THE EFFECT OF CHEMICAL FINISHING ON THE MICROBIAL TRANSFER FROM CARPETS TO HUMAN SKIN AND SELECTED FABRICS

by

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(Under the Direction of Dr. Karen Leonas)

ABSTRACT

Microorganisms are commonly found in carpets during normal use and they can be transferred from carpets to human skin and textile materials through direct contact. To reduce the transfer of microorganisms from the carpets, chemical treatments (Humectant A treatment, Freepel™ 1225 treatment, and Sulfated 2-EH treatment) were applied to the carpets and altered their surface properties such as surface energy. The transfer of four microorganisms (*Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae*, and *Aspergillus niger*) from the treated and untreated carpets (loop pile and cut pile) to the receptor fingers and receptor fabrics (compression fabric, cotton knit fabric, and cotton woven fabric) was investigated in this study.

The results show that the chemical treatment significantly influenced the microbial transfer. The transfer of microorganisms was decreased from carpets after the Humectant A treatment and the Sulfated 2-EH treatment. The transfer of microorganisms was increased from carpets after the Freepel™ 1225 treatment. The microbial species significantly influenced the transfer of microorganisms. *Klebsiella pneumoniae* was difficult to transfer from carpets. The transfer of *Aspergillus niger* was lower than that of *Staphylococcus aureus* and *Escherichia coli*.

The transfer of *Escherichia coli* was higher than that of *Staphylococcus aureus* from the untreated and the Freepel™ 1225 treated carpets. The type of receptor fabric significantly influenced the amount of microorganisms transferred from carpets. The transfer of microorganisms from carpets to the cotton knit fabrics was highest, followed by the compression fabrics, and then the cotton woven fabrics.

An airbrush method was developed to apply the microorganisms to the carpets. By examining LSCM images, most of the microorganisms were known to be located at the surface of carpets when applied to the carpet using this airbrush technique.

INDEX WORDS: Microbial transfer, Carpet, Receptor materials, Chemical treatment, Surface energy, Airbrush, LSCM, Microbial adhesion and release

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DEDICATION

This dissertation is dedicated to my father, Peiyue, my mother, Qianli, and my fiancé, Zhanli, for their forever love and supports.

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CHAPTER 1

INTRODUCTION

Carpet is a popular floor covering which has a three dimensional structure. It provides both decorative and functional advantages to interior spaces. The various colors and textures help to create an inviting environment. Its softness reduces leg and foot fatigue and prevents serious injuries due to slips and falls. Today carpet is a widely used floor covering in schools and hospitals.

Microorganisms are commonly found in the carpet during normal use. For example *Staphylococcus aureus*, a potentially pathogenic gram positive bacteria, is known to cause a wide range of infections including staphylococcal scalded skin syndrome (Gravesen, 1998). *Cladosporium herbarium* is a spore forming fungus. It is a common allergen and can cause cutaneous and subcutaneous infections (Tortora, 1998). Small children often sit and play on the carpet, thereby coming in direct contact with the microorganisms. The microorganisms can then be transferred to the children's hands. If the children happen to touch their mouth, nose, eyes or any opening in the skin before washing their hands, the microorganisms will enter the body. Once these transferred microorganisms enter the human body, the risk of disease is increased. Therefore, the study of reducing microbial transfer is of interest.

Microbial transfer is controlled by many factors which can be classified into three categories: 1) environmental factors, 2) characteristics of surfaces, and 3) characteristics of the microorganism. Environmental factors include variables such as temperature and humidity. Characteristics of the surfaces include physical characteristics such as smoothness, and chemical

characteristics such as the surface energy and static charge. The size, shape, cell wall structure, extra cellular layers like capsules, and hydrophobicity of microorganisms are the microbial characteristics of interest. According to Fletcher, the microbial adhesion and release, known to influence the transfer of microorganisms, is strongly dependent on the composition of the microbial surface and the presence of complementary chemistries on the surfaces (Fletcher, 1996).

To understand the process of microbial transfer from carpet to human skin, more information regarding how the factors previously stated influence the microbial transfer and whether surface modification could be used to enhance or inhibit the microorganisms release is needed. In this study, chemical treatments were applied to the carpet to increase or decrease the surface energy and to alter the static charge of the carpet. The microbial transfer from treated and untreated carpets was investigated and compared.

Significance of Study

A variety of microorganisms are found in carpets in schools, hospitals and other commercial settings. According to previous studies, microbial transfer from carpet to hands and textile materials does occur (Annis and Leonas, 2004). Once the microorganisms are transferred to the human and enter the body, the likelihood of infection is increased. Therefore the study of reducing microbial transfer to human hands can provide significant information about infection control.

Microbial transfer is controlled by microbial adhesion and release (Salerno *et al*., 2004). There are some studies that have evaluated the influence of surface energy and static charge of the surface on the microbial adhesion and release (Ranade, 1987). No publications were found

regarding the microbial transfer from carpets with repellent finishes which change the surface energy of the carpet. In this study the chemical treatments were applied to the carpet to alter selected surface characteristics (surface energy and static charge). The microbial transfer from treated and untreated carpets was investigated and compared. The results of this study will provide important information for carpet manufacturers and public agencies in selecting carpets for use in commercial settings including educational and health care facilities.

Purpose and Objectives

The purpose of this research is to study the influence of chemical finishes, which change the surface energy and electrostatic property of the carpets, on the transfer of microorganisms from carpets to the human skin and selected textile materials. The sub-objectives include:

1. Evaluate the impact of the carpet's surface energy (water repellent finishing and hydrophilic finishing) on the microbial transfer from carpets to human skin and selected fabrics,
2. Evaluate the impact of the electrostatic property of carpet (surfactant treatment) on the microbial transfer from carpets to human skin and selected fabrics,
3. Evaluate the impact of different methods to apply microorganisms to the carpet on the microbial transfer and evaluate the distribution of microorganisms in the carpets based on different methods of application using Laser Scanning Confocal Microscopy,
4. Evaluate the impact of different kinds of microorganisms (bacteria and fungi) on the microbial transfer from carpets to human skin and selected fabrics.

CHAPTER 2

LITERATURE REVIEW

To provide a general understanding of this study some basic information about carpets, microorganisms and their presence in carpets during normal use, and the general transfer of microorganisms is introduced in this chapter. Chemical finishing of textiles and the application of Laser Scanning Confocal Microscopy technology in the textile area are reviewed as well.

Carpet - Soft Floor Covering

Floor coverings are commonly classified into three categories of hard, semi-hard, and soft floor coverings. Hard floor coverings include natural stone and ceramic tile; semi-hard floor coverings include vinyl tile and cork; soft floor coverings include carpets and rugs. Carpet is defined as a securely fastened or anchored soft floor covering (Yeager and Teter-Justice, 2000).

Tufting is the most prevalent carpet construction method. Today more than 90% of the carpet produced in the United States is tufted (CRI, The Carpet Primer, 2003). Figure 2.1 shows the profile of a typical tufted carpet. The tufted carpet consists of the pile yarns (tuft) and the backing system, which give the carpet a three dimensional structure. Most backing systems include a primary backing fabric, an adhesive compound, and a secondary backing fabric.

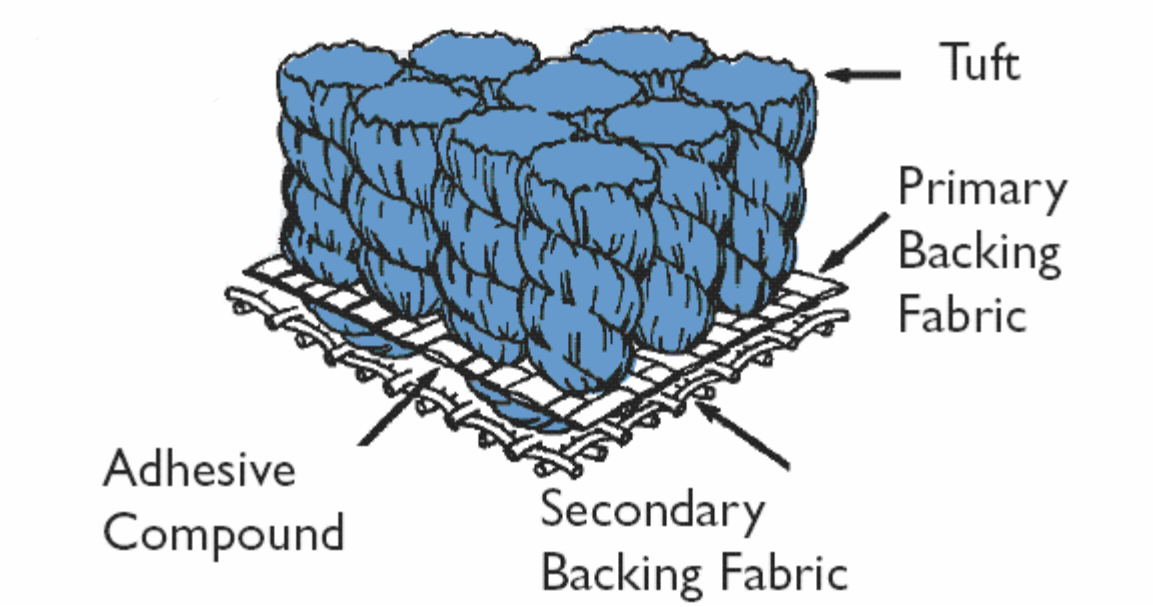
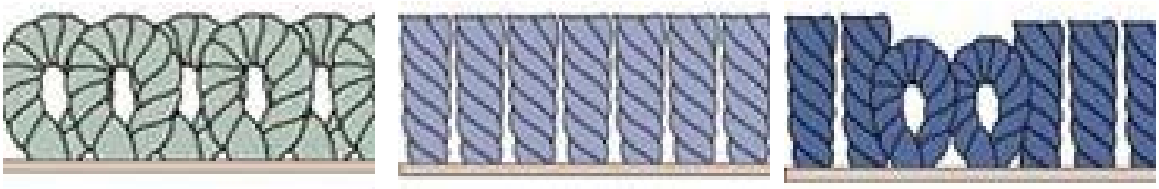


Figure 2.1 Typical cut pile carpet profile (CRI, The Carpet Primer, 2003)

Carpet texture is important when describing the properties of a carpet. It refers to the surface appearance of the carpet. The most common carpet textures include loop pile, cut pile, and the combination of loop and cut pile, which are shown in Figure 2.2.



(a) loop pile (b) cut pile (c) loop and cut pile

Figure 2.2 Carpet texture
(http://www.wisenbaker.com/Products/Carpet/CarpetConstructionStyling.htm)

Loop pile carpet includes level loop pile and multilevel loop pile. A level loop pile carpet has all the same height pile yarns, while a multilevel loop pile carpet has loops of different heights that create a sculptured surface. Cut pile carpets can be classified as plush, velvet, saxony, or frieze, depending on their pile height, yarn twist, and tuft density. The pile yarns in the plush

and velvet carpets have relatively low levels of twist. The pile height of velvet is shorter than that of plush. The pile yarns of saxony carpet are the same height and density as that seen in plush. However, the saxony yarns have more twist. In the frieze carpet the pile yarns are extremely highly twisted and heat set, which creates a "curly" textured surface. A combination of loop and cut pile is used to create tip-sheared and random-sheared textures. A tip-sheared texture has a level surface with cut and uncut loops. A random-sheared texture has multilevel loop piles with the highest loops shorn away (CRI, The Carpet Primer, 2003).

Some properties including pile height (pile thickness), pile yarn weight, tufts/inch^2, and pile construction density are critical for the carpet construction specification. Pile height (for cut pile carpet) is the length of the pile tufts above the backing. It is generally measured from the surface of the primary backing to the top of tufted yarn. The pile thickness has the same meaning as pile height, but is used for the loop pile carpet. The pile yarn weight refers to the amount of pile yarns, both above and embedded in the backing. Tufts per square inch is an important specification of the carpet. It is calculated by multiplying the gauge by the tufts or stitches per inch. Gauge is the distance between the tufting needles measured across the carpet width. Generally, the gauge is expressed in fractions of one inch. For example, 1/8 gauge has needles 1/8 of an inch apart. The tufts or stitches per inch refers to the number of tufts per inch in a single length-wise tuft row. Pile construction density is determined by the closeness of the tufts. The more closely spaced the tufts the higher the pile construction density is. The Figure 2.3 shows carpets in different pile construction densities.

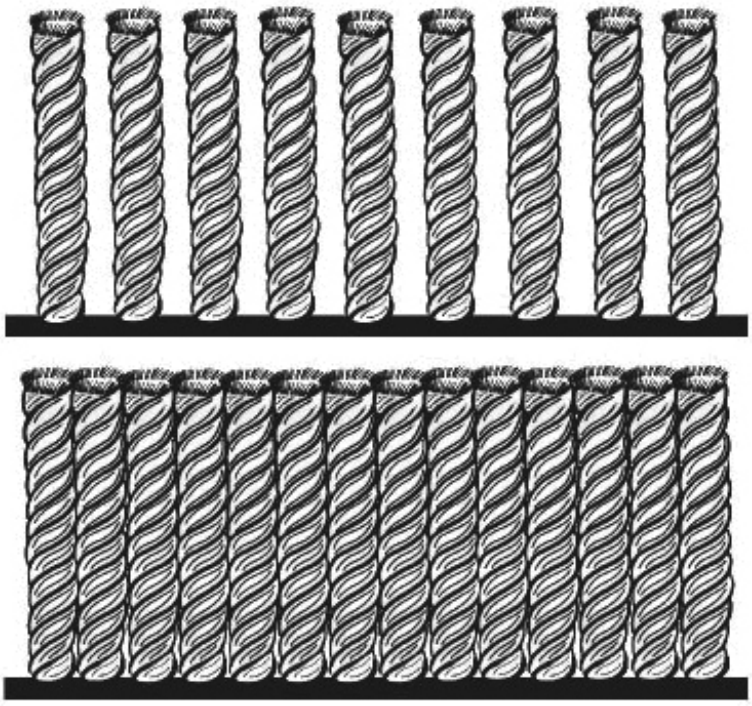


Figure 2.3 High and low pile construction density of carpets
(http://www.wisenbaker.com/Products/Carpet/CarpetConstructionStyling.htm)

Microorganisms - An Overview

Microorganisms are found almost everywhere on the earth. They are essential to the existence of life and are responsible for the natural recycling of material and energy in the environment. Some microorganisms are directly involved with maintenance of human health and others cause diseases.

Microorganisms are classified as bacteria, fungi (mold and yeast), viruses, algae and protozoa. Bacteria and fungi are two types of microorganisms which have commonly been found in the carpet and are of interest in this study.

Bacteria are generally smaller, simpler and more primitive than the fungi. Their nuclear material comprises a single, double-stranded, but very large DNA molecule without a structural nuclear membrane (Ryan, 1994). Bacteria can be classified as gram positive or gram negative

based on the cell wall structure. Approximately 90% of the gram positive bacteria cell wall is composed of peptidoglycan, while gram negative bacteria have a much thinner layer of peptidoglycan. The gram negative bacteria have phospholipids and negatively charged lipid A portion of lipopolysacccharide as components of their membranes. *Staphylococcus aureus, Staphylococcus epidermidis, Corynebacterium diphtheroides, Streptococcus pneumoniae* and *Mycobacterum tuberculosis* are the gram positive bacteria most commonly found in carpets. *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi* and *Shigella dysenteriae* are the gram negative bacteria most commonly found in carpets (Tortora, 1998).

Fungi have many cellular characteristics of both mammalian and plant cells: nuclear membranes, several chromosomes, mitotic apparatus, mitochondria, sterol-containing cell membranes, and in many cases, the ability to reproduce sexually (Ryan, 1994). Fungi are classified as eukarya because of the "true" nuclear structure. Many fungi form spores, which are relatively resistant to heat and chemicals. Some fungi growing as hyphae/mycelia are called molds. Some kinds of fungi may occur either as yeast or as mold under different circumstances. *Aspergillus niger, Penicillium funiculosum, Cladosporium herbarium, Candida albicans, Epidermophyton floccosum* and *Trichophyton rubrun* are the fungi most commonly found in carpets (Gravesen, 1998).

Microorganisms in Carpet

The microorganisms in a carpet are generated by a multiplicity of sources. They can be deposited on the carpet from the air (Burge, 2002). The majority of the indoor airborne microbial population is derived from outdoor sources. Through ventilation, the outdoor aerosols enter the

building. The concentration and composition of this population depends on the ventilation rate and air movement patterns in the space. The other primary source of microorganisms in a carpet is from the traffic on the carpet. People and pets always carry some microbes from outside into a building. When they walk on a carpet, they will drop some microorganisms. People also shed skin scales with microorganisms, and eject microorganisms in particles from the respiratory tract.

Carpet has a large specific surface area due to its three dimensional structure. Small particles, like microorganisms and dust, are adsorbed onto the carpet easily. Shaffer et al. reported that the number of microorganisms on the carpet is larger than that on a floor without carpeting (Shaffer *et al.*, 1966). Once the microorganisms settle onto the carpet, if the conditions are suitable, they will grow and reproduce.

The three dimensional structure of the carpet helps to hold free air containing oxygen and keep the temperature constant at about 20-25^{o}C. Many environmental bacteria and fungi grow very well in this temperature range. However, some microorganisms such as thermophiles and hyperthermophiles, cannot grow at room temperature. Therefore, the indoor environmental temperature limits the growth of some types of microorganisms. The pH value at the carpet surface is neutral (around seven), which is suitable for growth of most bacteria and some fungi (Prescott, 1996).

Microorganisms can degrade carpet components made of natural fibers. Before the 1970's most carpets were made of cotton, wool (pile yarns), and jute (backing) (CRI, 2003). Some bacteria and fungi release certain enzymes which degrade these fibers into glucose or amino acids. These organic compounds are then used by the microorganisms as nutrition. The microorganisms directly attack the carpets made of natural fibers. Since the 1960's carpet composition has changed dramatically. Most carpet pile yarns are now made of synthetic fibers

like nylon, polyester, or olefins, and the primary and secondary backings are made from polypropylene rather than jute. These synthetic materials themselves serve as a poor nutrient substrate for microbial growth. But if appropriate food and water are present in the carpet structure, the microorganisms can obtain sufficient nutrients for growth. Each carpet environment is unique and characterized by its own non-biological particle composition. These non-biological particles determine the type and number of the microorganisms which grow on the carpet. For example, some fungi like those of the *Aspergillus* species, need a high concentration of sugar or glycerol to grow. If these organic substances are not present in or on the carpet, *Aspergillus* cannot grow (Burge, 2002).

Water is the most important ingredient in the growth and reproduction of microorganisms. Once water is dropped onto a carpet, it may drain through to the backing and is difficult to remove. If the carpet in a building becomes sufficiently wet, it will support the growth of some organisms and will become a source of bio-aerosols (Burge, 1995). Occasionally microorganisms are present as a result of unique events such as water damage. Wet carpets can promote microbial growth and cause odor problems (Anderson *et al*., 1982).

Since the 1970's, many studies related to microorganism growth on the carpet have been published. Macher reported that four broad groups of microorganisms were isolated from carpet dust: mesophiliic and thermophilic bacteria like *Staphylococcus aureus*, and moderately hydrophilic and xerophilic fungi, like *Aspergillus niger* (Macher, 2001). Bakker and Faoagali investigated the effect of carpet on the number of microbes in a hospital environment. They found there was no difference in the types of organisms isolated from the air above a floor with carpet and a floor without carpet. They reported that one source of microorganisms in carpets was air, and the growth of microbes in carpet was related to the frequency of use and the quality,

intensity and frequency of cleaning (Bakker and Faoagali, 1977). Anderson R.L. et al. measured higher microbial counts per square inch for the floor with carpet than for the bare floor. He also found that air above carpeting contained more consistent concentrations of organisms than the air above bare flooring (Anderson *et al*., 1982). All these studies have indicated that room carpeting should be considered as a potential reservoir of microorganisms.

Transfer of Microorganisms from Carpet to Human Skin

Generally speaking, microorganisms have four transmission routes into the human body. The first is inhalation. Respiratory pathogens can enter the body through this pathway via droplet infections and aerosols. The second route is the transfer of microorganisms through direct contact. The microbes transferred to the human skin can enter, and be disseminated through the body if the physical barrier (skin) is broken. The third transmission route is arthropod borne. The entry of microorganisms is through an insect bite/wound via saliva injection, defecation or regurgitation into the wound. The last route is by ingestion (Prescott, 1996). For the microorganisms on the carpet, the most probable transmission route to the human body is direct contact.

The Carpet and Rug Institute (Dalton, GA) is currently funding a study at the University of Georgia to investigate the transfer of viable microorganisms from carpet to skin and skin-like materials (Annis and Leonas, 2006). To date, the following generalizations can be made: 1) the bacteria (*E.coli* and *S.aureus*) transfer more readily than fungi (*A.niger* and *C.herbarium*), 2) more *E.coli* is transferred than *S.aureus,* and 3) 75% of the time, the slide transfer method results in a higher microorganism transfer than the compression method (Leonas and Annis, 2004). A Materials Evaluator that simulates contact transfer from contaminated carpets has been

developed in this study. This is a mechanical transfer method from carpet to other fabrics. The variables, especially pressure, time and pattern of transfer, are better controlled when using the materials evaluator as compared to the human subject transmission method (Leonas and Annis, 2004).

Transfer of Microorganisms from Fabric to Skin and Other Textile Materials

Since the 1970's, many research studies related to the bacterial transfer from fabrics to human skin and from fabrics to other textile materials have been published in the medical journals (Marples and Towers, 1999; Satter *et al*., 2001; Mackintosh and Hoffman, 1994; Slayton *et al*., 1998). These studies indicated that the microbial contaminated surfaces might play a role in the spread of bacteria, and then disease (Rusin *et al*., 2002). For example, the bacteria may transfer from the hospital bedding and nurse's uniforms to the hand, and then cause the infection of both multiple patients and nurses. Numerous tests have been used to investigate this contact transfer. Some variables such as surface type, bacterial species, moisture level, pressure, friction, and inoculum size have been discovered to influence the bacterial transfer from one surface to the other.

Marples and Towers (1999) established a laboratory model for the investigation of contact transfer of *Staphylococcus saprophyticus*. The model was based on grasping a fabric-covered bottle contaminated with the bacteria, then grasping a sterile fabric-covered bottle, rinsing the contaminated fabric, plating the microorganisms, incubating and then counting the organisms transferred. The model showed more bacterial transfer when the moisture of the donor fabric was high (Marples and Towers, 1999).

Mackintosh and Hoffman (1994) measured the ability of the organisms to transfer from the contaminated fabrics to hands, from hands to sterile fabrics, and their ability to survive on the skin of hands. The results showed the difference of transfer was due to the bacterial species. *Staphylococcus saprophyticus* transferred well to the hand but not as well from hand to fabrics as the other species. It survived well on skin. *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Serratia macrescens* transferred moderately well and also survived on the skin. These results were different from those obtained with *Escherichia coli* and *Streptococcus pyogenes*. They were transferred well from donor fabric but did not survive well on the skin (Mackintosh and Hoffman, 1984).

Sattar *et al*. (2001) used *Staphylococcus aureus* as a target bacterium to develop a method to transfer the bacteria from fabrics to hands and other fabrics and determine the number of bacteria transferred. In this research, they found bacterial transfer from moist donor fabrics to recipients with moisture was always higher than that to and from those which were dry. They also reported that bacterial transfer from cotton blend fabric (cotton/polyester 65/35) was consistently higher than that from a 100% cotton fabric. Two transfer methods, compression and slide, were investigated and compared. Both of the methods simulated the microbial transfer through direct contact. It was reported that sliding increased the level of transfer from fabrics to finger pads by as much as five fold when compared with the compression method (Sattar *et al*., 2001).

Rusin *et al*. (2002) investigated the surface-to-hand and finger-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria and phage. This study also compared the impact of the surfaces, smooth vs. porous, on the microbial transfer. The results showed that the bacterial transfer from the material with a porous surface (such as sponge) was

less than that from a smooth surface. There were many deep crevices in the porous surface. The microorganisms could reside in these crevices and became less accessible to the human hand. However, a hard smooth surface like stainless steel did not offer these crevices for microorganisms to hide. Therefore the transfer of microorganisms through direct contact was much higher from the smooth surface than that from the porous surface (Rusin *et al*., 2002). The carpet can be seen as a material with porous surface because it has a three dimensional structure. The microorganisms are not always at the surface of the carpet. They are also distributed throughout the depth of the carpet and sometimes concentrated at the top or at the base of pile yarns. If increased pressure is applied when touching the carpet, the hand has more opportunities to contact the microorganisms below the surface, and the transfer is increased (Leonas and Annis, 2004).

There are many other research studies related to the transfer of bacteria and viruses (Montville and Schaffner, 2003; Noskin et al., 1995; Lu and Fenske, 1999; Brown *et al*., 1980). In these studies, exposure methods like hand press, hand drag, wipe, polyurethane coated rollers, and a vacuum system were developed.

Adhesion of Microorganism to Surfaces

According to Leonas and Annis, the transfer of microorganisms from carpet to skin and skin-like materials does occur, which is a potential danger to human health (Leonas and Annis 2004). Therefore, it is important to decrease this transfer.

The transfer of microorganisms is related to microbial adhesion to surfaces and its release from these surfaces. Salerno et al. reported that electrostatic and hydrophobic forces were generally recognized as important factors in bacterial adhesion. Electrostatic forces arise due to

charge groups on bacteria or the substrate surface. These forces can be attractive or repulsive, depending on the type of charge of the substrate and bacteria. Hydrophobic forces include acid–base interactions and Van Der Waals forces. These forces are related to the surface characteristics of the bacteria and substrate (Salerno *et al*., 2004).

Some reported research has shown that the surface properties of materials, particularly with respect to their surface energy and wettability, are determinants of initial cell spreading and adhesion to these surfaces. Simple linear relationships may not hold between relative surface energy and cell-substrate interactions. However, the surface energy seems to correlate to some biological responses, like the number of adsorbed cells, the strength of cell adhesion and the spread area of individual adsorbed cells (George *et al*., 2003; Perzon *et al*., 2004).

Bacterial properties which may influence adhesion are: 1) surface charge of bacterial cells; 2) fimbriae or pili; 3) capsule and slime polysaccharides; 4) the bacterial cell wall; and 5) bacteria with stalks and holdfasts (Sadamoto *et al*., 2004). The attachment and adsorption of some fungi are different from those of bacteria. The actual penetration by hyphae into an "open" substrate may serve to anchor the organisms to surfaces (Berkeley *et al*., 2004).

