MICROSCOPIC EVALUATION OF DAMAGE BY STAPHYLOCOCCUS AUREUS ON A KNITTED NYLON-COTTON FABRIC BEFORE AND AFTER LAUNDERING

by

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INTRODUCTION

Bacteria and fungi are the two main groups of microorganisms responsible for microbial decomposition of textile materials. Under optimum conditions of moisture and temperature, textile materials are susceptible to attack by both bacteria and fungi. However, the dominance of a microorganism at any given time is determined largely by the prevailing environmental conditions.

A suitable environment for microbial growth and survival can develop at various areas on a human body. Newburgh (28) stated that under a condition of high temperature accompanied with sweating, an environment can develop that may encourage microbial growth. As reported by Riley et al. (32) in their study of men in controlled environments, two areas of the body that became uncomfortable for subjects clothed in space suits were the groin and the feet. Thus, moist areas of the human body such as the groin and the feet may be subject to irritations and infections caused by microorganisms if the proper environmental conditions prevail.

Since little is known about the growth and survival of pathogenic microorganisms such as Staphylococcus aureus in textiles used for footwear, this organism was chosen for study on a knitted nylon-cotton footwear fabric. According to McNeil (26), the ability or inability of a potentially harmful microorganism to survive and multiply in a textile material could influence foot health and serviceability of the fabric. Conversely, microbial survival could be dependent upon the textile fabric itself.

Basu and Ghose (4) indicated that previous study of microbial degradation of textile materials has been concerned with identification of organisms and their properties, but little research has been accomplished concerning the mode of attack by microorganisms or the changes brought about in the fiber structure. They explained that microorganisms may be on the surface of the fabric, may be attached to the fibers, may be held mechanically in the interstices of the fibers, or may penetrate the fibers. Ultimately, microbial action may cause damage to fibers in the fabric structure, thereby, affecting the fabric's physical properties.

Microscopic examination and analysis of photomicrographs are helpful in understanding the breakdown of fibers, yarns, and fabrics. Microscopic analysis is needed to determine the effects of microbial action on fibers and to observe the ways that damage occurs.

This study was undertaken to examine fiber breakdown resulting from microbial attack on a knitted nylon-cotton footwear fabric before and after laundering. Photomicrographs taken of fibers exposed to Staphylococcus aureus for specified holding periods at selected environmental conditions were compared. Laundering of the footwear fabric was completed to simulate normal wearing conditions and to assess the susceptability of the footwear fabric to microbial attack after laundering.

The objective of this study was: to compare, by microscopic evaluation, the type and extent of fiber damage of uninoculated specimens to specimens subjected to contact with Staphylococcus aureus for specified holding periods at selected temperatures and humidity before and after laundering.

REVIEW OF LITERATURE

Apparel fabrics are frequently exposed during wear to conditions that cause degradation of the textile material. According to Handu et al. (15) and Kaswell (19), degradations of apparel fabrics may include abrasion, stress, cuts, stretching, microbial damage, chemical damage, laundering, and weathering. Of importance to this study is microbial damage to apparel, specifically nylon-cotton footwear.

Detection of damage is necessary in the practical control and elimination of microbial deterioration of textile materials. To seek means of eliminating the threat of microbial degradation, an understanding is needed about the requirements for microbial growth and survival; the intimate functioning of the degradative organisms, notably their physiological processes and enzymatic capabilities; and methods for controlling the deteriorating effects of microbial attack on textile materials.

Requirements for Microbial Growth and Survival

Microorganisms are exceedingly numerous and are widely distributed in soil, air, water, food, and decaying organic matter; and on clothing, skin, and innate objects of the environment. However, they are so inconceivably small that they cannot be seen with the human eye without the aid of a microscope.

Microorganisms differ markedly in the type of environment and nutrients required for their growth and survival. Gershenfeld (12) and Thompson (36) listed the requirements for microbial growth and survival

as nourishment; moisture; proper temperature; an adequate supply of oxygen; a suitable acid-base balance; and the absence of direct sunlight, retarding chemicals, and antagonistic organisms.

Microorganisms are dependent primarily on animal and vegetable matter for nourishment. However, Thompson (36) noted that to be usable a food constituent must be in a form capable of entering the bacterial cell or capable of being changed into a form that could enter the cell. Carpenter (7) stated that if cells cannot use proteins, cellulose, and fats directly, the nutrients are broken down for absorption by use of enzymes through a process known as digestion. Not all cells secrete the same enzymes; hence, there may be differences in the type of foods used and the products formed.

The speed of chemical reactions within the microbial cell is determined by the environmental temperature. Lawrence (20) suggested that for each microorganism there is a maximum, minimum, and optimum temperature to maintain growth. Optimum temperature is the temperature at which a microorganism will grow most rapidly.

Moisture requirements for microbial survival differ with each species of microorganism. Saturation, or one hundred per cent relative humidity, is optimum moisture for some microorganisms, while others require less moisture. Complete drying will kill many microorganisms, especially certain pathogenic forms.

The enzymatic functioning of all living cells is sensitive to the acidity or the alkalinity of the environment. Thompson (36) revealed that most microorganisms grow well in a nutritive medium that is

approximately neutral (pH 6.5 to 7.5), but some will grow in the range of pH 4.0 to 8.5.

Microorganism: Staphylococcus aureus

As cited by Thompson (36), microorganisms are usually considered harmful or undesirable when they cause disease, spoil foods, or deteriorate fibers. Included in this classification of microorganisms is Staphylococcus aureus (Micrococcus pyogenes var. aureus). Webster (37) stated that this Gram-positive microorganism can be identified by its grape-like cluster formations and its colonies that have a distinct golden color.

