12-1984

Growth characterization and control of fungi on polyvinyl chloride film

Tim Roberts

To the Graduate Council:

I am submitting herewith a thesis written by Tim Roberts entitled "Growth characterization and control of fungi on polyvinyl chloride film." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

P. Michael Davidson, Major Professor

We have read this thesis and recommend its acceptance:

Hugh O. Jaynes, John R. Mount

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by William Timothy Roberts entitled "Growth Characterization and Control of Fungi on Polyvinyl Chloride Film." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

P. Michael Davidson

P. Michael Davidson, Major Professor

We have read this thesis
and recommend its acceptance:

Hugh L. Haynes

John R Mount

Accepted for the Council:

Lee Minkel

The Graduate School

GROWTH CHARACTERIZATION AND CONTROL OF FUNGI ON POLYVINYL CHLORIDE FILM

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

William Timothy Roberts

December 1984

ACKNOWLEDGMENTS

In the course of this study many people have given freely of their time and knowledge. Appreciation and thanks are extended to Dr. P. Michael Davidson for serving as major professor and for his guidance and friendship throughout the preparation of this manuscript. Appreciation is also extended to Dr. Hugh O. Jaynes and Dr. John R. Mount for serving as committee members and for taking time to review and improve my manuscript. Special thanks are also extended to Dr. James T. Miles and the Department of Food Technology and Science staff for their assistance, advice, and friendship during the course of the author's graduate studies.

The author would also like to thank Dr. Theron E. Odlaug and Travenol Laboratories for making this study possible. Their financial support and provision of necessary research materials was greatly appreciated.

The author would also like to thank Jean Barwick for drawing the illustrations and Dennis Cline for taking the photographs in this manuscript.

The author also wishes to recognize and express appreciation to the following graduate students who contributed their time, friendship, and knowledge during preparation of this manuscript; Mohammad Amin, Effimia Bargiota, Jeff Durniak, Keith Moore and Doug Schoenrock.

Finally, I would like to thank my parents for their patience and support through my many years of school.

ABSTRACT

Polyvinyl chloride films are widely used packaging materials. These films are used to package food, pharmaceutical, and medical products. Since the occurrance of microbial growth on a PVC film could result in contamination of the product, this study was conducted to determine if a PVC film could support growth of fungi and to define chemical methods of inhibiting growth.

Polyvinyl chloride film was subjected to nine species of fungi in three separate growth studies to determine if the film could serve as a nutrient source. All mold species were found to be capable of utilizing the film for carbon or nitrogen when the other nutrient was supplied. Only two species, Aspergillus fischeri and Paecilomyces sp., were found to be capable of utilizing the film without added nutrient. The film components found to be utilized by the fungi as carbon sources included the epoxidized oil (plasticizer-stabilizer) and the Ca-Zn stearate (heat stabilizer). As a nitrogen source, the stearamide (lubricant) was not found to be an available nutrient source in the component study, but was found to be available in the PVC film.

To inhibit growth of the fungi on the PVC film, an initial screening test was conducted in vitro to determine the susceptibility of the fungi to the following antimicrobials: BHA (butylated hydroxyanisole), methyl paraben, propyl paraben, and potassium sorbate. The fungi were found to be inhibited by all of the

preservatives except potassium sorbate at low concentrations. However, when the preservatives were applied to the film the concentrations which inhibited growth in the in vitro study were not effective in inhibiting fungal growth on the film. Therefore, increased concentrations of the inhibitors were applied to the film. Potassium sorbate at 4000 ug/ml (0.4%) was found to totally inhibit growth of Fusarium sp. while BHA at 2000 ug/ml (0.2%) inhibited growth of Paecilomyces sp.

In conclusion, polyvinyl chloride films were found to be susceptible to microbial attack. The composition of the film, the microorganisms present, and the environmental conditions were shown to be determinants in the utilization of the film as a nutrient source. Therefore, it is important to determine if a PVC film can support growth of microorganisms under actual use conditions. If the film is susceptible, then appropriate methods such as controlling environmental conditions, reformulation of the film, or application of an antimicrobial agent can be used to prevent growth.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

CHAPTER I

INTRODUCTION

Several conditions must be met for a mold species to be able to utilize the components of and degrade a plastic film. Firstly, the mold must be present as a contaminant. Studies by Baribo et al. (8) and Sinell (96), with bacteria, showed that microorganisms are present on films at a rate of 1-20 per 1000 cm^2 and that films are contaminated by these microorganisms during manufacture due to electrostatic charges. Secondly, the mold species must be able to utilize the available substrates in or on the film. Lasman and Scullin (65) reported that certain plasticizers used for flexibility in plastic films were susceptible to microbial attack and degradation, if desirable environmental conditions existed. Other studies have shown that fungi such as Paecilomyces varioti and Aspergillus versicolor may utilize various mono- and dibasic-fatty acids of the plasticizers as carbon sources (12,82,102). Finally, environmental conditions, such as moisture and temperature, must be conducive to the growth of fungi (14,66,72,73).

The occurrence of fungal growth on plastic film could result in possible penetration of the viable organism into the package (49,86), production of toxic end-products which could penetrate and contaminate the product, or undesirable spotting of the packaging material (47,108,116). Therefore, the objectives of this study were:

1. to determine the characteristics of fungal growth on polyvinyl chloride (PVC) film; and

2. to test chemical methods for eliminating the growth of fungi on PVC film.

CHAPTER II

REVIEW OF LITERATURE

1. MICROORGANISMS

Microorganisms such as fungi, bacteria, actinomycetes, and yeasts have been reported to degrade PVC films (11,15,16,24,65,77, 108). Of this group, fungi have received the most attention primarily because they can grow at a relative humidity as low as 85% and are easily visible once established (27,61,118). This study was concerned with the occurrence of fungal growth on a PVC film. Following is a brief description of the morphology, physiology, and biochemical activities of fungi.

Fungi are eucaryotic, plantlike organisms that lack chlorophyll and thus rely on organic materials as nutrient sources. Fungi are composed of threadlike structures bounded by walls composed of cellulose and/or chitin (48,78,98). These tubular structures, called hyphae, may exist in a vegetative or reproductive form (101). Vegetative hyphae are composed of strands, known as mycelia, which may penetrate and secrete enzymes to degrade a substrate for nutrients. Fungi have been found to produce extracellular enzymes such as esterases which can degrade large molecular weight compounds into small fragments that can be transported into the mycelium by active transport (48). Reproductive hyphae extend from the mycelial mass and produce spores. Hyphae of most fungi contain crosswalls

(septa) which divide the mycelia into small segments similar to cells. Each segment (cell) tends to be porous to cytoplasmic movement of nuclei and organelles throughout the mycelia. This movement is important for uptake of nutrients, excretion of cellular wastes, and production of spores (48).

Fungi are widely distributed in the air, soil, and water. In the air fungi are present primarily as spores, whereas in the soil they may be in an active growing condition (2,87). When optimum environmental conditions exist, a fungal spore will swell and liberate a germ-tube (hyphal strain) (48,98). The germ-tube will initially obtain nutrients from the spore itself, but growth and development depend upon the presence of available nutrients in the substrate (2,48). The nutrient elements required for fungal growth include carbon, hydrogen, oxygen, nitrogen, sulfur, potassium, magnesium, and phosphorous. In some cases, small amounts of the trace elements, iron, copper, zinc, and manganese may be required. As long as desirable conditions exist, the fungus will grow and mycelia may branch laterally for long distances from the food source (2). Once an essential nutrient is depleted or an alteration in environment occurs, the fungus (hyphae) will undergo physiological and biochemical changes resulting in the production of spores (28,48,78,98). The spores may arise directly from apical hyphal cells by a "pinching" process forming a sac-like structure called a sporangium. The sporangium contains the fungal spores (sporangia) which can be released into the air by contact with external forces. Species of

Mucor and Rhizopus produce their asexual spores by this method. Asexual spores may also be produced on the tips of stalks or branches (sterigma) in chains. These spores, referred to as conidia, are produced by species of Aspergillus and Penicillium.

Environmental conditions that effect the growth of fungi include temperature, moisture, oxygen supply, and pH. Most fungi can grow over a wide temperature range. Aspergillus fumigatus and Paecilomyces varioti are capable of growth in excess of 45°C, while certain species of Penicillium, Fusarium, and Alternaria can grow below 18°C (72). The optimum temperature of fungi exists between 22 to 30°C (72,78). Fungi can obtain moisture for growth from the atmosphere and/or substrate (14,78). Relative humidity (RH) above 85% is conducive to fungal growth, although some fungi such as Aspergillus glaucus and Aspergillus versicolor can grow as low as 75% RH (2,72,73).

Fungi tend to be strictly aerobic in that they require an abundant supply of oxygen. Reduced oxygen tensions normally result in a reduction of growth, although some fungi, such as species of Mucor and Fusarium, have been found to grow in low oxygen concentrations (48). Most fungi are not dependent on pH and can grow in the range of 2.0 to 9.0 with an optimum pH of 5.6 (78).

The fungi are highly adaptive organisms that can utilize a wide variety of organic substrates as nutrient sources. These organic compounds can be used by the fungus as an energy source and/or as structural components. Fungi also have the ability to

survive prolonged periods of time under extreme conditions of temperature and humidity through the production of spores.

2. MICROBIAL SUSCEPTIBILITY OF PVC FILM COMPONENTS

Microorganisms are capable of attacking plasticized PVC film to utilize components of the film such as the plasticizer, lubricant and/or stabilizer (11,15,16,24,38,65,77,108). The PVC resin has been found to be resistant to microbial attack (2,11,16,18). Since the plasticizer makes up a large portion of a PVC film, it is the major contributing component for nutrition of microorganisms (60, 61,62). Brown (18) was the first to report on the susceptibility of plasticizers incorporated in synthetic polymers. Plasticizers such as adipates, laurates, oleates, ricinoleates, sebacates, vegetable oils, and other fatty acid derivatives tend to be susceptible, whereas phthalic and phosphoric acid derivatives were found not to support fungal growth. Stahl and Pessen (102) tested Aspergillus versicolor and Pseudomonas aeruginosa in a shake-flask culture to determine if these organisms could degrade plasticizers. These authors reported that the sebacates, with the exception of dimethyl sebacate, and ricinoleates tended to support growth. Citrates, aconitates, phosphates, and phthalates could not be utilized by A. versicolor, but slight growth was noted on some commercial phosphates and phthalates by P. aeruginosa. Berk et al. (12) confirmed these findings by testing the ability of 24 species

of fungi to utilize 127 compounds as the sole carbon source in mineral salts agar medium. The most commonly used plasticizers for PVC films and their vulnerability to microbial attack are shown in Table 1.

Other components such as stabilizers and lubricants may support growth on PVC films. The stabilizer dibutyltin dilaurate and certain sulfur-containing dibutyltin stabilizers are susceptible to microbial attack, whereas trialkyltin derivatives may actually exhibit antimicrobial activity (107,108). Common lubricants such as stearic acid, waxes and oils may support microbial growth (38, 59,107,108).

The composition of a PVC film will dictate the degree of susceptibility to attack by microorganisms. Resistant components may make a PVC film inert, but the complex interactions of the components within the polymer matrix may make the entire system susceptible. It is important to test a PVC film exposed to actual use conditions to determine susceptibility. Once growth characteristics of organisms have been obtained appropriate methods can be employed to prevent growth.

3. EFFECTS OF MICROBIAL GROWTH ON PVC FILM

Microorganisms can make a plasticized PVC film undesirable by degrading the film components such as the plasticizer. Removal of the plasticizer from a PVC film will cause the film to become

Table 1. Vulnerability of Plasticizer to Microbial Attack

Resistant plasticizers
Phthalic acid derivatives
DOP (di-2-ethylhexyl phthalate)
DIOP (di-isooctyl phthalate)
DIDP (di-decyl phthalate)
DBP (di-butyl phthalate)
Phosphoric acid derivatives
TCP (tri-cresyl phosphate)
TP (tri-phenyl phosphate)
TXP (tri-dimethylphenyl phosphate)
CDP (cresyl diphenyl phosphate)
Highly susceptible plasticizers
Azelaic acid derivatives
DOZ (di-2-ethylhexyl azelate)
DIOZ (di-isooctyl azelate)
Adipic acid derivatives
DIBA (di-isobutyl adipate)
DOA (di-2-ethylhexyl adipate)
DIOA (di-isooctyl adipate)
Sebacic acid derivatives
DOS (di-octyl sebacate)
DBS (di-butyl sebacate)
DIOS (di-isooctyl sebacate)
Succinic acid derivatives
DES (di-ethyl succinate)
DBS (di-butyl succinate)
Epoxy derivatives
Epoxidized soybean oil
Epoxidized octyl tallate

Source: Tirpak (108).

brittle, increase in tensile strength, and decrease in elongation (9,11,16,65,108). Berk (11) reported that a PVC film plasticized with a mixture of dibutyl sebacate and dioctyl phthalate would support growth of Trichoderma sp. on mineral salts agar. After six weeks of incubation the film had a 63% increase in tensile strength and a 67% loss in elongation when compared to the uninoculated control. The author concluded that the removal of plasticizer by the fungus attributed to the increase in tensile strength. In a more recent study, Booth and Robb (16) found PVC films plasticized with di-isooctyl esters of adipic, sebacic and azelaic acids to lose weight and become stiff when exposed to Pseudomonas and Brevibacterium species in soil.

Microbial growth on the surface of a PVC film does not necessarily mean that the organism is utilizing the film component(s). Growth may be a result of organic material on the film surface (9, 23,66). Microorganisms such as fungi, bacteria and actinomycetes may be capable of secreting pigments when growing on the surface of a plastic film (15,38,47,65,108,116). These pigments tend to be soluble in the plasticizer of most plastic formulations resulting in discoloration (38,65). First reports of this phenomenon were by Girard and Koda (47) who identified a Penicillium sp. that could discolor vinyls by producing a pinkish-red pigment. Booth et al. (15) reported that the bacterium, Brevibacterium sp. could degrade and pink stain PVC film plasticized with di- isoocotyl sebacate Streptomyces rubrireticuli has also been found to cause pink

discoloration of vinyls (116). The pigment produced by this organism has been identified as a mixture of two prodiginine pigments, undecylprodiginine and butylcycloheptyl-prodiginine (44). Tirpak (108) reported that species of Penicillium and Fusarium were found to stain vinyl films orange and yellow. The ability of a microorganism to stain a PVC film will depend upon the ability of the organism to produce a particular pigment, solubility of the pigment in the PVC film, and environmental conditions such as pH, temperature and moisture (44,47,108).

4. DEGRADATIVE MECHANISMS OF MICROORGANISMS ON PVC FILM

Rigid PVC is very resistant to microbial attack, whereas plasticized PVC films tend to be susceptible. The additives incorporated in PVC films include mixtures of vegetable oils, acids, waxes and fatty acid derivatives which tend to be excellent food sources for microorganisms (27,38,60,82,107,108). Reese et al. (82) reported various mold species could utilize plasticizers of vinyl films such as coconut oil, methyl acetyl ricinoleate, and dihexyl sebacate in nutrient broth.

Microorganisms are capable of utilizing the components of a PVC film by secreting enzymes. Enzymes are proteins that catalyze chemical reactions and have a high specificity toward a particular substrate (48,105). Berk et al. (12) noted a clear zone produced around fungal colonies grown on mineral salts agar supplied with a

plasticizer as a source of carbon. The zones were contributed to extracellular esterases which diffused beyond the colony and converted the insoluble esters into water-soluble compounds. Williams et al. (113) reported that esterases from mycelial acetone powders of Aspergillus glaucus could break down the plasticizer, dibutyl sebacate, to sebacic acid. The esterases were also found to degrade vinyl films plasticized with dibutyl sebacate.

The production of extracellular esterases (lipases) by fungi can hydrolyze oils (triglycerides) to glycerol and free fatty acids (82,105). The fatty acids can be broken down into low molecular weight compounds by Beta-oxidation and utilized for energy through the tricarboxylic acid (TCA) cycle (48,105). This would explain why epoxidized oils and fatty acid derivatives used as plasticizers and stabilizers are susceptible to microbial degradation.

The phthalate esters once thought to be very resistant may be prone to microbial attack. Using enrichment culture techniques, Klausmeier and Jones (62) noted that dibutyl phthalate was converted to monobutyl phthalate by a Fusarium sp. when an available carbon source such as glucose was present. The available carbon source induced an extracellular esterase capable of hydrolyzing one of the ester linkages. The de-esterification of dibutyl phthalate liberated butanol, which could serve as an energy source for the organism. Similar activity was observed for diethyl, dipropyl, and butyl isodecyl phthalate. Dioctyl phthalate, the most commonly used plasticizer in PVC films, was not found to support growth.

Klausmeier and Jones (62), however, have suggested that growth of fungi due to organic debris on the surface of a plastic film could possibly induce an esterase capable of degrading a resistant plasticizer such as dioctyl phthalate.

5. MICROBIAL TESTS FOR PVC FILM

Susceptibility of a PVC film to microbial attack depends upon the composition of the film, presence of microorganisms, and environmental conditions. These factors must be considered when devising a test procedure to determine if a PVC film can support growth of microorganisms. The most common tests include the agar-plate culture test (2,5,11,12,23,61,91), shake-flask culture technique (102), humidity cabinet test (23,61), soil burial test (16,23,59, 61,91), and manometric studies (21,22,24). The following discussion summarizes these testing procedures.

In the agar-plate culture test, a segment of PVC film is layered on the surface of nutrient agar that does not contain a carbon source. The plates are inoculated with the test organism(s) and incubated (usually three weeks). Individual film components, such as the plasticizer, may also be tested by dispersing them in the nutrient agar to serve as carbon sources. In either case, growth rates are measured according to the American Society for Testing Materials (ASTM) (5) by the percentage of agar surface covered with mycelium or colonies. The ASTM scale for determining microbial growth is shown in Table 2.

Table 2. ASTM Scale for Determining Microbial Growth

Observed Growth on Specimen	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

Alternative methods include placing a piece of PVC film on the bottom of a petri dish and covering with nutrient agar. After inoculation and growth, physical changes such as weight loss and tensile strength are measured. Clear zone tests are used to determine enzymatic activity on film components in nutrient agar. Secretion of esterases by fungi will result in a clear zone around the colony, because the non-polar film component becomes water soluble in the medium.

The shake-flask culture technique is used to determine if organisms degrade plasticizers and other film components. In this method, the desired component is dispersed in a mineral salts solution and transferred to volumetric flasks. The flasks are shaken on a reciprocating shaker during the incubation period to ensure a homogenous dispersion of the film component within the medium. Growth is measured by weighing mycelial mats or measuring turbidity photometrically.

In the humidity cabinet test, a piece of PVC film is placed in a chamber at approximately 30°C and 90-100% relative humidity. The film is inoculated by spraying with a mixture of fungal spores suspended in distilled water or in diluted mineral salts solution. Growth is evaluated by visual observation, or by removal of the film and measuring for changes in physical properties such as weight loss, tensile strength and elongation.

The soil burial test involves placing strips of PVC film in moist soil for a desired period of time. Samples are removed at various time intervals and deterioration is measured by changes in physical properties. This test is usually employed for PVC films that will be exposed to outdoor environments or similar conditions. The test is very harsh in that the film will be exposed to a mixed flora of microorganisms under optimum conditions. The soil may also cause chemical changes in the film resulting in increased susceptibility.

Most of the tests mentioned are time consuming and laborious. To overcome these testing procedures, Burgess and Darby (21,22) devised a manometric method for measuring the amount of oxygen consumed and carbon dioxide (CO_2) liberated when microorganisms were in contact with PVC films. Their results, however, could not be directly correlated with percent weight loss of the films. Cavett and Woodard (24) improved the method by measuring the oxygen uptake and $^{14}CO_2$ liberated from ^{14}C labeled Pseudomonas sp. cells when in contact with a plasticizer or PVC film in a Warburg respirometer.

In contrast to the method of Burgess and Darby, their results could be correlated with percent weight loss of PVC films in three days.

The correct test to determine susceptibility of a PVC film will depend on the actual use conditions of the product. Films that are not going to be exposed to outdoor environments would not need to be exposed to soil burial tests. The described tests will allow the film manufacturer to determine if an antimicrobial would be needed to provide protection against microbial degradation.

