NOTE TO USERS

Control of Fungal Growth on PVC Building Composites Blended with Residual Material (Lignin)

Rishi Kumar Vasudeva

A Thesis

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ABSTRACT

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Sustainable engineering uses residuals as components of new building materials. This research makes focus on resistance of such new materials to microbial growth. Microbial growth on polymer building composites renders the materials discoloured cracked; and it also causes loss in mechanical properties of material. Present work investigated best formulation of PVC (Poly Vinyl Chloride) composites blended with residual materials such as lignin. Four different types of plasticizers and three different types of lignins are used in PVC composite formulations. The spore suspension of five different fungal species was used to inoculate the series of vinyl formulations blended without lignin and with lignin. The specimens were kept for an incubation period of 28 days under the most favourable conditions for fungal growth. After fungal attack the specimens were studied under microscope to analyze the changes at the surface and tested for mechanical and thermal properties.

Results showed that all vinyl formulations are susceptible to fungal attack. Certain Vinyl – Lignin blends are having similar susceptibility to fungal attack as those vinyl formulations without lignin. Reduced plasticizer content in formulations from 35 phr to 30 phr provided better fungal resistance. The order of susceptibility of plasticizers to biodegrade with lignin blend can be seen as *2-45>DOP>Mesamoll>Lindol.*

Results of the study and testing of composite has shown that *Lindol* can be confirmed as best plasticizer and *Tomlinite* is the best lignin to be induced in the PVC composite formulations for their better resistance to fungal attack.

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NOMENCLATURE

Abbreviation	Description
δ	Solubility parameter
2-45	Di-ethylene glycol di – benzoate / Benzflex
ACTT	American Type Culture Collection
AL	Alcell
APME	Associated Press Managing Editors
ASTM	American Society of Testing Material
ATIR/FTIR	Attenuated Total Reflection Fourier Transformations Infrared Spectroscopy
BBP	Butyl benzyl phthalate
BOP	Butyl octyl phthalate
C=O	Carbonyl functional group
$CaCO_3$	Calcium carbonate
CaS	Calcium stearate
DBTL	Di-butyltin dilaurate
DEHP	Di (2-ethylhexyl) phthalate
DIDP	Di-isodecyl phthalate
DINP	Di-isononyl phthalate

Abbreviations	Description
DOP	Di-octyl phthalate
DSC	Differential Scanning Colorimeter
DTA	Differential Thermal Analysis
EHP	Environmental Health Perspectives
EPA	Environment Protection Agency
H	Hydrogen
HCl	Hydrochloric acid
ICSCs	International Chemical Safety Cards
IUPAC	International Union of Pure and Applied Chemistry
K-value	Fikentscher constant
Lindol	Trycresyl phosphate
Mesamoll	Alichil sulphonate
Min	Minutes
M_n	Number average molecular weight
MW	Molecular weight
M_w	Weight average molecular weight
OCH_3	Methoxyl group
OH	Hydroxyl group

Abbreviation	Description
OSHA	Occupational Safety and Health Administration
pH	Potential of hydrogen
phr	parts per hundred parts resin
PVC	Poly (vinyl chloride)
T_g	Glass transition temperature
UV	Ultraviolet
VAc	Vinyl Acetate
VC	Vinyl Chloride
VC-VAc	Vinyl Chloride and Vinyl Acetate
VF	Vinyl Flooring
VOCs	Volatile Organic Compounds

GLOSSARY

Taxonomy	The division of the natural sciences which treats the classification of plants and animals
Morphology	That branch of biology which deals with the structure of animals and plants, treating the forms of organs and describing their varieties, homologies, and metamorphoses
Anatomy	The science which treats the structure of organic bodies; anatomical structure or organization
Hyphae	Thread like filaments forming the mycelium of fungus
Apical growth	Growth at tip
Septum	Wall separating two cells
Chitin	Tough semi transparent horny substance; the principal component of the exoskeletons of arthropods and the cell walls of certain fungi
Catalase	Enzyme found in most of the plants and animals
Propagule	A runner terminated by a germinating bud
Vesicles	A small bladder like body in the substance of vegetable
Monopodium	A single and continuous vegetable axis
Cytoplasm	The substance of the body of a cell
Mycelium	The white threads or filamentous growth from which a

	fungus is developed
Sporangium	A spore case in the cryptogamous plants
Golgi body	A net-like structure in the cytoplasm of animal cells
Sporulation	Production of spores

CHAPTER 1 . INTRODUCTION

1.1. Sustainable Development and Environment

In Greek mythology, Pandora was given a gift box, containing all the ills and diseases, by Zeus. Sadly, the box was opened and the ills and diseases unleashed into the world leaving only hope at the bottom of the box. This is a fitting start to the examination of the environmental impacts of polymers and the ultimate hope of achieving sustainable development (Azapagic et al., 2003).

The first oil crisis in 1973 shook the world with the threat of depletion in natural resources. Thus, pessimistic predictions had been marking the era of resource scarcity, in the next century. However, the collapse of oil prices, proved this paradigm false (Meadows et al., 1972). As a result, a new concern had emerged over the future of the global environment. One of these concerns was the keen sense of human vulnerability to environmental changes. This would warrant a collaborative effort to maintain the economic development and to ensure the quality of life of all living beings for a definite period of time.

The idea of sustainable development was coined in the 1980's signifying the approach, and used in the World Conservation Strategy report by the International Union for the conservation of nature (IUCN, 1980). The Brundtland report was the pioneer of all future discussions of sustainable development and set forth a working definition of this 'meets the needs of present without compromising the ability of future generations to meet their own needs' (UN Commission, 1983). The essence of this report revolutionalized the current thinking, which promotes the economic growth while also

satisfies the needs of people and enhances the quality of life without depleting the environment.

Recently, the synthetic polymer has been made an issue for sustainability as consumption of polymer reached 100 million tones in 1995, and the total plastic consumption in Western Europe was 33.5 million tones (84 kg of plastics per person) of which 19 million tones were collected as waste (APME, 1999). Forty percent of plastics used for packaging have a very short lifespan as of the plastics used in the construction or automotive industry, which is reaching the waste stream quicker, thus turning the earth into a polymer dump yard. Once again, the concept of sustainable development has pointed fossil resources as an issue as most of the synthetic polymers are derived from them. In addition, this concept addresses the limited supply of fossil fuel as they will become depleted and their burning will cause global warming.

It is of paramount importance that problems and solutions are analyzed and implemented by adopting more holistic life cycle thinking. At one point, there was only an emphasis on the production of the polymers. However, if we consider the whole life cycle approach, which examines the consequences on the environment, from the cradle (polymer processing) to the grave (polymer disposal), then this matter becomes a serious concern, as few polymers are biodegradable (Azapagic, 2001). Once they reach a landfill, the polymers will continue to occupy space for a long time. Additives used to improve polymer properties leach out from landfills and contaminates the water table. The landfill burning of plastic waste can produce toxic substances, resulting in air pollution. Littering of the polymers in our cities, towns, and countryside, affects the aesthetic aspects of life.

Hence we need to identify more sustainable practices for polymeric materials and products.

Total 60% of the polymers used are vulnerable to microbial damage. Microscopic fungi detected in more than 80% of the microbial damage of materials whereas that of bacteria is only 16% (Gumargalieva et al., 2003).

Replacement of certain parts of synthetic polymer materials with a natural, less harmful, abundant, economic, residual and biodegradable ingredient is a major accomplishment to attain sustainable development in this vast field of research.

In this study, the idea of a better control of microbial growth by looking for most convenient formulation of PVC composite blended with lignin as a residual material. Further studies were conducted to establish good mechanical and thermal properties for implementing the optimized product as building, particularly flooring material. The resistance of this composite material to fungi attack was vigilantly researched.

The possible mechanism is that bound water molecules could disrupt the dipole–dipole interactions between relatively polar PVC chains effectively further plasticizing the PVC in the vinyl composites (Tsukruk et al., 2000). Additional plasticization would increase void volume within the PVC matrix, possibly increasing sorptive capacity, creating more suitable conditions for fungal growth.

Lignin is a residual material from pulp and paper industry. Annually large quantity of lignin is being produced from the pulp and paper industry. Piling of the lignin in huge quantity is an environment concern. Lignin is a natural polymer with complex chemical structure that makes it very resistant to degradation. Blending lignin in vinyl

composites and other building materials by replacing certain synthetic part of it helps to reduce the cost of the vinyl composites, further making this abundant waste utilizable and used to replace other organic components of composite material.

However, mechanical behaviour of the new formulation composite is unknown and required further investigation particularly when the fungal attack is considered (Hui et al., 2003).

Objectives

Objective of this research program includes the development of new composite material formulations including lignin in some parts to get an optimal product taking into consideration given aims as:

1. To control the susceptibility of vinyl composites to the microbial attack.

2. To use the abundant waste (lignin) as a filling material

3. To obtain PVC - Lignin blend with optimal mechanical properties.

Description

Formulating the new building material polymer composite in order to decrease the impact of the microbial attack (fungal growth), leading to lowest bioarosols hence improving indoor air quality.

To optimize different compositions, less susceptible to microbial attack. Whole research program can be divided into three main subjects:

Subject 1 - To test the new formulations of different components with wastes material (lignin) in order to produce sustainable building material composites

Subject 2- Test response of the formatting building material to fungal attack (using mixture of different ubiquitous fungi species)

Subject 3 - Evaluation of quality of material to optimize the formulation, which will give lowest susceptibility to microbial growth.

Organization of Thesis

The following chapter comprise the literature review (Chapter 2) related to the introduction to fungi and basic composite materials including PVC and PVC additives as well as lignin. Aspect of biodegradation of vinyl composite, predicted deleterious effects on indoor air quality and toxicity study are the crux of this chapter.

Chapter 3 describes the experimental methodology, which discusses preparation of specimens and their inoculation by mixed fungal spore suspension. Brief discussion is also presented regarding processing and testing equipment.

Chapter 4 consists of experimental results discussing visual effect of fungal growth, comparison with previous results. The analysis and explanation of results of mechanical, thermal properties are also included in this chapter.

Finally, conclusions (Chapter 5) drawn, recommendations for further research, and contribution in this field are mentioned.

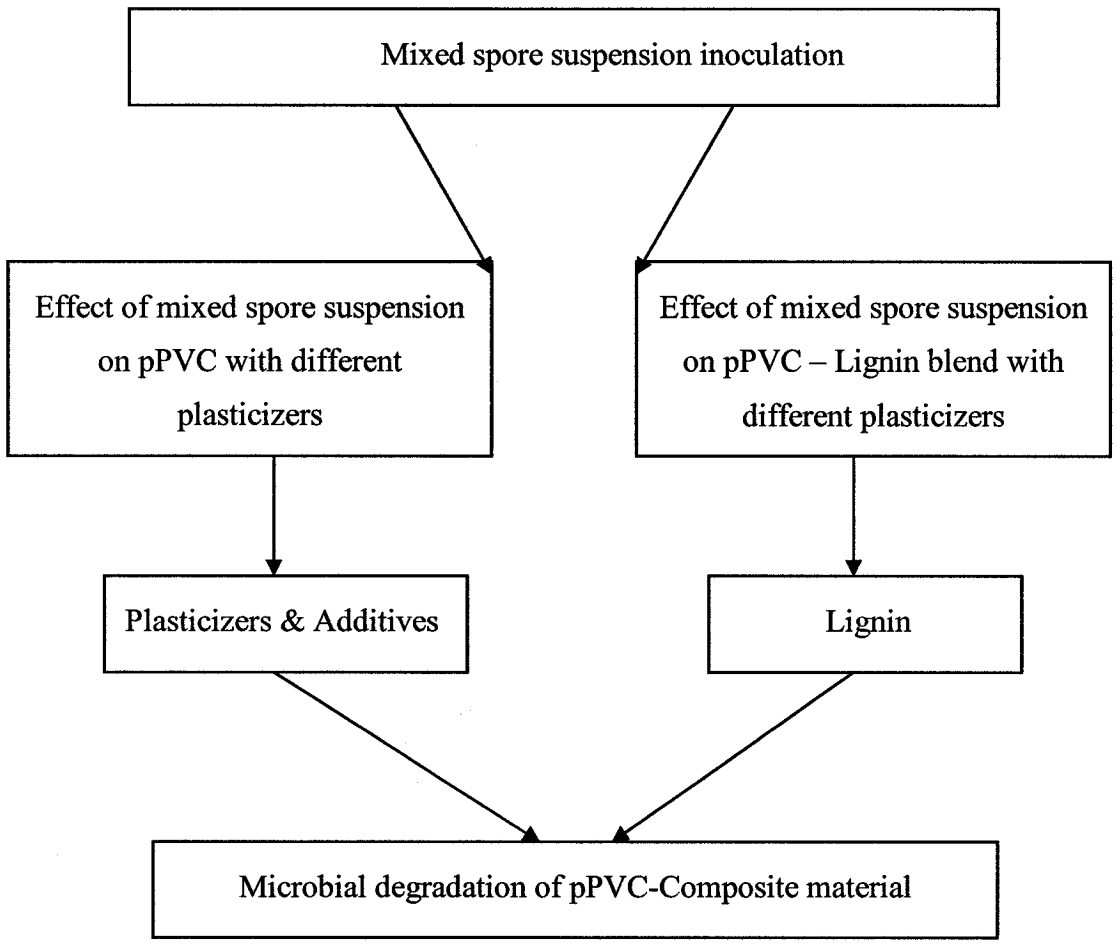

Figure 1.1 Sequence of research

CHAPTER 2 . LITERATURE REVIEW

2.1 Polymer Composites

The field of civil engineering and construction utilizes 30 % of the polymer produced each year. Chemical modification of polymers is of scientific and industrial importance as new polymers are providing an enhanced level of engineering properties. The polymers offer numerous advantages over the conventional materials, such as lightweight, resistance to corrosion, and the ease of processing (Dawson, 1990). The polymers can be combined with fibres to form composites that have enhanced properties, enabling them to be used as structural members and units (Feldman, 1989). Composites can be used in a variety of different forms, ranging from structural composites in the construction industry to high technology composites of the aerospace and space satellite industries. PVC is one of abundantly used polymer in polymer composite formulations over its almost 70 years of history. PVC including its copolymers is most versatile thermoplastic having wide range of compatibility with other products like plasticizers and impact modifiers. Simple and inexpensive production of PVC, wide variety of resin type's availability, compounding and processing versatility has encouraged research on possible further improvement of the material (Cravaer et al., 2000). Vinyl Flooring (VF) is a major composite material, developed from PVC. VF first time introduced in 1934 by Dynamit Nobel AG under the name of Nipolan. Nowadays, VF is widely used in form of tiles and wall covering used in commercial, institutional and residential buildings.

2.1.1 Vinyl Flooring

Vinyl flooring (VF) is a practical alternative in homes for many reasons. Vinyl flooring is hardwearing, resilient and easy to keep clean, and is not cold under ones feet compared to other materials. PVC, main constituent of VF is difficult to ignite and as such minimizes the fire hazards. PVC has the ease of joining separate sheets together by welding which then prevents water and moisture from seeping through the gaps. In design terms, it is available in a wide variety of colors and finishes which can simulate tiles, wood or stone.

2.1.2 Raw Material for Vinyl Composites

Vinyl composites are primarily composed of a mixture of poly vinyl chloride (PVC), inert fillers (usually calcium carbonate) $CaCO_3$, and organic plasticizer such as di-octyl phthalate (DOP). Other additives such as stabilizers, lubricants, antioxidants, and colorants are used in the processing. These additives improve the product functionality and appearance. In general, the plasticizers make up approximately 30 to 35 parts in the vinyl composite formulations.

2.1.2.1 PVC

PVC is a chlorinated hydrocarbon produced from the vinyl chloride having chemical formula CH_2=CHCl. It was Baumann (1872) who first obtained the solid product PVC in 1872. PVC has a major contribution in our daily merchandise and one of the most resourceful thermoplastics with wide range of use in daily life compared to other polymers. PVC is widely acknowledged for its versatile properties, such as recyclable nature, versatility, durability, safety, and cost-effectiveness. Wide range of PVC products

(rigid to plasticized) with adjustable mechanical properties can be obtained, as there are varieties of processing techniques available. PVC can be produced from the oil or from salt. Therefore PVC is less dependent on oil as compared to other plastics, and so makes better use of earth's finite resources (Anon, 2004a). This property has made PVC one of the most used polymers in the world market. In 1997, global sales of PVC were approximately 52 billion pounds (Craver et al., 2000).

The composition of PVC consists of 56.8% of chlorine, 38.4% of carbon, and 4.85% of hydrogen. Molecular weights of commercial PVC are, M_w (100,000-200,000) and M_n (45,000-64,000) respectively, where as $M_w/M_n \cong 2$ (Leaversuch, 1987). The PVC microstructure is mainly atactic, but sufficient quantities of syndiotactic portions are available with low fraction crystallinity (5%).

PVC structural units linked in a head to tail fashion. The structure of one of the major thermoplastic units linked in a head to tail fashion as in Figure 2.1. Where n is the number of repeating units of vinyl chloride monomer.

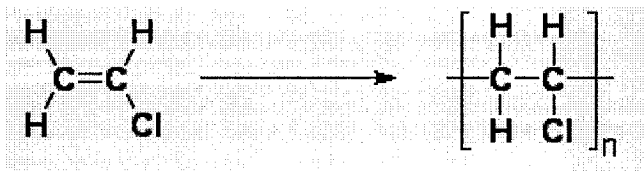

Figure 2.1 Chemical formula of PVC

In PVC homopolymers, the repeating units average around 400-1000. Commercial PVC is amorphous in nature showing a crystallinity ranging from 5-10%. The effect of low crystallization and amorphous nature has a deep impact on the performance properties of PVC in comparison to the other amorphous and atactic polymers. It has been reported that PVC can form at least three types of crystallites and

that these melt over a wide range of temperatures. Differential thermal analysis (DTA) results established that PVC could melt at temperatures ranging from 105 to 210°C. PVC is reported to have a lamellar crystal structure, but other revelations have demonstrated spherical crystallites of PVC as well (Witenhafer, 1970; Weing, 1978 and Blundell, 1979).

Commercially produced polymers differ not only in molecular weight and molecular structure but also in the particle characteristics like porosity, shape, size, and distribution. Basically, PVC has a long lasting life span of 40 years in 60% of its applications. Global consumption of PVC currently amounts to 26 M tones (Zweifel, 2001). European consumption of PVC has increased from 5.5 M tones, in 1999, to approximately 5.7 M tones, in 2001. The construction sector is the largest consumer, utilizing approximately 60% of the total PVC as shown in Table 2.1. PVC mainly utilized in the building construction sector, for use in window frames, doors, potable pipes, gravity pipes, flooring, wall covering, reservoir linings, sports stadiums and stadium seats (Tittow, 1984). Global PVC market has addressed to the issues regarding process developments, the product developments, applications, PVC and the environment.

Table 2.1 Consumption of PVC by the Western European Construction Industry (Anon, 2004a)

	1999	2001
Pipes and fittings	1.05 million tones	1.6 million tones
Profiles	1.01 million tones	1.32 million tones
Cables	0.34 million tones	0.47 million tones
Flooring	0.21 million tones	0.28 million

Approximately 800,000 tones of PVC were used in flooring and wall covering in 1999 in Western Europe. The PVC market is growing at an average annual rate of 3.9%, according to Business communication Company, and it is expected to reach 6.08 billion

kgs by 2005. The construction sector, biggest consumer showing an average annual growth rate of 7.1%, is the fastest growing segment and will reach 1.41 billion kgs by 2005. The pipe industry will remain the largest construction segment, reaching 3.04 billion kgs in 2005.

2.1.2.2 PVC Price

Presently PVC price is US $0.40/kg (Chemical, Market Reporter Journal, November, 2003). PVC producers announced a price increase of 1 cent per kg on Poly vinyl chloride implemented on January 1, 2003 due to the shortage of chlorine. The increase in prices was first declared by Shintech and was followed by other producers. Oxy Vinyls LP further increased the prices by 3 cents on Feb 1, 2003 (Anon, 2004a). Subsequently, it is worth to substitute some amount of PVC with another material during the formulation of a composite.

2.1.3 PVC Resin Production

In last 25 years, the availability of initiators with a short half-life period at polymerization temperature has revolutionized the polymerization process. The polymerization processes is initiated by free radicals. In 1980's premium quality resins available were only able to bring about 70% of conversions. However, the availability of better initiators, with short half-lives at polymerization temperatures, increases the production rate to a desirable level.

Commercial PVC is manufactured by suspension, emulsion, and solution polymerization processes. The vinyl chloride (VC) monomer is produced from treating the raw materials i.e. ethylene and chlorine. The ethylene dichloride (EDC) is the

intermediate product formed in this process. The process is carried out in three major steps. In the first step, ethylene dichloride is formed in direct chlorination unit. Next, the EDC is thermally broken down to VC monomer and hydrogen chloride. The hydrogen chloride is recycled for the feedback stock, whereas vinyl monomer is further purified for the production of PVC. In the third and final step, the recycled hydrogen chloride is reused in the reaction with the ethylene feed stock (Anon, 2004a).

The suspension polymerization process is the most widely acclaimed and utilized process in the production of the PVC. The polymer formed has the general chemical formula, $(CH_2\text{-}CHCl)_n$. This is a heterogeneous polymerization process. In the batch process, the VC monomer is polymerized in the presence of water, an initiator, and additives. This technique primarily involves the breaking down of the liquid monomer into fine, tiny droplets that form a large continuous mass of a known solvent suspension media by agitation. The monomer droplets are converted into the solid mass of PVC polymer. The unreacted monomer is stripped off from the polymer (Tittow, 1984). Water is then centrifuged off and the polymer is fluidized dried. The PVC produced is used virtually in extrusion, and injection-moulding sheet forming processes. The grading of the polymer is done on the basis of the melt flow index and suitability for rigid and flexible applications. PVC resins produced in suspension and mass polymerization have particle size specification >99.8% through a US 40 mesh sieve, having normal openings of 16.5 mils (420 microns). PVC resins produced by suspension and mass polymerization have been found to contain control porosities level between 0.18 and 0.57ml/g (Goodrich, 1987).

Emulsion polymerization has wide utility and applications in dipping or spreading. This process involves the emulsion of very fine droplets of vinyl chloride monomer in water. The surface tension is controlled by the addition of a surface tension regulator, using a long chain fatty acid or cetyl alcohol. The average size of the polymer particle ranges from 500-1500 Angstrom. After receiving the optimum polymerization, an inhibitor can be inserted into the process to stop the process. The polymer is isolated by breaking the emulsion, using salt or acid, and then followed by coagulating, filtering, washing and drying.

PVC resin is made in a variety of particle sizes, shape and internal structures, as per different purpose of utility. PVC industry prefers low porosity and high bulk density resin, to attain high rate of conversion (around 95%). The most general and special process of the PVC manufactured is by suspension and mass polymerizations. The particle porosities are controlled in a range of 0.18 to 0.57ml/g (Goodrich, 1987).

Some toxicological problems, which were main hindrance in the manufacturing and processing of PVC, were solved comprehensively many years ago. OSHA and EPA, in United States, has set the guidelines for PVC manufactures to strip down the residual vinyl chloride monomer to 1 - 5 ppm in their PVC resins produced by suspension and mass polymerization (Goodrich, 1987).

2.1.4 PVC Compounding

PVC compounds are prepared by blending PVC resin with additives that give the required properties in the fabricated finished product. These additives include plasticizers, stabilizers, lubricants, fillers, impact modifiers, and pigments that will make

the PVC either flexible or rigid. Compounds are produced in two physical forms, granules and dry blends. The PVC resin blend is fed to melt processing equipments. The molten composition is palletized and cooled, producing PVC compounds in a granular form.

Resins produced from the micro suspension differ in certain characteristics from the emulsion-polymerized resins, such as the lower surfactant content. A micro suspension resin shows greater clarity and low moisture sensitivity (Craver et al., 2000).

2.1.5 VC – VAc Copolymer

The polymer whose chain molecules are composed of more than one kind of repeating chemical units is commonly termed copolymer (Flory, 1963). Copolymers are widely used in flooring, copolymerized VC and VAc, as they give a lower softening point and better stability than pure PVC (Brydson, 1989). Copolymer provides an even molecular weight distribution, which provides proper clarity and excellent film forming properties in certain compositions. The blending of copolymers with homopolymer gives special performance results. The processing temperature of PVC can be substantially lowered by the incorporation of a plasticizer or co-monomer. Vinyl chloride – vinyl acetate (VC-VAc) copolymers contain approximately 3-20% vinyl acetate, and can be processed at 130-140°C. The copolymer, loaded with large proportions of filler and other essential ingredients, has significant use in flooring (Craver et al., 2000).

2.1.6 PVC Heat Stabilizers

In all PVC formulations, heat stabilizer prevents the decomposition of PVC by heat and shear during processing. They can also enhance resistance to daylight, weathering, heat, and aging of the PVC.

When PVC is processed at elevated temperatures, it is susceptible to dehydrohalogenation, leading to "unzipping effect", chain scission and cross-linking that causes degradation of the polymer. The dehydrohalogenation results with liberation of HCl (Tittow, 1984) and discoloration of polymer take place in two reactions: primary reaction involves formation of conjugated polyene sequences of 5-30 double bonds and followed by highly reactive conjugated polyenes crosslink and formation of benzene and condensed or trace amount of alkylated benzene takes place (Zweifel, 2001).

The choice of heat stabilizer depends on a number of factors. These factors are the technical requirements of the PVC product, such as non-toxicity, colorless, non-extractability, no migrating, and no plasticizing. Additionally, there are some major desirable parameters for stabilizers, which are their stability, the compatibility with PVC, heat and light stability, and environmental acceptability (Tittow, 1984). Organotins are the most widely used and popular type of the stabilizers in the rigid PVC application (US Patent, 1956), The Firestone Tire and Rubber Co. in Zweifel (2001).