There are many strains of Staphylococcus aureus, and all can be pathogenic under the proper environmental conditions. According to Gershenfeld (12), staphylococci need food, moisture of sixty per cent to one hundred per cent relative humidity, and temperature of 60° F. to 109° F. Optimum temperature for growth is 98.6° F. The lack of these conditions does not necessarily mean the death of the microorganism, because even during cold and dry environmental conditions, staphylococci can survive for months and are able to multiply rapidly when optimum temperature and moisture are present again.

The body of a human being produces a suitable environment of moisture and temperature for the growth and survival of Staphylococcus aureus. Burrows (6) concluded that since body temperature (98.6° F.) is optimum for their growth, staphylococci are constantly present on the body, especially in moist areas such as the skin, respiratory tract, feet, and

groin. These microorganisms can cause illness varying from an infection around a cut to boils and sties, food poisoning, mastitus, child bed fever, pneumonia, and death.

Microbial Degradation of Textiles

Most microorganisms are considered the natural flora of soils, and consequently, can be transferred readily to clothing and other textile materials. Apparel fabrics may act as a fomite in harboring microorganisms by supplying them with water, warmth, and nutrients on which to flourish and multiply. Kaswell (18) reported that all natural and regenerated hydrophilic vegetable and animal fibers are susceptible to microbial damage. Very little research concerning microbial degradation of synthetic fibers was found in the literature.

A few resources provided information about the physical characteristics of fibers and their relationship to the survival of microorganisms on textile materials. McNeil and Greenstein (27) presented evidence that microbial survival on fabrics for periods up to twenty days was of epidemiological significance. The scales of wool fibers, the roughness of cotton, and the relatively smooth fibers of synthetics undoubtedly influence the attachment of microorganisms. The fabric construction including the type of yarn and tightness of weave, as well as the moisture content of the fabric, must also be considered as factors that influence the number of microorganisms found on textiles and wearing apparel. McNeil (26) summarized the influences affecting the survival of microorganisms on fabric as generic differences, suspension media,

temperature, relative humidity, light, fiber type, fabric construction, and finish.

Cotton. Numerous researchers have observed that the important aerobic bacteria concerned in the deterioration of cotton textiles belong to the mesophilic group that has an optimum temperature range of 30^{o} C. to 37^{o} C. Basu and Bose (3) found the optimum temperature for several cellulosic-decomposing microorganisms to be about 30^{o} C.

Moisture is considered an essential factor in the maintenance of microbial growth. It was cited by Kaswell (19) that decomposition of cotton is not initiated unless the relative humidity is above eighty-two per cent. Above this value, the fibers are able to absorb enough moisture to permit growth of microorganisms.

Generally, microorganisms prefer a slightly alkaline pH. Lawrence (20) stated that cellulose-decomposing microorganisms prefer a pH between 7.0 and 8.0. Basu and Bose (3) observed that microorganisms preferred an initial pH near the neutral point, although the final pH turned toward alkalinity.

Siu (34) concluded from available evidence in his studies that the amphorous areas in the cotton fiber are more rapidly attacked by microorganisms than the crystalline areas. The loosely-packed amphorous areas allow for more rapid accessibility by the enzymes secreted from the microorganisms. Perlin et al. (29) also found that bacterial decomposition of cellulose was favored by a low crystalline structure.

Kaswell (19) explained that cotton and other cellulose-based fibers are subjected to breakdown where, by a process called glycolysis,

the cellulose is converted by the enzyme cellulase to celloboise. Celloboise then is converted to glucose that serves as food for the microorganism. Mandels and Reese (22) postulated some years ago that cellulose digestion occurs by a series of steps. The first step converts native cellulose into a reactive cellulose. There is increased moisture uptake, hydrating the cellulose and pushing apart the closely packed chains. The resultant reactive cellulose is acted upon by a group of enzymes that hydrolyze the glucosidic bonds producing reducing sugars.

Mandels and Reese (22) indicated that biological degradation of cotton fabric involves more than the enzymatic hydrolysis of the glycosidic linkage of the cellulose chain. Microorganisms can bring about changes in the structure of the fiber on which they are growing. From his studies, Siu (34) revealed the mode of breakdown of cotton fibers. When a fungus or bacterium lands on a cotton fiber it begins to germinate in the presence of adequate moisture and favorable temperature. First, digestion of the cuticle, or outer noncellulosic layer, is completed. The next step involves the degradation of the cellulosic portions of the fiber. Here the attack is not a general one as in the case of the cuticle, but is more localized. The fungus or bacterium apparently attacks the cellulosic wall at the point of immediate contact.

Some differences in the method of attack of fibers by fungi and bacteria were observed by Siu (34). Unlike fungi, bacteria do not grow in long filaments. Instead, they remain as small cells of protoplasm that multiply by fission. Of necessity, therefore, bacteria are not able to penetrate the fiber by means of germ tubes as fungi are able to

penetrate the fiber. Consequently, bacteria etch the surface, merely adhering to the fiber and pitting away. The attack is from the outside inward. The digestion of cellulose occurs apparently only at the point of immediate contact between the microorganism and the cotton fiber. There appears to be no degradation of cellulose at a distance from this point.

Mandels and Reese (22) noted structural damage to fibers after exposure to celluloytic-enzyme producing bacterium. A general description of damage included transverse cracks, helical cracks, spiral fissures, complete breaks, transverse holes, and corrosion. In similar studies, Abrams (1) and Porter et al. (30) reported that transverse cracking occurred on short exposure to bacterial enzyme, while Blum and Stahl (5) and Marsh (23) found spiral fissures evident after prolonged incubation of five days on cotton fibers.