6. PREVENTION OF MICROBIAL GROWTH

Microbial growth on a PVC film may be prevented by using resistant plasticizers, chemical modification of polymer, removal of organic materials on the surface, control of environmental conditions and/or application of an antimicrobial agent.

The plasticizer makes up 25% or more of a plastic film and tends to be the most susceptible component to microbial attack (53, 60,61,62). Therefore, the logical method to prevent microbial growth on a PVC film would be to incorporate a resistant plasticizer(s) such as dioctyl phthalate into the plastic formulation. This would increase the overall resistance of a PVC film, but would not guarantee the film to be completely inert to microorganisms. In contrast, the incorporation of a fatty oil plasticizer such as soybean oil may make a PVC film susceptible to microbial attack. Fatty oil plasticizers are commonly used because they are less expensive and impart low temperature flexibility to plastic films (38,102). Plasticizers compatible with a PVC film are listed in Table 1.

Microbial resistance of plastic films may be attained by internal plasticization in which no plasticizer has been added to the resin (27,87,103). Chemical modification of the resin has been found to reduce the inter- and intra-molecular forces duplicating the structural feature obtained from addition of plasticizers (87, 103). Plastic films internally plasticized have been found to resist microbial attack (103).

As mentioned previously, the presence of extraneous material on the surface of a PVC film may serve as a nutrient source for microorganisms. Therefore, it is important that the surface of a plastic film be free of excessive debris. Microbial growth on a film surface may not result in direct utilization of the film components, but production of pigments by these organisms can penetrate a plastic film making it undesirable (15,47,108,116).

Environmental factors, such as moisture and temperature, influence the growth of microorganisms. Microbial degradation of a plastic film is not possible without the presence of moisture. Fungi can tolerate a relative humidity as low as 75%, whereas bacteria will only grow in aqueous environments (14,78). Microorganisms grow best over a narrow temperature range. Fungi have an optimum temperature range of 22°C to 30°C, whereas bacteria grow best from 20°C to 37°C (78). Deviations from these optima will result in a reduction in metabolic activity and growth. In general, the growth of microorganisms can be inhibited or retarded by controlling one or more environmental factors conducive to microbial growth.

If none of the above methods are feasible to prevent growth of microorganisms, the plastics formulator should consider applying an antimicrobial to the plastic film. The use of antimicrobial agents to prevent growth on PVC film is discussed in the following section.

7. ANTIMICROBIALS

Plasticized PVC film may need protection against microorganisms when composed of resistant or non-resistant plasticizer(s). Organic debris such as dust can collect on the surface of a plastic film and provide nutrient for growth (9,23,66). Therefore, it is essential that an appropriate antimicrobial be applied as a coating to the film surface or be incorporated within the polymer matrix (18). The protection required will depend upon the susceptibility of the film, mainly the plasticizer, the potency of the antimicrobial, and the expected service life of the film (89).

Selection of an appropriate antimicrobial for a PVC film may be difficult and should be carefully considered. The following requirements have been recommended for antimicrobials to be used in vinyl films (18,20,23,61,65,75,107,118).

1. The antimicrobial must be compatible with the components of a PVC film; it should be soluble within the matrix and not affect the physical or chemical properties of the film;

2. The antimicrobial should be heat stable and not decompose or volatilize during the molding or extruding operations;

3. The preservative should exhibit a wide spectrum of antimicrobial activity against microorganisms throughout the expected life of the film;

4. The antimicrobial should be economical and non-toxic to humans.

Not all antimicrobials can meet the above criteria. For example, zinc and sulfur compounds are not used in PVC films because they may affect the durability of the plastic by reacting with salts of fatty acids commonly used as stabilizers in PVC formulations (75,108). Compromises are usually made in order to obtain the desired antimicrobial properties for a particular plastic.

Methods of Incorporation

Antimicrobials are usually added as formulation ingredients during the mixing and blending operations (20,23,65,104). They may be added as undiluted compounds or dispersed in organic solvents. Some antimicrobials can be purchased as solutions uniformly dispersed within the plasticizer or incorporated within the resin pellets (23). The degree of compatibility with the film components will determine the best method for incorporating an antimicrobial.

Activity of Antimicrobials in Vinyl Films

For an antimicrobial to be effective in a PVC film there must be a constant level of preservative on the film surface. Surface growth may occur if the antimicrobial cannot diffuse within a vinyl film. This growth may not affect the physical properties of the film, but may render the film undesirable if staining should occur.

As mentioned, the antimicrobial is dispersed in the plasticizer of most formulations. The function of the plasticizer is to impart flexibility by migrating throughout the vinyl film. If the plasticizer concentration is too high an oily layer may form on the film surface. This undesirable characteristic is referred to as "spewing" in the plastics industry (117,118). To prevent "spewing," formulators have developed systems to reduce mobility of the plasticizer. This restricted movement of the plasticizer will reduce the antimicrobial concentration on the film surface needed to prevent surface growth (23,61,117,118).

Environmental factors such as excessive heat and UV light have been reported to break the resin of PVC films causing a reorientation of the components. This reorientation may encapsulate the antimicrobial and prevent it from migrating to the film surface (61,118).

It is very important that an antimicrobial be capable of migrating throughout a vinyl film to prevent surface growth of microorganisms and/or utilization of susceptible film components.

Mechanism of Antimicrobial Activity

Antimicrobials prevent microbial growth by affecting cellular constituents such as the cell wall, cytoplasmic membrane, enzyme systems and/or genetic material (115). Surface active agents such as quaternary ammonium compounds are thought to interfere with membrane and cell wall activity (52), whereas metal containing compounds such as copper 8-quinolinolate and tributyltin oxide

inactivate enzymes by reacting with sulfhydryl groups (23). Antimicrobial activity depends upon the solubility and incorporation of the antimicrobial into the microbial cell (115).

Testing Antimicrobials in PVC Film

Numerous studies have been conducted to determine if PVC film treated with antimicrobial is subject to attack by microorganisms. The agar-plate and soil burial tests are the most commonly used methods for determining susceptibility of antimicrobial treated films (2,20,23,37,38,57,107). The agar-plate method involves placing PVC film treated with antimicrobial on the surface of agar (usually mineral salts agar supplied with an available carbon source such as dextrose) that has been inoculated with fungal spores. The plates are incubated for a desirable period of time to determine if growth will occur on the film. Antimicrobial activity is based on a clear zone of inhibition surrounding the test sample. If no zone appears and growth occurs on the sample, the antimicrobial is ineffective. Zones of inhibition should be interpreted carefully because there is no guarantee that the antimicrobial will exert activity over the service life of the film (23,37,38,107). Variations of this method have been used to determine antimicrobial efficiency. For instance, a susceptible film component such as the plasticizer plus antimicrobial may be incorporated into mineral salts agar as the sole carbon source (65). The plates are inoculated with single or mixed species of mold spores and incubated. If growth does not occur,

the antimicrobial is effective in protecting the film component. A study of this nature does not consider the complex interaction of all the components within a PVC film, but does give a good indication of antimicrobial activity.

In the soil burial test, thin strips of PVC film incorporated with antimicrobial are placed vertically in moist outdoor soil or soil within a humidity cabinet and inoculated with a pure or mixed microflora (20,22,57). The films are removed at various time intervals and physical properties such as weight loss, tensile strength, and elongation can be measured. The test gives a good indication of the degree of protection given by the antimicrobial. Environmental conditions such as temperature and moisture can be closely monitored in a humidity cabinet, whereas outdoor exposure test conditions will vary.

The test samples may be exposed to water for a period of time prior to exposure to the agar-plate or soil burial tests (2,57,107). This will indicate if the antimicrobial is susceptible to leaching from the PVC film. This practice is usually performed on vinyl films that are expected to be used in outdoor environments. The type of test to conduct will depend upon the desired protection and service-life of a vinyl film.

Antimicrobials Used in PVC Films

Table 3 lists some of the antimicrobials that have been incorporated into PVC films. These preservatives are compatible with

Table 3. Antimicrobials Used in PVC Films

Compound	Reference
Dibromosalicylanilide	108
Tribromosalicylanilide	108
N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide	20,23,65,104
N-(trichloromethylthio)phthalimide	20,23,57,65,104,108
N-(trichloromethylthio)tetrahydrophthalimide	104,108
10,10'-Oxybisphenoxarsine in epoxidized oil	20,23,38,65,104,108
Dodecyldimethylbenzyl ammoniumn naphthenate	20,23,108
2-n-Octyl-4-isothazolin-3-one	23,104
Tributyltin oxide	20,23,65,104,107,108
Copper 8-quinolinolate	23,57,65,75,104
Vinyzene BP-5	107
p-Hydroxybenzoic acid esters	20,23,65,104

the resin, soluble in the plasticizer(s), and heat stable. The following discussion concerns the most commonly used antimicrobials for PVC film.

Captan (N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide) and phaltan (N-(trichloromethylthio) phthalimide) have been widely used in PVC films exerting a wide spectrum of antimicrobial activity against bacteria and fungi. They can be used to make transparent as well as opaque compounds and have been used in plastisols (65). Captan is used as a fungicide for controlling plant and crop diseases. These compounds are primarily used as undiluted active compounds, but are available in plasticizers (23). Levels of 0.25 to 0.5% of the total formula weight have been applied to PVC films, but some reports have shown surface growth of fungi to occur (23,65). Kaplan et al. (57) reported phaltan and copper 8-quinolinolate to protect PVC film plasticized with methyl acetylricinoleate when exposed to four weeks of soil burial.

Copper 8-quinolinolate is a highly effective fungicide that has been used in PVC films where clear, colorless compounds are not desired. This compound imparts a yellowish-green color to the film and has been used extensively in military applications (23,57,65,75).

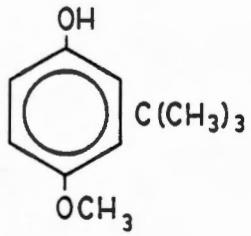
Vinyzene BP-5^{R} (Estabex ABF) is an arsine epoxy soybean adduct that can partially replace the epoxidized oil plasticizer in PVC formulations (107). It has been found to be relatively non-toxic and exhibit a broad antimicrobial activity against bacteria and fungi (107). Accelerated aging tests have shown the antimicrobial to be stable in PVC films (107).

Other compounds listed as preservatives for PVC films include di- and tri-bromosalicylamilide, N-(trichloromethylthio)tetrahydrophthalimide, 10,10'-Oxybisphenoxarsine, dodecyldimethylbenzyl ammonium naphthenate, 2-n-octyl-4-isothiazolin-3-one, tributyltin oxide, and p-hydroxybenzoic acid esters. Of these commercial antimicrobials only three have been approved by the Food and Drug Administration (FDA) as indirect additives for preserving paper and plastics in contact with foods (20). These are captan (N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide), copper 8-quinolinolate, and the esters of p-hydroxybenzoic acid (parabens). There is a need to develop safer antimicrobials for preserving plastics.

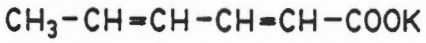
This study was concerned with preserving a PVC film with FDA approved antimicrobials such as esters of p-hydroxybenzoic acid and potassium sorbate. BHA (Butylated hydroxyanisole) is an FDA approved antioxidant that exerts antimicrobial activity and was also used in the study. The following sections discuss the chemical properties, antimicrobial activity, and applications in plastic films of these antimicrobials.

Butylated Hydroxyanisole

Butylated hydroxyanisole (BHA) is a phenolic antioxidant that has been found to possess antimicrobial activity (17,25,30,33). BHA (Figure 1) is a mixture of 2- and 3-isomers of tertiary-butyl-4-hydroxyanisole and has a molecular weight of 180.0 (30). The compound is insoluble in water, but soluble in ethanol and vegetable oils (30). BHA is manufactured as powders or tablets and is heat stable (3,30,42).

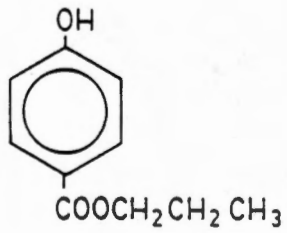


BUTYLATED HYDROXYANISOLE
(BHA)



POTASSIUM SORBATE

METHYL PARABEN



PROPYL PARABEN

Figure 1. Chemical structures of butylated hydroxyanisole, potassium sorbate and esters of p-hydroxybenzoic acid.

Chang and Branen (25) were the first to report the antifungal activity of BHA. They noted that mycelial growth and aflatoxin production of Aspergillus parasiticus were inhibited by 250 ppm of BHA (1,000 ppm for spores) in a glucose-salts medium. Sankaran (88) reported growth of Aspergillus flavus and Aspergillus fumigatus on potato dextrose agar to be inhibited by 750 ppm BHA in 5 days at 32°C. Fung et al. (42) found Aspergillus flavus growth and aflatoxin production to be inhibited by BHA on laboratory media. Similar results were obtained by Beuchat and Jones (13) who reported that BHA at 100 ppm prevented colony formation of heated and unheated conidia of A. flavus. Ahmad and Branen (3), in a comprehensive review, reported that 200 ppm of BHA totally inhibited growth of species of Penicillium, Aspergillus and Geotrichum in glucose-salts broth.

Studies of bacteria have shown Staphylococcus aureus, Clostridium perfringens, Escherichia coli, Salmonella typhimurium, Vibrio parahaemolyticus and species of Pseudomonas to be inhibited by BHA at a concentration of 400 ppm or less (6,25,32,34,63,85,94). The greatest antibacterial activity has been noted against Gram-positive bacteria. Chang and Branen (25) reported growth of S. aureus in nutrient broth to be inhibited by 150 ppm of BHA, while 400 ppm was required to prevent growth of E. coli. The greatest resistance of Gram-negative bacteria to BHA has been attributed to the solubility of BHA into the lipopolysaccharide layer of the bacterial cell (17). The lipopolysaccharide layer prevents BHA

from reaching the cytoplasmic membrane, the probable site of microbial inhibition.

BHA has been reported to have antimicrobial activity over a wide pH range depending on the type of microorganism tested (3,6,13,39). Beuchat and Jones (13) reported growth of A. flavus conidia to be inhibited by BHA to a greater extent at pH 3.5 than at pH 5.5. In contrast, Ahmad and Branen (3) found BHA to be inhibitory to A. flavus from pH 4.0 to 9.0.

The mechanism(s) by which BHA exerts antimicrobial activity may be directly related to phenol (33,79). BHA and other phenolic compounds have been found to affect the permeability of the cell membrane which may cause leakage of intracellular constituents such as nucleotides and amino acids (31,35,55,58,92,111). Also, BHA may affect some of the membrane-bound enzymes of the cytoplasmic membrane which are responsible for energy coupling reactions (31) or inhibit synthesis of genetic material (17,106).

In the plastics industry phenolic antioxidants, such as BHA and BHT (butylated hydroxytoluene), have been added to plastic resins and plasticizers to prevent oxidative degradation (54,59,104, 114). Since BHA also has antimicrobial properties, it could possibly serve a dual purpose in plastics. BHA may be dissolved in the plasticizer (vegetable oil) of plastic formulations to inhibit microbial attack of film components and prevent undesirable surface growth. Although this seems feasible, BHA may not be effective as an antimicrobial in plastic films. In separate studies, Klindworth

(63) and Ahmad and Branen (3) reported that the antimicrobial activity of BHA against C. perfringens and A. flavus was decreased when corn oil was added to the medium. Similarly, Rico-Munoz and Davidson (83) noted that the addition of 1.5 to 3.0% corn oil to solid media eliminated the antimicrobial activity of BHA against A. niger. Thus, it must be considered that BHA in a plastic film may be completely absorbed in the plasticizer (oil) and/or used up in preventing autoxidation of a susceptible film component. The loss or binding of BHA would render a plastic film susceptible to microbial attack. Tests need to be conducted to determine if BHA exhibits antimicrobial activity when incorporated into a plastic film. An alternative method would be to apply BHA as a surface coating to preserve plastic films.

Esters of p-Hydroxybenzoic Acid

Parabens (alkyl esters of p-hydroxybenzoic acid) are widely used compounds for preserving food, pharmaceutical, cosmetic and industrial products. They are white fluffy powders that are colorless, odorless, heat stable and non-volatile (1,26,30,109). Chemically, the parabens are composed of benzoic acid with an esterified carboxyl group para to a hydroxyl group (Figure 1). Antimicrobial activity of these compounds increases as the alkyl chain length increases, but water solubility decreases (1,26,30,51,68,93). At 25°C methyl paraben is slightly soluble in water (0.25 gm/100 ml), whereas propyl paraben is virtually insoluble (0.04 gm/100 ml) (26,30,109).

Both compounds are very soluble in ethanol and vegetable oils. These esters may be used in combination to increase water solubility and antimicrobial activity (1).

The parabens are highly effective against yeasts and molds, but not as effective against Gram-negative bacteria (1,26,30,33). Aalto et al. (1) reported that growth of Aspergillus niger in nutrient broth was inhibited by methyl paraben at 1,000 ppm and propyl paraben at 200 ppm, whereas Escherichia coli was inhibited by 2,000 and 1,000 ppm of methyl and propyl paraben, respectively. The antimicrobial effectiveness of these compounds is attributed to their stability over a wide pH range (3.0-8.0) (1,7,26,33,68,109). Bandelin (7) noted that the parabens could inhibit growth of molds on Sabourauds agar medium at acidic, neutral and slightly alkaline conditions. At neutral pH, methyl paraben at 1,000 ppm and propyl paraben at 400 ppm were found to inhibit growth of A. niger, whereas Penicillium citrinum was inhibited by 800 ppm of methyl paraben and 200 ppm of propyl paraben. The antimicrobial activity of these compounds depends upon the undissociated acid which can penetrate the cell wall of microorganisms. Parabens inhibit microbial growth by altering the structure of cytoplasmic membranes, inactivating intracellular enzymes and/or preventing uptake of nutrients (33,41,43,64,76,111).

In the United States, only the methyl, propyl and heptyl esters are approved by FDA as generally recognized as safe (GRAS) compounds that can be added directly to foods. Heptyl paraben is approved for use in beer at a level not to exceed 12 ppm, while methyl and propyl paraben may be used in food up to 1,000 ppm (26,30,33,109).

In packaging materials the esters can be incorporated into the package or applied as a surface coating to prevent microbial growth (26). Parabens may be uniformly dispersed in the plasticizer of plastic formulations in a range of 0.1 to 0.5% based on the solution weight (109). The level applied will depend on the susceptibility of the plastic, environmental conditions such as moisture and temperature, exposure to microorganisms and duration of protection required (109).

Potassium Sorbate

Potassium sorbate, the potassium salt of sorbic acid, is a straight chain trans-trans unsaturated fatty acid (Figure 1) (67, 99,100). The compound is manufactured as a white crystalline powder, as granules or in solution, and has approximately 75% of the antimicrobial potency of sorbic acid on a weight basis (71,74,100). At room temperature potassium sorbate is readily soluble in water (138 gm/100 ml) and slightly soluble in ethanol (2 gm/100 ml) (26,100). Potassium sorbate is stable to oxidation, has a mild characteristic odor and will decompose at 270°C (67,71,74).

Sorbates (Sorbic acid and salts) are safe, effective antimicrobials that have a broad spectrum of activity against yeasts and molds, but less activity against bacteria (26,67,68,90,99,100). The antimicrobial action of sorbates and other antimicrobials depends upon the pH of the substrate because only the undissociated form is effective. Sorbates exert antimicrobial activity up to pH 6.5 with

maximum effectiveness below pH 4.75 (pKa) (10,26,68,90,99). Bell et al. (10) tested the effect of sorbic acid on bacteria, yeasts and molds at different pH levels and found that 0.1% sorbic acid at pH 7.0 was not inhibitory in laboratory media. The yeasts and molds were inhibited on media containing 0.1% sorbic acid at pH 4.5, whereas lactic acid bacteria were inhibited at this concentration at pH 3.5. Bullerman (19) reported growth and aflatoxin production of Aspergillus parasiticus and Aspergillus flavus to be totally inhibited by 1,500 ppm (0.15%) of potassium sorbate in yeast extract sucrose agar at pH 5.0. The mechanism(s) of microbial inhibition by sorbates is related to their ability to inactivate cellular enzyme systems, suppress cellular adenosine triphosphate (ATP) levels, inhibit amino acid transport and/or depress fatty acid metabolism (50,70,71,80, 110,112,119).