PVC heat stabilizers are invariably supplied in the form of specific blends of metal soaps, metal salts, and organometallic compounds. These compounds provide a synergistic effect, and an enhancement of overall stabilizer performance. An indication of the typical proportion of organometals used in formulations is shown in Table 2.2.

Table 2.2 Typical metal content of PVC formulations (Anon, 2004)

Stabilizer Type	Principal Metal	Metal Content (%)	In PVC Formulation
Lead compounds	Lead		0.5-2.5
Organotins	Tin	0.1-0.2	0.3-0.5
Cadmium compounds (usage restricted by EC 91/338)	Barium Cadmium Lead		0.1-0.2 0.1-0.3 1.0-1.8
Barium/zinc compounds (only for plasticized applications	Barium Zinc		~0.1 <0.1
Calcium/zinc compounds	Calcium Zinc	0.1 <0.1	~0.1 <0.1

Every stabilizer has typical uses, although various types may be used in the same application sector. In the United States, the organotin stabilizers are usually used e.g. DBTL (dibutyl tin dilaurate). In Europe and the Far East, lead based stabilizers are the mainstay. Lead compounds are the most cost - effective and mostly used stabilizers in PVC. Organotin stabilizers, calcium/zinc, barium/zinc and cadmium are other commonly used stabilizers. The worldwide consumption of thermal stabilizers for PVC is estimated to be 450,000 tones (Philips Townsend Ass. Inc., 1997 in Zweifel, (2001)).

2.1.7 Plasticizers

A plasticizer as defined by IUPAC (1951), "A substance or material incorporated in a material (usually a plastic or elastomer) to increase its flexibility workability, or distensability. A plasticizer may reduce the melt viscosity, lower the temperature of a second order transition, or lower the elastic modulus of a product."

Commercial plasticizer for the PVC should be cost effective, readily dispersible, stable, colorless, and compatible with PVC; they should have low volatility, and good permanence. Other requirements such as solubility parameters, dielectric constants, dipole moments, hydrogen parameters are also considered (Wilson, 1995).

Dialkyl ortho-phthalates, dialkyl tere-phthalates, epoxides, aliphatic carboxylic diesters, polyesters-type polymerics, phosphate esters, trimellitate esters, benzoate and dibenzoate esters are the major plasticizers used in PVC compounding. Some of these plasticizers are not compatible; however the majority of them are compatible for PVC compounding.

General theories regarding the mechanisms of the plasticization of PVC, which have flourished, are the Lubricity, the Gel, and the Mechanistic and Free Volume theories (Sears et al., 1982).

Types of Plasticizers

Phthalates and terephthatles are the most widely used plasticizers. They are incorporated in hard PVC, which comprises approximately 70% of all plasticizers sold in the United States (Craver et al., 2000). Commercial plasticizers can be classified as general-purpose plasticizers, performance plasticizers, and specialty plasticizers (Krauskopf, 1993).

DOP (Di-octyl phthalate) has a long history of used plasticizer in the PVC industry due to its excellent balance properties. DIDP (Di-isodecyl phthalate), DEHP (Di-ethylhexyl phthalate) or DOP, BOP (Butyl octyl phthalate) are good example of PVC compatible platicizers. BBP (Butyl benzyl phthalate) is used in vinyl flooring formulations as it imparts superior stain resistance (Craver et al., 2000).

2.1.7.1 Environmental Effects of Plasticizers

The presence of plasticizers in the environment has always been a controversial issue. PVC plasticizers and other polymer plasticizers have been proven to have adverse

toxicological effects on some animal species. Allegation of toxicity regarding the behaviour of plasticizer has been put under strict scrutiny. Extensive study and scientific techniques have been applied to prove the relevancy of fact. DOP is the most stringently researched plasticizer. DINP (Di-isononyl phthalate) has also undergone extensive biological testing (Craver et al., 2000). Decrease in molecular weight increases plasticizer efficiency. Linearity of the alcohol chain also improves the plasticizer efficiency (Wilson, 1995).

Phthalates administrations on rodents cause proliferation of peroxisomes in the liver and are linked to liver tumours but there was no such symptoms found in non-rodents species. In 1990, the European Union Commission and the United States environment organizations concluded that DEHP is a non-carcinogen and non-irritant substance to human beings (CMA, 1998). Some extensive studies have established that commercial phthalate esters are not estrogenic and other studies have described non-endocrine disruptor and non-harmful character of DINP and DIDP (Zacharewaski et al, 1998).

2.1.8 Lubricants

The main function of the lubricants is to avoid sticking of the PVC composite during processing in the equipment. Therefore lubricants reduce the frictional and adhesive properties during the processing. External lubricants have limited compatibility such that they sweat out during processing and form a protecting film between bulk of the compound and metal surface. They promote metal release. Calcium stearate and lead stearate are often used as external lubricants. Internal lubricants are mainly intended to improve the flow of melt, or to reduce the melt viscosity. Wax derivatives, glyceryl

monostearate, and long–chain esters such as cetyl palmitate are used as internal lubricants (Zweifel, 2001).

2.1.9 Fillers

Fillers incorporation is done to increase the strength of the material and reduce the cost and improve the dimensional stability of the PVC composite, to improve the electrical insulation, mechanical and thermal properties, it enhances surface finish, glossy look and to reduce the tackiness of highly plasticized PVC composite.

Various calcium carbonates are most widely used fillers for PVC. Natural calcium carbonates are marketed in the form of chalk, limestone, or marble. Calcium carbonate occurs in most common form, calcite with crystalline form either trigonal-rhombohedral or trigonal–scalenohedral crystal lattice. Advantages of using calcium carbonate are that: low cost, non-toxic, non-corrosive (Zweifel, 2001).

2.1.10 Degradation of Plasticized PVC

Polymeric materials are very unique in chemical composition, physical forms, mechanical properties and applications. High versatility of the carbon to carbon and carbon to non-carbon (C–C, C–R and C–H) bonds and constituent groups, the possible configurations, stereochemistry and orientation provide basis for variations of chemical structures and stereochemistry (Odian, 1990). The microorganisms easily attack these bondings.

Degradation of any material is the significant effect and biological factors alters the structural and functional characteristics of the material (GOST, 1989), (Ilyichev, 1987). The interaction between the micro organism and material becomes a tight

closeness as spores precipitate on the surface, and fixation of cell take with the distance of hundreds Angstroms. The interacting stage between materials microbes can be explained on the basis of unit operations like adhesion, sorption, adsorption and fixing. Modern physics recognises this interaction as the phenomenon of adhesion (Ghumargalieva et al., 2003).

A general rule is that biologically synthesized polymers are readily biodegradable in natural environments and synthetic polymers are either less biodegradable or degraded very slowly. This widely accepted rule suggests that the degradation processes have evolved through time and complexity of biochemical pathways may increase with the structure diversification of polymeric materials. PVC comprises of C-C, C-H, and C-Cl structures (Kirbas et al., 1999). However, the rate of degradation is largely affected by the chemical structure, e.g., the C–C and other types of bonds, molecular weights, structures and configuration as well as the participating microorganisms and the environmental conditions. High molecular weight or long chain lengths molecules with less water solubility are less biodegradable or degrade at a slower rate than those with low molecular weights and short branch (Mulligan, 2002).

Deterioration of PVC results in reduced transparency of the sample, staining and cracking in presence of fungal spores and hyphal fragments. Plasticizers, softeners, fillers, lubricants, and other additives were termed potential carbon source of nutrition and energy and more readily colonized by microorganisms. Broadly, the susceptibility of plasticized PVC to microbial attack comes from the presence of plasticizers, commonly organic esters such as DOP, incorporated to improve the physical or mechanical properties. Bacteria and fungi both are capable of degrading ester-based plasticizers

(Webb et al., 2000). Studies of fungal colonization and biodeterioration of plasticized PVC show that fungi produce extra cellular esterase that degrade plasticizers and cause weight loss of the plasticized PVC substratum. It was also shown that plasticizers are utilized as a carbon source by *Aureobasidium pullulans* (Webb et al., 2000). It is likely that plasticizers enhance adhesion by directly influencing physicochemical interactions, such as hydrophobic or electrostatic forces, between blast spores and the PVC substratum. Surface smoothness and integrity influence the attachment of microorganisms to materials (Ghumargalieva et al., 2003).

The plasticized PVC degradation normally involves chemical, physical and microbiological factors. Generally, degradation of plasticized PVC results due to migration of some additives and thermo-oxidation, photo degradation, chemical degradation, and biodegradation (Schnabel, 1981).

Major factors that influence the rate of biodegradation include:

(a) The environment, e.g. oxygen availability, temperature, moisture, pH, pressure, light and the presence of trace metals and salts, nutrients and co-metabolites.

(b) The nature of the polymeric substrate, e.g. its molecular weight, stereochemistry and interactions with other components, branching, hydrophilicity and surface area.

(c) Microbial activity, e.g. the nature, levels, location and appropriateness of the enzymes that are produced, and the presence of inhibitors or inducers of enzymes (Mas-Castella, 1995).

Biodegradation is directly linked to the plasticizer content in the plasticized PVC. Plasticizers are not permanently bounded to the PVC polymer; it is bound by secondary bounding to the PVC polymer. They are free to associate with the polymer at different sites. Under certain conditions, the plasticizer can leave the flexible PVC. Volatilization, extraction, and migration are three common mechanisms by which the plasticizers ooze out of the PVC (Schnabel, 1981).

Volatilization

Plasticizer loss occurs at elevated temperatures and during processing. Volatilization is directly linked to the vapour pressure of plasticizer. The addition of one carbon number to the alcohol group in common series, of the esters, can hinder the plasticizer loss of volatilization to a great extent.

Migration

Migration of plasticizers takes place when plasticized PVC comes into contact with the surface of another material.

Extraction

The most common solvent is water, while there are other solvents that are capable of extracting the plasticizer from plastics. The rate of extraction is related to the solvating strength of solvent for plasticizer. The water extraction rate is quite slow, as compared to oil and other low molecular weight organic solvents (Wilson, 1995)

2.1.11 Conclusion of Chapter PVC

(a) PVC is not costly but it is produced from non - renewable resources.

(b) It is necessary to introduce sustainable development idea leading to substituting some part of synthetic component of building composite materials by a residual material from other processes.

(c) Some trend of use of residual materials is observed in different countries. However, no adequate research has been done on potential impact of residual materials, on microbial growth rate.

2.2 Fungi

2.2.1 Introduction

The fungi are an integrating part of the biosphere as they help in the decomposition of a substrate into the simpler molecules (carbon dioxide and water). Taxonomic characterization of fungi has been done on the basis of their properties or through comparison and contrasting attributes with other organisms. This can be done on the basis of their morphology, anatomy, ultra–structural, and biochemical sequence (Moore-Landecker, 1982). Fungi can be classified as mentioned in Table 2.3.

Fungi comprise of many filamentous-branched system of cells, which is known as hyphae and has apical growth. The cell comprises of hyphae, each of which is separated from each other by a cross wall, which is called the septum. The main component of the cell wall of fungi is chitin, in a vast majority of cases. There are however some cell walls that are made of cellulose (Moore-Landecker, 1982). The framework of the chitin and cellulose are micro fibrils that are present in a single layer while the remaining layers consist of amorphous protein and/or carbohydrates (Brunett et al., 1979). The fungal cells contain micro bodies that contain catalase as well as a variety of other enzymes.

2.1.2 Fungi Life Cycle

The life cycle of the fungi begins with the spore, the dormant state, and certain environmental conditions allow for the spores to germinate and grow with the production of biomass. Spores are unicellular formation of oval or spherical shape with diameter ranging form 1 to 50 μm in size (Aristovskaya, 1980). Moisture is assumed to be most important factor controlling reproduction in fungi. Requirement of certain quantity of moisture is always there for spore germination and reproduction as many fungal species are indifferent to temperature conditions (Wilkins and Harris, 1946). The life cycle of the fungi can described as

Propagule >>>>> Germination >>>>> Substrate exploitation >>>>> Sporulation

Microscopic fungi are spread into the atmosphere via spores. This is done by two ways. First, the spores are released from the mother tissue via the wind, insects, etc. Secondly, they can be released by the bursting of cells, which is then spread by water droplets or dispersed through swollen mucous mass of the cell (Moore-Landecker, 1982). The germination is a key process, which takes place in four major steps including breaking of spore dormancy, isotropic swelling, and formation of a germ tube and maintenance of polar growth (d`Enfert, 1997).

Table 2.3 Fungi classification (Nicklin, 2000)

Group:	Perforate septae +/-	Asexual sporulation	Sexual sporulation
'LOWER FUNGI'			
Zygomycotina	-	*Non-motile Sporangiospores*	*Zygospore*
Chytridiomycotina	-	*Motile zoospores*	*Oospore*
'HIGHER FUNGI'			
Ascomycotina	+	*Conidiospores*	*Ascospore*
Basidiomycotina	+	*Rare*	*Basidiospore*
Deuteromycotina	+	*Conidiospores*	*None*

True fungi, eumycota are divided into four (including one form class) major phyla subsequently depending upon their sexual reproduction and morphology as in Table 2.3. Latest information derived on the type of mitochondria and the DNA sequencing of ribosomal RNA. "Lower fungi vegetative mycelium is non-septate, and complete septa are only found in reproductive structures. Asexual reproduction is by the formation of sporangia, sexual reproduction by the formation of *Zygospores*". Whereas, "Higher fungi are a more complex mycelium with elaborate, perforate septa. They are divided into the *Ascomycotina* and the *Basidiomycotina*. Members of the *Ascomycotina* produce asexual *conidiospores* and sexual *ascospores* in sac-shaped cells called asci. Fungi from the *Basidiomycotina* rarely produce asexual spores, and produce their sexual spores from club-shaped *basidia* in complex fruit bodies" (Nicklin, 2000). Generally, fungi can reproduce both sexually and asexually, however, some species of fungi are not capable of having sexual stages. These species are placed under the artificial taxonomic group based on the asexuality and metaphoric stage (Table 2.3).

2.2.3 Cell Biology of Fungi

Hypha is the basic cellular unit of the fungus. Its tubular shape is protected by a rigid cell wall made of chitin. The hyphae contain a nucleus; mitochondria, ribosome, golgi and membrane bound vesicles within the plasma membrane that is surrounded by cytoplasm. A fine web of branches is formed by the hypha that is known as mycelium. The Endoplasmic reticulum and microtubules takes care of the sub cellular structures (Newcomb et al., 1975).

2.2.4 Fungal Metabolism

Most of the fungal species utilize aerobic metabolism and prefer a moist habitat. The fungus prefers a mesophyllic temperature range between 15-35°C. Fungal energy metabolism and biosynthesis is based on their ability to materialize their food source heterotrophically. Fungal food sources are generally acquired by one of three ways:

1. Parasitism of plants or animals;
2. Saprophytis: growing on dead animal, plant, or microbial biomass;
3. Symbiosis: growing together with algae, plants, or insects;

Fungi are unable to fix gaseous nitrogen; however they are equipped to utilize nitrate, ammonia, and some amino acids by direct uptake across the hyphal membrane (Nicklin, 2000).

Fungus consists of an apical cell of mycelium plus a few other cells. The cells beyond five cross walls are called septae, which are normally dead ends, unless they have survival functions. The cells are variable in size, ranging from 3-10 μm wide and 50 μm long, whereas the mycelium apical cell is usually from 300 to 400 μm long. Septae that separate the cell are usually perforated and have varying structures to protect them from spilling their contents when disrupted by the adjoining cell.

Food material is absorbed from all over the living region, mostly around the tip, that creates the concentration gradient around the mycelium. This creates the drive for mycelium to grow forwards from the tip to the concentration gradient of nutrients and towards the fresh substrate zone. Nutrient uptake by fungi is in direct contact with their food sources in the environment. Smaller molecules, such as the simple sugars and

amino acids, in solution in a watery film surrounding the hyphae can be directly utilized by the hyphae. However, in larger molecules, as some natural polymers like cellulose, preliminary degradation occurs before the fungi can utilize them. This process is carried out by extra cellular enzymes, which digest molecules that are too complex to be directly consumed by the fungi. Enzymatic metabolism of fungi on these complex materials usually entails hydrolysis (Keller and Hohn , 1997). This results in large molecules being broken down to simple molecules. The digestive enzymes are highly specific and are capable of carrying out the degradation and are also able to control the hydrolysis of a particular molecule. The degradation of large and complex materials involves a wide range of the enzymes. The reaction is carried out until a simple molecule is produced and is able to be taken up by the fungus. Once the cells absorb this smaller molecule, intercellular enzymes carry out the process further. Hence, the ability of the fungus to feed on large, complex molecules depends upon the enzymes the fungus is equipped with, which reflects their capability to digest certain molecules. Fungi have an array of such enzymes but remain idle until the fungus comes into contact with a substrate in which a particular enzyme is required. The growth rate of fungi is equal in almost all type of media, whether it contains simple or complex nutrients. However, there are instances when fungi may not grow and thrive due to a lack of enzyme(s) that are needed in a particular medium. In some cases, it has been observed that fungi have formed the adaptive enzymes that they need to survive (Moore-Landecker, 1982).

2.2.5 Physiological Characteristics of Fungi

Essential Elements

Fungi need some essential elements to be present in the medium for their growth. Absence of these essential constituents can inhibit the growth of the fungi no matter how abundant the other elements are. The essential elements can be subdivided into two groups macroscopic and microscopic. Carbon, hydrogen, nitrogen and oxygen are essential macroscopic elements required for fungal growth.

Microscopic constituent consists of phosphorus, magnesium, potassium and vitamins. Although high concentration of all elements should be present there, but some time excess of some nutrients affect their growth, for example, they require the vitamin D, but in excessive amount it would diminish fungal growth (Burnett, 1968, Robbins and Harvey, 1950). Fungi require traces amount of metals such as iron, zinc, copper, manganese and molybdenum. Specific metal intake in fungi differs from species to species (Steinberg, 1938).

Moisture Requirement

Fungi has liking for high humidity conditions. Moisture content provides favourable growing conditions for fungi. More than 70% for moisture create very conducive conditions growth. A few species are capable of growing at moisture content less then 65% (Ghumargalieva et al., 2003). The fungal growth can be prevented with help of controlling moisture.

Oxygen and pH

Most of fungi are aerobic in nature. They require oxygen supply for growth. The absence of oxygen ceases the fungal growth. Fungi utilize molecular oxygen in absence of atmospheric oxygen. Fungi can grow in wide range of hydrogen concentrations. Nutrient intake and enzymatic activity in fungi are pH dependent. Conducive pH is an essential requirement for fungi growth. Fungi are comfortable to grow in acidic medium. The optimum pH range for fungal growth lies in range 5 - 6.5. Some fungal species are capable of growing at pH below 3 and above pH 9 (Frazier et al., 1992).

Temperature

Temperature is an important factor affecting growth rate and enzymatic activity. Fungi grow well in ordinary (mesophillic) range of temperature. The optimal growth can be observed around 25 to 30°C. Some grow well 35 to 37 °C or above, e.g., certain *Aspergillus Spp* species. Some species that are capable of growing at relatively high temperatures are named as thermophilic. Fungi are also capable of growing well at temperatures of refrigeration and some others can grow slowly at temperature below freezing (Ingold, 1984).

Light

Growth rate and synthesis in some fungi might be controlled by light; otherwise, most of the other fungi apparently are not sensitive to light. Strong light may inhibit the growth of fungi as it destroys essential vitamins (Burnett, 1968; Robbins and Harvey, 1960). So fungi show different behaviour towards light. Some fungi apparently do not need light source, others requires light during period of sporulation (Moore-Landecker,

1982). Few species requires alternatively period of light and darkness. Fungi are able to produce spores in presence and in absence of light source as observed during this research.

2.2.6 Morphology

Most of fungi are filamentous labyrinth. The individual part is called as hypahe, while intertwined woolly structure is termed as mycelium. Rhizoids are specific kind of hyphae that establishes the fungus into substrate (Esser and Kuenen, 1967). Observation under electron microscopy has shown that the fungal cell walls are made up of a complex of fibrillar materials with an amorphous matrix. The structures comprising β 1-4 linkages permit microfibrils to form many parallel molecules aligning. The microfibrils can grow between 10-25 nm in diameter. They are normally embedded in the amorphous matrix.

2.2.7 Growth

Growth occurs at the hyphal tip by the fusion of characteristic membranes-bound vesicles derived from the golgi apparatus or vesicles. These vesicles accumulate in the apical 10 μm of the hyphal tip. The organization of these vesicles varies in the different taxonomic groups (Nicklin, 2000).

2.2.7.1 Cell wall Synthesis

With the accumulation of biomass, the size of the hypha increases and the cell becomes larger; this leads to septation and branching. Branching occurs at the place behind the first septum. Branching takes place at a precise angle to the parent hypha (Nicklin, 2000).

2.2.7.2 Kinetics of Growth

The measurements of yeast and bacterial growth employ the haemocytometer or the turbid metric measurements. The growth of fungus, however, is determined on the basis of measuring the mass change (m) with the time (T) under the excess of nutrient conditions. The specific growth (μ) of the culture can be calculated by using the formula:

$$Dm/DT=\mu M$$

where D represents derivative rate of change with respect to time.

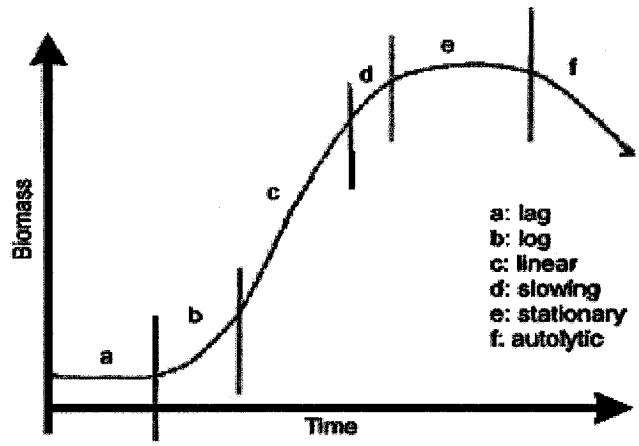

Figure 2.2 Different phases of fungal growth (Nicklin, 2000)

Figure 2.2 represents the distinguished phases in microbiology of microorganism's development (Shelegel, 1985). Intensity and development duration of microorganisms on certain substrates varies according to their ability to adapt the nutrition sources. Exponential growth occurs for a brief period as the hypha branching is initiated and the new hypha extends at linear rate into uncolonised regions of substrate. Hyphal tips contribute to the extension of growth and, the old hyphae produce the sporing structure.

The hyphal growth can be observed under a microscope, measuring the tip growth and branching rates of mycelium. The fungal growth can be calculated from this data. "The hyphal growth unit (G), which is the average length of the hypha required to support tip growth, is defined as the ratio between the total length of the mycelium (L_T) and total number of tips (N_M)" (Nicklin, 2000).

Total number of tips/Total length of the mycelium = hyphal growth unit (G)

Growth is a highly regulated process, which is significantly affected by light, heat, pressure, changes in osmolarity, and the ionic strength of the media. Nuclear division and cross wall formation induces growth. Apical compartments are multinucleate with as much as 50 nuclei. The mitotic division occurs rapidly at an approximate rate of four minutes, as the hypha concomitantly grows forwards. Fungal colonies can be divided into different zones starting from margin to center: the extension zone, the productive zone, and the fruiting zone.

The Extension Zone

The branching patterns of hyphal growth on the fresh media stay at a right angle to the monopodium. The branches are arranged in an efficient manner to utilize and colonize the substrate optimally. The growth orientation takes place at safe distance not to disturb the other adjoining hyphal growth. The mechanism of the growth is mysterious but it might be related to the food ingredients and the toxin gradients. Experimentally, it is observed that the growth rate depends on the type of the food and toxins present. If the substrate is a complex structure or if there is toxin on the food media, the growth begins away from the toxin on the media. This observation was made during this research. Hence, it can be concluded that the toxic presence affects the growth rate of the fungi.

The Productive Zone

In this zone, there is a major increase in the biomass. The formation of aerial mycelium and thickening of the hyphae take place here.

The Fruiting Zone

The biomass that is gained stops in this zone and spores are formed. This zone can be equated to the stationary phase.

The Aging Zone

Due to the formation of the fruiting bodies, the empty hyphal remains back. This zone is equivalent to the decline phase (Nicklin, 2000).

2.2.8 Reproduction in the Fungi

Reproduction in the fungi normally takes place in two processes, asexually and sexually (Ingold, 1984).

2.2.8.1 Asexual Reproduction

The asexual reproduction of fungi is done by budding or by binary fission.

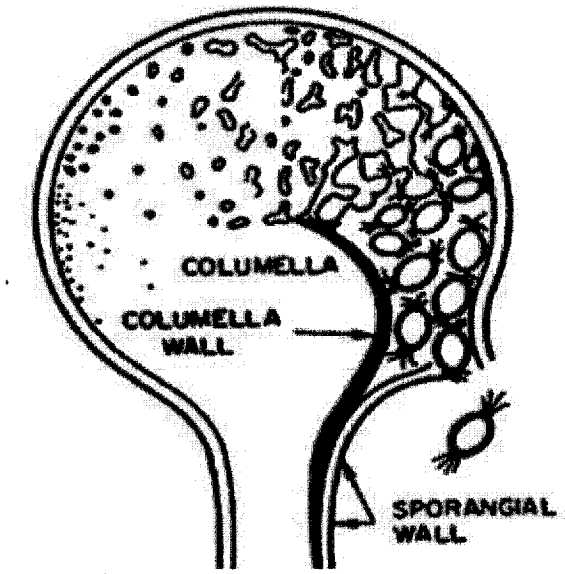

Figure 2.3 Sporangium formation (Nicklin, 2000)

The onset of fission is carried out by nuclear mitosis of the cells. Asexual reproduction is known as the metaphoric or anamorphic phase, whereas the sexual reproduction is known as teleomorphic phase. The onset of the asexual production is regulated by many factors, such as the nutrient level, CO_2 levels, light levels, and some environmental factors, which influence the production of spores. Other than these factors, fungi have their own internal clock, which is programmed for time and the season for sporulation. The formation and development of the sporangium occur with time as shown in the Figure 2.3. The nuclei undergo repeated mitosis, and thus the golgi produces vesicles surrounded by a membrane which are filled with the material that form spore cells. Then they coalesce around the nuclei to form spores and are eventually released from the sporangium as shown in Figure 2.3. The spores released from the

sporangium can be thrown to a distance of 1 to 1.5 centimetre (Ingold, 1934). The size of the sporangia depends upon the species.