The electrostatic properties and hydrophilicity of the bacterial cells are the two most important microbial characteristics which influence microbial adhesion and release. Most bacterial cell surfaces possess a net negative electrostatic charge because of ionized phosphoryl and carboxylate substituents on the outer cell envelope which are exposed to the extra cellular environment (Robert A. and Freitas Jr., 2003). The peptidoglycon in the cell wall of gram positive bacteria influences the surface electro negativity through the phosphoryl groups located in the teichoic and teichuronic acid residues and unsubstituted carboxylate groups. However the peptidoglycon of gram negative bacteria is sequestered within the periplasmic space inside the

outer membrane. Therefore, it is not exposed to the extracelluar environment. Negative electrostatic surface charges in these microorganisms are due to the phosphoryl and 2-keto-3-deoxyoctonate carboxylate groups of lipoplysaccharide located in the outer leaflet of the outer membrane. Some surface layers found external to the cell walls of bacteria are also known to affect cell surface charge properties. For example, extra cellular polysaccharides, which may exist as relatively compact capsules attached to the cell surface, or as diffuse slime layers only loosely associated with the cell surface, are typically acidic in nature (Wilson *et al*., 2001). They impart a negative charge to the cell surface.

Some bacteria are positively charged at physiological pH, for example, *Stenotrophomonas maltophilia* 70401 (Jucker *et al*. 1996). The positive charge probably originates from proteins located in the outer membrane. The electrostatic properties of bacteria can be altered in the presence of the chloride salts of heavy metals, like Cd, Cr, Cu, and Ni. The hydrolyzed forms of these metals bind on the cell surface and alter the net charge of the cell. This change in charge could affect various physiological functions of the cell, as well as its interactions with other cells and inanimate particulates in the environment (Collins and Stotzky, 1992).

So far there is no method for directly determining surface charge. Net cell surface charge can be assessed on the basis of zeta potential which is the electrical potential of the interfacial region between the bacterial surface and the aqueous environment. Zeta potential can be estimated by measuring cellular electrophoretic mobility in an electric field. The currently existing methods include microelectrophoresis, electrostatic interaction chromatography, aqueous two-phase partitioning, isoelectric equilibrium analysis, and electrophoretic light scattering. Among all these methods for estimating zeta potential, the technique of

electrophoretic light scattering was the most advanced. It offers the measurement distinct advantages in accuracy, measurement time, and ease of use (Wilson *et al*., 2001).

The hydrophobic and hydrophilic properties of bacteria are also determined by the nature of the cell surface. The bacterial cell envelope consists of acidic mucopolysaccharides. Hydrophilic sites are composed of positively charged amino groups and negatively charged carboxyl-, phosphate-, and guanidyl-groups. Lipids and lipopolysaccharides represent the hydrophobic sites of the bacterial cell surface. According to Stoderegger and Herndl, the hydrophilicity in gram-negative bacteria was much higher than that in gram-positive bacteria (Stoderegger and Herndl, 2004).

A number of methods for investigating hydrophilic and hydrophobic properties of bacteria have been reported in the research publications. These methods include binding of hydrocarbon and fatty acid to cells and cell components, measurement of the force required to remove hydrocarbon-bond cells, partitioning of bacteria in aqueous polymer two-phase systems, hydrophobic interaction chromatography, and contact angle measurements of dried cell layers (Rosenberg *et al*., 1980).

Microbial Transfer Test Method

There are currently no standard methods to test the transfer of microorganisms from carpets to human hands or textile materials. However, there is a standard method, AATCC Test Method 174-1999; Antimicrobial Activity Assessment of Carpets (Technical Manual of the AATCC, 2002), which is used for evaluating the antimicrobial effectiveness of the carpets. Some procedures in this standard involving the extraction of microorganisms from the carpet and the quantification of the microorganisms from the carpet, can be used in the testing of microbial

transfer from carpets. In some previous studies, the microbial transfer from fabric and the survival of microorganisms on the fabric were investigated by using these procedures (Neely and Maley, 2000; Harrison, 2003; Scott and Bloomfield, 1990). The microorganisms were grown in nutrient broth at 37^{o}C. A certain volume of 18-24 hours broth bacterial inoculum adjusted 1-2 $\times 10^{5}$ Colony Forming Units / milliliter (CFU's/ml) was inoculated to the fabric surface evenly using a sterile pipette. After the transfer, the microorganisms were washed off from the receiving materials such as fingers or fabrics. Then a serial dilution was made and the solution was plated in nutrient agar. The CFUs were counted after appropriate incubation (Neely and Maley, 2000; Harrison, 2003; Scott and Bloomfield, 1990).

According to Wendy Dustman (personal communication) another method for applying microorganisms to carpet can be used. First, microorganisms are freeze-dried or centrifuged. Then the freeze-dried microorganisms or pellet formed after centrifuging is applied to the surface of carpet using an airbrush. The airflow can be adjusted to control the amount of microorganisms applied to the carpet.

The airbrush is an atomizer that sprays small particles by means of compressed air. It is widely used in car and truck murals and painting of crafts and t-shirts. The airbrush tool usually consists of a paint container and the airbrush gun. The air compressor and airbrush gun are connected with a tube. Once the air flow is turned on, the switch or knob on the airbrush can be depressed to let the air flow through and the paint can be forced by pressure from the container and out through the nozzle (Melspray Equipment Industry, http://melspray.com/info/airbrush.htm).

There are two types of airbrushes, single action and double action (Figure 2.4). The single action airbrush is the simplest and least expensive type of airbrush available. It has a

single on/off trigger for the air supply. There is no control over the amount of air drawn into the airbrush or the ratio of air to paint while in use. The double action airbrush has a trigger that gives a complete control over the amount of air flowing through the airbrush and the amount of paint. Some models have an adjustable screw or ring that fixes the trigger at a certain point so that it can be used as a fixed double action airbrush. This allows for the presetting of a fixed spray volume (Melspray Equipment Industry, http://melspray.com/info/airbrush.htm).

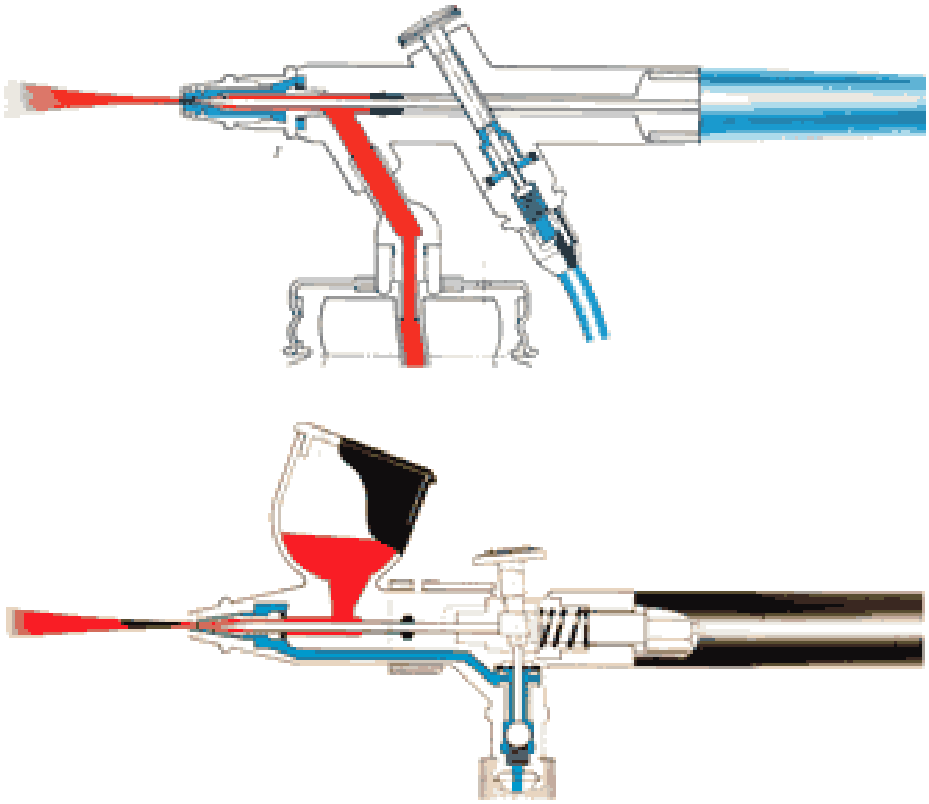


Figure 2.4 Single action airbrush (top) and double action airbrush (bottom) (Melspray Equipment Industry, http://melspray.com/info/airbrush.htm)

A standard test method of dermal transfer of microorganisms from carpet is currently being developed by Annis and Leonas (Annis and Leonas, 2006). The test method provides a

procedure to measure the transfer of microorganisms from a carpet surface to a skin substitute using a mechanical transfer tester. By substituting an artificial surface for human skin, measurements can be made with a high degree of precision and can be made using pathogenic microorganisms (Annis and Leonas, 2006). The human exposure to microorganisms is reduced using the mechanical tester. During the whole testing process, the operator does not need to contact the microorganisms directly. Therefore, the risk of getting microbial infection and disease can be reduced.

Chemical Finishing

Chemical finishing is defined as the use of chemicals to achieve a desired fabric property. Several chemical finishes commonly used by the industry are applied to the carpet resulting in a change in surface energy. Repellent finishes decrease the carpet's surface energy and can impart water and even oil repellency to the carpet. The applications of hydrophilic finishing chemicals result in the increase of the surface energy and hydrophilicity of the carpet.

Mechanisms of Repellency

The critical surface energy of a solid is defined as the surface tension of a liquid that just completely spreads on the surface. The relationship between the surface energy of the solid and the surface tension of the liquid determines whether the solid is repellent to that liquid. Liquids with surface tensions below the critical surface energy of a solid will spread on the surface of the solid. When a drop of liquid on a solid surface does not spread it appears to be constant on the surface and exhibits an angle θ which is called contact angle. The relationship between contact angle (θ) and the surface tension are described by Young's Equation: $\gamma_{SV}-\gamma_{SL}=\gamma_{LV}COS\ \theta$, where

γ_{SV} represents the surface tension at the interface of the solid and vapor, γ_{SL} represents the surface tension at the interface of the solid and liquid, and γ_{LV} represents the surface tension at the interface of the liquid and vapor (Figure 2.5). When θ is greater than 90^o it is relatively difficult for the liquid to spread on the surface and the solid is generally considered as repellent to this liquid. When θ is less than 90^o the liquid can spread on the surface and the solid is generally considered as non-repellent to this liquid (Schindler and Hauser, 2004).

There are two kinds of contact angles, advancing contact angle and receding contact angle, known as the dynamic contact angles. During the process of contact angle measurement, the sample is immersed to a set depth in the water and then is reversed. The advancing contact angle is calculated from the data generated as the sample advanced into the water and the receding contact angle is calculated from the data generated as the sample retreated from water. The advancing contact angle is considered as the "wetting angle" because the liquid is ready to wet the sample during the measurement. The receding angle is on the opposite, which is considered as the "de-wetting angle" because the sample is removed from the liquid.

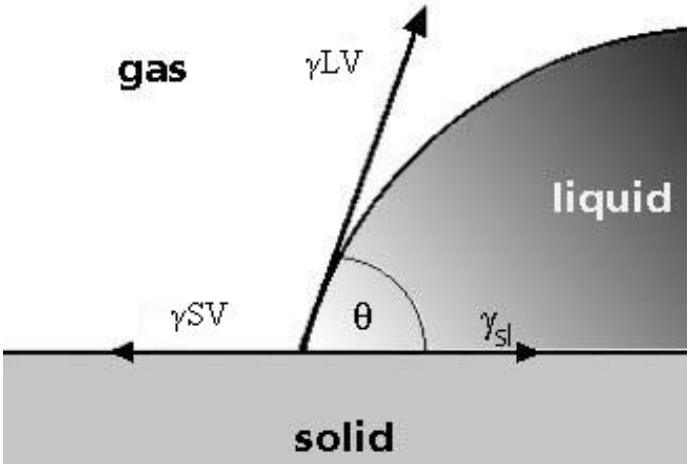


Figure 2.5 Contact angle of liquid on the solid surface
(http://ciks.cbt.nist.gov/~garbocz/captrans/node12.html)

Some fabrics are made of inherently repellent (hydrophobic) fibers and have low surface energies. The inherently repellent fibers include olefin and polyester. These fabrics have repellent properties without adding chemical finishes. However, fabrics made of hydrophilic fibers, such as cotton, require the addition of repellent finishes to achieve repellent properties. There are different methods to impart low energy surfaces (repellent finishes) to textiles. The first method is the mechanical incorporation of the water repellent chemicals in or on the fiber and fabric surface, in the fiber pores, and in the spacing between the fibers and yarns. The other method is the chemical reaction of the repellent material with the fiber surface (Schindler and Hauser, 2004).

Mechanisms of Hydrophilic Finishing

The mechanism of hydrophilic finishing is similar to that of repellent finishing. The hydrophilic finishes increase the surface energy instead of decreasing it. The chemicals for this finishing contain many –OH or –COOH groups in their molecular chain which adsorb water molecules. Thus, the hydrophilicity is increased.

Finishing Application Method

The application of finishes to textile materials is usually completed through a wet process. There are two methods of wet chemical processing primarily used in textile finishing: pad-dry-cure and exhaustion. The most commonly used finishing application method is the pad-dry-cure method. The chemicals are applied to the fabrics with a padder. The fabrics are then dried and cured. The exhaustion method includes immersing the textile samples into a chemical solution, removing the excess liquid, drying and curing the samples. Carpet has a three dimensional

structure. When it passes through the padder the pile structure will be crushed resulting in the non-uniform distribution of chemicals. Therefore, the exhaustion method rather than pad-dry-cure method is commonly used for carpet finishing. There is also a third method that is used occasionally in the chemical treatment of carpets that involves spraying the chemicals evenly on the carpet surface and then drying and curing the carpet at high temperatures.

Laser Scanning Confocal Microscopy

Laser Scanning Confocal Microscopy (LSCM) is a valuable tool for obtaining high resolution images and 3-D reconstructions of a variety of specimens. Today it has been extensively used in numerous applications of cellular biology. LSCM has had limited uses in the textile area. In 1999, Leonas discussed the history, principles and advantages of LSCM, and studied the feasibility of using LSCM to investigate the three dimensional structure of fabric (Leonas, 1999). In the same year, Huang and Leonas used the LSCM to locate particles and tagged microorganisms in surgical gowns and drapes during their transmission (Huang and Leonas, 1999). In 2005, Shen proved that LSCM could be used to optically section the fabrics to determine the depth of particle penetration (Shen, 2005). Locating dye diffusion is the other application of LSCM in the textile area. In 2001, McFarland et al. observed the dye, disperse blue 3, in a nylon 6,6 film with LSCM and developed a new technique based on LSCM for obtaining concentration profiles at much higher concentrations where the effects of absorption of the incident light cannot be ignored (McFarland, E.G. et al. 2001).

LSCM has several advantages over the conventional optical microscopy. They are: (Shen, 2005)

1. Contrast and resolution are improved since out of focus information is greatly reduced,

2. Images can be acquired as single planes to produce three-dimensional representations of serial optical sections,
3. LSCM can use a variety of excitation illuminations and change the scan pattern,
4. Non-destructive examination of surface topography can be done using LSCM.

However, LSCM has two disadvantages: (Shen, 2005)

1. The real time imaging is impossible because the raster pattern is established by physically moving mirrors,
2. The operator can only see the resultant image as it is presented on the CRT.

The basic concept of confocal microscopy was originally developed by Martin Minsky in the mid-1950s (patented in 1961) when he was a post doctoral student at Harvard University. However, the lack of an adequate light source prevented full development of the confocal microscope at that time. In the late 1960s M. David Egger and Mojmir Petran fabricated a multiple-beam confocal microscope by using a spinning (Nipkow) disk to examine unstained brain sections and ganglion cells. In 1973 Egger developed the first mechanically scanning confocal laser microscope, and published the first recognizable images of cells. During the late 1970s and 1980s, advances in computer and laser technology and new algorithms for digital manipulation of images led to a growing interest in confocal microscopy. The first commercial instruments appeared in 1987. In the 1990s, advances in optics and electronics offered more stable and powerful lasers, light-efficiency scanning mirror units, high-throughput fiber optics, better thin film dielectric coatings, and low noise detectors (Claxton *et al*., 2005).

Modern confocal microscopes can be considered as completely integrated electronic systems, a computer, and several laser systems combined with wavelength selection devices and a beam scanning assembly.

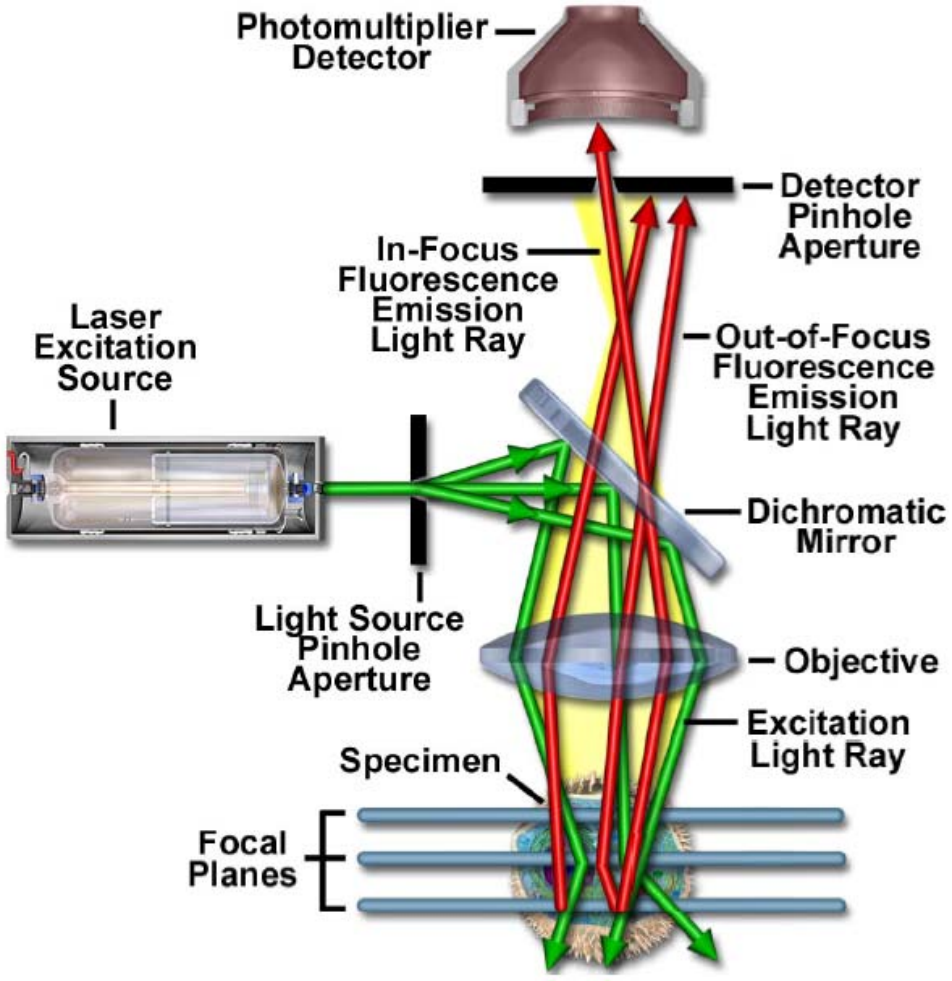


Figure 2.6 Schematic diagram of the optical pathway and principal components in a LSCM (Claxton *et al*., 2005)

The figure 2.6 shows the optics of a typical laser scanning confocal microscope. Coherent light emitted by the laser system passes through a pinhole aperture and is reflected by a dichromatic mirror. The light then passes through the objective lens and is focused on the specimen. As the laser is reflected by a dichromatic mirror and scanned across the specimen in a defined focal plane, secondly fluorescent light emitted from points on the specimen pass back

through the dichromatic mirror and is focused at the detector pinhole aperture as a confocal point (Claxton *et al*., 2005).

Laser Scanning Confocal Microscopy (LSCM) can be used to investigate the distribution or location of the microorganisms in the carpet structure, especially when the microorganisms have Green Fluorescent Protein (GFP). The green fluorescent light from the protein inside the bacteria observed in the LSCM images can help to locate the bacteria in the carpet.

CHAPTER 3

MATERIALS AND METHODS

MATERIALS

Carpets

Two tufted carpets obtained from Mohawk Industries were used in this study. One carpet is cut pile, and the other is loop pile. Characteristics of these two carpets are shown in Table 3.1.

Table 3.1 Carpet characteristics

Characteristics	Multilevel loop pile carpet	Cut pile carpet
Fiber content	Nylon 6, 6	Nylon 6
Pile height (inch)	0.175	0.237
Stitch per square inch	100	90
Mass per unit (oz/yd2)	61.83	62.16
Thickness (inch)	0.287	0.336

Microorganisms

Four microorganisms, including three bacteria and one fungus were chosen for use in this study. They are *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae*, and *Aspergillus niger*.

The first three microorganisms are bacteria. *Staphylococcus aureus* is a potentially pathogenic gram positive bacterium which may cause a wide range of infections such as staphylococcal scalded skin syndrome, impetigo, toxic shock syndrome, and pneumonia. *Escherichia coli* is gram negative. It can cause urinary tract infections, neonatal meningitis, and intestinal diseases. *Klebsiella pneumoniae* is also a gram negative bacterium. It can cause hospital-acquired urinary tract infections or burn wound infections. *Klebsiella pneumoniae* has a

capsule around the cell which makes its cell surface characteristics different from the other two bacteria used in this study. *Aspergillus niger*, is a spore forming fungus. It is less likely to cause disease than some other *Aspergillus* species, but if large amounts of the spores are breathed in, serious lung disease, aspergillosis, may occur. The characteristics of these four microorgansims are reported in Table 3.2. All microorganisms used in this study are commonly found in carpet during its normal use (Leonas, 2004)

Table 3.2 Microorganisms specification

Microorganism	*Staphylococcus aureus* (Gram positive bacteria)	*Escherichia coli* (Gram negative bacteria)	*Klebsiella pneumoniae* (Gram negative bacteria)	*Aspergillus niger* (Fungus)
Size (μm)	0.5-1.0	1.0-3.0	1.0-3.0	4-20 (spores)
Shape	Round	Rod	Rod	Round spores and irregular hyphae
Extra layer	N.A.	N.A.	Capsule	N.A.

Receptor Materials

In this study the term receptor materials is used to identify either the fabrics or human skin which the microorganisms are transferred to from the carpet. Their characteristics are critical to the microbial transfer. Four different receptor materials were used in this research: bare fingers (human skin); PET/latex comfortwear® compression fabric, which was a double warp knit; 100% cotton knit fabric, which was a single jersey; and 100% cotton woven fabric which was a twill weave. The characteristics of receptor fabrics are presented in Table 3.3.

In the preliminary study the compression fabric was used as the primary receptor material. The transfer of microorganisms from carpet to compression fabric has been well studied (Leonas and Annis, 2004). The compression fabric was considered as the skin-like fabric and used as a control receptor fabric in this study. The transfer of microorganisms from carpets

to the compression fabric wrapped fingers and the bare fingers was performed by a single individual. The transfer of microorganisms from carpets to the compression fabric, the cotton knit fabric, and the cotton woven fabric was performed by the materials evaluator. The single jersey cotton fabric and twill weave cotton fabric are two commonly used fabrics for T-shirts and pants respectively. They were chosen to investigate the impact of different fabric construction on the microbial transfer from carpet.

Table 3.3 Characteristics of receptor fabrics

Variable		Compression fabric	Cotton knit fabric	Cotton woven fabric
Fabric count (stitches or loops per inch)	Thread count[a] (warp)	N.A. [c]	N.A.	52±1
	Thread count[a] (filling)	N.A.	N.A.	109±5
	Gauge[b]	52±6 (warp), 58±3 (filling)	30±5	N.A.
Yarn size	Twist/inch	19.6±2.1	0	0
	Fineness (Denier)	N.A.	282±16	522±19
Thickness (mm)		0.603±0.05	0.837±0.08	0.528±0.1
Weight (g/m^2)		276±6	206±9	242±10

a: thread count is for woven fabric; b: Gauge is for knit fabric; c: Not avaiable

Chemicals

Three chemicals were used in this study to change the surface characteristics of the carpet: Humectant A (Manufactures Chemicals, L.P.), Freepel^{TM} 1225 water repellent (Freedom Textile Chemicals Co.), and Sulfated 2-EH (60%) (Manufactures Chemicals, L.P.).

Humectant A is a blend of glycols and potassium acetate. Freepel^{TM} 1225 water repellent is the paraffin wax and cationic polymeric emulsifier. These two chemicals could alter the surface energy of carpet, which has been known to influence the microbial adhesion and release (George et al., 2003). Humectant A was expected to decrease the surface energy and Freepel^{TM} 1225 water repellent was expected to increase the surface energy.

Sulfated 2-EH is an anionic surfactant which could alter electrostatic property of the carpet. The electrostatic property of the carpet fiber influenced the adhesion and release of microorganisms, and then the transfer of microorganisms.

METHODS

Percentage Add-on of Finishes

The add-on level of the chemical finish is critical to impart the desirable properties to products. In the preliminary work three add-on levels, 3%, 6%, and 9%, were selected for the Humectant A and Freepel^{TM} 1225 treatments. Since both chemicals altered the surface energy of carpet, the contact angle of pile yarns, related to the surface energy of carpet, was measured to assess which add-on level offered the carpet desired repellent properties.

The concentration of chemicals necessary in the solution to achieve the desired add-on level was determined by using following formula:

$$g/l = \frac{add-on(\%)}{wet\ pick-up(\%)} \times 1000. \qquad 3\text{-}1$$

The wet pick-up was determined by the formula:

$$wet\ pick-up(\%) = \frac{wet\ weight - dry\ weight}{dry\ weight} \times 100 \qquad 3\text{-}2$$

In this study the wet pick-up was controlled at 100%. The concentration of each chemical was calculated according to the wet pick-up and desired add-on level. Concentrations of chemicals at 30g/l, 60g/l, and 90g/l were prepared to obtain 3%, 6%, and 9% add-on levels respectively.

The yarn treatment and contact angle measurement were performed as follows. The pile yarns were pulled out from loop pile carpet. Before they were treated with chemicals the latex materials that had adhered to the yarns were removed. The pile yarns were immersed into the

chemical solutions with the different concentrations as stated above. After ten minutes with constant agitation, the yarns were removed from the solution and centrifuged for 10 seconds to achieve 100% wet pick-up. Finally, the yarns were dried and cured at 100^{o}C for 60 minutes. The contact angle between de-ionized water and the treated yarns was measured using the Dynamic Contact Angle Analyzer manufactured by CAHN Instruments.

The percentage add-on level used for Sulfated 2-EH was 2%, which was recommended by the manufacturers.

Chemical Application

Carpet samples, cut into circular specimens with 40 mm or 130 mm diameter, depending on the use for finger transfer or mechanical transfer, were immersed in the chemical solution and constantly agitated for ten minutes. Then the carpet samples were removed from the solution and centrifuged in a washing machine for 5 seconds (for 40 mm samples) or 30 seconds (for 130 mm samples), to achieve a wet pick-up of 100%. The carpet samples were then dried and cured at 100^{o}C in a laboratory oven (Econotherm) for 90 minutes. The percentage add-on was controlled by the concentration of chemical in the solution.