There have been reports of certain molds which are capable of degrading sorbates. Melnick et al. (70) postulated that sorbic acid, a fatty acid, could be utilized by fungi by Beta-oxidation. Marth et al. (69) reported that certain Penicillium species could degrade potassium sorbate by decarboxylation to 1,3-pentadiene. Finol et al. (40) confirmed these findings by reporting that Penicillium species isolated from cheese could deplete potassium sorbate from media when the preservative was present initially from 3,000 to 12,000 ppm. These results suggested that sorbates were not desirable in preserving highly contaminated products.

Sorbates have been used in preserving foods, food wrapping materials, pharmaceuticals and cosmetics (26,67,71,100). In packaging materials for foods, sorbates are bound to the wrappers with adhesive agents or impregnated within the wrapper at a concentration of 1 to 6 gm per square meter (45,46,71). Fungistatic wrappers are commonly used to preserve margarine and cheese in the food industry (29,71,74,97). Smith and Rollin (97) reported that natural American cheese could be protected from mold growth when packaged with thermoplastic-coated cellophane wrappers dusted with 2.5 to 5.0 gm of sorbic acid per 1,000 square inches of wrapper. Ghosh et al. (46) noted that the grease-proof paper coated with 2% carboxymethyl cellulose (CMC) and 3% sorbic acid (2 gm of sorbic acid per square meter on paper surface) could be used for preserving bread. The author found bread removed directly from the oven, cooled for two to three minutes and packaged in sorbate coated wrappers was resistant to mold growth for 15 days. The amount of preservative required to prevent growth will depend upon the moisture content, initial microbial load, and the type of microorganisms encountered (71,74).

Sorbates are generally recognized as safe (GRAS) compounds that have been approved by FDA for preserving food products and packaging materials (26,71,99,100). The amount added to foods will vary, but the normal range is from 0.01 to 0.3% (26,99). Sorbates are non-toxic and can be metabolized in the body like other naturally occurring fatty acids (26,36,68).

CHAPTER 3

MATERIALS AND METHODS

1. TEST ORGANISMS

All of the fungal species tested were provided by Travenol Laboratories, Inc., Morton Grove, IL. The genera tested and the form in which they were received is shown in Table 4. All species were stored at 4°C prior to testing.

2. POLYVINYL CHLORIDE FILM

Plasticized polyvinyl chloride film was provided by Travenol Laboratories, Inc., Morton Grove, IL. The film was supplied as 0.318 mm thick sheets in bundles within a plastic wrapper. In addition to the PVC resin, the film contained dioctyl phthalate (plasticizer), epoxidized oil (plasticizer-stabilizer), calcium-zinc stearate (heat stabilizer) and a stearamide (lubricant). The film was stored at room temperature and was not treated with a cleaning solution prior to analysis.

3. PREPARATION OF SPORE SUSPENSIONS

Tubes containing 10 ml of sterile glucose-salts-amino acids broth (95) were inoculated with a mold isolate and incubated at 30°C for 5 days. Following incubation, the tubes were vortexed and 1 ml of each suspension was transferred to three prepoured plates

Table 4. Fungi Utilized in the Growth and Inhibition Studies

Fungi	Source
Aspergillus fischeri	Paper Disk
Aspergillus niger	PDA[a] Plates
Cladosporium sp.	PDA Plates
Fusarium sp.	PDA Plates
Paecilomyces sp.	PDA Plates
Penicillium sp.	0.9% Saline
Penicillium citrinum	PDA Plates
Trichoderma sp.	PDA Plates
Verticillium sp.	PDA Plates

[a]Potato Dextrose Agar.

of acidified potato dextrose agar (PDA). The inoculum was spread with a sterile glass rod and incubated at 30°C until microscopic examination verified sporulation.

Seed plates were harvested by dispensing 10-15 ml of sterile 0.005% Triton-X 100 into each plate, loosening spores with a sterile glass rod and transferring with a 10 ml pipet into a sterile dilution bottle. One ml of this suspension was dispensed into each of 10 prepoured PDA plates, spread with a glass rod, and incubated at 30°C until sporulation occurred.

Crop plates were harvested into 10-15 ml of sterile 0.005% Triton-X 100 by loosening the spores with a glass rod and transferring the suspension into sterile dilution bottles. These spore suspensions were checked for purity prior to filtration by streaking prepoured plates of trypticase soy agar (TSA) (BBL, Cockeysville, MD) and by inoculating yeast extract-sucrose (YES) broth (2% yeast extract; 4% sucrose) with 0.1 ml of the appropriate spore suspension. Plates and tubes were incubated at 30°C for 3-5 days and checked for contaminants.

Uncontaminated suspensions were cleaned by filtering through sterile nylon filters (25 and/or 54 nm pore size), centrifuging at 12,000 x g for 8 minutes, decanting the supernatant, and resuspending the spores in sterile 0.005% Triton-X 100. This process was continued until microscopic examination revealed a clean crop free of vegetative debris. Clean spores were finally suspended in 0.9% saline. Suspensions were checked for purity as before by streaking

TSA and inoculating YES broth. Purified spore suspensions were adjusted to approximately 1.0 x 10^6 spores/ml using a microscopic counting chamber (Hauser Scientific, Blue Bell, PA) and stored at 4°C.

4. INOCULATION TECHNIQUES

Preliminary tests showed that polyvinyl chloride film with added plasticizer was very nonpolar. Adding aqueous spore suspensions and nutrients to the surface of the film was a major problem. When 0.1 ml of 5% dextrose was applied to the film, a large bead formed that could not be spread uniformly with a glass rod. Inoculation of the spores directly onto the dried bead resulted in little or no contact with the film. Non-ionic surfactants, such as Triton-X 100 and Antifoam emulsion B, could be used to reduce the contact angle between the liquid/film interface, but an unaccountable variable would be introduced. Therefore, a series of decreased volume inoculation levels was applied to the film in an attempt to determine an optimum. Ten microliters of aqueous spore suspension, applied with a 10 ul Eppendorff pipet (Brinkmann Instruments, Inc., Westbury, NY) was determined to be the best inoculation level for the film growth studies.

5. GROWTH OF FUNGI ON PVC FILM IN PETRI PLATES

Whatman No. 4 filter paper was cut to the shape of a sterile petri dish and holes were punched in the paper for visual observation

of growth. The filter paper was autoclaved at 121°C for 15 minutes and aseptically placed in a sterile petri plate. Plasticized polyvinyl chloride film was aseptically cut into 2 inch strips. Film to be heat treated was layered onto Whatman No. 4 filter paper with sterile forceps, covered with foil, and autoclaved at 121°C for 18 minutes. The film was heat treated to determine if sterilization would increase susceptibility of the film to fungal attack. Unheated film was placed directly onto the surface of the sterile filter paper in the petri plate (Figure 2).

Sterile nutrient solutions of 5% (w/v) dextrose, 1% ammonium sulfate and 5% (w/v) dextrose + 1% (w/v) ammonium sulfate were applied to the surface of the unheated and heat-treated film using a 10 ul Eppendorff pipet (Figure 2). In one inoculation site, either 5% dextrose was supplied as the sole carbon source or 1% ammonium sulfate was supplied as the sole nitrogen source; a second site was inoculated with 5% dextrose + 1% ammonium sulfate which supplied both nutrients; no nutrients were supplied in the third site. The nutrients were dried onto the surface of the film by heating 60 minutes at 30°C. The appropriate spore suspension (10^6 spores/ml) was applied to the dried surface of the nutrient solutions and to the film alone in 10 ul aliquots (10^4 spores per inoculation site).

For controls, nutrients and spores were inoculated onto medium drop wet mount glass slides (Fisher Scientific Co., Fair Lawn, NJ) instead of the film. These slides were cleaned in a strong base

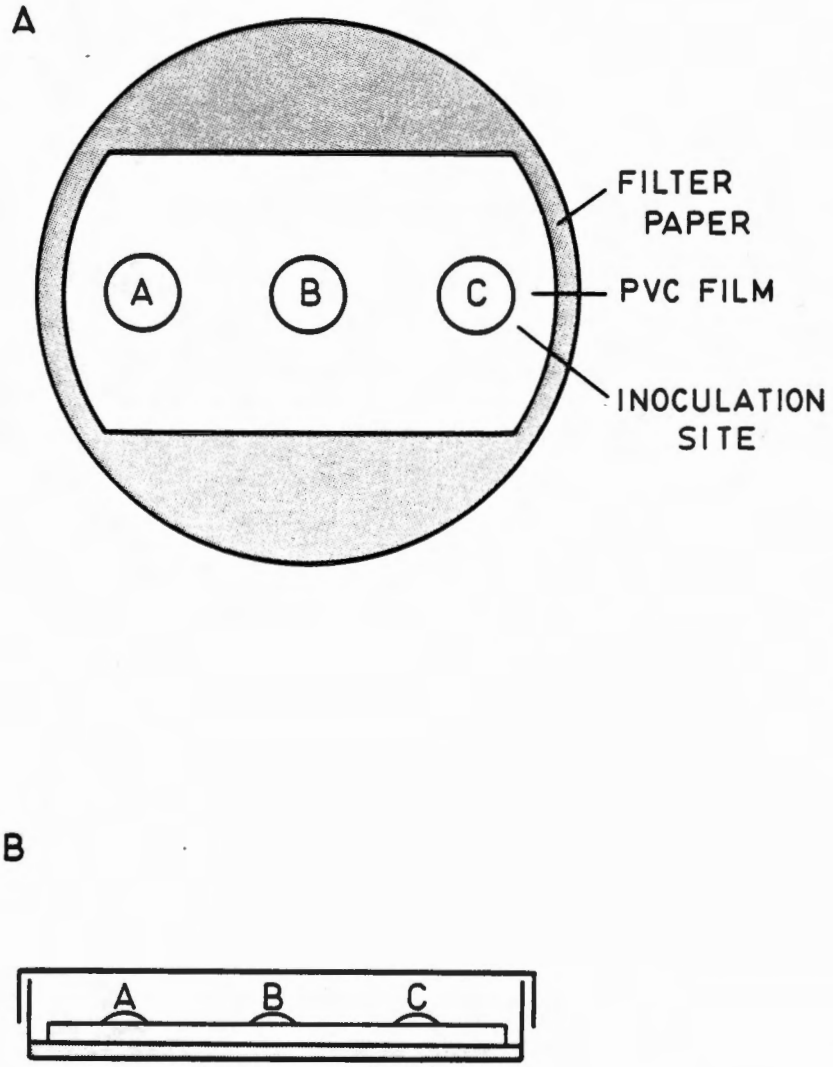


Figure 2. Top view (A) and side view (B) of petri plate containing polyvinyl chloride film layered on the surface of sterile filter paper.

solution consisting of 200 g KOH, 50 g Na_2HPO_4 (tech grade), 5 g Na_2H_2EDTA diluted to 1 liter (4) and a strong acid, 3N HCl, to insure that no residues were present. These slides were thoroughly rinsed with distilled water, dried at 45°C, and autoclaved at 121°C for 15 minutes.

After inoculation, the systems were activated by adding sterile distilled water to the filter paper in the petri dish and incubating at 30°C. At 48 hour intervals, the filter paper was re-moistened with 1 ml of sterile distilled water to maintain a high relative humidity and each plate was observed for growth using a stereo microscope. The plates were incubated up to six weeks and mold growth was evaluated subjectively on the following scale:

0 = No growth
1 = Very slight growth (just detectable)
2 = Slight growth (mycelia present)
3 = Moderate growth (easily detectable)
4 = Abundant growth (detectable without magnification)

Fusarium and Cladosporium sp. were used to determine the influence of initial number of spores on growth of the fungi on the PVC film. Spores of each species were inoculated at a rate of either 10,000 or 100 spores per site and plates incubated 6 weeks at 30°C. Growth was evaluated as above.

6. GROWTH OF FUNGI ON PVC FILM IN TEST TUBES

Thin strips of heated and unheated PVC film were aseptically transferred to sterile acid/base treated test tubes. Tubes were inoculated with 9.9 ml of combinations of sterile 5% (w/v) dextrose,

1% (w/v) ammonium sulfate and water (Figure 3). Mold spores were inoculated into the tubes to obtain a final level of 10^4 spores/ml. Controls consisted of inoculating test tubes without PVC film (Table 5).

Tubes were incubated 18 weeks at 30°C in order to obtain sufficient growth. Mold growth was evaluated subjectively every two weeks using the following scale:

0 = No growth
1 = Very slight growth (< 25% of film covered)
2 = Slight growth (25%-50% of film covered)
3 = Moderate growth (50%-75% of film covered)
4 = Abundant growth (> 75% of film covered).

7. GROWTH STUDIES USING THE PARABIOTIC CHAMBERS

A third type of growth study involved using a parabiotic chamber (Bellco Glass, Inc., Vineland, NJ) to determine if nutrients could diffuse across the PVC film and support fungal growth on the outside.

The PVC film was aseptically cut into 1 inch diameter circles and heat sterilized at 121°C for 18 minutes. Aliquots (10 ul) of each spore suspension (10^6 spores/ml) were applied to the surface of the film in a sterile petri plate and dried for approximately 60 minutes at 30°C.

The parabiotic chamber was assembled by sandwiching the PVC film with dried spores between the U-tubes and clamping (Figure 4). Ten milliliters of 5% dextrose was then added to the chamber on the opposite side of the film that had been inoculated with dried spores.

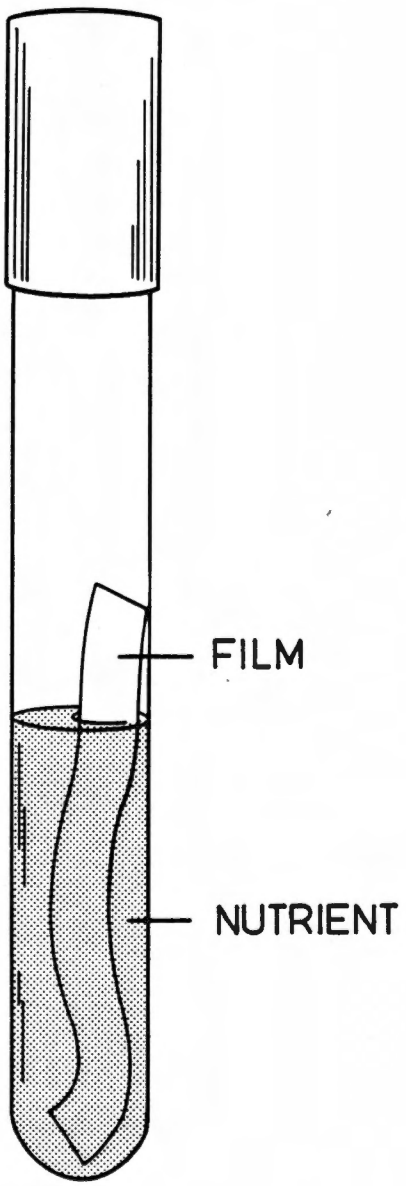


Figure 3. Growth system for fungi on polyvinyl chloride film in test tube.

Table 5. Treatments for Growth Studies on PVC Film in Test Tubes

Treatment	Nutrient Supplied	Nutrients Film Supplied for Growth
Film + 5% Dextrose + Spores[a]	Carbon	Nitrogen
Film + 1% $(NH_4)_2SO_4$ + Spores	Nitrogen	Carbon
Film + H_2O + Spores	-	Both
Controls		
5% Dextrose + Spores	Carbon	-
1% $(NH_4)_2SO_4$ + Spores	Nitrogen	-
5% Dextrose + 1% $(NH_4)_2SO_4$ + Spores	Both	-
H_2O + Spores	-	-

[a]Inoculated with 10,000 spores/tube.

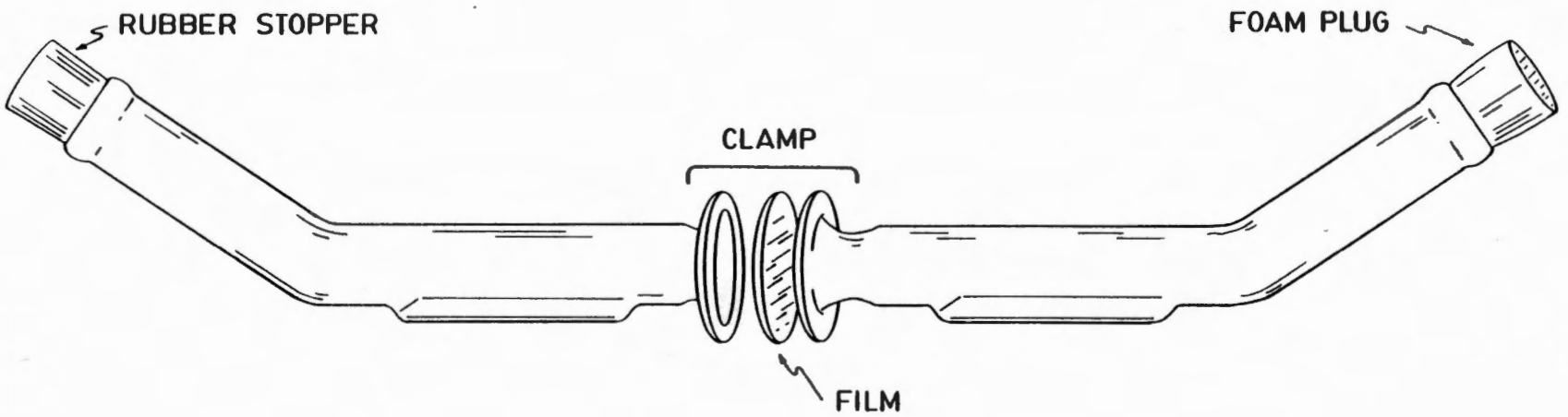


Figure 4. Polyvinyl chloride film placed between the U-tubes of a parabiotic chamber.

Sterile distilled water (0.1 ml every 48 hours) was added to the well of the spore containing chamber to provide moisture for growth. This chamber was stoppered with a foam plug to allow for aerobic conditions. Parabiotic chambers were incubated at 30°C for 6 weeks, disassembled, and microscopically observed for growth.

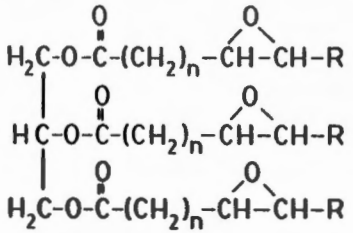
Mold growth was evaluated subjectively on the following scale:

0 = No growth
1 = Very slight growth (0-25% covered)
2 = Slight growth (25%-50% covered)
3 = Moderate growth (50%-75% covered)
4 = Abundant growth (> 75% covered)

8. ABILITY OF FUNGI TO UTILIZE COMPOUNDS IN PVC FILM

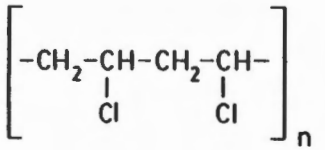
Based upon growth studies and literature reports of microbial degradation of plasticized polymer films (9,11,15,16,18,38,65,77,108), a study was conducted to determine which film components, if any, could support fungal growth. The film components and their chemical structures are presented in Figure 5.