2.2.8.2 Sexual Reproduction

Sexual reproduction introduces variation into the population, which is the reason why most fungi have the sexual phase. The presence of two haploids (n + n) or a diploid is required for sexual production. The fusion of the two haploid nuclei produces one diploid, which then undergoes further meiosis (Figure 2.4). This further division brings more variations to the progeny. The processes involved in the production of spores can withstand the harshest of conditions.

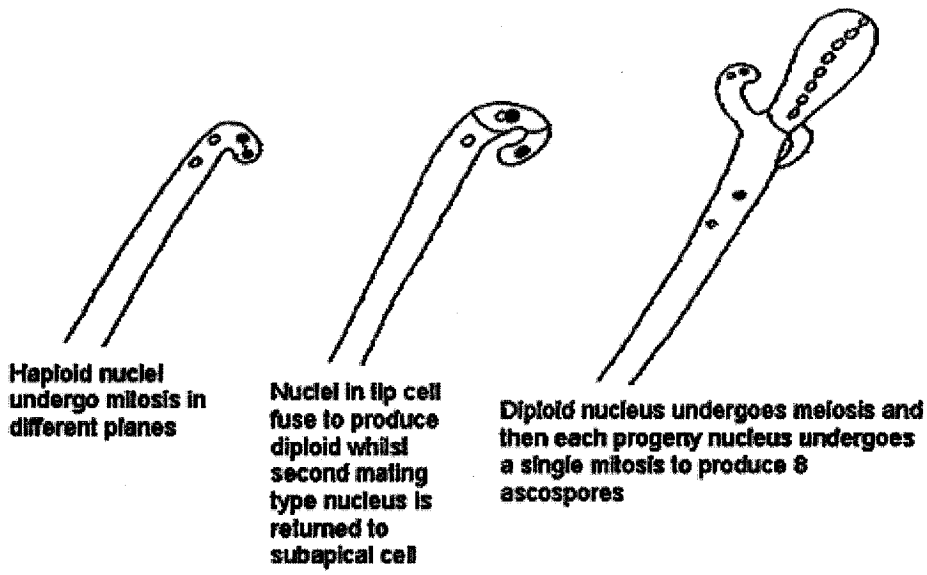

Figure 2.4 Sexual reproduction in spores (Nicklin 2000)

2.2.9 Spores

Spores are more recalcitrant structure of microorganisms, which can survive the harshest condition that includes desiccation, high UV, low temperatures, and starvation.

Mainly they have two main functions: dispersion and survival. The spores are the most heat resistance structure of microorganisms that can survive even the sterilization temperatures. Wet spores generally comprise of 25% protein and 20% fat and comparatively have less moisture content as compared to mycelium. The spore cell wall is made of melanin and has ornamentations (Nicklin, 2000).

Survival of the spores in the harshest condition is related to their low respiration rate (1- 4%) in comparison to mycelium and their storage of essential reserves including lipids, phospholipids that include sugar alcohol. Period of survival obviously depends upon the reserves.

2.2.9.1 Dormant Spores

Dormancy is the stage in which the spores do not germinate after formation. This is a break in life cycle of the spore. There are two types of dormancy, endogenous (constitutive) and exogenous (induced). In endogenous dormancy, spore is unable to metabolize on water and nutrient. Probably this stage occurs due to some metabolic hindrance or an inhibitor. In other instance the self-inhibition persists and spores are unable to germinate in dense suspensions due to their excessive sensitivity to change in the oxygen level or carbon dioxide, nutrient competition and mostly due to the presence of the inhibitors. Some volatile inhibitors include ethanol and acetaldehyde (Robinson et al., 1968). There can be physical barrier to germination i.e. deposition of protein around spore which prevents the uptake of water. These inhibitors have to be leached away before germination takes place.

Exogenous dormancy occurs because of certain external environmental conditions. As these external conditions fade away, the spores start the germination process (Nicklin, 2000).

An optimal environmental condition ends inhibition and triggers the germination. Some chemicals and host compounds stimulate the germination. Germination begins with the absorption of water and cells swell 3 to 20 times from their normal size and with the emergence of the germ tube, followed by the rupture of the cell wall and liberation of the new germ with a new cell wall (Sussman et al.; 1966).

2.1.9.2 Air Spore

Fungi have very dynamic mechanism to disperse in the environment and use of air is one of the best ways to do spread. The spores are very dry and friable, that means they are very light to be blown away by the gust of turbulent air above the boundary layer. The movement of spores is linked to their size; light spores can travel long distance and vice versa (Ingold, 1984). A sample of air can contain 200,000 fungal spores per cubic meter; 10,000 per cubic meter are a more general figure. Some spores have the active guns to fire the spores in the atmosphere. The types of the spore flora vary from place to place. Species like *Penicillium* and *Cladosporium* are normally isolated from agricultural saprophytes of the plants and decaying matter (Nicklin, 2000). Normally in homes and in workplaces the quantity of spores varies from hundred per cubic litre of the air or higher. *Aspergillus Spp* can be important flora in the dry and warm areas. Spore inhalation can pose a potential health hazard as the spore count increases.

2.3. Toxicity

Jaakkola et al. (1999) carried out a study that linked occurrence of bronchial obstruction in young children to PVC flooring. In a matched case study conducted in Oslo, researchers observed that children with bronchial obstruction were more likely to have PVC flooring in their homes than were children without PVC flooring in their homes.

In another study made by Jaakkola et al. (1999) also observed that plasticizer migrate from PVC floor to sediment house dust. Since plasticizers have high affinity for particles, in this case the risk of bronchial obstruction in children is more likely due to vinyl flooring than vinyl wallpapers.

Additionally, when plasticizer desorbs to the surface of the vinyl flooring, it comes in the contact with high moisture contents from the environment, a conducive pH that subsequently creates a favourable environment condition for the fungal growth. Fungal attack causes brittleness, discoloration and cracking of flooring material, which results in VOC emissions and deleterious conditions indoor that were observed during this research work.

Semi-volatile organic compounds (SVOC) are known as frequent pollutants of the indoor air environment. SVOC concentration is relatively high at surface level as compared to indoor airspace. Plasticized PVC formulated with phthalic esters as plasticizers, suspected to cause the potential health hazards (Uhde et al., 2001).

It was Uhede et al. (2001) who found that the level of phthalic esters VOC from plasticized PVC in chamber air was very low (between 0.12-1.22μg/kg body weight/day)

and far below the tolerable intake of 37-66/μg/kg body weight /day which is based on studies of oral toxicity.

Some observations made by Gumargalieva et al. (2003) suggested that loss of plasticizer from PVC under the influence of surface biodegradation by a microscopic fungus *Aspergillus niger* is much faster than loss without fungal growth. When there is no fungal growth, the loss of the plasticizer takes only by volatility of plasticizer whereas in the presence of fungus it is limited to the diffusion process.

Webb et al. (1999) observed that, plasticizer may accelerate the bio-degradation processes occurring on plasticized PVC by enhancing fungal adhesion. They investigated the effect of plasticizer on the adhesion of the deteriogenic fungus *Aureobasidium pullulans* to PVC.

2.3.1 Fungi Mycotoxins

According to Wicklow (1981), it's a basic tenet of the fungal ecology that mycotoxins are concentrated within spores. The production of toxins and volatiles by fungal species and such as seasonal effects are equally important. The lethality of the fungal spore depends upon size and shape of the invading organism. The level of mycotoxins in fungal spores and propgules might be very high. The size of some species of fungal spore is around 5 micrometers, which is quite in the range of respiration size particle; hence, they can accumulate in the alveoli (Day, 1986). Mycotoxins are inhaled easily by the mucous membrane of the respiratory tract due to their small size. Mycotoxins (trichothecenes, giotoxin, and alfatoxins) can affect immune system of humans.

As shown in the Figure 2.2, growth of fungi can be categorized in main three phases: the rapid growth phase, stationary phase and death phase. Mycotoxins are secondary metabolites and are produced in the third phase when mycelium faces deficiency in the nutrients. All mycotoxins are not secondary metabolites but some of them result of the primary metabolism too and while others are not defined till now (Hale, 1985).

Volatiles Chemicals Produced by Fungi

Fungi volatiles are a complex smell of alcohols and esters, which may affect the immune system. Diversity and number of the propagules in indoors represent the mycoflora present in outer environment. *Aspergillus, Penicillium* and *Trichoderma* in addition to *Cladosporium, Alternaria, Aureobasidium* are the most commonly present in the indoor air, their average quantity counted in number of propagules of each species can reach from 0 to 104 /m^3. Number of micro flora can be higher than that, because a quite few of them can be found hidden in moist places under carpet, walls, humidification and ventilation systems (Bernstein et al., 1983; Solomon, 1975).

Aspergillus niger is ubiquitous, but does not create deleterious conditions at low concentrations but toxic at high concentrations, while *Aspergillus fumigatus* another species of *Aspergillus* causes health hazard to human beings as it is allergic and pathogenic capable of producing some mycotoxins (Miller et al.; 1982).

Few species still are not established for their ability to produce the volatiles, and variations in production of volatiles are linked to specific genus and species (Collins, 1976). Fungal volatiles have harmful affects on the humans; they can be inhaled at low concentrations.

Non – volatile Chemicals and Mycotoxins Produced by Fungi

Mycotoxins are the complex organic chemicals with molecular weight generally greater than 200 mass units. They are usually associated directly with the fungal spores or hyphae. Spores of toxigenic species produce large amount of mycotoxins. Studies revealed that alfatoxins concentrations in some spores and scelerotia of *Aspergillus flavus*, *A. parasiticus,* could reach 200000 ppb (Wicklow et al., 1983). Spores of *Stachybotrys* and *Trichoderma* are also reported to accumulate mycotoxins (Miller et al., 1981)

2.3.2 Human Symptoms and Epidemiology

Allergic reactions are the most immediate symptoms and, may be developed in few minutes or occur in period of 4-8 h. These reactions can be considered as initial stage towards the asthemic disease. Repeated exposure to certain concentrations of the antigens can lead to pneumonia. Infection starts from inhalation, and disease begins with the lung infection. From there, fungal infection can migrate to other organs including heart, brain and kidneys. Infection can cause disseminated systematic blast mycosis that may involve liver, spleen and long bones; coccidiomycosis that can result in meningitis, spondylytis and otomycosis; and histoplasmosis. Acute histoplasmosis is usually fatal in children (Day, 1986).

2.3.3 Fungi and Indoor Air Quality

Fungi are ubiquitous and comprise of around 25% biomass on earth. They have great importance in the ecosystem, as they are essential decomposers of organic mass on

the earth and help to sustain the animal and human life (McNeel et al., 1996 and Ammann, 2001).

The most of the fungi found indoors are saprotrophic, meaning they have versatility in growing on all type of dead moist organic compound. Extent of their attacking capability can be judged, as they are able to feed on glass and concrete structures.

Fungi are able to germinate in time span of 12 h. The fungal concentration indoor is directly linked to their concentration in outdoor atmosphere. Fungi type and nature are also linked to their outside source and prevailing condition in environment. Moisture is one of the major culprits that leads to the fungal infestation indoors. Inadequate ventilation and insufficient drying of flooded area leads to fungal growth very rapidly. In addition, poor indoor cleaning habits also enhance the indoor fungal growth (Anon, 2004b).

The fungi are reported to produce the VOCs, chemicals responsible for the musty odour produced by fungi. Exposure to high level of VOCs in indoors or in industrial work places can irritate the mucous membranes, which disturbs the central nervous system causing nausea, headache, lack of concentration, dizziness, and other infections (Richerson, 1990).

According to McNeel (1996) the fungi produced VOCs are only little fraction of the total VOCs emitted indoors. The fungal spore having allergens, produce the allergic reactions, causing running nose, running eyes, coughing and sneezing to more serious problems of sinusitis and asthma.

Mycotoxins

Some specific fungi like *Aspergillus niger, Cladosporium sp., Chaetomium sp., Penicillium sp., Alternaria sp.,* are able to produce mycotoxins according to specific types of the environmental conditions. Appropriate conditions are a needed for the production of mycotoxins (Yang, 2001). EPA suggested that some of the mycotoxins recognized have tendency to concentrate on the spores; still very little information is available regarding mycotoxins release from spores.

2.3.3.1 Potential Health Hazard of Fungi

Irritation in eyes, dermatitis, respiratory distress, asthma has been proven resulting from the mold species. Other reported health effects are fever and disfunction, regular blood from nose, dizziness, nausea, and damage and impairing of active immune system (McNeel et al., 1996).

2.4 Lignin

2.4.1 Source and Structure of Lignin

Lignocellulose is the most abundant natural organic product on earth. It amounts to 50% of the total biomass and its annual production is approximately $50x10^{12}$ tons. Lignin is the most important constituent of lignocellulose (Lynch, 1987).

Lignin is a complex biopolymer, with different functional groups and linkages that are not easily defined. This polyphenolic material is the result of enzymatic

dehydrogenative polymerization of three phenyl propane monomers, namely coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol Figure 2.5.

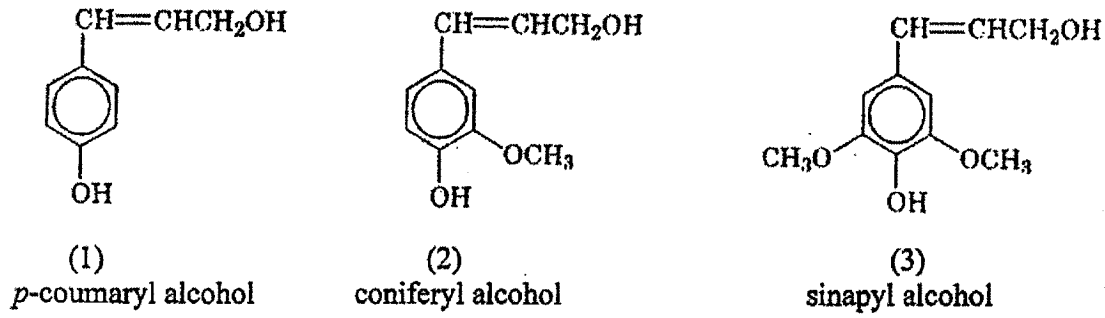

Figure 2.5 Different lignin precursors

These monomers are naturally bonded by random linkages and by coupling reactions, which result in a three dimensional heterogeneous structure. They have a great number of branches, with molecular weights ranging from 1000 to12000. Lignin, a phenyl-propane, has interconnections with at least ten different types of linkages (Feldman et al., 2001).

Now lignin is confirmed as a source of renewable materials. Earlier, lignin had been considered a waste product and was merely dumped, but the 1970 oil crisis propelled its use in extracting energy and using it as energy source (Vazquez, 1991).

Lignin is the most recalcitrant part of the plant cell wall. It behaves like the binder between wood cells. It regulates the flow of water across the cell wall and plays an important role in the internal transport of the water, nutrients, and other metabolites. Lignin also acts as an antioxidant, a UV stabilizer, and it impedes the penetration of destructive enzymes into the cell wall. Lignin is particularly difficult to biodegrade, and

checks the bioavailability of other wood contents, for the lignin molecule reduces the area available to enzymatic penetration and activity (Hawkins et al., 1997).

The complexity of the chemical structure has proven lignin to be the most resistant natural polymer, of which, the chemical structure has not been established to date (Bushwell and Odier, 1987). Typically, lignin comprises of approximately 25% of the total weight of wood. The quantity of the lignin in dry softwoods and temperate zone hardwoods varies between range of 24-33% and 19-28%, respectively. The variability of lignin content in wood is due the variety and location of the wood. It is known that some species of fungi are equipped with enzymes, which are capable of degrading lignin. The white rot fungi were the first species discovered to degrade lignin (Kirk and Farrel, 1987). Lignin degradation is primarily an aerobic process but under anaerobic conditions lignin can persist for a long time (Kuhad et al., 1997).

2.4.2 Lignin Properties

Commercially, lignins are available in powder form. The lignin polymer is amorphous in nature, as there is no crystal formation observed under the electron microscope. Lignins have densities of 1.3-1.4 g/cm^3 with molecular weight in the range of 1000 to 12000. The molecular weight can vary according to the location and the type of wood. The Glass Transition temperature T_g value of the lignin is between 100 and 180, which is higher than the commercial synthetic polymer. Lignin has a good thermal stability up to 200 °C. Thermal degradation begins as temperatures above 200 °C, with a slight structural change occurring at 300 °C. At very high temperatures (up to 400 °C), the lignin macromolecules undergo depolymerization, decarboxylation, dehydration, and become low molecular products (Feldman et al., 2001).

2.4.3 Delignification

As mentioned earlier, lignin is obtained as a waste product of the paper and pulp industry. Delignification is the process in which lignin component is separated from the other parts of the wood such as the cellulose. Delignification is carried out in different processes in the pulp and paper industry.

Some of the main conventional processes

1) Kraft process, 2) Sulphite process, 3) Soda process, and 4) Organosolv process

In the Kraft process woodchips are digested at high temperatures in presence of the alkali solution. After the completion of digestion, the cellulose is removed from the residual liquor, which is known as the black liquor from papermaking. Lignin is commonly available in the black liquor, which is generated from wood pulping (Harvey, 1986).

The organosolv process is a new delignification process that is based on the aqueous organic solvents. This organosolv process had been established a decade ago to replace the conventional chemical processes.

2.4.4 Utilization of Lignin

In the last 20 years, the use of lignin as a source of energy has been increasing as a result of the oil crisis in 1970. For the development of commercially valuable products, lignin is being used in the renewable composite material because of its availability, lower cost, and unique properties. It is being used in products such as polymers, adhesives, and resins and reinforcement of rubbers. Despite these revelations, lignin is still underutilized.

2.4.5 Biodegradation of Lignin

Lignin is termed as the most resistant natural polymer to undergo biodegradation. Despite the numerous studies performed on the biodegradation of the lignin polymer during the last two decades, only a handful of studies were able to establish lignin mineralization by microbes. Some bacteria and fungi species are capable of degrading the lignin substrate. Studies have indicated that fungi are a superior decomposer of lignin (Perestelo et al., 1994). "White rot wood decay fungi have a unique capacity for degrading wood and its basic constituents, cellulose and lignin. These fungi use the cellulose fraction as a source of carbon and have the ability to completely degrade lignin in order to have access to the cellulose molecule (Hatakka, 2001). *Basidiomycetes* species have been extensively studied due to their high degradation capacity" (Hatakka, 2001). The biodegradation of lignin results in a major carbon constituent recycling step on the earth, which amounted to 150,000 million tons of wood (Steinlin, 1979). It is well known that cellulose is easily utilized by variety of organisms, and its hydrolysis is hampered by this recalcitrant lignin. However, white rot fungi, a species of *Basidiomycetes*, have a remarkable ability to decompose lignin (Higuchi, 1997). White rot fungi comprise of 100 heterogeneous groups of *Basidiomycetes* species. They have the capability of mineralizing the cell wall component. This particular species degrade lignin selectively by secreting an enzyme, which is widely distributed, as per the ecology factor (Kirk and Farrel, 1987).

Fungal attacks were first carried out in plant tissue on the parenchymal cells and consequently followed on xylem cells. In the non-selective attacks, erosion of plant tissues and the formation of the cavities filled with fungal cells were observed. The

pattern of attack on the fungi can also be correlated with the kind of the lignin present. Syringyl units (Figure 2.5) degraded faster relative to the guaiacyl units (Figure 2.5). For instance, white rot fungi attack angiosperm, cells which are rich in the syringyl units (Gilbertson, 1980). Electron microscopy has demonstrated erosions of the cell wall by fungal hyphae at a distance, which then confirms the presence of an extra cellular enzyme. White rot fungi secretes the enzymes that breaks up the linkages between aromatic rings. This results in Ca-Cß, ß-ethyl ether, C_1-Ca, Ca and Ca= Cß oxidations, hydroxylation and a demethoxylations of the aromatic ring which leads to the complete mineralization of lignin (Janshekher and Fiechter, 1983).

The degradation of the lignin macromolecule is challenging job for the enzymes (Kirk and Cullen, 1998). On the basis of the enzyme production pattern, they divided the white rot fungi into four major groups (Table 2.4). The first group is the LiP - MnP, which contains the most prolific degraders of the lignin that also produce laccase. The second group is the MnP - Laccase, which comprises a selective band of the lignin decomposers. The third group is the LiP - Laccase and LiP-Phenoloxidase, which are poor degraders of lignin. The forth group is the Laccase - Aryl alcohol oxidase groups (Hatakka, 1994) Table 2.4

Table 2.4 Enzyme involved in the lignin degradation and their main reactions (Hatakka, 1994)

Enzyme activity, Abbreviation	Cofactor or Substrate	Main Effect or Reaction
Lignin peroxidase (LiP)	H_2O_2,veratryl alcohol	aromatic ring oxidized to cation redical
Manganese peroxidase (MnP)	H_2O_2 Mn,organicacids as chelator thiols unsaturated lipids,	Mn(II)oxidized to Mn(III);chelated Mn(III) oxidizes phnolic compounds to phenoxyl radicals.
Laccase (Lacc)	O_2;mediators,e.g. hydroxybenzotriazole	Rxn in presence of the mediators as phenols oxidized phenoxyl redicals
Glyoxal oxidase (Glox)	glyoxal,methyl gyloxal	glyoxal oxidized to glyoxylic acid; H_2O_2 production
Aryl alcohol oxidase (AAO)	Aromatic alcohol Scompound(anisyl,veratrylalcohol)	aromatic alcohols oxidized to aldehydes; H_2O_2 production
Other H_2O_2 producing Enzyme	many organic compounds	O_2 reduced to H_2O_2

Brown rot fungi are also a *Basidiomycetes* species capable of degrading wood but lignin degradation is limited. Lignin is chemically modified by demethylation of its phenolic and nonphenolic units (Kirk and Farrell, 1987; Eriksson et al., 1990). Lignin has limited aromatic hydroxylation, and ring cleavage of lignin also occurs (Kirk and Farrell, 1987). The characteristic of the attack is similar to that of the white rot fungi; however, the brown rot fungi do not form cavities but attacks on the layers that are deficient in lignin. Further research is still underway to determine the optimal functioning conditions of these less known fungi. Brown-rot fungi degrade cellulose and hemicelluloses in wood extensively, but the lignin degradation is limited. Brown-rot fungi are able to mineralize the methoxyl groups of lignin, but the mineralization of other parts is much less (Buswell and Odier, 1987; Kirk and Farrell, 1987). Soft-rot fungi, such as *Ascomycotina* or *Deuteromycotina,* degrade both hardwood and softwood, although hardwoods are degraded to a greater extent than softwoods (Kuhad et al., 1997).

Soft-rot fungi consist of *Ascomycetes* and fungi *Imperfecti*. Soft rot fungi work under moderate moisture conditions, particularly in fresh water and marine environments. However soft-rot fungi are more intense in degrading lignin as compared to the brown-rot fungi, which are slower then white rot fungi.

The degradation of lignin to CO_2 is optimal under high oxygen tension. High production of CO_2 has been obtained under 100% oxygen atmosphere. Some time even these conditions do not guarantee that fungi will grow well in this type of climate (Hatakka et al., 1983). Fungi then utilize lignin for their growth and convert it to CO_2, water, and new fungal biomass (Kirk and Cullen, 1998). Studies also indicate that this degradation is a secondary metabolism, which is unfavourable to the growth metabolism. Both intercellular and extra cellular degradation of lignin by fungi came into picture under natural conditions.

2.4.6 Lignin Blends with Other Polymers

Advances in science and technology have required a variety of new polymers with good performance and low cost. However, it has become more difficult to find the necessary properties in the limited number of homopolymers available. Thus, polymer blends or alloys and composite materials have been considered to be the more promising approach to the production of new materials and have been studied extensively. Such materials in PVC industries are well known, and polymer blends in engineering plastics have attracted much recent attention.

Polymer blending is generally done to reduce the cost of the product, incorporation of materials like fibers, wood to improve the mechanical properties and performance of the polymer.

Lignin is abundant natural polymer, its hydrophilic nature pauses a problem to make a blend of lignin with hydrophobic polymers. Considering the lignin recalcitrant character, cheap price and sustainable nature lured more research work. Lignin incorporation in polymers is under research to find a suitable formulation for building materials. In addition, lignin is very stable in nature with other aspect as it helps the plant to reduce the chemical stress by inhibiting the atmospheric degradation acting as the antioxidant and UV light stabilizer. Lignin composed of phenyl propane unit's tri or tetra substituted with hydroxyl or methoxyl group. Phenyl propane linked with variety of linkages that is a complex intertwined structure. This matrix numerous functional groups linked to each other, which impart the macromolecule a high polarity. Taking in account the above-mentioned properties of the lignin and the functional groups and commercial use of the PVC, it sounded reasonable to judge the contribution lignin can make to the poly blends with the lignin and PVC. Purpose of this blending is to produce dark colored and cost effective blend with the PVC for indoor and outdoor applications in the building construction materials.

