point (°C)	22-24
Boiling point or range (°C)	205 at 1.3kPa
Specific gravity (density)	1.1
Flash point (°C) (close cup)	179
Solubility in water	none

3.3.1.4 Filler

Calcium carbonate ($CaCO_3$), obtained from Steep Rock Resources in Perth, Ontario, was used as the filler in this study. The grade was Snowhite 12. Calcium carbonate has strong resistance to biodegradation (Pankhurst *el al*, 1966). Its properties are listed in Table 3.12.

Table 3.12 The properties of $CaCO_3$, Snowhite 12 (Technical sheet from Steep Rock Inc., 2001)

Typical physical properties:	
Bulk density (loose), g/cm^3	0.8
Bulk density (packed), g/cm^3	1.4
Specific gravity	2.71
Typical particle size:	
% retained, 325 mesh	0.1
Mean particle size	12 microns
Typical chemical analysis:	
$CaCO_3$ (%)	96.0
$MgCO_3$ (%)	2.0
Acid insoluble (%)	2.0

3.3.1.5 Lubricant

In this research, calcium stearate grade L-155 (CaSt), obtained from Blachford LTD, (Canada), was used as a lubricant. Calcium stearate is a compound of calcium with a mixture of solid organic acids obtained from fats. CaSt consists primarily of calcium stearate, calcium palmitate, and the equivalent of 9.0 to 10.5% of calcium oxide. Its molecular formula is $C_{36}H_{70}CaO_4$ An experiment undertaken by Pankhurst *el al* (1968) demonstrated calcium stearate is susceptible to microorganism attack. The physical and chemical properties are listed in Table 3.13.

Table 3.13 Physical and chemical properties of calcium stearate (Loyd V. Allen, 2000)

Molecular formula	$Ca(CH_3(CH)_{16}COO)_2$
Mean molecule weight	607
Physical state	Fine Flowing Powder
Colour	White to yellowish white
Bulk Density g/ml Max	0.16-0.38
Melting point (°C)	155 - 165
Soluble in water max	0.2
Typical particle size % Pass through, 300 Mesh	100

3.3.1.6 Lignin

An organosolv-type Alcell lignin, supplied by Alcell Technologies Inc., Miramachi, New Brunswick, was used in this research. The product is obtained through a new delignification process. The properties of Alcell lignin are provided in Table 3.14.

Table 3.14 Properties of Alcell lignin (Technical sheet from Alcell Technologies Inc., 2001)

Mw	<2,000
Mn	800-900
specific gravity	1.27
Softening temperature (ring and ball, ASTM E28), (°C)	145
Median particle size, μm	20-40
Solubility parameter δ, $(cal/cm^3)^{1/2}$	13.7

3.3.2 Specimens preparation

3.3.2.1 Stage 1

The specimens used in Stage 1 were prepared mainly for testing mechanical properties, and are produced based on the same formulation as the finished product for flooring material. The formulations are provided in Table 3.15. Four specimen groups were prepared with different plasticizers and ten sheets of samples for each specimen group were prepared. Among them, five sheets were inoculated with fungi and another five sheets, which were not inoculated with fungi, were to control the effects caused by specimen sterilization and high humidity.

All ingredients of specimens were mixed in a Haake Rheomix 600 equipped with roller blades at 150 °C and the time of mixing was 8 minutes. Next, the melt mixing was ground to a size of about 3-5 mm. Sheets in the thickness of 2 mm were made by

compression at 158°C, for 8 minutes, in a laboratory press equipped with a temperature control. After cooling with air and of being under pressure for 20 minutes, the sheets were cut with a cutting die in shoulder shaped specimens. Fifteen specimens were prepared for each formulation. The samples were stored in the dark.

Table 3.15 Formulation of specimens for Stage 1

Ingredient	**Composition**	
	PVC control(phr)	**PVC-Lignin blend (phr)**
Resin: VC-VAc copolymer	100	80
Lignin: Alcell lignin	0	20
Plasticizer: DOP or Benzoflex 2-45 or Lindol or Mesamoll	35	35
Heat stabilizer: DBTL	3.0	3.0
Lubricant: CaSt	1.5	1.5
Filler: $CaCO_3$	200	200

3.3.2.2 Stage 2

In this stage, film specimens were prepared for determining the changes of chemical structures of plasticizers, PVC, and lignin. In order to identify the changes more clearly, filler was excluded. A heat stabilizer and a lubricant were still maintained to keep the processing characteristics. The formulations of specimens are given in Table 3.16. Four specimen groups were prepared with different plasticizers.

Table 3.16 Formulation of specimens for Stage 2

Ingredient	Composition	
	PVC control (phr)	PVC-Lignin blend (phr)
Resin: VC-VAc copolymer	100	80
Lignin: Alcell lignin	0	20
Plasticizer: DOP or Benzoflex 2-45 or Lindol or Mesamoll	35	35
Heat stabilizer: DBTL	3.0	3.0
Lubricant: CaSt	1.5	1.5

Specimens of Stage 2 were films with a thickness of 0.1mm and in the diameter of about 1.6 cm. At first, all the ingredients of a sample were prepared according to a formulation mentioned above. The compound was melted in a Haake Rheomix 600 equipped with roller blades. This step was operated at 150°C and lasted 8 minutes. After mixing and cooling, the compound was ground into small beads. According to the required thickness of the film and the density of the compound, the required weight of well-ground compound was calculated and weighed. The ground compound was placed between two sheets of aluminum foil which were placed between two plates of a Universal Film Maker. Then the compound was heated for 4 minutes at 135°C and under the pressure of one ton. Afterwards, the pressure was maintained for 3 minutes without heating. At the end, it was cooled to the room temperature under the atmospheric pressure. The obtained specimen film was formed and removed from the two sheets of aluminum foil. Finally, the film was placed in Petri dishs before the test and was stored in the dark to avoid photodegradation. All the specimens with different formulations were prepared following the procedure mentioned above.

3.3.2.3 Stage 3

Generally, native lignin is highly resistant to fungi attack except wood-decay fungi. However, the structure of industrial lignin is different from the native lignin. The intent of this experiment was to investigate the biodegradability of the specific lignin in the presence of plasticizers. The formulations of specimens are provided in Table 3.17. Four specimen groups were prepared with different plasticizers.

Specimens of this stage were formed by melting the components in an oven at the temperature of 145°C for 8 minutes, and then cooled at the room temperature. After finishing the experiment, the fungi were removed from the specimens, and the specimens were ground into powder, and then processed in films. The procedure of preparing the films was the same as that of Stage 2.

Table 3.17 Formulation of specimens for Stage 3

Ingredient	**Composition**	
	Lignin control (phr)	**Lignin-plasticizer blend (phr)**
Lignin: Alcell lignin	100	100
Plasticizer: DOP or Benzoflex 2-45 or Lindol or Mesamoll	0	35
Filler: $CaCO_3$	200	200

3.4 Experimental procedure

3.4.1 Stage 1

In Stage 1, specimens of PVC controls and the PVC-lignin blends containing various plasticizers were tested by inoculation with a mixed fungi spore suspension

(Series 1) or different single fungus spore suspensions (Series 2). In the first step, the solidified substrate was prepared. A solution of 10mL nutrient-salt agar was moved into a Petri dish with a pipette. After the agar was solidified, the specimens were placed on the surface of the agar. A Petri dish contained five specimens with the same composition. Before the specimens were placed into Petri dishes, they were sterilized by UV light for two minutes and were merged in 70% ethanol for two minutes. The mixed spore suspension was moved into a sterilized atomizer (TLC reagent sprayer 422530-series) by a pipette. Subsequently, the surface of the agar and the specimens were inoculated with the mixed spore suspension by spraying the spore suspension from the atomizer with 5 kPa nitrogen, and the entire surface was covered with the spore suspension. Nitrogen had been checked to confirm that it was not contaminated prior to being used, and the gas tube also had been sterilized. Finally, these Petri dishes were incubated at 28°C and 100% relative humidity. In order to correct the influence caused by high humidity, a control experiment (Series 2) was carried out at the same temperature and humidity, but without fungi. The procedure of Series 2 was the same as Series 1, with the exception that individual fungus spore suspensions instead of the mixed fungi spore suspension. The experimental system is illustrated in Figure 3.2. After a 28-day incubation, the specimens were immersed in an aqueous solution of mercuric chloride for 5 minutes. The weight ratio of mercuric chloride to water was 1:100. The specimens were rinsed in water and air-dry overnight at room temperature.

The specimens were placed in the laboratory for 48 days before being tested.

PVC control PVC-lignin blend

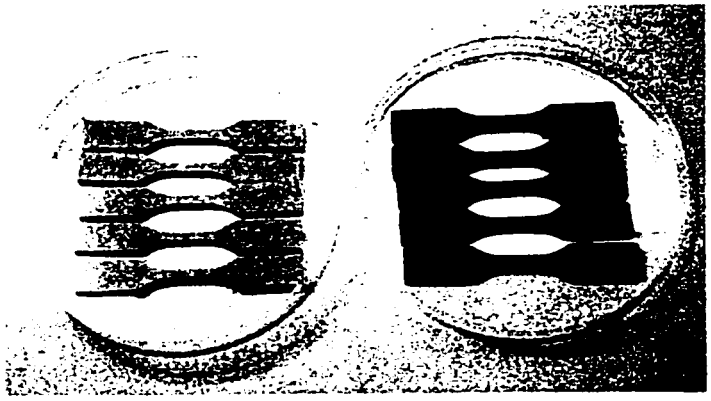


Figure 3.6 Experimental system of Stage 1

3.4.2 Stage 2 and Stage 3

In the two stages, the specimens had been tested by inoculating with the spore suspension of *Aspergillus niger*. *Aspergillus niger* was chosen due to its rapid growth which had been detected in Stage 1. The experimental procedure was the same as Stage 1. The test systems are shown in Figure 3.3. After the fungi were removed, the specimens were kept in a room for 10 days before analysis.

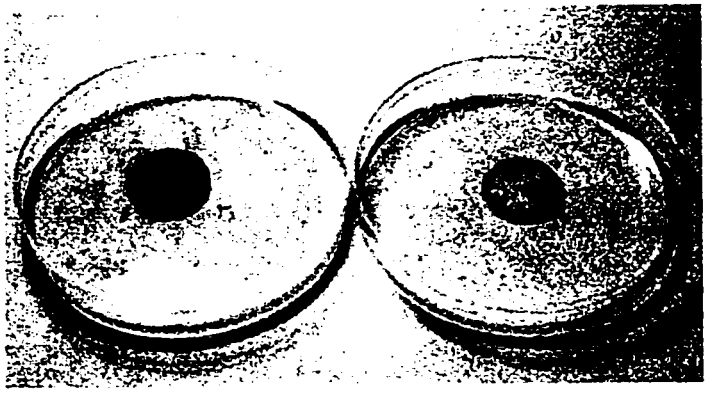


Figure 3.7 Experimental systems of Stage 2 and Stage 3

3.5 Analysis of specimens

In order to assess the impact of fungi development on PVC controls and PVC-lignin blends, a combination of several methods was applied. All samples of Stage 1, Stage 2, and Stage 3 were submitted to evaluation, and the rates of fungi growth were performed by an optical microscope at 50x magnification (Wild M5A, Wild Heerbrugg).

For Stage 1, the fungal growths were documented on a series of photographs under an optical microscope (Wild MP551 microscope with Leitz Periplan GF 10x lens) equipped by a camera with Wild Photoautomat MPS 45 at 500x magnification. The surfaces of specimens after fungi attack were also investigated using an optical stereoscopic microscope (Leica MZ-12 Stereomicroscope with Planapo 1.6x lens) equipped with Videocamera US522. The photographs were taken by Polymer Engineering Company Ltd., Vancouver.

In this research, the specimens of Stage 1 were examined by ATR (Attenuated Total Reflectance) / FTIR (Fourie Transform Infrared Spectroscopy) spectra. Infrared spectra were done with a Nicolet FTIR (Magna 550) combined with an ATR. The ATR crystal, a rectangular trapezoid composed of ZnSe, has a 45° angle of incidence. The device can analyze liquid and solid samples. The spectra of PVC, lignin, plasticizers, and their blends, before and after fungal attack, were obtained through ATR/FTIR and were analyzed by the program of Omnic1.2 a. All spectra were collected at a spectral resolution of 32 cm^{-1}.

Specimens of Stage 2 and Stage 3 were characterized by FTIR spectroscopy and also analyzed by the program Omnic 1.2a. All spectra were collected at a spectral resolution of 4 cm^{-1}. Weight losses were measured in Stage 2 before and after fungi growth to determine the changes of specimens.

CHAPTER 4 RESULTS AND DISCUSSION

The present research investigates the growth of fungi and their impact on plasticized PVC and plasticized PVC-lignin blends. The experimental results will be presented and discussed in three parts. The composites are expressed in the following manner: i) the PVC control (PVC with different plasticizers and other additives), ii) the PVC blends (PVC with Alcell lignin, different plasticizers and other additives), iii) or others, for example, "the DOP control", which means the composite contains PVC, plasticizer DOP and other additives. Likewise, "the DOP blend" contains PVC, Alcell lignin, DOP, and other additives.

4.1 Stage 1

In this stage, specimens, which were PVC blends with various plasticizers, the lubricant, the filler, the heat stabilizer, and with lignin or without lignin, were inoculated with cultures consisting of five individual fungi or a single fungus.

4.1.1 Observation of the fungi growth

After a 28-day incubation, fungi significantly grew on all samples. Using an optical microscope with a 500x magnification, it was found that there were more abundant growths on the samples containing lignin than on those without lignin (see Figures 4.1 to 4.4). The observation is coordinative to all the blend samples containing various plasticizers, and it is also coordinative to all the blend samples which were inoculated with mixed fungi or were inoculated with different single fungus species. Fungi grown on the samples containing plasticizer 2-45 had evidently sporulated. The growths of the five single fungi were also much higher on the samples

containing 2-45 than other samples. Figures 4.5 to 4.9 show the growths of individual fungus on the 2-45 controls (PVC with plasticizer 2-45 and other additives) and the 2-45 blends (PVC with plasticizer, lignin, and other additives), observed by a stereomicroscope at 500x magnification. *Aspergillus niger* exhibited a slight rich growth than other fungi and had an obvious sporulation. In addition, it was found that the fungi grew richly in the edges of all the samples. The traces of the mixed fungi growths were confirmed by an optical microscope (Wild M5A) at 50x magnification. The rates of the fungi growths listed in Table 4.1 were evaluated by using the standard of ASTM G21-96.

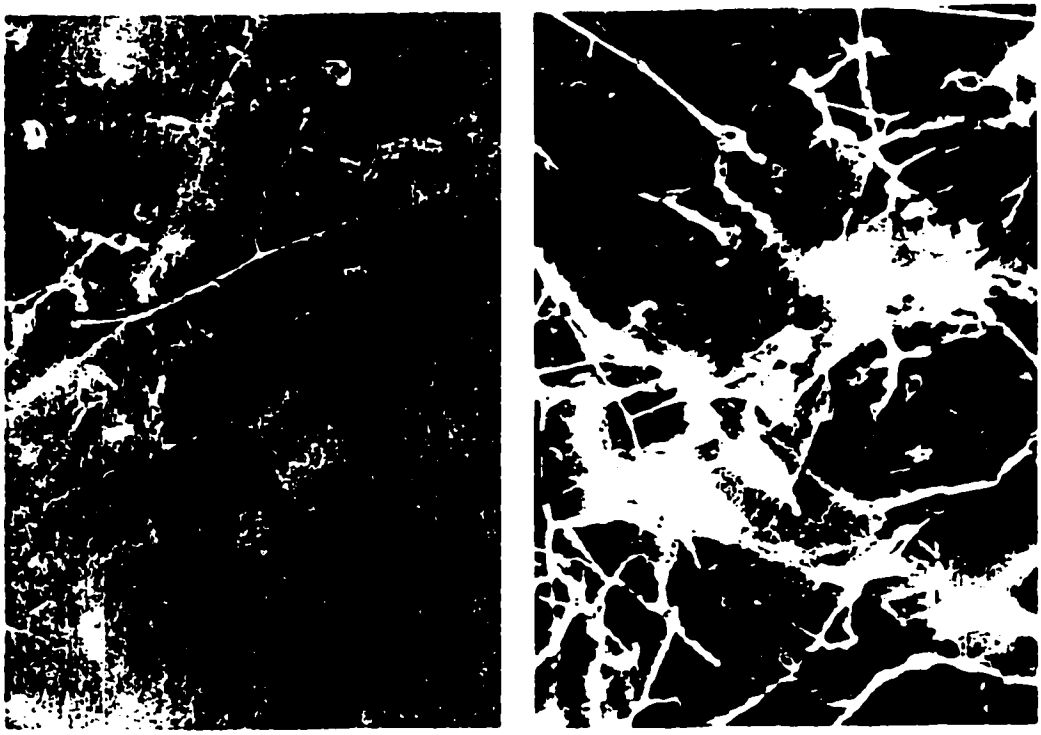


Figure 4.1 Mixed fungi growth on the DOP control sheet (left) and the DOP blend sheet (right)

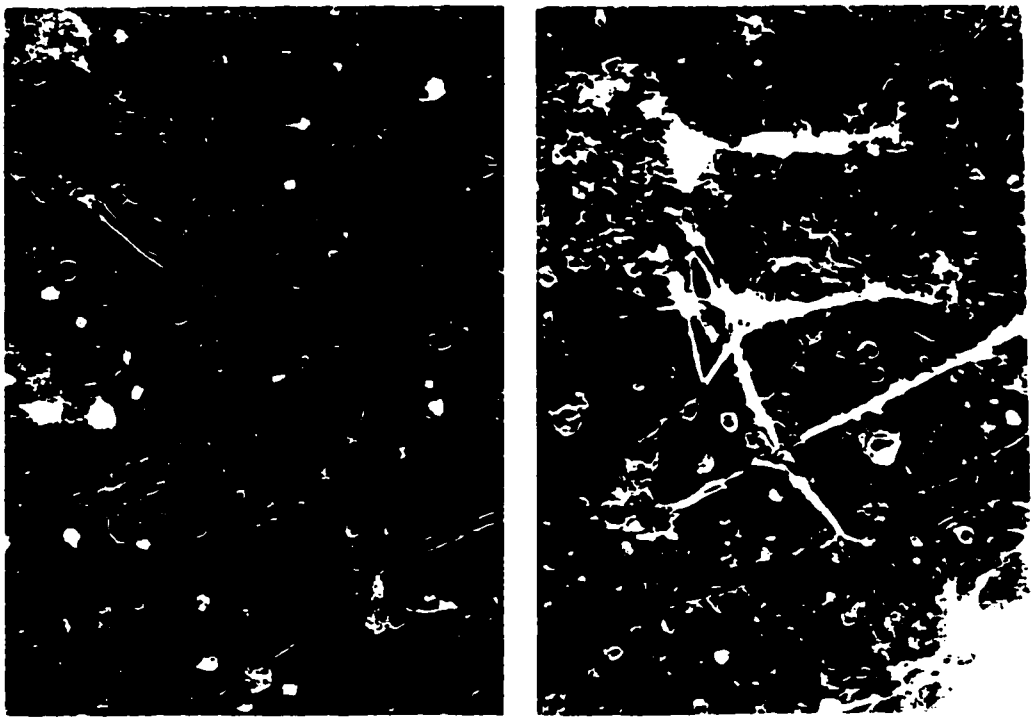


Figure 4.2 Mixed fungi growth on the 2-45 control sheet (left) and the 2-45 blend sheet (right)