Antimicrobial Test of Chemicals

One objective of this study was to determine if the number of microorganisms transferred from carpets to human skin and textile materials could be reduced after surface modification of carpets using chemical treatments. Some chemicals have antimicrobial properties that kill the microorganisms or inhibit their growth. In this study, it was critical that chemicals selected for carpet treatment did not kill the microorganisms, inhibit or enhance their growth. Otherwise, it

would be difficult to conclude if the change of microbial transfer was solely due to the change of carpets surface characteristics after chemical treatment. Therefore, the impact of chemicals on the microorganisms and their growth was evaluated to ensure that the chemicals selected had no influence on the microorganisms. Two different methods were used to evaluate the antimicrobial effectiveness of chemicals. One method was to measure the bacterial growth curve in the liquid media (minimal media broth for *Escherichia coli* and *Klebsiella pneumoniae,* and nutrient broth for *Staphylococcus aureus*) to determine if the chemicals encourage the microbial growth. The components of minimal media broth are presented in Table 3.4. The other method was to measure the antimicrobial activity of the treated carpets.

Table 3.4 Minimal media broth

Ammonium sulfate	2.0g
Potassium phosphate dibasic	14.0g
Potassium phosphate monobasic	6.0g
Sodium citrate	1.0g
Magnesium sulfate heptahydrate	0.2g
Dextrose*	5.0g
Water	1000g

* Dextrose was autoclaved separately and then added aseptically to the media

To measure the growth curve, the 24-hour bacterial culture and four 250ml flasks containing 40 ml liquid media (minimal media broth [MM] or nutrient broth [NB]) were prepared. Specified amounts of the Humectant A, Freepel^{TM} 1225 water repellent, and Sulfated 2-EH were added to the flasks. The concentrations of chemicals in the liquid media were the same as those used for carpet treatment. Three milliliters of 24-hour bacterial culture were transferred into the flask and the culture was mixed in thoroughly. The flasks with mixed bacterial culture and target chemicals were placed in the water bath at 37^{o}C to determine the effects on the growth and/or survival of the bacteria. The optical density was measured every 30 or 60 minutes thereafter. Standard plate counts were prepared every 60 minutes as well. The

growth curve of *Aspergillus niger* was not measured in this study because the optical density of *Aspergillus niger* culture was not available.

To test the antimicrobial activity of carpets after they were treated with chemicals, AATCC Test method 174, Part I-1999: antimicrobial activity assessment of carpets (AATCC, 2002) was used. Both treated and untreated carpets were cut into samples 25 × 50 mm in size. In accordance with the test method, one loop of the microbial culture was transferred to the surface of the sterile agar plate by making one long streak of approximately 75 mm in length cross the center of the plate. The carpet specimen was then gently pressed transversely across the inoculum streak to ensure intimate contact with the agar surface. The plates were then incubated at 37^{o}C for 24 hours. Growth was documented by photographing the inverted plates.

Microorganisms Application to Carpet

Microbial Culture Preparation

The microbial culture was prepared in accordance with standard microbiology procedures. Bacteria were grown in 200 ml nutrient broth for 18-24 hours. Each 25 ml bacterial culture was transferred to a 50 ml centrifuge tube. The tubes were centrifuged at 4000 r/min for 10 minutes to obtain a pellet of bacteria which formed at the bottom of the tube. The liquid was then decanted, leaving the pellet in the tube. One milliliter of nutrient broth was then added to each pellet containing tube to re-suspend the bacteria and form a concentrated bacterial culture. The concentrated bacterial cultures of eight tubes were combined and were prepared for application to the carpet. Ten-fold serial dilutions in 0.85% saline solution were made until the number of bacteria plated was valid (30-300 colony forming units/plate) to quantify the bacteria in the concentrated culture.

Fungal spores and hyphae were harvested from inoculated Potato Dextrose Agar plates. Ten milliliters of 0.1% sodium lauryl sulfate (SLS) were applied to the surface of fungi in the plates. A sterile loop was used to harvest the spores into the SLS solution. The SLS solution with spores and hyphae was ready for the application to the carpet. Ten-fold serial dilutions in 0.85% saline solution were made to determine the density of spores and hyphae.

Application of Microorganisms to Carpet

Before the application of microorganisms, the carpets were sterilized by exposing to UV light for 15 minutes on each side. After sterilization the carpet samples were placed into the empty petri dishes. The concentrated bacterial culture or fungal spores and hyphae solution was applied to the carpet surface using either the pipette method or airbrush method. The pipette method involved transferring microbial culture to the carpet surface evenly using a sterilized pipette. The airbrush method involved spraying the microbial culture to the carpet surface using airbrush equipment (1102 Dual Action Airbrush). During the airbrush process the air pressure was set at 10 psi and the spray time was 2 seconds for small carpet (40 mm) and 30 seconds for large carpet (130 mm). Each time about same amount of microorganisms were applied to the both loop pile and cut pile carpets. After application of the microorganisms by the pipette or airbrush method, the petri dishes with carpet samples inside were closed and placed into an incubator ($37\pm2^{o}C$). The transfer of microorganisms from carpets to fingers was performed after zero hours, six hours, twelve hours, twenty-four hours and forty-eight hours respectively. The advantage and disadvantage of two methods were investigated and compared in this study.

Transfer of Microorganisms and Determination of Transferred Microorganisms (Method Description)

Microbial Transfer from Carpet to fingers and Compression Fabric Wrapped Fingers

Two methods of transfer were investigated in this study: sliding and compression. Sliding transfer was performed by sliding the top finger pads on the middle and ring fingers, both bare and wrapped with the compression fabrics, over the inoculated area in the carpet (40 mm diameter) for 20 seconds at the pressure of 2.5±0.5 lbs (Leonas and Annis, 2004). The slide pattern is shown in Figure 3.1.

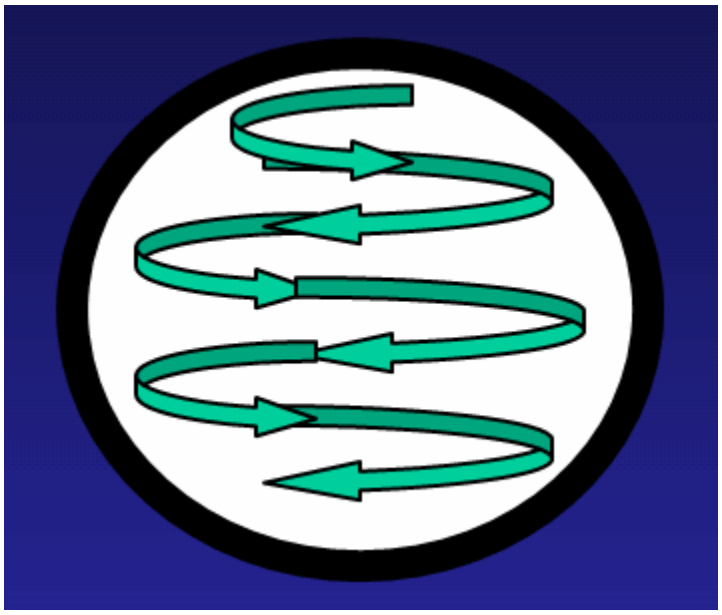


Figure 3.1 Slide pattern on the carpet

Compression transfer was completed by placing the top fingers pads of the middle and ring fingers, or with the compression fabrics wrapped on those two fingers, on the middle of inoculated carpet (40mm diameter) for 20 seconds at a pressure of 2.5±0.5 lbs (Leonas and Annis, 2004).

An area (0.5 square inch) of the receptor material from each finger was eluted separately by placing the contaminated part of the finger over a small test tube (13 X 100 mm) containing one milliliter of 0.1% SLS solution and inverting the tube 20 times within 20 seconds. The eluted solutions were combined and vortexed, and serial dilutions were made in 0.85% saline solution. One-tenth milliliter of each dilution was plated in the appropriate media and inoculated for the proper time for that microorganism. After incubation the colonies on the agar plates were counted to determine the number of microorganisms for each transfer (Leonas and Annis, 2004).

Microbial Transfer from Carpet to Receptor Fabrics

Microbial transfer from the carpet to the different receptor fabrics was investigated and compared. The materials evaluator developed by Annis and Leonas was used to perform this mechanical transfer.

The fabrics were scoured before being used for transfer. The scouring solution was composed of 1g/l Tween 80 and 1g/l sodium carbonate. The fabrics were immersed into the scouring solution and treated for 30 minutes at 70^{o}C. After that the fabrics were removed from the solution and rinsed four times with the same mixture at room temperature. Another plain water rinse was needed before the fabrics went to air dry.

The scoured fabrics were sterilized by the exposure to UV light for 15 minutes on each side. Then each fabric was mounted separately to a sterilized cylindrical specimen holder unique to the equipment. The receptor fabric was moved by the instrument across the carpet surface for 20 seconds at a pressure of 2.5 psi. After transfer, the specimen holder with the receptor material still attached was placed over the mouth of a sterilized jar containing 25 ml of 0.1% SLS solution. The lid was screwed tightly onto the jar, and the container was vigorously shaken for 30

seconds. The solution was then decanted off and serially diluted by 1/10 into a series of 0.85% saline solutions. One-tenth milliliter of each dilution was plated in the appropriate media and inoculated for the proper time for the specific microorganism. After incubation the CFU's were counted to evaluate the microbial transfer (Leonas and Annis, 2004).

Distribution of Microorganisms in the Carpet:

The previous study (Leonas and Annis, 2004) has shown that only those microorganisms on the pile yarns at or near the surface of carpet can be transferred to the human skin or other materials because the receptor does not go deeply into the carpet structure. Therefore, it is important to investigate the distribution of the microorganisms in the carpet structure, especially in the depth direction. LSCM was used to investigate the microbial distribution.

GFP *Escherichia coli* were the bacteria whose distribution in the carpet was observed under the microscope. Their green fluorescent signals can help to locate the bacteria in the carpet. GFP *Escherichia coli* were applied to the carpet using the pipette method and airbrush method respectively, as described before. The exposed carpet samples were placed in the incubator for 24 hours. After that, a few pile yarns from a single tuft were randomly picked up from the contaminated area of the carpet and placed on a glass slide, and then covered with a cover slip. Three different locations along the pile yarns, as shown in Figure 3.3, were examined using a Leica TCS SP2 Spectral Confocal Microscope at the Center for Ultrastructural Research at the University of Georgia. The locations were the surface or near the surface of carpet, middle of the carpet, and the base of carpet close to the primary backing. For each carpet sample, three specimens were observed.

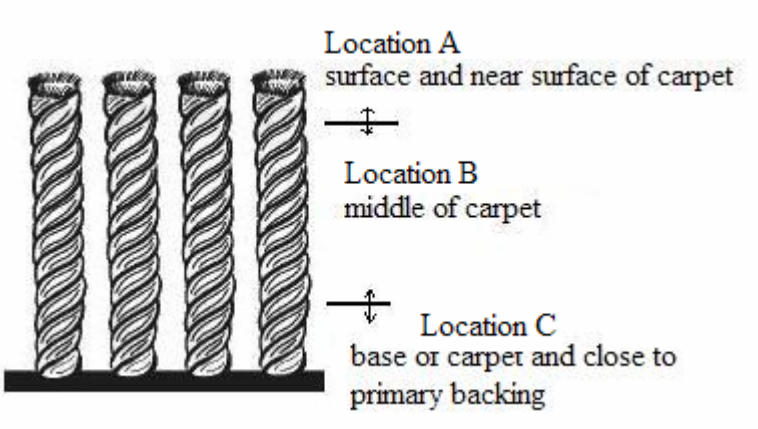


Figure 3.2 Three locations along pile yarns in the carpet for LSCM examination

Bacterial Hydrophobicity

The cell surface hydrophobicity, related to the adhesion of microorganisms to the solid surface, can be defined most simply as the relatively tendency of a microorganism to adhere to a non-polar material compared with that to water (Rosenberg, 1980). In this study, the hydrophobicity of three bacteria was measured in accordance with a method described by Rosenberg (Rosenberg, 1980). Bacteria (*Staphylococcus aureus, Escherichia coli*, and *Klebsiella pneumoniae*) were grown at 37^{o}C in the nutrient broth for 18-24 hours. The bacterial cultures were washed twice by centrifuging at 3500 r/min for 10 minutes, decanting the nutrient broth, and re-suspending in the Fish Gram-Pac Buffer (pH=7.41). Various volumes (0.5, 1.0, 1.5, 2.0, 2.5 ml) of hydrocarbon (hexadecane, octane, and dodecane) were added to the round-bottom test tubes (13 X 100 mm) containing 2 ml of washed bacterial culture. The mixture was vortexed for 120 seconds and allowed to stand for 15 minutes for the hydrocarbon phase to rise completely. The aqueous phase was then removed and transferred to the cuvettes (Elkay Ultra-Vu®, Disposable). The light absorbance at 600 nm was measured on the spectrophotometer (Spectronic® 20 Genesys^{TM}).

Surface Energy of Carpet

Since the chemical treatment altered the surface tension of the carpets in this study, it is important to measure this change on the carpet. There is no such standard test method for carpet. Therefore, an indirect measurement of the contact angles between water and the carpet pile yarns was used to evaluate the change of the surface energy.

The pile yarns were pulled out from loop pile carpet. Before they were treated with chemicals the latex materials adhered to the yarns were removed. The pile yarns were immersed into the chemical solutions. After ten minutes, under constant agitation, the yarns were removed from the solution and centrifuged for 10 seconds to achieve 100% wet pick-up. Finally, the yarns were dried and cured at 100^{o}C for 60 minutes. The percentage add-on used for the yarn treatment was determined in the preliminary work. The contact angles between de-ionized water and the untreated and treated pile yarns were measured using Dynamic Contact Angle Analyzer manufactured by CAHN Instruments.

Statistical Analysis

To study the impact of microorganism, carpet texture, and chemical treatment on the microbial transfer from carpets to human skin and fabric, analysis of variance (ANOVA) was used to test the significance of each variable. However, the use of ANOVA is based on the following assumptions: (1) the samples should be independent, (2) the populations from which the samples were obtained should be normally or approximately normally distributed, and (3) the variances of the populations should be equal. If any of the three assumptions did not fit with this study a generalized linear model was established instead and the analysis of deviance was used to test the significance of each variable. The generalized linear model can be used both for

dependent variables with non-normal distributions and for dependent variables which are nonlinearly related to the independent predictors. The statistical analyses were conducted using a 1% significant level.

When the p value for the analysis of deviance was less than 0.01, post hoc pairwise comparison was performed for the further significance analysis. If the data were in normal distribution the Dunnett's test was used. If the data were in non-normal distribution Wilcoxon's rank sum test was used in this study.

CHAPTER 4

RESULTS AND DISCUSSION

The results and discussion are presented in five sections as follows:

Section I: Determination of the chemical add-on level necessary to alter the surface energy of carpets;

Section II: Impact of chemicals on the microorganisms and their growth;

Section III: Distribution of GFP *Escherichia coli* in the depth of carpet based on different methods to apply the bacteria to carpets (pipette method and airbrush method);

Section IV: Microbial transfer from carpets to fingers (human skin), including the variables of time, microbial species, chemical treatments, transfer methods, carpet texture, carpet surface energy, and hydrophobicity of bacteria; and

Section V: Microbial transfer from carpets to different receptor materials.

Section I. Percentage Add-on of Chemical Treatment

The contact angles between water and carpet pile yarns with varying add-on levels of finishes are presented in Tables 4.1-4.4. The results include two contact angles, advancing contact angle and receding contact angle. The advancing contact angle was used for the statistical analysis in this study. An advancing contact angle of 90 degrees is the critical value to evaluate the interaction between the surface and the liquid. If it is greater than 90 degrees, the liquid is repelled by the surface, and if it is less than 90 degrees, the liquid is absorbed by the surface.

Table 4.1 Advancing contact angles between the water and the Freepel^{TM} 1225 treated carpet pile yarns

	Advancing contact angle (degrees)			
	Add-on Level 0%	Add-on Level 3%	Add-on Level 6%	Add-on Level 9%
Rep. #1	88.39	93.31	92.32	97.36
Rep. #2	88.37	96.46	97.35	95.41
Rep. #3	89.27	95.07	94.94	93.45
Rep. #4	89.35	96.40	94.84	97.85
Rep. #5	89.76	93.08	97.29	93.70
Rep. #6	88.72	95.73	93.59	94.67
Rep. #7	89.07	96.41	96.17	95.07
Rep. #8	87.98	97.32	94.81	95.99
Rep. #9	89.21	96.57	94.59	96.03
Rep. #10	88.49	97.03	95.03	94.62
Mean	88.86	95.74	95.10	95.42
Std. Dev.	0.56	1.48	1.54	1.43
C.V. (%)	0.63	1.54	1.62	1.50

Table 4.2 Receding contact angles between the water and the Freepel^{TM} 1225 treated carpet pile yarns

	Receding contact angle (degrees)			
	Add-on Level 0%	Add-on Level 3%	Add-on Level 6%	Add-on Level 9%
Rep. #1	73.77	84.55	84.62	76.63
Rep. #2	74.35	81.34	81.47	81.75
Rep. #3	75.35	82.66	83.98	81.86
Rep. #4	71.29	81.06	83.72	80.68
Rep. #5	76.96	82.12	81.86	77.32
Rep. #6	74.19	82.56	82.63	81.72
Rep. #7	73.25	83.11	82.72	82.38
Rep. #8	74.37	83.44	81.92	82.84
Rep. #9	75.07	82.16	82.04	83.01
Rep. #10	74.43	84.07	83.11	80.78
Mean	74.30	82.71	82.81	80.90
Std. Dev.	1.46	1.12	1.04	2.21
C.V. (%)	1.97	1.33	1.25	2.73

Table 4.3 Advancing contact angle between the water and the Humectant A treated carpet pile yarns

	Advancing contact angle (degrees)			
	Add-on Level 0%	Add-on Level 3%	Add-on Level 6%	Add-on Level 9%
Rep. #1	88.39	86.87	86.92	82.41
Rep. #2	88.37	87.82	86.44	83.53
Rep. #3	89.27	88.84	87.21	82.62
Rep. #4	89.35	86.13	86.42	80.42
Rep. #5	89.76	87.11	86.44	83.84
Rep. #6	88.72	86.96	85.98	82.43
Rep. #7	89.07	87.43	86.13	81.79
Rep. #8	87.98	88.14	87.33	83.08
Rep. #9	89.21	87.78	87.40	82.56
Rep. #10	88.49	86.99	86.89	82.41
Mean	88.86	87.43	86.72	82.51
Std. Dev.	0.56	0.73	0.50	0.95
C.V. (%)	0.63	0.84	0.58	1.15

Table 4.4 Receding contact angle between the water and the Humectant A treated carpet pile yarns

	Receding contact angle (degrees)			
	Add-on Level 0%	Add-on Level 3%	Add-on Level 6%	Add-on Level 9%
Rep. #1	73.77	75.78	77.60	68.82
Rep. #2	74.35	75.83	78.21	69.23
Rep. #3	75.35	74.33	77.58	69.39
Rep. #4	71.29	77.38	78.98	67.55
Rep. #5	76.96	77.38	78.32	69.48
Rep. #6	74.19	76.11	76.45	70.98
Rep. #7	73.25	77.08	77.38	71.24
Rep. #8	74.37	76.53	76.48	69.47
Rep. #9	75.07	75.98	77.15	69.58
Rep. #10	74.43	76.14	79.53	70.14
Mean	74.30	76.25	77.78	69.59
Std. Dev.	1.46	0.91	0.99	1.05
C.V. (%)	1.97	1.20	1.27	1.51

The advancing contact angle between water and carpet pile yarns was increased after the yarns were treated with Freepel^{TM} 1225 (Table 4.1). The mean advancing contact angle between water and the untreated yarns was 88.86 degrees, less than 90 degrees and the carpet pile yarns did not show repellency to water. When a 3% add-on level was applied to the pile yarns, the mean advancing contact angle increased to 95.74 degrees, greater than 90 degrees, indicating that the treated yarns became water repellent. When 6% and 9% add-on levels were applied to the pile yarns, the mean advancing contact angles were 95.10 degrees and 95.42 degrees, respectively, which were not significantly different from that with 3% add-on. Therefore, increasing the add-on level of Freepel^{TM} 1225 did not offer a greater advancing contact angle or better repellency than those yarns with an add-on level of 3%. So the 3% add-on level was selected for use in this study.

The advancing contact angle between water and carpet pile yarns decreased after the yarns were treated with Humectant A (Table 4.3). The higher the add-on level of the Humectant A, the less the advancing contact angle between water and the treated carpet yarns. At a 3% add-on level the mean advancing contact angle was 87.43 degrees, only about one and a half degrees less than that between water and the untreated pile yarns (control), 88.86 degrees. At a 6% add-on level the mean advancing contact angle was 86.72, only about two degrees less than the control. When the add-on level was increased to 9%, the mean advancing contact angle decreased to 82.41 degrees, 6.45 degrees less than the control. For Humectant A treatment, 9% was the lowest add-on level, of those used in this study, which resulted in a significantly different advancing contact angle ($p=3.7\times10^{-6} < 0.01$). Therefore, the 9% add-on level of Humectant A treatment was selected for use in this study.

The different advancing contact angles between water and carpet pile yarns after the chemical treatments indicated differences in the surface energy of carpets. The greater the advancing contact angle, the lower the surface energy of the carpet. In this study, the Humectant A treated carpet had the highest surface energy, followed by the untreated carpet, and then the Freepel^{TM} 1225 treated carpet (Tables 4.1 and 4.3). In addition, the Humectant A treated carpet had a hydrophilic surface and the Freepel^{TM} 1225 treated carpet had a hydrophobic surface. Therefore, different chemical treatments imparted to the carpet different surface energies and different surface characteristics. Figure 4.1 shows the interaction between the water and the treated carpets.

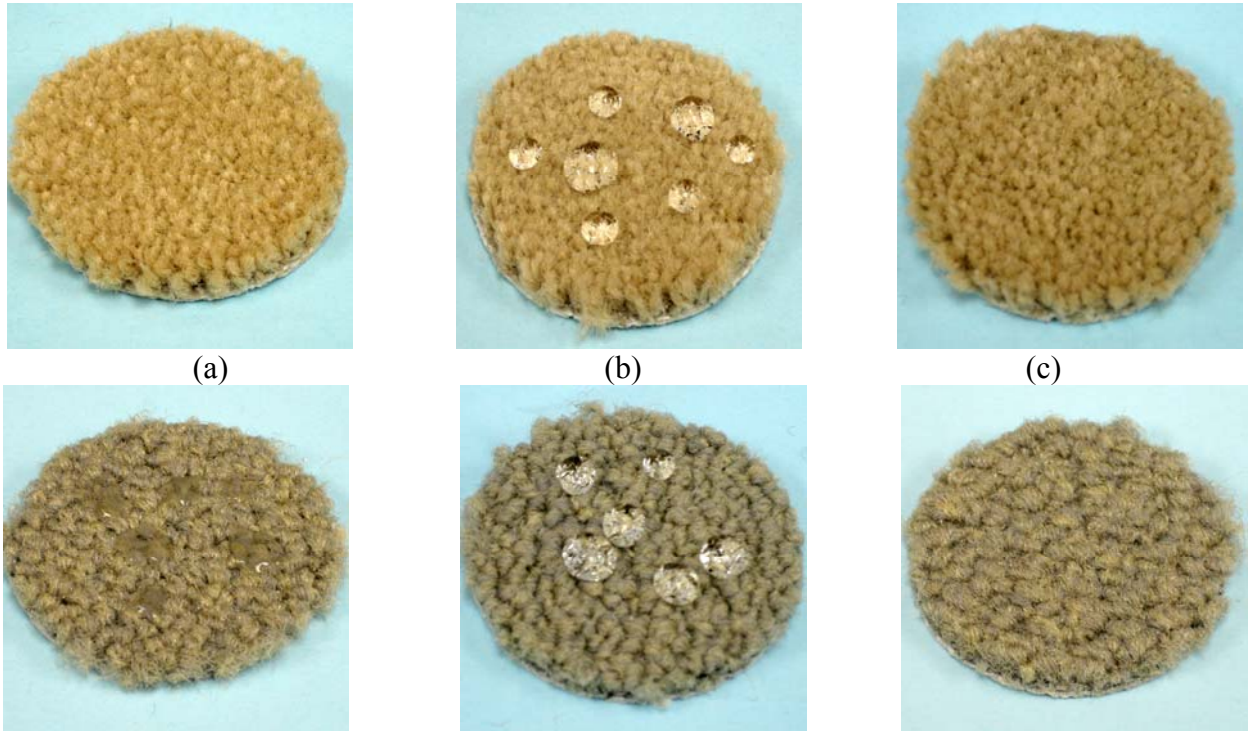


Figure 4.1 Interaction between water and treated cut pile carpet: (a) untreated cut pile carpet; (b) Freepel^{TM} 1225 treated cut pile carpet; (c) Humectant A treated cut pile carpet; (d) untreated loop pile carpet; (e) Freepel^{TM} 1225 treated loop pile carpet; (f) Humectant A treated loop pile carpet

Section II. Impact of Chemicals on Microbes and Their Growth

Growth Curve of Bacteria in the Media Containing Chemicals for Carpet Treatment

Optical density measurements were used to evaluate the influence of Humectant A on bacterial growth. However, the optical density of bacteria culture containing Freepel™ 1225 and Sulfated 2-EH could not be accurately measured in this study because the media became cloudy after the selected chemicals were added and the optical density was unreadable. Therefore, the bacteria counts in the media containing Freepel™ 1225 and Sulfated 2-EH instead of optical density were measured during 24 hours incubation using serial dilution. Figures 4.2, 4.4, and 4.6 are the growth curves of bacteria in the blank media (without chemicals) and the media containing Humectant A. They show the change of optical density of the bacterial cultures during a 24 hours incubation period. Figures 4.3, 4.5, and 4.7 are the growth curves of bacteria in the blank media (without chemicals) and the media containing Freepel™ 1225 and Sulfated 2-EH.