Porter et al. (30) attributed extensive variation in the amount of damage observed in small areas of a fiber surface to the uneven growth of microorganisms on exposed fibers. Bacterial colonies, growing in the folds of the primary wall, caused diagonal and longitudinal splitting of the fiber surface. As degradation became more extensive, bacteria remained embedded in the fiber surfaces, surrounded by limited areas of extensive damage. Porter and his colleagues pointed out that as the microorganism penetrated deep into the secondary layers, they created more disorder in the fibrillar structures than observed in surface replicas of celluloytic degraded fibers. Fragmentation patterns indicated a continued breaking down of the fibrillar structure into small

fragments and particles.

Nylon. The synthetic fibers, including nylon, are formed of chemical substances that do not occur naturally. Mauersberger (24) and Kaswell (19) agreed that microorganisms will not readily attack synthetic fibers, since these fibers have little or no tendency to absorb water, a necessary component required by all microorganisms for survival.

Control of Microbial Deterioration of Textiles

Undesirable effects of microbial action on textile materials often can be thwarted and controlled if precautions are taken to insure against such deterioration. A relationship between textile materials and undesirable microbial action has been recognized for a number of years.

Microbial transfer. There has been an increased awareness of the transmission of microorganisms by textiles and clothing, resulting from a recent concern about Staphylococcus aureus infections. Hare and MacKenzie (16) reported that microorganisms, including staphylococci, may survive for relatively long periods of time on clothing and bedding. Duguid and Wallace (10), using a slit air sampler, found that Staphylococcus aureus was liberated more consistently and in greater numbers from clothing than from the respiratory tract.

Of some importance to this study were the findings of Hamburger and Green (14). They indicated that many more hemolytic streptococci are expelled by blowing the nose than by sneezing, coughing, or breathing. As the carrier blows his nose, the expelled microorganisms rapidly

contaminate the hands. The hands ultimately transfer the organisms to secondary environmental reservoirs of personal clothing, bedding, or anything the hands touch, which also may harbor or transmit the microorganisms.

Control of microbial transmission. It has been suggested by McNeil and Greenstein (27) that laundering is one method in the control of microbial transmission and textile deterioration. As early as 1926, Guernsey (13) discussed the influence of temperature and chlorine bleach on the sanitation of laundering. McCulloch (25) stated that laundering depends upon the bactericidal action of hot water, mild alkali, soap, and low concentrations of chlorine in the bleach. Mechanical action also assists in reducing bacterial contamination by washing out organic matter and microorganisms.

Cohen and Linton (8) proposed that a microorganism can be killed by the same general methods as for all living things. They can be starved from lack of nutrition; they can be deprived of water; and they can be killed by contact with various chemical agents. Staphylococcus aureus, in particular, can be destroyed in several ways. Conditions for their destruction include high temperature, chemical attack, and ultraviolet or nuclear irradiations. The two most effective and most often used means are those of high temperature and chemical attack.

Carpenter (7) explained that heat is a reaction where the protein of the microbial cell is coagulated. Depending on the strain, Staphylococcus aureus can be killed from temperatures of 140° F. for ten minutes to 175° F. for thirty minutes. Dry staphylococci require higher

temperatures and a longer period of time to be killed. Arnold (2) stressed that low temperature operations (100^{o} F.) would ultimately present bacterial problems.

An effective detergent is a primary asset in laundering sanitation. Ridenhour (31) related that surface active agents, such as detergents, disrupt the cell membranes of microorganisms by combining with the proteins of the cells and eventually destroying the cell membranes.

Lidwell and Lowbury (21) found that the death rate of most bacteria increased with a decrease in humidity. Kaswell (18) noted that if storage conditions are maintained below eighty per cent relative humidity, no fiber damage will result, even though spores of the organism are present. If drops of moisture accumulate, the relative humidity of the atmosphere immediately surrounding the spores will be sufficient to initiate germination, and the subsequent development of the organism will deteriorate the fiber.

Thompson (36) mentioned that drying increased the death rate of microorganisms, especially when oxygen was present. Thus, drying is a valuable inhibiting agent, since microorganisms require water for their life activities. The destructive action of drying depends on many variables such as kind of organism, number of cells, thickness of layer, temperature, and presence or absence of oxygen.

The need for examination of the bacteriology of home laundering was revealed in an investigation by McNeil (26). Many home washing machines now have a "warm" water wash temperature (100^{o} F.) as well as a "hot" water wash temperature (120^{o} F. to 130^{o} F.), which is still too low

for bactericidal action. Furthermore, some machines recirculate water, and unless the water temperature is very high, this would result in an introduction of large numbers of microorganisms into the washer. McNeil also noted an increase in the use of commercial laundry facilities both in multiple-unit dwellings and in self-service laundries. Sanitary practices are not always observed in the use of these facilities.

Soiled clothes and other textiles may contain from few to millions of microorganisms including pathogenic forms. According to Ridenhour (31), the microorganisms may be tightly absorbed on the fabric even in the absence of soil. In some cases, soil-bound bacteria are removed more easily than soil-free bacteria. A complete laundering operation with detergent only will remove from a low percentage to ninety-nine per cent of the bacteria present on textile materials, as cited by Ridenhour (31). The range of removal is dependent upon the presence or absence of soil, the amount of detergent, and the type of washer. Approximately ninety-five per cent of microbial removal in a complete laundering operation takes place in the wash cycle. The rinse action may or may not account for a small additional removal. When moist heat sanitization is used on fabrics, practically all organisms are destroyed at 145^{o} F. within three minutes with a pH of 8.0 or above.