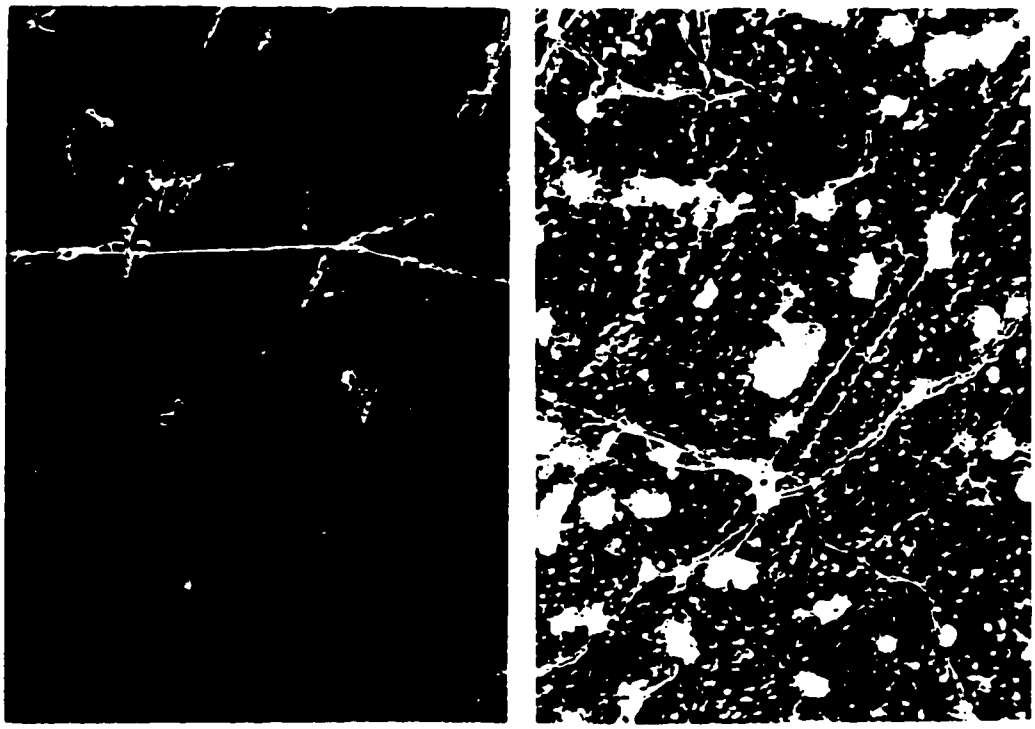


Figure 4.3 Mixed fungi growth on the Lindol control sheet (left) and the Lindol blend sheet (right)

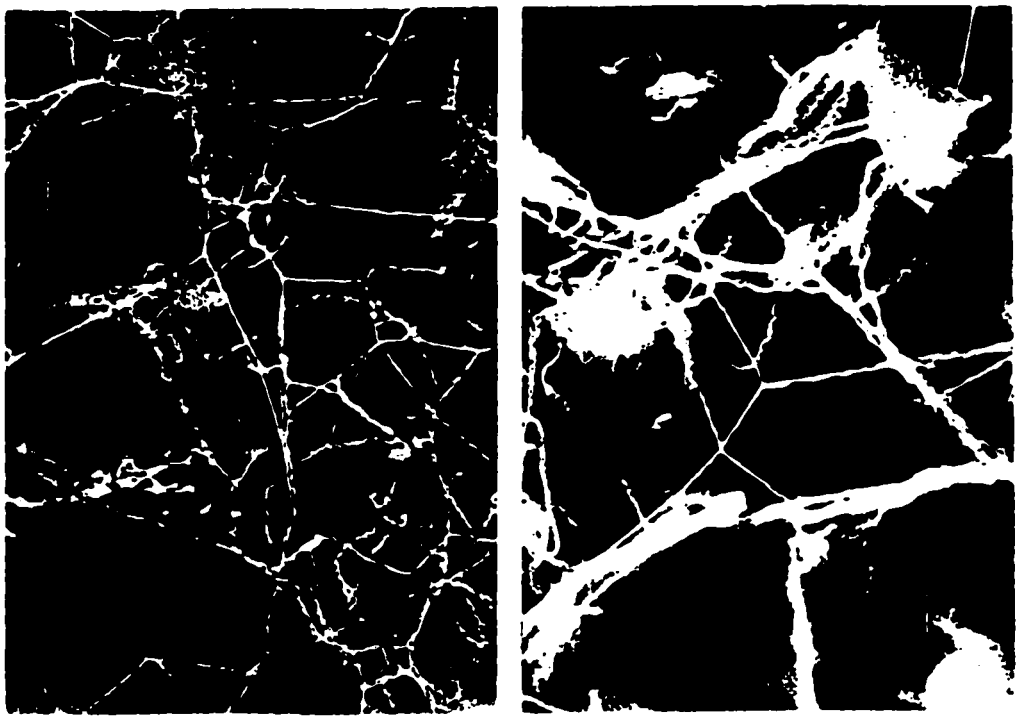


Figure 4.4 Mixed fungi growth on the Mesamoll control sheet (left) and the Mesamoll blend sheet (right)

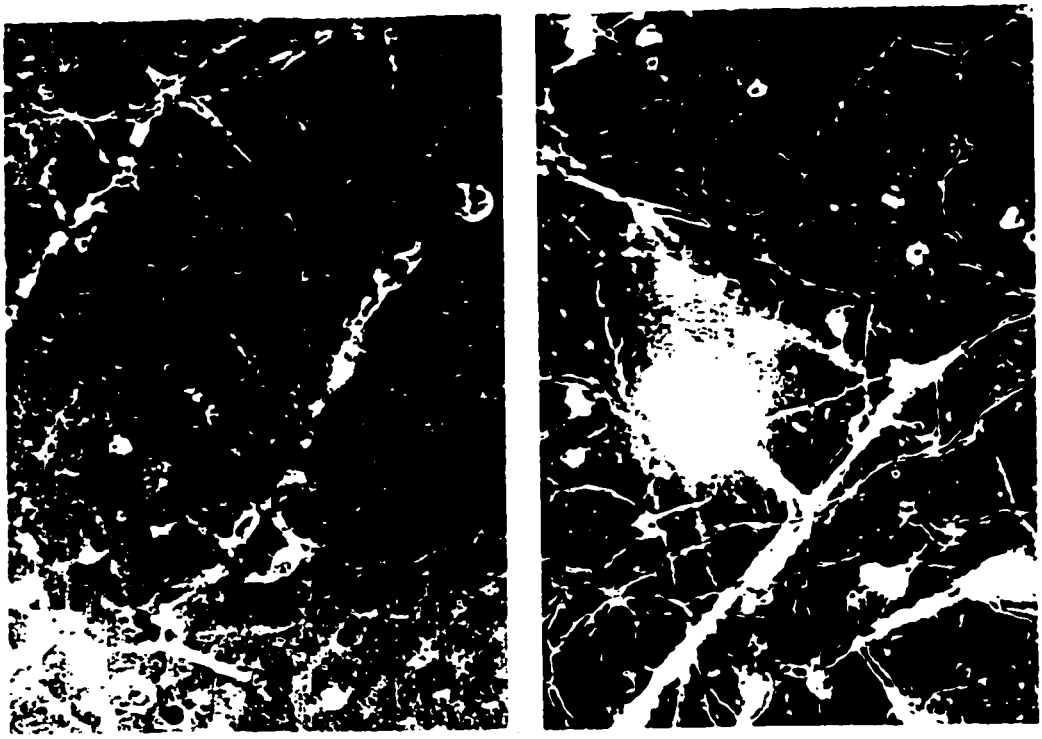


Figure 4.5 *Aspergillus niger* growth on the 2-45 control sheet (left) and the 2-45 blend sheet (right)

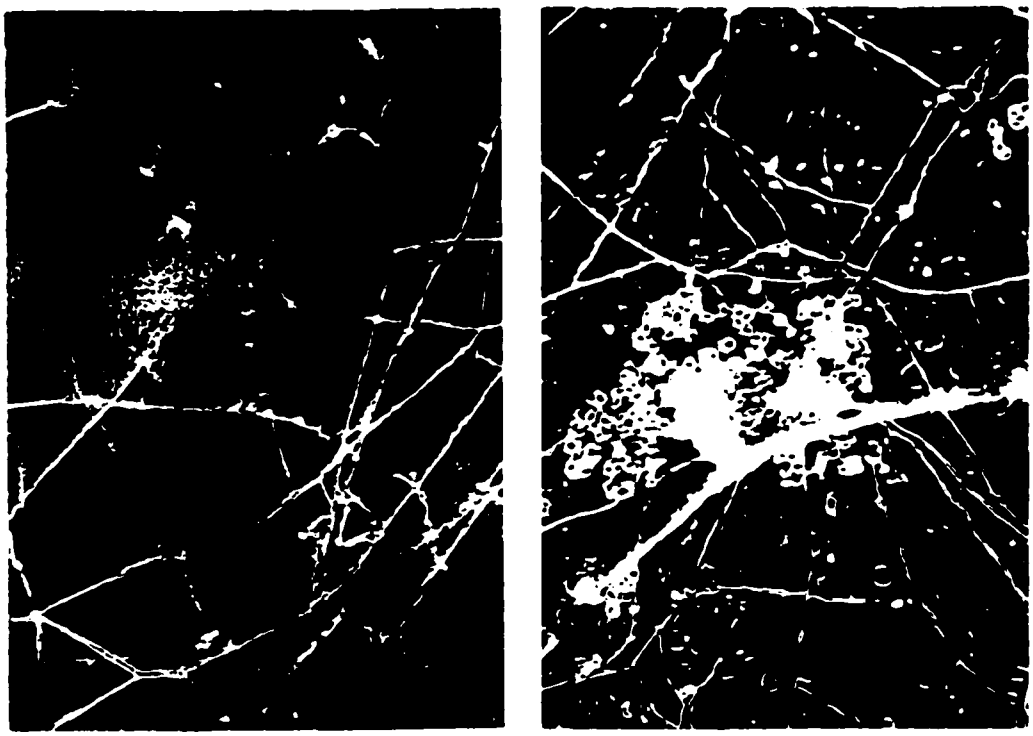


Figure 4.6 *Penicillium pinophilum* growth on the 2-45 control sheet (left) and the 2-45 blend sheet (right)

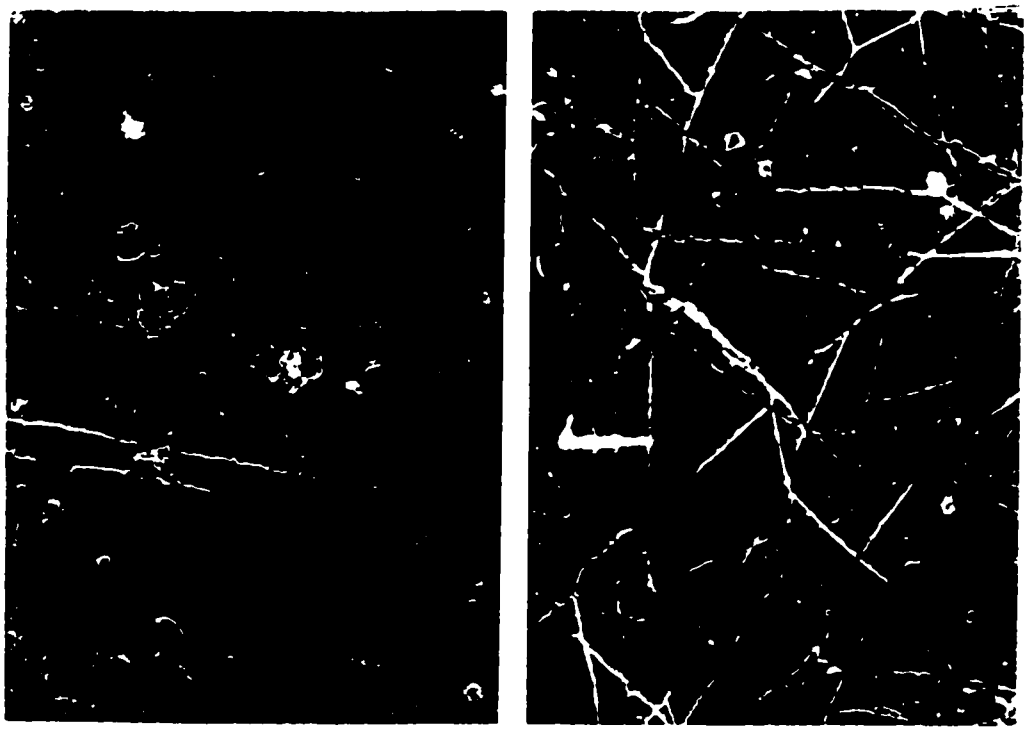


Figure 4.7 *Chaetomium globosum* growth on the 2-45 control sheet (left) and the 2-45 blend sheet (right)

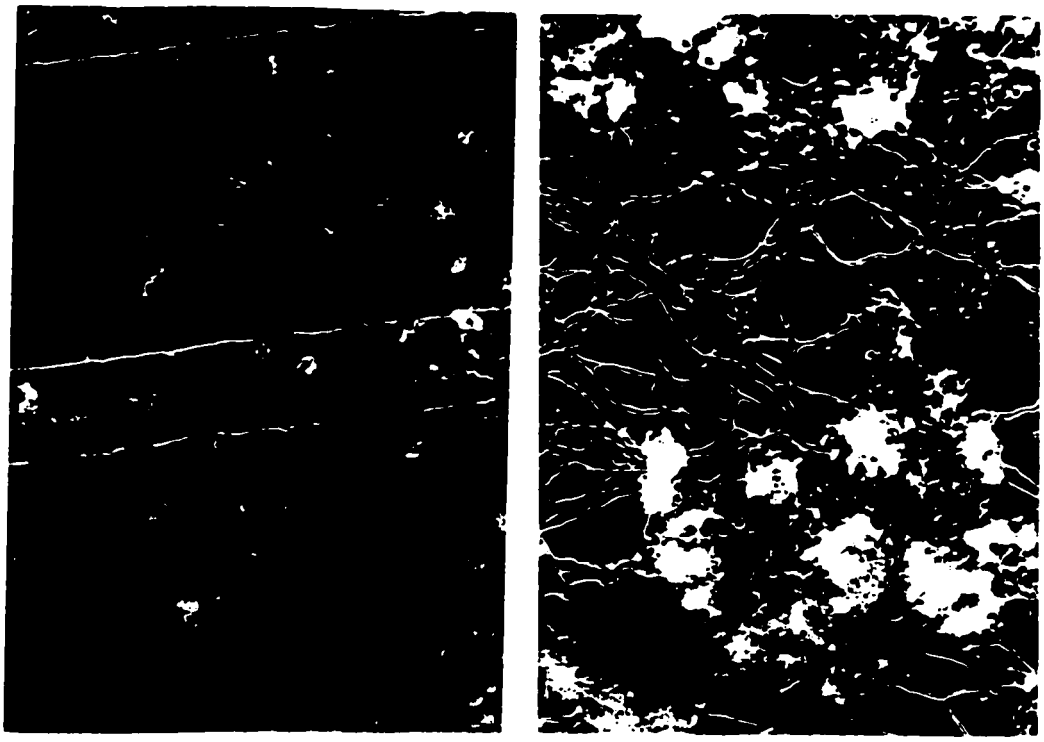


Figure 4.8 *Gliociadium viren* growth on the 2-45 control sheet (left) and the 2-45 blend sheet (right)

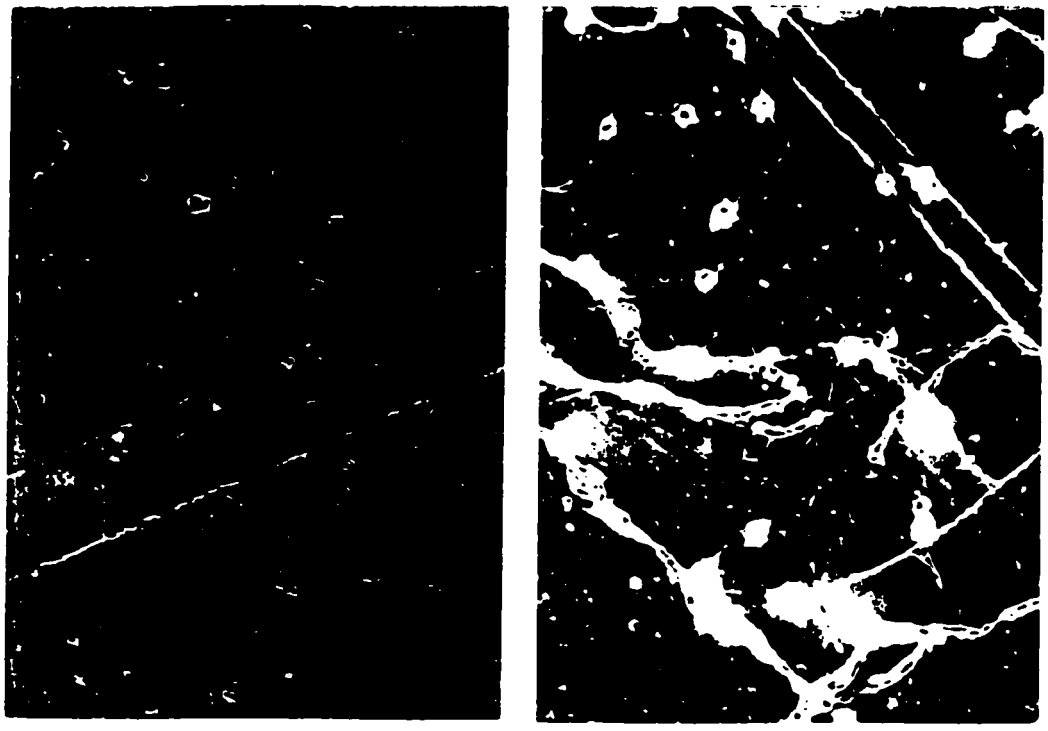


Figure 4.9 *Aureobasidium pulluians* growth on the-45 control sheet (left) and the 2-45 blend sheet (right)

Table 4.1 Ratings of fungi growth on the surfaces of the specimens (Stage 1)

Specimen	Rating of Growth							
	DOP		2-45		Lindol		Mesamoll	
	Control	Blend	Control	Blend	Control	Blend	Control	Blend
Mixed fungi	2	3	3	4	2	3	2	3
Aspergillus niger	2	3	3	4	2	3	2	3
Penicillum pinopnilum	2	3	3	4	2	3	2	3
Chaetomium globosum	2	3	3	4	2	3	2	3
Gliocladium virens	2	3	3	4	2	3	2	3
Aureobasidium pulluians	2	3	3	4	2	3	2	3

Note: 2-light growth (10% to 30%)

3-medium growth (30 to 60%)

4-heavy growth (60% to complete coverage)

After the fungi were washed from the surfaces of these samples, it was observed that all the samples slightly lost their shine. The only exception was the 2-45 blends, which showed a serious discolouration, and some light yellow spots appeared on the surfaces of the samples. Figure 4.10 shows the appearances of these samples, before and after fungal growth. We can see in Figure 4.10 that all the 2-45 blends exhibit the discolouration after being attacked by the five individual fungi or by the mixed fungi.