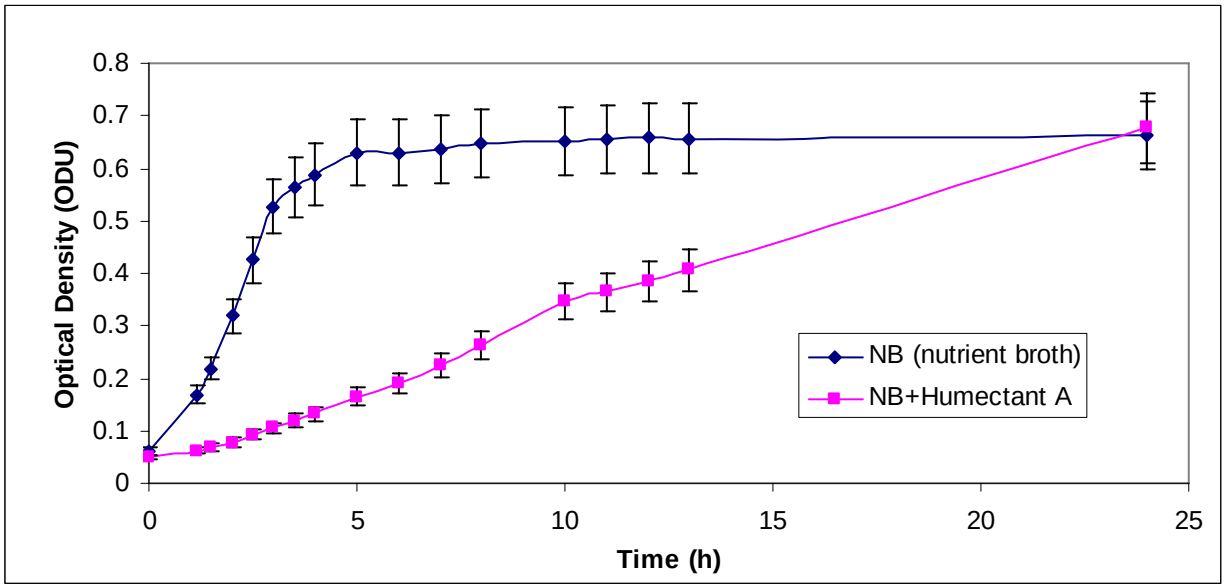


Figure 4.2 Growth curve of *Staphylococcus aureus* (mean of three replications) in the nutrient broth (NB): change of the optical density in 24 hours

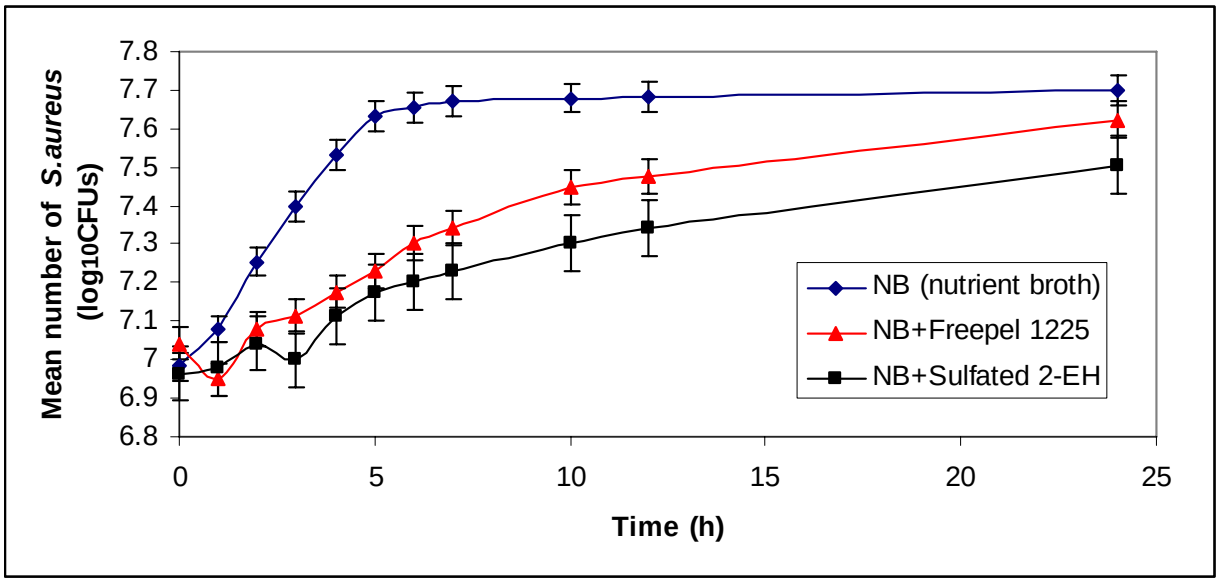


Figure 4.3 Growth curve of *Staphylococcus aureus* (mean of three replications) in the nutrient broth (NB): change of the bacterial amount in 24 hours (log plot)

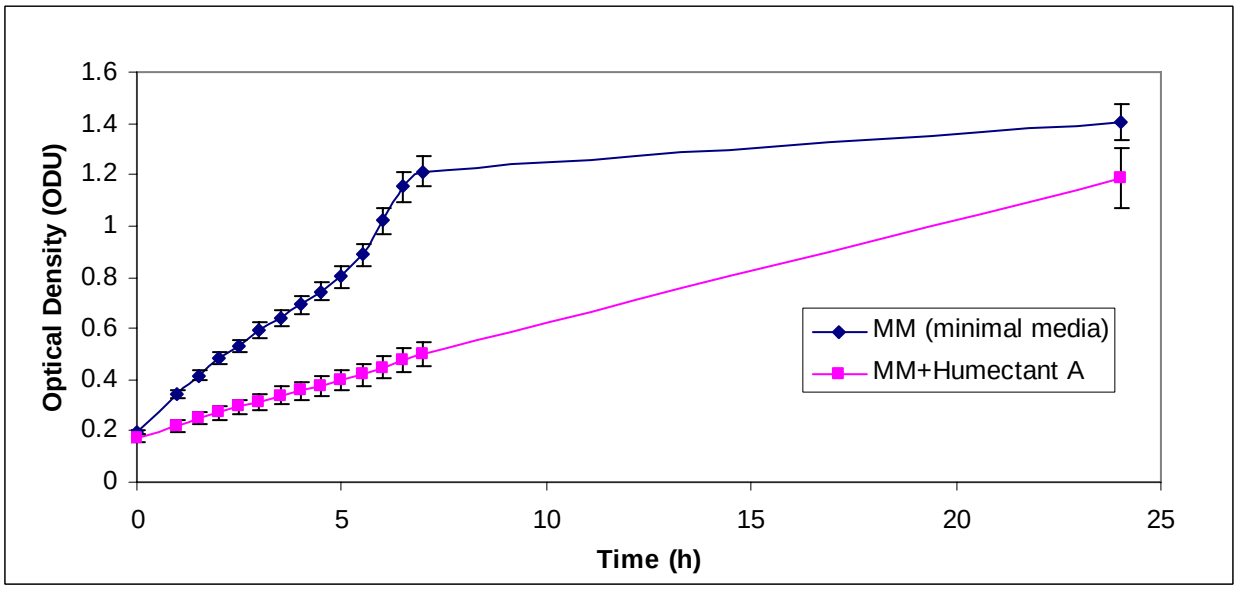


Figure 4.4 Growth curve of *Escherichia coli* (mean of three replications) in the minimal media (MM): change of the optical density in 24 hours

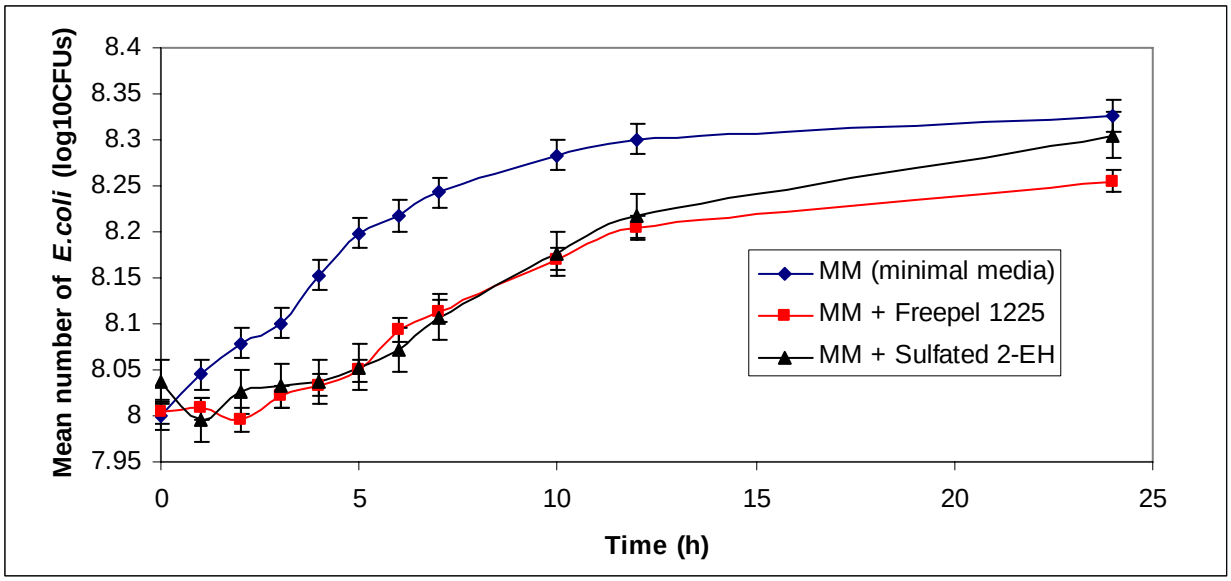


Figure 4.5 Growth curve of *Escherichia coli* (mean of three replications) in the minimal media (MM): change of the bacterial amount in 24 hours (log Plot)

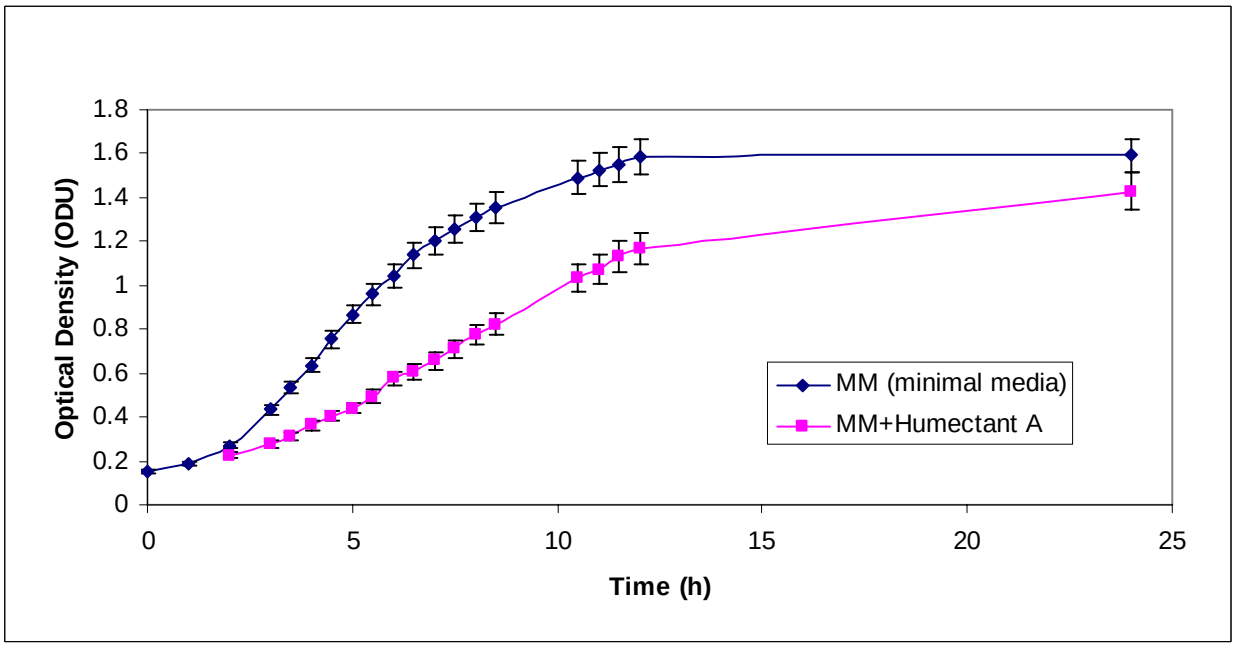


Figure 4.6 Growth curve of *Klebsiella pneumoniae* (mean of three replications) in the minimal media (MM): change of the optical density in 24 hours

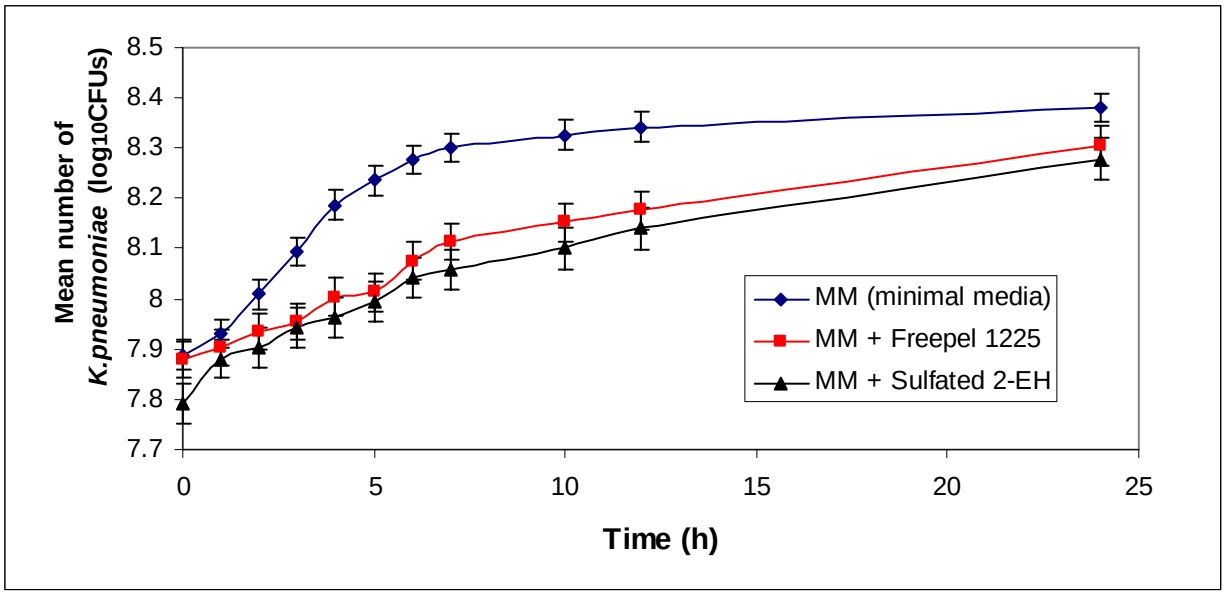


Figure 4.7 Growth curve of *Klebsiella pneumoniae* (mean of three replications) in the minimal media (MM): change of the bacterial amount in 24 hours (log plot)

The growth curve of *Staphylococcus aureus* in the nutrient broth (blank media) was different from that in the nutrient broth containing Humectant A used for carpet treatment (Figure 4.2). The optical density of bacterial culture in the nutrient broth increased much faster than that in the Humectant A containing nutrient broth in the first 5 hours of incubation. During this period, *Staphylococcus aureus* grew and divided at a constant rate. The optical density and the number of bacteria in the culture increased as time increased. After 5 hours the growth curve in the control media became horizontal which indicated the balance between cell division and cell death was reached. For the growth curve of *Staphylococcus aureus* in the Humectant A containing nutrient broth, an approximately linear relationship between optical density and incubation time was observed. The bacteria grew and divided at a constant rate. At the end of the incubation (24 hours) the bacterial cultures in both nutrient broth and Humectant A containing nutrient broth had the similar optical density at 0.7 ODU. This result suggests that

Humectant A was not providing nutrients to enhance the growth of *Staphylococcus aureus* nor did it have a prolonged antimicrobial effect on the bacteria. Adding Humectant A to the nutrient broth did not encourage the bacterial growth, but did slow down the growth rate. There may be several reasons for this. Humectant A may block a normal biochemical synthesis pathway and some other backup pathways may then become reactive. It may also affect the DNA replication and protein synthesis. Therefore, the growth rate of *Staphylococcus aureus* in the Humectant A containing nutrient broth was slower than that in the blank nutrient broth. Exactly, how the Humectant A retarded the bacterial growth rate is still unknown.

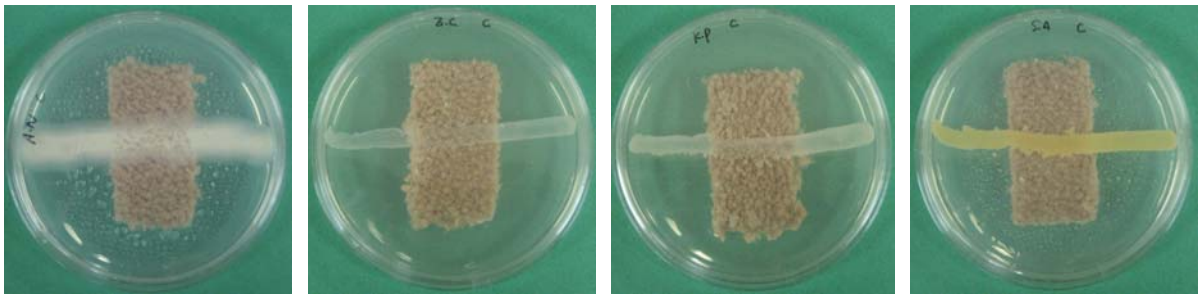
The growth curves of *Staphylococcus aureus* in the minimal media and the minimal media containing Freepel^{TM} 1225 and Sulfated 2-EH are shown in Figure 4.3. The number of bacteria in the minimal media containing chemicals (Freepel^{TM} 1225 and Sulfated 2-EH) increased more slowly than that in the minimal media without chemicals. However, the final number of CFU's in different media (with and without chemicals) after 24 hours incubation was not significantly different from each other ($p=0.074\text{-}0.084 > 0.01$). The results show that the chemicals, Freepel^{TM} 1225 and Sulfated 2 EH, did not have an antimicrobial activity to *Staphylococcus aureus*, inhibit the growth (in the overall 24 hour incubation period), or provide nutrients to enhance its growth.

The growth curves of *Escherichia coli* in the media with and without Humectant A, Freepel^{TM} 1225, and Sulfated 2-EH are shown in Figures 4.4 and 4.5. The growth curves of *Klebsiella pneumoniae* in the respective media are shown in Figures 4.6 and 4.7. These growth curves had the similar shapes and trends as those in Figure 4.2 and 4.3, which indicated that the optical density and bacteria counts of *Escherichia coli* and *Klebsiella pneumoniae* in the media containing chemicals increased more slowly in the 24 hours incubation than that in the media

without chemicals. Therefore, three chemicals, Humectant A, Freepel^{TM} 1225, and Sulfated 2-EH, neither were effective bacteriocides for *Escherichia coli* and *Klebsiella pneumoniae* nor enhance their growth during incubation. However, the growth rates of these bacteria were slowed and thus the chemicals (at the concentrations of conditions tested) did demonstrate a low level of bacteriostatic activity.

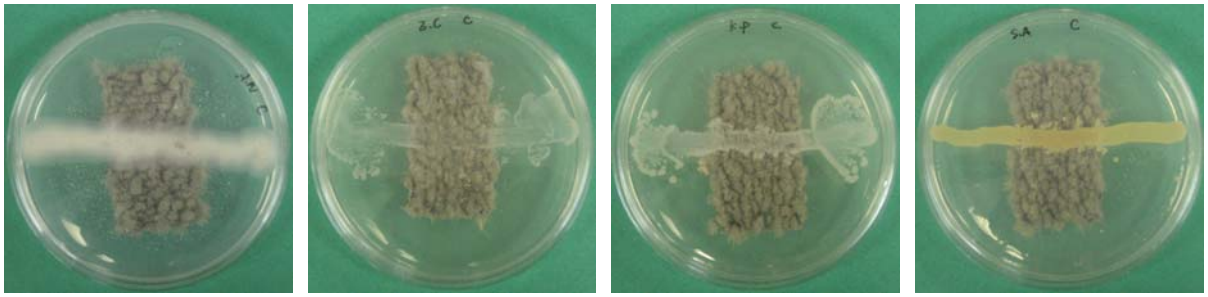
Antimicrobial Testing for Untreated and Treated Carpets

The results of antimicrobial activity assessment of carpets are presented in the images of Figures 4.8-4.15 and Table 4.5. If the chemicals inhibited or killed the microorganisms a clear zone of interrupted growth underneath and along the sides of the carpet specimen would be present. By examining the images in Figures 4.8-4.15, no such clear zones were found in the agar plates, which indicated that all three chemicals used in this study, Freepel^{TM} 1225, Humectant A, and Sulfated 2-EH, did not impart any antimicrobial activity to the carpets. The treated carpets did not inhibit or kill the target microorganisms used in this study.



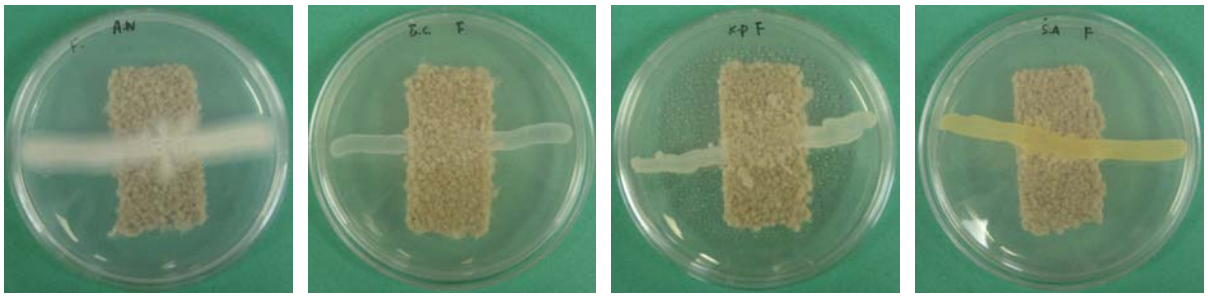
(a) (b) (c) (d)

Figure 4.8 Representatives of antimicrobial activity assessment of untreated cut pile carpet; (a) *A.niger*, (b) *E.coli*, (c) *K.pneumoniae*, (d) *S.aureus*



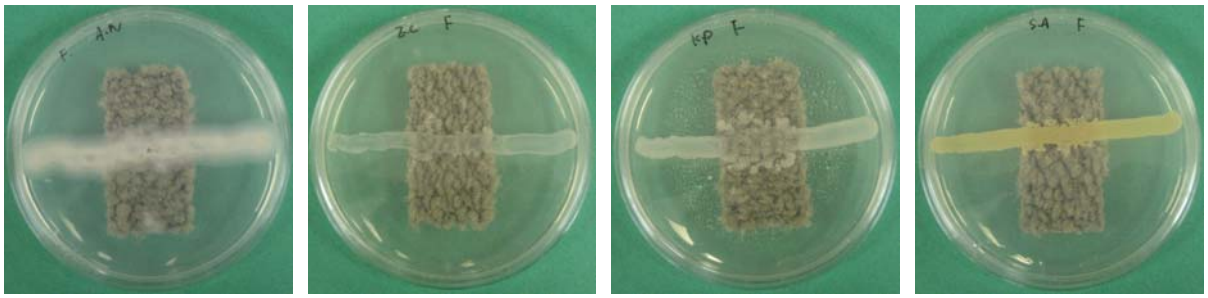
(a) (b) (c) (d)

Figure 4.9 Representatives of antimicrobial activity assessment of untreated loop pile carpet; (a) *A.niger*, (b) *E.coli*, (c) *K.pneumoniae*, (d) *S.aureus*



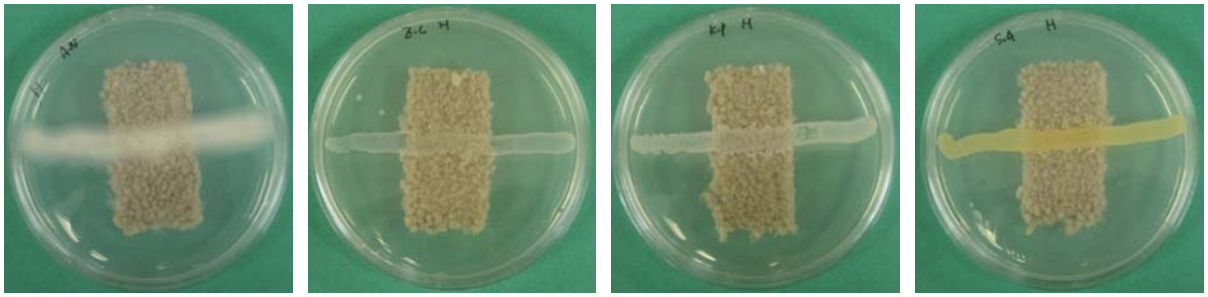
(a) (b) (c) (d)

Figure 4.10 Representatives of antimicrobial activity assessment of Freepel^{TM} 1225 treated cut pile carpet; (a) *A.niger*, (b) *E.coli*, (c) *K.pneumoniae*, (d) *S.aureus*



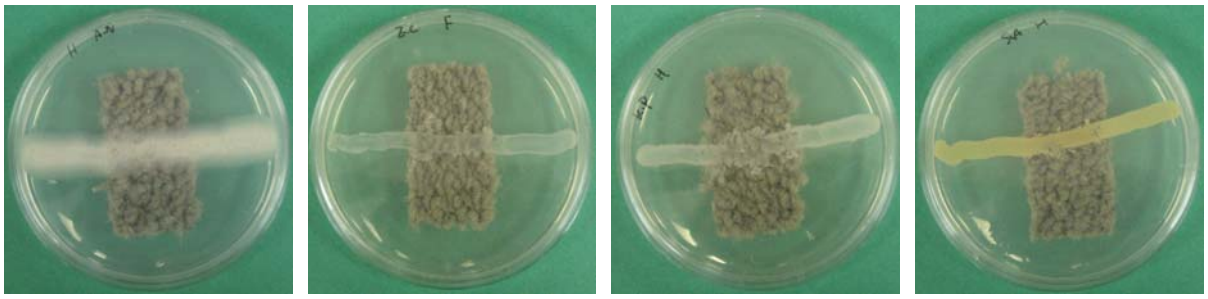
(a) (b) (c) (d)

Figure 4.11 Representatives of antimicrobial activity assessment of Freepel^{TM} 1225 treated loop pile carpet; (a) *A.niger*, (b) *E.coli*, (c) *K.pneumoniae*, (d) *S.aureus*



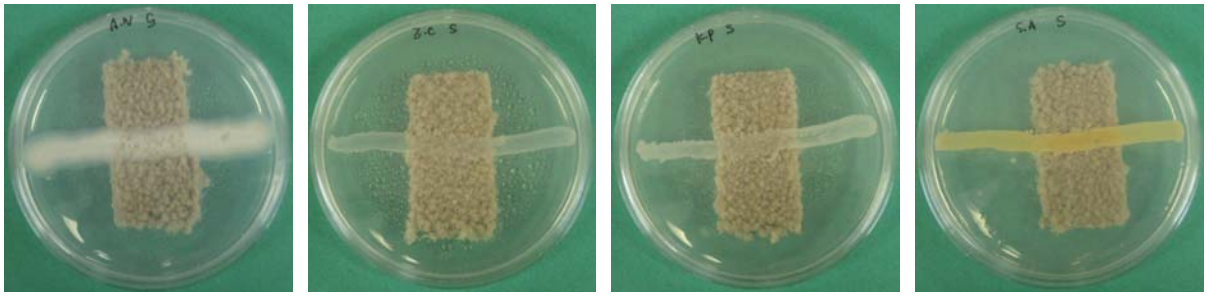
(a) (b) (c) (d)