Mineral salts agar (MSA) was used as the basal medium for determining the ability of fungi to utilize plasticizers and related organic compounds as carbon and nitrogen sources. The medium as reported by Berk et al. (12) consisted of 1.0 g NH_4NO_3, 0.005 g NaCl, 0.002 g $FeSO_4$-$7H_2O$, 0.002 g $ZnSO_4$-$7H_2O$, 0.7 g K_2HPO_4, 0.7 g KH_2PO_4, 0.7 g $MgSO_4$-$7H_2O$, 0.001 g $MnSO_4$-$7H_2O$, and 15 g agar (Difco, Detroit, MI) per liter of deionized water. Each film ingredient was

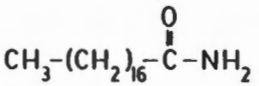


R= hydrocarbon chain

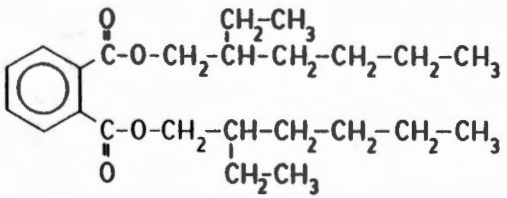
EPOXIDIZED OIL



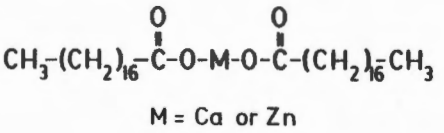
POLYVINYL CHLORIDE (PVC)



STEARAMIDE



DIOCTYL PHTHALATE (DOP)



Ca-Zn STEARATE

Figure 5. Chemical structures of the polyvinyl chloride film components.

incorporated into the MSA at a level of 0.5% to determine if fungi could utilize the compound as a carbon source. A non-ionic silicone surfactant, Antifoam emulsion B, was incorporated at a level of 0.1% to obtain a homogenous dispersion of the film components in the MSA. Exoxidized oil (plasticizer-stabilizer), and stearamide (lubricant), were the only film compounds found to contain nitrogen by the Kjeldahl nitrogen method (4) (Table 6). Therefore, for possible utilization as a nitrogen source, the ammonium nitrate was replaced with 0.5% of either epoxidized oil or the stearamide, while 0.5% dextrose was used as an available carbon source. The mineral salts agar containing the appropriate film ingredient was steamed and autoclaved for 18 minutes at 121°C.

Approximately 30 ml portions of dispersed medium were poured into 9 cm sterile petri dishes and allowed to solidify at room temperature. The pH of the mineral salts agar was 6.2 and was only slightly altered by the addition of the film components. Solidified agar plates were inoculated with a 3 mm loop in the center of the plates using a spore suspension containing 10^6 spores/ml. Controls consisted of inoculated potato dextrose agar (PDA) and MSA without the film ingredients. Plates were incubated in an inverted position for 28 days at 30°C. Results were reported as the diameter of the colony growth in mm.

9. INHIBITION OF FUNGI IN VITRO

The first step in inhibiting growth of fungi on the PVC film was to determine the susceptibility of the fungi to preservatives

Table 6. Kjeldhal Nitrogen Determination of Compounds in PVC Film

Compound	Nitrogen (%)
Epoxidized Oil	0.006
Dioctyl Phthalate	0.000
Calcium-Zinc Stearate	0.000
Stearamide	4.576
PVC Resin	0.000
- - - - -	
Whole Film	0.037

in a nutrient medium. Potassium sorbate, methyl and propyl paraben, and butylated hydroxyanisole (BHA) were tested in yeast extract sucrose broth (YES), consisting of 2% yeast extract and 4% sucrose, to determine the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) against the molds.

Butylated hydroxyanisole (Eastman Chemical Products, Inc. Kingsport, TN), propyl paraben and methyl paraben (Fisher Scientific) were dissolved in 95% ethanol to a final concentration of 10 and 15% (w/v), respectively. Potassium sorbate (Monsanto Co., St. Louis, MO) was dissolved in deionized water to a final concentration of 20% (w/v). These solutions were filter sterilized using a 0.45 um sterilization filter (Sybron/Nalge, Inc., Rochester, NY) and stored in the dark at 4°C. Appropriate dilutions were made in sterile 95% ethanol or deionized water to give the desired test concentrations. Testing ranges were determined based upon prior studies of the antimicrobial agents (1,3,25,33,68,81,84,99).

Test medium preparation included autoclaving YES broth at 121°C for 15 minutes and dispensing into 9.8 ml sterile tubes. A 0.1 ml aliquot of the appropriate antimicrobial was added followed by inoculation with 0.1 ml of the spore suspension (10^6/ml) to obtain a final concentration of 10^4 spores per ml. Controls were run using sterile 95% ethanol or water depending upon the antimicrobial.

Inoculated tubes were incubated at 30°C for 5 days and observed for growth. The concentration which inhibited growth was the minimum inhibitory concentration (MIC), while the minimum lethal concentration

(MLC) was determined by subculturing tubes with negative growth (0.1 ml) into tubes containing only YES (10 ml). These tubes were incubated at 30°C for 5 days to determine the minimum concentration required to kill the spores.

10. APPLICATION OF PRESERVATIVES TO PVC FILM

The chemical preservatives found to be most effective in inhibiting growth of fungi *in vitro* were applied to the film in an ethanol solution to determine a concentration that would inhibit growth on the PVC film.

Whatman No. 4 filter paper was cut to the shape of a sterile petri dish and holes were punched in the paper for visual observation of growth. The filter paper was autoclaved at 121°C for 15 minutes and aseptically placed in a sterile petri plate. Polyvinyl chloride film was cut into 2 inch strips, sterilized at 121°C for 18 minutes, and aseptically placed onto the surface of the sterile filter paper in the petri plate.

Chemical preservatives used in the *in vitro* study were applied to the film by a dip or spread method. In the dip method, appropriate antimicrobial dilutions in 95% ethanol were prepared in 250 ml beakers. The film was immersed for 30 seconds and transferred to the petri plate. In the spread method, 0.2 ml of the appropriate dilution was applied and spread with a sterile glass rod.

Sterile nutrient solutions of 5% (w/v) dextrose and 5% (w/v) dextrose + 1% (w/v) ammonium sulfate were applied to the film as

described in the growth studies. Fusarium sp. or Paecilomyces sp. were inoculated onto the dried surface of the nutrients and to the film alone in 10 ul aliquots (10^4 spores/inoculation site) (Figure 2, page 38). Controls consisted of heated film that had not been treated with antimicrobial.

Once inoculated, the systems were activated by the addition of sterile distilled water to the filter paper in the petri dish and incubation at 30°C. At 48 hour intervals, 1 ml of sterile distilled water was applied to each plate. Plates were observed weekly up to six weeks and results were reported on the scale used in the growth studies.

11. STATISTICAL ANALYSIS

In the growth studies, fungal growth on the controls was subtracted from growth on the test film to determine possible utilization of the film as a nutrient source. When growth on the control was greater than that on the film, a zero value was assigned. All tests were done in duplicate.

The data was analyzed using analysis of variance (ANOVA) with a split plot design, when time was a factor, to detect significant differences in main effects and interactions. Sample means were separated by Duncan's Multiple Range Test ($\alpha = 0.05$).

CHAPTER IV

RESULTS AND DISCUSSION

1. GROWTH CHARACTERISTICS OF FUNGI ON PVC FILM

Growth Studies in Petri Plates

Growth studies in petri plates showed that most of the mold species tested were capable of growth on the heated and unheated polyvinyl chloride films with and without added nutrients (Tables 7 and 8). When 5% (w/v) dextrose was applied to the film as a sole carbon source, all species grew, but to varying extents. Trichoderma sp. showed the least amount of growth, while Cladosporium sp. grew abundantly over the six week storage period. Growth also was observed on a majority of the glass slides (controls) inoculated with the dextrose. Comparison of the amount of growth on the film and the controls indicated there was no significant difference ($P>0.05$) in growth of the fungi when supplied with 5% dextrose (Table 9). When 1% (w/v) ammonium sulfate was applied to the film as a nitrogen source, most species again were able to grow on the film and the controls (Tables 7 and 8). Aspergillus fischeri, Aspergillus niger, and Fusarium sp. showed a significantly greater amount of growth on the film compared to their respective controls, and, therefore, may be capable of utilizing the film as a carbon source. When no nutrient was applied to the film, all mold species were found to be capable of utilizing the film as a carbon and nitrogen source

Table 7. Mean Growth Levels of Selected Fungi on Heated (121°C, 18 min) PVC Film in Petri Plates (10,000 Spores/Site)

Organism	Week	Dextrose[a]		Am. Sulfate[b]		Dextrose Am. Sulfate[c]		No Nutrient	
		Film	Cont[d]	Film	Cont	Film	Cont	Film	Cont
		Growth Level[e]							
A. fischeri	2	1.0	3.0	1.5	1.5	1.5	3.0	0.5	0.0
	4	2.0	3.5	2.5	1.5	2.5	4.0	1.5	0.0
	6	3.0	4.0	2.5	1.5	3.0	4.0	2.0	0.0
A. niger	2	1.5	0.5	1.5	1.0	1.5	0.5	0.0	0.0
	4	3.0	1.5	2.5	1.0	3.0	2.0	0.0	0.0
	6	3.0	1.5	2.5	1.0	4.0	3.5	0.5	0.0
Cladosporium sp.	2	2.5	1.5	1.5	1.5	2.5	3.0	1.5	0.5
	4	3.5	2.0	2.0	1.5	3.0	3.0	2.0	0.5
	6	4.0	2.0	2.0	1.5	4.0	3.5	2.0	0.5
Fusarium sp.	2	1.5	1.5	1.0	0.5	1.0	3.0	1.5	2.0
	4	2.0	2.5	1.5	0.5	2.0	4.0	1.5	3.0
	6	2.5	2.5	2.5	0.5	3.5	4.0	2.5	3.0
Paecilomyces sp.	2	0.5	0.0	0.0	3.0	1.0	2.0	1.5	0.0
	4	1.5	0.0	0.5	3.5	2.5	4.0	2.5	0.0
	6	2.0	0.0	1.0	3.5	3.0	4.0	2.5	0.0
Penicillium sp.	2	1.5	0.0	0.0	0.0	1.0	2.5	1.0	0.0
	4	1.5	0.0	0.0	0.0	2.0	3.5	2.0	0.0
	6	2.0	0.0	0.0	0.0	2.0	4.0	2.0	0.0
P. citrinum	2	0.5	0.5	0.5	1.5	1.0	3.0	1.0	0.0
	4	2.0	2.0	1.5	1.5	2.5	4.0	1.5	0.0
	6	2.5	2.0	2.0	1.5	3.5	4.0	1.5	0.0
Trichoderma sp.	2	0.5	0.5	0.0	0.5	0.5	1.0	0.0	0.0
	4	0.5	0.5	0.0	0.5	0.5	2.0	0.0	0.0
	6	1.0	0.5	0.0	0.5	1.0	3.0	0.0	0.0
Verticillium sp.	2	0.0	1.0	0.0	0.0	0.0	2.0	1.0	0.0
	4	1.0	1.5	0.0	0.0	1.5	3.5	1.5	0.0
	6	2.0	1.5	0.0	0.0	2.0	4.0	1.5	0.0

[a]5% Dextrose solution.

[b]1% Ammonium sulfate solution.

[c]5% Dextrose + 1% ammonium sulfate solution.

[d]Control.

[e]0 = no growth; 1 = very slight growth; 2 = slight growth; 3 = moderate growth; 4 = abundant growth.

Table 8. Mean Growth Levels of Selected Fungi on Unheated PVC Film in Petri Plates (10,000 Spores/Site)

Organism	Week	Dextrose[a]		Am. Sulfate[b]		Dextrose Am. Sulfate[c]		No Nutrient	
		Film	Cont[d]	Film	Cont	Film	Cont	Film	Cont
		Growth Level[e]							
A. fischeri									
	2	1.0	3.0	2.0	1.5	1.5	3.0	0.5	0.0
	4	2.0	3.5	2.5	1.5	2.0	4.0	1.5	0.0
	6	2.0	4.0	2.5	1.5	3.0	4.0	1.5	0.0
A. niger									
	2	1.5	0.5	1.0	1.0	2.0	0.5	0.0	0.0
	4	2.5	1.5	2.5	1.0	3.5	2.0	0.0	0.0
	6	3.0	1.5	2.5	1.0	4.0	3.5	0.5	0.0
Cladosporium sp.									
	2	2.5	1.5	1.5	1.5	2.0	3.0	1.0	0.5
	4	3.5	2.0	2.0	1.5	3.0	3.0	2.0	0.5
	6	4.0	2.0	2.0	1.5	3.0	3.5	2.0	0.5
Fusarium sp.									
	2	1.5	1.5	1.0	0.5	1.5	3.0	1.0	2.0
	4	2.5	2.5	1.0	0.5	2.5	4.0	2.0	3.0
	6	2.5	2.5	1.5	0.5	3.5	4.0	2.0	3.0
Paecilomyces sp.									
	2	0.5	0.0	0.0	3.0	1.0	2.0	1.0	0.0
	4	2.0	0.0	0.0	3.5	2.5	4.0	2.0	0.0
	6	2.0	0.0	0.0	3.5	2.5	4.0	2.0	0.0
Penicillium sp.									
	2	1.5	0.0	0.5	0.0	1.0	2.5	1.0	0.0
	4	1.5	0.0	1.0	0.0	1.5	3.5	2.0	0.0
	6	2.0	0.0	1.0	0.0	2.0	4.0	2.0	0.0
P. citrinum									
	2	0.0	0.5	0.5	1.5	1.0	3.0	1.0	0.0
	4	1.5	2.0	1.5	1.5	2.5	4.0	2.0	0.0
	6	2.5	2.0	2.0	1.5	3.5	4.0	2.5	0.0
Trichoderma sp.									
	2	0.5	0.5	0.5	0.5	0.5	1.0	0.0	0.0
	4	0.5	0.5	0.5	0.5	1.0	2.0	0.0	0.0
	6	1.0	0.5	0.5	0.5	1.0	3.0	0.0	0.0
Verticillium sp.									
	2	1.0	1.0	0.0	0.0	1.0	2.0	1.0	0.0
	4	1.5	1.5	0.0	0.0	1.5	3.5	1.5	0.0
	6	2.0	1.5	0.0	0.0	2.5	4.0	2.0	0.0

[a]5% Dextrose solution.

[b]1% Ammonium sulfate solution.

[c]5% Dextrose + 1% ammonium sulfate solution.

[d]Control.

[e]0 = no growth; 1 = very slight growth; 2 = slight growth; 3 = moderate growth; 4 = abundant growth.

Table 9. Analysis of Variance for Growth of Fungi on PVC Film in Petri Plates When Supplied With Various Nutrients Measured as a Function of Organism and Time

Source	df	Nutrient Supplied		
		Dextrose	Am. Sulfate	No Nutrient
		MS		
Replication (R)	1	19.17^{ns}	0.45^{ns}	9.48^{ns}
Organism (O)	8	4.62^{ns}	2.15^{*}	6.21^{ns}
Film (F)	1	0.00^{ns}	0.00^{ns}	0.01^{ns}
O X F	8	0.04^{ns}	0.27^{ns}	0.12^{ns}
Error A[a]	17	5.40	0.59	3.00
Time (T)	2	3.42^{*}	1.75^{*}	5.09^{*}
O X T	16	0.32^{*}	0.26^{*}	0.28^{*}
F X T	2	0.02^{ns}	0.03^{ns}	0.10^{ns}
Error B	52	0.16	0.08	0.14

$^{*}P<0.05$.

$^{ns}P>0.05$.

[a] R X O; R X F; R X O X F.

with the possible exceptions of Trichoderma sp., Fusarium sp. and Aspergillus niger. There was no significant difference ($P>0.05$) in the amount of growth of the fungi on the PVC film and their respective controls (Table 9). The 5% dextrose + 1% ammonium sulfate served as a viability control in the growth study. All mold species were found to grow when supplied with both a carbon and nitrogen source. Results also indicated that there was not a significant difference ($P>0.05$) in growth of the fungi on the heated and unheated PVC film (Table 9).

Possible reasons for the slight amount of growth on the controls (glass slides) may have been due to one or more of the following factors:

1. a transfer of nutrient residues produced during spore suspension preparation, which could have supplied a limited amount of carbon and nitrogen;

2. impurities in the dextrose or ammonium sulfate used for the growth studies; and

3. carbon or nitrogen compounds in the water.

Growth studies of Fusarium sp. and Cladosporium sp. on PVC film at different inoculation levels showed that both organisms could grow on the heated and unheated PVC film without added nutrients at a level of 10,000 spores per inoculation site (Tables 10 and 11). When the inoculum was reduced to 100 spores no growth was observed on the PVC film. Therefore, these organisms did not have the ability to utilize the film components for growth during the observation

Table 10. Effect of Number of Spores on Mean Growth Levels of Fungi on Heated (121°C, 18 min) PVC Film in Petri Plates

Organism	Spores	Week	Dextrose[a] Film	Dextrose[a] Cont[c]	Dextrose Am. Sulfate[b] Film	Dextrose Am. Sulfate[b] Cont	No Nutrient Film	No Nutrient Cont
			Growth Level[d]					
Fusarium sp.	100	2	1.5	0.0	1.0	3.0	0.0	0.0
		4	2.5	0.0	2.0	4.0	0.0	0.0
		6	3.0	0.0	2.5	4.0	0.0	0.0
	10,000	2	1.5	1.5	1.0	3.0	1.5	2.0
		4	2.0	2.5	2.0	4.0	1.5	3.0
		6	2.5	2.5	3.5	4.0	2.5	3.0
Cladosporium sp.	100	2	0.5	0.5	0.5	1.5	0.0	0.0
		4	1.0	1.5	2.0	2.0	0.0	0.0
		6	1.0	2.0	2.0	2.5	0.0	0.0
	10,000	2	2.5	1.5	2.5	3.0	1.5	0.5
		4	3.5	2.0	3.0	3.0	2.0	0.5
		6	4.0	2.0	4.0	3.5	2.0	0.5

[a] 5% Dextrose solution.

[b] 5% Dextrose + 1% ammonium sulfate solution.

[c] Control.

[d] 0 = no growth; 1 = very slight growth; 2 = slight growth; 3 = moderate growth; 4 = abundant growth.

Table 11. Effect of Number of Spores on Mean Growth Levels of Fungi on Unheated PVC Film in Petri Plates

Organism	Spores	Week	Dextrose[a]		Dextrose Am. Sulfate[b]		No Nutrient	
			Film	Cont[c]	Film	Cont	Film	Cont
			Growth Level[d]					
Fusarium sp.								
	100	2	1.0	0.0	0.5	3.0	0.0	0.0
		4	2.0	0.0	1.5	4.0	0.0	0.0
		6	2.0	0.0	2.5	4.0	0.0	0.0
	10,000	2	1.5	1.5	1.5	3.0	1.0	2.0
		4	2.5	2.5	2.5	4.0	2.0	3.0
		6	2.5	2.5	3.5	4.0	2.0	3.0
Cladosporium sp.								
	100	2	0.0	0.5	0.5	1.5	0.0	0.0
		4	0.0	1.5	2.0	2.0	0.0	0.0
		6	0.0	2.0	2.0	2.5	0.0	0.0
	10,000	2	2.5	1.5	2.0	3.0	1.0	0.5
		4	3.5	2.0	3.0	3.0	2.0	0.5
		6	4.0	2.0	3.0	3.5	2.0	0.5

[a] 5% Dextrose solution.

[b] 5% Dextrose + 1% ammonium sulfate solution.

[c] Control.

[d] 0 = no growth; 1 = very slight growth; 2 = slight growth; 3 = moderate growth; 4 = abundant growth.

time at the low inoculation levels. In the presence of 5% dextrose, there was no apparent difference in the amount of Fusarium sp. growth when it was inoculated onto the film at a rate of 10,000 or 100 spores per site. In contrast, Cladosporium sp. showed much more growth when inoculated at a rate of 10,000 versus 100 spores per site. Therefore, as the initial number of spores increased the amount of growth generally increased, but this was dependent on the species tested.

Growth Studies of Fungi in Test Tubes

Growth of fungi on PVC film in test tubes generally showed that mold species could grow on the heated and unheated film when supplied 5% (w/v) dextrose or 1% (w/v) ammonium sulfate (Tables 12 and 13). Aspergillus fischeri (Figure 6), A. niger (Figure 7), Fusarium sp. (Figure 8) and Paecilomyces sp. (Figure 9) all grew over the entire surface of the film within the 18 week incubation period. Other organisms such as Cladosporium sp., P. citrinum, Penicillium sp. and Trichoderma sp. grew to a moderate extent covering approximately 25% to 75% of the film surface. Verticillium sp. was not found to grow in the 5% dextrose + 1% ammonium sulfate which served as a viability control in the growth study. These results indicated that most of the fungi tested have the ability to utilize the film for either a carbon or nitrogen source when the other nutrient is present.