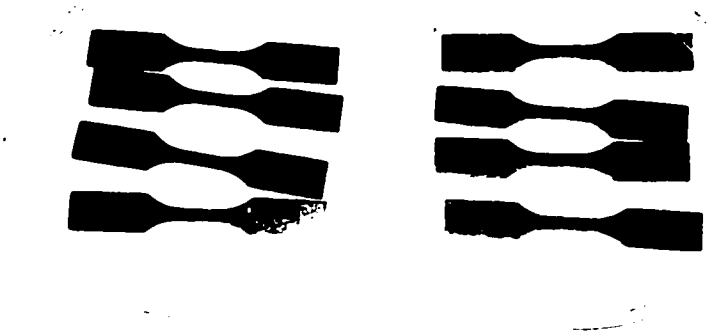


Figure 4.10 The appearances of the 2-45 blends before (left) and after fungi attack (right)

4.1.2 Observation of the specimens' surfaces

In order to understand the changes on the surface of each specimen after fungi assault, visual observation were made using an optical stereoscopic microscope at 500x magnification. Upon observing the surfaces of the samples, some lignin mass was found in the samples of DOP and Mesamoll blends. It may hint that the two composites are not homogenous. 2-45 and Lindol blends seem to be more homogenous than DOP and Mesamoll blends. Comparing the surfaces of the specimens with and without fungi colonization (both with a same formulation), some changes were detected and are summarized in Table 4.2.

Table 4.2 Changes of the surfaces of the specimens after fungi growth

Specimens		Changes of Surfaces after fungi growth
DOP	Control	insignificant
	Blend	insignificant
2-45	Control	white spot and small holes on the surface (see Figures 4. 10 and 4.12)
	Blend	many yellow spots (see Figures 4.13 and 4.14)
Lindol	Control	insignificant
	Blend	insignificant
Mesamoll	Control	insignificant
	Blend	insignificant

Figure 4.11 and Figure 4.12 show the surfaces of the 2-45 controls before and after fungal attack, at 25 x magnification and 300 x magnification respectively. Some white spots are found on the surface of the sample that was attacked by fungi, and no significant change is found on the surface of the sample without fungi growth. It means the composite of 2-45 control was degraded by fungi and caused a obviously appearance change. Figure 4.13 illustrates the change of the 2-45 blends before and after fungi attack. Remarkable discolouration is detected on the surface of sample which was attacked by fungi. Because lignin is the only component with colour, the discolouration of 2-45 blend may be explained as the change of chemical structure of lignin. The surfaces of the DOP blend, Lindol blend, and Mesamoll blend are shown in Figures 4.14 to 4.16 respectively. No considerable change is found. Based on the results of observation, the composites of 2-45 control and blend exhibit more visible changes than other composites, namely, they are more susceptible to fungi colonization than other composites. Although fungi also grew on the samples of DOP, Lindol, and Mesamoll controls and blends, the fungi growth had not caused visible change on the surfaces.

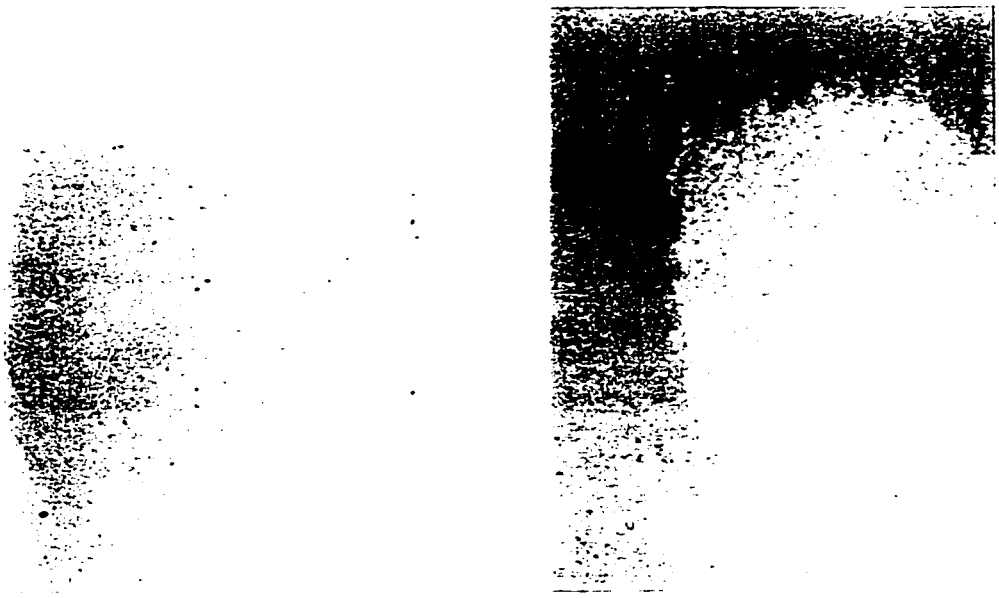


Figure 4.11 The surfaces of the 2-45 controls before (left) and after (right) fungi growth, 25 x magnification

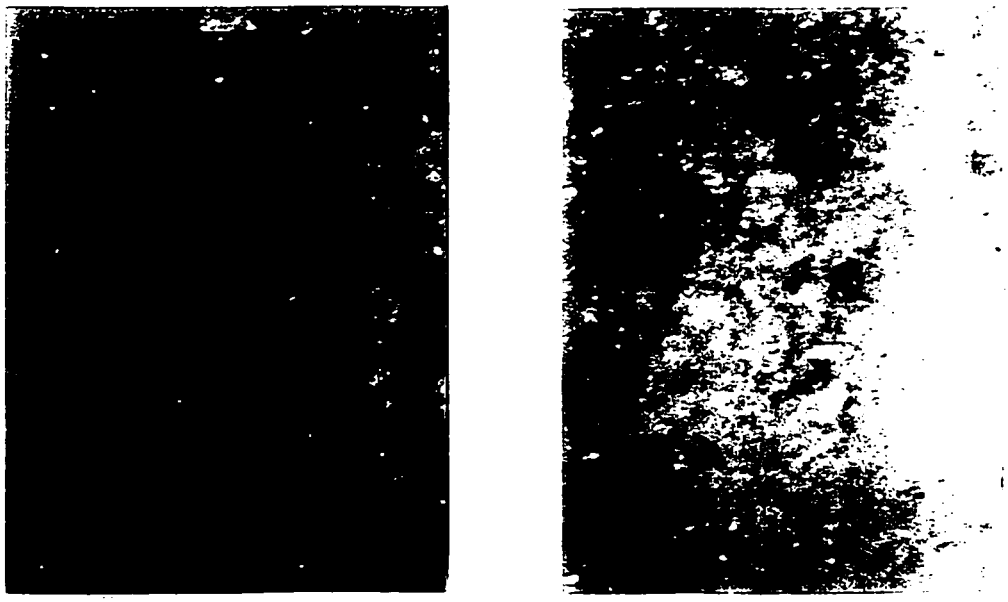


Figure 4.12 The surfaces of the 2-45 controls before (left) and after (right) fungi growth, 300 x magnification

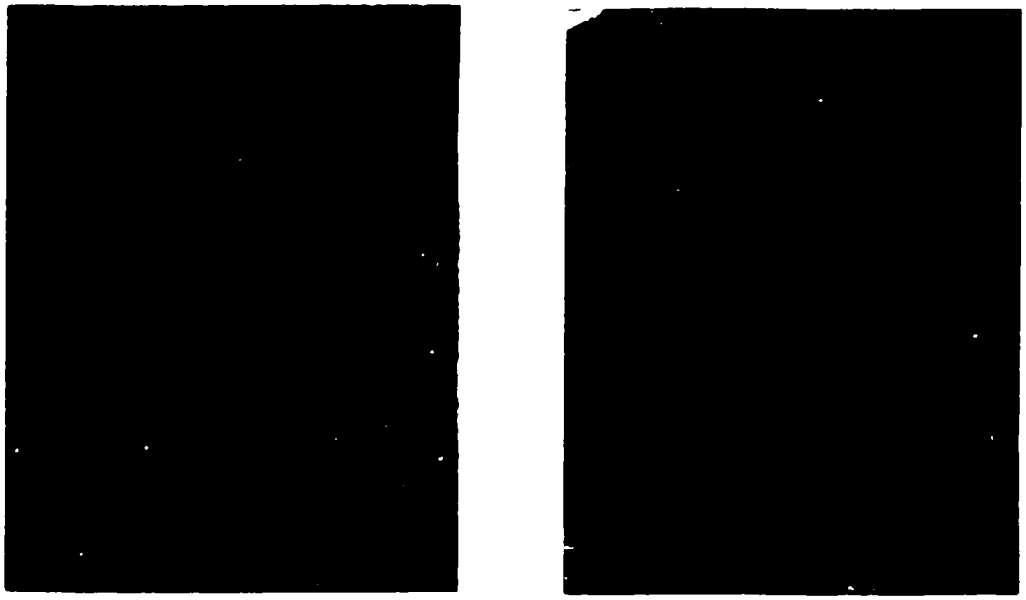


Figure 4.13 The surfaces of the 2-45 blends before (left) and after (right) fungi growth, 25 x magnification

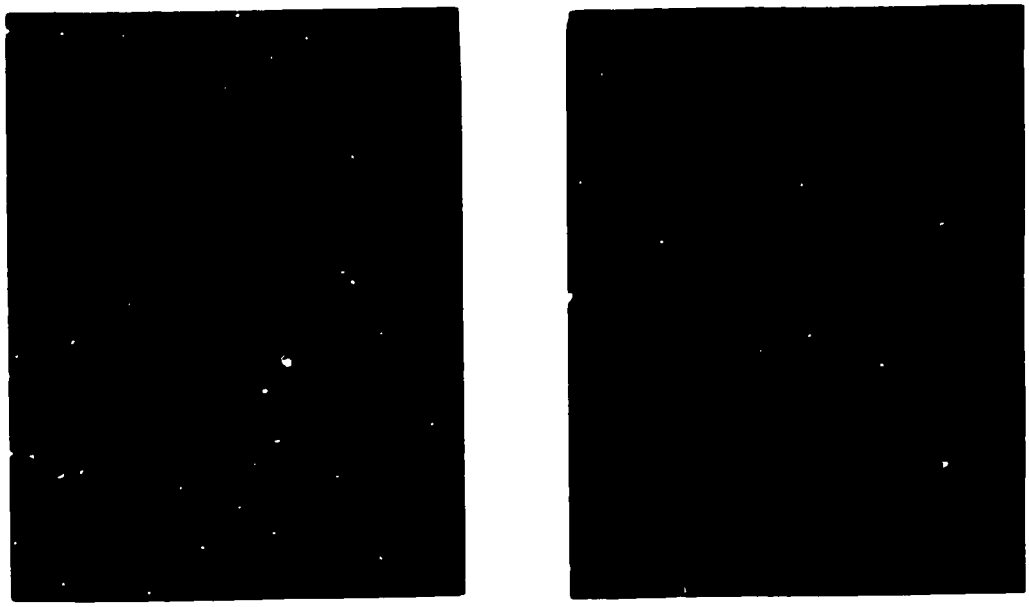


Figure 4.14 The surfaces of the DOP blends before (left) and after (right) fungi growth, 300 x magnification

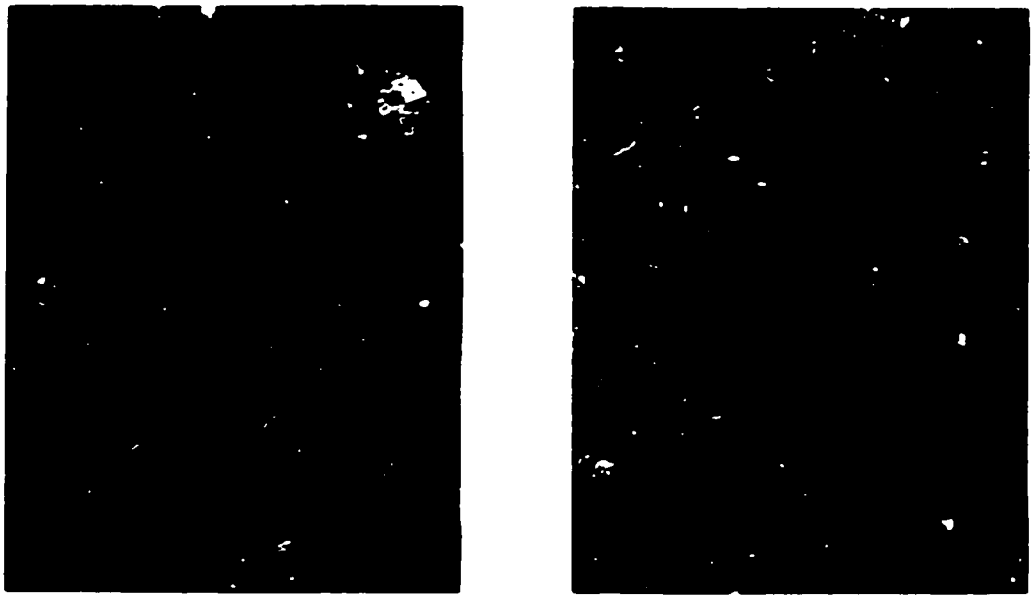


Figure 4.15 The surfaces of the Lindol blends before (left) and after (right) fungi growth 300 x magnification

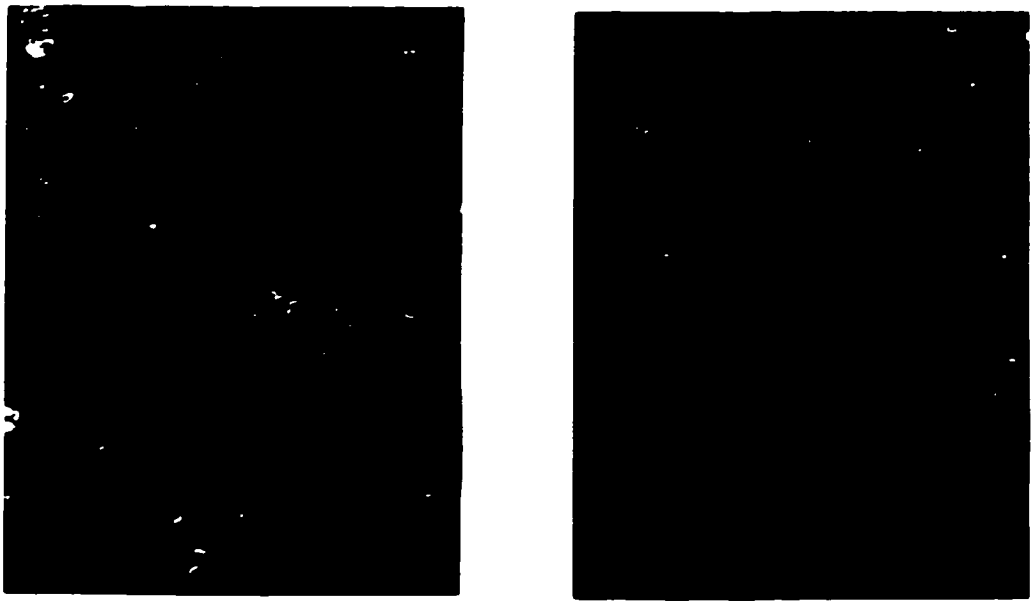


Figure 4.16 The surfaces of the Mesamoil blends before (left) and after (right) fungi growth, 300 x magnification

4.1.3 Changes of chemical structures

In order to understand the changes in the chemical structures of materials after fungi colonization, ATR correction of FTIR spectra were employed. The ATR/FTIR spectra of the samples were collected and presented in Figures 4.17 to 4.20. The most intensive and characteristic bands in these spectra are listed in Table 4.3. The wave numbers of representative bands of individual components and their composites are also shown in different columns of Table 4.3. The intention of these bonds is expressed as strong, medium, or weak. In this table, the subscript "v" means a bond is very strong or very weak, and the subscript "b" represents a broad band.

Table 4.3 The bands (cm^{-1}) and the assignment in the ATR/FTIR spectra of specimens

Copolymer VC-VAc	$CaCO_3$	Lignin	DOP	DOP control blend	2-45	2-45 control blend	Lindol	Lindol control blend	Mesamoll	Mesamoll control blend	Assignments[d]
			2959s		2953w						$\nu_{as}(CH_2)$
2930m		2915m	2931s	2925vs		2922m	2922w	2922m	2925vs	2926s	$\nu_{as}(CH_2)$
2850m		2830w							2854s	2861m	$\nu_{s}(CH_2)$
1730s			1727vs	1725s	1712vs	1723m		1738w		1740m	ν (C=O)
		1700m									
		1590m			1602m		1610s	1592w	1600m	1590w	ν (C-H_{arom})
							1585s				ν (C-H_{arom})
		1500s					1504vs		1490s		ν (C-H_{arom})
1428s	1430vsb	1430s		1425s							$\delta_{as}(CH_2)$
		1415s				1420s		1420s		1421	$\delta_{as}(CH_2)$
1370m		1320m	1380w	1380s		1377s		1380s			$\delta_{s}(CH_2)$
									1364s	1355vs	ν_{as}(S=O)
							1300s	1303w			ν(P=O)
			1267s	1256s	1260s	1256vs					ν_{as}(COO-C_{arom})
1235vs		1220s					1239s[a]	1235vs[a]		1237s	δ (C-H), ν (C-O)
1124w			1120s[b]	1122w[b]	1105s[b]	1105m[b]	1138vs	1142w			ν (C-C)
1100m		1100s	1070s	1075vw							ν (C-C)
970m							966vs[c]	966s[c]			γr (CH2)
								867m	857s	862s	ν_{s}(S=O)
			741m		707	700m					

Notes: s - strong, m - medium, w - weak, v - very, b – broad

ν_{as} and ν_{s} - asimmetric and simmetric stretching vibrations, δ_{as} and δ_{s} - asymmetric and symmetric deformation vibration

γ_{r} - rocking vibrations

a - attributed to ν_{as} (P-O-C_{arom}), b - to ν_{s} (COO-C_{arom}), c - to ν_{s} (P-O-C_{arom}), d - except $CaCO_3$