Figure 4.12 Representatives of antimicrobial activity assessment of Humectant A treated cut pile carpet; (a) *A.niger*, (b) *E.coli*, (c) *K.pneumoniae*, (d) *S.aureus*



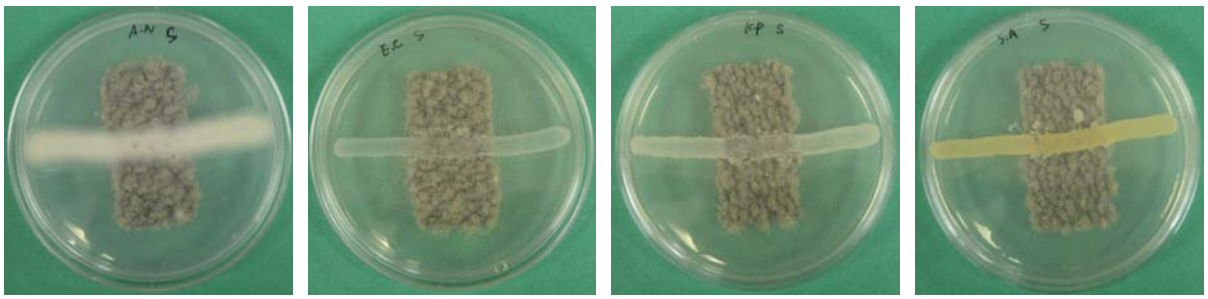
(a) (b) (c) (d)

Figure 4.13 Representatives of antimicrobial activity assessment of Humectant A treated loop pile carpet; (a) *A.niger*, (b) *E.coli*, (c) *K.pneumoniae*, (d) *S.aureus*



(a) (b) (c) (d)

Figure 4.14 Representatives of antimicrobial activity assessment of Sulfated 2-EH treated cut pile carpet; (a) *A.niger*, (b) *E.coli*, (c) *K.pneumoniae*, (d) *S.aureus*



(a) (b) (c) (d)

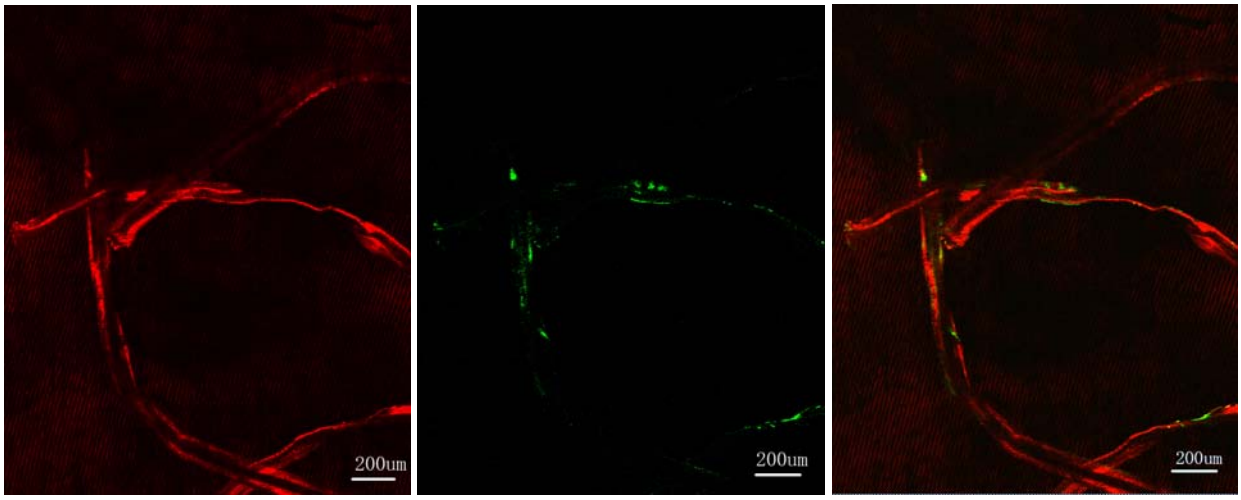
Figure 4.15 Representatives of antimicrobial activity assessment of Sulfated 2-EH treated loop pile carpet; (a) *A.niger*, (b) *E.coli*, (c) *K.pneumoniae*, (d) *S.aureus*

Table 4.5 Antimicrobial effectiveness of carpets

Carpet		Width of zone of inhibition (mm)			
		Aspergillus niger	*Escherichia coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
Cut pile	No treatment (Control)	0.0	0.0	0.0	0.0
	Freepel™ 1225 treated	0.0	0.0	0.0	0.0
	Humectant A treated	0.0	0.0	0.0	0.0
	Sulfated 2-EH treated	0.0	0.0	0.0	0.0
Loop pile	No treatment (control)	0.0	0.0	0.0	0.0
	Freepel™ 1225 treated	0.0	0.0	0.0	0.0
	Humectant A treated	0.0	0.0	0.0	0.0
	Sulfated 2-EH treated	0.0	0.0	0.0	0.0

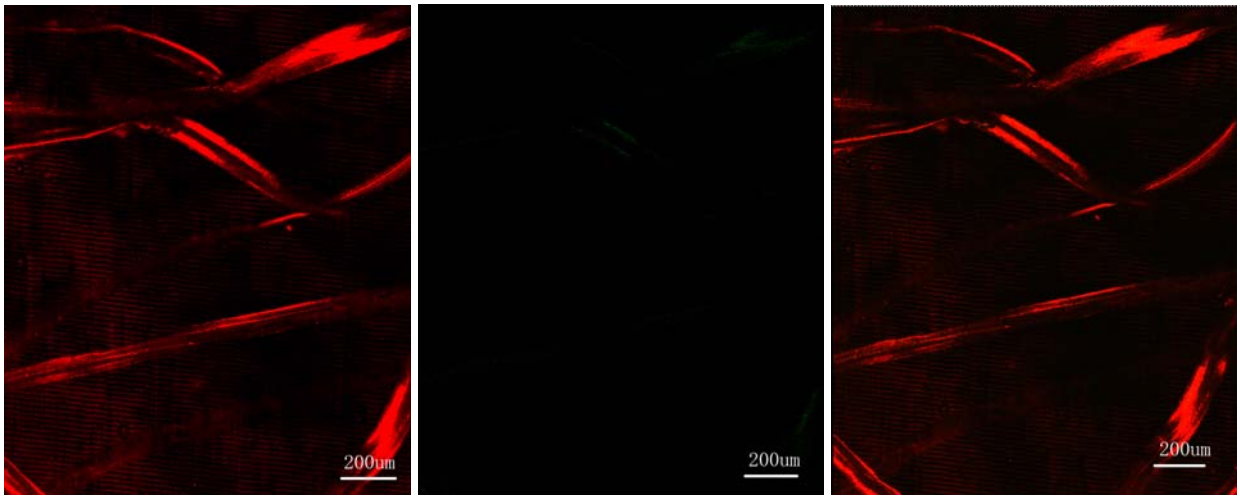
Section III. The Distribution of GFP *Escherichia coli* in Carpets Based on Different Application Methods (Qualitative Assessment)

Three different locations along the pile yarns in the carpet were examined under the microscope (Figure 3.4, P40). These three locations were identified as (1) the surface and near the surface of the carpet (Location A), (2) the middle of the carpet (Location B), and (3) the base of the carpet close to the primary backing (Location C). The LSCM images were taken for each location and are presented in Figures 4.16-4.21.



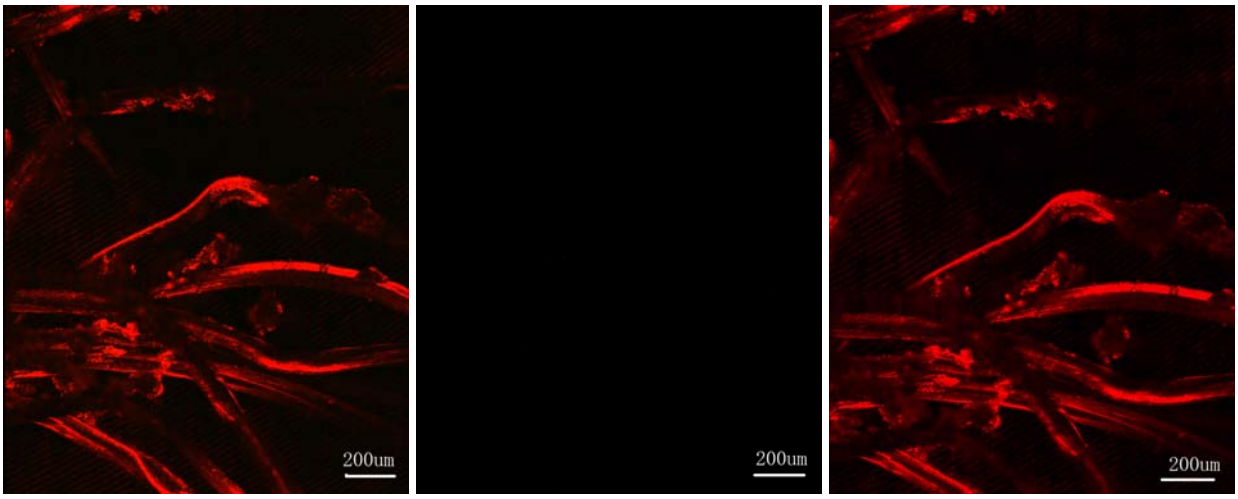
(a) (b) (c)

Figure 4.16 Representatives of LSCM images for Location A in the carpet with GFP *Escherichia coli* applied using airbrush method; (a) image obtained by PMT one, (b) image obtained by PMT two, and (c) merged image



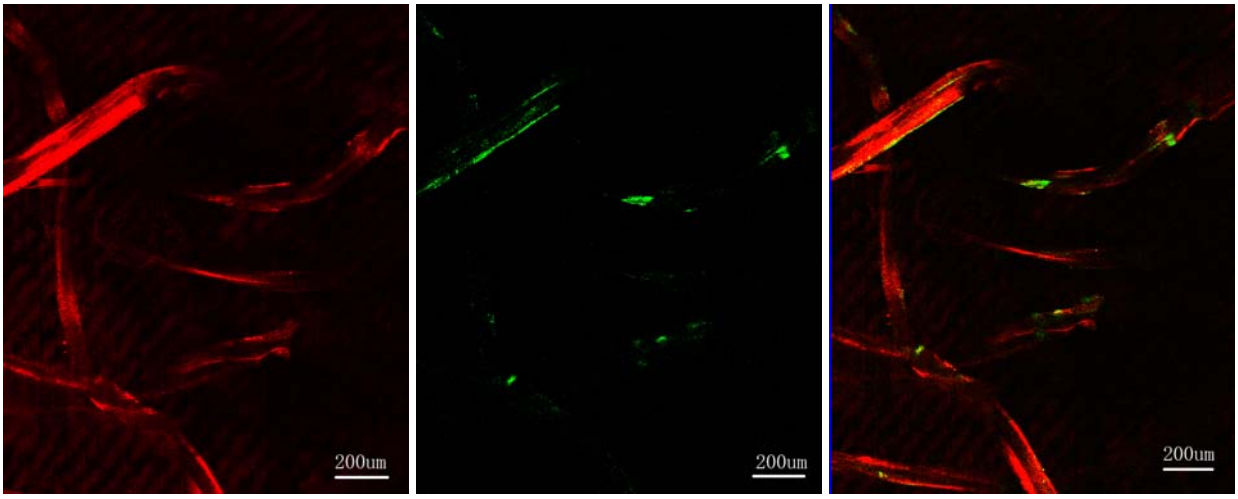
(a) (b) (c)

Figure 4.17 Representatives of LSCM images for Location B in the carpet with GFP *Escherichia coli* applied using airbrush method; (a) image obtained by PMT one, (b) image obtained by PMT two, and (c) merged image



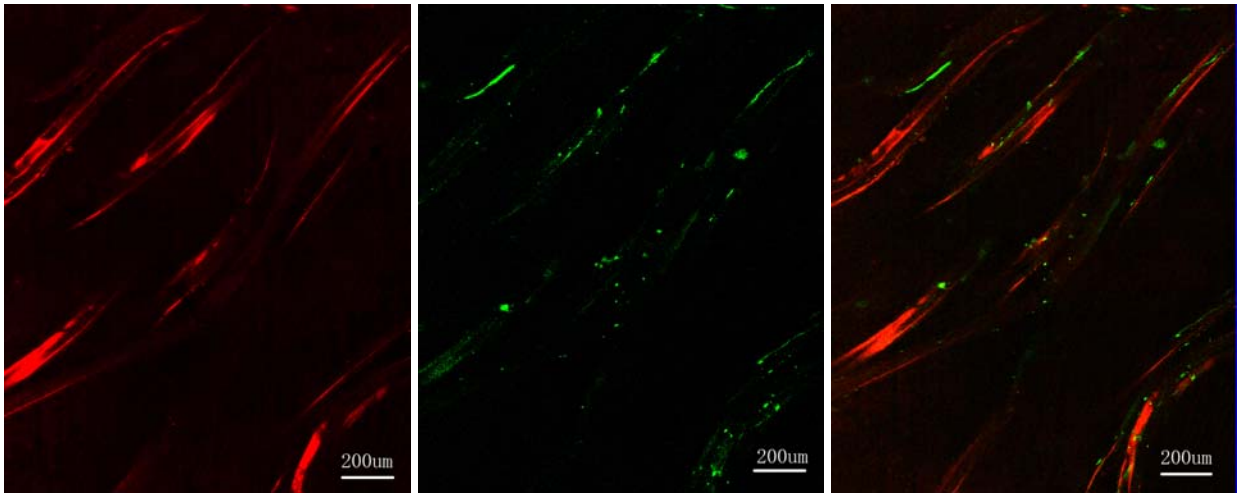
(a) (b) (c)

Figure 4.18 Representatives of LSCM images for Location C in the carpet with GFP *Escherichia coli* applied using airbrush method; (a) image obtained by PMT one, (b) image obtained by PMT two, and (c) merged image



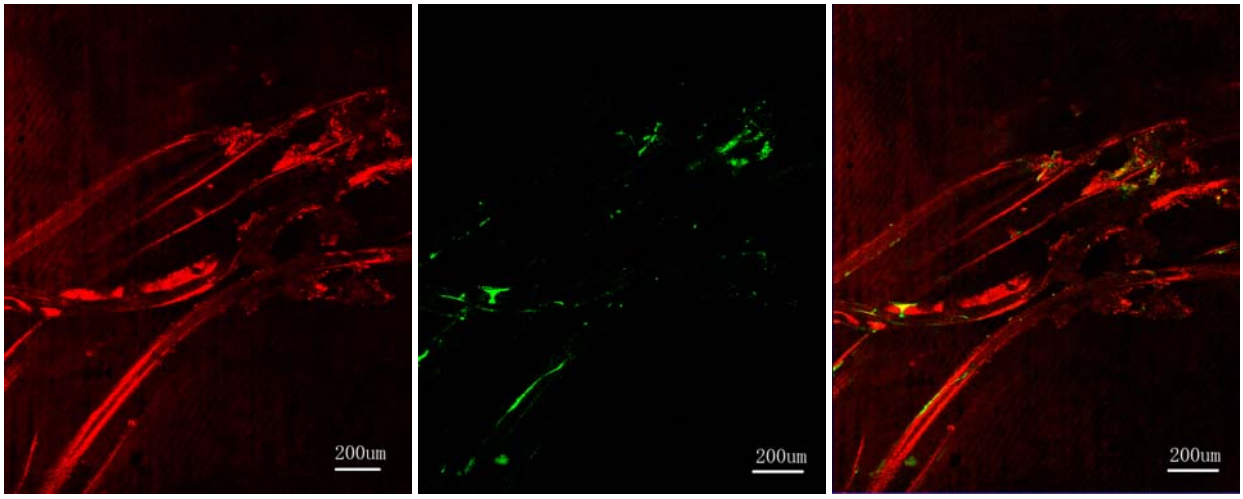
(a) (b) (c)

Figure 4.19 Representatives of LSCM images for Location A in the carpet with GFP *Escherichia coli* applied using pipette method; (a) image obtained by PMT one, (b) image obtained by PMT two, and (c) merged image



(a) (b) (c)

Figure 4.20 Representatives of LSCM images for Location B in the carpet with GFP *Escherichia coli* applied using pipette method; (a) image obtained by PMT one, (b) image obtained by PMT two, and (c) merged image



(a) (b) (c)

Figure 4.21 Representatives of LSCM images for Location C in the carpet with GFP *Escherichia coli* applied using pipette method; (a) image obtained by PMT one, (b) image obtained by PMT two, and (c) merged image

The images (a) in Figures 4.16-4.21 show the pile yarns of carpet which are represented by red. The images (b) in Figures 4.16-4.21 show the green fluorescent signals detected from GFP *Escherichia coli*. The bacteria are represented by green in these color micrographs. The images (c) in Figures 4.16-4.21 are the merged images that show the location of bacteria in the carpet structure. If any green signals were observed in images (c), GFP *Escherichia coli* were indicated to be at that location. If no green signals were observed, no bacteria were present.

The location of GFP *Escherichia coli* in the depth of carpet after the bacteria were applied to the carpet using airbrush method is shown in Figures 4.16-4.18. Strong green signals were observed in images (b) and (c) of Figure 4.16, indicating that GFP *Escherichia coli* were located at the surface and near the surface of the carpet. Week green signals were observed in images (b) and (c) of Figure 4.17, which meant some GFP *Escherichia coli* were located in the

middle of carpet. No green signals were observed in images (b) or (c) of Figure 4.18, indicating that no GFP *Escherichia coli* were located at the base of the carpet close to the primary backing.

In summary, by examining Figures 4.16-4.18, most of the bacteria were located at the surface and near the surface of carpet after application using the airbrush method. In this application process, the bacterial culture was atomized producing thousands of fine bacteria containing droplets. These droplets (with bacteria inside) were so fine that they were adsorbed onto the pile yarns immediately after the application. As the liquid evaporated, the bacteria contacted and adhered to pile yarns at the carpet surface. The LSCM images also show that small amounts of bacteria were located in the middle of the carpet. This was expected, as the droplets of bacterial culture after atomization could penetrate the carpet surface through the open spaces between yarns or fibers. However, the carpet has a three dimensional structure. The depth of carpet and the closely packed tufts in the carpet played an important role in the penetration process, which resulted in the bacteria containing droplets being trapped in the middle of carpet before reaching the primary backing.

The location of GFP *Escherichia coli* in the carpet structure after application of bacteria to the carpet using pipette method was evaluated using LSCM techniques (Figures 4.19-4.21). Strong green signals were observed in images (b) and (c) of Figures 4.19-4.21, indicating that the GFP *Escherichia coli* were located at all three locations examined here: the surface and near the surface of the carpet, the middle of the carpet, and the base of the carpet close to the primary backing.

When the bacterial culture was applied to the carpet by the pipette method, the bacterial culture was a liquid rather than the fine atomized droplets. The liquid moved in the carpet and reached the base of the carpet due to gravity and capillary action. Therefore, the bacteria

transmitted through the depth of the carpet with the movement of liquid media. They were stopped at those three locations in the carpet during transmission and distributed throughout the depth of the carpet.

The location of microorganisms in the carpet influenced the transfer of microorganisms. According to Leonas and Annis, the receptor materials had less opportunity to contact the microorganisms below the carpet surface and those microorganisms below the carpet surface were not available for transfer (Leonas and Annis, 2004). Using the airbrush method to apply microorganisms to the carpets, the number of microorganisms in the middle and at the base of the carpet were reduced and most of the microorganisms available for transfer were located at and near the carpet surface. Therefore, the airbrush method was selected for use in this study.

Another disadvantage of using the pipette method for microorganism application was related to the interaction of the microbial culture and the surface energy of carpet. The surface energy of carpet treated with Freepel^{TM} 1225 was decreased and the treated carpet had repellency to water (Figure 4.1). The microbial culture was in the water-based liquid which had a surface tension similar to that of water. After microorganisms application to the Freepel^{TM} 1225 treated carpet surface using pipette method, the microbial culture rolled into a sphere and remained constant for over a 10 minute period as shown in Figure 4.22. The microorganisms only adhered to the pile yarns at the carpet surface which was in contact with the microbial culture. Therefore, the microorganisms were concentrated at the surface of carpet where the spheres had been. The Humectant A treatment imparted a hydrophilic surface to the carpet. The microbial culture penetrated the carpet surface and reached the base of the carpet very easily because of the high surface energy of the treated carpet. The microorganisms appeared to be concentrated at the base of Humectant A treated carpet (Figure 4.22). The pipette method resulted in a different

distribution of microorganisms in the carpets with different surface energies and this would bring the interference to the final results. Therefore, the pipette method was not suitable for this study.

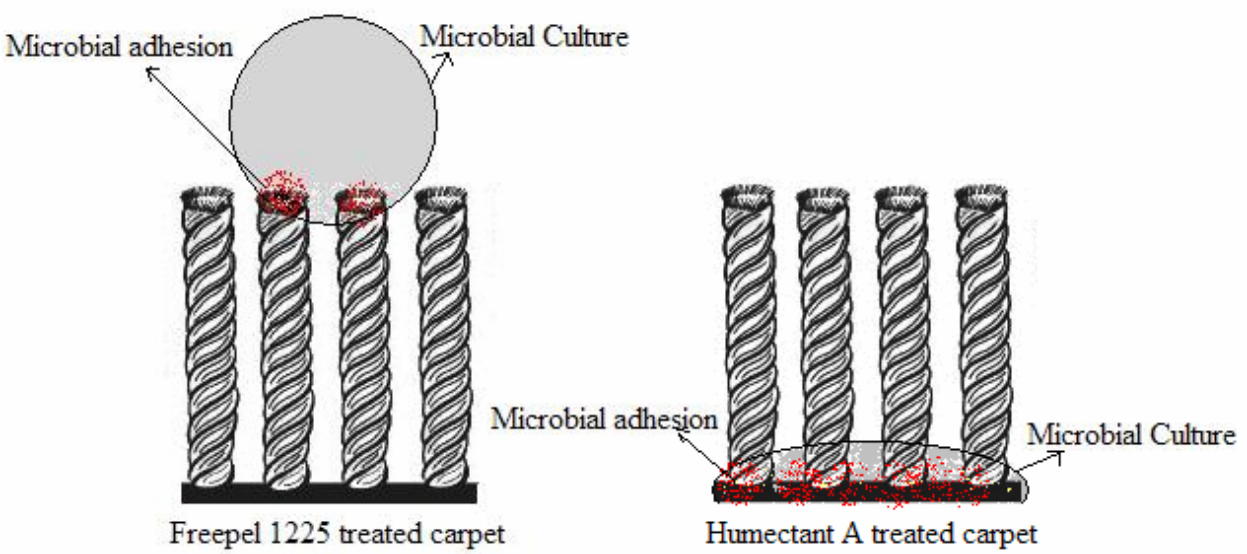


Figure 4.22 Adhesion of microorganisms on the pile yarns of the Freepel^{TM} 1225 treated and Humectant A treated carpets after the microorganisms were applied using pipette method

Section IV. Transfer of Microorganisms from Carpet to Fingers (Human Skin)

Statistical Analysis of Impact of Microorganism, Carpet texture, Chemical Treatment on Microbial Transfer from Carpets to Fingers

The Shapiro-Wilk test was completed to determine whether the samples were approximately normally distributed. The results are summarized in Table 4.6 and the "R" programs and outputs are presented in Appendix A.

Table 4.6 Statistical results of Shapiro-Wilk test*

Variables		W	P Value
Bacteria	*Staphylococcus aureus*	0.4831	2.2×10^{-16}
	Escherichia coli	0.3604	2.2×10^{-16}
	Klebsiella pneumoniae	0.4457	2.2×10^{-16}
	Aspergillus niger	0.68	2.2×10^{-16}
Carpet Texture	Cut Pile	0.145	2.2×10^{-16}
	Loop Pile	0.2103	2.2×10^{-16}
Chemical Treatment	Control (no treatment)	0.2037	2.2×10^{-16}
	Freepel™ 1225	0.2175	2.2×10^{-16}
	Humectant A	0.1521	2.2×10^{-16}
	Sulfated 2-EH	0.2016	2.2×10^{-16}

* Significant level: $p\leq0.01$

The p values for all variables are less than 0.01 indicating that the samples were not normally distributed and the assumptions for using ANOVA were not met. Therefore, the Chi square test was used to analyze the impact of each variable on the microbial transfer from carpets to fingers. The results are summarized in Table 4.7 and the "R" programs and outputs are presented in Appendix B. The following null hypotheses were tested:

Hypothesis 1: There were no significant differences of microbial transfer from carpets to fingers when different microorganisms, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Aspergillus niger*, were used.

The p value of the influence of microorganisms (hypothesis 1) is 4.506×10^{-43} (< 0.01) (Table 4.7). Therefore, the hypothesis 1 was rejected. It was concluded that different microorganisms significantly influenced the microbial transfer from carpets to fingers.

Hypothesis 2: There was no significant difference of microbial transfer from the cut pile carpet and the loop pile carpet to fingers.

The p value of the influence of carpet texture (hypothesis 2) is 0.03659 (> 0.01) (Table 4.7). Therefore, the hypothesis 2 was not rejected. It was concluded that carpet texture (loop pile or cut pile) did not significantly influence the microbial transfer from the carpets to fingers.

Hypothesis 3: There were no significant differences of microbial transfer from carpets to fingers after the carpets were treated with Humectant A, Freepel^{TM} 1225 water repellent, and Sulfated 2-EH.

The p value of the influence of chemical treatment (hypothesis 3) is 3.665×10^{-8} (< 0.01) (Table 4.7). Therefore, the hypothesis 3 was rejected. It was concluded that the chemical treatment significantly influenced the transfer of microorganisms from the carpets to fingers.

Table 4.7 Results of Chi Square test (analysis of deviance)*

	P(>|Chi|)
Null	---
Microorganism	4.506e-43
Carpet texture	0.03659
Chemical treatment	3.665e-08

* Significant level: p≤0.01

After the Chi square test, the Wilcoxon's rank sum test was performed for the further significance analysis of microorganisms and chemical treatments. The results of this statistical analysis are presented in table 4.8 and the "R" programs and outputs are presented in Appendix C. The following null hypotheses were tested:

Hypothesis 4: There was no significant difference between the transfer of *Staphylococcus aureus* and the transfer of *Escherichia coli* from the carpets to fingers.

Hypothesis 5: There was no significant difference between the transfer of *Staphylococcus aureus* and the transfer of *Klebsiella pneumoniae* from the carpets to fingers.

Hypothesis 6: There was no significant difference between the transfer of *Staphylococcus aureus* and the transfer of *Aspergillus niger* from the carpets to fingers.