When no nutrient was supplied, only A. fischeri and Paecilomyces sp. grew on the film surface (Figure 6 and 9). Therefore, these

Table 12. Mean Growth Level of Fungi on Heated (121°C, 18 min) PVC Film in Tubes (10,000 Spores/Tube)

Organism	Dextrose[a] Film	Dextrose[a] Cont[c]	Am. Sulfate[b] Film	Am. Sulfate[b] Cont	No Nutrient Film	No Nutrient Cont
	Growth Level[d]					
A. fischeri						
6 Weeks	3.0	1.5	2.0	1.0	1.0	0.0
12 Weeks	4.0	2.0	4.0	1.0	3.0	0.0
18 Weeks	4.0	2.0	4.0	1.0	3.0	0.0
A. niger						
6 Weeks	3.0	1.0	2.0	0.0	0.0	0.0
12 Weeks	4.0	1.0	3.0	0.0	0.0	0.0
18 Weeks	4.0	2.0	4.0	0.0	0.0	0.0
Cladosporium sp.						
6 Weeks	3.0	1.0	3.0	0.0	0.0	0.0
12 Weeks	3.0	1.0	3.0	0.0	0.0	0.0
18 Weeks	3.0	1.0	3.0	0.0	0.0	0.0
Fusarium sp.						
6 Weeks	3.0	1.0	4.0	0.0	0.0	0.0
12 Weeks	3.0	1.0	4.0	0.0	0.0	0.0
18 Weeks	4.0	1.0	4.0	0.0	0.0	0.0
Paecilomyces sp.						
6 Weeks	2.5	1.0	1.5	1.0	0.0	0.0
12 Weeks	4.0	2.0	3.0	1.0	2.0	0.0
18 Weeks	4.0	2.0	3.0	1.0	2.0	0.0
Penicillium sp.						
6 Weeks	2.0	1.0	1.0	0.0	0.0	0.0
12 Weeks	3.0	1.0	3.0	0.0	0.0	0.0
18 Weeks	3.0	1.0	3.0	0.0	0.0	0.0
P. citrinum						
6 Weeks	2.0	1.0	2.0	0.0	0.0	0.0
12 Weeks	2.0	1.0	3.0	0.0	0.0	0.0
18 Weeks	2.0	1.0	3.0	0.0	0.0	0.0
Trichoderma sp.						
6 Weeks	1.0	1.0	0.0	0.0	0.0	0.0
12 Weeks	1.0	1.5	1.0	0.0	0.0	0.0
18 Weeks	2.0	1.5	2.0	0.0	0.0	0.0
Verticillium sp.						
6 Weeks	0.0	0.0	0.0	0.0	0.0	0.0
12 Weeks	0.0	0.0	0.0	0.0	0.0	0.0
18 Weeks	0.0	0.0	0.0	0.0	0.0	0.0

[a] 5% Dextrose solution.

[b] 1% Ammonium sulfate solution.

[c] Control.

[d] 0 = no growth; 1 = <25% covered; 2 = 25 - 50% covered; 3 = 50 - 75% covered; 4 = > 75% covered.

Table 13. Mean Growth Level of Fungi on Unheated PVC Film in Tubes (10,000 Spores/Tube)

Organism	Dextrose[a]		Am. Sulfate[b]		No Nutrient	
	Film	Cont[c]	Film	Cont	Film	Cont
	Growth Level[d]					
A. fischeri						
6 Weeks	3.0	1.5	2.0	1.0	1.0	0.0
12 Weeks	4.0	2.0	4.0	1.0	3.0	0.0
18 Weeks	4.0	2.0	4.0	1.0	3.0	0.0
A. niger						
6 Weeks	2.5	1.0	2.0	0.0	0.0	0.0
12 Weeks	4.0	1.0	4.0	0.0	0.0	0.0
18 Weeks	4.0	2.0	4.0	0.0	0.0	0.0
Cladosporium sp.						
6 Weeks	3.0	1.0	3.0	0.0	0.0	0.0
12 Weeks	3.0	1.0	3.0	0.0	0.0	0.0
18 Weeks	3.0	1.0	3.0	0.0	0.0	0.0
Fusarium sp.						
6 Weeks	3.0	1.0	4.0	0.0	0.0	0.0
12 Weeks	3.0	1.0	4.0	0.0	0.0	0.0
18 Weeks	4.0	1.0	4.0	0.0	0.0	0.0
Paecilomyces sp.						
6 Weeks	2.5	1.0	2.0	1.0	0.0	0.0
12 Weeks	4.0	2.0	3.0	1.0	1.0	0.0
18 Weeks	4.0	2.0	3.0	1.0	1.5	0.0
Penicillium sp.						
6 Weeks	2.0	1.0	1.0	0.0	0.0	0.0
12 Weeks	3.0	1.0	2.5	0.0	0.0	0.0
18 Weeks	3.0	1.0	3.0	0.0	0.0	0.0
P. citrinum						
6 Weeks	2.0	1.0	2.0	0.0	0.0	0.0
12 Weeks	2.0	1.0	3.0	0.0	0.0	0.0
18 Weeks	2.0	1.0	3.0	0.0	0.0	0.0
Trichederma sp.						
6 Weeks	0.5	1.0	0.0	0.0	0.0	0.0
12 Weeks	1.0	1.5	1.0	0.0	0.0	0.0
18 Weeks	2.0	1.0	2.0	0.0	0.0	0.0
Verticillium sp.						
6 Weeks	0.0	0.0	0.0	0.0	0.0	0.0
12 Weeks	0.0	0.0	0.0	0.0	0.0	0.0
18 Weeks	0.0	0.0	0.0	0.0	0.0	0.0

[a] 5% Dextrose solution.

[b] 1% Ammonium sulfate solution.

[c] Control.

[d] 0 = no growth; 1 = < 25% covered; 2 = 25-50% covered; 3 = 50-75% covered; 4 = > 75% covered.

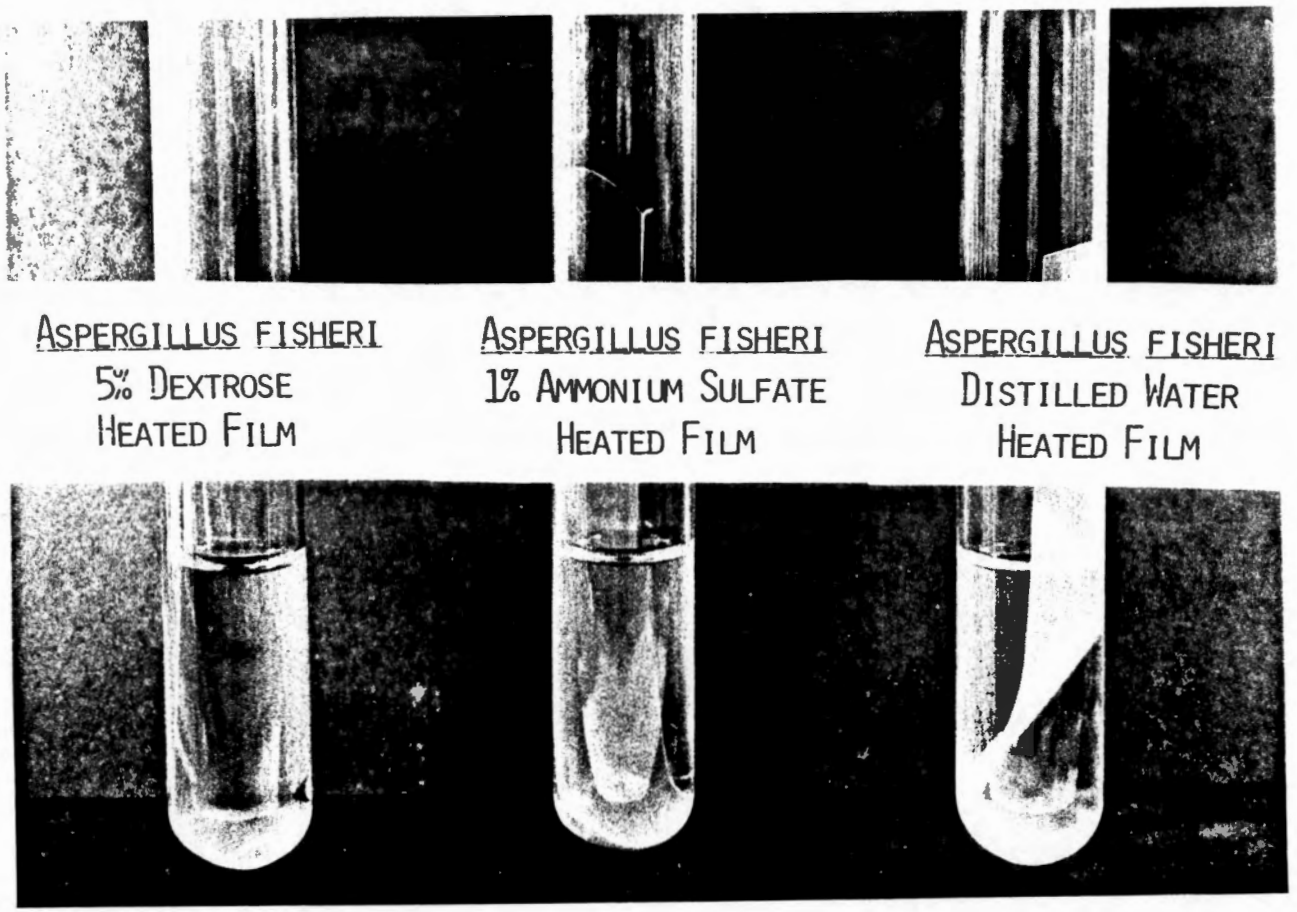


Figure 6. Growth of Aspergillus fischeri on heated polyvinyl chloride film supplied with 5% dextrose, 1% ammonium sulfate or distilled water.

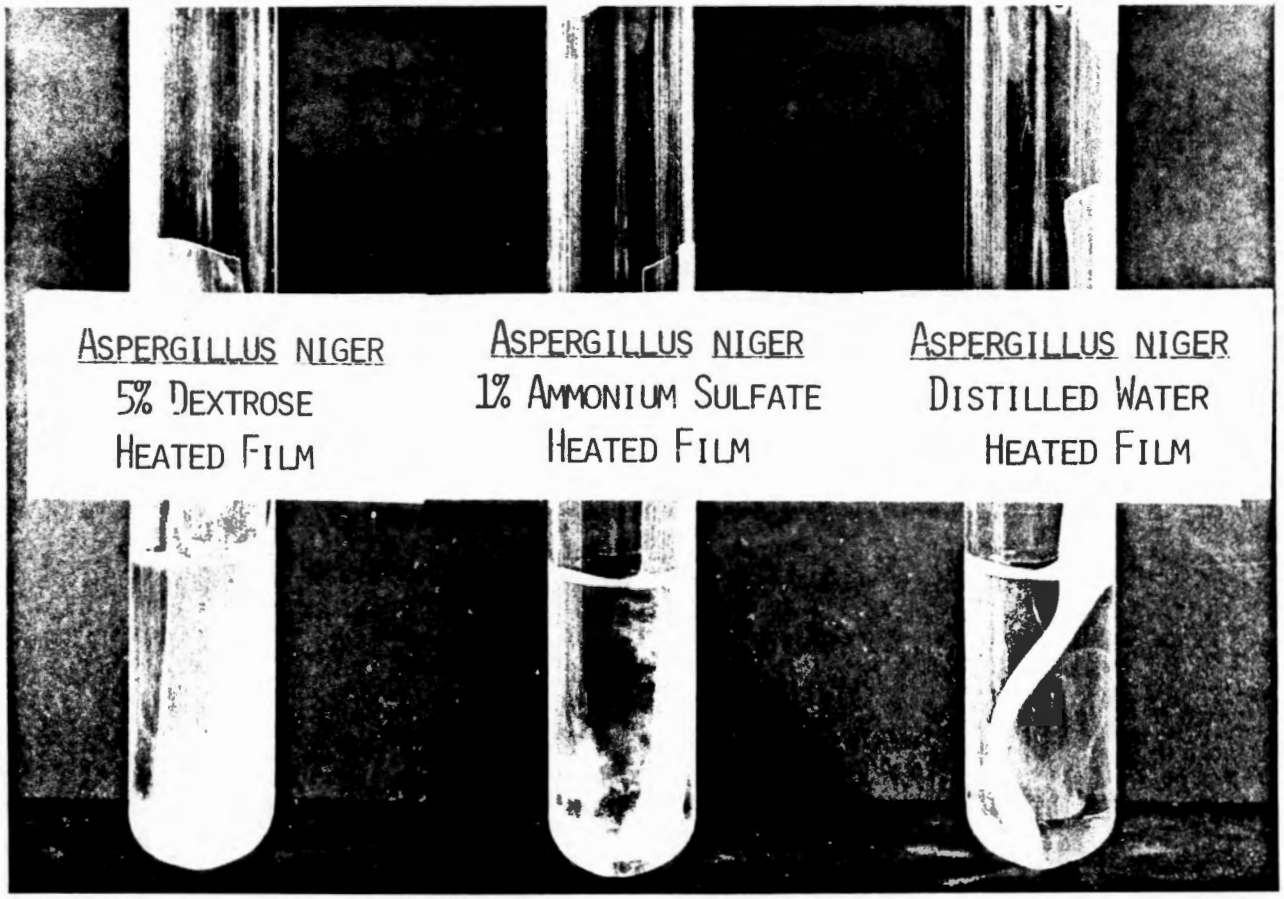


Figure 7. Growth of Aspergillus niger on heated polyvinyl chloride film supplied with 5% dextrose, 1% ammonium sulfate or distilled water.

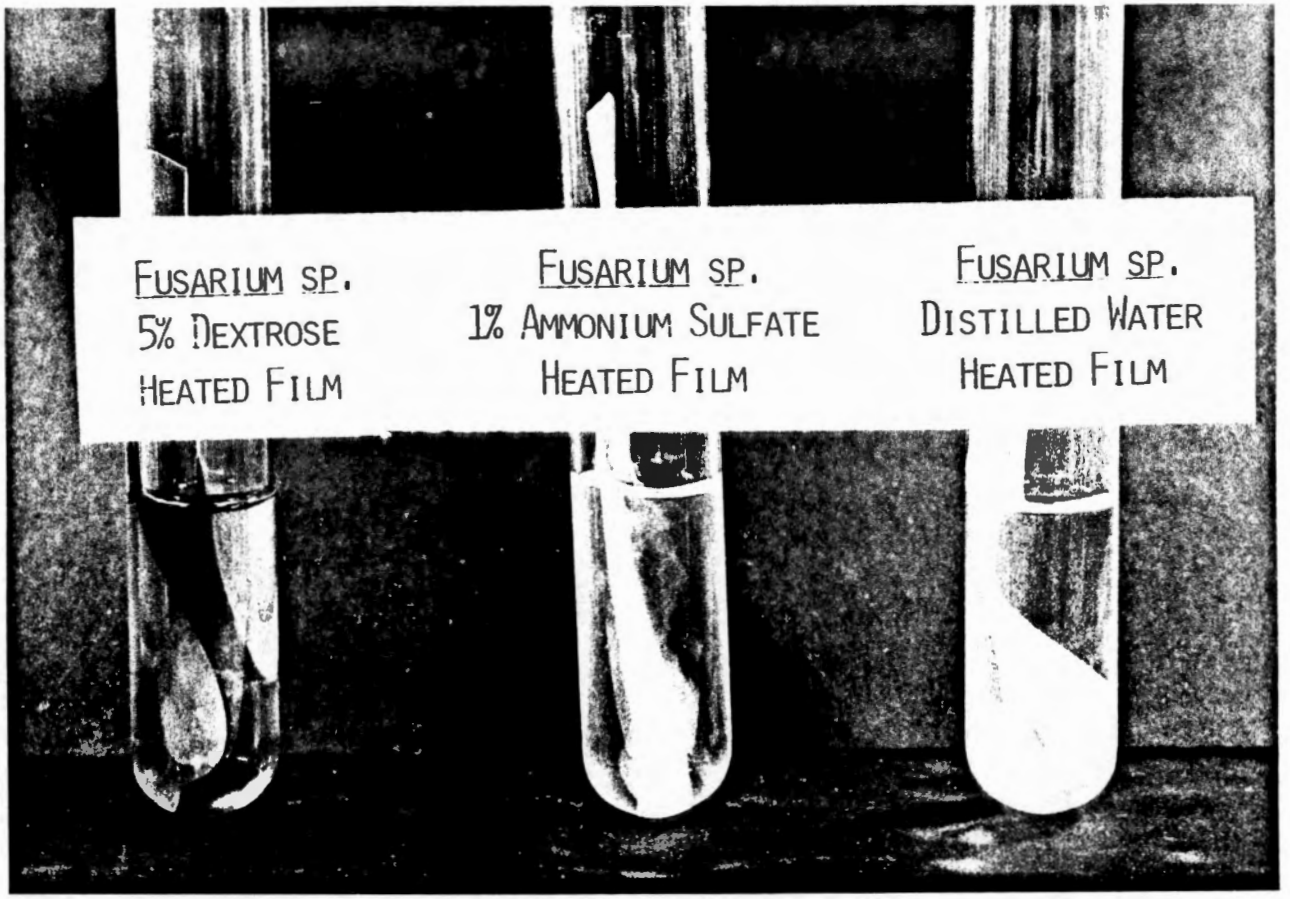


Figure 8. Growth of Fusarium sp. on heated polyvinyl chloride film supplied with 5% dextrose, 1% ammonium sulfate or distilled water.

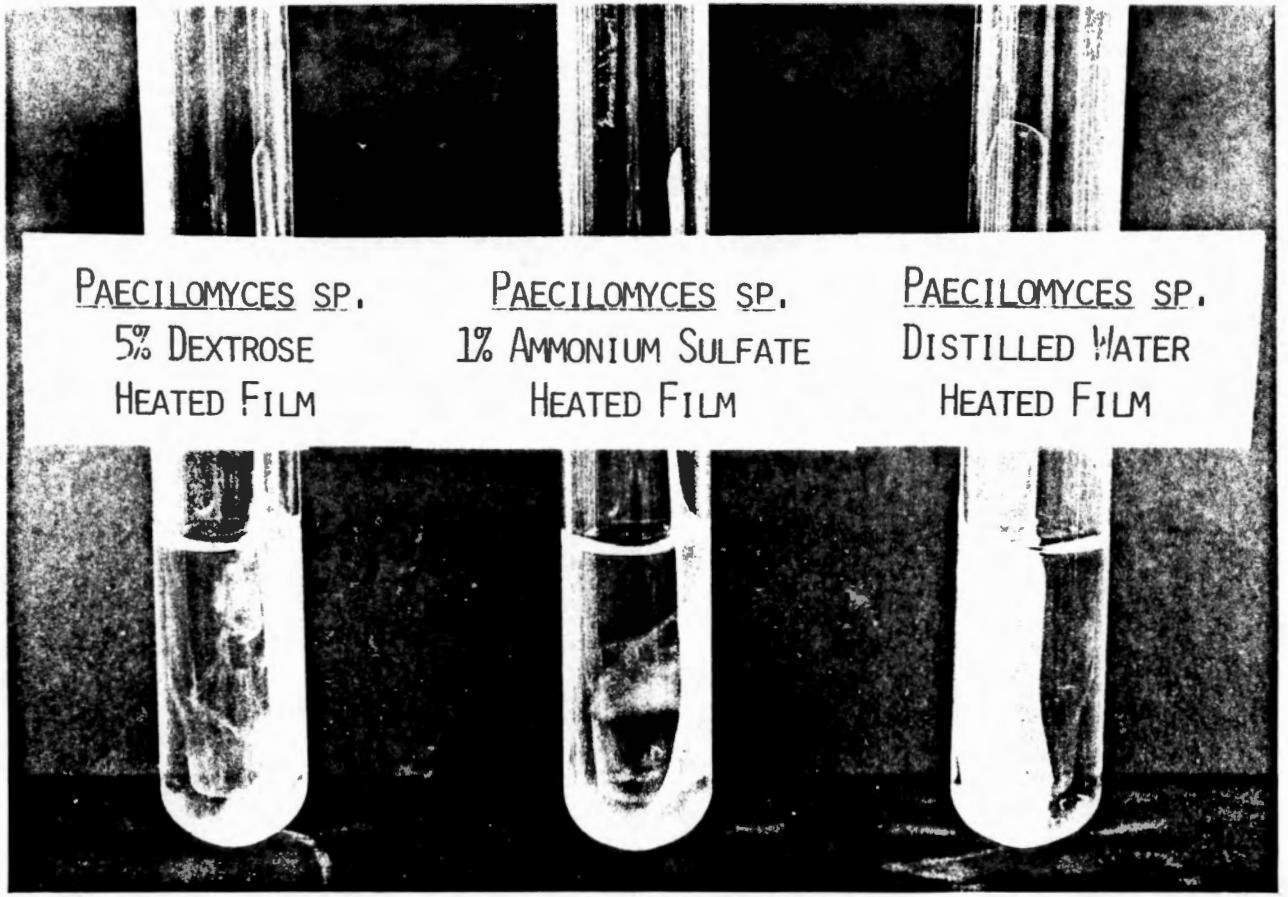


Figure 9. Growth of Paecilomyces sp. on heated polyvinyl chloride film supplied with 5% dextrose, 1% ammonium sulfate or distilled water.

two species appear to be able to utilize the film for both a carbon and nitrogen source.