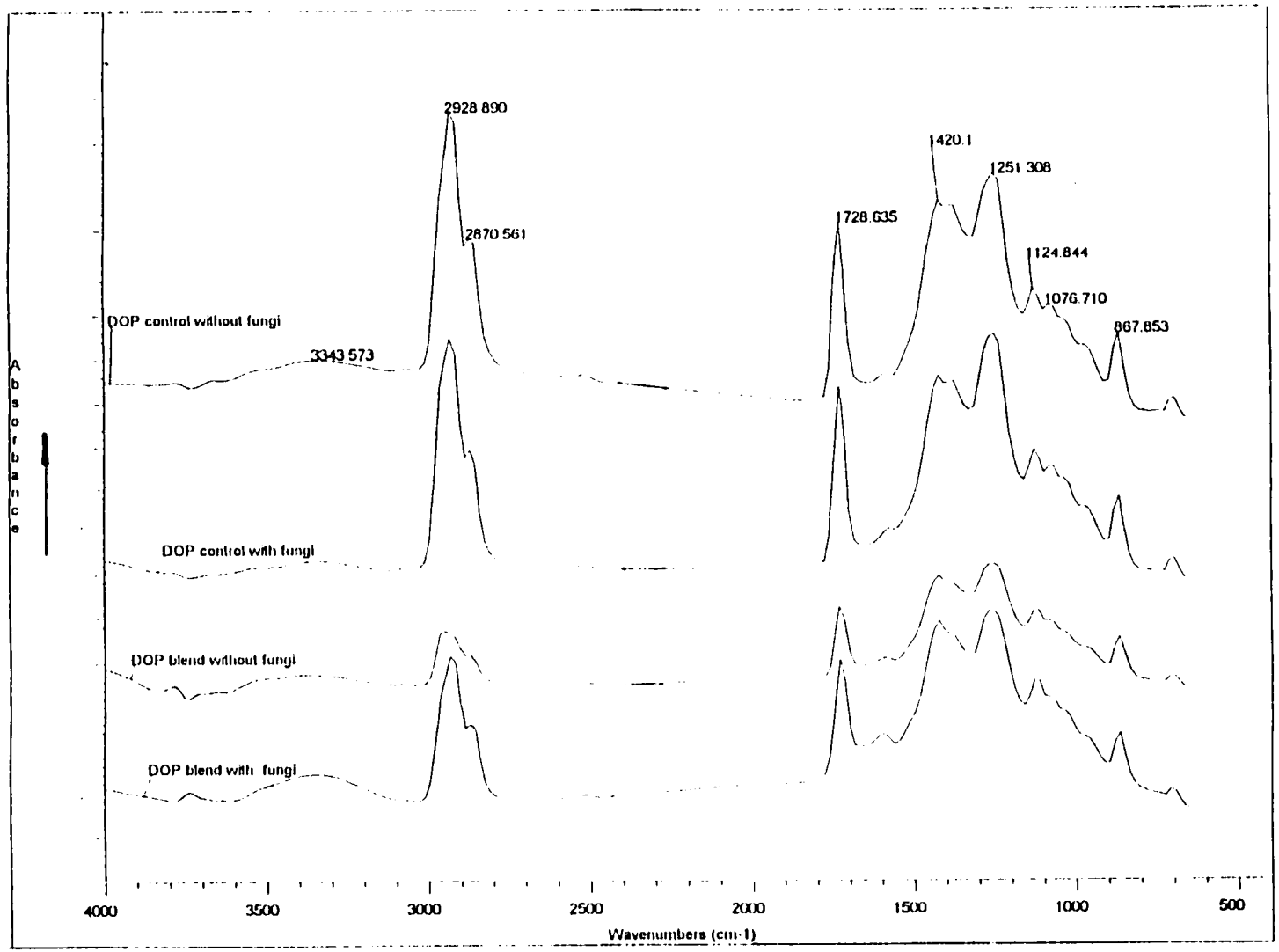


Figure 4.17 The ATR/FTIR spectra of the DOP control and the DOP blend with 32 scan at a resolution of 32cm^{-1}

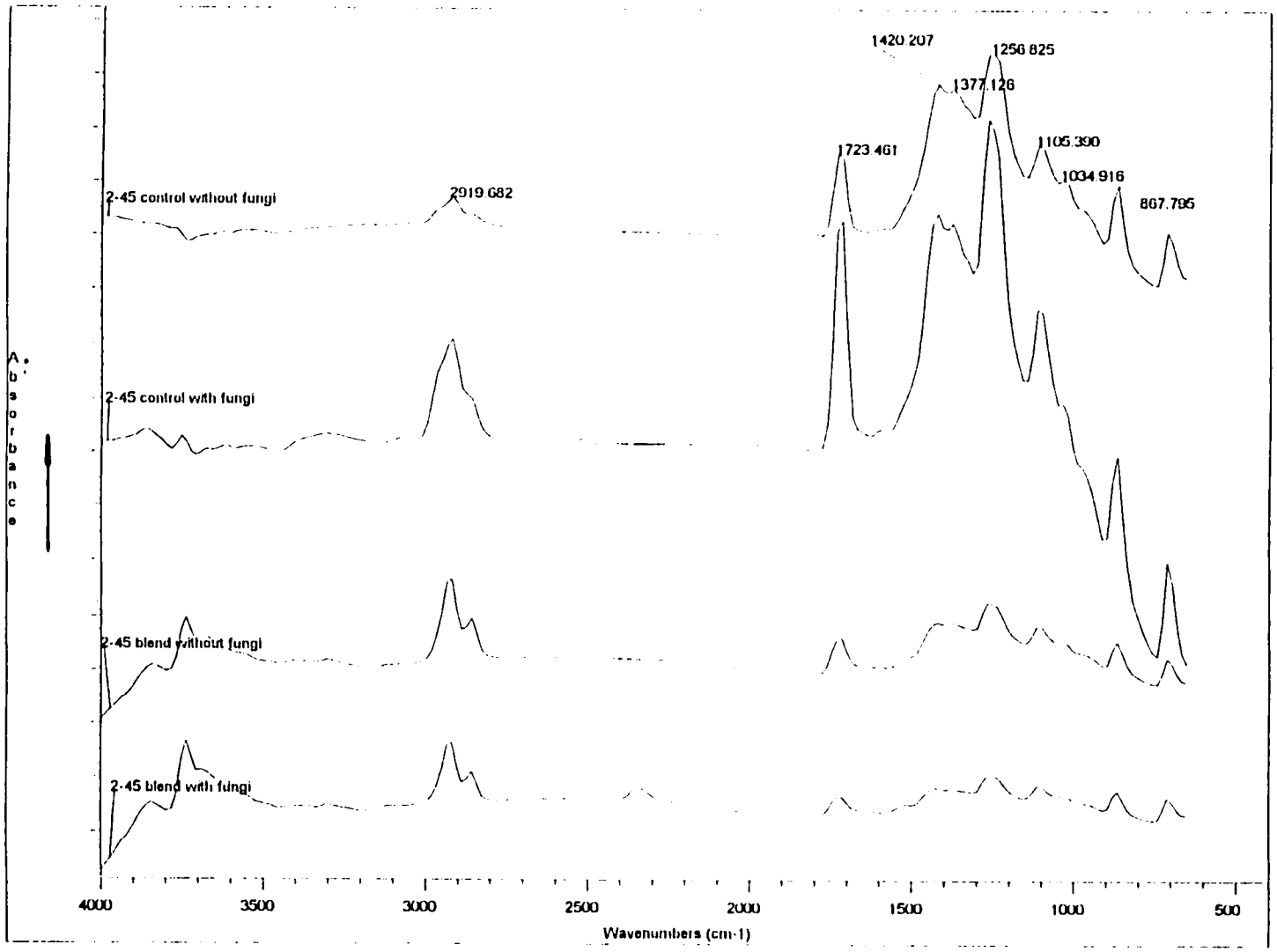


Figure 4.18 The ATR/FTIR spectra of the 2-45 control and the 2-45 blends with 32 scan at a resolution of 32cm^{-1}

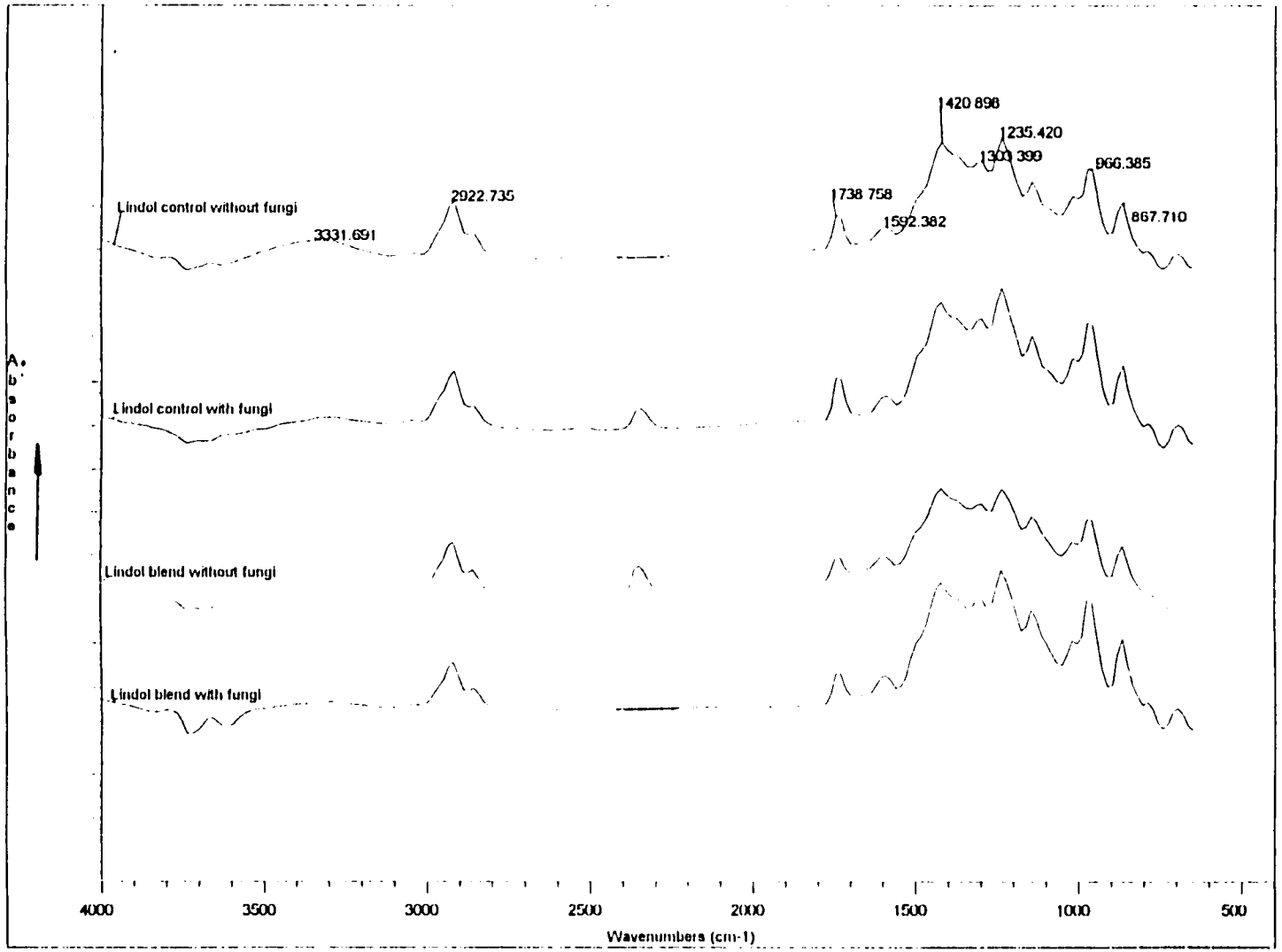


Figure 4.19 The ATR/FTIR spectra of the Lindol control and the Lindol blend with 32 scan at a resolution of $32cm^{-1}$

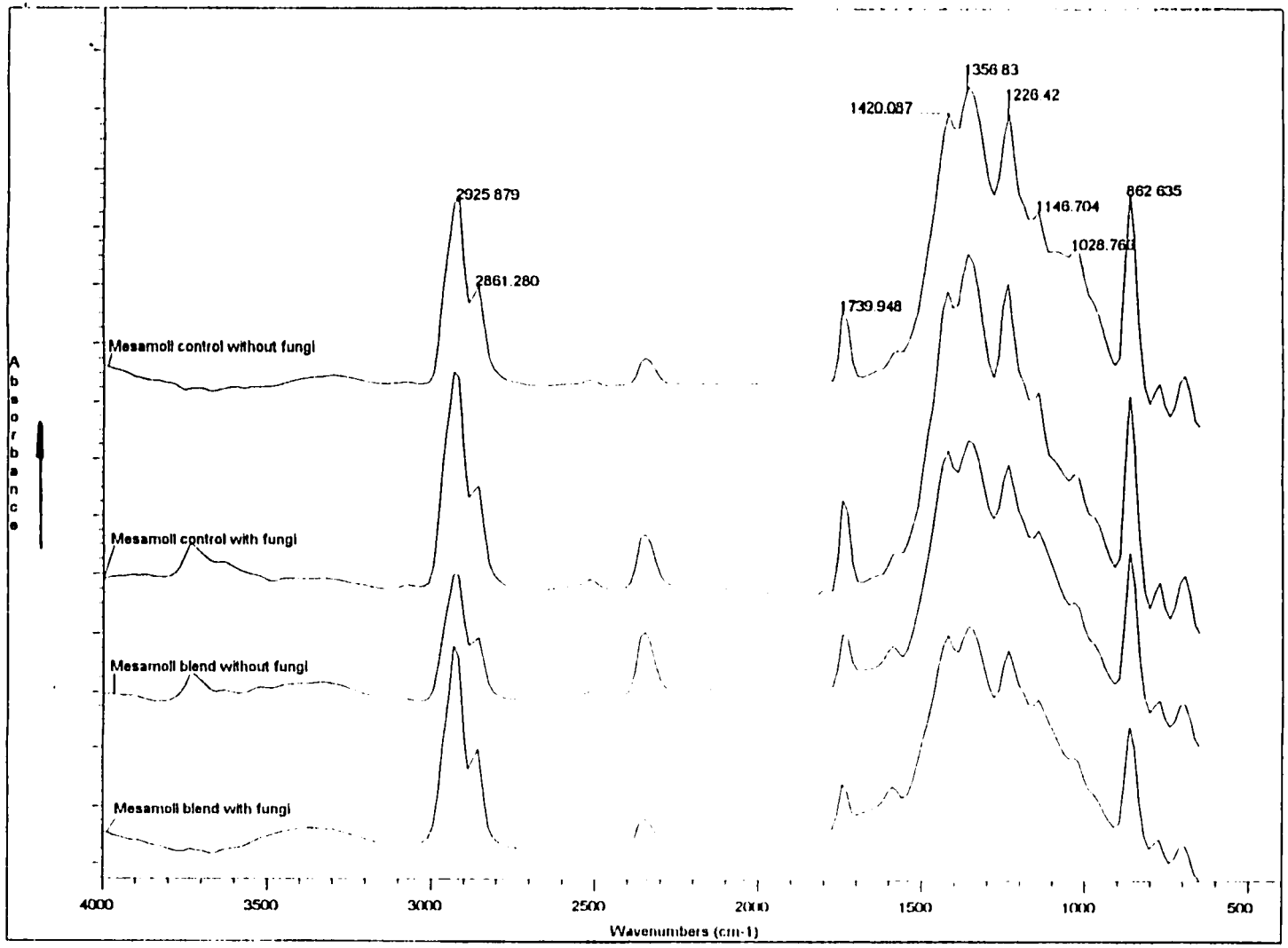


Figure 4.20 The ATR/FTIR spectra of the Mesamoll control and the Mesamoll blend with 32 scan at a resolution of 32cm^{-1}

The spectra shown in Figures 4.17 to 4.20 are similar because the main part of each belongs to the spectrum of PVC, and because many bands attributed to the plasticizers and lignin overlap the bands of PVC.

The strongest and broadest band found in the spectra of $CaCO_3$ is at 1430 cm^{-1}, which is hidden in the spectra of other ingredients of the composites in the region of 1000-1500 cm^{-1}. However, the presence of $CaCO_3$ can be identified by the strong and sharp band at 877 cm^{-1}. Since the content of $CaCO_3$ is constant and has not been degraded by fungi, as mentioned in Chapter 3, the band at 877 cm^{-1} is used as an internal standard to evaluate the absorbance values of the other bands.

Comparing the spectra of the DOP controls and the blends with the spectra of the 2-45 controls and blends, it was found that the bands related to stretch vibrations of aliphatic C-H bonds at 2925 cm^{-1} are stronger in the spectra of the DOP than in the spectra of 2-45 (see Fig 4.17 and Figure 4.18). Based on the chemical structures of DOP and 2-45, it is known that DOP contains more C-H bonds. Therefore, the strong absorbance of C-H bonds in the spectra of the DOP control and blend could be considered as an indication of the DOP in the composite.

Lindol is characterized by the band at 966 cm^{-1} (ν_s P-O-C_{arom}). Mesamoll is identified by the band at 1364 cm^{-1} (ν_{as} S=O) and the band at 857 cm^{-1} (ν_s S=O).

It is difficult to identify the bands that belong to lignin in the spectra of PVC-lignin blends. Therefore, the assessment of the impact of lignin on the biodegradability of the PVC-lignin blended materials might be indirect. The effects of fungi attack are studied by comparison of ATR/FTIR spectra of the PVC controls and the PVC-lignin blends before and after fungal growth.

Comparing to the spectra of the samples not attacked by fungi, no new bands were found in the spectra of samples exposed to fungi. Nevertheless, the changes in

absorbance of some bands are observed.

To standardize the calculation of the absorbance values of these bands (A_v), the ratio called relative absorbance is used:

$$A_v / A_{877} \qquad [4.1]$$

where: A_v - the absorbance of the band at v cm^{-1},

A_{877} - the absorbance of the band at 877 cm^{-1}, which is considered as the internal standard.

The relative values of band absorbance of different composites are illustrated in Figures 4.21to 4.24.