Hypothesis 7: There was no significant difference between the transfer of *Escherichia coli* and the transfer of *Klebsiella pneumoniae* from the carpets to fingers.

Hypothesis 8: There was no significant difference between the transfer of *Escherichia coli* and the transfer of *Aspergillus niger* from the carpets to fingers.

Hypothesis 9: There was no significant difference between the transfer of *Klebsiella pneumoniae* and the transfer of *Aspergillus niger* the from carpets to fingers.

Hypothesis 10: There was no significant difference in microbial transfer between using the untreated carpet or the Freepel™ 1225 treated carpet.

Hypothesis 11: There was no significant difference in microbial transfer between using the untreated carpet or the Humectant A treated carpet.

Hypothesis 12: There was no significant difference in microbial transfer between using untreated carpet or Sulfated 2-EH treated carpet.

Hypothesis 13: There was no significant difference in microbial transfer between using the Freepel™ 1225 treated carpet or the Humectant A treated carpet.

Hypothesis 14: There was no significant difference in microbial transfer between using the Freepel™ 1225 treated carpet or the Sulfated 2-EH treated carpet.

Hypothesis 15: There was no significant difference in microbial transfer between using the Humectant A treated carpet or the Sulfated 2-EH treated carpet.

Table 4.8 Statistical results of Wilcoxon's test*

Variables		P value
Microorganisms	*Staphylococcus aureus* vs. *Escherichia coli*	0.0002294
	Staphylococcus aureus vs. *Klebsiella pneumoniae*	0.002054
	Staphylococcus aureus vs. *Aspergillus niger*	3.646e-11
	Escherichia coli vs. *Klebsiella pneumoniae*	2.22e-16
	Escherichia coli vs. *Aspergillus niger*.	<2.2e-16
	Klebsiella pneumoniae vs. *Aspergillus niger*.	0.00445
Chemical Treatment	Control (no treatment) vs. Freepel™ 1225 treatment	<2.2e-16
	Control (no treatment) vs. Humectant A treatment	4.885e-15
	Control (no treatment) vs. Sulfated 2-EH treatment	<2.2e-16
	Freepel™ 1225 treatment vs. Humectant A treatment	<2.2e-16
	Freepel™ 1225 treatment vs. Sulfated 2-EH treatment	<2.2e-16
	Humectant A treatment vs. Sulfated 2-EH treatment	1.596e-05

* Significant level: $p \leq 0.01$

The results of Wilcoxon's test show that the p values of all variables tested were less than 0.01 (Table 4.8). Therefore the null hypotheses 4-15 described above were rejected and it was concluded that microorganisms and chemical treatments of carpets were significantly different from each other in influencing the microbial transfer.

Transfer of Microorganisms from Carpet to Fingers (Human Skin)

Figures 4.23-4.38 show the results of microbial transfer from carpets to fingers (human skin). The microbial transfers were performed 0, 6, 12, 24, and 48 hours after the microorganisms were applied to the carpets using the airbrush method.

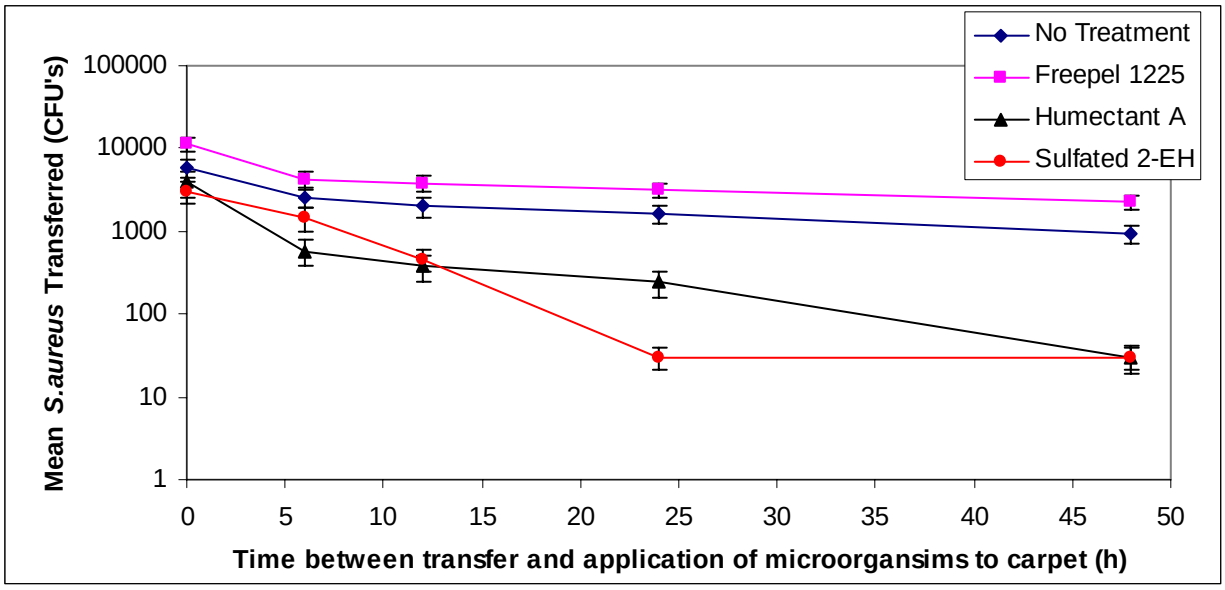


Figure 4.23 Transfer of *Staphylococcus aureus* (mean of three replications) from cut pile carpet to fingers using compression method (log scale)

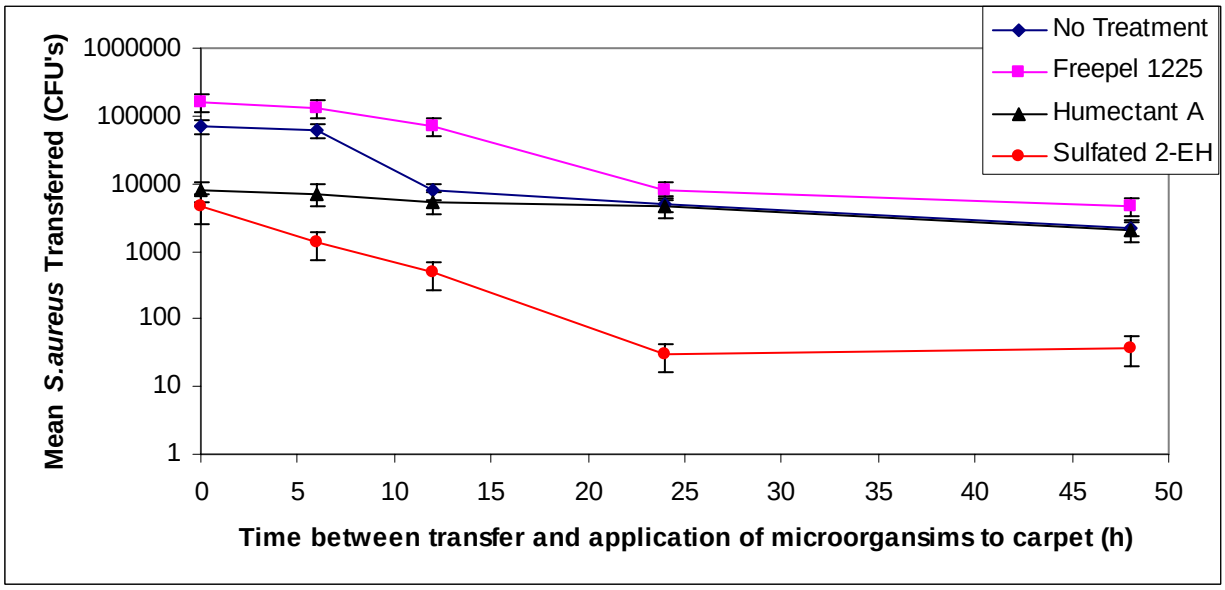


Figure 4.24 Transfer of *Staphylococcus aureus* (mean of three replications) from cut pile carpet to fingers using slide method (log scale)

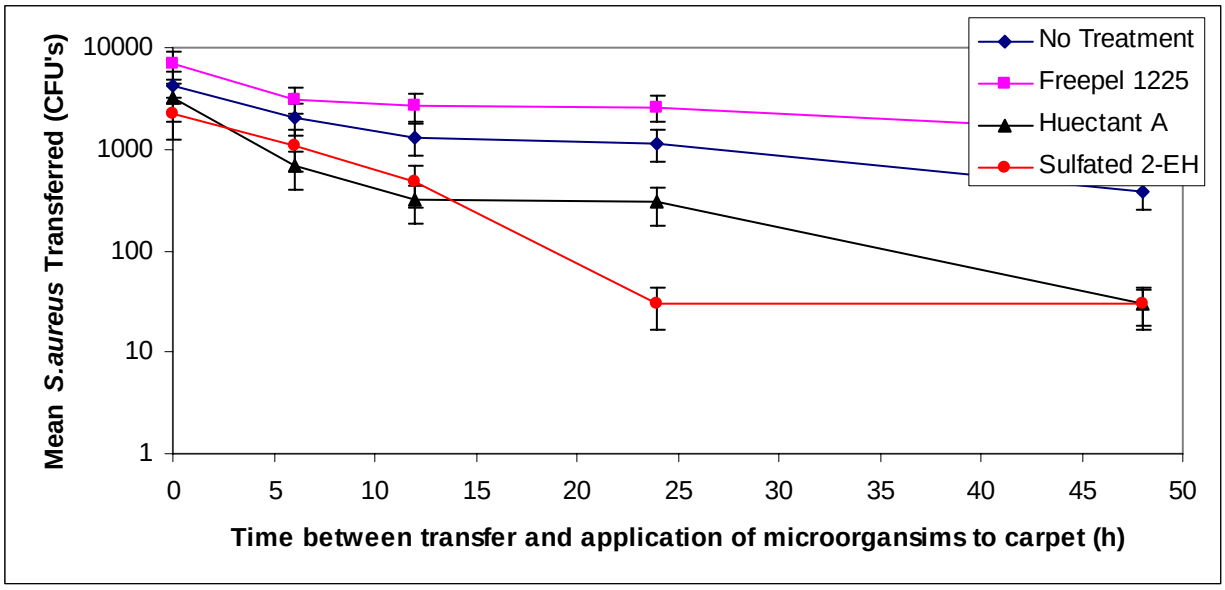


Figure 4.25 Transfer of *Staphylococcus aureus* (mean of three replications) from loop pile carpet to fingers using compression method (log scale)

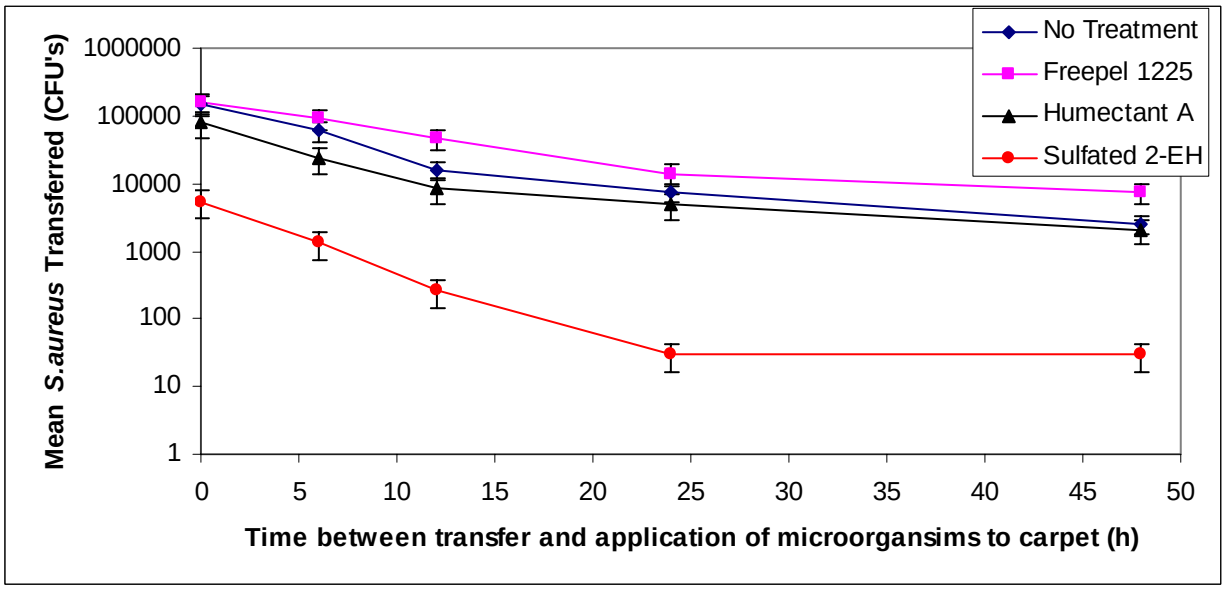


Figure 4.26 Transfer of *Staphylococcus aureus* (mean of three replications) from loop pile carpet to fingers using sliding method (log scale)

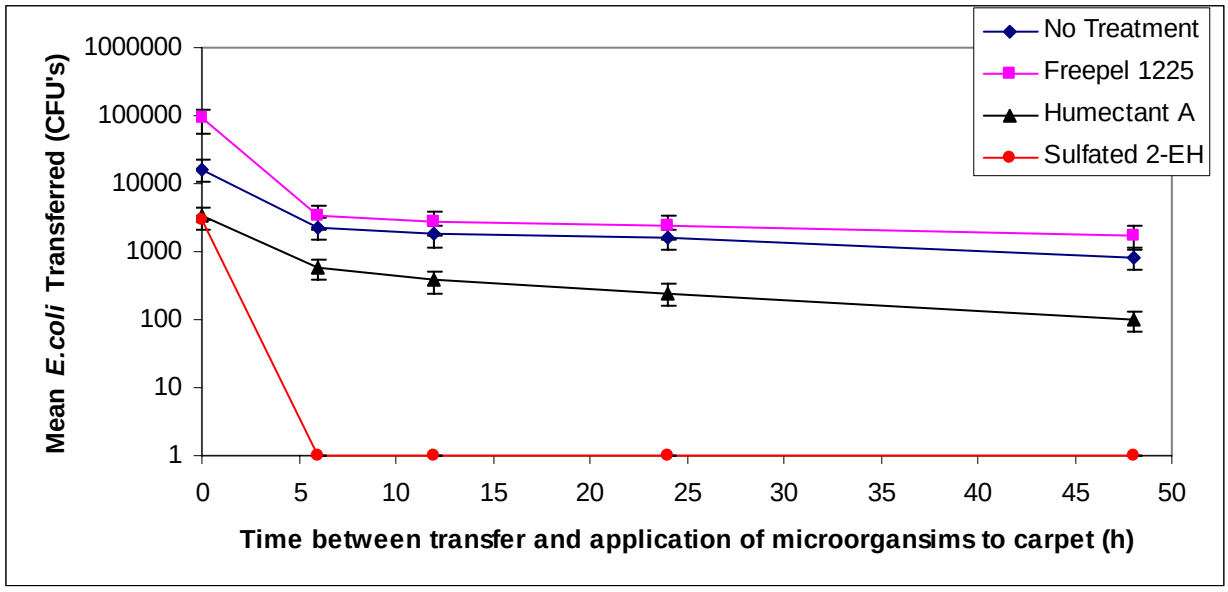


Figure 4.27 Transfer of *Escherichia coli* (mean of three replications) from cut pile carpet to fingers using compression method (log scale)

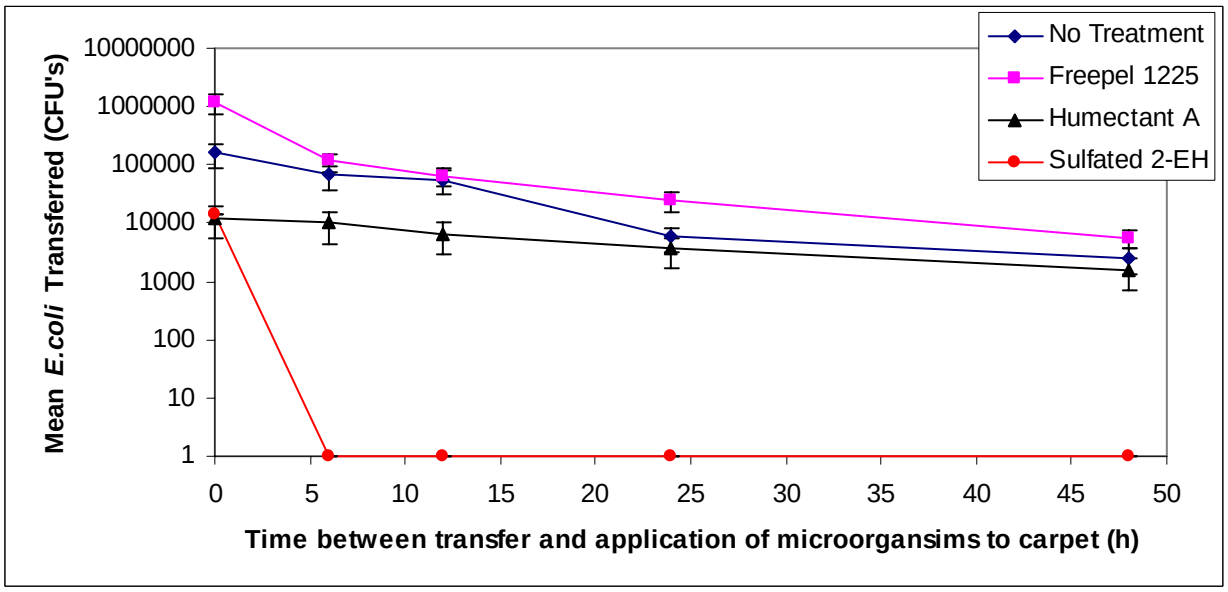


Figure 4.28 Transfer of *Escherichia coli* (mean of three replications) from cut pile carpet to fingers using slide method (log scale)

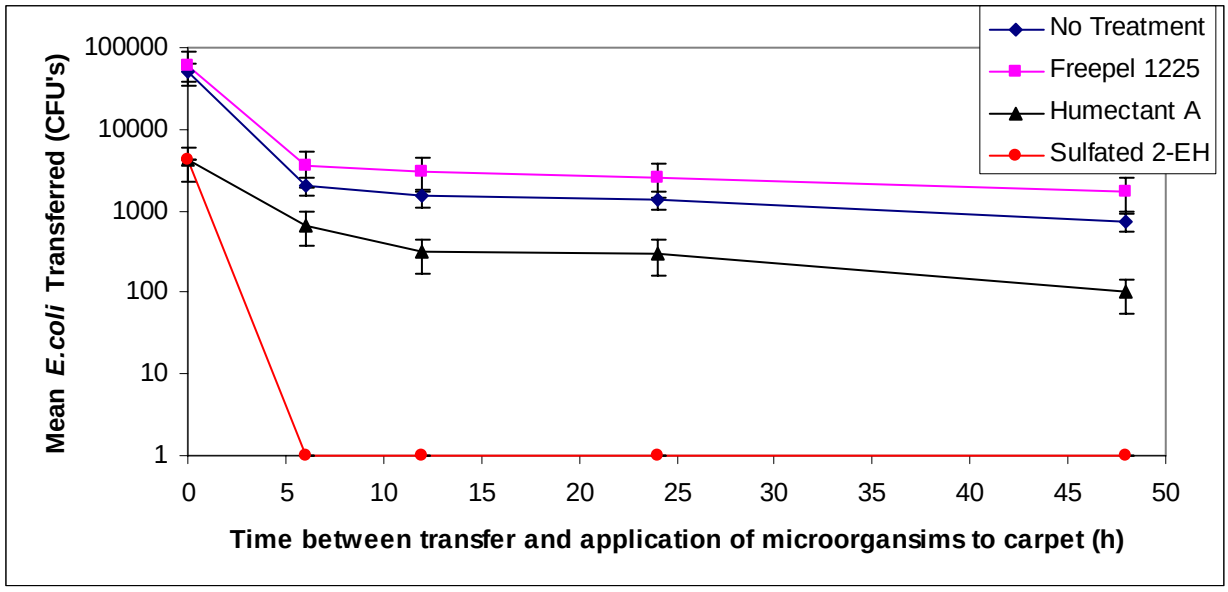


Figure 4.29 Transfer of *Escherichia coli* (mean of three replications) from loop pile carpet to fingers using compression method (log scale)

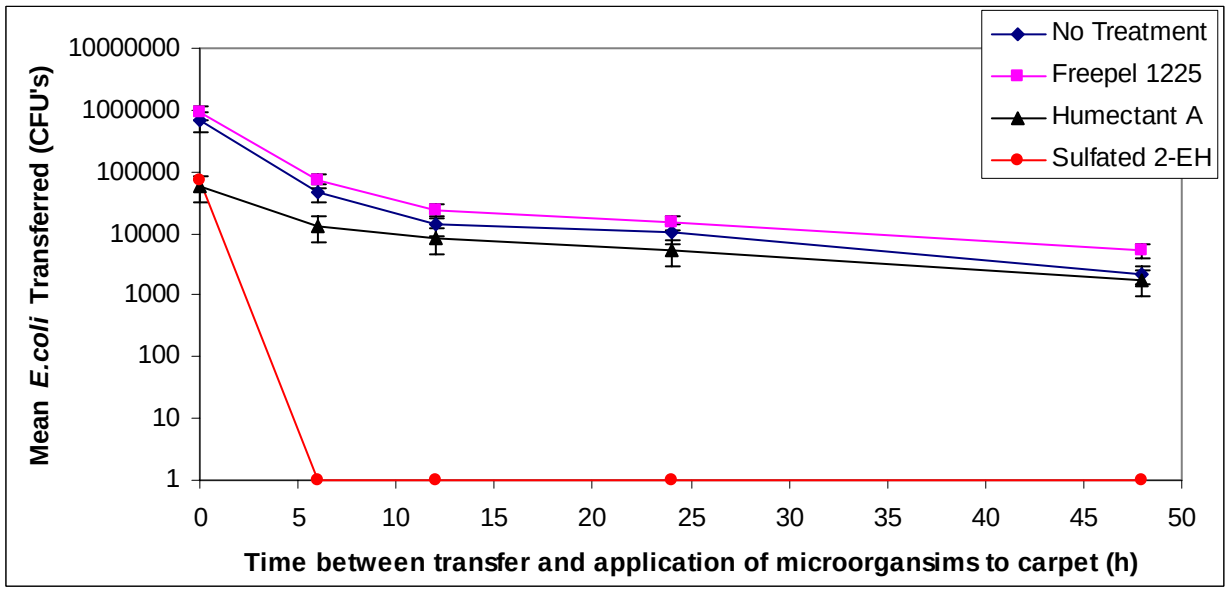


Figure 4.30 Transfer of *Escherichia coli* (mean of three replications) from loop pile carpet to fingers using slide method (log scale)

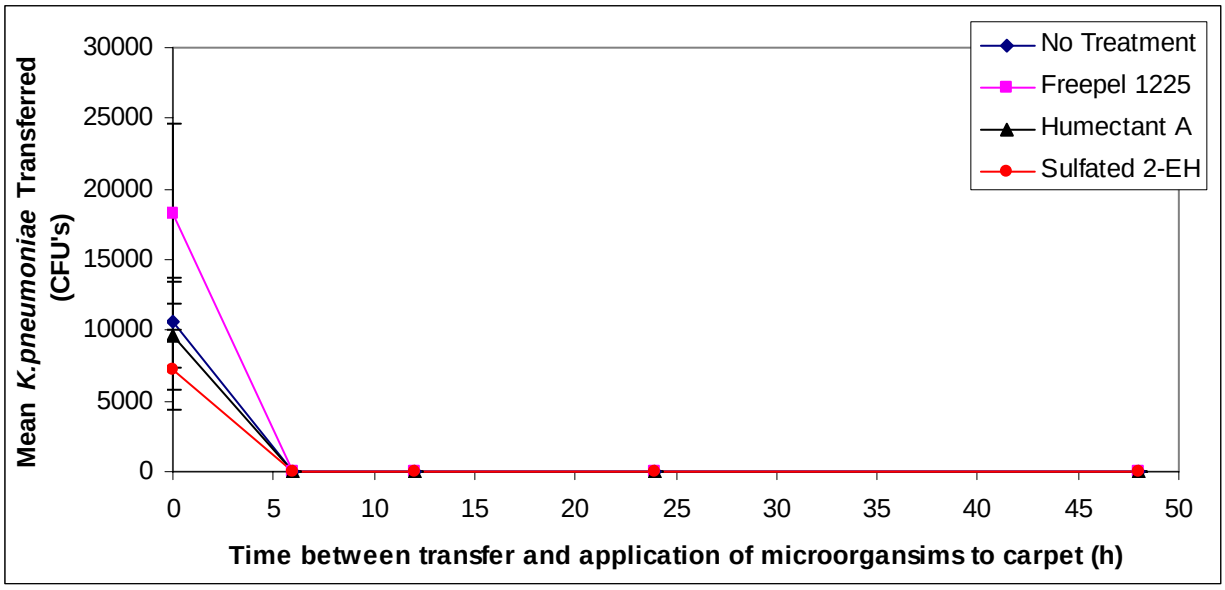


Figure 4.31 Transfer of *Klebsiella pneumoniae* (mean of three replications) from cut pile carpet to fingers using compression method

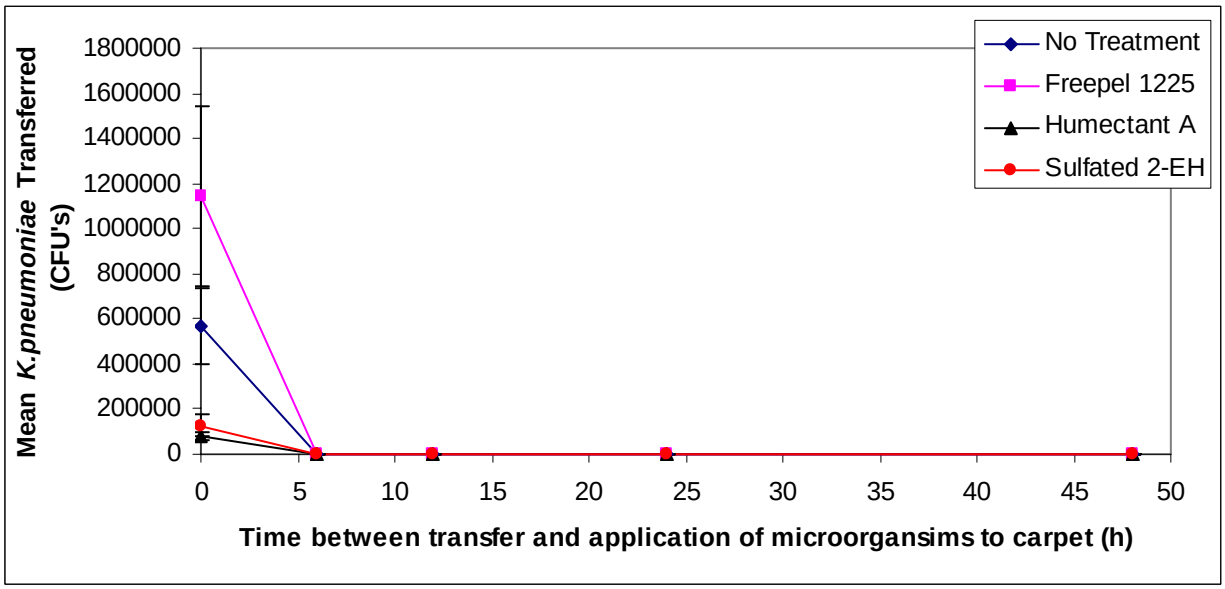


Figure 4.32 Transfer of *Klebsiella pneumoniae* (mean of three replications) from cut pile carpet to fingers using sliding method