Blends of Lignin with PVC

At the Department of Building, Civil and Environmental Engineering of Concordia University, studies have been undertaken to examine the feasibility of the incorporation of the lignin in the vinyl flooring because of the abundance of lignin, its chemical composition, and its various properties. Previous research in this field has

established the evolution of a blend with PVC and different types of lignins (Feldman et al., 1996). The data suggested that there are interactions between two polymers occurring between the OH group and the ?-hydrogen of PVC. The mechanical properties of the Lignin - PVC matrix reinforcement (10:100) have indicated an increase in the Young's Modulus. But on other hand, substantial losses in both strength and elongation at break, as well as in impact strength was observed. Results of an experiment carried out in Concordia University compared with plasticized VA-VAc copolymer – Lignin blends with plasticized VA-VAc copolymer controls. The results illustrated that PVC – Lignin blends exhibited a slight increase in stiffness accompanied by the moderate losses in strength and elongation at break. The polymer blend morphology has also shown some impact on the mechanical properties. It was experimentally observed that PVC – Lignin blend has the homogeneous structure (Feldman et al., 2001).

CHAPTER 3 . METHODOLOGY

3.1 Introduction

This research program was carried out to formulate PVC composite to control microbial growth. Susceptibility of various plasticized PVC composites samples formulated with natural polymer lignin called PVC - Lignin blend (substituting certain parts of the synthetic PVC polymer with lignin), and without lignin PVC control. In order to assess the mentioned objective of research, a series of vinyl composite samples were prepared and subjected to fungal growth. Three types of lignins (AL, Indulin and Tomlinite) were used. Some samples were also prepared by incorporating the purified AL lignin (washed with water) to differentiate the growth rate between washed and unwashed AL lignin. Further, four different types of plasticizer are used for the vinyl composites.

PVC blend formulation comprises of 80-phr (per hundred parts of resin) PVC or VC-VAc copolymer, 20- phr (AL lignin purified and unwashed, Tomlinite, Indulin) 200 - phr of calcium carbonate, 3 -phr of heat stabilizer, and 1.5-phr lubricant. Plasticizers DOP, 2-45, Lindol and Mesamoll were used in quantity from 30 to 35 phr of PVC or PVC- Lignin blend. Whereas PVC control samples consists of 100 phr of PVC or VC-VAc copolymer and all above mentioned additives except lignin. Composite specimens PVC or VC-VAc - Lignin blend were kept for 28 days incubated in an environment with a relative humidity of 98% and temperature 28°C. High moisture and temperature combination provided the most favourable conditions for fungal growth. The experiments are carried out in the absence of light source.

The spore suspension of five different ubiquitous fungal species *Aspergillus niger, Penicillium pinophilum, Chaetomium globosum, Gliocladium virens, Aurebasidium pullulans* were used to inoculate the PVC control and PVC – Lignin blend, composite sample specimens (dog bone shape and as sheet form) surfaces. These fungal species used for inoculation of composite specimens are most abundant in our outdoor and indoor environment (ASTM G21-96). Fungi are heterotrophic organisms, which feeds on the carbon sources. The additive present in vinyl formulations such as: plasticizers, heat stabilizers, lubricant and or natural polymer lignin serves as the potential source of carbon.

It is well known fact that normally fungi are unable to metabolize on the synthetic vinyl polymer. However, additives and lignins are main constituent for their feeding. It is established fact that plasticizers are more susceptible to microbial attack, as their simple structure is easily catabolised by microorganisms.

Assessment of fungal growth on vinyl composite specimens was done by visual examination, loss of weight and change in mechanical and thermal properties. To assess the change in mechanical properties of a specimen due to a biotic process, one set of reference specimens without inoculation were kept under the same temperature and moisture condition in incubator for 28 days as inoculated specimens were kept.

Previous research results established that PVC – Lignin (Al) blend is more vulnerable to fungal attack as compared to the PVC without lignin. Alcell lignin was used with 35 parts of different plasticizers in her research work (Hui, 2002).

In this thesis, the analysis of several plasticized vinyl homopolymer/copolymer blended with different lignins (Alcell, Indulin and Tomlinite) was done. The study

focuses on the influence of different plasticizers as well as influence of partial replacement of vinyl homopolymer/copolymer with different kinds of lignins on fungal growth. Simultaneously the changes in thermal and mechanical properties of the composites were tested. Experimental outline is shown on next page.

Phase 1

- Preparation of specimen composite (sheet): 1.5mm, with formulation comprising PVC homopolymer, PVC additives, plasticizers 35 phr, lignin (AL)

Experiment Procedure:

- Inoculation and incubation of specimens with mixed spore fungi suspension. Specimens incubated without fungal spore suspension (controls), to check the effect of high humidity and temperature on the specimen
- Analysis after fungal attack: visual analysis; appearance, growth rate

Phase 2

Formulations with VC-VAc copolymer and 30 phr of plasticizer

- Preparation of specimen composite (sheet): 1.5mm, with formulation comprising of VC-Vac copolymer, PVC additives, plasticizers 30phr, lignin AL and purified AL)

Experiment Procedure: as mentioned in Phase 1

Analysis after fungal attack: visual analysis: appearance, growth rate, weight loss

- Thermal Properties: Tg of the composites

Phase 3

Formulations with three different types of lignins and 30 phr of plasticizer

- Preparation of specimen composite (sheet): 1.5mm, with formulation comprising VC-VAc copolymer, PVC additives, plasticizers 30 phr, lignin (AL,, Indulin , Tomlinite).

Experiment Procedure: as mentioned in Phase 1

Analysis after fungal attack: visual analysis: appearance, growth rate, weight loss

- Mechanical Properties: modulus, tensile strength at yield and break and elongation
- Thermal Properties: Tg of the composites

Phase 4

Formulation with PVC homopolymer with 35 phr plasticizers and different types of lignins

- Preparation of specimen composite (Sheet): 1.5 mm, with formulation comprising PVC Homopolymer, PVC additives, plasticizers 35 phr, lignin (Alcell , Indulin , Tomlinite)

Experiment Procedure: as mentioned in Phase 1

Analysis after fungal attack: visual analysis: appearance, growth rate, weight loss

Figure 3.0 Experimental outlines

3.2 Raw Material Constituents of Vinyl Composite

3.2.1 PVC

The PVC polymer used in the sample preparation includes vinyl chloride (Oxy 185) and vinyl chloride (VC) – vinyl acetate (VAc) copolymer (Oxy 1810), supplied by Occidental Chemical Corporation, TX, USA. Synthetic polymer PVC is not susceptible to biodegradability while studies have revealed the biodegradation of VC-VAc copolymer.

VC-VAc Copolymer

The synthetic polymer resin is used in the program, Oxy 1810, a vinyl chloride – vinyl acetate copolymer. The properties of this resin are presented in Table 3.1

Table 3.1 Properties of the Oxy 1810 VC-VAc copolymer (Occidental Chemical Corporation, 2001)

K –Value	57
M_w	54000
M_n	26000
Specific gravity	1.37
Bulk density g/cm^3	0.63
Particle size % retained, 40 mesh (in micro meter) % through, 200 mesh (in micrometer)	 10 -
Volatiles, %	1
Bound vinyl acetate, wt %	9.7

PVC Homopolymer

The PVC homopolymer Oxy 185 was procured from Oxy Vinyl, LP Texas. PVC homopolymer reported not biodegradable.

Table 3.2 Properties of PVC homopolymer PVC (Occidental Chemical Corporation, 2001)

K –Value	56
Specific gravity	1.4
Bulk density g/cm^3	0.59
Relative Viscosity	1.82
Volatiles, %	1
Bound vinyl acetate, wt %	9.7

3.2.2 Additives

3.2.2.1 Plasticizer

Four types of plasticizers were used in preparing the samples Table 3.3. The details about their chemical composition are given in Figure 3.1 to 3.3.

Table 3.3 Plasticizer types used in the research

Plasticizer/Trade name	Abbreviation	Supplier
Di-octyl-phthalate	DOP	Fisher Scientific
Di-ethylene glycol dibenzoate/Benzoflex 2-45	2-45	Velsicol
Tricresyl-phosphate/Lindol	Lindol	Akzo Nobel
Alchil phenol/Mesamol	Mesamol	Bayer AG

Di-octyl –phthalate (DOP)

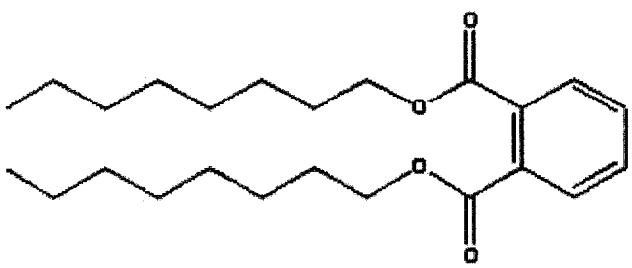

Figure 3.1 Chemical structure of DOP

Chemical Formula: $C_{24}H_{38}O_4$

DOP mainly released in the atmosphere from industrial effluents. The compound was found in the soil, sludge, sediment and water due to the release from plastic waste. Physical and chemical properties of the di-octyl phthalate are shown in Table 3.4.

Table 3.4 Physical and chemical properties of di-octyl phthalate (DOP) (Fisher Scientific)

Molecular Formula	$C_{24}H_{38}O_4$
Relative molecular mass	390.57
Physical state	Liquid
Color	Colorless yellow oily liquid
Odour	Slightly aromatic
Solubility in water	0.24-.34mg/l
Octanol-water partition coefficient	4.88

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

Di –ethylene glycol benzoate is a high solvating plasticizer used in vinyl flooring. Plasticizer 2-45 is clear colorless to slight straw colored, having faint aromatic odour, and is slightly soluble in water. Plasticizer 2-45 is produced from the benzoic acid and belongs to the benzyl ester group. The Food and Drug Administration (FDA) legally approved this plasticizer to be used in the US for use in the plastic and glues in food packaging (Anon, 2002c).

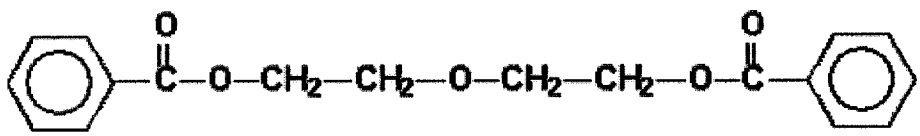

Figure 3.2 Chemical structure of 2-45

Table 3.5 Physical and chemical properties (Velsicol Corporation technical data sheet)

Molecular Formula	$(C_6H_5CO_2CH_2)_2O$
Relative molecular mass	314.4
Physical state	Liquid
Color	Clear colorless
Odour	Mild ester odour
Solubility in water	38.3mg/l at 30°C
Octanol-water partition coefficient	3.0

Biodegradability of the 2-45:

Information available from the Velsicol Corporation limited document and test performed showed that 2-45 is biodegradable in aerobic conditions. The CO_2 evolution test (modified Sturm test) was conducted and showed that mean CO_2 production by mixtures of Benzoflex 2-45 was equivalent to 16% of the theoretical value (Total CO_2 106.4 mg CO_2) after 2 days of incubation and 63% after 10 days; a mean level of 83% degradation was achieved by the end of the test on day 29 (Velsicol Corporation technical data sheet).

Lindol - (Trycresyl phosphate)

Lindol is a fire resistant plasticizer, which is widely used in the vinyl flooring. Lindol has a relatively low volatility and good characteristics of resistance to extraction by oil. As information from the (Akzo-Nobel, 2004), Lindol is a flame retardant plasticizer used in vinyl and cellulosic plastics and cellulose nitrate and ethyl cellulose coatings (Anon, 2004d).

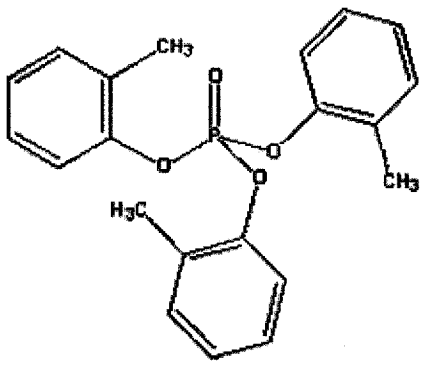

Figure 3.3 Chemical structure of Lindol

Table 3.6 Physical and chemical properties of the Lindol (anon, 2004d 1)

Molecular Formula	$C_{21}\ H_{21}\ O_4\ P$
Relative molecular mass	368.4
Physical state	Liquid
Color	Colorless
Odour	Very slightly aromatic
Solubility in water (mg/l)	0.36
Octanol-water partition coefficient	5.11

Biodegradability of Lindol:

A 200-µg portion of Lindol was completely degraded within four days in 200 ml of Mississippi River (USA) water at room temperature. The degradation pathway for Lindol most probably involves a stepwise enzymatic hydrolysis to orthophosphate and phenolic moieties. The phenol would then be likely to undergo further degradation. The major metabolite extracted with ethyl ether from the aqueous phase was identified as *p*-hydroxybenzoic acid by thin-layer chromatography and gas chromatography-mass spectrometry, while two other radioactive spots remained unidentified (Anon, 2004d1).

Mesamoll

Mesamoll is a high solvating, saponification resistance, and highly compatible plasticizer for vinyl flooring. Its high resistance to saponification makes it more beneficial in the articles, which come into contact with water and alkali. Mesamoll also has an outstanding resistance to weathering and light. Mesamoll has excellent dielectric properties that give added benefit to plasticized PVC for outstanding weldability (Anon 2004e). Physical properties of Mesamoll are shown in Table 3.7.

Table 3.7 Physical and chemical properties of the Mesamoll (technical sheet from Bayer)

Molecular Formula	$C_{15}H_{31}S\ O_3\ C_6H_5$
Specific gravity	1.03-1.07
Physical state	Liquid
Color	Colorless
Odour	Very slightly aromatic
Solubility in water	Not miscible in water
Octanol-water partition coefficient	Soluble in common organic solvents

Biodegradability of Mesamoll:

Mesamoll has high resistance to degradation as it is having very high saponification resistance.

3.2.2.2 Heat Stabilizer

Dibutylin dilaurate (DBTL) was employed as a heat stabilizer, which was supplied by Sigma Aldrich Canada Ltd (2001). The physical properties are shown in Table 3.8.

Table 3.8 Physical and chemical properties of DBTL (International Chemical Safety Cards, ICSC: 1171

Molecular Formula	$(C_4H_9)_2Sn(OOC(CH_2)_{10}CH_3)_2/C_{32}H_{64}O_4Sn$
Relative molecular weight	631.6
Physical state	Oily liquid or waxy crystals
Color	Yellow
Specific gravity	1.1
Solubility in water	Not miscible in water
Boiling point / range (°C)	205 at 1.3 KPa

3.2.2.3 Filler

Calcium carbonate $CaCO_3$, is used as the filler for the formulations. The filler was supplied by the Steep Rock Resources in Perth, Ontario. The snow-white grade 12 was used. The properties are shown in table 3.9.

Table 3.9 Properties of the $CaCO_3$ (Snow white 12)

Typical physical properties	Bulk density (loose), g/cm^3	0.8
	Bulk density (packed), g/cm^3	1.4
	Specific gravity	2.71
Chemical analysis	$CaCO_3$ (%)	96
	$MgCO_3$ (%)	0.2
	Acid Insoluble	0.2
Particle size	% Retained, 325 mesh	0.1
	Mean particle size	12 μ

3.2.2.4 Lubricant

Calcium stearate (CaS) lubricant grade L-155, was obtained from Blachford Ltd. Canada, was used in the formulation of composite samples. Generally, as the name indicates, it is used to reduce the friction between the particles to the mixture it is added. Although it is mainly used in food industry, CaS is also an important constituent of mould releasing agents in the plastic processing industry and a stabilizer in PVC resin (Anon, 2002f). Physical properties are presented in Table 3.10.

Table 3.10 Physical and chemical properties of Calcium stearate

Molecular Formula	$Ca(CH_3(CH)_{16}COO)_2$
Mean molecular weight	607
Physical state	Fine flowing powder
Color	White to yellowish white
Bulk density/g/ml max	0.16-0.38
Melting point (°C)	179
Solubility in water g/100ml	0.2

As per the description of its contents, this lubricant is biodegradable.

3.2.2.5 Lignin

Three types of lignins used in this formulation were Alcell (Tab 3.11), Indulin (Tab 3.12), and Tomlinite (Tab 3.13). Alcell an organosolv-type, supplied by Alcell Technologies Inc., Miramachi, New Brunswick, was utilized in the VF formulation. Alcell lignin was used in two forms, washed (purified) and unwashed.

Purified Alcell Lignin

Washed Al lignin - 50 grams of Al was taken in a one litre flask and stirred in hot water for 7 to 8 hours at a temperature 55 °C. The approximate water quantity used in

washing was 700 ml. After completion of the process, the solution was filtered through Whatman filter paper with a Buckner funnel. The filtrate was removed under a vacuum and the remaining residue on the filter paper was dried in an oven at 95°C, and kept in a desiccators. The residue was weighed and dried again until no significant difference was noted. Washing of the lignin was done in order to remove volatile organic contents and water-soluble impurities from the lignin.

Table 3.11 Properties of the Alcell lignin (Technical sheet from Alcell Technologies Inc.)

M_w	<2000
M_n	800-900
Specific gravity	1.27
Softening temperature (ring and ball, ASTM E28), (°C)	145
Median particle size, micrometer	20-40
Solubility parameter δ,$(Cal/cm^3)^{1/2}$	13.7

Indulin (Kraft Pine Lignin)

Indulin AT is a purified form soft wood Kraft lignin. It contains no hemicelluloses and ideal for the use in a wide range of polymeric applications where solid dispersant or adsorption properties are required. Indulin was obtained from Westvaco Chemicals, Canada.

Table 3.12 Physical properties of Indulin (Wastvaco chemicals)

M_w	<2000
M_n	800-900
Specific gravity	1.27
Softening temperature (ring and ball, ASTM E28) (°C)	145
Median particle size, micrometer	20-40
Solubility parameter δ, $(Cal/cm^3)^{1/2}$	13.5

Tomlinite Lignin

Tomlinite (hard wood lignin) used in experiment is a purified form of Kraft lignin (Table 3.13).

Table 3.13 Properties of Tomlinite lignin(Wastvaco chemicals)

M_w	<2000
M_n	800-900
Specific gravity	1.295
Softening temperature (ring and ball, ASTM E28) (°C)	145 °C
Median particle size, micrometer	20-40
Solubility parameter δ, $(Cal/cm^3)^{1/2}$	16

3.3 General Procedure Involved in Experiment

3.3.1 Preparation of the Mixed Fungal Spore Suspension

In this research program fungi species listed below are utilized. The fungi species were obtained from the American Type Culture Collection (ATCC) in a freeze-dried powder in the double vials.

Table 3.14 Types of fungi and optimum media (ATCC, 2002)

Fungi	ATCC No.	Optimum Medium
Aspergillus niger	9642	360 potato dextrose agar
Penicillium pinophilum	11797	360 potato dextrose agar
Chaetomium globosum	6205	329 potato malt agar
Gliocladium virens	9645	360 potato dextrose agar
Aurebasidium pullulans	15233	28 Emmons modification of Sabouraud agar

The preparation of the fungal suspension conducted on the basis of the Standard Practice was used to determine the resistance of the synthetic polymeric materials to fungi (ASTM G21-96). In order to revitalize the freeze-dried culture, 0.5 ml of the sterile water is added to the culture and placed in an inner vial cavity and the mixture is then transferred to a 5 ml test tube, which contains 5 ml sterilized water.

After keeping the contents for one night of rehydration, the cultures are moved to their respective optimum media as described in Table 3.14. The rest of the cultures are kept in the refrigerator at 5°C. Further, for preparation of a spore suspension, the subcultures of each species were inoculated on the respective media plate. These plates were incubated under the most favourable condition for the fungal growth i.e. temperature of 28 °C and humidity of 100 % (Hui, 2002).

Table 3.15 Composition of nutrient salt agar solution and nutrient salt agar (ASTM, 1996)

360-potato dextrose agar (PDA)	Diced potatoes, 300.0 g Glucose, 20.0 g Agar, 15.0 g
28 Emmons modifications of Saburauds agar	Sabouraud glucose agar 65g Distilled water,1.0 L Adjusted pH to 6.8-7.0 Autoclave at 121°C for 15 min Sabourauds glucose agar
329 Mineral salts agar	$NaNO_3$, 2.0 g $MgSO_3$, 0.5 g KCl, 0.5 g Fe_2 (SO) $_3.H_2O$, 0.01g KH $_3PO_4$.0.14 g Adjust pH to 7.2 Autoclave at 121°C /20 min Distilled water, 1.0 L Yeast extract, 0.02 g Agar, 15.0 g K_2HPO_3, 1.2 g

The fungal spores that were obtained for the preparation of the spore suspension were taken from the fungal slants prepared earlier. They were kept under refrigerated conditions. The fungal slants were revitalized after three weeks. These slants served as the initial inoculums source for the fungal spores. The potato dextrose agar and sabouraud agar were prepared in distilled water according to above-mentioned formulation. Both media were dissolved in the warm water and further heated up to boiling temperature to achieve a clear solution in the flask. The media were then sterilized at high temperatures with the time combination of 121°C/15 min. The media were poured into petridishes by sterilized pipette.

After a few hours, the media hardened after cooling. The whole process was done under strict sterilized conditions in a laminar flow cabinet to avoid contamination from the outside environment. Petridishes containing the media were inoculated with fungi from the slants with the help of platinum wire. To avoid contamination, the platinum wire was sterilized on the flame (gas burner) each time after taking out particular species of fungi. These inoculated petridishes were then incubated at a temperature of 28°C where humidity of 98-100% was maintained. During the next 15 days, there was profound fungal growth on the media. Normally it takes two to three weeks for them to grow well.

A 10 ml sterile solution of 0.05g/l of sodium dioctyl sulfosuccinate was then poured into the petri dish with sporulated growth to harvest the fungal spores. The surface was then gently scraped to break the spore carrying bodies called sporangium. Subsequently, the spores were transferred to sterile stopped plastic vials having conical bottoms containing 12 solid glass beads, 5 mm in diameter. In order to break down the

fruiting body and spore clumps to release the spores, the tubes were vigorously shaken on the shaker. The suspension was then filtered through the sterilized funnels stuffed with a thin layer glass wool filter media. The whole suspension was then carefully filtered into another 50 ml-sterilized tube. The spore suspension was centrifuged in International Equipment Company, MA., USA, HN-S11 centrifuge equipment for 15 minutes at 4000 rpm. The upper portion of water was cautiously removed from the tube and settled residue resuspended in 50 ml of sterile water. This step was repeated three times and the final residue was resuspended with nutrient-salts agar solution constituting contents as in Table 3.16 except agar. The final washed residue was diluted with sterile nutrient salt solution in such a manner that the resultant spore suspension was counted to contain approximately 100000 spores /ml, as determined by the counting chamber by microscope.

This process was repeated for each organism used in the test and was blended in equal volumes based on their concentrations, to make resultant mixed spore suspension. Spore suspension prepared could be used for four days keeping it at room temperature.

Viability Control Test

To confirm the viability of the spore suspension, the suspension was sprayed with the sterilized atomizer on the hardened nutrient-salts agar in a separate petridish, so that the entire surface could be moistened. Inoculated petridishes were then incubated at 28-30 °C at a relative humidity of 95-100% and examined after 14 days. Copious growth on media indicated the viability of the spore suspension. The same procedure was performed in all three stages of the experiment. Mixed spore suspension was used in all the three stages of the experiment.

Table 3.16 Composition of the nutrient –salt agar solution and nutrient salts agar

Compounds	Weight/Litre
Potassium dihydrogenorthophosphate(KH_2PO_4)	0.7g
Magnesium sulfate ($MgSO_4$, $7H_2O$)	0.7g
Ammonium Nitrate (NH_4NO_3)	1.0g
Sodium Chloride (NaCl)	0.005g
Ferrous Sulfate ($FeSO_4$, $7H_2O$)	0.002g
Zinc Sulfate ($ZnSO_4$, $7H_2O$)	0.002g
Manganous Sulfate ($MnSO_4$, H_2O)	0.001g
Agar	15g
Potassium monohydrogenorthophosphate (K_2HPO_4)	0.7g
Distilled water	As per requirements

Visual Assessment Test Preparation

The visible examination was performed after the samples were removed from the incubator and then rated on a scale 0 to 4.

Table 3.17 Observation scale for visible effects

Observed growth on the specimen rating method sporulating and non sporulating or both	Rating
None	0
Traces of growth (less than 10%)	1
Light growth (10 to 30%)	2
Medium Growth (30 to 60%)	3
Heavy growth (60% to complete coverage)	4

3.3.2 Preparation of Sample

The preparation of samples was done in four stages. In all stages, different types of formulations were used. In first stage, the PVC homopolymer was used, and in next two stages the VC-VAc copolymer was used, and in last stage again the PVC homopolymer was used. The formulations were prepared with different types of plasticizers and lignins. In every stage, control samples (those without lignin) were also

prepared. The purpose of the controls was to determine whether the fungal growth rate was different than that of blended samples.

The preparation of the specimens was carried out in few steps. The ingredients were melted, mixed in the twin extruder mixing system, grounded, compressed, moulded into sheet formation, cut into dog bone shape/used as whole sheet form. Further specimens were conditioned, inoculated, incubated, visually inspected, washed, dried, and finally ready for mechanical and thermal properties testing.

3.3.2.1 Processing Technique

The specimens prepared in first phase stage were used to determine the visual fungal growth rate only. In the first phase of the experiment, five specimen groups were prepared. The samples were prepared using the four types of plasticizers with all lignin types mentioned in the Table 3.18 and Table 3.19. Only one control sample without lignin was prepared using the same plasticizers. Sample sheets were formed and subsequently, each sheet was cut into a dog bone shape. Six dog bone specimens were taken from each sheet. Among them, three specimens were inoculated with fungal spores and the other three samples were kept without inoculation. This was done to determine an effect on the samples in equal conditions, adjusting for temperature and humidity. The control samples that were incubated under these conditions also helped in distinguishing the changes that occurred after the fungal attack.