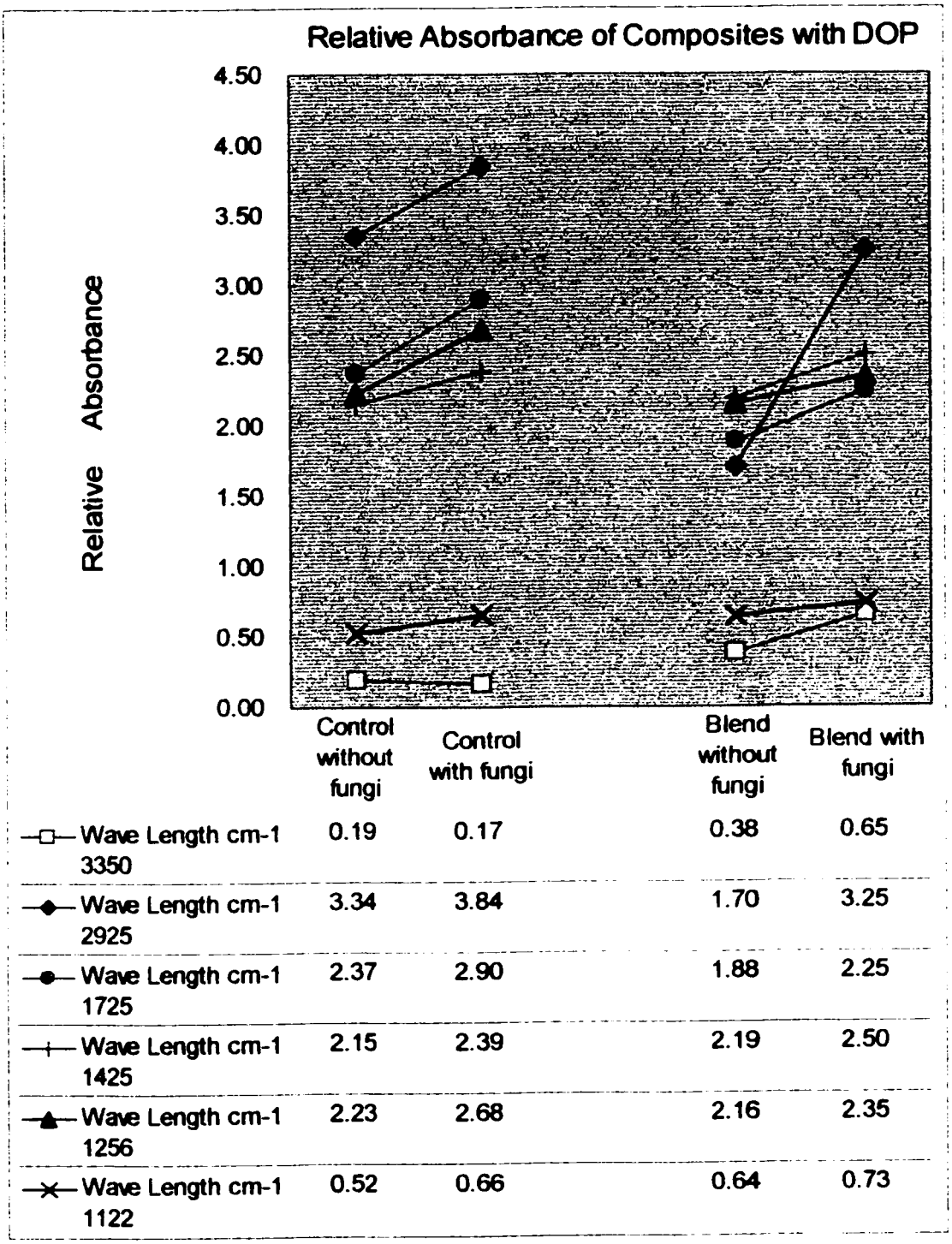


Figure 4.21 Relative absorbance of the DOP control and blend

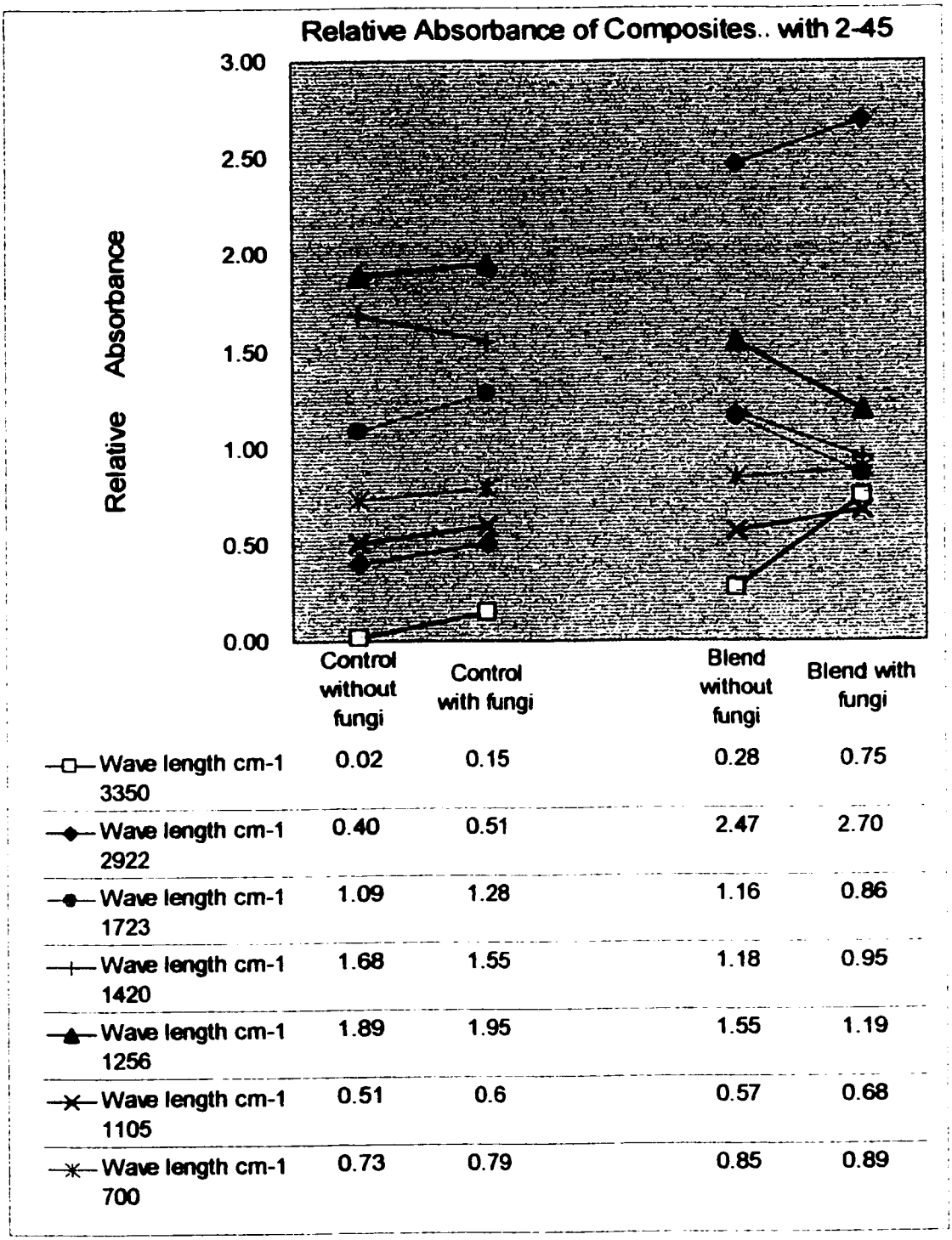
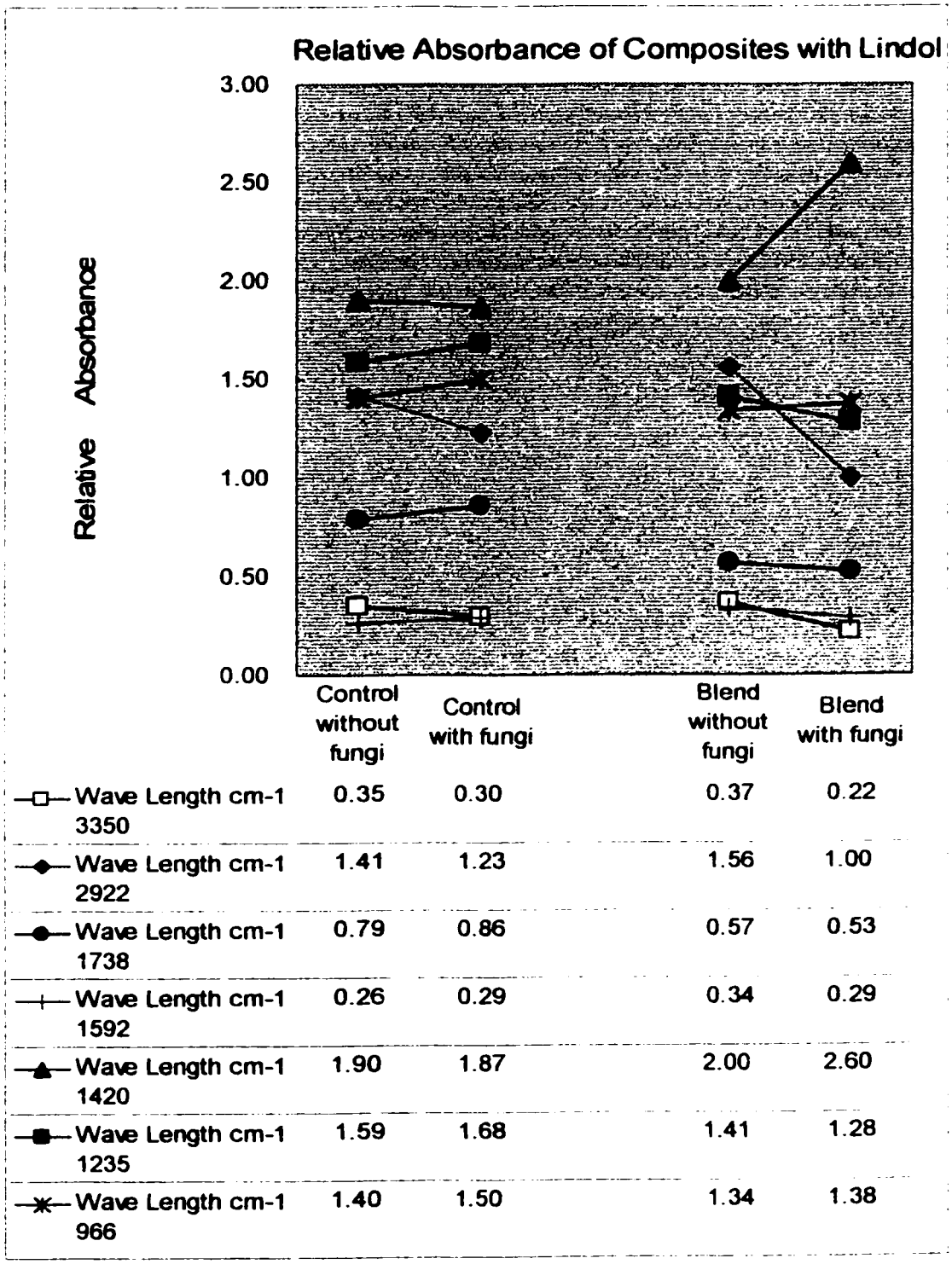
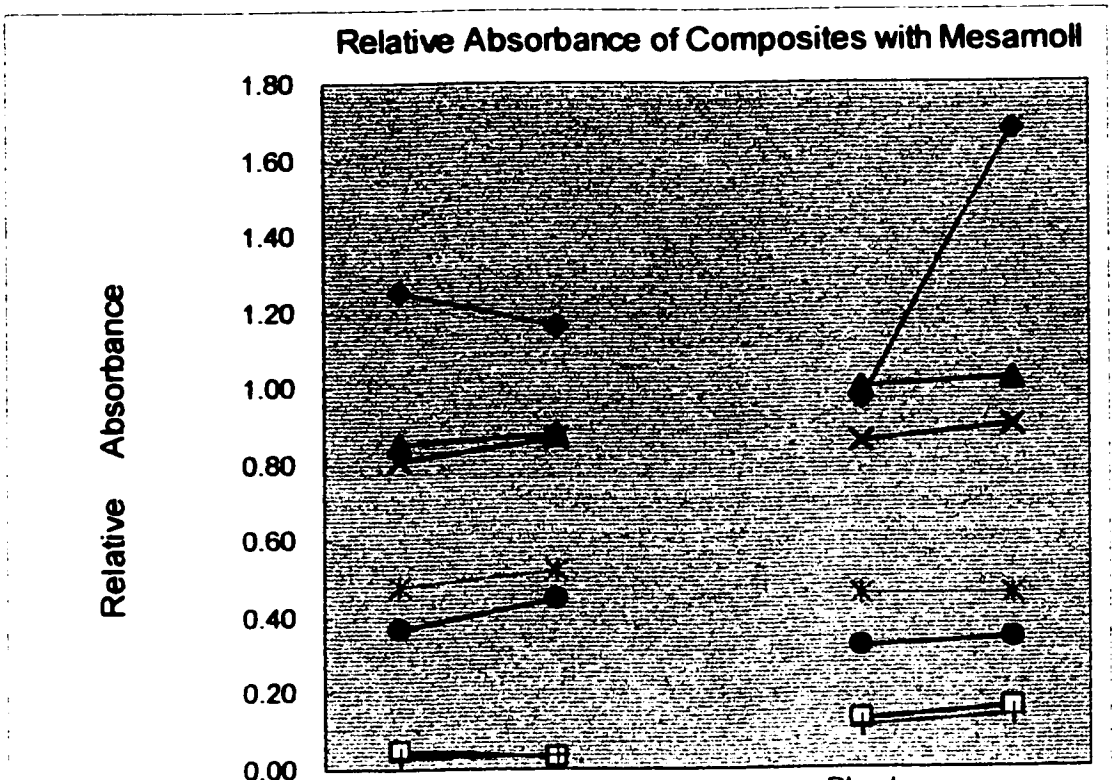


Figure 4.22 Relative absorbance of the 2-45 control and blend



	Control without fungi	Control with fungi	Blend without fungi	Blend with fungi
Wave Length cm-1 3350	0.35	0.30	0.37	0.22
Wave Length cm-1 2922	1.41	1.23	1.56	1.00
Wave Length cm-1 1738	0.79	0.86	0.57	0.53
Wave Length cm-1 1592	0.26	0.29	0.34	0.29
Wave Length cm-1 1420	1.90	1.87	2.00	2.60
Wave Length cm-1 1235	1.59	1.68	1.41	1.28
Wave Length cm-1 966	1.40	1.50	1.34	1.38

Figure 4.23 Relative absorbance of the Lindol control and blend



	Control without fungi	Control with fungi	Blend without fungi	Blend with fungi
Wave Length cm-1 3350	0.05	0.03	0.13	0.16
Wave Length cm-1 2925	1.25	1.16	0.97	1.68
Wave Length cm-1 1739	0.37	0.45	0.32	0.34
Wave Length cm-1 1590	0.032	0.036	0.11	0.14
Wave Length cm-1 1420	0.85	0.88	1.00	1.02
Wave Length cm-1 1355	0.81	0.87	0.86	0.90
Wave Length cm-1 1238	0.48	0.52	0.46	0.46

Figures 4.21 to 4.24 present the changes of absorbance which denote the changes in chemical structure. The difference (ΔF) between the relative absorbance values of a band in the spectra of a sample attacked by fungi [A_{Rv} (F)] and that in the spectra of a sample not attacked by fungi [A_{Rv} (NF)] is obtained.

$$\Delta F = A_{Rv}(F) - A_{Rv}(NF) \qquad [4.1]$$

The results for the PVC controls and the PVC-lignin blends with four plasticizers DOP, 2-45, Lindol, and Mesamoll are listed in Table 4.4.

Table 4.4 The values of ΔF for PVC controls and PVC-lignin blends with different plastisizers

Specimens		**Bands at wavelength (cm^{-1})**						
DOP		3350	2925	1725	1425	1256	1122	
	Control	0	0.5	0.5	0.2	0.4	0.1	
	Blend	0.3	1.5	0.4	0.3	0.2	0	
2-45		3350	2922	1723	1420	1256	1105	700
	Control	0	0.1	0.2	-0.1	0.1	0.1	0.1
	Blend	0.5	0.2	-0.3	-0.2	-0.3	-0.1	-0.2
Lindol		3350	2922	1738	1592	1420	1235	966
	Control	0	-0.2	0.1	0	0	0.1	0.1
	Blend	-0.1	-0.6	0	0	0	0.1	0
Mesamoll		3350	2926	1740	1590	1421	1355	1237
	Control	0	-0.1	0.1	0	0	0.1	0
	Blend	0	0.5	0	0	0	0	0

According to the results presented in Table 4.4, the attacks of fungi on the DOP control and the blend introduce the most considerable changes in comparison to samples with the three other plasticizers. The absorbance of the bands attributed to the aliphatic C-H bonds at 2925 cm^{-1} and the carbonyl groups at 1725 cm^{-1} increases in both the PVC control and the PVC-lignin blend significantly. The absorbance of the

band at 1256 cm^{-1} is attributed to stretching asymmetric vibrations of the COO-C_{arom} bond in the ester group increased. The absorbance of a band of the PVC-lignin blend at 3350 cm^{-1} (vOH) increases more obviously. The increase of absorbance of the bands could be interpreted as a result of migration of DOP to the surface of the specimens. The increase of the DOP blend at 1256 cm^{-1} is stronger than that of the control, which is attributed to the weak compatibility of DOP with lignin (Hui Zhu, 2000). In other word, DOP migrate to the surface from the PVC-lignin blend bulk much easier than from the PVC control bulk. The increase of absorbance at 3350 cm^{-1} (vOH) might indicate hydrolysis of the ester group of DOP.

The 2-45 control and blend are more sensitive to fungi attack than other composites. Hydrolysis occurring in the 2-45 control and the blend are more evident than that in the other composites. This fact is reflected by the increase of absorbance of the band at 3350 cm^{-1}. The absorbance of the bands at 1723 cm^{-1} and 1256 cm^{-1} decline remarkably that suggests the destruction of the relative functional groups. The control seems to be less sensitive than the blend because the changes occurring in the blend are more evident than that in the control.

The specimens with Lindol and Mesamoll attacked by fungi have undergone quite different processes. Small or no changes were observed on the bands at 3350 cm^{-1} and 1740 cm^{-1}. In these spectra the band of 1740 cm^{-1} is evidently attributed to v C=O of the VAc part of the copolymer because Lindol and Mesamoll do not have this bond. The bands at 1590 cm^{-1} (v C-H_{arom}), 1235 cm^{-1}, 966 cm-1 (v P-O-C_{arom}), and 1355 cm^{-1} (v S=O), which are attributed to the plasticizers, have little or no changes. However, the absorbance of the bands at 2922 cm^{-1} and 2926 cm^{-1} has changed, and the changes in the blends are more significant than in the controls.

Since the specimens are not transparent, the absorbance of ATR/FTIR was very

weak; therefore, the changes of chemical structures were not very evident. In order to investigate the effects of fungi on the PVC controls and the PVC-lignin blends, the experiment of Stage 2 was conducted.

4.2 Stage 2

In Stage 2, film specimens following the formulations shown in Table 3.16 were tested. The specimens were weighed and the FTIR spectra of these specimens were collected prior to being inoculated with *Aspergillus niger*. After incubating with *Aspergillus niger* for 28 days at temperature 28°C and 100% relative humidity, the samples were treated in the same way as outline in Stage 1. In this stage, the observation of visible effect, weight loss, and FTIR spectroscopy were employed to evaluate the effects of potential biodegradation.

4.2.1 Observation of visible effects

Ratings of fungi growth observed by an optical microscope at 50x magnification are shown in Table 4.5. The rating order of fungus growth on PVC-lignin blend samples was found to be as follow:

2-45 blend > Mesamoll Blend > DOP blend > Lindol blend.

As in Stage 1, the sample of 2-45 blend in Stage 2 had a slight discolouration while the appearances of other samples showed negligible changes. In addition, in Stage 2, all the samples expressed a remarkable dominant fungi growth on the edges of the samples (see Figure 3.3).

Table 4.5 Ratings of fungus growth on the surfaces of the specimens (Stage 2) (ASTM G21-96)

Growth on specimen	DOP		2-45		Lindol		Mesamoll	
	Control	Blend	Control	Blend	Control	Blend	Control	Blend
Rating	30%	40%	40%	80%	20%	40%	20%	50%

4.2.2 Change of weight

Table 4.6 introduces the weight losses and the percent of changes of the samples. After fungi attack, all the samples showed weight losses. Consequently, it was concluded that all of the samples were available for fungi. The data shows that the weight losses of all the blends are much higher than the corresponding controls. The ratio of the weight loss percentage of the blends to the percentage of the corresponding controls ranges between 1.73 and 2.67. It was assumed that the presence of Alcell lignin increases the bio-availability of plasticized PVC material. Among the various plasticizers, 2-45 (both the control and the blend) were much more susceptive to fungi attack than other plasticizers by 2.45 % and 5.33 % respectively. Lindol showed the highest resistance to fungi attack, less than 0.5 % for control. Furthermore, comparing the weight-loss percentages of all the blends to that of the corresponding controls, it clearly shows that the blends are more sensitive to biodegradation than controls.

The order of weight loss for PVC controls is as follow:

2-45 > Mesamoll > DOP > Lindol.