In comparing the mean growth levels of the organisms, A. fischeri, Fusarium sp. and A. niger were found to be the most efficient ($P<0.05$) at utilizing the PVC film as a nutrient source. In contrast, P. citrinum and Trichoderma sp. were found to produce significantly less ($P<0.05$) growth than any of the other organisms tested (Table 14).

The film was stained a yellow color by the growth of A. fischeri and a dark purple color by the Fusarium sp. only in the presence of 5% dextrose (Figure 6 and 8). This was due to the organisms producing metabolic by-products (pigments) that were soluble in the plasticizer(s) during utilization of the film as a nutrient source (38,47,65,108, 116).

Results also indicated that there was not a significant difference ($P>0.05$) in the ability of the fungi to utilize the heated and unheated PVC film (Table 15). This result is in agreement with that found in the petri plate study.

Slight growth was observed in the 5% dextrose control tubes for most fungi (Table 12 and 13). A majority of this growth was observed within the first two weeks of the study. The growth could have been attributed to small amounts of nitrogen in the water used to suspend the film.

Table 14. Mean Growth Levels of Various Fungi on PVC Film in Test Tubes After 18 Weeks of Incubation at 30°C

Organism	Growth Level[a]
A. fischeri	2.17[b]
Fusarium sp.	2.11[b]
A. niger	1.92[b]
Cladosporium sp.	1.67[c]
Paecilomyces sp.	1.50[cd]
Penicillium sp.	1.31[de]
P. citrinum	1.22[e]
Trichoderma sp.	0.39[e]

[a]0 = no growth; 4 = > 75% covered.

[b-e]Means with different letters are significantly different.

Table 15. Analysis of Variance for Growth of Fungi on PVC Film in Test Tubes

Source	df	MS	F-value
Organism (O)	7	6.07	44.63*
Film (F)	1	0.01	0.07ns
Nutrient (N)	2	52.29	384.50*
Time (T)	2	8.28	60.88*
O X F	7	0.01	0.07ns
O X N	14	3.59	26.40*
O X T	14	0.59	4.34*
Error	96	0.14	

*$P<0.05$.

ns$P>0.05$.

Growth Studies Using the Parabiotic Chambers

Aspergillus fischeri and *Paecilomyces* sp. grew abundantly on the PVC film in the parabiotic chamber (Table 16). In contrast, *A. niger*, *Cladosporium* sp., *Penicillium* sp. and *Trichoderma* sp. showed little or no growth. These results were very similar to those of the test tube study in which no nutrients were supplied to the growing fungi (Table 12). *Aspergillus fischeri* and *Paecilomyces* sp. were shown to have the ability to utilize the film without added nutrients in the test tube study, while the other organisms had to have either a carbon or nitrogen source present to grow. Since the only organisms which could grow to any extent on the film in the parabiotic chambers were already known to be capable of utilizing the film in test tubes without added nutrients, it was concluded that no additional nutrients were diffusing across the plasticized PVC film to provide for the growth of any additional species. Therefore, the film was impermeable to the aqueous 5% dextrose solution.

Ability of Fungi to Utilize Components of the PVC Film

Another study was done to determine the ability of the fungi to utilize the components of the polyvinyl chloride film as possible carbon sources. The film components (Figure 5, page 45) were incorporated into mineral salts agar (MSA) and the fungi were allowed to grow for up to four weeks (Tables 17 and 18). All of

Table 16. Mean Growth Level of Fungi on PVC Film in Parabiotic Chambers After 6 Weeks of Incubation at 30°C

Fungi	Growth Level[a]
A. fischeri	3.0
A. niger	0.5
Cladosporium sp.	0.0
Fusarium sp.	1.0
Paecilomyces sp.	3.5
Penicillium sp.	0.0
P. citrinum	1.5
Trichoderma sp.	0.0
Verticillium sp.	2.0

[a]0 = no growth; 1 = > 25% covered; 2 = 25-50% covered; 3 = 50-75% covered; 4 = > 75% covered.

Table 17. Ability of Fungi to Utilize Components of PVC Film as Carbon Sources (2 Weeks at 30°C)

Organism	PDA[b]	MSA[c]	Film Component[a]				
			EO	DOP	CZS	SA	PVC
	---------- Mean Colony Diameter (mm) ----------						
A. fischeri	90	0	90	0	90	90	0
A. niger	80	0	69	0	64	39	0
Cladosporium sp.	33	0	22	0	26	0	0
Fusarium sp.	72	0	90	0	90	0	0
Paecilomyces sp.	90	0	71	0	90	0	0
Penicillium sp.	90	0	21	0	0	0	0
P. citrinum	90	0	65	0	0	0	0
Trichoderma sp.	90	0	90	0	90	0	0
Verticillium sp.	11	0	48	0	0	0	0

[a]EO = Epoxidized Oil; DOP = Dioctyl Phthalate; CZS = Ca/Zn Stearate SA = Stearamide; PVC = PVC Resin.

[b]PDA = Potato Dextrose Agar.

[c]MSA = Mineral Salts Agar.

Table 18. Ability of Fungi to Utilize Components of PVC Film as Carbon Sources (4 Weeks at 30°C)

Organism	PDA[b]	MSA[c]	Film Component[a]				
			EO	DOP	CZS	SA	PVC
	Mean Colony Diameter (mm)						
A. fischeri	90	0	90	0	90	90	0
A. niger	90	0	85	0	81	77	0
Cladosporium sp.	69	0	32	0	55	0	0
Fusarium sp.	90	0	90	0	90	0	0
Paecilomyces sp.	90	0	90	0	90	0	0
Penicillium sp.	90	0	74	0	0	0	0
P. citrinum	90	0	90	0	0	0	0
Trichoderma sp.	90	0	90	0	90	0	0
Verticillium sp.	25	0	73	0	0	0	0

[a]EO = Epoxidized Oil; DOP = Dioctyl Phthalate; CZS = Ca/Zn Stearate SA = Stearamide; PVC = PVC Resin.

[b]PDA = Potato Dextrose Agar.

[c]MSA = Mineral Salts Agar.

the fungal species grew on potato dextrose agar (PDA), which served as the viability control. Mineral salts agar containing only 0.1% $(NH_4)NO_3$ as a nitrogen source was used as the negative control, and was found not to support fungal growth. With epoxidized oil as a carbon source, all of the fungal species showed growth within two weeks of incubation. Three species, A. fischeri, Fusarium and Trichoderma, grew to the maximum 90 mm within the 2 week incubation time. After 4 weeks, Paecilomyces sp. and P. citrinum had also reached 90 mm in diameter. Verticillium sp. was actually shown to grow to a greater extent on the MSA plus epoxidized oil than on the PDA. These results indicated that all the species were able to efficiently utilize the epoxidized oil as a carbon source.

With the exception of Penicillium sp., P. citrinum, and Verticillium sp., all of the organisms were also able to utilize the calcium-zinc stearate as a carbon source within the four week incubation period (Tables 17 and 18). Again A. fischeri, Fusarium and Paecilomyces along with Trichoderma sp. grew to the maximum 90 mm in a 2 week period. The three species which could not grow in the presence of the stearate were apparently not able to enzymatically adapt and utilize the component for carbon even after 28 days of incubation. The presence of the calcium or zinc could also have influenced the growth of the fungi.

Only Aspergillus fischeri and A. niger were shown to be able to utilize the stearamide component of the PVC film as a carbon source.

None of the other species showed growth in the presence of this component after four weeks of incubation.

The plasticizer, dioctyl phthalate, and the polyvinyl chloride resin were found not to serve as carbon sources for any of the fungal species tested. These results are in agreement with previous studies on susceptibility of PVC film components (2,11,12,18,62,108).

A comparison of the growth of the fungi on all the PVC film components showed that A. fischeri grew to a greater extent ($P<0.05$) than the other organisms with a mean colony diameter of 49.3 mm (Table 19). Cladosporium sp. and Verticillium sp. were found to grow significantly less than the other fungi tested with mean colony diameters of 15.4 and 10.4 mm, respectively. In comparing the susceptibility of the PVC components, epoxidized oil was found to be utilized to a significantly greater extent than the calcium-zinc stearate or the stearamide (Table 20).

Growth of A. niger, A. fischeri, and Cladosporium sp. on the PVC film components over time is presented in Figures 10, 11, and 12, respectively. With A. niger, an initial lag period was apparent when the organism was grown with epoxidized oil and the stearamide (Figure 10). After 28 days, however, all the plates with the film components were nearly equivalent to the PDA control. Aspergillus fischeri did not demonstrate any appreciable lag plase with any of the film components (Figure 11). After only 14 days, all of the treatments were equal to the PDA control. Cladosporium sp. was found to grow at a slower rate than the two Aspergillus species, even on

Table 19. Mean Colony Growth of Various Fungi on The PVC Film Components in Mineral Salts Agar for 4 Weeks at 30°C

Organism	Colony Diameter (mm)
A. fischeri	49.30[a]
A. niger	39.62[b]
Trichoderma sp.	38.57[b]
Fusarium sp.	35.60[c]
Paecilomyces sp.	35.41[c]
P. citrinum	22.25[d]
Penicillium sp.	18.21[e]
Cladosporium sp.	15.42[f]
Verticillium sp.	10.40[g]

[a-g]Means with different letters are significantly different.

Table 20. Susceptibility of the PVC Components to Fungal Growth in a Mineral Salts Agar at 30°C for 4 Weeks

Film Component	Colony Diameter (mm)
Potato Dextrose Agar[a]	73.95[c]
Epoxidized oil	66.71[d]
Calcium/Zinc Stearate	50.02[e]
Stearamide	15.25[f]
Dioctyl Phthalate	0.00[g]
PVC Resin	0.00[g]
Mineral Salts Agar[b]	0.00[g]

[a]PDA = Viability Control.

[b]MSA = Negative Control.

[c-g]Means with different letters are significantly different.

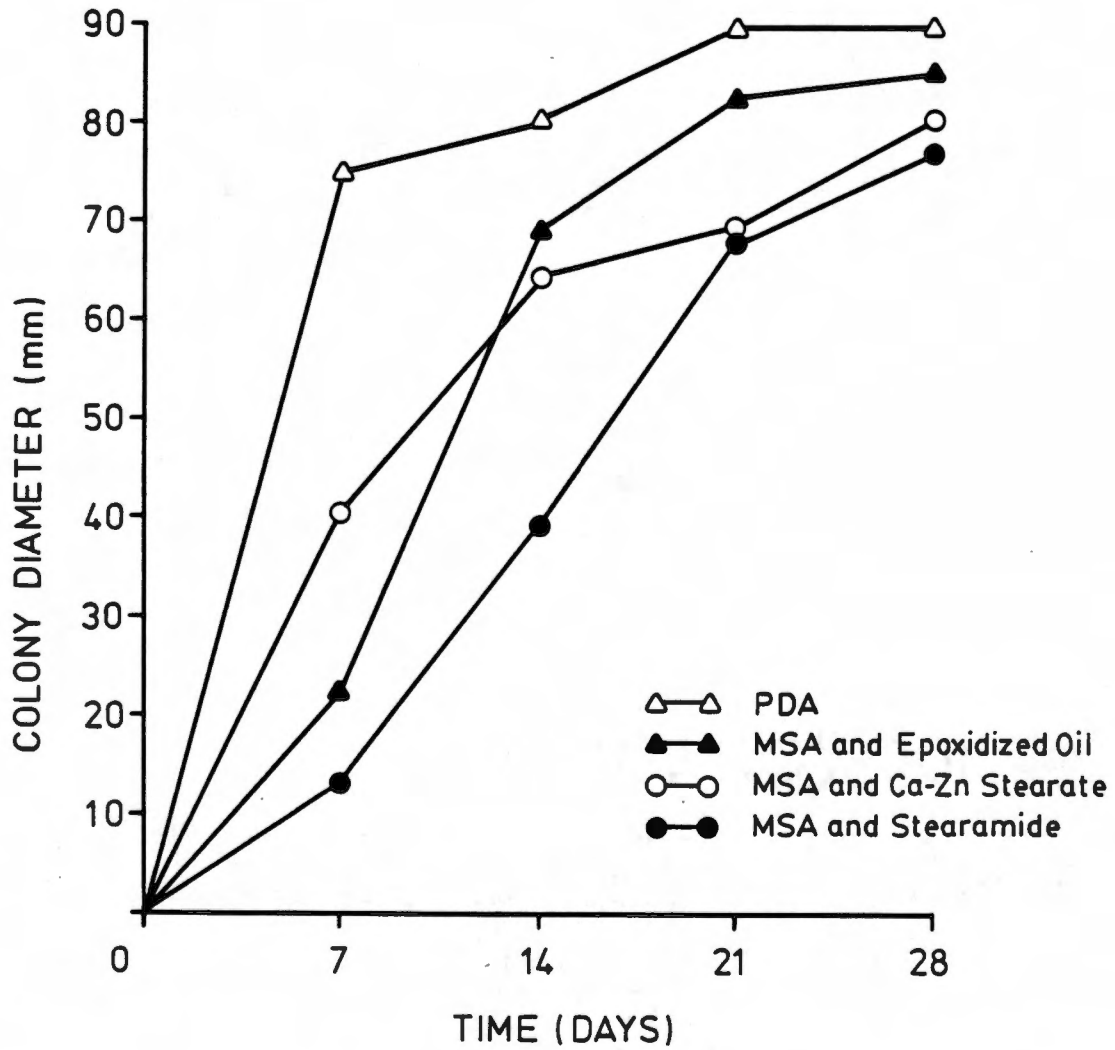


Figure 10. Growth of Aspergillus niger on potato dextrose agar (PDA) or mineral salts agar (MSA) containing 0.5% of each film component as a carbon source.

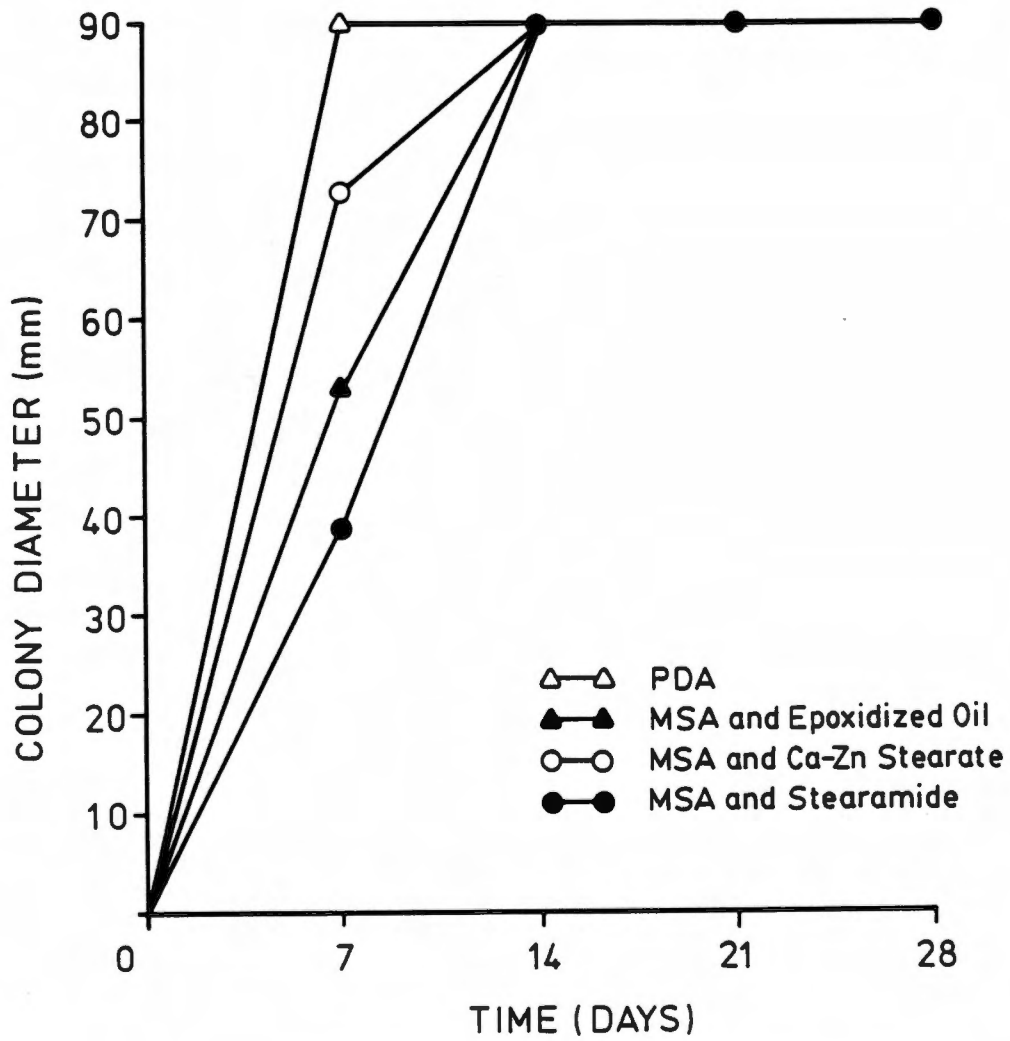


Figure 11. Growth of Aspergillus fischeri on potato dextrose agar (PDA) or mineral salts agar (MSA) containing 0.5% of each film component as a carbon source.