All constituents of the formulation were accurately weighed, and the powdered constituents were mixed and made into dough with the liquid contents. The dough was mixed thoroughly to form a homogeneous compound. This compound gradually shifted for melt-mixing chamber equipped with two kneaders. The transfer of the material was

carried out in the stipulated time period of less then 45 seconds. The filling coefficient was 67% for the net chamber volume of 60 cm^3.

Subsequently, the melt mix was homogenized further for another 8 minutes at 145 °C, with a rotor at the speed of 65 rpm in the Haake Rheomix 600 powered by the variable speed motor. The Haake Rheomix 600 is batch-type mixer that is equipped with a couple of kneaders. The temperature in the chamber is controlled with high precision thermocouple. The mixture is thermally heated and cooled with the cooling sockets provided with in the mold. The torque in the mixer is controlled by the torque rheometer, the Haake Rheocord-M300, and it is reflective of the torque throughout the mixing process. The thermocouple is connected to a digital displaying temperature recorder.

Once mixing time of 8 minutes elapses, the machine stops and material is place on a clean surface quickly to avoid over heating of sample that can degrade temperature sensitive constituent of the materials, like PVC. Several batches for each formulation were prepared. The material was procured from the melt-mixing machine, immediately grounded, and cut in to small pieces, size of approximately 2-3 mm in width and maximum length of 10 mm.

The small chips were compression moulded into sheets of 1.5 mm thickness at a temperature of 155°C and under a pressure of 5 MPa. The compression moulding is done on the Carvey Laboratory Press equipped with the digital temperature displayer and temperature controller. Mould consisted of two platens, one of which is movable and other is fixed. Both platens are fitted with the thermocouple for temperature control in the system. The platens are allowed to heat for some time to attain the required temperature. The polymer chips are placed on the metal plate after weighing certain

quantity of material according to density of the polymer material; and the hollow mould of rectangular shape is placed on the chips. Further, the material is covered with the other metal platen to close the mould. Then mould is transferred to the movable hot platen carefully. The metal plates covering the mould from the side are enveloped in the foil so the two get a smoother and glossy surface on the sheet. A heating period of 6 min with no pressure was allowed. The compression press was attached with a manual hydraulic system to close the platens tightly towards each other. The pressure can be read on the gauge on the machine. The pressure is applied for a period of 2 min, followed by cooling in the cooling press for 5 min under a pressure of 4 MPa. The Cooling Press is similar to the compression-moulding press as it has thick metal platens attached with manual hydraulic system without a heating mechanism. Formulation for control samples is given in Table 3.18.

Table 3.18 Formulation for control sample in Phase 1

Formulation for Phase 1(35 phr Plasticizer)	
Ingredients	Composition (phr)
Resin PVC homopolymer	100
Plasticizer (DOP,Lindol,2-45,Mesamoll)	35
Filler	200
Heat Stabilizer	3
Lubricant	1.5

Sheets formed were then cut into a dog bone shaped specimen with a cutting die. The samples were kept at room temperature 23 ± 2°C, with a relative humidity of 50 ± 5% for one week prior to their inoculation with the fungal spore solution. The samples were kept under dark condition to avoid photodegradtion (Schnabel, 1981).

Normally, lignin is resistance to fungal attacks, although wood decaying fungi are able to degrade the lignin. The formulation given in Table 3.19 was used for the PVC blend with lignin.

Table 3.19 PVC blend with four types of lignin in Phase 1

Formulation for Phase 1 (35 phr Plasticizer)	
Ingredients	Composition (phr)
Resin PVC homopolymer	80
Lignin (Alcell)	20
Plasticizer (DOP,Lindol,Mesamoll,2-45)	30
Filler	200
Heat Stabilizer	3
Lubricant	1.5

Several batches were prepared for each type of formulation listed below. After melt mixing in the twin-screw extruder mixer, the product was grounded to 2-3 mm particle size. Subsequent sheets of 1.5 mm thickness were moulded by compression at 153°C and 4 MPa pressure for 4 min and 6 MPa for 2 min afterwards. The moulded sheets were cooled in a mould at room temperature, at a cooling rate of 10/min under pressure of 4 MPa.

3.3.2.2 Experimental Procedure - Phase 1

In the experiment procedure of the PVC control with different plasticizers and the specimen with PVC – Lignin blend (formulations mentioned in Table 3.18 and 3.19) were inoculated by the mixed fungal spore suspension.

In the beginning of this microbiological step, the nutrient agar salt solution was prepared. The experiment was carried out in a laminar flow cabinet. The cabinet is properly cleaned with a 70% ethyl alcohol solution and further sterilized with the UV

light to make it free from any microbial contamination. The blower was turned on two create an air curtain between the cabinet and the outer environment. The flame inside the cabinet was turned on to sterilize the surface of the apparatus used in process. The sterilized nutrient agar solution as mentioned in Table 3.16 was poured into the petri dishes aseptically with a sterlized pipette.

The petridishes with agar solution were allowed to harden for a period of time. Then three specimens with same composition were placed on petridish containing agar media for fungal spore spraying and the three specimens were placed in same condition as control samples without fungi. Before putting the sample in the petridish, the specimens were sterilized with the 70% ethanol by just mopping their surface.

The same procedure was followed by putting the specimens with the other composition in the petridish. Now some nutrient salt solutions were transferred into a sterilized atomizer (TLC reagent sprayer 422530 series) by pipette. Whole glass apparatus of atomizer and the tubing used for nitrogen supply were sterilized before use. The salt solution sprayed on the specimens without fungi at pressure of 5 KPa with nitrogen gas pressure was sprayed to create the same moist condition on the control samples as on the samples with fungi.

The spore solution was carefully poured into the sterilized atomizer. Similarly, the surface of agar and specimens were inoculated with the mixed spore suspension by spraying. Nitrogen had been checked to confirm that it was not contaminated prior to being used. The samples both with fungal spray and without fungi were then incubated in the incubator for 28 days at 28°C and 100% relative humidity.

Samples were taken out for the visual examination after 28 days. The visual examination was done under the microscope at 50x magnification (Wild M5A, Wild Heerbrugg) and the rating of the specimens done according to growth as mentioned in the ASTM G21-96. Microscope (Leitz Erogolox) system attached to photo automat MP45 was used to take the pictures. The focusing was adjusted with a timer. The light falls on the surface of the specimen and the timer adjusted accordingly from the absorbance of light on the surface.

After the visual examination the samples were washed with mercuric solution for one minute, the weight ratio of mercury chloride to water was 1:1000. Then the specimens were further rinsed in water and air dried before disposal.

PVC – Lignin blend PVC Control

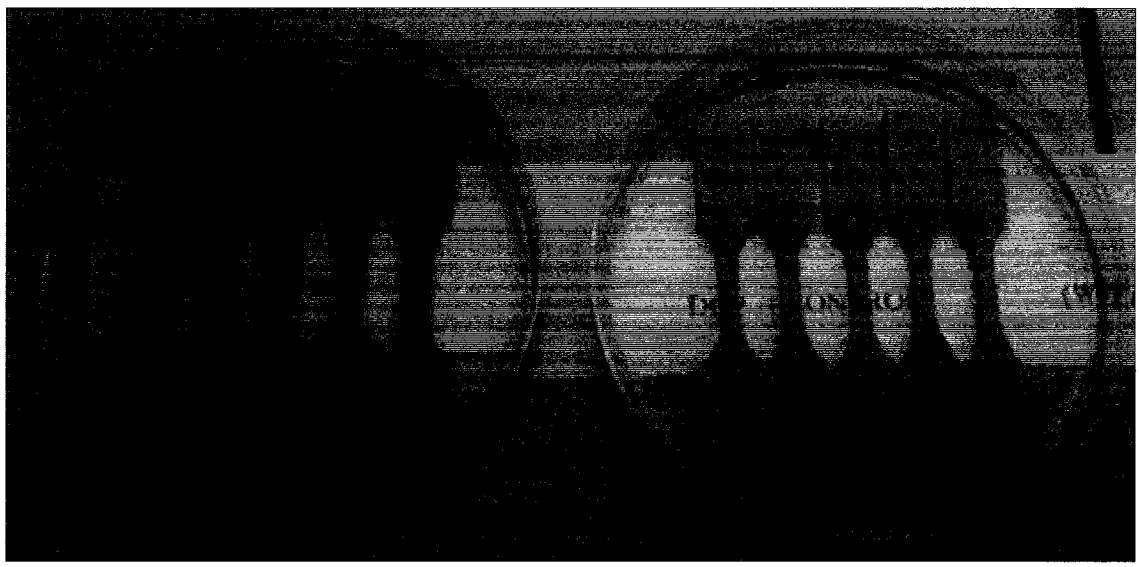

Figure 3.4 Experimental system before incubation

3.3.2.3 Sample Sterilization Process

Earlier research work done in this project illustrated that prior to inoculating the samples with the fungal spores, the samples were sterilized by dipping them in to the 70% alcohol solution for 2 min and further irradiating with the UV light. It becomes necessary to irradiate because the preparation of samples involves too much handling and there is an increase risk of contamination. Hand touch and the keeping of the sample in the open environment make it susceptible to indoor bacteria and fungi. Random fungi and bacteria species from the indoor environment can affect natural growth of the fungal species employed in the experiment. Occasionally, fungal mycelia and bacterial colonies contamination were observed in the control sample. The use of alcohol and UV light is adequate to sterilize the surface of the specimen, but they can also affect the morphology of the sample specimen. For instance, alcohol is capable of extracting the plasticizer content from the polymer material, whereas UV light can cause the chain scission, which break down bonds and cause the release of chloride ion and the oozing out of plasticizer to the surface (Schnabel, 1981). These alterations make the materials more susceptible to the fungal attacks. Considering these factors, the samples in the second phase of the experiment were mopped with alcohol rather than dipping them in alcohol. By doing this, the time of exposure to the alcohol was decreased.

In the third phase of the process, step of sterilizing the samples with alcohol was removed and new process of sterilizing specimen was involved. Although this method was pre-existing, it revolutionized procedures by introducing sterilize bags to store samples in the form of sheets.

Sterilization is a heat treatment that is carried out at high temperatures of 121°C for a certain time, depending upon the material and its ability to be liberated from microorganisms. However, spores can still survive as heating is followed by immediate cooling of samples.

Following the standard procedures, there were new processes that have been incorporated in the experiment. After the completion of melt mixing, the product was grounded into 2-3 mm particle size and then subsequently sheets were prepared at the temperature of 153° C. At this point, the process is tapped and the mould is taken out of the compression mould and cooled to room temperature in a cooling press. The mould plates were not opened in the room but the entire mould and enclosing sheets were transferred to the laminar flow cabinet, which was a sterilized chamber. The polymer sheet was then removed from the platens and aseptically relocated in the sterilized bags. The bags were sealed off in the same laminar flow cabinet. With this newly introduced process, pressing the sample at a higher temperature assumed the sterilization process. The samples were kept for 7 days at room temperature for aging and then weighed inside the laminar flow cabinet before inoculating them with the fungal spore solution.

3.3.2.4 Experiment - Phase 2

The new formulation was used in the second phase of the experiment. The PVC was replaced by a VC-VAc copolymer. The plasticizer content was reduced to 30 phr.

All four types of plasticizers (DOP, Lindol, Mesamoll and 2-45) were used with the lignin AL purified and AL. The sterilization of the samples was done by the above-

mentioned technique. Samples were kept in sterilized bags in dark before and after incubation. No light source was used in the experiment.

The control samples with all types of plasticizers were prepared. The formulation was as given in Table 3.20.

Table 3.20 Formulation used in the phase 2: VC-VAc copolymer control)

Formulation for Phase 2 (30 phr Plasticizer)	
Ingredients	Composition (phr)
Resin VC-VAc copolymer	100
Plasticizer (DOP, Lindol, Mesamoll,2-45)	30
Filler	200
Heat Stabilizer	3
Lubricant	1.5

The composite blend was prepared with all types of lignins. The formulation used is the phase 2 given in Table 3.21. Visual analysis of the specimen was carried out after 28 days of incubation.

Table 3.21 PVC blend with Alcell lignin

Formulation for Phase 2 (30 phr Plasticizer)	
Ingredients	Composition (phr)
Resin VC-Vac copolymer	80
Lignin (Alcell purified,Alcell)	20
Plasticizer (DOP, Lindol, Mesamoll,2-45)	30
Filler	200
Heat Stabilizer	3
Lubricant	1.5

3.3.2.5 Experiment - Phase 3

In third phase of the experiment, the samples were prepared for (Table 3.22) testing mechanical properties by visual examination.

Table 3.22 Formulation used in the phase 3: VC-VAc copolymer control

Formulation for Phase 3 (30 phr Plasticizer)	
Ingredients	Composition (phr)
Resin VC-VAc copolymer	100
Plasticizer (DOP, Lindol, Mesamoll, 2-45)	30
Filler	200
Heat Stabilizer	3
Lubricant	1.5

The DOP, Lindol, 2-45 and Mesamoll were used in the formulation with the four Lignins (Alcell, Alcell purified, Indulin, and Tomlinite). The samples were prepared in the form of rectangular sheets the size of the mould and the same sterilization process was used as mentioned in experiment step 2 and samples were kept in sterilized bags.

Table 3.23 PVC blend with three types lignin

Formulation for Phase 3 (30 phr Plasticizer)	
Ingredients	Composition (phr)
VC-VAc copolymer	80
Lignin (Alcell purified,Alcell,Indulin,Tomlinite)	20
Plasticizer (DOP, Lindol, Mesamoll,2-45)	30
Filler	200
Heat Stabilizer	3
Lubricant	1.5

To analyze the weight loss from the sample, the sheets were weighed before and after the incubation period of 28 days. Sheets were cut into dog bone shaped samples. After one week after washing of samples with mercuric chloride solution, the samples analyzed for the mechanical properties. The samples were kept in dark before and after incubation.

3.3.2.6 Experiment - Phase 4

The new formulation was used in the fourth phase of the experiment. The VC-VAc copolymer was replaced by a PVC homopolymer. All three types of plasticizer (DOP, Lindol, Mesamoll) were used with the lignin Indulin and Tomlinite.

The control samples with all types of plasticizers were prepared. The formulation was as given in Table 3.24

Table 3.24 Formulation used in the Phase 4: PVC homopolymer control

Formulation for Phase 4 (35 phr Plasticizer)	
Ingredients	Composition (phr)
Resin PVC homopolymer	100
Plasticizer (DOP, Lindol, Mesamoll, 2-45)	30
Filler	200
Heat Stabilizer	3
Lubricant	1.5

The composite blend was prepared with all type of lignins. The formulation used is given in Table 3.25.

Table 3.25 PVC blend with Lignins: Indulin and Tomlinite

Formulation for Phase 4 (35 phr Plasticizer)	
Ingredients	Composition (phr)
Resin PVC homopolymer	80
Lignin (Indulin, Tomlinite)	20
Plasticizer (DOP, Lindol, Mesamoll, 2-45)	30
Filler	200
Heat Stabilizer	3
Lubricant	1.5

The effects on mechanical and thermal properties of the composite material were determined after fungal growth was identified with the different lignins. Several batches were prepared for each type of formulation. After melt mixing in the twin-screw extruder

mixer, the product was grounded to 2-3 mm particle size. Subsequent sheets of 1.5 mm thickness were moulded by compression at 153 ° C and 4 MPa pressure for 4 min and 6 MPa for 2 min afterwards. The moulded sheets were cooled in a compression mold at room temperature, at a cooling rate of 10/min under pressure 4 MPa. After cooling, the samples were not cut to shape of dog bone, but rather they were stored in sterilized bags in sheet form for aging. The new step of sterilizing the sample was used in this phase of research.

The visual examination was carried out for this formulation

3.4 Mechanical Properties

The three groups of samples from each composition were tested for tensile strength. This was conducted in accordance to procedure condition laid by the (ASTM D638, 1993) using an Instron Universal Testing Machine Model 1125. Tensile test in a broad sense is measurement of tensile elongation and tensile modulus. It is a measurement of capacity of the material to hold the forces that tends to pull it apart and to determine the extent of stretching tolerated before the material breaks. Tensile modulus is an indication of the relative stiffness of a material. The mechanical properties in the composite material after the fungal attack were compared on the basis of tensile strength, elongation and tensile modulus data. The tensile strength data was used to select a particular type of the plastic material.

The tensile testing machine was set at a constant rate of crosshead movement equipped with a fixed head. This machine comprised of a grip and a movable member, carrying a second grip. Self-aligning grips were employed for holding the test specimen

between the fixed member, and the movable member prevents alignment problems. The control velocity drive mechanism was also used. The load indicated by the mechanism shows the total tensile load that was used. The extensometer gave the reading of the elongation of the specimen. The software in the extensometer performed the stress, the elongation, the modulus, the energy, and statistical calculations automatically. The tensile test properties were carried out at the standard recommended conditions of a temperature of 23 ± 2°C and 50 ± 5 % relative humidity.

Tensile Test

The tensile test was conducted at a speed of 0.2 in/min for each of the specimens. The tensile strength was calculated by under given formula

$$\text{Tensile strength} = \frac{\text{Force (Load) (kg)}}{\text{Cross section area (cm}^2\text{)}}$$

3.5 Thermal Properties

Thermal behaviour of the polymer blend was studied by determining their Tg value using the Digital scanning colorimeter (DSC) tests. DSC method used the differences in heat capacity of a reference and the sample material analyzed over a predetermined time, at a programmed temperature. The reference specimen and samples to be analyzed were placed separately and heated in individual controlled units. The differential temperature of the specimens was monitored by area thermocouples located on the bottom of the disc beneath each specimen pan. The differential power or energy required to achieve this state of affairs was recorded against the programmed temperature of the system. For transitions involving latent heat such as fusion, the heat of transition

was determined by integrating the heat energy input over the time interval covering the transition.

The thermal properties of the blends were measured using a 912 Dupont differential scanning calorimeter connected with a Dupont DSC Standard Data Analysis Program version 4.0 at heating rate of 20 °C /min under nitrogen atmosphere between 50 to 160 °C. The reported Tg values were estimated from the second scan and the transition midpoint temperatures. The glass transition (Tg) value is also known as the primary transition. This transition arises as result of excitation of rotation of segments of main chains.

At least two specimens of 14-15 mg were scanned at a heating rate of 20°C/min, under nitrogen atmosphere between -40 °C/min, and 160°C for 30 phr formulations. The reported Tg value represents the inflection point of heat flow versus temperature curve.

Analysis of data and discussion of results are presented in Chapter 4.

CHAPTER 4 RESULTS AND DISSCUSSION

Samples in triplicate prepared with different vinyl composite formulations were subjected to fungal growth as described in Chapter 3. The results from testing the above mentioned formulations were compared with previous research on resistance of vinyl composite material to fungi attack (Hui, 2002):

- VC-VAc Copolymer 100 parts for controls, VC-VAc Copolymer 80 parts and AL 20 parts for blends

- Plasticizer 35phr (DOP or 2-45 or Lindol or Mesamoll)

- Heat stabilizer (dibutyl tin dilaurate) 3.0 phr

- Lubricant (calcium stearate) 1.5 phr

- Filler (calcium carbonate) 200 phr

Sheets, of 1.5mm thickness, from each formulation were prepared and tested according to ASTM specifications. Films of 0.1mm thickness were also prepared from the same formulations without filler for Fourier Transform Infrared Spectroscopy (FTIR) examination and loss in weight determination.

The visual and microscopic examination of the specimens showed that the mixed fungi and five individual fungi significantly grew on both plasticized control and plasticized blend specimens. The results of mixed fungi growth are summarized in Table 4.1

Table 4.1 Rating of fungi growth on surface of VA-VAc control and blend (Hui, 2002)

	Rating of Growth							
	DOP Plasticizer		2-45 Plasticizer		Lindol Plasticizer		Mesamoll Plasticizer	
Specimen	Control	Blend	Control	Blend	Control	Blend	Control	Blend
Mixed fungi	2	3	3	4	2	3	2	3

Rating: 0=none; 1=trace of growth (less than 10%); 2=light growth (10 to 30%); 3= medium growth (30 to 60%); 4=heavy growth (60% to complete coverage)

FTIR results indicated that VC-VAc copolymer, as well as the AL, were not remarkably affected by the fungi. The FTIR spectra showed that the fungi growth lead to some degradation of the plasticized controls and blends. The plasticizers were the main targets of the fungi attack, 2-45 being the most and Lindol being the least susceptible to attack. Although the AL alone was not remarkably affected by fungi attack, the fungi growth was higher on the blends than on the control surfaces. The PVC films used for FTIR showed the loss in weight after 28 days of incubation.

The order of weight loss of films was as follow:

Controls: 2-45 = (2.45%)>Mesamoll= (1.30%)≅DOP= (1.28%)>Lindol= (0.46%)

Blends: 2-45= (5.33%)>Mesamoll= (2.68%)≅DOP= (2.22%)>Lindol=1.23%

All the experiments showed that the used fungi species were able to grow on VC-VAc control and VC-VAc-AL blend specimens. Fungi biodegradation, of the surface of specimen was established through visible surface changes, weight losses and modification of the chemical structure of the plasticizers that were used. The biodegradation of the plasticized blends was more intense than that of the controls. This may be attributed to the more complex system of lignin dispersion in PVC and to a different morphology of each pair plasticizer-lignin in the blends.

The order of sensitivity to fungi attack in these experiments as function of type of plasticizer was as follows:

2-45 > DOP > Mesamoll > Lindol

For ease in processing and to enhance flexibility of the formulation, a vinyl resin VC-VAc copolymer was utilized in the study, which contains ester groups. These groups are having weak links that provide favourable sites to fungi growth due to the ability of fungi to secrete esterase enzyme. So vinyl acetate co monomer may act as a biodegradable component and may influence the fungi ability to degrade the VC-VAc copolymer. On the other hand the ability of fungi to degrade in a higher measure VC-VAc blends may be caused by the presence of traces of low molecular weight products in AL (an organosolv lignin) such as furfural and acetic acid present in wood extraction solvents, and which could not be completely removed from the final product.

Subsequently to improve composite resistance to fungi new experiment must be introduced.

1. Replacing the VC-VAc copolymer with PVC homopolymer

2. Decreasing the plasticizer content in PVC and VC-VAc formulations from 35 phr to 30 phr

3. Preparing and assessing VC-VAc copolymer blends with 30-phr plasticizer and purified AL

4. Preparing and assessing VC-VAc copolymer controls and blends with 30 phr plasticizer and other lignin

5. Preparing and assessing PVC homopolymer controls and blends with 35 phr plasticizers and other lignins.

4.1.1 Replacing the VC-VAc Copolymer with PVC Homopolymer

In order to find out that the PVC homoplymer has a higher resistance to fungi attack than the VC-VAc copolymer, the flooring formulation in controls and blends was the same as in the previous work with the only exception that VC-VAc was replaced with a PVC homopolymer, having a close molecular weight and specific gravity with that of copolymer.

The specimens were cut into dog bone shape and five samples of each kind were kept in the agar containing petridishes as shown in figure 4.1. The specimens were inoculated by mixed spore suspension of the five fungi species. Visual observation of specimens carried out after 28 days period of incubation. The growth on some of blend specimens was abundant; where as on control samples very minor growth was observed. The observations were made on the basis of grading according to ASTM G21-96 protocol and defined in Table 3.17 in Chapter 3. Results are shown in Table 4.2.

Figure 4.1 Placing of PVC blend (left) and PVC control (right) specimens on the agar media before fungal attack

Table 4.2 Rating of fungi growth on surface of control and blend specimens prepared with PVC homopolymer

	Rating of Growth							
	DOP Plasticizer		2-45 Plasticizer		Lindol Plasticizer		Mesamoll Plasticizer	
Specimen	Control	Blend	Control	Blend	Control	Blend	Control	Blend
Mixed fungi	2	3	3	4	2	3	2	3

Results of Table 4.2 and Table 4.1 indicate that the replacement of homopolymer by copolymer doesn't make any difference in the resistance of formulations to fungi. Fungal growth is shown in Figure 4.3 and Figure 4.2. The plasticizers and AL seem to remain the main target of fungi attack.

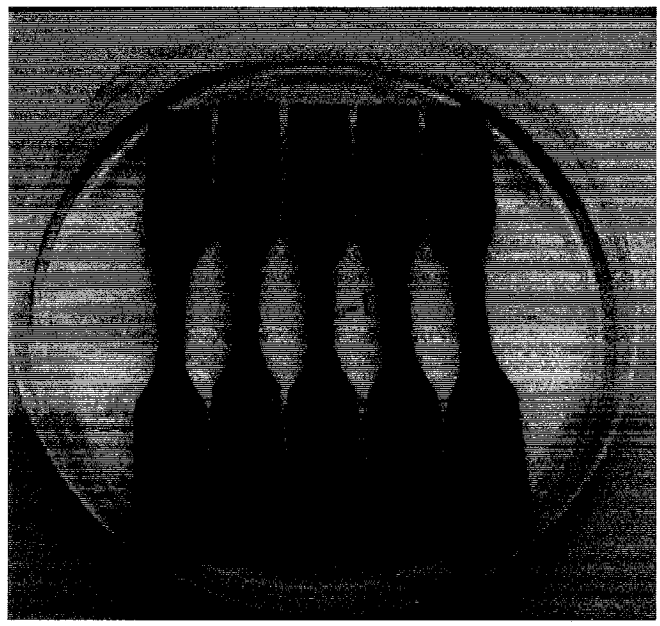

Figure 4.2 PVC Blend specimen after fungal attack

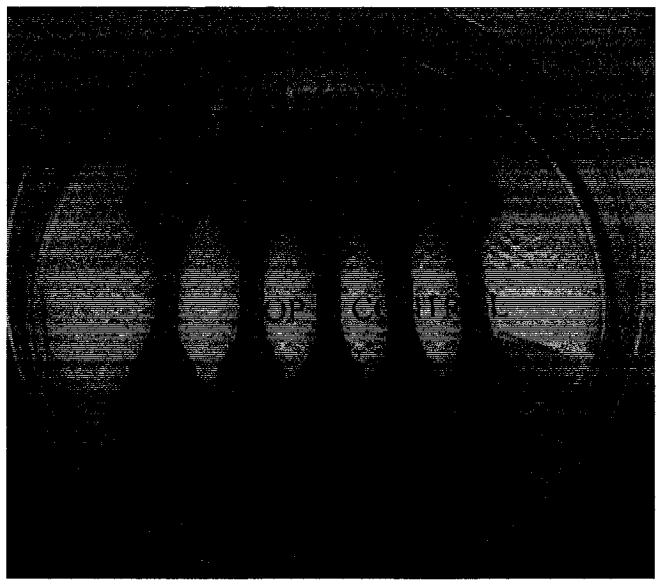

Figure 4.3 PVC Control specimen after fungal attack

In subsequent sets of experiments, the plasticizer concentration in the formulations was reduced from 35 phr to 30 phr.