Microscopic Examination

The microscope plays an important and ever increasing role in textile testing and in research. In addition to the identification of fibers, yarns, and fabric structures, Schwarz (33) reported that various

types of damage to fibers during processing, storage, and use can be determined by microscopic methods. Skinkle (35) noted other advantages of microscopic evaluation are that only small specimens are needed and that these specimens are not destroyed by analysis. Photomicrographic records can assist further in the identification of variations of fibers.

Microbiological damage to textile fibers cannot be assessed without the use of a microscope. Since microbial damage to fibers is invisible to the naked eye, the microscope can be used to produce a magnified image of the fibers to reveal details. Heyn (17) recommended microanalysis as an effective means for revealing the type and extent of fiber damage.

Through microscopic analysis there have been extensive investigations of microbial degradation to textile fibers, especially the cellulose-based fibers. From a few studies it was concluded that synthetic fibers are not susceptible to microbial attack. The literature revealed little information about the growth of pathogenic microorganisms, specifically Staphylococcus aureus, on cellulose-based fibers and their mode of fiber decomposition.

METHOD OF PROCEDURE

A knitted nylon-cotton fabric, complying with military specifications for men's ribbed stretch-type socks, was utilized (9). The same fabric is being used in the Themis Project No. 45, Performance and Life Support in Altered Environments, conducted by the Institute for Environmental Research for the United States Air Force. The fabric was composed of sixty per cent stretch-type 70-denier nylon and forty per cent carded and combed mercerized cotton. The knitting yarns were two-ply of a nylon filament yarn and a cotton staple yarn. Only the plain knitted structure of the sole, heel, and toe portions of the footwear were used, since microbial growth and survival were expected to be greater in these areas than in other areas of the footwear. The fabric was black without any applied finishes.

Tubular lengths of the knitted fabric were cut into rectangular swatches, six by twelve inches. Table I, page 49, shows the treatments for which the specimens were cut and coded for identification. Following preparation, the swatches were inoculated with Staphylococcus aureus before and after laundering, held at selected environmental conditions, and evaluated under the microscope as described in the following sections and illustrated in Table II, page 50.

Laundry Procedure

Preliminary interviews and testing were completed for the Themis Project No. 45 to help establish a laundry procedure that would simulate a home laundering operation. Interviews with managers of supermarkets in

Manhattan, Kansas, revealed that a high sudsing synthetic detergent with whitening agents and enzymatic reactives was purchased most often by consumers in this area. Therefore, this commercial detergent was used in the laundry procedure. From the same interviews, it was found that few consumers used a bactericidal agent in their home laundries. Of the bactericidal agents available to the consumer, chlorine bleach was purchased most frequently. However, in pre-laundry tests, chlorine bleach used in recommended quantities caused rapid deterioration of the fabric. Consequently, no bleach or other disinfectant was used in the study.

Preliminary testing indicated that the water used for the laundry procedure had a hardness of five grains and a pH of 7.0 to 8.0. The water temperature was recorded throughout each laundering. Fluctuations in temperature, which are normal for home laundries, ranged from 130° F. to 145° F. with an average temperature of 140° F.

Home laundry procedures of washing and drying were adopted. The conventional automatic washer and electric dryer were utilized with settings for a normal wash and wear cycle. The weight of each laundry load was held constant at eight pounds.

Following establishment of the laundry procedure, the prepared swatches were divided and used for the treatments in Table I, page 49. One group of swatches were unlaundered and uninoculated. Another group of swatches were uninoculated, but laundered and drawn after zero, one, five, ten, and fifteen launderings. A final group of swatches were laundered and drawn after zero, one, five, ten, and fifteen launderings. After laundering, these swatches were inoculated with Staphylococcus

aureus one time.

Inoculation Procedure

All fabric swatches were premoistened in a synthetic soil solution for twenty minutes before testing to simulate the soiling of a fabric during a normal wearing period and to enhance recovery of the microorganism. The synthetic soil solution consisted of fifteen grams of all-purpose flour, fifteen grams of cornstarch, fifteen grams of cane sugar, one gram of powdered carbon, fifteen milliliters of vegetable oil, fifteen milliliters of mineral oil, one hundred milliliters of evaporated milk, and two hundred fifty milliliters of water (31). The ingredients for the synthetic soil were mixed in a standard commercial blender for five minutes to form a relatively stable emulsion. The pH of the prepared synthetic soil was approximately 6.2.

Following premoistening, the laundered-Staphylococcus aureus inoculated swatches were seeded with the staphylococci. Fifteen milliliters of physiological saline suspension, containing 110 X 10^8 staphylococci per milliliter, were used to inoculate every ten swatches held at 37^{o} C. and one hundred per cent relative humidity. For every ten swatches held at 21^{o} C. and one hundred per cent relative humidity, fifteen milliliters of physiological saline suspension, containing 377 X 10^6 staphylococci per milliliter, were used for inoculation. Aseptically handled, the specimens were placed in a Chromotocab airtight chamber and suspended for a period of twenty minutes. The specimens were inoculated by means of aerosalization of the microbial suspension in the nebulizer

chamber.

The laundered-Staphylococcus aureus inoculated swatches as well as the unlaundered-uninoculated swatches and the laundered-uninoculated swatches were placed in an environmental chamber that had been pre-sterilized with paraformaldehyde gas. The swatches were held in the chamber for specified holding periods at selected temperatures and humidity as noted in Table I, page 49.

Following completion of the inoculation procedure, survival of the staphylococci was assayed. Mannitol-salt agar was utilized for isolation of the staphylococci. Colonies of the microorganism were enumerated with the aid of a Quebec colony counter. Further identification was made in accordance with standard microbial procedures. All inoculation and microbial survival work was completed by personnel in the Department of Infectious Diseases.