The order of weight loss for PVC-lignin blend is the same as the controls.

Table 4.6 The weight changes of the samples after fungal attack

Specimen		Weight (g)		Percentage of weight loss %	Ratio of weight loss percentage (Blend / Control)
		before attack	after attack		
DOP	Control	0.0391	0.0386	1.28	1.73
	Blend	0.0361	0.0353	2.22	
2-45	Control	0.0408	0.0398	2.45	2.18
	Blend	0.0413	0.0391	5.33	
Lindol	Control	0.0432	0.0430	0.46	2.67
	Blend	0.0407	0.0402	1.23	
Mesamoll	Control	0.0386	0.0381	1.30	2.04
	Blend	0.0411	0.0400	2.68	

4.2.3 Changes within the chemical structure of composites

The FTIR spectra of specimens of this stage (see Table 3. 16), collected with 32 scans at a resolution of 4 cm^{-1}, are presented in Figures 4.25 to 4.32. The main changes of the absorbance of the bands are summarized in Tables 4.7 to 4.10. The absorbance of some bands was measured; in addition, the differences of the absorbance ($\Delta F'$) before and after fungi growth are calculated and shown in Tables 4.7 to 4.10.

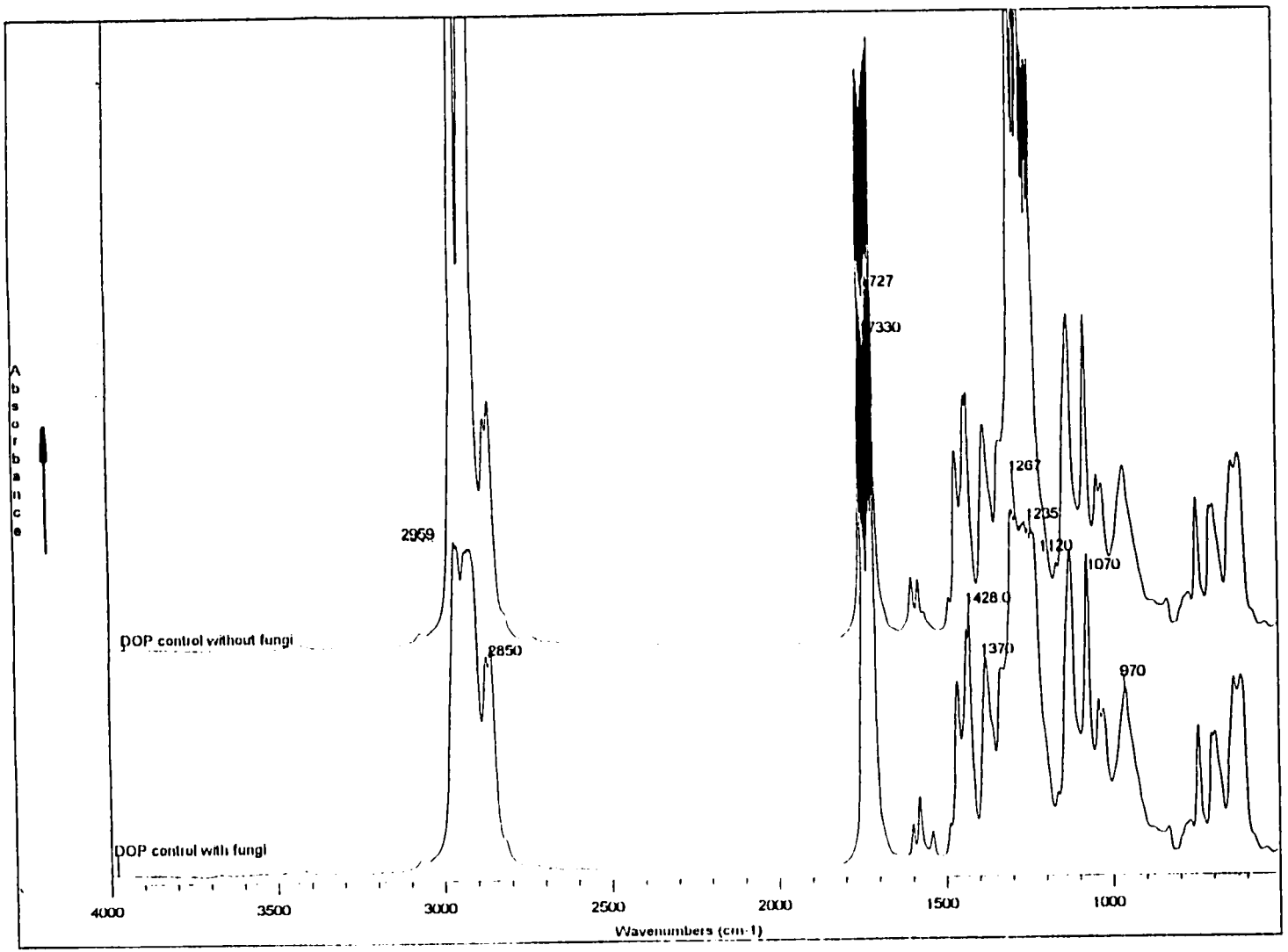


Figure 4.25 The FTIR spectroscopy spectra of the DOP control

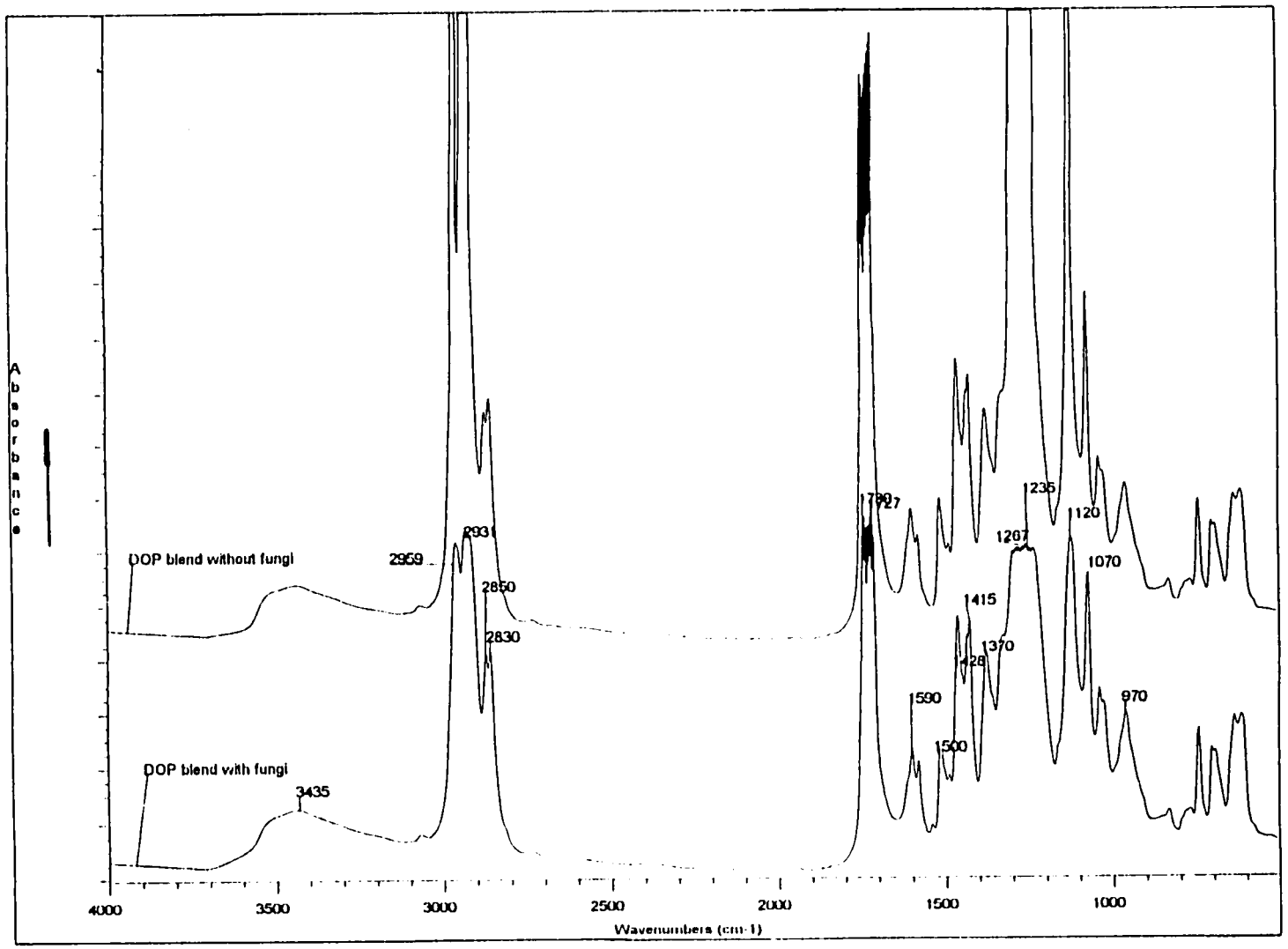


Figure 4.26 The FTIR spectroscopy spectra of the DOP blend

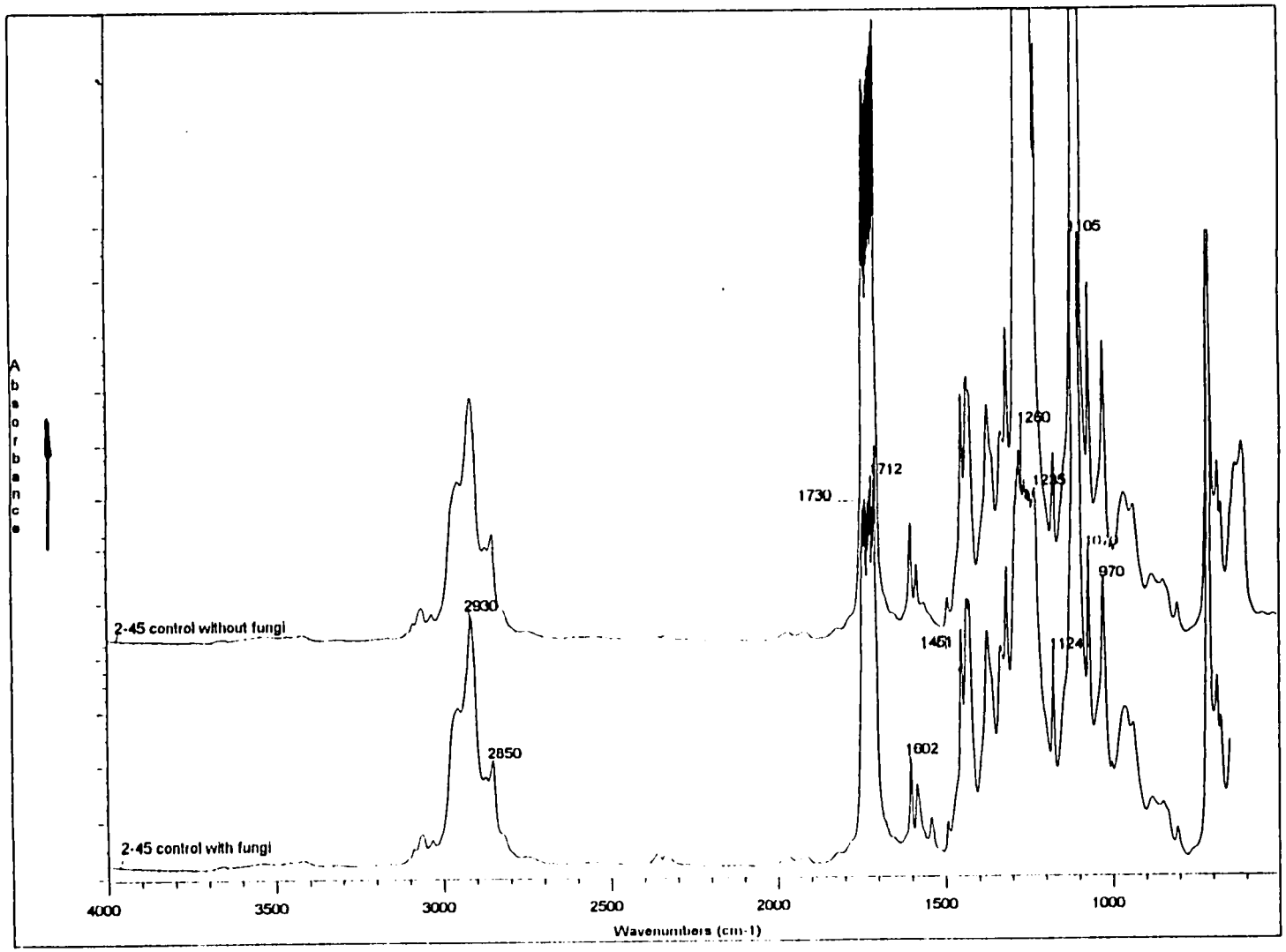


Figure 4.27 The FTIR spectroscopy spectra of the 2-45 control.