4.1.2 Decreasing the Plasticizer Content in PVC and VC-VAc Copolymer Formulations from 35 phr to 30 phr

The previous result indicated that the plasticizers and lignins are the main target of fungal attack. In order to prove, that a lower plasticizer concentration would improve the resistance of the materials to fungi growth, formulations containing PVC and 30 phr of the same plasticizers were prepared and tested for resistance to fungi attack, as well as for mechanical properties.

Due to the high content of the filler and the higher viscosity of the homopolymer, there were difficulties in processing the formulations with PVC at the temperature recommended by the producer. Some degradation was observed on material surface with increase in the melt-mixing temperature by 5 °C as discussed in the Chapter- 3. Taking into account that the resistance of VC-VAc copolymer formulations to fungi attack is similar to that of PVC homopolymer with 35 phr plasticizer content and the fact that copolymer is more appropriate for highly filled formulations than homopolymer, further experiments were performed with copolymer only.

Consequently, in a preliminary experiment, control and blends with 20 parts AL formulated with the same plasticizers as before, with a content of 30 phr. were prepared and evaluated for resistance to fungi, by visual examination only. The rating of fungi growth of these new formulations is given in Table 4.3

Table 4.3 Rating of fungi growth on the surfaces of the control and blend specimens prepared with VC-VAc copolymer and 30 phr plasticizer

	Rating of Growth							
	DOP Plasticizer		2-45 Plasticizer		Lindol Plasticizer		Mesamoll Plasticizer	
Specimen	Control	Blend	Control	Blend	Control	Blend	Control	Blend
Mixed fungi	1	2	2	4	1	2	1	2

The data from Table 4.3 illustrated that decreasing the plasticizer concentration from 35 phr to 30 phr improved the resistance of the formulations to fungal growth except for 2-45 blend. As a result, the fungal growth changed from light growth (10-30%) to trace growth (less than 10%) in the controls, and from medium growth (30-60%) to light growth (10-30%) in the blends. This was true for all of the plasticizers except 2-45. However considering the above results, it can be clearly confirmed, as the previous results illustrated, that the VC-VAc copolymer blend are more vulnerable to fungal attack as compared to VC-VAc control. There was no copious amount of growth observed except sample plasticized DOP with purified AL with and AL with 2-45 plasticizers. The pigmentation of the samples shows the effect of fungal growth. All the samples lost their gloss after fungal attack. But there were no sign of serious discoloration. The specimens (dog bone shape) have more prevalent growth on sides and rough surfaces as compared to the smooth surfaces. Fungal mycelium was observed under the microscope on the specimen surfaces.

Specimens blend with AL and 2-45 were wearing some yellow marks, which can be seen in the Figures 4.4-4.20.

As was discussed in the previous work, the ability of fungi to degrade in a higher measure in blend than the controls may be due to the presence of trace of low molecular

weight products present in AL, like wood extraction solvents, which could not be completely removed from the lignin final product.

Consequently, washing of AL lignin with water was done, in order to determine if this procedure has the ability to remove traces of low molecular weight products, to improve its fungal resistance. The washing of AL was done according to some existing literature data (Yoshida et al., 1987).

4.1.3 Preparation of VC-VAc Copolymer Blends with Purified AL

In preliminary experiment, formulations with VC-VAc copolymer controls and blends with AL and purified AL, containing 30 phr of DOP plasticizer were prepared. After inoculation, and the incubation period, the specimens were evaluated for fungal growth.

The entire blend specimens lost their original luster. On the sides they became duller colored. The specimens with DOP with purified AL observed with the copious amount of growth as we can see in the Figure 4.4. Some white and yellow pigmentation was observed on the surface. The results are as shown in Table 4.4.

Table 4.4 Fungal growth rate on DOP with AL and purified AL

Specimen Type	Fungal growth Rating
DOP control	1
DOP –AL blend	2
DOP purified AL blend	3

Based on these preliminary results, the subsequent experiments consisted of the preparation and complete evaluation of VC-VAc blends done with two other commercial lingnins such as Tomlinite and Indulin.

4.1.4 Preparation and Evaluation of VC-VAc Controls and Blends other Lignins with 30 phr

The VC-VAc copolymer control and the blend formulations were prepared with non-purified AL. In other formulations, the AL (20 parts) was replaced with the same amount of Tomlinite (hard wood) or Indulin (soft wood) lignins. These lignins were obtained by alkaline delignification of wood, and presumed to contain less low molecular weight products than the organosolv AL lignin. Their molecular weights and specific gravities were close to those of AL. The rating of fungi growth on blends formulated with Tomlinite, Indulin, and AL in comparison to the controls is shown in Table 4.5

Table 4.5 Rating of growth on surface of VC-VAc blends formulated with 30 phr plasticizer and various lignins, compared to controls

Plasticizer	Control	Alcell	Alcell washed	Tomlinite	Indulin
DOP	1	2	3	1	1
2-45	2	4	-	4	4
Lindol	1	2	-	1	2
Mesamoll	1	2	-	1	1

The analysis of microbial damage to the material was scientifically studied based on the consideration, which suggests an actual interaction between the microrganism destructors and the material. The process is divided into three main stages, which are associated with each other.

1. Adhesion of microbial cells transferred to material surface;

2. Growth of the microbial cells on the material surface;

3. Variations in material properties under impact of the microorganims (Gumargalieva et al., 2003).

The visual examination confirmed the copious fungal growth on VC-VAc copolymer (blend and control) formulation with plasticizer 2-45. Whole surface of the specimen was badly discoloured with yellow plaque formation. The change in the color of polymer specimen is due to the capacity of the microorganisms to produce various types of acids, which confirms Gumargalieva et al., (2003) observations.

Another study revealed that the change in color suggests a chemical modification in lignin structure as effect of fungi attack. Literature data have shown that peroxy radicals are intermediates in the degradation and discoloration process of lignin. Usually they are formed as effect of UV light, mainly from the aryl -α carbonyl structure and structures containing conjugates double bonds. Further phenoxy radicals may lead to the formation of compounds such as alcohols, aldehydes and ketones (Gellerstodt et al., 1977).

As the dark color of lignin is due to the presence of the above-mentioned structures, it should be possible that the various types of acids produced by micro organisms are able to break, those structures or part of them, which may explain the blends discoloration.

A tentative to correlate the resistance of different blends to the fungi attack is presented. The Tg of studied lignins is 97°C for Alcell, 133 °C for Tomlinite and 142 °C for Indulin. These values are high compared to Tg of most synthetic polymers, and also

when related to the quite small molecular weight of lignins i.e. ≅ 200-for Alcell, 2400 for Indulin and 2800 for Tomlinite. The high Tg_s are due, in large parts, too strong intermolecular secondary bonds caused by the presence of hydroxyl groups in lignins.

Studies showed that it is possible to decrease the degree of association of lignin oligomers through the use of specific plasticizers (Feldman et al., 2001). The effect of plasticizers efficiency on the lignin's utilized in this study, i.e. the extent to which their Tg values were lowered, was determined by DSC and the data are the following Table 4.6. (Feldman et al., 2004, unpublished data)

Table 4.6 Tg of lignins with 35 phr plasticizer mixtures and the extent of Tg lowering (ΔTg)

Plasticizer types	Alcell		Indulin		Tomlinit	
	Tg (°C)	ΔTg (°C)	Tg (°C)	ΔTg (°C)	Tg (°C)	ΔTg (°C)
2-45	32	65	62	80	47	86
Mesamoll	83	14	121	21	130	3
DOP	-	-	126	16	123	10
Lindol	42	55	114	28	55	78

If we combine the above data Table 4.5 with the data presented in the Table 4.6 the following Table 4.7 received.

Table 4.7 Combining the data presented in the Table 4.5 and Table 4.6 the resultant data given in table

Plasticizer Types	Alcell		Indulin		Tomlinite	
	Rating	ΔTg (°C)	Rating	ΔTg (°C)	Rating	ΔTg (°C)
2-45	4	65	4	80	4	86
Mesamoll	2	14	1	21	1	3
DOP	2	-	1	16	1	10
Lindol	2	55	2	28	1	78

The sensible differences in the extent of Tg reduction of the different pairs lignin-plasticizer suggest that the magnitude and the mode of changes in lignins' chains

mobility are different. A high value of ΔTg, or a low value of Tg indicates that the degree of association of lignin molecules was reduced. The dissociated lignin, which has a lower molecular weight, as mentioned above, can be an easier target for fungi attack than the lignin with a higher degree of association.

It can be clearly seen that the ranking of fungi attack is higher in the blends obtained from lignin-plasticizer pairs, whose extent of Tg lowering is high. The only exception is noticeable for the Alcell and Tomlinite blends plasticized with Lindol.

Formulation with Lindol has shown better resistance to fungal attack. Very minute growth was observed on the specimen's surface.

To make more precise results specimens were a scrutinized under microscope for better interpretation of results. Pictures were taken with help of Microscope camera (Lietz Ergoloux) system connected to photo automat MP45 figures attached herewith. Pictures lucidly show mycelia web growth on the specimen's surface. The fungal growth is shown in the Figures from 4.4-4.20 on different specimen formulations.

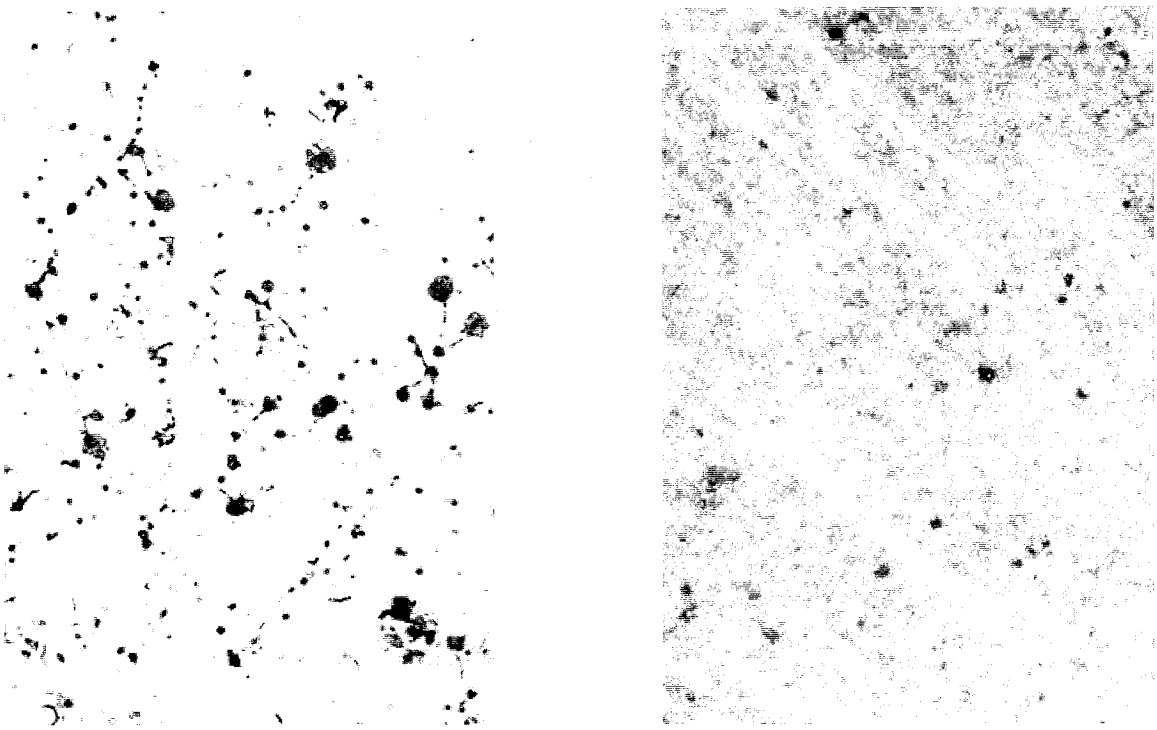

Figure 4.4 Mixed fungal growth on agar surface (left) and surface without fungal attack (right)

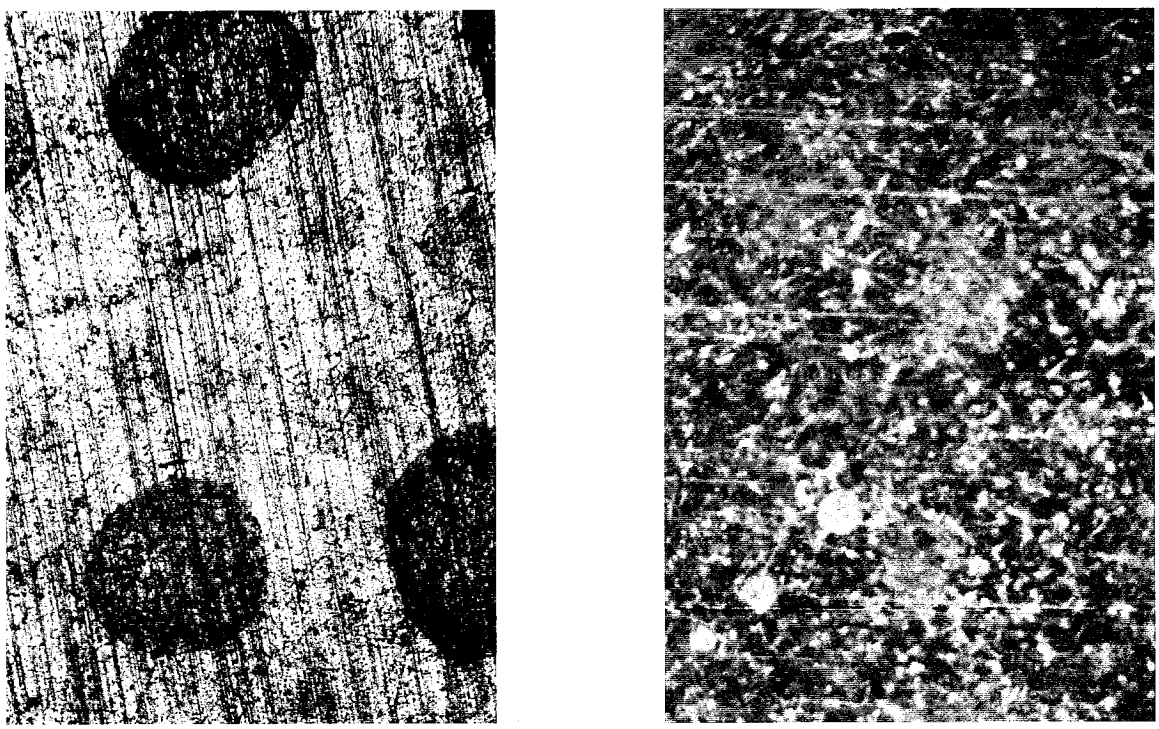

Figure 4.5 Mixed fungal growth on surface of the control specimen (left) and blend with AL purified (right) specimens plasticized with DOP

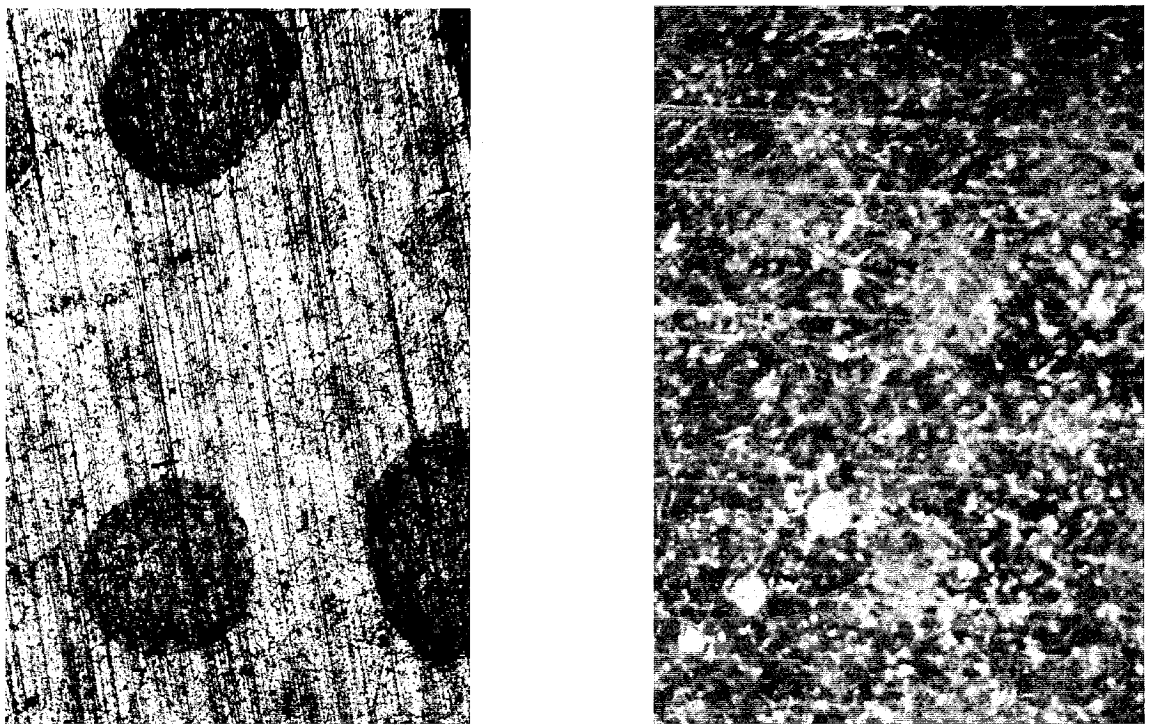

Figure 4.6 Mixed fungal growth on surface of control specimen (left) and blend with AL unwashed specimens plasticized with DOP

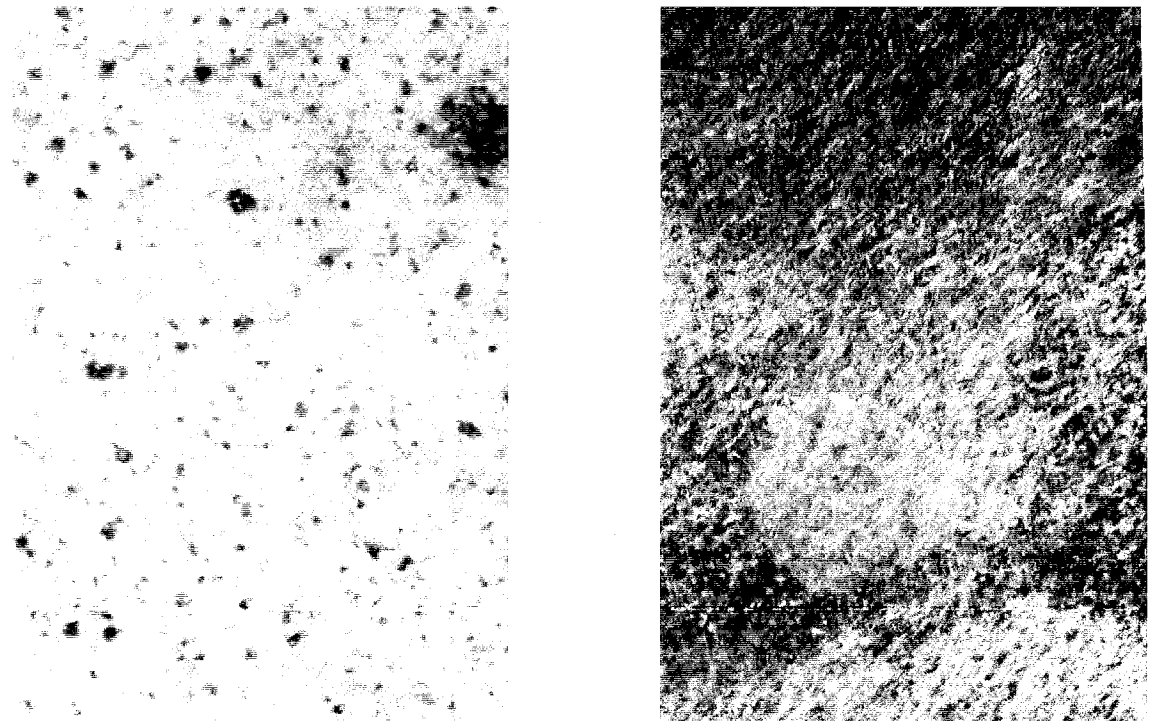

Figure 4.7 Mixed fungal growth on surface of control specimen (left) and blend with Tomlinite (right) specimens plasticized with Lindol

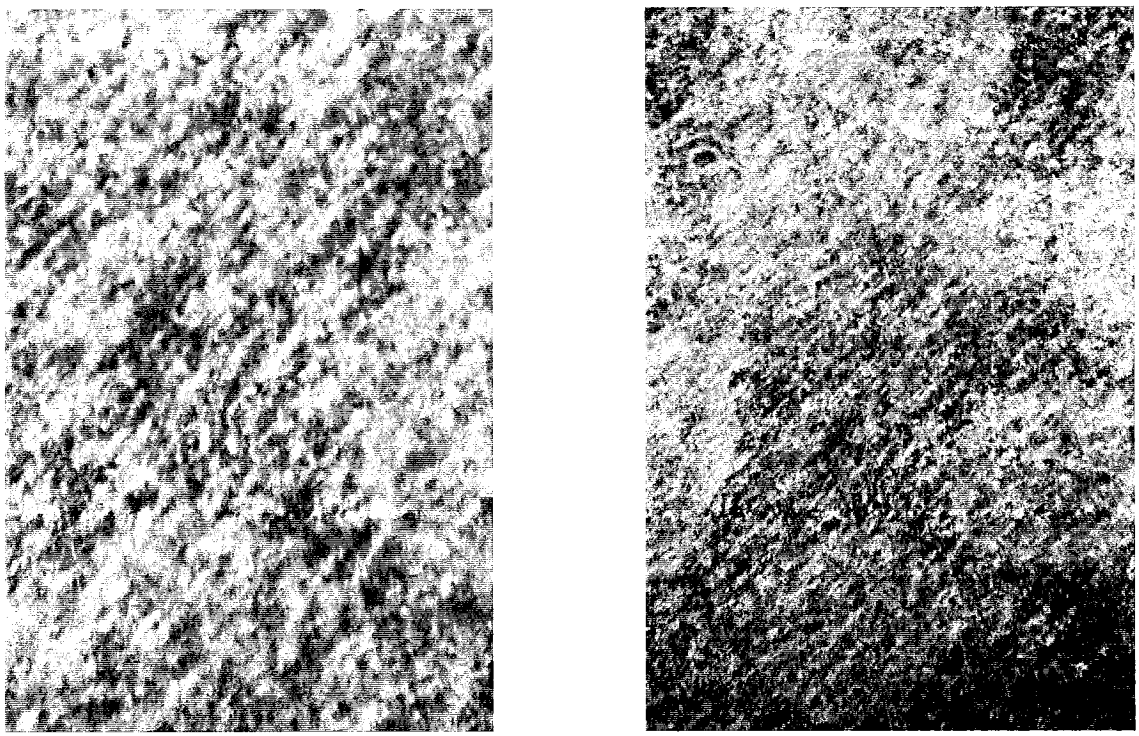

Figure 4.8 Mixed fungal growth on Lindol plasticized Tomlinite blend surface specimen both left and right

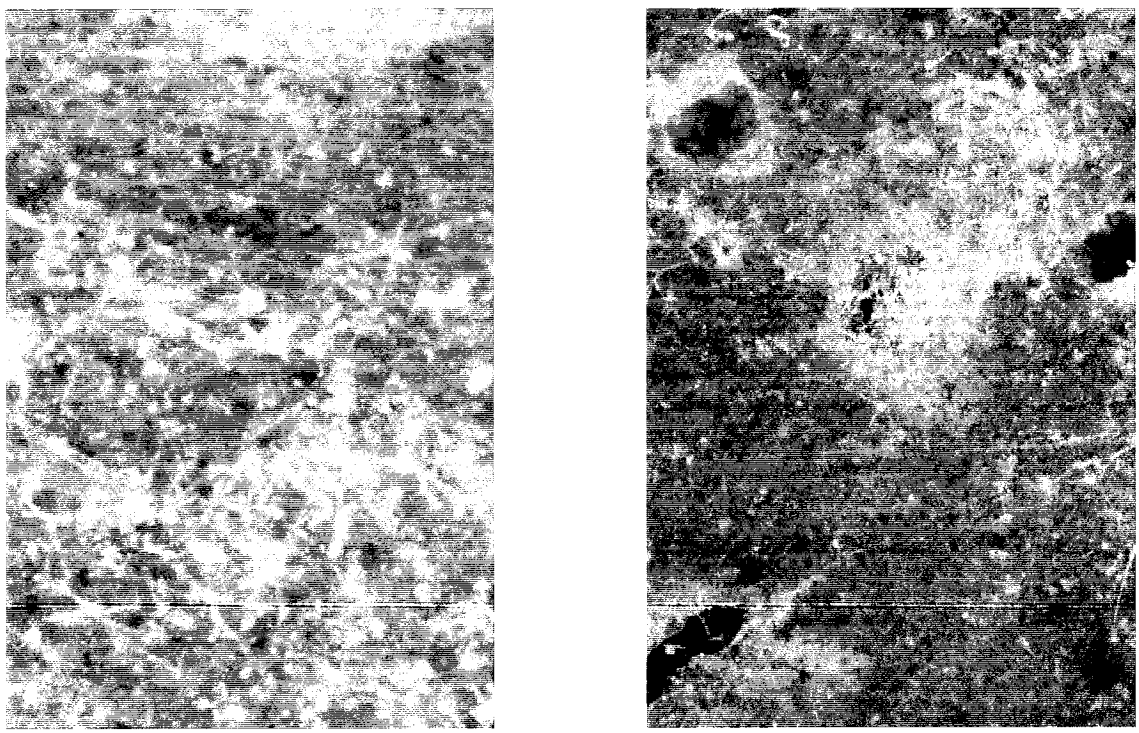

Figure 4.9 Mixed fungal growth on Mesamoll - Indulin specimen (left) Lindol - Indulin specimen (right)