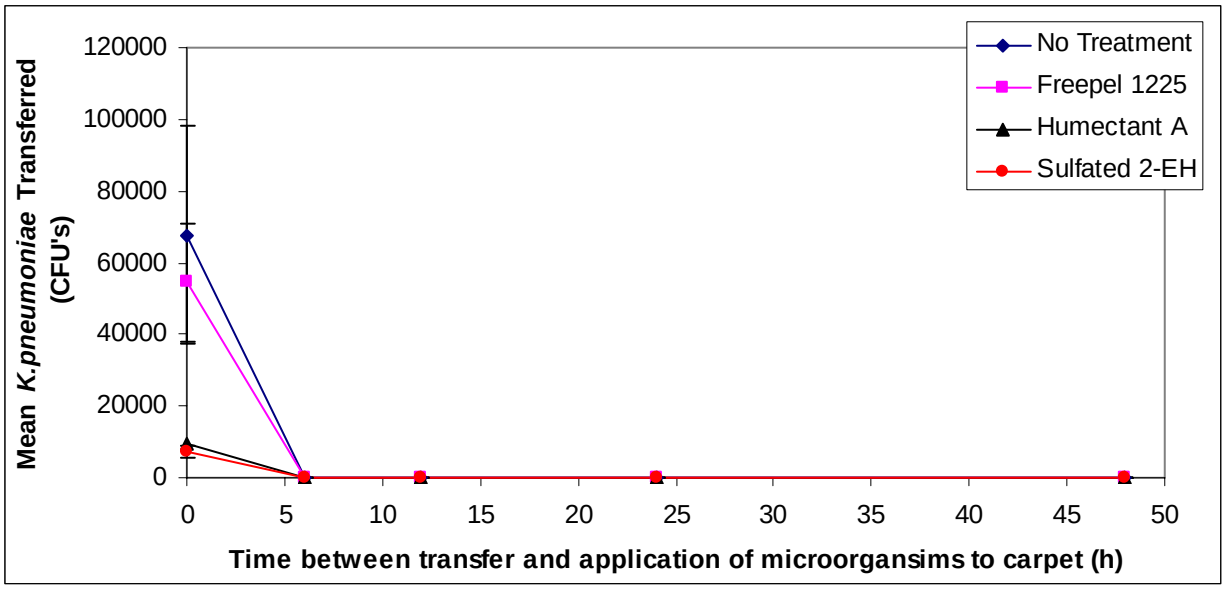


Figure 4.33 Transfer of *Klebsiella pneumoniae* (mean of three replications) from loop pile carpet to fingers using compression method

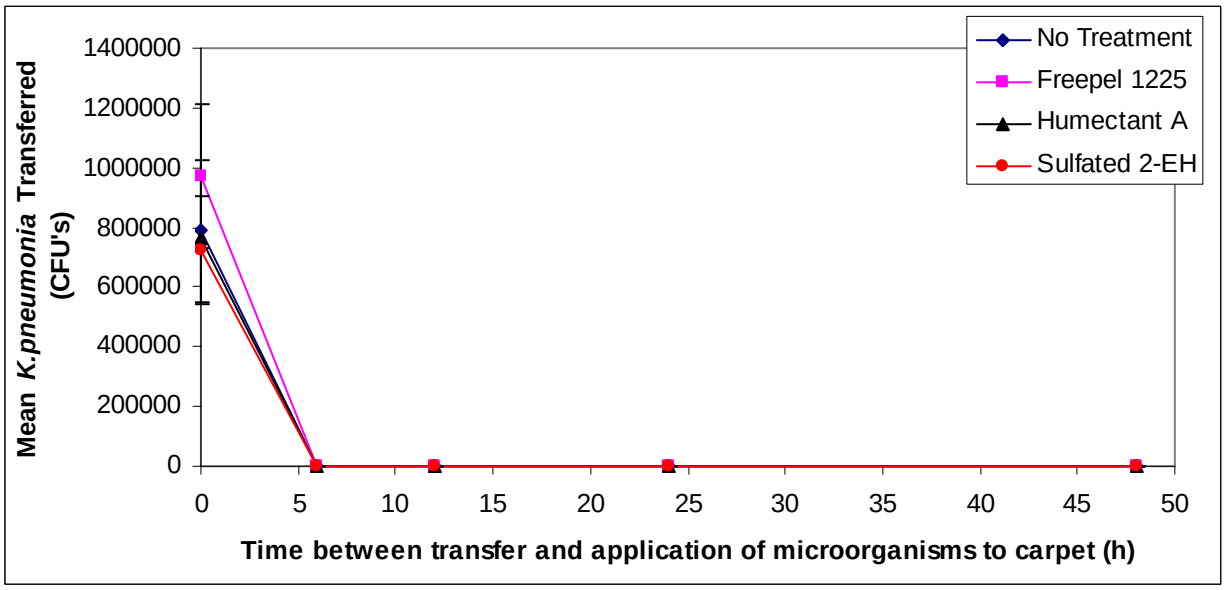


Figure 4.34 Transfer of *Klebsiella pneumoniae* (mean of three replications) from loop pile carpet to fingers using sliding method

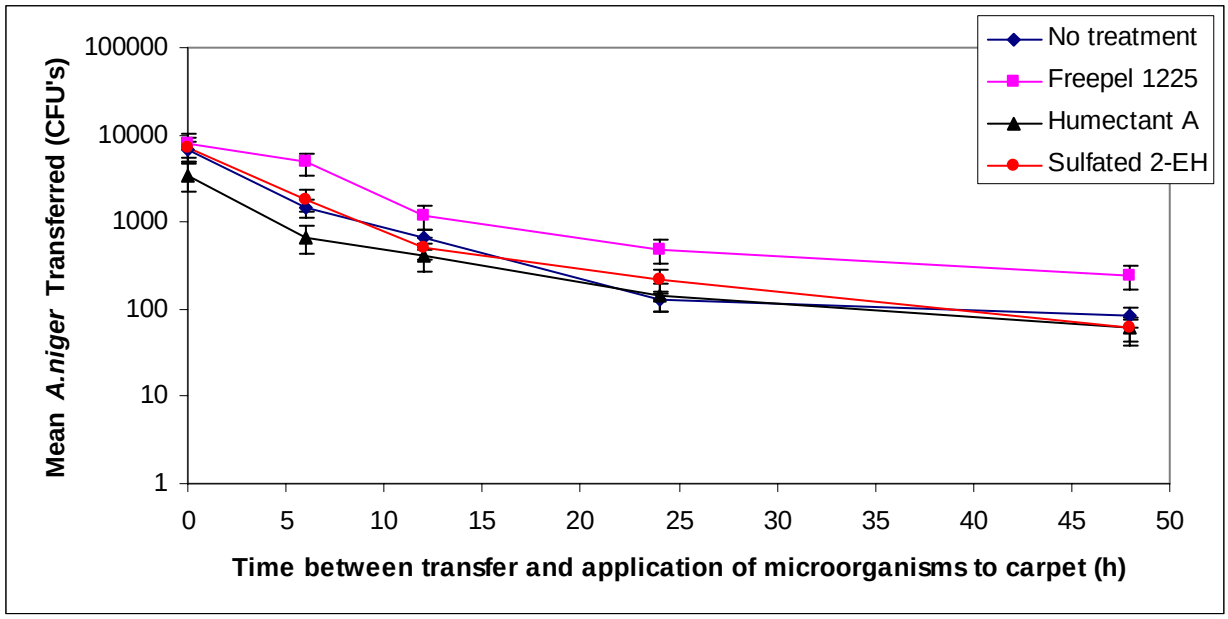


Figure 4.35 Transfer of *Aspergillus niger* (mean of three replications) from cut pile carpet to fingers using compression method (log scale)

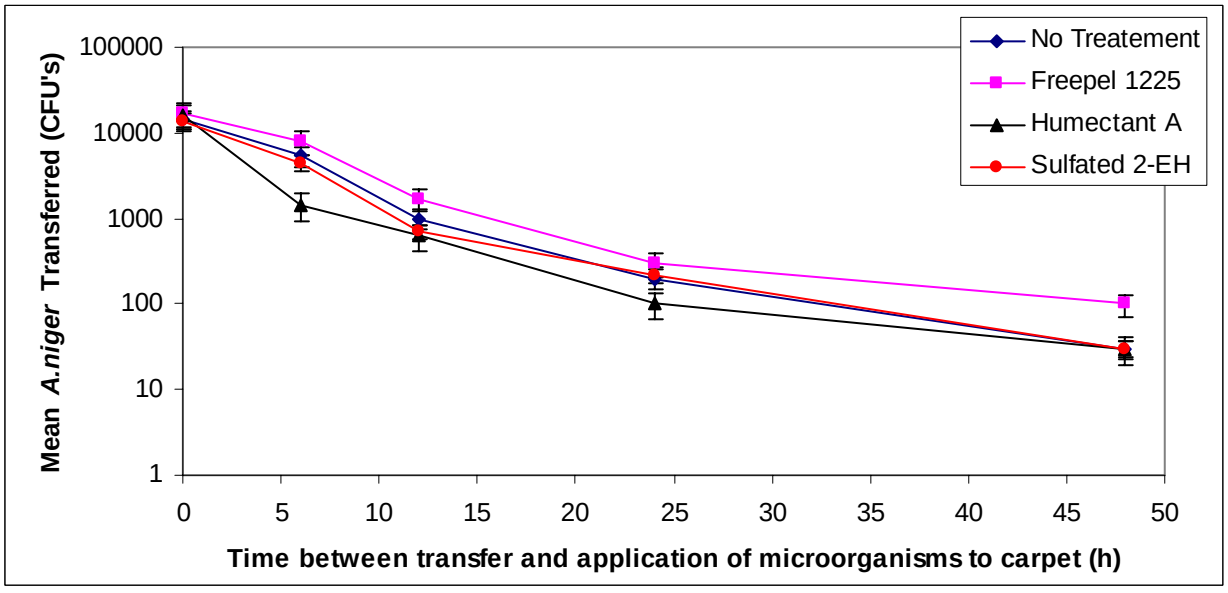


Figure 4.36 Transfer of *Aspergillus niger* (mean of three replications) from cut pile carpet to fingers using slide method (log scale)

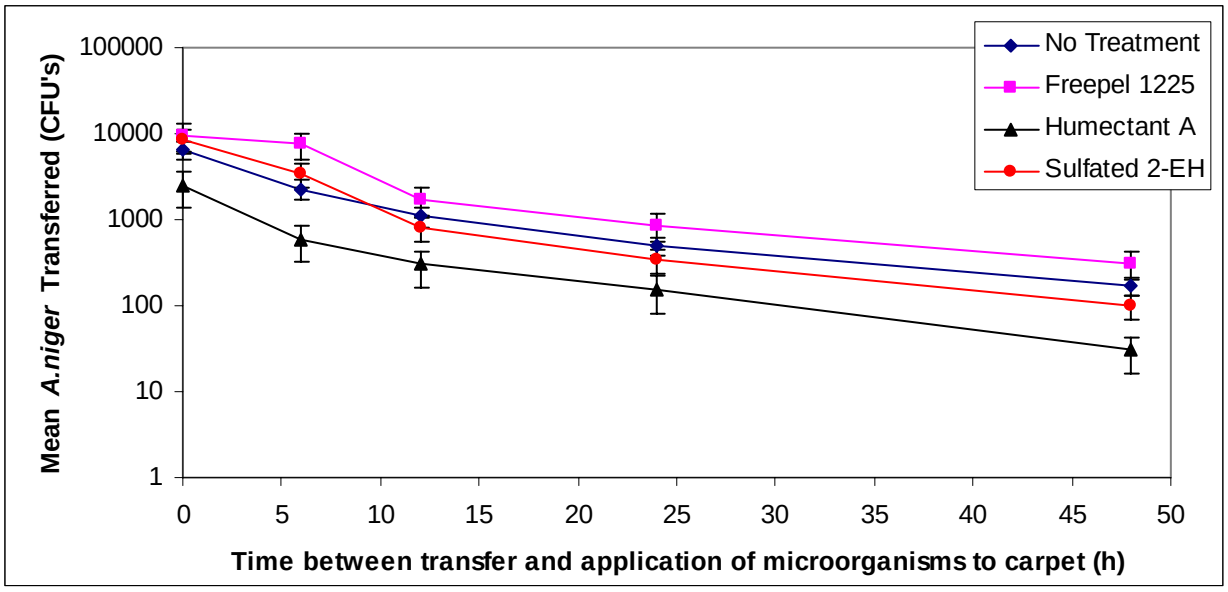


Figure 4.37 Transfer of *Aspergillus niger* (mean of three replications) from loop pile carpet to fingers using compression method (log scale)

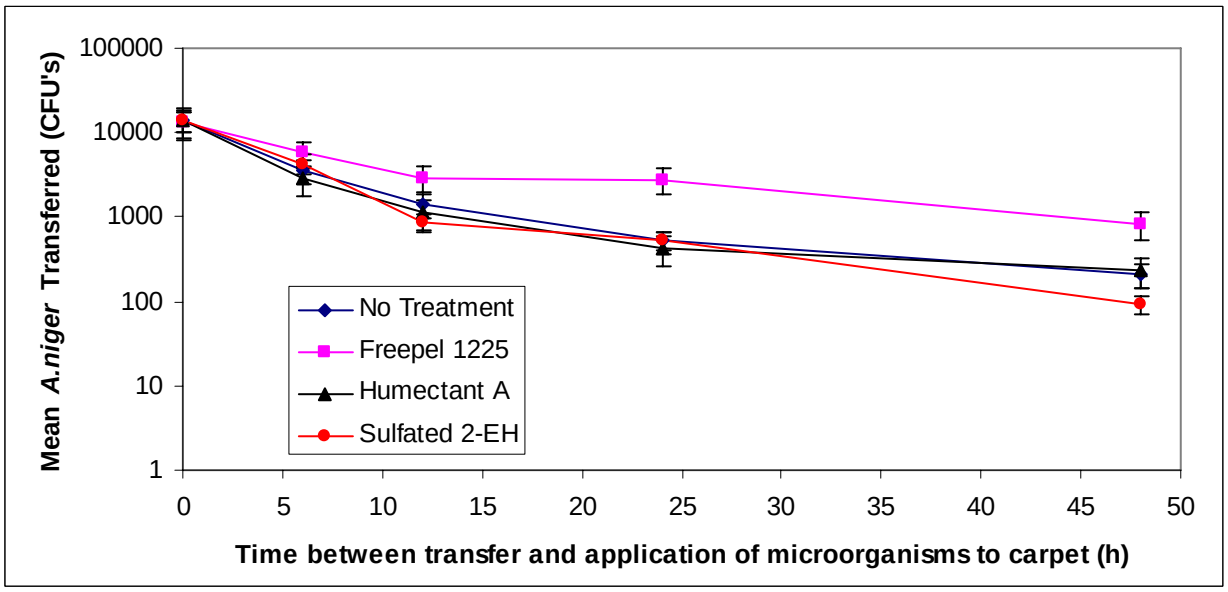


Figure 4.38 Transfer of *Aspergillus niger* (mean of three replications) from loop pile carpet to fingers using slide method (log scale)

In general, the transfer of microorganisms from carpets to fingers decreased as the time between application of microorganisms to the carpet and transfer increased (Figures 4.23-4.38). The number of microorganisms transferred from carpets was the largest when the transfer was performed immediately after the application of microorganisms. At this point, most of the microorganisms might be still in the microbial culture and have not adhered to the carpet pile yarns. They were easily transferred from carpets to fingers through direct contact. In addition, the liquid in the microbial culture increased the moisture level on the carpet surface. Marples and Towers found that increased moisture of donor surface increased the transfer of microorganisms from one surface to the other (Marples and Towers, 1979). Therefore, the transfer from carpets to fingers was the highest when the transfer was performed immediately following the application of the microorganisms to the carpets.

As the time between the application of the microorganisms and transfer increased, more and more microorganisms adhered to the carpet pile yarns. The microbial release and transfer then became more difficult. Therefore, the number of microorganisms transferred from carpets to fingers decreased as the time increased. Another factor which could have influenced in the decrease of microbial transfer with time was the death of microorganisms in carpets. In the preliminary work, a test was completed to extract the microorganisms from the carpet 0 and 24 hours after the application. The results show that up to 50% of the total amount of microorganisms in the carpets was reduced after 24 hours (Table 4.9). The microorganisms died possibly due to lack of nutrients and water in the carpet. The longer the microorganisms stayed on the carpet, the lower the number of viable microorganisms available for transfer. Therefore, the microbial transfer from carpet to fingers decreased gradually in 48 hours.

Table 4.9 Reduction of the number of viable microorganisms in the carpet

Time (hour)	Number of microorganisms extracted from cut pile carpet (CFU's) (S.D.)			
	Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Aspergillus niger*
0	2.675×10^7 (5414795)	5.5×10^7 (5000000)	8.25×10^7 (2500000)	3.5×10^7 (8144528)
24	5.75×10^6 (642910)	9×10^6 (1000000)	1.0×10^6 (312249)	1.2×10^6 (577350)

Impact of Different Microorganisms on Microbial Transfer from Carpets to Fingers

The microbial species significantly influenced the transfer of microorganisms from the carpets to fingers (Table 4.7). In general, the average transfer of *Escherichia coli* from the carpets to fingers was the highest, followed by *Staphylococcus aureus*, *Aspergillus niger*, and then *Klebsiella pneumoniae*. The microorganisms' characteristics, cell wall structure, size, and shape, might result in the difference in microbial transfer.

Transfer of Klebsiella pneumoniae

The transfer of *Klebsiella pneumoniae* was only observed when the transfer was performed immediately after the application of bacteria to the carpet, and for the rest of study, no transfer of *Klebsiella pneumoniae* occurred from the carpets to fingers (Figures 4.31-4.34). *Klebsiella pneumoniae* is gram negative and in a rod shape. The difference between *Klebsiella pneumoniae* and the other two bacteria used in this study is that *Klebsiella pneumoniae* has a capsule around its cell. The capsule is highly hydrated, containing as much as 98% water and it has a slimy or gelatinous consistency (Wilkinson, 1958). The capsule is usually composed of polysaccharide consisting of a number of monosaccharides joined by glycosidic links. Compared with the bacteria cell wall composed of the complexes of peptidoglycan, lipid, and protein like found in *Escherichia coli* and *Staphylococcus aureus*, the bacteria capsule contains more

hydroxyl groups in its polysaccharide molecular chains (Wilkinson, 1958). The hydroxyl groups may form hydrogen bonds with the amine group in the nylon fiber which is one type of adhesion force known to contribute to the adherence of the microorganisms to the surface. The more hydrogen bonding that occurs, the more firmly the microorganisms may adhere to the surface. The adhesion force between *Klebsiella pneumoniae* and the carpet pile yarns might be larger than that of the other microorganisms because of more hydroxyl groups in the capsule of *Klebsiella pneumoniae* resulting in the increased potential for hydrogen bonding. The adhesion force could not be overcome through the mechanical action of direct contact during the transfer process. Therefore, *Klebsiella pneumoniae* was difficult to transfer from carpets to fingers once they had adhered to the carpet pile yarns.

Transfer of Aspergillus niger

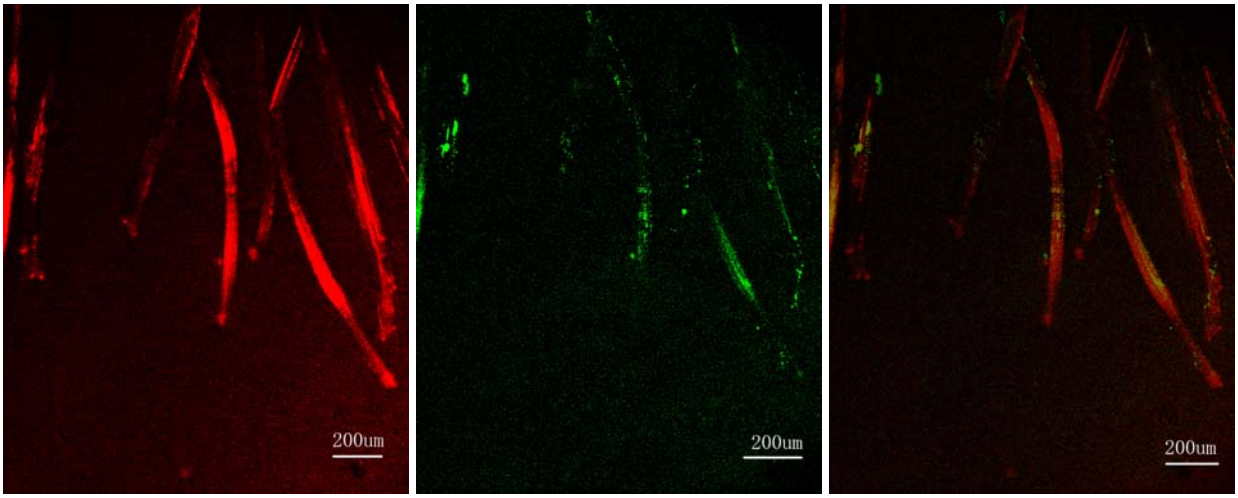
The transfer of *Aspergillus niger* from the carpets to fingers was significantly less than *Staphylococcus aureus* and *Escherichia coli*. *Aspergillus niger* is a fungus whose spores and hyphae used for transfer have a much larger size than the bacteria studied here. The small microorganisms have more surface area per weight bases than large ones. Therefore, the fingers have more chances to contact the small microorganisms during the transfer process, which resulted in the higher transfer of small microorganisms. In this study the spores and hyphae of *Aspergillus niger* were larger than two of the bacteria in size. Therefore, the transfer of fungal spores and hyphae was significantly lower than the transfer of bacteria (except *Klebsiella pneumoniae*).

Transfer of Staphylococcus aureus and Escherichia coli

The transfer of *Staphylococcus aureus* and the transfer of *Escherichia coli* from carpets to fingers were significantly different from each other (Table 4.8). More *Escherichia coli* transferred from the untreated carpet to fingers than *Staphylococcus aureus* (Figures 4.23-4.30). The shape and size of *Staphylococcus aureus* and *Escherichia coli* are different. *Staphylococcus aureus* has a round shape and *Escherichia coli* has a rod shape. The size of *Escherichia coli* is 1.1-1.5 μm in width by 2.0-3.0 μm in length, which is larger than *Staphylococcus aureus* which has a diameter of about 1 μm. Many factors significantly influenced the transfer of these two bacteria. For example, the orientation of bacteria might result in the different contact area between bacteria and carpet yarns. *Escherichia coli* have a rod shape. If it adhered to the yarns along the length of bacteria, the contact area would be larger than that along the width and that of *Staphylococcus aureus*. Therefore it would be expected that the transfer of *Escherichia coli* would be lower than that of *Staphylococcus aureus.* However, the results showed that more *Escherichia coli* transferred from untreated carpet to the fingers than *Staphylococcus aureus* (Figures 4.23-4.30). This can be explained by the different locations of bacteria in the carpets. It was indicated in the previous study (Section III) that most of microorganisms applied to the carpet with airbrush were located at the surface of the carpet. This was based on LSCM examination of the carpet after application of GFP *Escherichia coli* (Figures 4.17-19). However, there were still small amounts of bacteria that penetrated the carpet surface through the open spaces between the yarns and/or fibers and were trapped in the middle of the carpets.

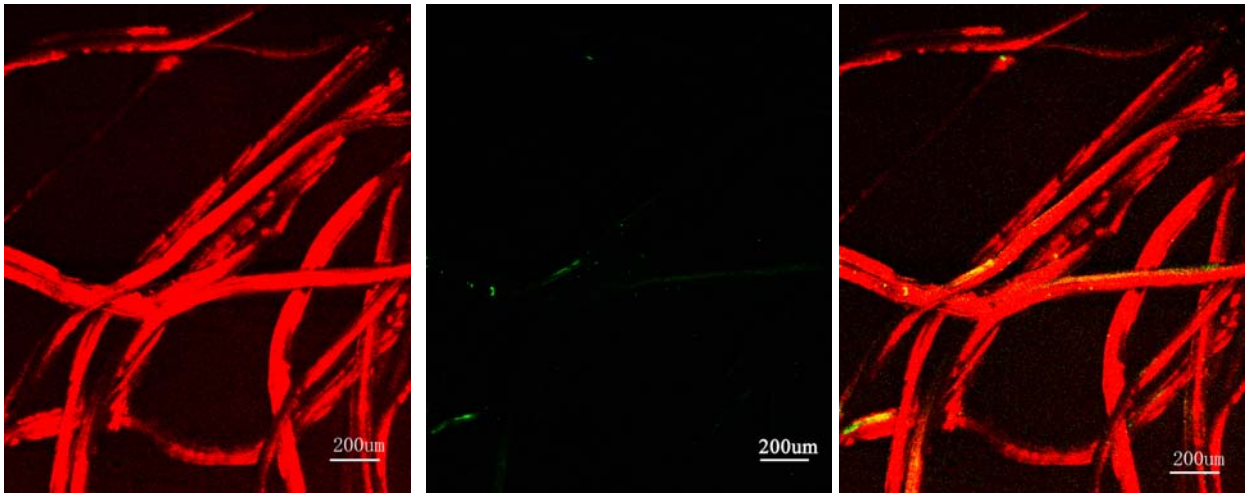
The LSCM images in Figures 4.39-4.41 show the distribution of fluoresbrite^{TM} carboxylate micropheres in the carpet structure after they were applied to the carpet using the airbrush. The average size of these microspheres is 1.0 micron and they are round in shape,

which was similar to that of *Staphylococcus aureus*. The surface characteristics of microspheres were not the same as *Staphylococcus aureus.* Therefore the adhesion between the spheres and carpet may be different from that of the bacteria and carpet. This was useful in evaluating the influence of the size and shape. By examining the LSCM images, the microspheres were located at the surface and in the middle of carpet, but not at the base of the carpets or close to the primary backing. Comparing Figures 4.40 with 4.17 (Figure 4.40 is the LSCM image of microspheres located in the middle of the carpet and Figure 4.17 is that of GFP *Escherichia coli*.), more microspheres than GFP *Escherichia coli* were located in the middle of carpets because more green signals from microspheres were observed in Figure 4.40. The microspheres, which were spherical in shape and smaller than GFP *Escherichia coli*, penetrated the carpet surface much more easily than *Escherichia coli* which are rod shape and have a higher ratio of length to width. Since the size and shape of the microspheres were similar to that of *Staphylococcus aureus*, it is expected that more *Staphylococcus aureus* than *Escherichia coli* penetrated the carpet surface and were trapped in the middle of the carpet. Therefore, more *Escherichia coli* remained at the surface of carpet and available for transfer than *Staphylococcus aureus*. This contributed to the higher transfer of *Escherichia coli* when compared with the transfer of *Staphylococcus aureus*.