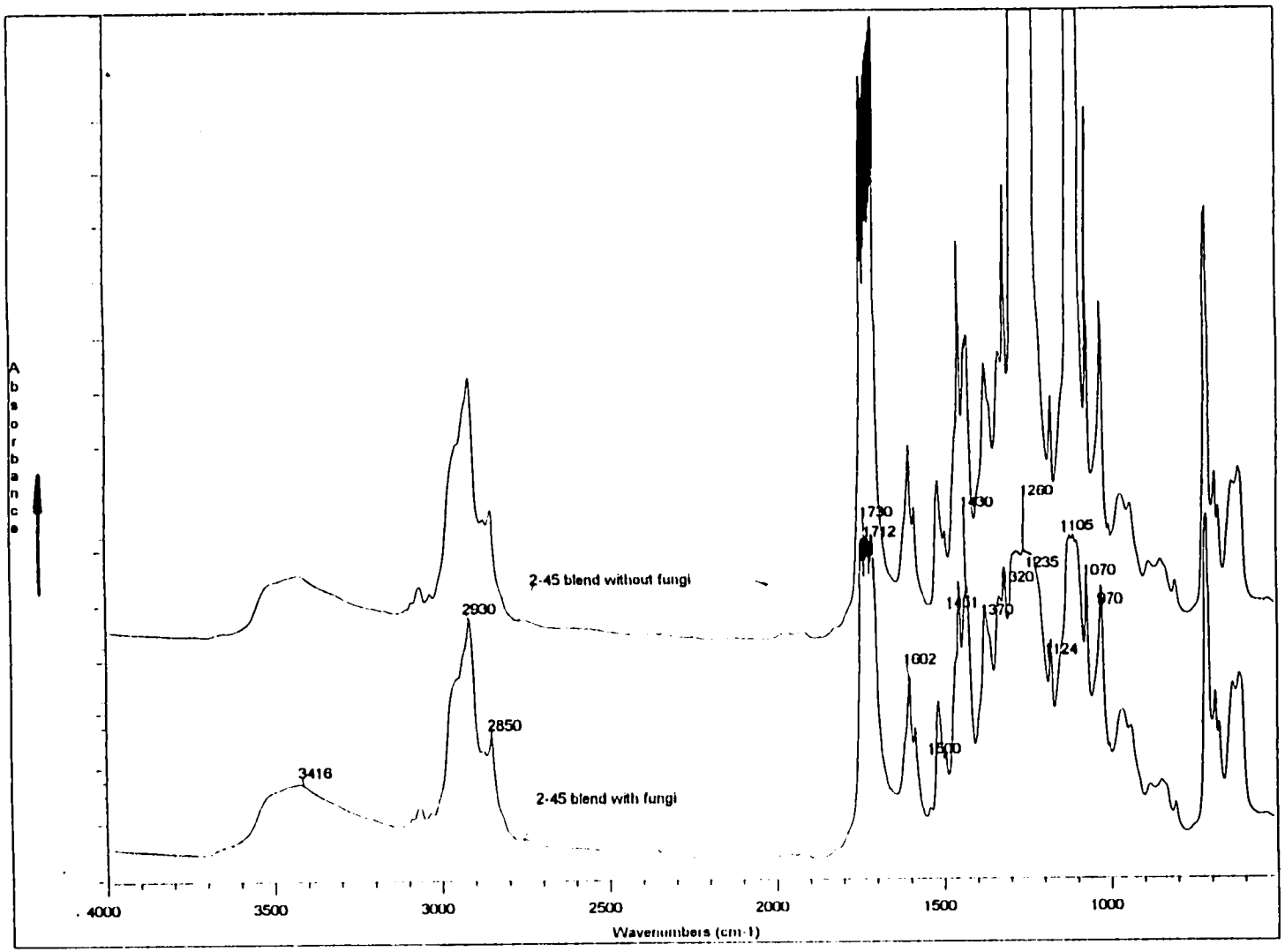


Figure 4.28 The FTIR spectroscopy spectra of the 2-45 blend

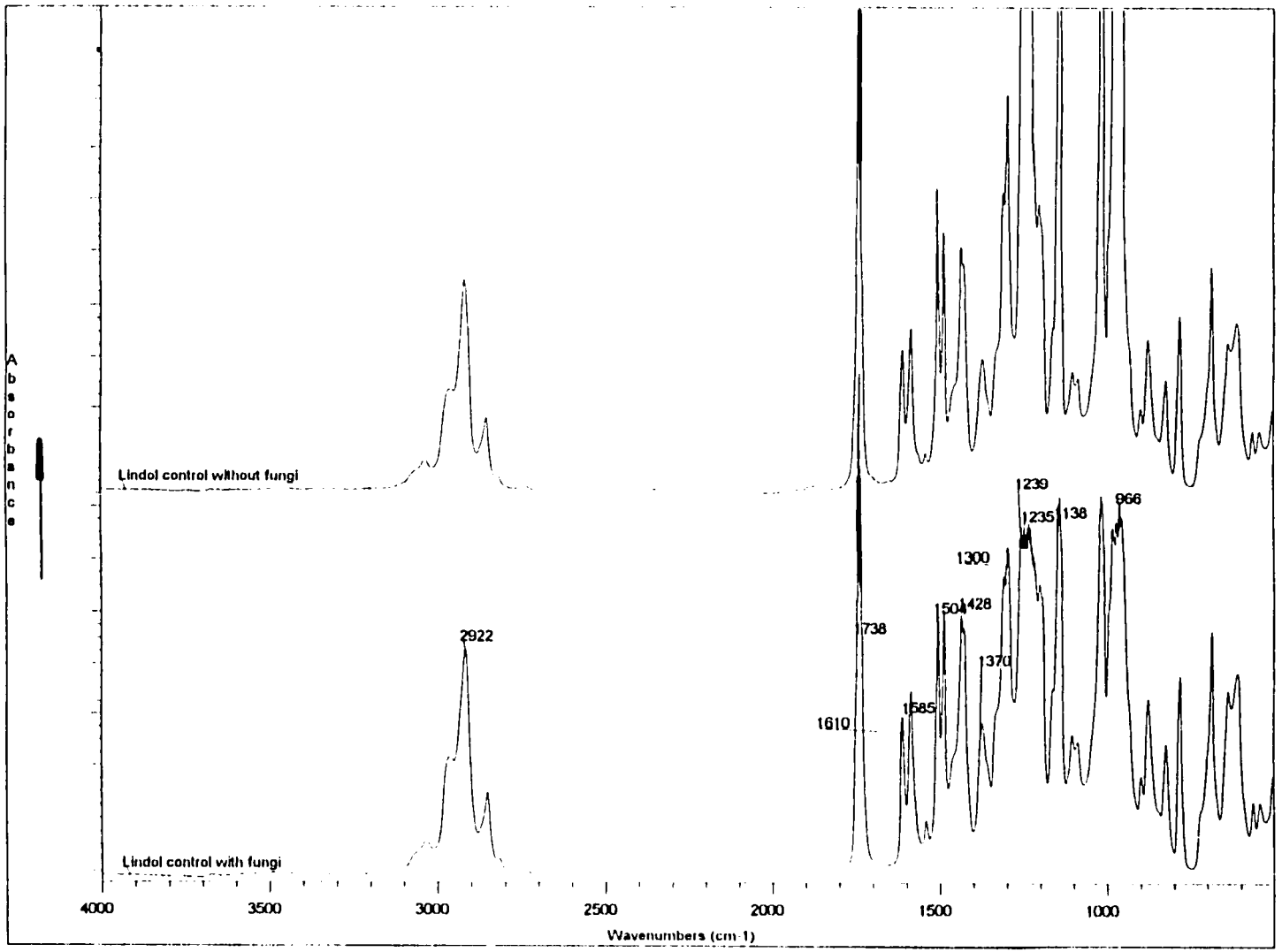


Figure 4.29 The FTIR spectroscopy spectra of the Lindol control

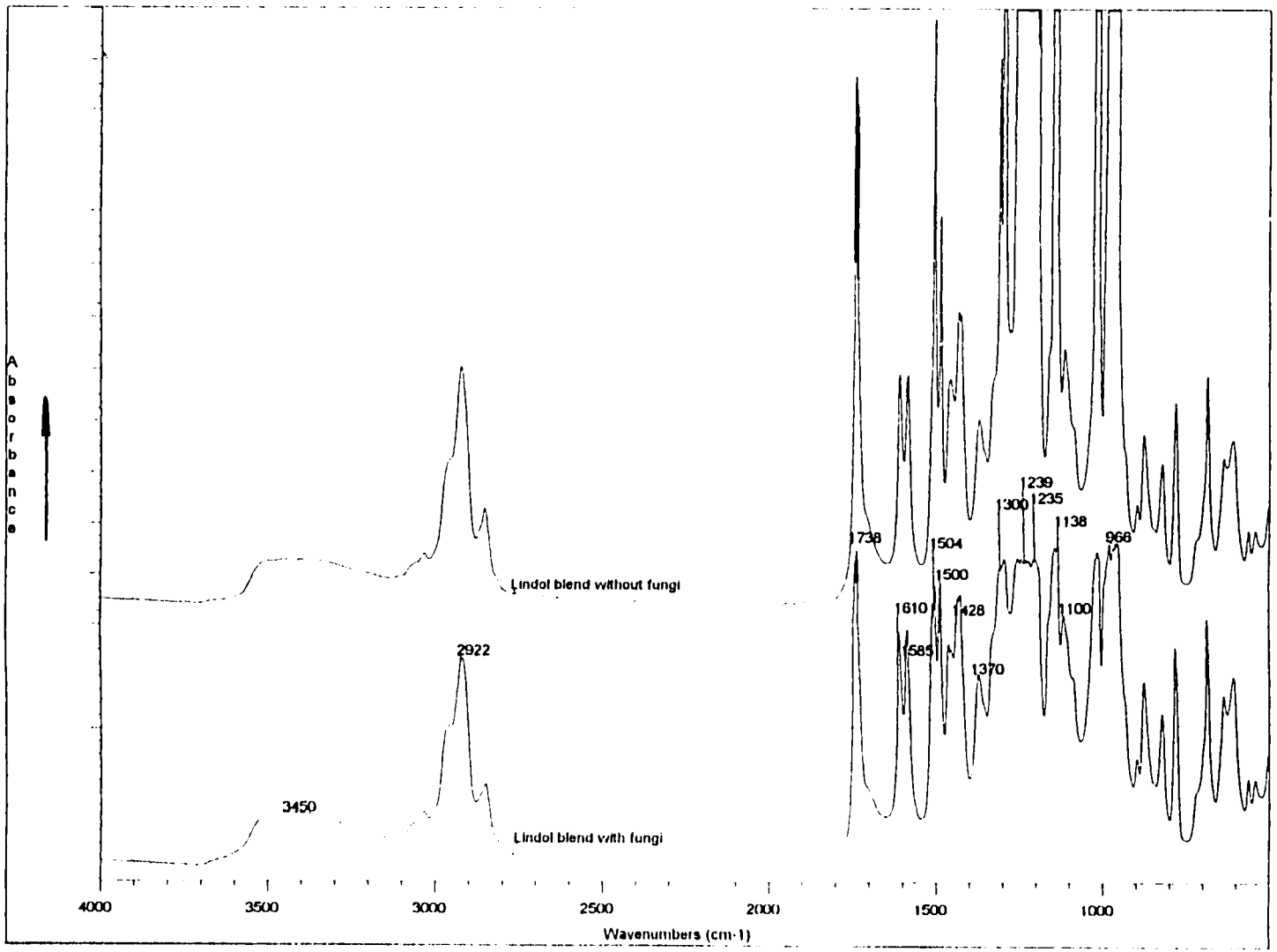


Figure 4.30 The FTIR spectroscopy spectra of the Lindol blend

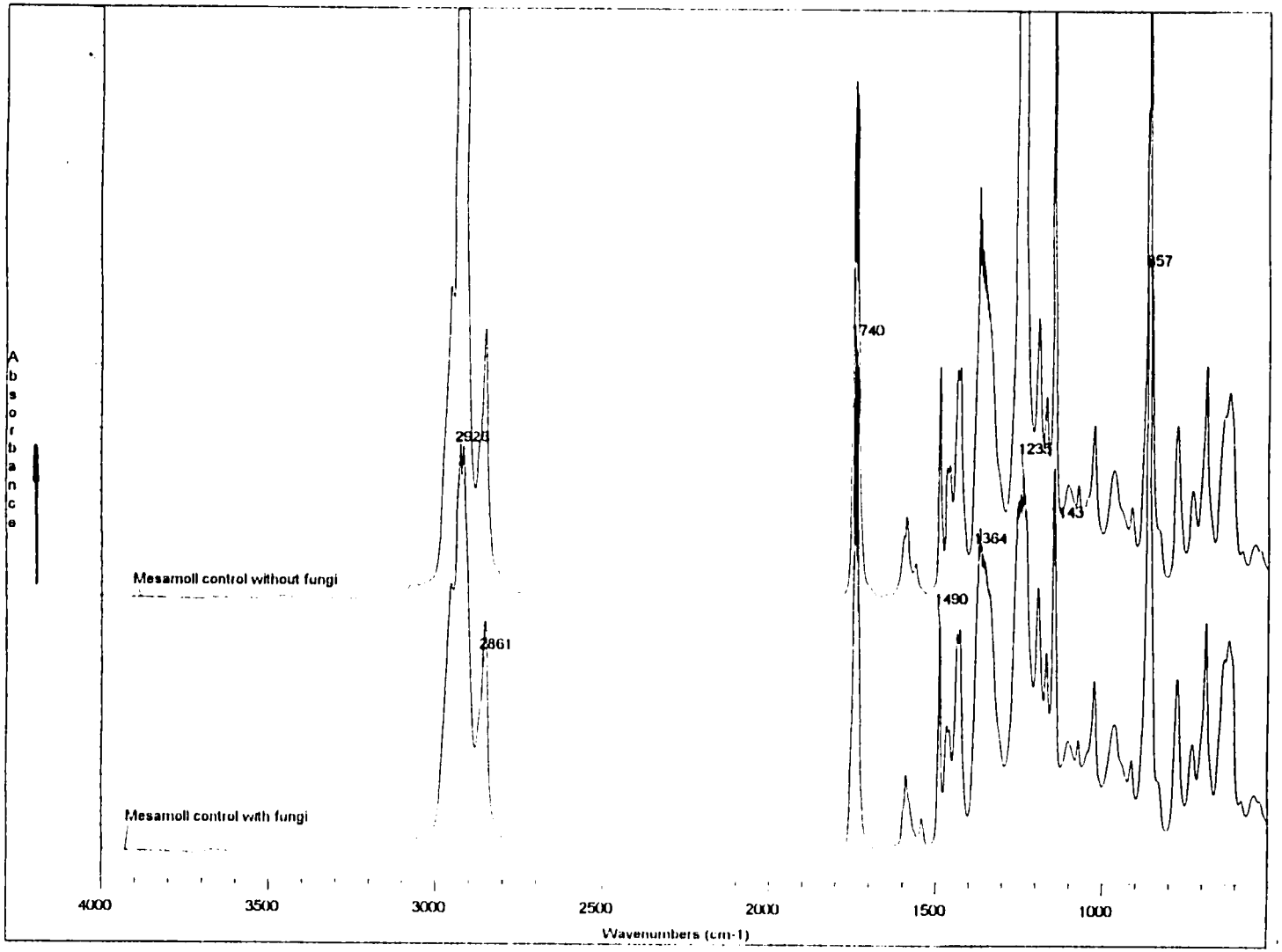


Figure 4.31 The FTIR spectroscopy spectra of the Mesamoll control

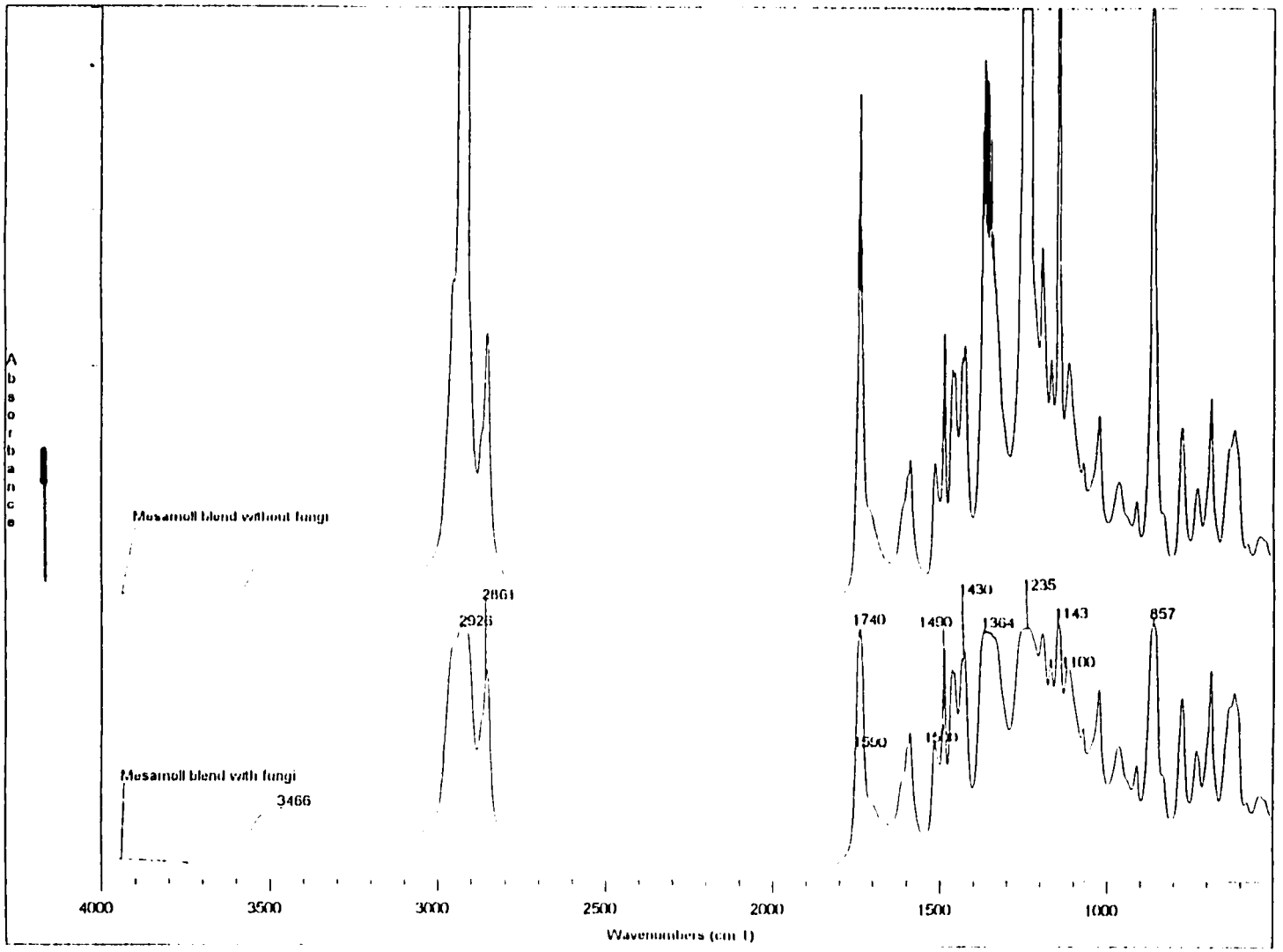


Figure 4.32 The FTIR spectroscopy spectra of the Mesamoll blend

Table 4.7 The changes of absorbance of bands (cm^{-1}) in the FTIR spectra of the DOP control and the blend specimens

Wavenumber (cm^{-1})	Assignment (band)	Assignment (component)	Observation of change in control specimen	Observation of change in blend specimen
3450	ν(OH)	lignin, hydrolysate of ester	insignificant	increase, $\Delta F'=0.2$
2959_s, 2931_s	$\nu_{as}(CH_2)$	DOP	remarkable decrease	remarkable decrease
2850_m	$\nu_s(CH_2)$	Copolymer (VC/VAc)	insignificant	insignificant
2830_w	$\nu_s(CH_2)$	lignin		not significant
1727_{vs}	ν(C=O)	DOP	remarkable decrease	remarkable decrease
1730_s	ν(C=O)	VAc	remarkable decrease	remarkable decrease
1590_m, 1500_s, 1430_s	$\nu(C\text{-}H_{arom})$	lignin		insignificant
1428_s,	$\delta_{as}(CH_2)$	Coplymer (VC/VAc)	insignificant	insignificant
1415_s	$\delta_{as}(CH_2)$	lignin		insignificant
1370_m	$\nu_s(CH_2)$	Copolymer (VC/VAc)	insignificant	insignificant
1267_s	δ_{as} (COO-Carom)	DOP	remarkable decrease	remarkable decrease
1235_{vs}	δ(C-H), ν(C-O)	VAc	remarkable decrease	remarkable decrease
1120_s	ν(C-C)	DOP	insignificant	decrease
1070_s	ν(C-C)	DOP	insignificant	insignificant
970_m	$\gamma_r(CH_2)$	Copolymer (VC/VAc)	insignificant	insignificant
$<800_m$	ν(C-Cl)[a]	Copolymer (VC/VAc)	insignificant	insignificant

a: (Garton, 1992)

Table 4.8 The changes of absorbance of bands (cm^{-1}) in the FTIR spectra of the 2-45 control and blend specimens

Wavenumber (cm^{-1})	Assignment (band)	Assignment (component)	Observation of change in control specimen	Observation of change in blend specimen
3416	ν(OH)	lignin, hydrolysate of ester	insignificant	increase $\Delta F'$=0.15
2930_m	$\nu_{as}(CH_2)$	Copolymer (VC/VAc)	insignificant	insignificant
2850_m	$\nu_s(CH_2)$	Copolymer (VC/VAc)	insignificant	insignificant
1730_s	ν(C=O)	VAc	remarkable decrease	remarkable decrease
1712_{vs}	ν(C=O)	2-45	remarkable decrease	remarkable decrease
1602_m	ν(C-H_{arom})	2-45	insignificant	insignificant
1500s	ν(C-H_{arom})	lignin		insignificant
1451	$\delta_{as}(CH_2)$	2-45	insignificant	decrease $\Delta F'$= - 1.0
1428,1430	$\delta_{as}(CH_2)$	lignin, Copolymer (VC/VAc)	insignificant	insignificant
1370_m	$\delta_s(CH_2)$	Copolymer (VC/VAc)	insignificant	insignificant
1320_m	$\delta_s(CH_2)$	lignin		insignificant
1260_s	ν_{as} (COO-Carom)	2-45	decrease remarkable	decrease remarkable
$1235_{vs.}$	ν(C-O) δ(C-H)	VAc	decrease remarkable	decrease remarkable
1124_w	ν(C-C)	Copolymer (VC/VAc)	insignificant	insignificant
1105_s	ν_s (COO-Carom)	2-45	insignificant	decrease remarkable
1070_s	ν(C-C)	2-45	decrease $\Delta F'$= - 0.64	decrease $\Delta F'$= - 2.1
970_m	$\gamma_r(CH_2)$	Copolymer (VC/VAc)	insignificant	insignificant
$<800_m$	ν(C-Cl)	Copolymer (VC/VAc)	insignificant	insignificant

Table 4.9 The changes of absorbance of bands (cm^{-1}) in the FTIR spectra of the Lindol control and the blend specimens

Wavenumber (cm^{-1})	Assignment (band)	Assignment (component)	Observation of change in control specimen	Observation of change in blend specimen
3450	ν(OH)	lignin hydrolysate of ester	insignificant	increase $\Delta F'=0.1$
2922_m	$\nu_{as}(CH_2)$	Copolymer (VC/VAc) Lindol	insignificant	decrease $\Delta F'= - 0.3$
1738_s	ν(C=O)	VAc	insignificant	decrease obviously $\Delta F'= - 2.5$
1500_s, 1590_m	$\nu(C\text{-}H_{arom})$	lignin		not significant
1610_s, 1585_s	$\nu(C\text{-}H_{arom})$	Lindol	insignificant	insignificant
1504_{vs}	$\nu(C\text{-}H_{arom})$	Lindol	medium decrease	remarkable decrease
1428_s,	$\delta_{as}(CH_2)$	Copolymer (VC/VAc)	insignificant	insignificant
1430_s,1415_s, 1415_s	$\delta_{as}(CH_2)$	lignin		insignificant
1370_m	$\nu_s(CH_2)$	Copolymer (VC/VAc)	insignificant	insignificant
1300_s	ν(P-O)	Lindol	remarkable decrease	remarkable decrease
1239_s	ν_{as} $(P\text{-}O\text{-}C_{arom})$	Lindol	remarkable decrease	remarkable decrease
1235_{vs}	δ(C-H), ν(C-O)	VAc	remarkable decrease	remarkable decrease
1138_{vs}	ν(C-C)	Lindol	remarkable decrease	remarkable decrease
1124_w	ν(C-C)	Copolymer (VC/VAc)	insignificant	insignificant
1100	ν(C-C)	lignin, Copolymer (VC/VAc)	insignificant	insignificant
966_{vs}	ν_{as} $(P\text{-}O\text{-}C_{arom})$	Lindol	remarkable decrease	remarkable decrease
$<800_m$	ν(C-Cl)	Copolymer (VC/VAc)	insignificant	insignificant

Table 4.10 The changes of absorbance of bands (cm^{-1}) in the FTIR spectra of the Measmoll control and the blend specimens

Wavenumber (cm^{-1})	Assignment (band)	Assignment (component)	Observation of change in control specimen	Observation of change in blend specimen
3466	ν(OH)	lignin	not significant	not significant
2926_s	$\nu_{as}(CH_2)$	Measmoll	decrease remarkably	decrease remarkably
2861_m	$\nu_s(CH_2)$	Mesamoll	medium decrease	medium decrease
1740_s	ν(C=O)	VAc	decrease remarkably	decrease remarkably
1590_m, 1500_s, 1430_s	ν(C-H_{arom})	lignin	not significant	not significant
1490_s,	ν(C-H_{arom})	Measmoll	little decrease	little decrease
1364_s	δ_{as}(S=O)	Measmoll	decrease remarkably ΔF'= - 3.5	decrease remarkable ΔF'= - 7.0
1370_m,1320m , 1415s,1428	$\delta_s(CH_2)$ $\delta_{as}(CH_2)$	Copolymer (VC/VAc) lignin	not significant	not significant
1235_{vs}	δ(C-H), ν(C-O)	VAc	decrease remarkably	decrease remarkably
1143s	ν(C-C)	Mesamoll	decrease remarkably	decrease remarkably
1100_m	ν(C-C)	lignin, Copolymer (VC/VAc)	not significant	not significant
857_s	ν_s(S=O)	Measmoll	decrease remarkably	decrease remarkably
$<800_m$	ν(C-Cl)	Copolymer (VC/VAc)	not significant	not significant

The results showed that DOP had been severely biodegraded seriously both in the control and in the blend specimens. The decrease of absorbance of the band at 1267_s cm^{-1} means $COO\text{-}C_{arom}$ in the ester group had been partially broken. No change was detected at the band of C-C (1120_s) in the PVC control specimen, while a proonounced change of this band was observed in the PVC-lignin blend. This result indicates the extent of DOP degradation in the blend was greater than in the control. Since the bonds of C-H and C-Cl in PVC were not changed, PVC is considered not to have been attacked. However, the decrease of the C=O (1730_s) band, which is attributed to VAc, shows that the ester bond of VAc had been attacked. Negligible changes occured in the lignin bonds. It could be concluded that lignin was not significantly attacked by fungi. It is well known that the degradation of the ester-plasticizer starts from hydrolysis. This was proven during the experiment with the strengthening of the absorbance at 3450_m [v (OH)]. In contrast, this change is not identified in the spectra of the control. It would suggest that hydrolysis was a control stage of biological degradation. Once the OH group was produced by hydrolysis, it was subsequently oxidized. This observation agrees with the result of another experiment of biodegradation of plasticizers (Nalli, 2000). Furthermore, this indicates that hydrolysis was quicker in the blend than in the control specimen.