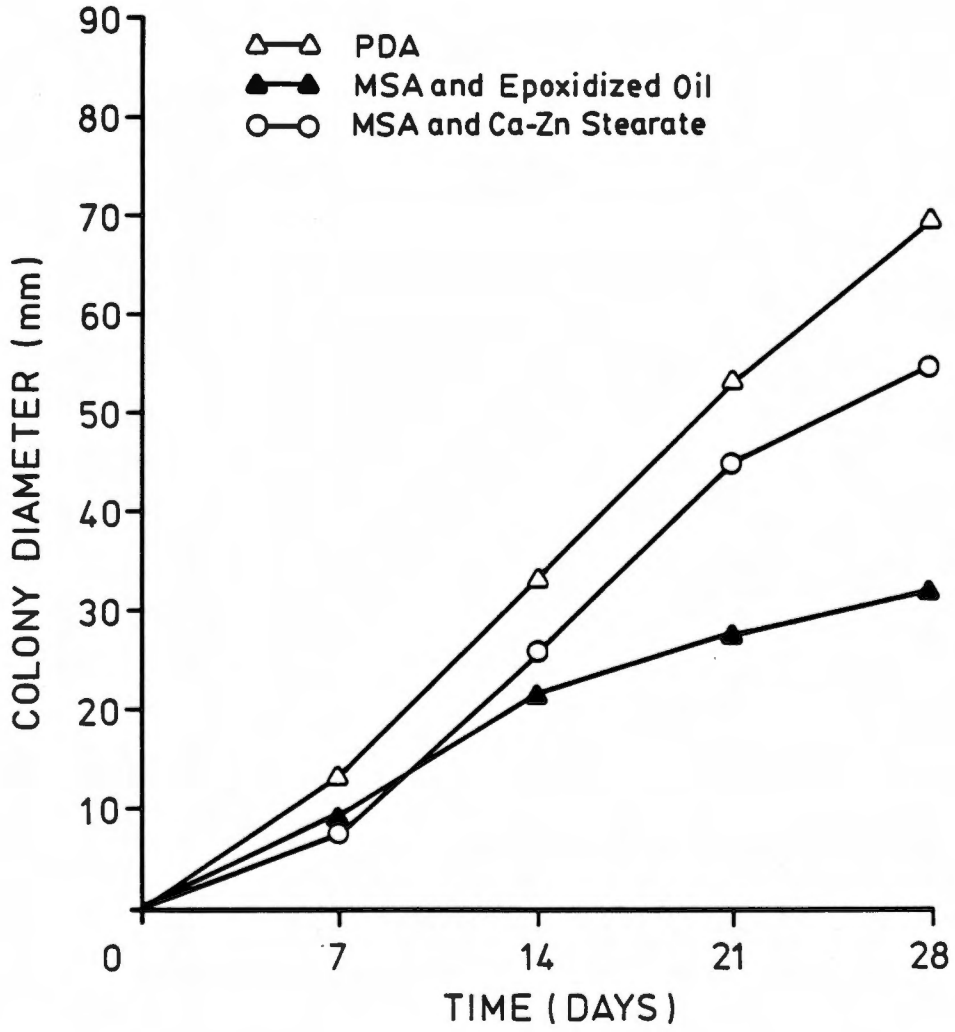


Figure 12. Growth of Cladosporium sp. on potato dextrose agar (PDA) or mineral salts agar (MSA) containing 0.5% of each film component as a carbon source.

the PDA control (Figure 12). This organism appeared to utilize the stearate at a rate similar to the control, but was somewhat slower in metabolizing the epoxidized oil.

As was stated previously, the only film components which contained nitrogen were the epoxidized oil and the stearamide (Table 6, page 47). Therefore, to determine whether the fungi could use the film components as nitrogen sources, 0.5% (w/v) of epoxidized oil or stearamide was incorporated into MSA containing 0.5% (w/v) dextrose as a carbon source. Results indicated that the fungi were not able to utilize either component of the film as a nitrogen source since only slight growth occurred on the control and test plates. Growth of fungi on the PVC film in test tubes strongly suggested that the film could be utilized as a nitrogen source. While these components were not found to be individually utilized for nitrogen in the ingredient study, there still exists the possibility that they may be an available source of nitrogen in the whole film. Brown (18) reported that combinations of plasticizers with small quantities of lubricant tended to be more susceptible as a nutrient source than either one alone. Nitrogen could also have been available from other unknown sources such as water, contaminants in the medium ingredients, diffusion from the air, or a combination of these.

2. INHIBITION OF FUNGI ON PVC FILM

Inhibition of Fungi In Vitro

The *in vitro* inhibitory effectiveness of the chemical preservatives, as measured by the minimum inhibitory concentration (MIC), was found to be very good for butylated hydroxyanisole (BHA), methyl and propyl parabens (Table 21). The BHA was effective in controlling the growth of the species tested at a level of 100-225 ug/ml, while the range for methyl and propyl parabens was 375-725 ug/ml and 100-225 ug/ml, respectively. These results are in agreement with other studies on the antifungal activity of these compounds (1,3, 26,33,56,109). The potassium sorbate was found not to be effective as an antifungal agent under the conditions of this test. Only *Verticillium* sp. and *Cladosporium* sp. were shown to be inhibited by potassium sorbate at concentrations of 1,775 and 1,850 ug/ml, respectively. The reason for the ineffectiveness of potassium sorbate may be due to the fact that the activity of this compound, along with many other antimicrobial agents, is highly dependent upon the degree of dissociation of the acid. Therefore, potassium sorbate is only effective at low pH's (6.0-6.5 or lower). Because the pH of the yeast extract-sucrose broth used in the present study was 6.4, potassium sorbate was not found to be effective in preventing fungal growth.

The most resistant species tested in the antimicrobial study were *A. niger*, *A. fischeri* and *Paecilomyces* sp. In contrast,

Table 21. Mean Minimum Inhibitory Concentrations (MIC) of Selected Antimicrobials Against Various Fungi in Yeast Extract Sucrose Broth

Fungi	BHA	Propyl Paraben	Methyl Paraben	Potassium Sorbate[a]
	MIC (ug/ml)			
A. fischeri	225	200	638	>2000
A. niger	213	225	725	>2000
Cladosporium sp.	100	100	388	1775
Fusarium sp.	150	175	450	>2000
Paecilomyces sp.	150	225	663	>2000
Penicillium sp.	150	200	525	>2000
P. citrinum	138	125	400	>2000
Verticillium sp.	150	100	375	1850

[a]Tested in concentrations as high as 2000 ug/ml.

Verticillium sp. and Cladosporium sp. were the most susceptible (Table 22).

The minimum lethal concentrations (MLC) of the antifungal agents used are presented in Table 23. These values are those that will actually destroy the mold spores. As with the MIC, the most resistant to the effects of BHA were the Aspergillus species with MLC's ranging from 375-475 ug/ml. The most susceptible species to BHA was Fusarium, which demonstrated both inhibition and lethality at 150 ug/ml. Propyl paraben and methyl paraben showed similar results for both the MIC and MLC studies. An MLC was not determined for potassium sorbate since little or no inhibition was achieved even in the MIC study.

Application of Preservatives to Film

The concentrations of the antimicrobials found to inhibit the in vitro growth of the fungi were found not be be effective when applied to the surface of the film. Therefore, a series of tests was run with increased concentrations of the antimicrobials applied to the film. The two species tested were chosen because they had shown high (Paecilomyces), or low (Fusarium) resistance in the in vitro testing. Fusarium growth on heated PVC film treated with antimicrobials was only slightly inhibited with BHA spread at 2,000 ug/ml (0.2%), whereas potassium sorbate at 4,000 ug/ml (0.4%) was totally inhibitory (Table 24). Propyl paraben was inhibitory at 2,000 ug/ml when no nutrient was applied, but only slightly inhibitory when

Table 22. Overall Minimum Inhibitory Concentration (MIC) of Antimicrobials (Butylated Hydroxyanisole, Methyl Paraben, Propyl Paraben and Potassium Sorbate) Necessary to Inhibit the Growth of Various Fungi for 5 Days at 30°C

Organism	MIC (ug/ml)
A. niger	790.63[a]
A. fischeri	765.63[a]
Paecilomyces sp.	759.38[ab]
Penicillium sp.	718.75[bc]
Fusarium sp.	693.75[cd]
P. citrinum	665.63[d]
Verticillium sp.	618.75[e]
Cladosporium sp.	590.63[e]

[a-e]Means with different letters are significantly different.

Table 23. Mean Minimum Lethal Concentrations (MLC) of Selected Antimicrobials Against Various Fungi in Yeast Extract Sucrose Broth

Fungi	BHA	Propyl Paraben	Methyl Paraben	Potassium Sorbate[a]
	MLC (ug/ml)			
A. fischeri	375	350	1425	nd
A. niger	475	375	1275	nd
Cladosporium sp.	150	125	675	nd
Fusarium sp.	150	250	725	nd
Paecilomyces sp.	200	300	>1500	nd
Penicillium sp.	375	500	>1500	nd
P. citrinum	175	263	888	nd
Verticillium sp.	175	150	650	nd

[a]nd = Not Determined.

Table 24. Mean Growth Levels of Fusarium sp. on Heated PVC Film Treated (Spread) with Selected Antimicrobials (Incubated 6 weeks at 30°C)

Conc (ug/ml)	Dextrose[a]		Dextrose Am. Sulfate[b]		No Nutrient	
	Film	Cont[c]	Film	Cont	Film	Cont
	Growth Level[d]					
Butylated Hydroxyanisole						
250	3.0	3.0	2.5	3.5	2.0	2.5
500	3.0	3.0	3.0	3.5	1.5	2.0
1000	3.0	3.0	3.0	3.0	1.0	2.0
2000	2.5	3.0	2.5	3.0	1.0	1.5
Propyl Paraben						
250	3.0	3.0	3.0	3.5	1.5	2.5
500	3.0	3.0	3.0	3.5	1.5	2.0
1000	3.0	3.0	2.5	3.0	1.0	2.0
2000	2.5	3.0	2.0	3.0	0.0	1.5
Methyl Paraben						
750	3.0	3.0	3.0	3.5	2.0	2.5
1500	3.0	3.0	3.0	3.5	2.0	2.0
2000	2.5	3.0	2.5	3.0	1.0	2.0
4000	2.5	3.0	2.0	3.0	0.0	1.5
Potassium Sorbate						
1000	3.0	3.0	3.5	3.5	2.5	3.0
2000	3.0	3.0	2.5	3.0	1.0	1.5
4000	0.0	3.0	0.0	3.0	0.0	1.5

[a] 5% Dextrose solution.

[b] 5% Dextrose + 1% ammonium sulfate solution.

[c] Control.

[d] 0 = no growth; 1 = very slight growth; 2 = slight growth; 3 = moderate growth; 4 = abundant growth.

nutrients were present. Methyl paraben showed similar results when spread at 4,000 ug/ml.

Growth of Paecilomyces sp. on film treated with antimicrobial showed that BHA was inhibitory at 2,000 ug/ml (Table 25). Potassium sorbate was inhibitory at 4,000 ug/ml with no nutrient and when 5% dextrose were supplied. Propyl paraben and methyl paraben showed similar results when spread at 2,000 and 4,000 ug/ml, respectively. With both organisms, there was no significant difference ($P>0.05$) in the dip and spread methods in applying the antimicrobials to the surface of the film (Table 26).

Due to the nonpolar nature of the PVC film, application of the antimicrobials in aqueous solutions to the film surface was a major problem. The antimicrobials could not be uniformly spread over the film surface. Wetting agents, such as Triton-X 100 and Antifoam emulsion B, were found to be ineffective in reducing the surface tension between the liquid/film interface. Based on these initial observations and the solubility of the antimicrobials in ethanol, an attempt was made to apply the preservatives to the film surface in an ethanol solution. In the dip and spread methods, the ethanol-antimicrobial solutions could be applied over the film surface. The ethanol volatilized leaving a residue of the preservative on the film surface. Comparison of the results of the effectiveness of the antimicrobials in nutrient broth and on the PVC film, revealed that much higher concentrations were needed to prevent growth on the film. Therefore, to obtain the desired antimicrobial concentration

Table 25. Mean Growth Levels of Paecilomyces sp. on Heated PVC Film Treated (Spread) with Selected Antimicrobials (Incubated 6 weeks at 30°C)

Conc (ug/ml)	Dextrose[a]		Dextrose Am. Sulfate[b]		No Nutrient	
	Film	Cont[c]	Film	Cont	Film	Cont
	Growth Level[d]					
Butylated Hydroxyanisole						
250	1.5	2.0	1.5	2.5	1.0	1.5
500	1.0	1.5	1.0	2.5	0.5	1.0
1000	0.5	1.5	0.5	3.0	0.0	1.0
2000	0.0	1.5	0.0	3.0	0.0	1.0
Propyl Paraben						
250	1.5	2.0	2.0	2.5	1.0	1.5
500	1.5	1.5	1.5	2.5	0.5	1.0
1000	1.0	1.5	2.0	3.0	0.0	1.0
2000	0.0	1.5	0.5	3.0	0.0	1.0
Methyl Paraben						
750	1.5	2.0	1.5	2.5	0.5	1.5
1500	1.0	1.5	1.5	2.5	0.0	1.0
2000	0.5	1.5	1.0	3.0	0.0	1.0
4000	0.0	1.5	0.5	3.0	0.0	1.0
Potassium Sorbate						
1000	2.0	2.0	2.5	3.0	1.5	1.5
2000	1.0	1.5	3.0	3.0	0.5	1.0
4000	0.0	1.5	2.0	3.0	0.0	1.0

[a] 5% Dextrose solution.

[b] 5% Dextrose + 1% ammonium sulfate solution.

[c] Control.

[d] 0 = no growth; 1 = very slight growth; 2 = slight growth; 3 = moderate growth; 4 = abundant growth.

Table 26. Analysis of Variance for Growth of Fungi on PVC Film Treated With Antimicrobials

Source	df	MS	F-value
Replication (R)	1	0.01	0.03^{ns}
Organism (O)	1	8.71	26.32*
Antimicrobial (A)	3	0.16	0.48^{ns}
Application (AP)	1	0.01	0.03^{ns}
Concentration (C)	6	5.95	17.98*
Nutrient (N)	2	7.91	23.90*
O x A	3	5.71	15.62*
O X AP	1	0.04	0.12^{ns}
O X C	6	1.78	5.38*
O X N	2	5.23	15.80*
Error	333	0.33	

$^{*}P<0.05$.

$^{ns}P>0.05$.

on the film surface, an adhesive agent may be desirable. An adhesive agent may ensure a uniform dispersion of the antimicrobial on the film surface and may prevent the antimicrobial from leaching from the film when in contact with water.

The antimicrobial level found to inhibit growth on a film surface must be present throughout the service-life of the film in order to prevent undesirable surface growth. Partial or complete removal of the antimicrobial may allow for possible fungal growth.

An alternative method for applying an antimicrobial to a PVC film would be to incorporate the preservative into the plasticizer of the film during formulation. Levels found to inhibit growth on the surface may or may not be as effective when incorporating into the film. Therefore, additional tests would be required to determine inhibition.

CHAPTER V

CONCLUSIONS

The first objective of this study was to determine growth characteristics of fungi on the polyvinyl chloride film. Growth studies indicated that Aspergillus fischeri and Paecilomyces sp. could utilize the film as a carbon and nitrogen source. Most of the other fungi tested grew on the film when supplied either 5% (w/v) dextrose, as a carbon source, or 1% (w/v) ammonium sulfate, as a nitrogen source. Epoxidized oil and Ca-Zn stearate were found to be utilized by most fungi as carbon sources, whereas the stearamide was not found to be utilized as a nitrogen source. To be available for the growth of the fungi, nitrogen may possibly have to be supplied through water, air, the stearamide in the heated plasticized PVC film or a combination of the three.

The second objective of the study was to test chemical methods for eliminating growth of fungi on the PVC film. Interestingly, the fungi which were found to be the most versatile at growing on the film, such as Aspergillus fischeri and Paecilomyces, also were generally the most resistant to the antimicrobials tested. The more hydrophobic antimicrobials, including BHA and methyl and propyl paraben, were most effective in the in vitro inhibition of the mold species. While potassium sorbate was not effective in inhibiting growth in vitro, it was found to be an effective preservative when applied to the film. At 4,000 ug/ml (0.4%) the growth of Paecilomyces

sp. was partially inhibited while Fusarium sp. growth was totally inhibited with and without added nutrients. BHA was also found to be an effective antimicrobial when applied to the film. At 2,000 ug/ml (0.2%), BHA was found to totally inhibit growth of Paecilomyces sp. and partially inhibit growth of Fusarium sp.

From the results of this study polyvinyl chloride films are susceptible to attack by microorganisms. The degree of susceptibility will depend upon the composition of the film, the type of mirco-organisms present, and the environmental conditions. Polyvinyl chloride films are used for packaging foods such as fresh meats and produce. In meat packaging, PVC films have several desirable characteristics such as abundant O_2 permeability, excellent elastic properties, outstanding optics and good sealability (53), but the major function of the film is to protect the food from microbial attack. Therefore, it is important to consider the possible bio-deterioration of the packaging material itself. There is a possibility that the natural microflora of shelf-stable foods packaged in PVC films may be capable of utilizing not only the food product, but also the film used to package the food. Migration of susceptible film components in contact with the food could also provide additional nutrients for growth of microorganisms. Growth on the surface of a PVC film could also result in contamination of the product. These factors should be considered when using PVC films for packaging food.

Incorporation of antimicrobials into PVC films would increase overall resistance to microbial attack. The amount applied will depend upon the potency of the antimicrobial, the susceptibility of the film, and the microorganisms encountered.

LITERATURE CITED

LITERATURE CITED

1. Aalto, T.R., M.C. Firman, and N.E. Rigler. 1953. p-Hydroxybenzoic acid esters as preservatives. I. Uses, antibacterial and antifungal studies, properties and determination. J. Am. Pharm. Assoc. 42:449-456.

2. Abrams, E. 1948. The microbiological deterioration of organic materials: Its prevention and methods of test. Nat. Bur. Stds. Misc. Publ. 188.

3. Ahmad, S., and A.L. Branen. 1981. Inhibition of mold growth by butylated hydroxyanisole. J. Food Sci. 46:1059-1063.

4. A.O.A.C. 1980. Official Methods of Analysis, 13th ed. Association of Official Analytical Chemists, Washington, D.C.

5. A.S.T.M. 1982. Standard practice for determining resistance of synthetic polymeric materials to fungi, p. 1153-1157. In American Society for Testing Materials Standards, Part 35. American Society for Testing Materials, Philadelphia.

6. Ayaz, M., L.D. Luedecke, and A.L. Branen. 1980. Antimicrobial effect of butylated hydroxyanisole and butylated hydroxy-toluene on Staphylococcus aureus. J. Food Prot. 43:4-6.

7. Bandelin, F.J. 1958. The effect of pH on the efficiency of various mold inhibiting compounds. J. Am. Pharm. Assoc., Sci. Ed. 47:691-694.

8. Baribo, L.E., J.S. Avens, and R.D. O'Neill. 1966. Effect of electrostatic charge on the contamination of plastic food containers by airborne bacterial spores. Appl. Microbiol. 14:905-913.

9. Bejuki, W.M. 1966. Microbiological degradation, p. 716-725. In Encyclopedia of Polymer Science and Technology, vol. 4. Interscience Publ., New York.

10. Bell, T.A., J.L. Etchells, and A.F Borg. 1959. Influence of sorbic acid on the growth of certain species of bacteria, yeasts, and filamentous fungi. J. Bacteriol. 77:573-580.

11. Berk, S. 1950. Effect of fungus growth on the tensile strength of polyvinyl chloride films plasticized with three plasticizers. ASTM Bull. 168:53-55.

12. Berk, S.B., H. Ebert, and L. Teitell. 1957. Utilization of plasticizers and related organic compounds by fungi. Ind. Eng. Chem. 49:1115-1124.

13. Beuchat, L.R., and W.K. Jones. 1978. Effects of food preservatives and antioxidants on colony formation by heated conidia of Aspergillus flavus. Acta Aliment. Acad. Sci. Hung. 7:373-384.

14. Block, S.S. 1953. Humidity requirements for mold growth. Appl. Microbiol. 1:287-293.

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

16. Booth, G.H., and J.A. Robb. 1968. Bacterial degradation of plasticized PVC - Effect on some physical properties. J. Appl. Chem. 18:194-197.

17. Branen, A.L., P.M. Davidson, and B. Katz. 1980. Antimicrobial properties of phenolic antioxidants and lipids. Food Technol. 34:42-53.

18. Brown, A.E. 1946. The problem of fungal growth on synthetic resins, plastics, and plasticizers. Mod. Plastics 23:189-195.

19. Bullerman, L.B. 1983. Effects of potassium sorbate on growth and aflatoxin production by Aspergillus parasiticus and Aspergillus flavus. J. Food Prot. 46:940-942.

20. Buono, F.J., and G.A. Trautenberg. 1976. Biocides, p. 95-115. In Encyclopedia of Polymer Science and Technology, vol. 1. Interscience Publ., New York.

21. Burgess, R., and A.E. Darby. 1964. Two tests for the assessment of microbial activity on plastics. Brit. Plast. 37:32-37.

22. Burgess, R., and A.E. Darby. 1965. Microbiological testing of plastics. Brit. Plast. 38:165-169.

23. Cadmus, E.L. 1978. Biocides, p. 219-231. In R.B. Seymour (ed.), Additives For Plastics: State of The Art, vol. 1. Academic Press, Inc., New York.