Evaluation of Microbial Damage Using the Microscope and Photomicrographs

Microscopic analysis of nylon-cotton fibers exposed to Staphylococcus aureus at selected environmental conditions before and after laundering was completed using an American Optical Company Series 4 Microstar trinocular microscope. After preliminary experimentation, the longitudinal sections of the fibers were studied using the ten power and forty-three power objectives, ten power eyepieces, and substage illumination with blue filter. The fibers were mounted on slides with Permount, a permanent mounting media.

The ten power objective, producing a magnification of 150X, was

used to study the amount of microbial damage, while the forty-three power objective, with a magnification of 645X, was used to show fine detail of selected areas of fiber damage. To analyze the scope of microbial damage to fibers at a magnification of 150X, fifteen microscopic fields were selected randomly from each group of slides of the laundered-uninoculated fibers and the laundered-Staphylococcus aureus inoculated fibers. The numbers of microscopic fields showing observable damage to fibers were recorded and are found in Table III, page 51.

Photomicrographs with magnifications of 150X and 645X were made of representative fibers obtained from unlaundered-uninoculated swatches, laundered-uninoculated swatches, and laundered-Staphylococcus aureus inoculated swatches to reveal any surface damage occurring to the fibers. A 35 mm camera, attached to the verticle tube of the trinocular body of the microscope, and Kodak Plus X Pan Black and White Panchromatic film were used for the photographic work.

FINDINGS

Photomicrographs of unlaundered-uninoculated fibers, laundered-uninoculated fibers, and laundered-Staphylococcus aureus inoculated fibers of a knitted nylon-cotton footwear fabric were made at magnifications of 150X and 645X. The photomicrographs were compared to evaluate the type and extent of fiber damage after exposure to Staphylococcus aureus for specified holding periods at selected temperatures and humidity and to assess the susceptibility of the footwear fabric to microbial attack before and after progressive laundering.

Magnification, 150X

Magnification of 150X was used to study possible microbial damage to several fibers. Close examination of the cotton fibers selected for the photomicrographs in Plate I (Figs. 2 and 4) may suggest some evidence of damage. However, the fibers are not defined well enough to distinctly reveal type and extent of fiber damage. Damage to nylon fibers could not be detected at a magnification of 150X.

There is limited depth of focus of both the microscope and the camera used; thus, all details in the fibers were not clearly seen in the photomicrographs. The thickness of the fibers, their twist, and the manner in which the fibers layed all combined to make it difficult to photograph desired details.

PLATE I

Photomicrographs of Cotton Fibers from Laundered-

Staphylococcus aureus Inoculated Fabric Held at

37° C. and 100% R.H. for 1, 4, and 8 Days

With Magnification, 150X

Fig. 1 Inoculated, held 4 days before laundering

Fig. 2 Inoculated, held 1 day after 1 laundering

Fig. 3 Inoculated, held 8 days after 5 launderings

Fig. 4 Inoculated, held 4 days after 10 launderings

Fig. 5 Inoculated, held 8 days after 15 launderings

PLATE I

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Magnification, 645X

Nylon. The unlaundered-uninoculated nylon fiber (Plate II, Fig. 1) represented the uniform and smooth cylindrical appearance that is typical of synthetic fibers. Dyeing of the fiber caused the nylon to appear grayed. The presence of delustrants, peppery black dots, in the fibers was also noted.

There was little observed difference in the general appearance among the unlaundered-uninoculated nylon fiber (Plate II, Fig. 1), the laundered-uninoculated nylon fibers (Plate III, Figs. 1-5), and the laundered-Staphylococcus aureus inoculated nylon fibers (Plate IV, Figs. 1-5). As exposure time (one to eight days) increased and optimum growth conditions (37° C. and 100% relative humidity) were created for the microorganism, there did not seem to be any visible change in appearance of the nylon fibers.

A few fibers were seen to deviate from the general characteristics of nylon. Fading and bubbles were noted occasionally after the tenth laundering. Bubbles were noted by Ford (11) in his study of strain-induced effects on synthetic fibers. Their presence in fibers was assumed to be a result of the normal stresses encountered in fiber production, fabric construction, and physical wear.

Cotton. The natural form of cotton fibers, often described as a twisted ribbon, consists of an outer wall, or cuticle, and of a central canal, or lumen, extending throughout its length (Plate II, Fig. 2). Dyeing of the fibers resulted in a grayed appearance, making the cuticle and lumen indistinguishable.

PLATE II

Photomicrographs of Nylon and Cotton Fibers

from Unlaundered-Uninoculated Fabric

With Magnification, 645X

Fig. 1 Nylon

Fig. 2 Cotton

Fig. 1

Fig. 2

PLATE III

Photomicrographs of Nylon Fibers from Laundered-Uninoculated Fabric Held at 37^{o} C. and 100% R.H. for 1, 4, and 8 Days With Magnification, 645X

Fig. 1 Held 4 days before laundering

Fig. 2 Held 1 day after 1 laundering

Fig. 3 Held 8 days after 5 launderings

Fig. 4 Held 4 days after 10 launderings

Fig. 5 Held 8 days after 15 launderings

PLATE III

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

PLATE IV

Photomicrographs of Nylon Fibers from Laundered-

Staphylococcus aureus Inoculated Fabric Held at

37° C. and 100% R.H. for 1, 4, and 8 Days

With Magnification, 645X

Fig. 1 Inoculated, held 8 days before laundering

Fig. 2 Inoculated, held 8 days after 1 laundering

Fig. 3 Inoculated, held 1 day after 5 launderings

Fig. 4 Inoculated, held 1 day after 10 launderings

Fig. 5 Inoculated, held 4 days after 15 launderings

PLATE IV

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Laundering alone caused little surface damage to the cotton fibers, as indicated by the photomicrographs in Plate V and Plate VI. Generally, the fibers remained flattened tubes characterized by irregular twists, or convolutions. Microscopic illumination and the subsequent shadowing produced varying visual effects on the fibers. Black specks on the fibers (Plate V, Figs. 4 and 5, and Plate VI, Fig. 5) were believed to be debris.