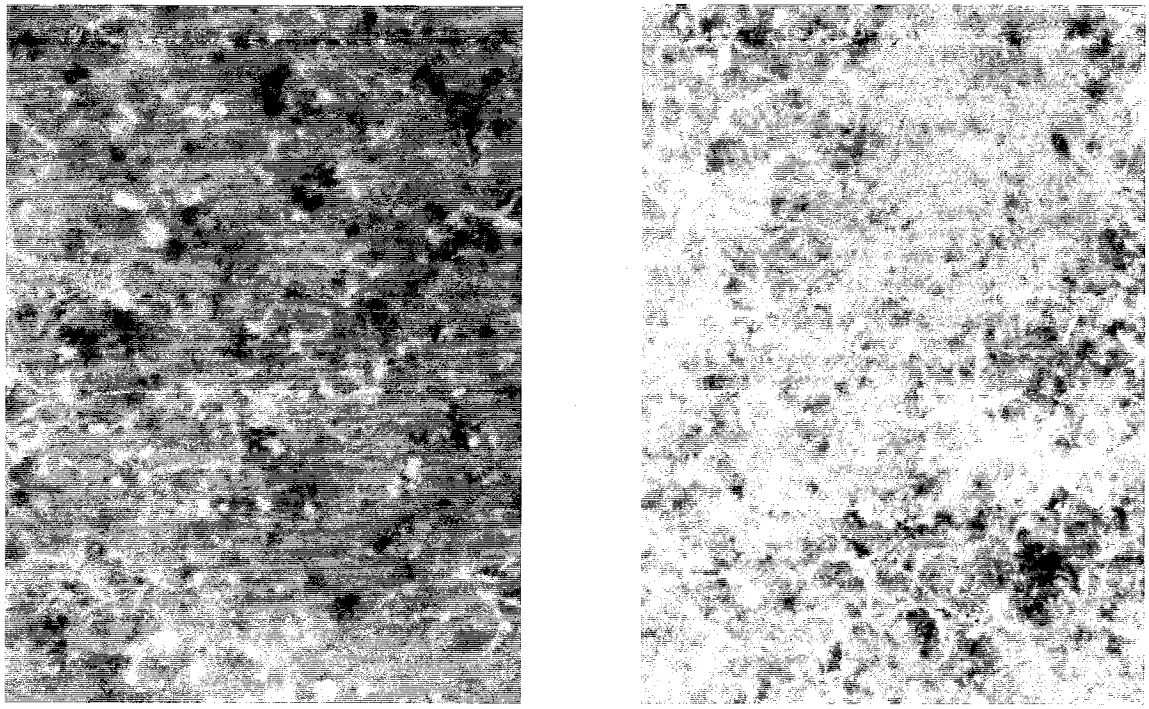

Figure 4.10 Mixed fungal growth on surface of DOP- Indulin specimen (left and right)

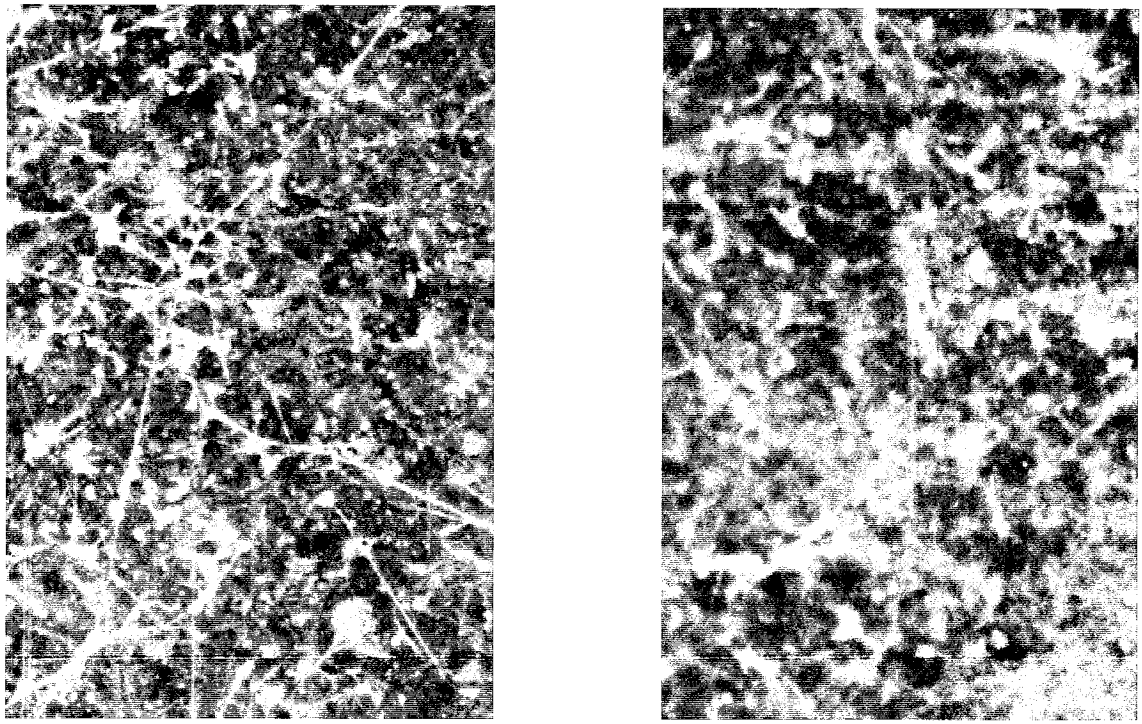

Figure 4.11 Mixed fungal growth on surface of Mesmoll - Tomlinite specimen (left and right)

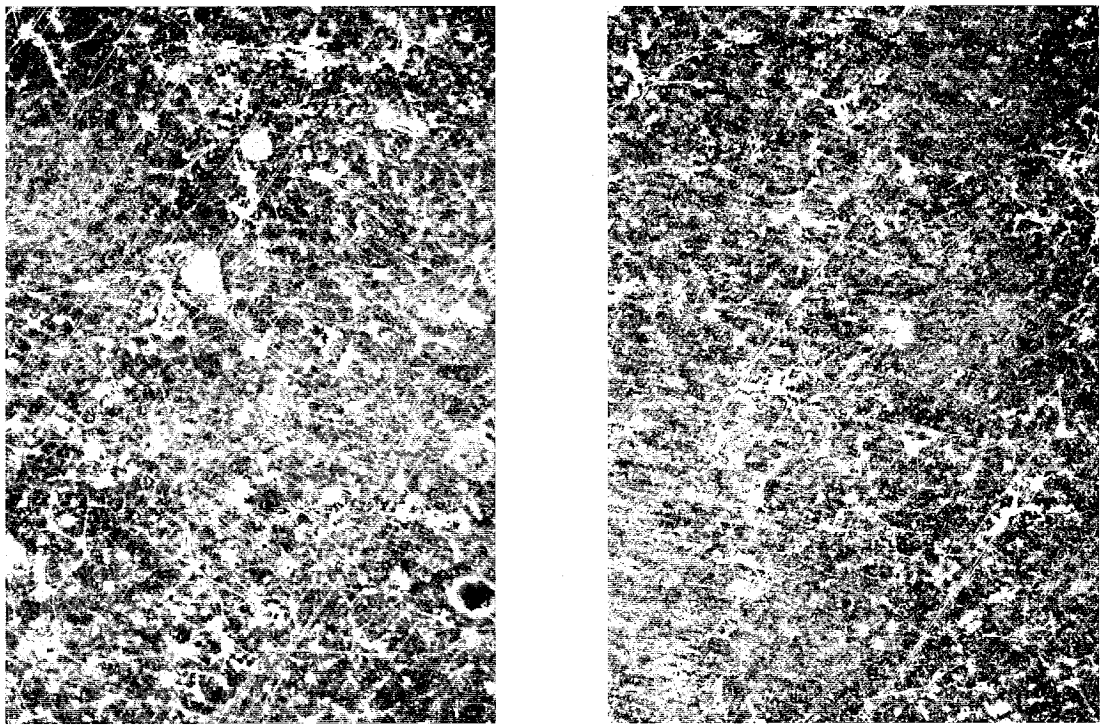

Figure 4.12 Mixed fungal growth on Mesamoll – AL specimen (left) Lindol - AL specimen (right)