24. Cavett, J.J., and M.N. Woodrow. 1968. A rapid method for determining degradation of plasticized PVC by micro-organisms, p. 162-169. In A.H. Walters and J.J. Elphick (ed.), Biodeterioration of Materials: Microbiological and Allied Aspects. Elsevier Publ. Co., New York.

25. Chang, H.C., and A.L. Branen. 1975. Antimicrobial effects of butylated hydroxyanisole (BHA). J. Food Sci. 40:349-351.

26. Chichester, D.F., and F.W. Tanner. 1972. Antimicrobial food additives, p. 115-184. In T.E. Furia (ed.), Handbook of Food Additives, 2nd ed. CRC Press, Cleveland, Ohio.

27. Coscarelli, W. 1972. Biological stability of polymers, p. 377-399. In W.L. Hawkins (ed.), Polymer Stabilization. John Wiley and Sons, Inc., New York.

28. Dalhberg, K.R., and J.L. Van Etten. 1982. Physiology and biochemistry of fungal sporulation. Ann. Rev. Phytopathol. 20:281-301.

29. Daniels, R. 1973. Edible coatings and soluble packaging. Noyes Data Corporation, Park Ridge, NJ.

30. Davidson, P.M. 1983. Phenolic compounds, p. 37-74. In A.L. Branen and P.M. Davidson (ed.), Antimicrobials in Foods. Marcel Dekker, Inc., New York.

31. Davidson, P.M., and A.L. Branen. 1980. Antimicrobial mechanisms of butylated hydroxyanisole against two Pseudomonas species. J. Food Sci. 45:1607-1613.

32. Davidson, P.M., and A.L. Branen. 1980. Inhibition of two psychrotrophic Pseudomonas species by butylated hydroxyanisole. J. Food Sci. 45:1603-1606.

33. Davidson, P.M., and A.L. Branen. 1981. Antimicrobial activity of non-halogenated phenolic compounds. J. Food Prot. 44:623-632.

34. Davidson, P.M., C.J. Brekke, and A.L. Branen. 1981. Antimicrobial activity of butylated hydroxyanisole, tertiarybutylhydroquinone, and potassium sorbate in combination. J. Food Sci. 46:314-316.

35. Degre', R., and M. Sylvestre. 1983. Effect of butylated hydroxyanisole on the cytoplasmic membrane of Staphylococcus aureus Wood 46. J. Food Prot. 46:206-209.

36. Deuel, H.J., Jr., R. Alfin-Slater, C.S. Weil, and H.F. Smyth, Jr. 1954. Sorbic acid as a fungistatic agent for foods. I. Harmlessness of sorbic acid as a dietary component. Food Res. 19:1-12.

37. DiBenedetto, J.L. 1978. Antimicrobials. Plastics Eng. 34:26.

38. Eisenschiml, R., and W.H. Bauer. 1968. Microbial degradation of plasticized vinyl films. Advan. Chem. Ser. 85:250-271.

39. Eubanks, V.L., and L.R. Beuchat. 1982. Effects of antioxidants on growth, sporulation and pseudomycelium production by Saccharomyces cerevisiae J. Food Sci. 47:1717-1722.

40. Finol, M.L., E.H. Marth, and R.C. Lindsay. 1982. Depletion of sorbate from different media during growth of Penicillium species. J. Food Prot. 45:398-404.

41. Freese, E., C.W. Sheu, and E. Galliers. 1973. Function of lipophilic acids as antimicrobial food additives. Nature 241:321-325.

42. Fung, D.Y.C., S. Taylor, and J. Kahan. 1977. Effects of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on growth and aflatoxin production of Aspergillus flavus. J. Food Safety 1:39-51.

43. Furr, J.R., and A.D. Russell. 1972. Some factors influencing the activity of esters of p-hydroxybenzoic acid against Serratia marcescens. Microbios 5:189-198.

44. Gerber, N.N., and D.P. Stahly. 1975. Prodiginine (Prodigiosin-like) pigments from Streptoverticillium rubrireticulli, an organism that causes pink staining of polyvinyl chloride. Appl. Microbiol. 30:807-810.

45. Ghosh, K.G., A.N. Srivatsava, N. Nirmala, and T.R. Sharma. 1973. Development and application of fungistatic wrappers in food preservation. Part I. Wrappers obtained by impregnation method. J. Food Sci. Technol. 10:105-110.

46. Ghosh, K.G., A.N. Srivatsava, N. Nirmala, and T.R. Sharma. 1977. Development and application of fungistatic wrappers in food preservation. Part II. Wrappers made by coating process. J. Food Sci. Technol. 14:261-264.

47. Girard, T.A., and C.F. Koda. 1959. Pink discoloration of vinyls. Mod. Plastics 36:148.

48. Griffin, D.H. 1981. Fungal Physiology. John Wiley and Sons, Inc., New York.

49. Hartman, M.W., J.J. Powers, and D.E. Pratt. 1963. Bacterial permeability of selected food packaging films. Food Technol. 17:1172-1174.

50. Hunter, D.R., and I.H. Segel. 1973. Effect of weak acids on amino acid transport by Penicillium chrysogenum: Evidence for a proton or charge gradient as the driving force. J. Bacteriol. 113:1184-1192.

51. Huppert, M. 1957. The antifungal activity of a homologous series of parabens. Antibiot. Chemotherapy 7:29-36.

52. Isquith, A.J., E.A. Abbott, and P.A. Walters. 1972. Surface-bonded antimicrobial activity of an organosilicon quaternary ammonium chloride. Appl. Microbiol. 24:859-863.

53. James, D.G. 1984. Films, polyvinyl chloride, p. 74-75. In The 1984 Packaging Encyclopedia. Cahners Publ. Co., Chicago.

54. Johnson, J.C. 1975. Antioxidants: Syntheses and Applications. Noyes Data Corporation, Park Ridge, NJ.

55. Judis, J. 1963. Studies on the mechanism of action of phenolic disinfectants. II. Patterns of release of radioactivity from Escherichia coli labeled by growth on various compounds. J. Pharm. Sci. 52:125-131.

56. Jurd, L., A.D. King, K. Mihara, and W.L. Stanley. 1971. Antimicrobial properties of natural phenols and related compounds. I. Obtusastyrene. Appl. Microbiol. 21:507-510.

57. Kaplan, A.M., M. Greenberger, and T.M. Wendt. 1970. Evaluation of biocides for treatment of polyvinyl chloride film. Polymer Eng. Sci. 10:241-246.

58. Kaye, R.C., and S.G. Proudfoot. 1971. Interactions between phosphatidylethanolamine monolayers and phenols in relation to antimicrobial activity. J. Pharm. Pharmacol. Suppl. 23:223.

59. Kelen, T. 1983. Polymer Degradation. Van Nostrand Reinhold Company, Inc., New York.

60. Klausmeier, R.E. 1968. Deterioration of vinyl resin systems - The current plasticizer deterioration study. Develop. Ind. Microbiol. 9:218-221.

61. Klausmeier, R.E., and C.C. Andrews. 1981. Plastics, p. 431-474. In A.H. Rose (ed.), Microbial Biodeterioration: Economic Microbiology, vol. 6. Academic Press, Inc., New York.

62. Klausmeier, R.E., and W.A. Jones. 1961. Microbial degradation of plasticizers. Develop. Ind. Microbiol. 2:47-53.

63. Klindworth, K.J., P.M. Davidson, C.J. Brekke, and A.L. Branen. 1979. Inhibition of Clostridium perfringens by butylated hydroxyanisole. J. Food Sci. 44:564-567.

64. Kroll, R.G., and G.D. Anagnostopoulos. 1981. Potassium leakage as a lethality index of phenol and the effect of solute and water activity. J. Appl. Bacteriol. 50:139-147.

65. Lasman, H.R., and J.P. Scullin. 1977. Miscellaneous modifying additives, p. 801-845. In L.I. Nass (ed.), Encyclopedia of PVC, vol. 2. Marcel Dekker, Inc., New York.

66. Leutritz, J. Jr. 1948. The effect of fungi and humidity on plastics. ASTM Bull. 152:88-90.

67. Lueck, E. 1976. Sorbic acid as a food preservative. Int. Flavors Food Addit. 7:122-125.

68. Lueck, E. 1980. Antimicrobial Food Additives, edited by G.F. Edwards. Springer-Verlag, Inc., New York.

69. Marth, E.H., C.M. Capp, L. Hasenzahl, H.W. Jackson, and R.V. Hussong. 1966. Degradation of potassium sorbate by Penicillium species. J. Dairy Sci. 49:1197-1205.

70. Melnick, D., F.H. Luckmann, and C.M. Gooding. 1954. Sorbic acid as a fungistatic agent for foods. VI. Metabolic degradation of sorbic acid in cheese by molds and the mechanism of mold inhibition. Food Res. 19:44-58.

71. Miles Laboratories, Inc., Citro-tech Products. 1982. Potassium sorbate, F.C.C., N.F. Elkhart, IN.

72. Mislivec, P.B. 1979. Specific requirements and physical factors affecting fungal growth and multiplication, p. 20-28. In M.E. Rhodes (ed.), Food Mycology. G.K. Hall and Co., Boston.

73. Mislivec, P.B., C.T. Dieter, and V.R. Bruce. 1975. Effect of temperature and relative humidity on spore germination of mycotoxic species of Aspergillus and Penicillium. Mycologia 67:1187-1189.

74. Monsanto 1978. Sorbic acid and potassium sorbate: For preserving food freshness and market quality. Monsanto Chemical Co., St. Louis, MO.

75. Nowak, M. 1967. Fungicides, p. 245-247. In H.R. Simmons and J.M. Church (ed.), Encyclopedia of Basic Materials for Plastics. Reinhold Publ. Corp., New York.

76. Oka, S. 1960. Studies on the transfer of antiseptics to microbes and their toxic effect. Part III. Adsorption of esters of p-hydroxybenzoic acid on yeast cell and their toxic effect. Bull. Agri. Chem. Soc. Japan 24:412-417.

77. Osman, J.L., R.E. Klausmeier, and E.I. Jamison. 1970. The ability of selected yeast cultures to degrade plasticized polyvinyl systems. Develop. Ind. Microbiol. 11:447-452.

78. Pelczar, M.J., Jr., R.D. Reid, and E.C.S. Chan. 1977. Microbiology, 4th ed. McGraw-Hill, Inc., New York.

79. Prindle, R.F., and E.S. Wright. 1977. Phenolic compounds, p. 219-251. In S.S. Block (ed.), Disinfection, Sterilization, and Preservation. Lea and Febiger, Philadelphia.

80. Przybylski, K.S., and L.B. Bullerman. 1980. Influence of sorbic acid on viability and ATP content of conidia of Aspergillus parasiticus. J. Food Sci. 45:375-376.

81. Ray, L.R., and L.B. Bullerman. 1982. Preventing growth of potentially toxic molds using antifungal agents. J. Food Prot. 45:953-963.

82. Reese, E.T., H. Cravetz, and G.R. Mandels. 1955. Activity of fungi on oils. Fawlowia 4:409-421.

83. Rico-Munoz, E., and P.M. Davidson. 1983. Effect of corn oil and casein on the antimicrobial activity of phenolic antioxidants. J. Food Sci. 48:1284-1288.

84. Robach, M.C. 1980. Use of preservatives to control microorganisms in food. Food Technol. 34:81-84.

85. Robach, M.C., L.A. Smoot, and M.D. Pierson. 1977. Inhibition of Vibrio parahaemolyticus 04:K11 by butylated hydroxyanisole. J. Food Prot. 40:549-551.

86. Ronsivalli, L.J., J.B. Bernsteins, and B.L. Tinker. 1966. Method for determining the bacterial permeability of plastic films. Food Technol. 20:1074-1075.

87. Rosato, D.V. 1968. Other properties and characteristics, p. 733-1102. In D.V. Rosato and R.T. Schwartz (ed.), Environmental Effects on Polymeric Materials. John Wiley and Sons, Inc., New York.

88. Sankaran, R. 1976. Comparative antimicrobial action of certain antioxidants and preservatives. J. Food Sci. Technol. 13:203-204.

89. Sarvetnick, H.A. 1969. Polyvinyl Chloride. Van Nostrand Reinhold Co., New York.

90. Sauer, F. 1977. Control of yeasts and molds with preservatives. Food Technol. 31:66-67.

91. Schnabel, W. 1981. Polymer Degradation: Principles and Practical Applications. Macmillan Publ. Co., Inc., New York.

92. Sgaragli, G., L.D. Corte, M. Rizzotti-Conti, and A. Giotti. 1977. Effects of monocyclic compounds on biomembranes. Biochem. Pharmacol. 26:2145-2149.

93. Shibaski, I. 1969. Antimicrobial activity of alkyl esters of p-hydroxybenzoic acid. J. Ferment. Technol. 47:167-177.

94. Shih, A.L., and N.D. Harris. 1977. Antimicrobial activity of selected antioxidants. J. Food Prot. 40:520-522.

95. Shih, C.N., and E.H. Marth. 1973. Aflatoxin production by Aspergillus parasiticus when incubated in the presence of different gases. J. Milk Food Technol. 36:421-425.

96. Sinell, H.J. 1980. Packaging, p. 193-204. In Microbial Ecology of Foods, vol. 1. International Commission on the Microbiological Specifications for Foods. Academic Press, Inc., New York.

97. Smith, D.P., and N.J. Rollin. 1954. Sorbic acid as a fungistatic agent for foods. VII. Effectiveness of sorbic acid in protecting cheese. Food Res. 19:59-65.

98. Smith, J.E., and D.R. Berry. 1974. An Introduction to Biochemistry of Fungal Development. Academic Press, Inc., New York.

99. Sofos, J.N., and F.F. Busta. 1981. Antimicrobial activity of sorbate. J. Food Prot. 44:614-622.

100. Sofos, J.N., and F.F. Busta. 1983. Sorbates, p. 141-175. In A.L. Branen and P.M. Davidson (ed.), Antimicrobials in Foods. Marcel Dekker, Inc., New York.

101. St. George, R.A., T.E. Snyder, W.W. Dykstra, and L.S. Henderson. 1954. Biological agents of deterioration, p. 175-233. In G.A. Greathouse and C.J. Wessel (ed.), Deterioration of Materials: Causes and Preventative Techniques. Reinhold Publ. Corp., New York.

102. Stahl, W.H., and H. Pessen. 1953. The microbiological degradation of plasticizers. I. Growth on esters and alcohols. Appl. Microbiol. 1:30-35.

103. Stahl, W.H., and H. Pessen. 1954. Funginertness of internally plasticized polymers. Mod. Plastics 31:111-112.

104. Stepek, J., and H. Daoust. 1983. Additives for Plastics. Springer-Verlag, Inc., New York.

105. Stryer, L. 1981. Biochemistry, 2nd ed. W.H. Freeman and Co., San Francisco.

106. Surak, J.G., R.L. Bradley, A.L. Branen, and E. Shrago. 1976. Effects of butylated hydroxyanisole on Tetrahymena pyriformis. Food Cosmet. Toxicol. 14:277-278.

107. Sweitser, D. 1968. The protection of plasticized PVC against microbial attack. Rubber and Plast. Age 49:426-430.

108. Tirpak, G. 1970. Microbial degradation of plasticized PVC. SPE Journal 26:26-30.

109. Tri-K Industries, Inc. No date. Technical data, esters of parahydroxybenzoic acid (parabens). Westwood, NJ.

110. Troller, J.A. 1965. Catalase inhibition as a possible mechanism of the fungistatic action of sorbic acid. Can. J. Microbiol. 11:611-617.

111. Vas, K. 1953. Mechanisms of antimicrobial action. Interference with cytoplasmic membrane. Agrokenn Talajtan 2:1-16 [Chem. Abstr. (1954) 48:794 d].

112. Whitaker, J.R. 1959. Inhibition of sulfhydryl enzymes with sorbic acid. Food Res. 24:37-44.

113. Williams, P.L., J.L. Kanzig, and R.E. Klausmeier. 1969. Evaluation and production of esterases on plasticizer substrates by fungal species. Develop. Ind. Microbiol. 10:177-182.

114. Wyatt, D.M., and E.R. Sherman. 1979. Analysis of BHA in polyethylene by direct-sampling gas chromatography. Food Technol. 33:46-49.

115. Wyss, O. 1948. Microbial inhibition of food preservatives. Adv. Food Res. 1:373-393.

116. Yeager, C.C. 1962. Pink staining in polyvinyl chloride. Plast. World 20:14-15.

117. Yeager, C.C. 1968. Deterioration of vinyl resin systems - Special considerations. Develop. Ind. Microbiol. 9:222-225.

118. Yeager, C.C. 1968. Laboratory and service tests for PVC, p. 151-161. In A.H. Walters and J.J. Elphick (ed.), Biodeterioration of Materials: Microbiological and Allied Aspects. Elsevier Publ., Co., New York.

119. York, G.K., and R.H. Vaughn. 1964. Mechanisms in the inhibition of microorganisms by sorbic acid. J. Bacteriol. 88:411-417.

APPENDIX

Table 27. Analysis of Variance for Growth of Fungi on PVC Film in Petri Plates With and Without Added Nutrient Measured as a Function of Inoculation Level and Time

Source	df	Dextrose	No Nutrient
		MS	
Replication (R)	1	1.69^{ns}	6.75^{ns}
Organism (O)	1	2.08^{ns}	6.75^{ns}
Film (F)	1	0.33^{ns}	0.02^{ns}
Inoculation Level (I)	1	1.33^{ns}	6.75^{ns}
O X F	1	0.33^{ns}	0.02^{ns}
O X I	1	24.08^{ns}	6.75^{ns}
F X I	1	0.08^{ns}	0.02^{ns}
Error A[a]	8	5.38	2.54
Time (T)	2	1.63*	0.19^{ns}
T X O	2	0.19^{ns}	0.19^{ns}
T X F	2	0.04^{ns}	0.02^{ns}
T X I	2	0.04^{ns}	0.19^{ns}
Error B	24	0.18	0.09

$^{*}P<0.05$.

$^{ns}P>0.05$.

[a] R X O; R X F; R X I; R X O X F; R X O X I; R X F X I; O X F X I; R X O X F X I.

Table 28. Analysis of Variance for Growth of Fungi on PVC Film Components Measured as a Function of Component and Time

Source	df	MS	F-value
Replication (R)	1	964.30	15.30*
Organism (O)	8	8525.39	135.30*
Component (C)	6	69258.25	1089.97*
O X C	48	2960.80	46.98*
Error A[a]	62	63.02	
Time (T)	3	3098.67	77.60*
T X O	24	111.71	2.80*
T X C	18	615.50	15.41*
Error B	270	39.93	

*P<0.05.

[a]R X O; R X C; R X O X C.

Table 29. Analysis of Variance for The Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) Against Various Fungi in Yeast Extract Sucrose Broth

Test	Source	df	MS	F-value
MIC				
	Replication (R)	1	87.89	0.05^{ns}
	Organism (O)	7	41349.05	22.70^{*}
	Antimicrobial (A)	3	11611259.76	6373.65^{*}
	O X A	21	7241.91	3.98^{*}
	Error	31	1821.76	
MLC				
	Replication (R)	1	833.33	0.23^{ns}
	Organism (O)	7	233028.27	65.13^{*}
	Antimicrobial (A)	2	3463684.90	968.08^{*}
	O X A	14	60768.30	16.98^{*}
	Error	23	3577.90	

$^{*}P<0.05$.

$^{ns}P>0.05$.

VITA

William Timothy Roberts was born on September 3, 1960 in Elizabethton, Tennessee to Mr. and Mrs. William H. Roberts. Upon graduation from Hampton High School, Hampton, Tennessee in June 1978, he entered The University of Tennessee, Knoxville and in June 1982 graduated with a Bachelor of Science degree in Agriculture with a major in Food Technology and Science.

The author enrolled in graduate school at The University of Tennessee, Knoxville in September 1982 and began work toward a Master of Science degree in Food Technology and Science. During his graduate work, he worked on a research grant supported by Travenol Laboratories, Morton Grove, Illinois and held a research assistantship under the direction of Dr. P. Michael Davidson. The author is a member of the Institute of Food Technologists and the American Society for Microbiology.