Mercerization of the cotton somewhat altered the microscopical appearance of the fibers. The mercerized cotton fibers of the footwear fabric had fewer twists, or convolutions, than would be found in the natural form of cotton.

Microscopic observation of the laundered-Staphylococcus aureus inoculated fibers revealed damage to cotton (Plate VII and Plate VIII). The fiber degradation is noted by comparing Plates VII and VIII with Plates V and VI.

The Staphylococcus aureus attacked the cotton fibers in a similar manner at both 37° C. and 21° C. with one hundred per cent relative humidity. Damage, at both holding temperatures, increased progressively as laundering and exposure time increased. The staphylococci essentially attacked areas of the fibers with roughened cuticle and areas with less convolutions. Any variation in the amount of damage may be attributed to the uneven growth of the microorganism on exposed fibers (30).

There was no evidence of microbial damage to unlaundered fibers exposed to the staphylococci for one day (Plate VII, Fig. 1, and Plate VIII, Fig. 1). Only slight damage was observed when the unlaundered

PLATE V

Photomicrographs of Cotton Fibers from Laundered-Uninoculated Fabric Held at 37° C. and 100% R.H. for 1, 4, and 8 Days With Magnification, 645X

Fig. 1 Held 4 days before laundering

Fig. 2 Held 1 day after 1 laundering

Fig. 3 Held 4 days after 5 launderings

Fig. 4 Held 8 days after 10 launderings

Fig. 5 Held 8 days after 15 launderings

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

PLATE VI

Photomicrographs of Cotton Fibers from Laundered-Uninoculated Fabric Held at 21° C. and 100% R.H. for 1, 4, and 8 Days With Magnification, 645X

Fig. 1 Held 1 day before laundering

Fig. 2 Held 1 day after 1 laundering

Fig. 3 Held 8 days after 5 launderings

Fig. 4 Held 4 days after 10 launderings

Fig. 5 Held 8 days after 15 launderings

PLATE VI

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

PLATE VII

Photomicrographs of Cotton Fibers from Laundered-

Staphylococcus aureus Inoculated Fabric Held at

37^{o} C. and 100% R.H. for 1, 4, and 8 Days

With Magnification, 645X

Fig. 1 Inoculated, held 1 day before laundering

Fig. 2 Inoculated, held 4 days before laundering

Fig. 3 Inoculated, held 8 days before laundering

Fig. 4 Inoculated, held 1 day after 1 laundering

Fig. 5 Inoculated, held 4 days after 1 laundering

Fig. 6 Inoculated, held 8 days after 1 laundering

Fig. 7 Inoculated, held 1 day after 5 launderings

Fig. 8 Inoculated, held 4 days after 5 launderings

Fig. 9 Inoculated, held 8 days after 5 launderings

Fig. 10 Inoculated, held 1 day after 10 launderings

Fig. 11 Inoculated, held 4 days after 10 launderings

Fig. 12 Inoculated, held 8 days after 10 launderings

Fig. 13 Inoculated, held 1 day after 15 launderings

Fig. 14 Inoculated, held 4 days after 15 launderings

Fig. 15 Inoculated, held 8 days after 15 launderings

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

PLATE VIII

Photomicrographs of Cotton Fibers from Laundered-Staphylococcus aureus Inoculated Fabric Held at 21° C. and 100% R.H. for 1, 4, and 8 Days With Magnification, 645X

Fig. 1 Inoculated, held 1 day before laundering

Fig. 2 Inoculated, held 4 days before laundering

Fig. 3 Inoculated, held 8 days before laundering

Fig. 4 Inoculated, held 1 day after 1 laundering

Fig. 5 Inoculated, held 4 days after 1 laundering

Fig. 6 Inoculated, held 8 days after 1 laundering

Fig. 7 Inoculated, held 1 day after 5 launderings

Fig. 8 Inoculated, held 4 days after 5 launderings

Fig. 9 Inoculated, held 8 days after 5 launderings

Fig. 10 Inoculated, held 1 day after 10 launderings

Fig. 11 Inoculated, held 4 days after 10 launderings

Fig. 12 Inoculated, held 8 days after 10 launderings

Fig. 13 Inoculated, held 1 day after 15 launderings

Fig. 14 Inoculated, held 4 days after 15 launderings

Fig. 15 Inoculated, held 8 days after 15 launderings

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

fibers were exposed to the microorganism for four and eight days. Transverse and helical cracking and slight fissures were noted as the beginning of microbial degradation (Plate VII, Fig. 3, and Plate VIII, Figs. 2 and 3).

The amount of fiber damage generally doubled after one laundering (Table III, page 51). Slight fissures began to appear after one day of exposure (Plate VII, Fig. 4, and Plate VIII, Fig. 4). Damage after four days of exposure included transverse and helical cracks (Plate VII, Fig. 5) and longitudinal fissures (Plate VIII, Fig. 5). It was apparent by the eighth day of exposure that the staphylococci were attacking the cuticle, or outer non-cellulosic layer of the fiber (Plate VII, Fig. 6, and Plate VIII, Fig. 6). Partial dissolution of the cuticle was observed after only four days exposure (Plate VII, Fig. 5). Once the cuticle has been removed, degradation of the inner cellulosic portions can begin.