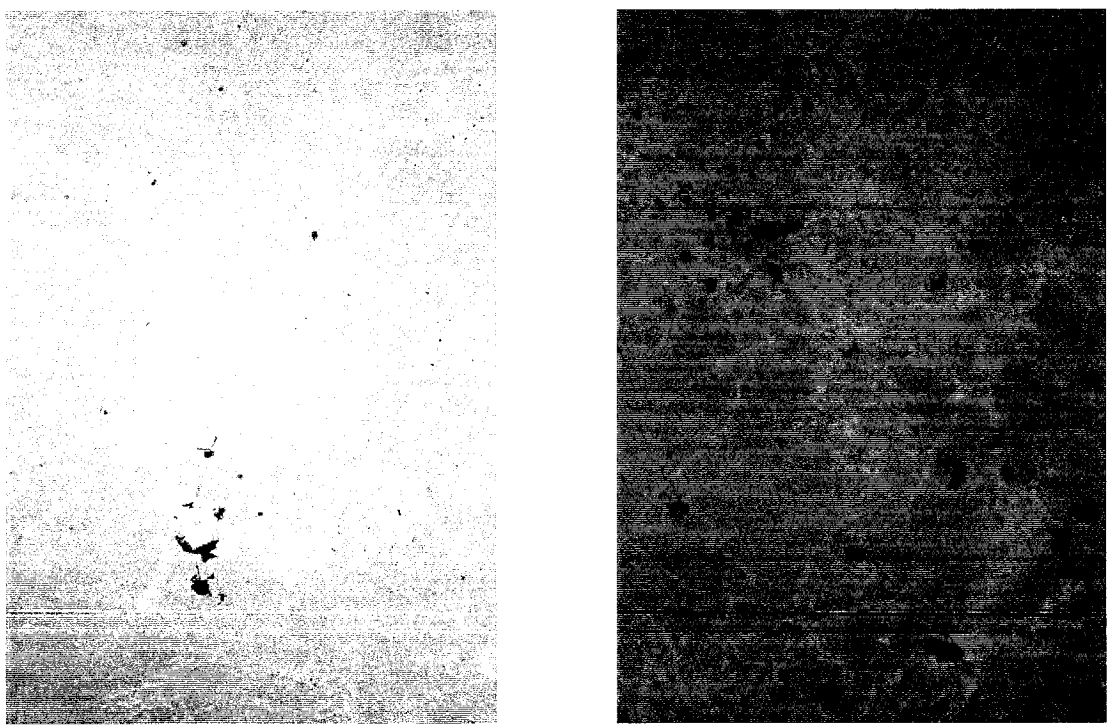

Figure 4.13 Mixed fungal growth on surface of 2-45 control specimens

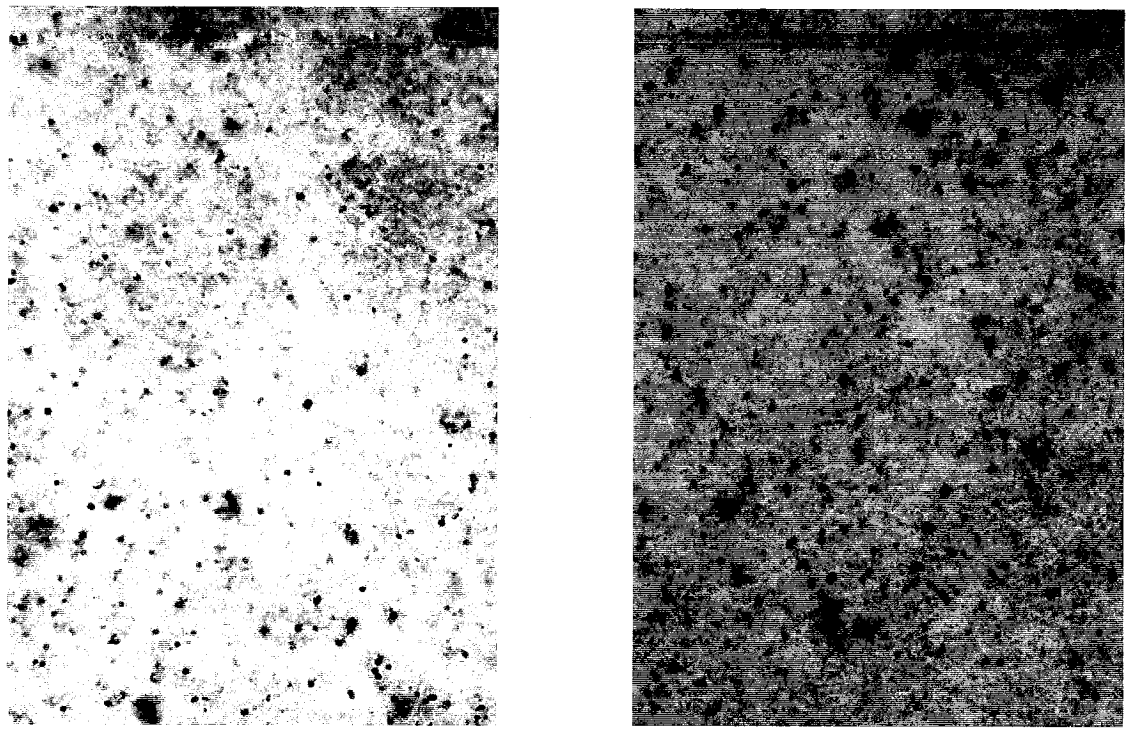

Figure 4.14 Mixed fungal growth on surface of 2-45 - Indulin specimens

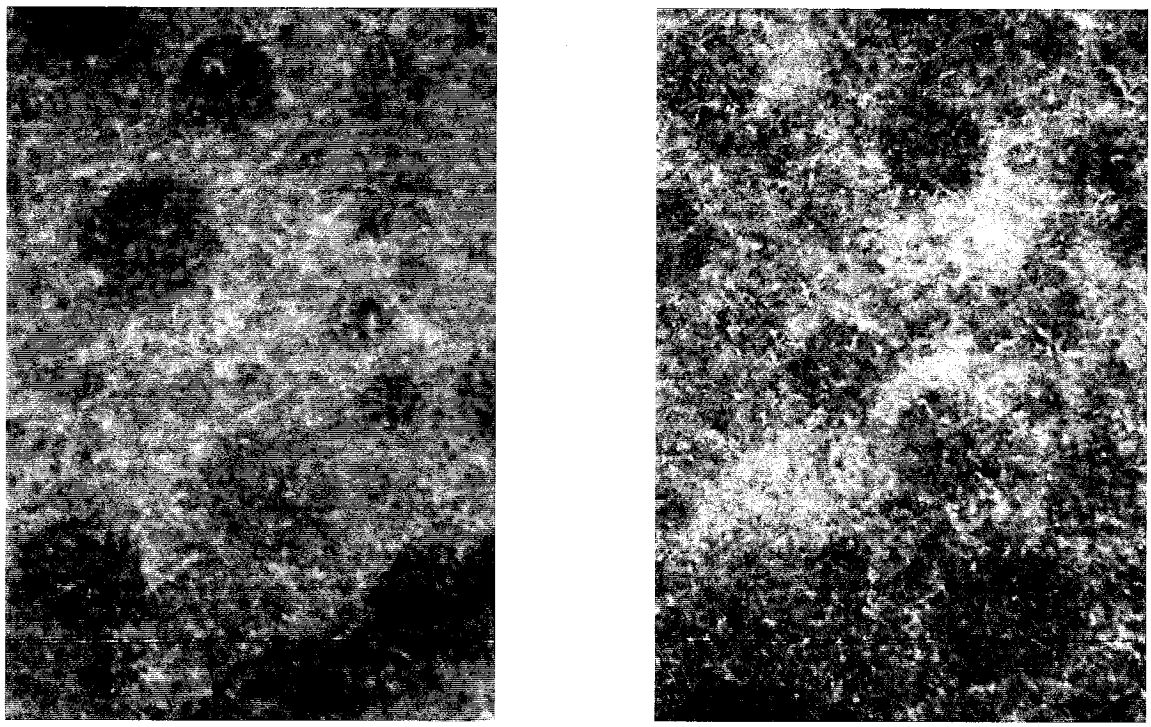

Figure 4.15 Mixed fungal growth on surface of 2-45 - Tomlinite specimens

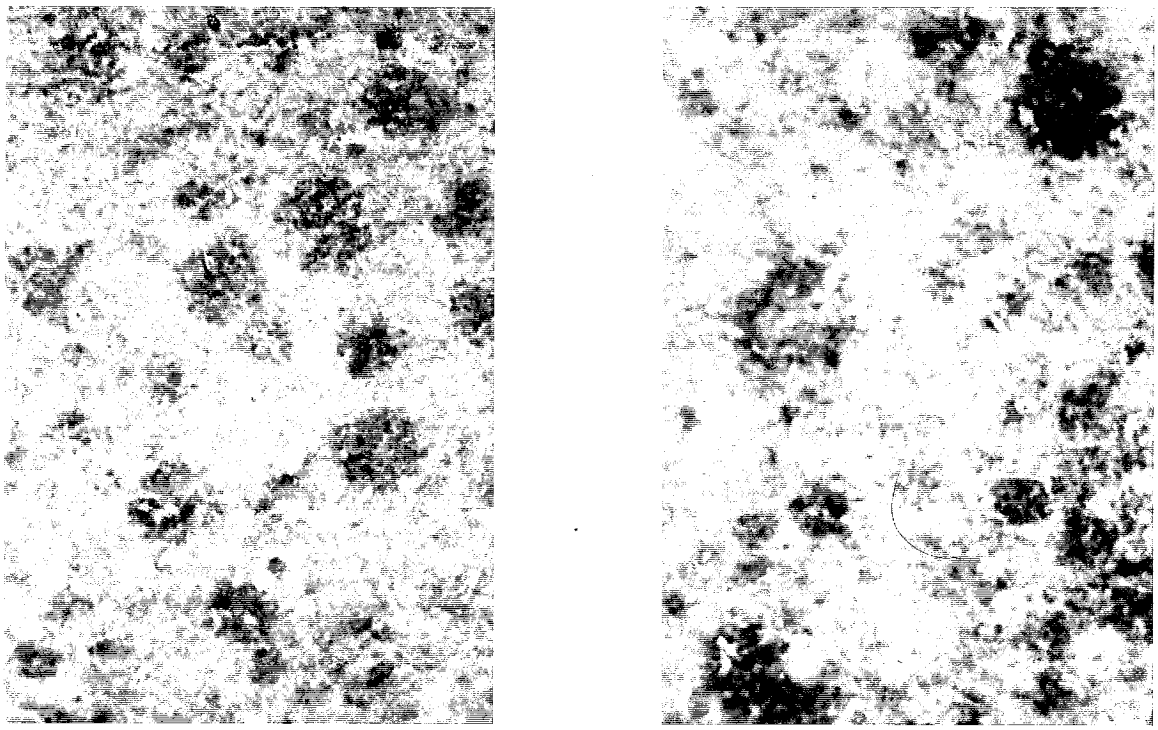

Figure 4.16 Mixed fungal growth on surface of 2-45 -AL specimens

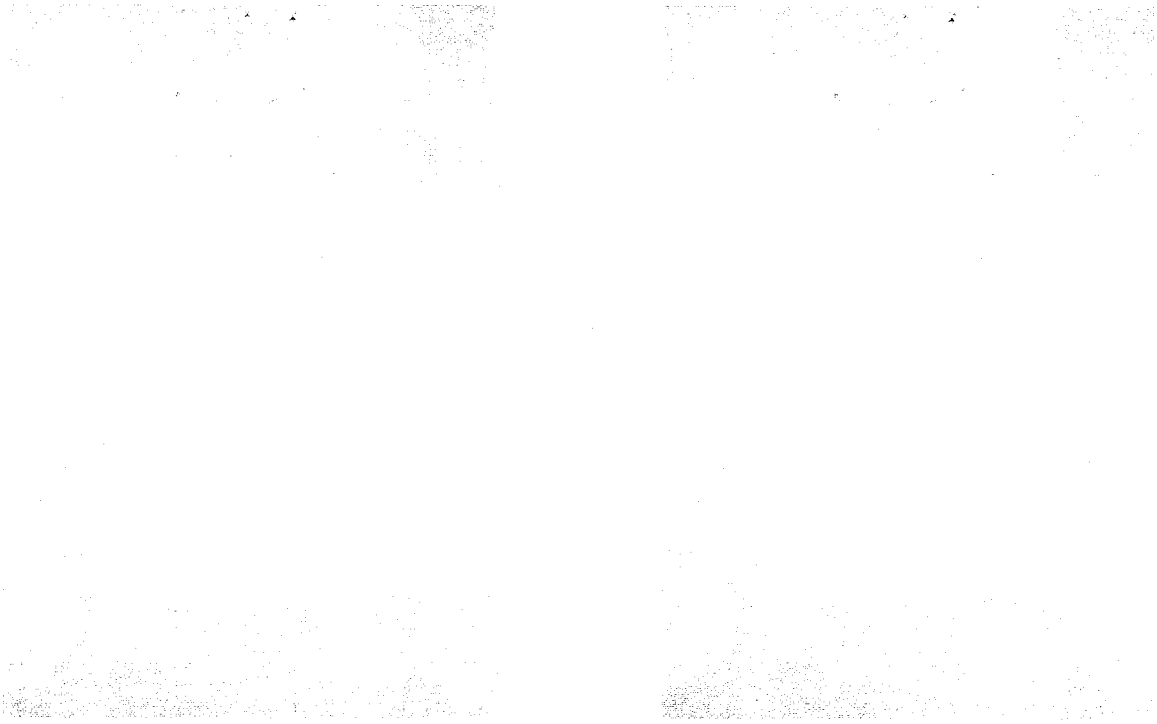

Figure 4.17 Mixed fungal growth on surface of Lindol - Indulin control specimens

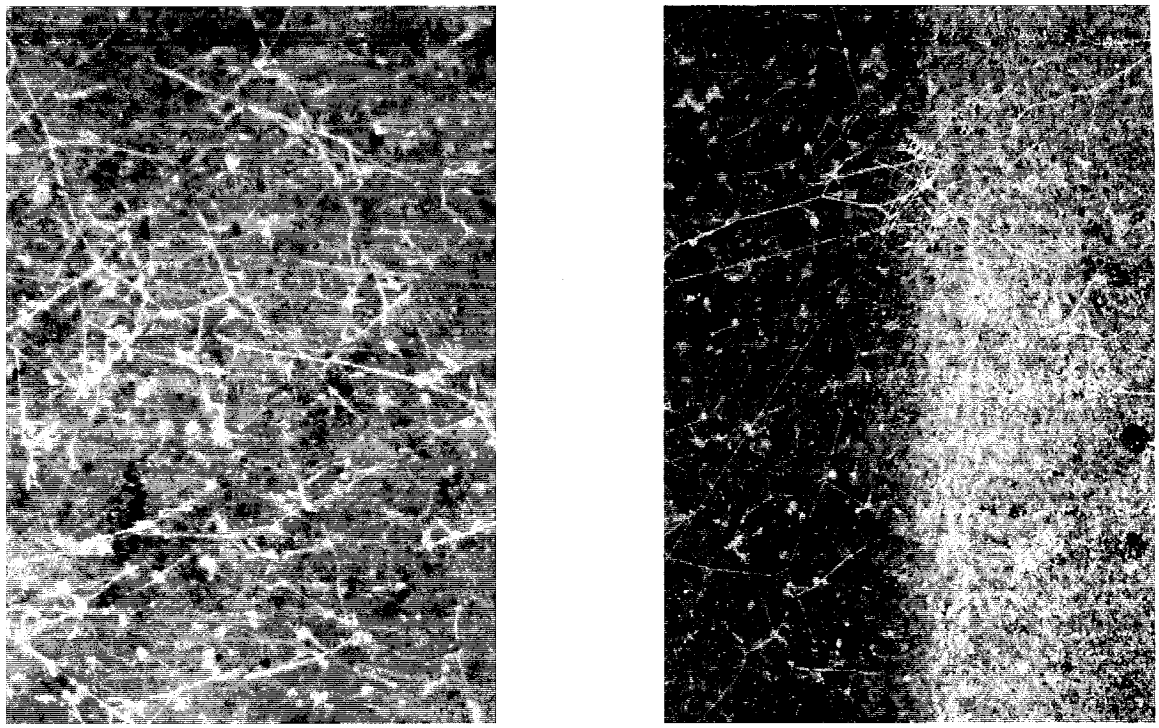

Figure 4.18 Mixed fungal growth on surface of Lindol - Indulin specimen

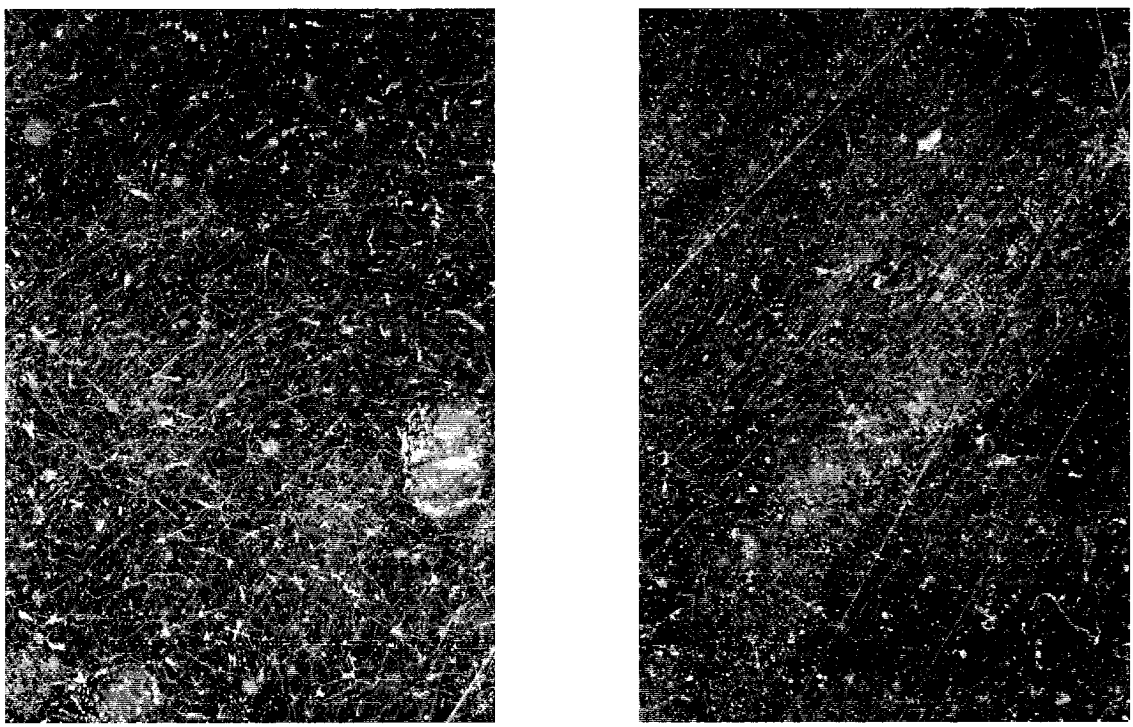

Figure 4.19 Mixed fungal growth on surface of Lindol - AL specimen

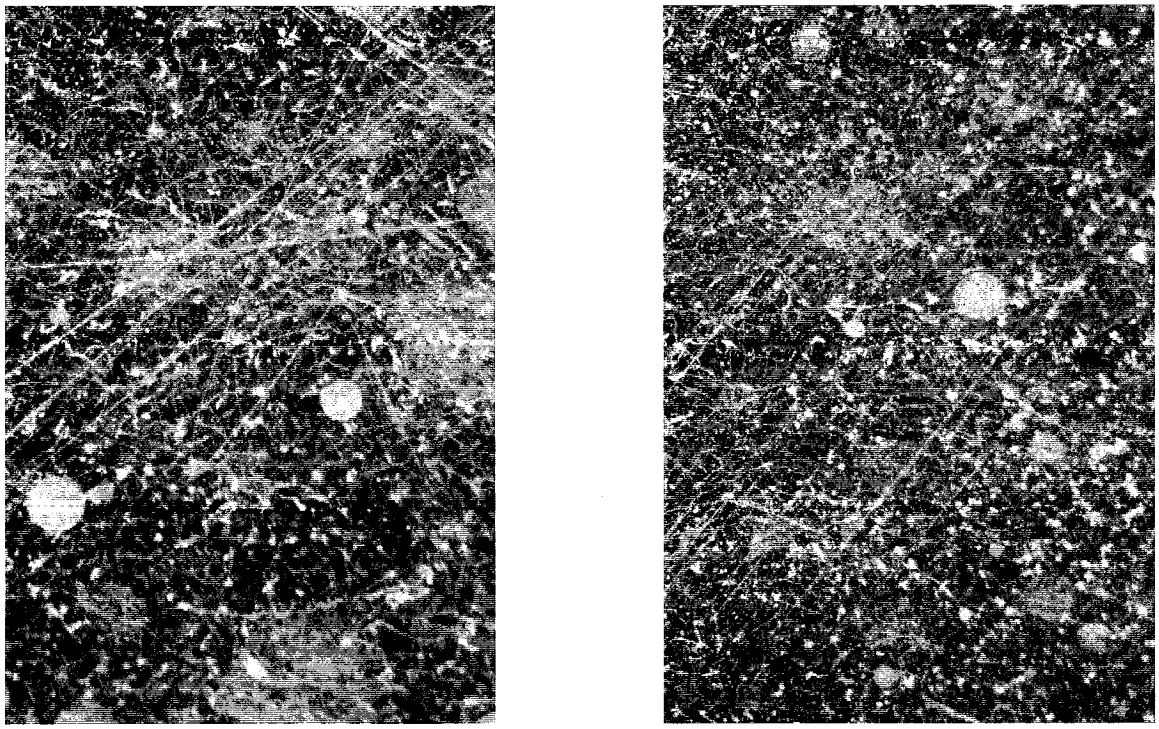

Figure 4.20 Mixed fungal growth on surface of Lindol - Tomlinite specimen

These data showed that the formulations with 30 phr plasticizer of Tomlinite blends have similar resistance to fungi attack as those of the controls. The higher resistence of Tomlinite in comparison with other lignins may be due to the fact that being a hard wood lignin is structurally more complex than soft wood lignins. Soft wood lignins contain, mostly guiacyl units (i.e. trisubstituted phenyl propane) whereas hard wood lignins contains guaycyl and syringyl units (i.e. tetrasubstituted phenyl propane). Similar observations were noted in Indulin blends plasticized with Mesamoll. These results are very encouraging because they indicated that DOP-Tomlinite, DOP-Indulin, and Mesamoll-Tomlinite blends formulated with VC-VAc copolymer and 30 phr of plasticizer could be successfully utilized for flooring formulations. Their mechanical properties were quite close to those of the controls, formulated with 30 phr of plasticizer,

2. After 28-day incubation period for non-inoculated specimens. This would establish whether the conditions of relative elevated temperature and high humidity level, as well as intimate contact with the non-nutrient agar layer (which contains a great deal of OH functional groups) would produce changes in the mechanical and thermal properties. These specimens represented sterile controls.

3. After 28 days incubation of inoculated specimens. These specimens represented inoculated specimens.

The data obtained for the mechanical tests are shown in Tables 4.8, 4.9 and 4.10. In these tables, the percentage represented the differences between the values of inoculated specimens and sterile controls and was divided by the values of sterile controls, and multiplied by 100.

$$\text{Percentage change} = \frac{Wa - Wb}{Wb} * 100$$

where Wa = Value of inoculated specimens

Wb = Value of sterile control

The differences in °C of inoculated specimens and sterile controls Tg as well as the Tg of zero controls, sterile controls and inoculated specimens are indicated in Table 4.11.

Table 4.9 Changes in modulus and elongation at break after 28 days incubation of inoculated specimens of VC-VAc copolymer control and blends

Specimen Identification	Modulus (MPa)				Elongation at break (%)			
	Zero Control	Sterile Control	Inoculated Specimen	Change%	Zero Control	Sterile Control	Inoculated Specimen	Change %
DOP Control	120	121	137	+13.2	278	258	255	-1.1
DOPBlend washed Alcell	82	167	148	-11.4	252	208	182	12.5
DOP Blend Alcell	73	190	197	+3.7	230	195	184	-5.6
DOPBlend Indulin	62	85	91	+7.1	291	253	253	0
DOPBlend Tomlinite	59	97	104	+7.2	304	217	228	+4.8
Lindol Control	391	424	415	-2.1	198	152	155	+1.9
LindolBlend Alcell	322	394	448	+13.7	193	-	-	-
Lindollend Indulin	308	365	377	+3.3	180	-	-	-
Lindol Blend Tomlinite	251	419	425	+1.4	113	-	-	-
Mesamoll Control	123	121	154	+27.0	310	296	249	-15.9
Mesamoll Blend Alcell	95	197	239	+21.3	257	169	139	-17.8
Mesamoll Blend Indulin	72	103	112	+8.7	297	242	220	-9.1
Mesamoll Blend Tomlinite	81	119	165	+14.6	240	226	195	-13.7

Table 4.10 Change in tensile strength at yield and break after 28 days incubation of incubation of inoculated specimens of VC-VAc copolymer control and blends

Specimen Identification	Yield Strength (MPa)				Break Strength (MPa)			
	Zero Control	Sterile Control	Inoculated Specimen	Change %	Zero Control	Sterile Control	Inoculated Specimen	Change%
DOP Control	5.03	5.17	5.28	+2.1	6.49	6.63	6.71	+4.4
DOP Blend washed Alcell	4.45	4.44	4.41	-0.68	4.86	4.80	4.73	-1.5
DOP Blend Alcell	3.95	4.61	4.26	+7.6	4.26	4.94	4.68	-5.3
DOP Blend Indulin	4.07	5.58	3.68	+2.8	3.92	4.03	4.14	+2.7
DOP Blend Tomlinite	4.06	4.19	4.06	+3.1	4.00	4.15	4.23	+5.6
Lindol Control	7.82	8.42	8.74	+3.7	7.27	7.95	8.26	+3.9
Lindol Blend Alcell	5.67	No yield	No yield	-	5.31	8.44	8.25	-2.3
Lindol Blend Indulin	6.22	No yield	No yield	-	5.26	6.76	6.11	-9.6
Lindol Blend Tomlinite	6.60	No yield	7.82	-	5.73	6.19	6.02	-2.7
Mesamoll Control	5.16	5.44	5.63	+3.5	6.88	7.36	7.18	-2.4
Mesamoll Blend Alcell	4.33	5.36	5.45	+1.7	4.76	5.04	5.31	+5.4
Mesamoll Blend Indulin	4.12	3.95	3.90	+1.3	4.23	4.53	4.24	-6.4
Mesamoll Blend Tomlinite	4.40	4.47	4.60	+2.9	4.03	4.63	4.40	-5.0

Table 4.11. Changes in Tg after 28days incubation of sterile controls and inoculated specimens of VC-VAc copolymer controls and blends

Specimen Identification	Zero Control	Sterile Controls		Inoculated Specimens	
	(°C)	(°C)	Change °C	(°C)	Change °C
DOP Control	25.3	27.1	+1.8	6.7	-0.4
DOP Blend washed Alcell	20.9	22.5	+1.6	21.8	-0.7
DOP Blend Alcell	20.4	22.9	+2.5	21.5	-1.4
DOP Blend Indulin	20.4	20.7	+0.3	20.3	-0.4
DOP Blend Tomlinite	20.5	21.1	+0.7	21.5	+0.4
Lindol Control	36.9	38.4	+1.5	37.3	-1.1
Lindol Blend Alcell	30.4	34.0	+3.6	34.7	+0.7
Lindol Blend Indulin	31.2	33.9	+2.7	33.1	-0.8
Lindol Blend Tomlinite	31.0	32.7	+1.7	33.9	+1.2
Mesamoll Control	28.7	30.1	+1.4	29.7	-0.4
Mesamoll Blend Alcell	21.7	23.5	+1.8	23.4	-0.1
Mesamoll Blend Indulin	22.5	21.6	+0.1	22.1	+0.5
Mesamoll Blend Tomlinite	21.5	21.7	+0.2	22.2	+0.5

From data in Table 4.8 it can be seen that when compared to the sterile controls, the modulus of the inoculated specimens had increased and their elongation had decreased. However percentages of increase or decrease were not in agreement with the visual assessment of the fungi growth (Table 4.5). From Table 4.8, it was apparent that the tensile strength at yield of the inoculated specimens was slightly higher than that of sterile controls. However, the changes in the tensile strength of inoculated specimens were erratic, presenting varied values in comparison to their respective sterile controls.

As the inoculated specimens had higher moduli and lower elongations than the respective sterile controls, it was expected that their Tg values should be higher than those of sterile controls.

From the data in Table 4.11, it can be seen that in the case of control formulations the Tg values of the inoculated specimens are slightly lower than those of the respective sterile control; whereas the blend formulations the Tg values of the inoculated specimens are slightly higher or lower than those of the respective sterile controls. However, for the most formulations of inoculated control and blend specimens (eight out of thirteen), the Tg values are lower than those of sterile control. Although in disagreement with the modulus and elongation data, the lower Tg values may suggest the presence of lower molecular weight products resulting from the plasticizer attack by fungi (alcohols, acids and/or possibly their oxidation products).

Data presented in Tables 4.8-4.10 illustrated that after the incubation period, the sterile controls presented a high degree of embrittlement. Their slight increase in modulus and Tg, as well as a slight decrease in elongation, as can be seen from Table 4.9, suggests a partial loss of plasticizer. These results could be attributed to a plasticizer migration that took place from the polymer formulations to the surrounding medium, respectively the non-nutrient agar layer that contains a great deal of OH groups. Subsequent hydrogen bonding may have taken place between the carbonyl or other ester group of plasticizers and some OH functional groups of agar. It should be noted that similar embrittlement of sterile controls in relation to zero controls, was reported by other authors as well for some unfilled plasticized PVC formulations (Hitz, 1967). Taking into account the chemical composition, MW, degree of interaction between each plasticizer and VC-VAc

copolymer, filler, lignins involved, as well as the different degrees of compatibility of each plasticizer with lignins, the possible migration of each plasticizer from each formulation into the agar layer would be different. The literature indicates that the plasicizer migration from a polymer formulation will create a diffusion gradient in the bulk of the formulations. Thus, the plasticizer concentration in material will decrease, and the water penetration will be facilitated (Fleming, 1988). Hence, for each formulation we will have a different decrease in plasticizer concentration and a different degree of water absorption. If, in addition to the different degrees of plasticizer migration to the agar layer, and different degrees of water absorption for each formulation we are taking into account the plasticizers attack by fungi, it appear quite clear that a correlation between the changes in mechanical properties and fungi attack is difficult to be done. High moisture content presents in time of incubation affects the properties of the polymeric material. Water itself act as a plasticizer and decreases the strength and stiffness, but increases toughness (Dyson, 1990). Some literature data concerning degradation of plasticized PVC by fungi, in similar conditions as for our tests, indicated too a disagreement between the visual examination of specimens and changes in the mechanical properties (Hitz, 1967).

The changes in weight of sterile controls (Table 4.12) and inoculated specimens (Table 4.13) all presented an increase in weight, which was still maintained after 96 hrs of drying at room temperature. This demonstrated that water absorption indeed took place, and that the water penetrated quite deep in the material because the specimens were difficult to dry at room temperature.

In order to find out that indeed the water penetrates deeply in the specimens during the incubation period, two controls and AL blends were prepared with 2-45 and Lindol as plasticizers. They represent the worst and the best plasticizers from point of view of resistance to fungi attack. Similar formulations were also prepared without filler to find out if some water could penetrate by capillarity in the calcium carbonate filler.

Sheets of (7x12.7x0.15) cm^3 of these formulations were inoculated with fungi and incubated as usual. After the incubation period, the ranking of fungi growth was done by visual examination. Specimens weight before and after incubation was recorded. The specimens were dried at room temperature for 48 hrs, their weight recorded again and thereafter they were kept in an oven with air circulation (2 L/min) at 32°C. The weight of the specimens was recorded every 24 hrs till constant weight. The initial weight of the specimens, the weight after incubation, the final weight after drying, as well as the loss in weight expressed in milligrams and as percentages of the initial weight is presented in Table 4.13. The fungal growth ranking is also presented in this table. It should be noted that the drying period was of two days at room temperature and nine days in the oven for the controls and of two days at room temperature and eleven days in the oven for the blends regardless their content in filler.

Several observations result from the analysis of data presented in Table 4.13:

1. All the specimens present an increase in weight after the incubation period as effect of water absorption. The increase in weight is higher in the blends than in the controls due probably to the high polarity of lignin.

2. The drying period is very long: two days at room temperature followed by nine and eleven days for the controls and blends respectively. Consequently, in all the

specimens, who were tested for the mechanical properties and dried only four days at room temperature, small quantities of water were still present. The water presence may explain the difficulties encountered in the correlation of the data obtained by visual examination and mechanical and thermal data.

3. The loss in weight data obtained with the above mentioned specimens seems to give helpful information: example the loss in weight is higher for the formulations without filler where the plasticizer content is of 25.1% in contrast to the filled formulations where the plasticizer content is only of 10.3%; also it is higher in the blends formulated without filler where the lignin content is of 14.3% although in the filled blends where the lignin content is 5.9%

4. The loss in weight data are somehow difficult to correlate with the fungi growth ranking established by visual examination,

5. It looks likely that the loss in weight determination after the incubation period, although a long lasting test, would give a better evaluation of the susceptibility of the different formulations to the fungi attack.

The literature data (Hitz, 1967) indicated that the fungi growth on the surface of plastic materials can be invisible or uneven. Furthermore, as their experiments showed, the extent of fungal growth is not necessarily related to the degree of biodegradation.

Table 4.12 Percentage change of modulus, elongation and weight of sterile controls of VC-VAc copolymer controls and blends after 28 days incubation

Specimen Identification	Modulus Changes (%)	Elongation Changes (%)	Weight Changes (%)
DOP Control	+0.8	-7.1	+0.121
DOP Blend washed Alcell	+103.6	-15.8	+0.272
DOP Blend Alcell	+160.2	-15.2	+0.257
DOP Blend Indulin	+85	-13.1	+1.221
DOP Blend Tomlinite	+97	-28.6	+0.428
Lindol Control	+8.4	-23.2	+0.230
Lindol Blend Alcell	+22.4	-	+0.440
Lindol Blend Indulin	+18.5	-	+1.850
Lindol Blend Tomlinite	+66.9	-	+0.361
Mesamoll Control	-0.8	-4.5	+0.268
Mesamoll Blend Alcell	+107.3	-34.2	+0.219
Mesamoll BlendIndulin	+31	-18.5	+1.929
Mesamoll Blend Tomlinite	+46.4	-5.8	+1.249

For DOP-Tomlinite, DOP-Indulin, Lindol-Tomlinite and Mesamoll-Tomlinite blends, the resistance to fungi attack is similar to that of respective controls. It is pointing out that the presence of these lignins in certain conditions creates the same resistance to fungi as the copolymer. Probably plasticizer, in these particular blends is the main fungi target. As a confirmation of these particularities the fungal growth rating was evaluated for PVC homopolymer blends formulated with 35 phr plasticizers, DOP, Lindol and Mesamoll and Indulin or Tomlinite, in comparison with the respective controls. The obtained results are presented in Tables 4.14 and 4.15

Table 4.13 Change in weight after 28 days incubation of inoculated specimens of VC-VAc copolymer blend control and blends

Specimen Identification	Weight Changes (%)
DOP Control	+0.132
DOP Blend washed Alcell	+0.235
DOP Blend Alcell	+0.277
DOP Blend Indulin	+1.283
DOP Blend Tomlinite	+0.463
Lindol Control	+0.242
Lindol Blend Alcell	+0.443
Lindol Blend Indulin	+2.081
Lindol Blend Tomlinite	+0.413
Mesamoll Control	+0.240
Mesamoll Blend Alcell	+0.212
Mesamoll Blend Indulin	+1.402
Mesamoll Blend Tomlinite	+0.451

In these formulations only Tomlinite blends plasticized with Lindol and Mesamoll have similar resistance to fungi attack as the respective controls, pointing out the higher resistance of Tomlinite than that of Indulin to fungi attack in PVC homopolymer formulations. This may be due to a different dispersion of Indulin in PVC homopolymer formulation in comparison with respective copolymer formulation, as well as to a different PVC-Lignin bonding in homopolymer formulation.

Table 4.14 Rating of fungi growth on surfaces of PVC blend with Indulin and Tomlinite formulated with 35 phr plasticizer DOP, Lindol and Mesamoll in comparison to PVC control

Plasticizer	Control	Indulin blend	Tomlinite Blend
DOP	2	4	3
Lindol	2	3	2
Mesamoll	2	3	2

The fact that the fungal growth was similar for all the control formulations plasticized with DOP, Lindol or Mesamoll, may indicate that other additives present in these formulations could be the targets of the fungi. It is well established that the alkyl sulphonic phenyl ester group present in Mesamoll has a higher resistance to degradation during exposure to microorganisms than the aryl phosphate ester present in Lindol, and a much higher resistance than the alkyl carbonyl ester present in DOP. The heat stabilizer dibutyl tin dilaurate and the calcium stearate lubricant, which are both derivatives of fatty acid, could enhance adhesion of fungi on the exposed surfaces because of the high affinity of fungi for fatty acids derivatives.

Table 4.15 Fungi growth ranking, initial weight, weight after incubation, loss of the initial weightafter 28 days incubation.

Sample Identification	Fungi growth ranking	Initial weight(g)	Weight After incub. (g)	Weight after drying (g)	Loss in weight	
					(mg)	%
2-45 control filler	3	25.193	25.222	25.118	7.5	0.298
2-45 control no filler	NA	16.803	16.830	16.730	7.6	0.434
Lindol control filler	2	25.705	25.765	25.696	0.9	0.035
Lindol control no filler	NA	16.801	16.869	16.779	2.2	0.131
2-45 blend filler	4	24.987	25.263	24.841	14.6	0.58
2-45 blend no filler	NA	16.207	16.315	16.074	13.3	0.82
Lindol blend filler	3	25.133	25.246	25.092	4.1	0.163
Lindol blend no filler	NA	16.347	16.434	16.299	4.8	0.293

Control and AL blends specimen formulated with 35 phr 2-45 and Lindol plasticizer with or without filler

CHAPTER 5 . CONCLUSIONS, RECOMMENDATIONS AND CONTRIBUTION

5.1 Conclusions

In present research the conclusion were divided in two main categories

5.1.1. Fungal Growth Impact

1. The five fungi species used in this research were capable of growing on all composite formulations;
2. After fungal attack all specimens lost their shine, and 2-45 blend specimen had been discoloured severely;
3. All plasticizers used in the vinyl-flooring composite were susceptible to fungal attack. Light growth observed on the control samples with DOP, 2-45, Lindol and Mesamoll control, and medium to high growth of mycelia web exhibited by the 2-45 control specimen;
4. PVC – Lignin blend with plasticizer DOP, 2-45, Lindol, and Mesamol are also susceptible to fungal attacks. Light growth was observed on Lindol blend (with all lignin types) specimens, whereas medium growth was found on the DOP, Mesamol blend specimens. Exceptionally high fungal growth was detected on the surface of 2-45 blend specimens;
5. The order of susceptibility of the plasticizers to biodegrade with lignin blend can be seen as 2-45>DOP>Mesamol>Lindol;

6. At the same concentration of plasticizer (35 phr), the fungal growth is similar for the flooring materials formulated with VC-VAc copolymer or PVC homopolymer;
7. When the content of plasticizer is reduced from 35 to 30 phr in PVC or VC-VAc copolymer control formulations, the fungal growth reduced from high to light growth in all formulations. This observation is not applicable for the formulations plasticized with 2-45;
8. Among specimens containing Tomlinite, Indulin, Alcell, Alcell lignin is most susceptible to fungi growth and Alcell purification of water washing was not an efficient method for improving specimen resistance to fungal attack;
9. DOP-Tomlinite, DOP-Indulin and Mesamoll -Tomlinite blends formulated with 30 phr plasticizes can successfully replace the controls formulated with 35 phr plasticizer, because of the similar mechanical properties while maintaining a higher resistance to fungal growth.

5.1.2. Change in Material Properties

1. The modulus of the inoculated specimens had increased and their elongation had decreased as compared to the sterile controls. Tensile strength at yield of the inoculated specimens was slightly higher than that of sterile controls;
2. All fungi inoculated specimens showing increased modulus and low elongation as compared to sterile and zero controls due to plasticizer loss;
3. In case of control formulations the Tg values of the inoculated specimens are slightly lower than those of the respective sterile control; whereas the blend

formulations the Tg values of the inoculated specimens are more to lower side as those of the respective sterile controls;

4. The loss of weight in inoculated lignin blended is more as compared to the inoculated control samples. Shows more susceptibility of certain blended formulations to fungal attack;

5. Further of loss in weight is higher for the formulations without filler where the plasticizer where as the content is of 25.1% in contrast to the filled formulations where the plasticizer content is only of 10.3%; also it is higher in the blends formulated without filler where the lignin content is of 14.3% although in the filled blends where the lignin content is 5.9%.

5.2 Recommendations:

The recycled residual material such as lignin could be used satisfactory in replacement of a part of PVC within composite building material.

The replacement with recycled residues change the properties of new materials, therefore it is recommended that new formulations would be always tested not only for mechanical properties but also for their response to microbial activities.

In further work, the formulation of new composite material might include environmental friendly biocides.

5.3 Contribution:

Formulation of new building material is based on sustainable development characteristics such as:

1. Use of abundant residual waste material such as lignin (pulp and paper industry by-product);
2. Decreasing the use and preservation of non renewable resources by reducing the use of synthetic polymer;
3. Formulation of a testing protocol for different properties, which can be used as reference in case when synthetic material will be substituted with residual material;
4. Discovering that residual materials can change the internal structure of the composite and create higher accessibility of degradable components to fungal attack;
5. Discovering that dispersion of constituents is responsible for higher fungi growth of blended material. Using different plasticizer decrease the accessibility to degradable components;
6. Successfully replacing susceptible 35 phr plasticizer composite building material formulation with other building material in order to decrease fungal growth.
7. Successful formulation of 30 phr plasticizer - PVC building material composite.
8. New formulation of blended PVC-Lignin building composite material that decreased fungal growth.
9. Concluded that source of changes within physical properties of composite are complex, and are not directly related to fungal growth rate.

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