The spectra of the 2-45 control and the blend exhibited the same result as the spectra of DOP. The weakening of the spectra of the 2-45 control and blend demonstrated the two composites were susceptive to fungal attack. Lignin and PVC did not show obvious change. Hydrolysis was more intense in the blend than in the control. The band of 2-45 at 1105_s [$\nu_s(COO\text{-}C_{arom})$] was modified significantly in the blend while no change was discovered in the control. The reduction in absorbance for the C-C bond of the blend was also more drastic than that of the control. Furthermore,

in the spectra of the DOP control and blend, no change of this band was observed. It would suggest 2-45 is more susceptible to fungal attack than DOP.

Based on the spectra of the Lindol control and blend specimens, it is evident that both of the composites are sensitive to biological degradation. In addition, PVC and lignin were not affected by fungi because the bonds attributed to PVC and lignin have no evident change. VAc was decayed, and the ester bond was degraded in the blend more seriously than in the control specimen. Lindol was decayed both in the control and in the blend as reflected by the weakening of bands at 1300_s [ν (P-O)], 1138_{vs} [ν(C-C)], 1239_s [ν_{as} (P-O-C_{arom})], and 966_{vs} [ν_{as} (P-O-C_{arom})]. The more considerable reduction in C-H_2 (2922_m), C=O (1738_s), and C-H_{arom} (1504_{vs}) that occurred in the blend shows that the blend has less resistance to fungi attack than the control. Likewise, the hydrolysis of the blend specimen was also more intensive than that of the control. A significant difference is that the degradation of the bond at 1738_s (C=O) attributed to VAc only developed in the blend, but no change was detected in the control. In contrast to the composite with Lindol, this bond was decayed in the composites with DOP and 2-45, both in the controls and the blends. This phenomenon may show that Lindol is more resistant to fungi attack than DOP and 2-45.

The spectra of the Mesamoll control and the blend show that these two formulations are sensitive to biodegradation. It is very clear that Mesamoll had been degraded to some extent. The result comes from the reduction of absorbance of some bands which is attributed to Mesamoll, for example, S=O (1364_s), C-C (1143s), and S=O (857_s). It is the same as other formulations with DOP, 2-45, and Lindol; no conclusive evidence proves lignin and PVC were degraded. However, VAc was attacked undoubtedly. In addition, the Mesamoll blend was more susceptible than the control. Mesamoll demonstrated lower resistance to fungi attack than Lindol because

the changes of VAc that occurred in the Mesamoll composite were larger than that in the Lindol composite.

In summary, the results obtained from Stage 1 and Stage 2 indicate the following:

1. The PVC control and the PVC-lignin blend specimens, both plasticized with DOP, 2-45, Lindol, or Mesamoll, are susceptible to fungi attack. Plasticizers are the prime targets of fungi attack. Plasticizers DOP, 2-45, Lindol, and Mesamoll are all degraded by fungi to different extent;
2. PVC-lignin blends are more susceptible than their corresponding PVC control;
3. The order of resistance to fungi assault of the plasticizers used in this research is: Lindol > DOP > Mesamoll> 2-45;
4. Among the four plasticizers studied, Lindol is the most proper plasticizer for PVC-lignin formulation;
5. Based on the FTIR spectra, no structural change is discovered in PVC and Alcell lignin. Vinyl acetate comonomer is degraded by *Aspergillus niger*;
6. All samples showed obvious growth domination on the edges;
7. *Aspergillus niger, Penicillum pinopnilum, Chaetomium globosum, Gliocladium virens*, and *Aureobasidium pulluians* have similar growth abilities on plasticized PVC control and plasticized PVC-lignin blend specimens.

The reasons for result 1, as mentioned in Chapter 2, are due to the presence of the biodegradable plasticizers. When the fungi colonize on the surfaces of the samples, they utilize these plasticizers as a carbon source to support their growth and reproduction. Therefore, the concentrations of plasticizers are decreased. A concentration gradient is formed, thereby promoting the diffusion of the plasticizer to

the surface from the bulk and maintaining degradation.

Additionally, the experiment was undertaken at a high humidity. The high concentration of water vapour, acting as an extractor, also promotes the loss of plasticizers. Another reason is probably that the resin used in this study was VC-VAc polymer which contains the ester group. Such groups are weak links that provide favourable sites to fungi because they can secrete esterase.

The PVC-lignin blends have shown to be more susceptible to biodegradation than the PVC controls. This may be caused by the chemical complexity of the formulation. The chemical structures of the blends are more complicated and irregular than the controls. This makes them more unstable than that of the controls. As mentioned in Chapter 2, PVC and plasticizers are more susceptible to bio-attack in the composites than on their own. Moreover, plasticizers DOP and Mesamoll do not have a good compatibility with lignin, established in Stage 1 and by another experiment (Zhu, 2000), although they have an excellent compatibility with PVC. Due to this weak compatibility, Mesamoll and DOP in blends migrate to surfaces from blends much easier than from the controls. On the other hand, Alcell lignin may be biodegradable because it is modified during wood processing and it is different from the native lignin.

The order of the resistance of plasticizers to biodegradation agrees well with the conclusion described in Chapter 2.2. Plasticizer 2-45 has two ester groups that contain the favourite bond for fungi. Furthermore, 2-45 has a linear chemical structure. The two characteristics make it very susceptible to fungal attack. Mesamoll should have a high resistance to biodegradation because of its chemical structure. However, in the experiment of Stage 2, it exhibited a relatively high susceptibility. This is due to the fact that the Mesamoll-lignin composite is not a homogenous one. Consequently, Mesamoll migrates to the surface of the sample, and then it is degraded by the fungus.

Therefore, compared to other plasticizers, Lindol is suitable for PVC-lignin formulation because of its relatively high resistance to fungal degradation and its good compatibility with lignin.

PVC and lignin have high resistance to biodegradation, as mentioned in Chapter 2. However, because of the presence of the ester group, vinyl acetate acts as a biodegradable material. This may influence the resistance of the VC/VAc copolymer to fungal attack.

The reason for edge-dominant growth is that fungi grown on the edges can gain appropriate carbon sources from the samples and the sufficient minerals from the nutrient salt agar, both being necessary for fungal growth and reproduction. In the centre of these samples, because of lack of the mineral nutrition, fungi could not grow appreciably.

In order to determine the resistance to biodegradation of Alcell lignin, a further experiment was necessary and this experiment has been carried out in Stage 3.

4.3 Stage 3

In order to further prove the biodegradability of the Alcell lignin, the experiment of this stage was conducted. The specimens were thin clod prepared by following the formulations shown in Table 3.17. It was found that DOP or Mesamoll could not adhere with lignin. The formulation was very brittle and very difficult to take out. On the contrary, Lindol and 2-45 exhibited excellent formation characteristics with lignin.

After incubation with fungi for 26 days at 28°C and 100% relative humidity, a rating of fungi growth observed by an optical microscope at 50x magnification is shown in Table 4.11. The fungi were then washed from the samples and were ground

into powder and kept in an oven at 45 °C for two days to dry.

Table 4.11 Rating of fungi growth on the surfaces of the specimens (Stage 3)

Growth on specimens	DOP blend	2-45 blend	Lindol blend	Mesamoll blend	Alcell Lignin
Rating	50%	90%	50%	60%	30%

Based on the experiment, it was found that Alcell lignin was attacked by fungi as a sole carbon source. The most probable reason is that the chemical structure of the Alcell lignin is modified in the delignification process, and its molecular weight is much lower than that of the native lignin and other industrial lignins, as mentioned in Chapter 2 and Chapter 3. These characteristics may make it more biodegradable than other lignins. However, the result may be caused by another reason, namely, the presence of furfural, acetic acid and other carbohydrates. Furfural and acetic acid also are by-products of the Alcell process. In the Alcell process, furfural, acetic acid, and lignin are extracted together by alcohol. Although lignin is separated by clarification, precipitation, and centrifugation, some furfural and acetic acid are still maintained in lignin (Muurinen, 2000). Both furfural and acetic acid are very susceptible to fungi attack. Hence, Alcell lignin is biodegradable due to the presence of these low molecular weight organic compounds. In addition, the result of this experiment proved the order of resistance to fungi attack of different plasticizers that was demonstrated in the experiment of Stage 2.

In order to compare the chemical structures before and after fungi attack, FTIR spectra were collected and illustrated in Figure 4.33. No obvious change was found. It may be because the proportion of biodegradable component was very low, compared to the total weight of lignin.

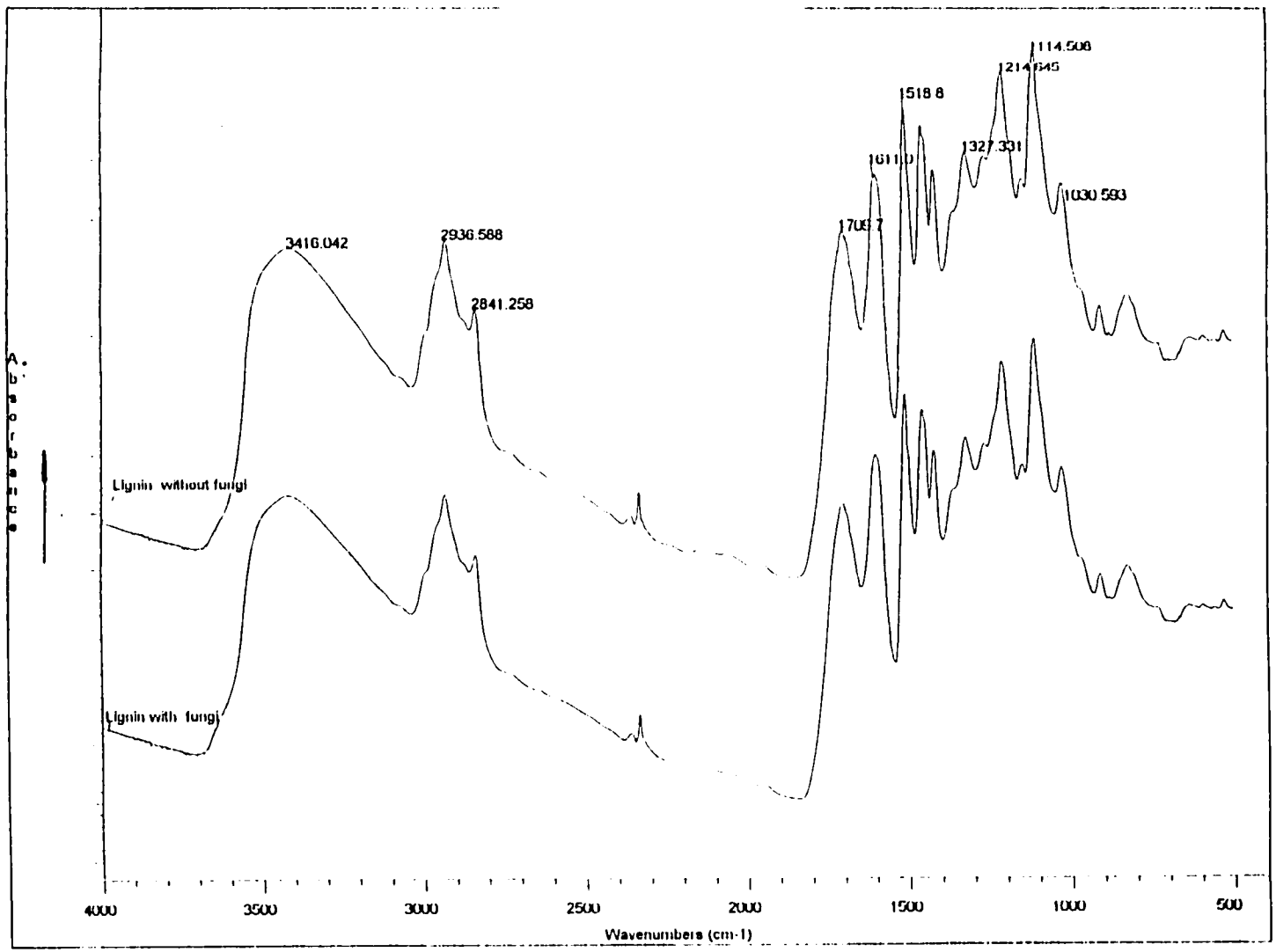


Figure 4.33 The FTIR spectroscopy spectra of Lignin

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The present research was carried out to evaluate the resistance of the PVC (copolymer VC-VAc)-lignin blend to biodegradation, and subsequently, to evaluate the feasibility of Alcell lignin as a partial substitute of PVC for PVC flooring. In addition, the research was aimed to obtain a suitable plasticizer for the formulation of PVC-lignin flooring.

In this research, the formulation was 80 phr PVC, 20 phr Alcell lignin, 200 phr calcium carbonate, 3 phr heat stabilizer, and 1.5 phr lubricant. DOP, 2-45, Lindol, and Mesamoll were used as plasticizers at a level of 35 phr per 100 parts PVC-lignin. Three test series were performed. Observation of the visible effects, optical microscopy, optical stereomicroscopy, FTIR and FTIR/ATR spectroscopy, loss of weigh were employed to evaluate the effects caused by fungi attack. The following conclusions are formulated based on the experiment:

1. The five fungi species used in this research can grow on all the composites and showed similar growth on both plasticized PVC control specimens and plasticized PVC-lignin blend specimens;
2. PVC with plasticizer DOP, 2-45, Lindol, and Mesamoll are susceptible to fungi attack. In three test stages, fungi grew on all the specimens' surfaces. The fungi

exhibited a light growth on the surfaces of DOP, Lindol, and Mesamoll control specimens, and exhibited a medium growth on the surface of 2-45 control specimen;

3. The PVC-lignin blend with plasticizer DOP, 2-45, Lindol, and Mesamoll are also susceptible to fungi attack. Moderate fungi growths were observed on the surfaces of DOP, Lindol, and Mesamoll blend specimens, and heavy growth was detected on the surface of the 2-45 blend specimen;
4. After fungi growth, all the specimens lost shine, and the 2-45 blend specimen had been discoloured severely;
5. Based on the observation by an optical stereoscopic microscope at 500x magnification, no considerable change was found on the surfaces of all the specimens except some white spots and yellow spots were found on the surface of the 2-45 control and the 2-45 blend specimens respectively;
6. After fungi attack, the weights of all the samples decreased. The PVC-lignin blends lost more weight than the PVC controls, approximately twice the weight loss of the control specimens. Compared to other formulations, the weight loss of the 2-45 control and the 2-45 blend is greater;
7. Plasticizers were the main targets of fungi attack. The chemical structures of plasticizers were changed after bio-attack, both in the PVC controls and the PVC-lignin blends. The chemical structures of PVC and Alcell lignin were not changed after fungi attack. However, the discolouration of 2-45 blend specimen may demonstrate the change of chemical structure of Alcell lignin to some extent;

8. The order of plasticizer susceptibility to biodegradation is 2-45 > Mesamoll > DOP > Lindol;
9. Lindol seems to be the most suitable plasticizer for the PVC-Alcell lignin formulation;
10. The presence of Alcell lignin decreases the resistance of the polymer to biodegradation because the PVC-lignin blend specimens exhibited much greater fungi growth, much more weight loss, and more appreciable changes in chemical structure;
11. Alcell lignin could be utilized as the sole carbon source by fungi probably because of the presence of low molecular weight components such as: furfural and acetic acid.

In general, plasticized PVC controls and PVC-lignin blends are sensitive to fungi assault. Alcell lignin promotes the biodegradation of the polymer. Lindol is an acceptable plasticizer for a PVC-Alcell flooring formulation since it shows stronger resistance to biodegradation than other plasticizers. Since Alcell lignin is biodegradable, it can be used in biodegradable plastic materials.

5.2 Recommendations

The present research established that PVC-Alcell lignin blends are more susceptible to fungal attack than the PVC control. Further research should be conducted to choose an appropriate fungicide to gain a valuable formulation of

PVC-lignin flooring with a satisfactory durability. Moreover, in all cases, even if the purpose is to manufacture biodegradable PVC, degradation of the PVC during manufacture, storage, and use must be prevented.

Taking into account that this research was done on the formulation with VC-VAc copolymer, further research has to be done on a formulation with PVC which does not contain VAc.

Alcell lignin is biodegradable, but it is not very clear that its biodegradability is caused by itself or by other materials maintained in lignin during the process of delignification. Further study may be carried out to determine which is the case.

To choose other kind of lignin to act as a substitute of PVC may be a better approach. On the other hand, it is also attractive to perform research on the application of Alcell lignin as a main additive to biodegradable plastics. At present, the cheapest biodegradable plastics are starch-based plastics. Unfortunately, they are still 10 percent more expensive than other non-degradable plastics. Furthermore, only 6 percent of starch can be incorporated into plastics (http://www.age.psu.edu/extension/factsheets/c/C17.pdf). Therefore, considering the low price of lignin, lignin-base biodegradable plastic materials may have an attractive future and it may develop a wide utilization in the single-use plastics industry.

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