The extent of microbial damage to the cotton increased only slightly after five launderings when compared to the amount of damage after one laundering (Table III, page 51). However, more critical damage to the cotton fibers appeared upon short exposure to the microorganism. Cracks and fissures were visible after one day of exposure (Plate VII, Fig. 7, and Plate VIII, Fig. 7). Fissures became numerous, increasing in width and depth as exposure time increased (Plate VII, Figs. 8 and 9, and Plate VIII, Figs. 8 and 9).

Photomicrographs of cotton fibers after the tenth laundering revealed extensive damage. The extent of microbial damage increased almost three times as much as before laundering (Table III, page 51).

Damage continued to include transverse and helical cracking, longitudinal fissures, and cuticle dissolution (Plate VII, Figs. 10, 11, and 12, and Plate VIII, Figs. 10, 11, and 12). Surface etching (Plate VIII, Figs. 10 and 11) and pitting (Plate VII, Fig. 10) were noted after short exposure.

Damage continued to increase by the fifteenth laundering (Table III, page 51). Fiber damage included the largest amounts of cracks and fissures that had been observed. Pitting was quite evident (Plate VII, Fig. 15). Partial dissolution of the cuticle occurred after one day of exposure (Plate VII, Fig. 13), becoming severe by the eighth day of exposure (Plate VII, Fig. 15, and Plate VIII, Fig. 15).

CONCLUSIONS AND RECOMMENDATIONS

This investigation was designed to evaluate the type and extent of damage to fibers of a knitted nylon-cotton footwear fabric before and after exposure to Staphylococcus aureus at selected environmental temperatures and humidity for specified holding periods. The fibers were analyzed microscopically, and photomicrographs with magnifications of 150X and 645X were studied for the type and scope of fiber damage. Unlaundered-uninoculated fibers, laundered-uninoculated fibers, and laundered-Staphylococcus aureus inoculated fibers were compared before and after progressive laundering to assess the susceptibility of the fibers to microbial attack.

Conclusions

Magnification, 150X. Photomicrographs with a magnification of 150X were taken to reveal the extent of microbial damage to several fibers. After analysis of the photomicrographs, it was concluded that a magnification of 150X was not powerful enough to show distinct areas of damage. The physical nature of the fibers and the depth focus of the microscope and camera combined to make it difficult to photograph distinct details of the fibers. However, magnification of 150X was used to estimate the scope of microbial damage to fibers by viewing a predetermined number of microscopic fields and recording the fields with observable fiber damage.

Nylon. Nylon fibers did not show any type of surface damage at a

magnification of 150X or a magnification of 645X. The nylon fibers maintained the same general smooth cylindrical appearance throughout the progression of laundering before and after exposure to Staphylococcus aureus. This comparison would indicate a resistance by nylon fibers to attack by the staphylococci. Slight physical damage in the form of fading and occasional bubbles was noted after the tenth laundering.

Cotton. Cotton fibers revealed surface damage when subjected to attack by Staphylococcus aureus before and after laundering. Similar damage was observed after exposure at 37° C. and 21° C. with one hundred per cent relative humidity. The staphylococci generally attacked areas of the cotton fibers where the cuticle had been roughened or where there were few convolutions. These areas may have resulted from fiber mercerization or from physical breakdown of the fibers caused by repeated laundering. Any variation in the amount of damage was attributed to the microorganisms's uneven growth patterns on the exposed fibers.

Laundering alone caused little observable surface damage to the cotton fibers, but when the fibers were exposed to staphylococci, damage increased progressively with each successive laundering. Removal of the fibers' wax coating after one laundering doubled the amount of surface damage when compared to the amount of surface damage before laundering. Severe damage was noted after the tenth laundering, suggesting that possible physical breakdown may have allowed for greater accessibility of the fibers to microbial attack than before the tenth laundering. By the fifteenth laundering surface damage had increased to almost four times the amount of damage as before laundering.

Damage increased with increased exposure time. Microscopic examination of cotton fibers revealed microbial damage in the form of transverse and helical cracks, fissures, surface etching, pitting, and partial or complete dissolution of the outer wall (cuticle). Upon short exposure (one to four days) to the microorganism, the cotton fibers showed transverse and helical cracks, slight fissures, and surface etching. Longer exposure (four to eight days) caused cracking, pitting, wide fissures, and partial or complete dissolution of the cuticle, ultimately leading to localized internal degradation of the cellulosic portions of the fiber.

Recommendations

Since this investigation was exploratory in nature, an attempt was made to find satisfactory methods to study fiber breakdown occurring as a result of microbial attack. However, further research is needed to substantiate this study's findings of physical and microbial damage to cotton.

This study's procedures and microscopic methods of analysis could be used to study microbial damage after exposure to different microorganisms. Another investigation might compare the effects of various temperatures and relative humidities to the extent of microbial damage to exposed fibers. A large sample study could be done to determine the amount of fiber damage after various exposure times to a microorganism. Correlation of microbial growth and survival counts with the amount of microbial damage to fibers is a possible research project. It would be

interesting to study cross sections of the fibers, stained to reveal microbial damage. A study of staining techniques used in biological and pathological laboratories might reveal dyes that could prove useful in staining the fibers.

It is suggested that in further studies of this nature that a magnification of 645X be used and that the use of polarized light be investigated. Although the electron microscope will show minute detail, it is not readily available to all laboratories. Thus, it is recommended that further work be done with the relative inexpensive and accessible microscope. Experimentation with photomicrographic techniques and with exposure time of the film, especially when studying dyed fibers, is recommended.

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APPENDIX

TABLE I

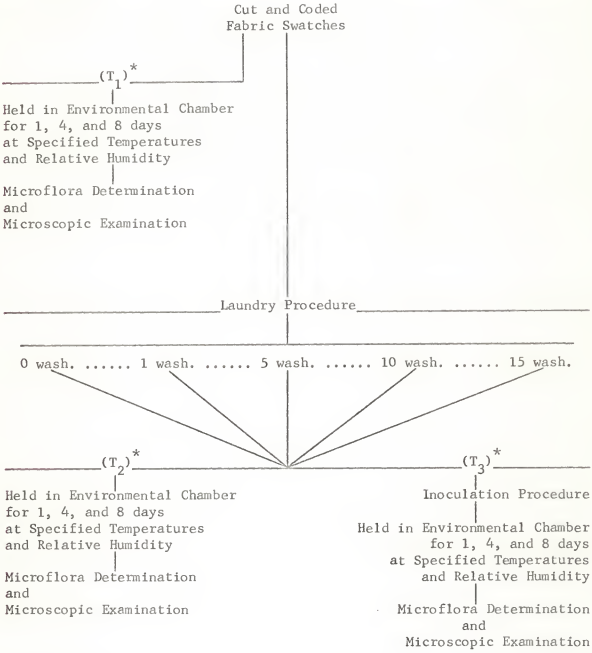
UNLAUNDERED-UNINOCULATED FABRIC SWATCHES, LAUNDERED-UNINOCULATED FABRIC SWATCHES, AND LAUNDERED-STAPHYLOCOCCUS AUREUS INOCULATED FABRIC SWATCHES HELD FOR SPECIFIED PERIODS AT SELECTED ENVIRONMENTAL CONDITIONS

Environmental Conditions	Fabric Swatch Treatments		
	Treatment 1 (Unlaundered-Uninoculated)	Treatment 2* (Laundered-Uninoculated)	Treatment 3* (Laundered-Staphylococcus Inoculated)
Holding Temperatures	21° C. and 37° C.	21° C. and 37° C.	21° C. and 37° C.
Holding Humidity	100% R.H.	100% R.H.	100% R.H.
Holding Periods	1, 4, and 8 days	1, 4, and 8 days	1, 4, and 8 days

*Laundered-uninoculated fabric swatches and laundered-Staphylococcus aureus inoculated fabric swatches were tested for the selected holding periods and environmental conditions after zero, one, five, ten, and fifteen launderings. After laundering, the laundered-Staphylococcus aureus inoculated fabric swatches were inoculated one time before testing under the selected environmental conditions.

TABLE II

FLOW CHART OF TEST PROCEDURES



*T_1, T_2, T_3 refer to the treatments given in Table I.

TABLE III

COMPARISON OF THE EXTENT OF MICROBIAL DAMAGE OF LAUNDERED-UNINOCULATED COTTON FIBERS TO LAUNDERED-STAPHYLOCOCCUS AUREUS INOCULATED COTTON FIBERS FROM FIFTEEN RANDOMLY SELECTED MICROSCOPIC FIELDS FROM EACH GROUP OF PREPARED SLIDES

Environmental Conditions for Laundered Fabric	Number of Fifteen Microscopic Fields Showing Staphylococcus aureus Damage to Cotton Fibers				
	Zero Laund.	One Laund.	Five Laund.	Ten Laund.	Fifteen Laund.
Uninoculated					
37° C. and 100% R.H.					
Held 1 day	0	0	0	0	0
Held 4 days	0	1*	0	0	0
Held 8 days	0	2*	0	0	0
21° C. and 100% R.H.					
Held 1 day	0	0	0	0	0
Held 4 days	0	0	0	0	0
Held 8 days	0	1*	0	0	0
Staphylococcus Inoculated					
37° C. and 100% R.H.					
Held 1 day	3	5	7	8	12
Held 4 days	3	8	10	11	13
Held 8 days	5	11	10	13	15
21° C. and 100% R.H.					
Held 1 day	4	6	6	10	12
Held 4 days	3	7	10	10	14
Held 8 days	7	9	11	13	14

*Uninoculated fabric laundered one time was held in the environmental chamber with staphylococci inoculated fabric laundered one time.

MICROSCOPIC EVALUATION OF DAMAGE BY STAPHYLOCOCCUS AUREUS ON A KNITTED NYLON-COTTON FABRIC BEFORE AND AFTER LAUNDERING

by

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B. S., Ohio University, 1966

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MASTER OF SCIENCE

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1969

Through microanalysis and photomicrographic evaluation, this study was designed to examine the type and extent of damage to fibers of a knitted nylon-cotton footwear fabric after exposure to Staphylococcus aureus for specified holding periods at selected temperatures and humidity. A comparison of photomicrographs of unexposed fibers to microbial-exposed fibers was made to assess the susceptibility of the footwear fabric to microbial attack before and after progressive laundering.

The footwear fabric was composed of sixty per cent stretch-type 70-denier nylon and forty per cent carded and combed mercerized cotton without applied finishes. The fabric was laundered zero, one, five, ten, and fifteen times before inoculation with Staphylococcus aureus. Following inoculation, the fabric was transferred to an environmental chamber and held for one, four, and eight days at 37^{o} C. and 21^{o} C. with one hundred per cent relative humidity.

Photomicrographs with a magnification of 645X showed detail of selected areas of fibers that could be studied for microbial damage. Nylon fibers showed resistance to Staphylococcus aureus with little or no surface damage observed. However, microanalysis disclosed microbial damage to cotton fibers. Damage increased progressively as laundering and exposure time increased. Transverse and helical cracks, slight fissures, and surface etching were apparent after one to four days of exposure to the microorganism. Prolonged exposure (four to eight days) caused pitting, wide fissures, and partial or complete dissolution of the outer wall (cuticle), ultimately leading to internal damage.