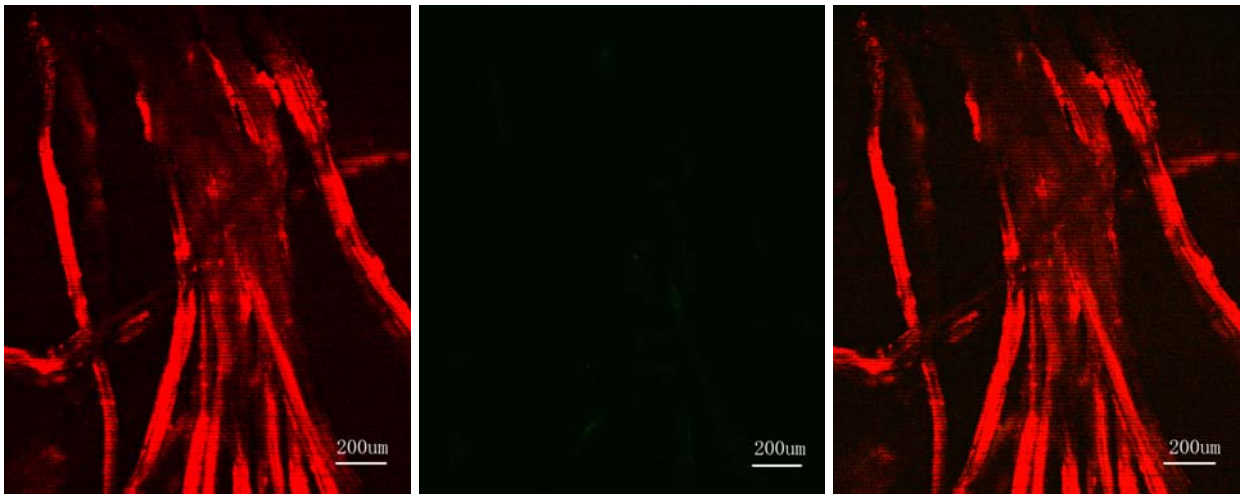
(a) (b) (c)

Figure 4.39 Representatives of LSCM images for Location A in the carpet with microspheres applied using airbrush method; (a) image obtained by PMT one, (b) image obtained by PMT two, and (c) merged image



(a) (b) (c)

Figure 4.40 Representatives of LSCM images for Location B in the carpet with microspheres applied using airbrush method; (a) image obtained by PMT one, (b) image obtained by PMT two, and (c) merged image



(a) (b) (c)

Figure 4.41 Representatives of LSCM images for Location C in the carpet with microspheres applied using airbrush method; (a) image obtained by PMT one, (b) image obtained by PMT two, and (c) merged image

Impact of Chemical Treatment on Microbial Transfer

Impact of Freepel^{TM} 1225 Treatment and Humectant A Treatment on Microbial Transfer

Chemical treatments of carpets significantly influenced the microbial transfer from carpets to fingers (Table 4.7). In general, the transfer of microorganisms was significantly increased from the Freepel^{TM} 1225 treated carpets compared with the untreated carpet except for the *Klebsiella pneumoniae* (Figures 4.23-4.38). The transfer of microorganisms was significantly decreased from the Humectant A treated carpet when compared with the untreated carpet except for the *Klebsiella pneumoniae* (Figures 4.23-4.38).

The surface energy of the carpets influenced the microbial adhesion and release which were closely related to the microbial transfer. Bakker et al. (2003) found that the microorganisms with lower hydrophobicity adhered preferentially to the surfaces with higher surface energy,

whereas the microorganisms with higher hydrophobicity showed a greater preference to adhere surfaces with lower surface energy. *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger* used in this study are hydrophilic microorganisms (Ryoo, D. and Choi, CH. 2001), which have a strong interaction with the surface whose surface energy was high. The high energy surface could induce the great and rapid hydrophilic cell spreading and adhesion as a result of this strong interaction. Conversely, a low energy surface gave minimal cell spreading and adhesion. In this study the Freepel^{TM} 1225 treatment decreased the carpet surface energy while Humectant A treatment increased the carpet surface energy (Tables 4.1 and 4.3). The adhesion of microorganisms to the Humectant A treated carpet was increased as the increasing of surface energy after treatment. Therefore, the microbial transfer was significantly decreased. When the surface energy of the carpet was decreased after the Freepel^{TM} 1225 treatment, the results showed low microbial adhesion and high microbial transfer.

Impact of Sulfated 2-EH Treatment on Microbial Transfer

The influence of the Sulfated 2-EH treatment on microbial transfer from carpets to fingers was very complicated. The transfer of *Staphylococcus aureus* from the Sulfated 2-EH treated carpets was less than that from the untreated carpets (Figures 4.23-4.26). There was no *Escherichia coli* transferred from the Sulfated 2-EH treated carpets if the transfer was performed 6 hours or longer after application to the carpet surface (Figures 4.27-4.30). The number of *Aspergillus niger* transferred from the Sulfated 2-EH treated carpets was not significantly different from that from the untreated carpets (Figures 4.35-4.38). The explanation for these results is quite unknown and needs further study. Sulfated 2-EH was an anionic surfactant. When the microbial culture was applied to the carpet surface the chemicals on the pile yarns may react with water molecule in the liquid culture and have resulted in the carpet pile yarns becoming

negatively charged. Most microorganisms, including those used in this study were also negatively charged (Robert and Freitas, 2003). Therefore, the repulsive force between the microorganisms and pile yarns of the Sulfated 2-EH treated carpets (due to their negative charges) should result in the microorganisms' difficulty to adhere to the carpet pile yarns and an increase in the transfer of microorganisms should be observed from the Sulfated 2-EH treated carpets than that from the untreated carpets. However, the results in this study showed that the transfer of microorganisms from the Sulfated 2-EH treated carpets was less than that from the untreated carpets. It seemed that the repulsive force could be overcome resulting in the firm adhesion between microorganisms and Sulfated 2-EH treated carpet pile yarns. Some other unknown factors may also influence these results.

Impact of Interaction between Bacteria and Carpets with Different Surface Energy on Microbial Transfer

In general the average transfer of *Escherichia coli* was highest from the Freepel^{TM} 1225 treated carpets to fingers, followed by *Staphylococcus aureus*, and then *Aspergillus niger* (Figures 4.42 and 4.43) (*Klebsiella pneumoniae* was not discussed here because it did not transfer after it had adhered to the carpet yarns). However, the transfer of *Escherichia coli* and *Staphylococcus aureus* from the Humectant A treated carpet was not significantly different from each other and the transfer of *Aspergillus niger* was still the lowest (Figures 4.42 and 4.43). Therefore, the following discussion focuses on the difference between the transfer of *Staphylococcus aureus* and *Escherichia coli* from the carpets with different surface energies.

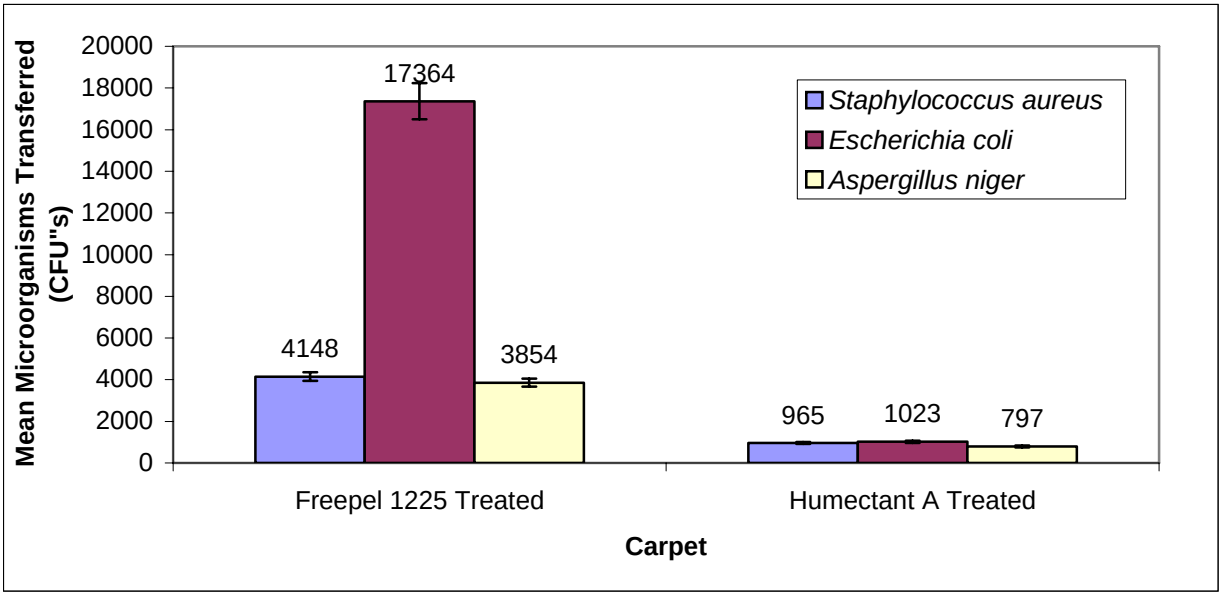


Figure 4.42 Mean of transfer of microorganisms from the treated carpets with different surface energies using compression method

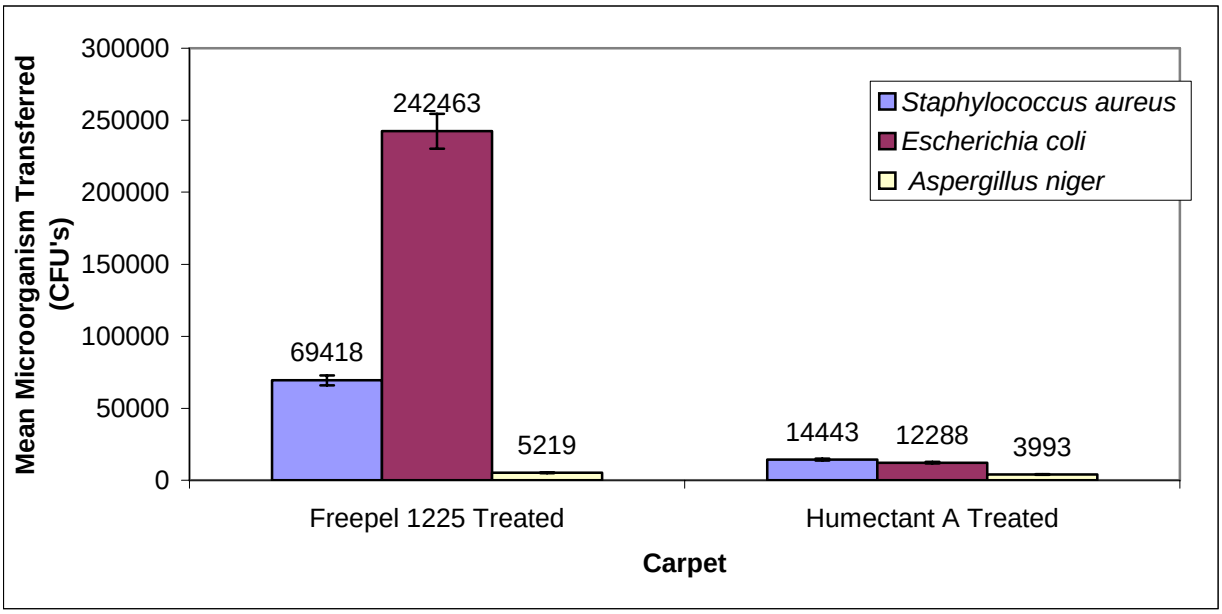


Figure 4.43 Mean transfer of microorganisms from the treated carpets with different surface energies using sliding method

It was the hydrophobicity of *Staphylococcus aureus* and *Escherichia coli* that resulted in their different transfer from treated carpets with different surface tensions. The hydrophobicity, which can be defined most simply as the relatively tendency of a bacteria to adhere to a non-polar material compared with that to water, was determined by the cell wall characteristics of bacteria. The results of hydrophobicity testing are presented in Figures 4.44-4.46.

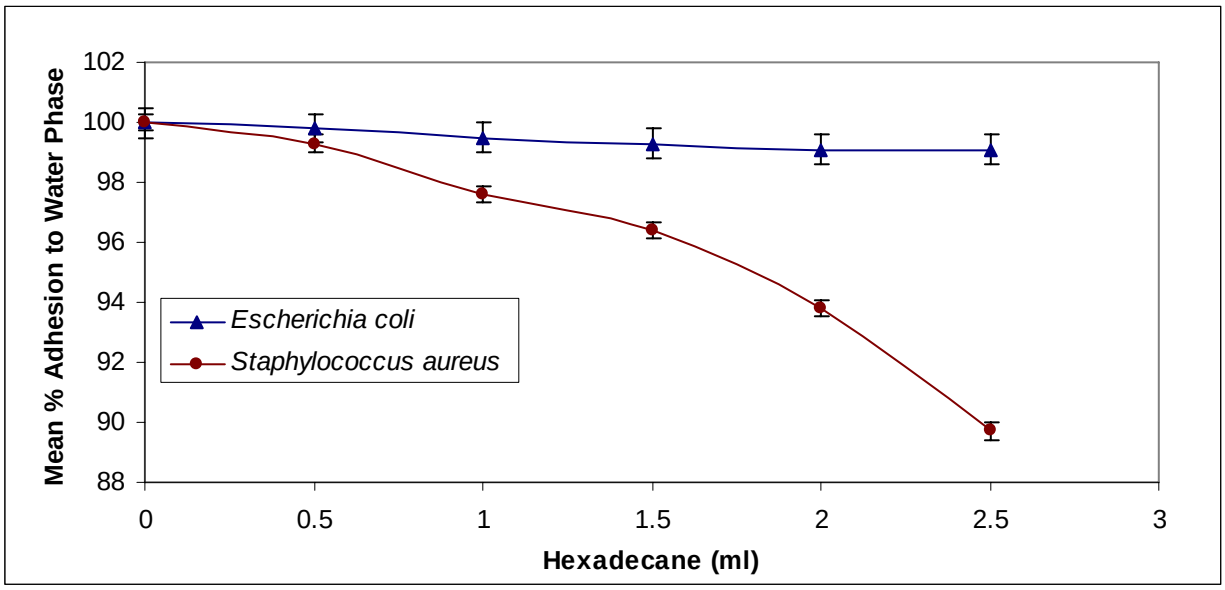


Figure 4.44 Adhesion of bacteria to water phase in the mixture of water and hexadecane (mean of three replications)

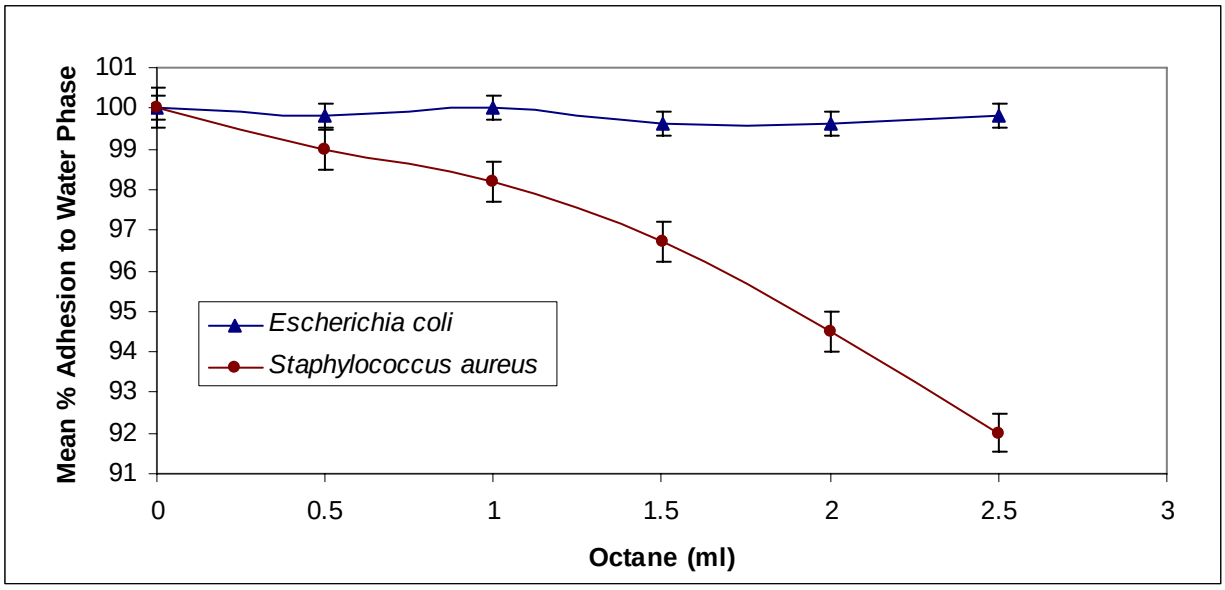


Figure 4.45 Adhesion of bacteria to water phase in the mixture of water and octane (mean of three replications)

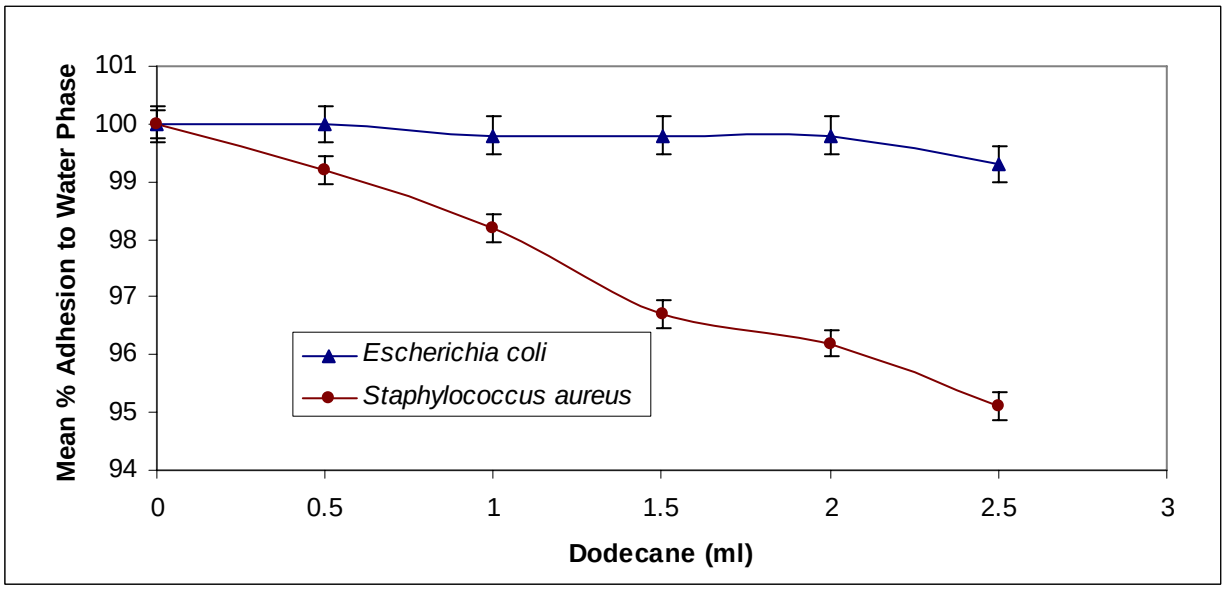


Figure 4.46 Adhesion of bacteria to water phase in the mixture of water and dodecane (mean of three replications)

Both bacteria have hydrophilic properties because more than 90% of the bacteria adhered to the water rather than the hydrocarbons (Figures 4.44-4.46). *Escherichia coli* had little tendency to adhere the hydrocarbon, while 4-5% of *Staphylococcus aureus* adhered to the hydrocarbon phase when there was 2 ml hydrocarbon in the mixture (Figures 4.44-4.46). Therefore, the hydrophobicity of *Escherichia coli* was lower than that of *Staphylococcus aureus*.

Rad, et al. identified the bacteria with higher hydrophobicity adhered more to the hydrophobic surfaces (low surface energy) and vice versa, which is in accordance with most studies in bacterial adhesion (Rad, A.Y. et al., 1998). In this study the Freepel^{TM} 1225 treated carpet had a hydrophobic surface (Figures 4.1). Since the hydrophobicity of *Escherichia coli* was lower than that of *Staphylococcus aureus*, they adhered less than *Staphylococcus aureus* to the Freepel^{TM} 1225 treated carpet. Therefore, more *Escherichia coli* could be transferred from the Freepel^{TM} 1225 treated carpet to fingers than *Staphylococcus aureus*.

The Humectant A treated carpet had a hydrophilic surface and *Escherichia coli* adhered to this surface more firmly than *Staphylococcus aureus*. Therefore, the release and transfer of *Escherichia coli* from the Humectant A treated carpet to fingers was less than that of *Staphylococcus aureus*.

Multiple factors may have influence on the transfer of *Staphylococcus aureus* and *Escherichia coli* from carpets to fingers. As discussed previously, it is possible that more *Staphylococcus aureus* penetrate the carpet piles and be trapped in the middle of the carpet than *Escherichia coli* due to their smaller size and round shape. It was concluded from this that more *Escherichia coli* might be located at the surface of the carpet than *Staphylococcus aureus*, therefore more *Escherichia coli* was available for transfer than *Staphylococcus aureus*. However, the hydrophobicity of these two bacteria and the hydrophilic property of the Humectant A treated

carpet yarns showed that more *Staphylococcus aureus* than *Escherichia coli* could be transferred from the treated carpets to fingers. As a result of these two factors the transfer of *Staphylococcus aureus* from the Humectant A treated carpets to fingers was not significantly different from that of *Escherichia coli*.

Impact of Transfer Method on Microbial Transfer from Carpets to Fingers

Two transfer methods, compression and sliding, were investigated in this study. In general sliding increased the transfer of microorganism from carpets to fingers except in several combinations (*Escherichia coli*/Sulafted 2-EH treated carpet/6, 12, 24, 48h; *Klebsiella pneumoniae*/any carpet/6, 12, 24, 48h) (Figures 4.23-4.38). In the sliding method, the fingers had more opportunities to come in contact with the microorganisms for contamination when moving on the carpet surface. Therefore, more bacteria could be transferred using sliding method. In the combinations indicated above, the sliding method and compression method did not result in the significant difference of microbial transfer because the adhesion force between the microorganisms and carpet pile yarns was too large to be overcome by either sliding or compression.

Impact of Carpet Texture

The different carpet textures, loop pile and cut pile, did not significantly influence the microbial transfer from carpet to fingers (Table 4.7). In this study the microorganisms were applied to carpets using airbrush. Each time about the same amount of microorganisms were applied to the both loop and cut pile carpets. By examining LSCM images, most microorganisms available for transfer were located at the surface of the carpet. Therefore, the number of

microorganisms transferred from these two carpets with different textures was not significantly different from each other.

Section V. Transfer of Microorganisms from Carpet to Different Receptor Materials

In this study, four different receptor materials were investigated. They could be classified into two categories, receptor fingers (bare fingers and compression fabric wrapped fingers) and receptor fabrics (compression fabric, cotton knit fabric, and cotton woven fabric). The transfer of microorganisms to the receptor fingers was performed by an individual, while the transfer of microorganisms to the receptor fabrics was performed using the materials evaluator. It was difficult to directly compare the results due to the different mechanical transfer methods. Therefore, the compression fabric was used as a control receptor fabric. The sliding transfer performed by an individual was completed to compare the microbial transfer from carpets to the compression fabric wrapped fingers with that of the bare fingers. The mechanical transfer, performed using the materials evaluator by sliding, was completed to compare the transfer of microorganisms from carpets to compression fabric, cotton knit fabric, and cotton woven fabric.

Microbial Transfer from Carpets to Compression Fabric Wrapped Fingers and Bare Fingers by Sliding

The results of microbial transfer from carpets to compression fabric wrapped fingers and bare fingers using the sliding method are presented in Tables 4.10-4.11. No transfer of *Klebsiella pneumoniae* was observed from the carpets. The Wilcoxon's signed rank test was completed to analyze the effect of compression fabric wrapped fingers and bare fingers on the transfer of

Staphylococcus aureus, *Escherichia coli*, and *Aspergillus niger* (Appendix D). The following null hypotheses were tested:

Hypothesis 16: There was no significant difference in *Staphylococcus aureus* transfer from the carpet to the compression fabric wrapped fingers and to the bare fingers.

The p value of the influence of compression fabric wrapped fingers and bare fingers on *Staphylococcus aureus* transfer (hypothesis 16) is 7.63×10^{-6} (< 0.01) (Appendix D). Therefore, hypothesis 16 was rejected. The transfer of *Staphylococcus aureus* from carpets to the compression fabric wrapped fingers was significantly different from that to the bare fingers.

Hypothesis 17: There was no significant difference in *Escherichia coli* transfer from carpet to the compression fabric wrapped fingers and to the bare fingers.

The p value of the influence of compression fabric wrapped fingers and bare fingers on *Escherichia coli* transfer (hypothesis 17) is 2.742×10^{-6} (< 0.01) (Appendix D). Therefore, the hypothesis 17 was rejected. The transfer of *Escherichia coli* from carpets to the compression fabric wrapped fingers was significantly different from that to the bare fingers.

Hypothesis 18: There was no significant difference in *Aspergillus niger* transfer from carpets to the compression fabric wrapped fingers and to the bare fingers.

The p value of the influence of compression fabric wrapped fingers and bare fingers on *Aspergillus niger* transfer (hypothesis 18) is 0.2511 (>0.01) (Appendix D). Therefore, the hypothesis 18 could not be rejected. The transfer of *Aspergillus niger* from carpets to the compression fabric wrapped fingers was not significantly different from that to the bare fingers.

The transfer of *Staphylococcus aureus* from carpets to the compression fabric wrapped fingers was significantly lower than that to the bare fingers under most of the testing conditions in this study. The only exception was its transfer from the Sulfated 2-EH treated carpet to the

compression fabric wrapped fingers where it was the same as that to the bare fingers (Tables 4.10 and 4.11). The transfer of *Escherichia coli* from carpets to compression fabric wrapped fingers was significantly higher than that to the bare fingers under most testing conditions. The only exception was its transfer from the Humectant A treated carpet to compression fabric wrapped fingers where it was lower than that to bare fingers (Tables 4.10 and 4.11). The transfer of *Aspergillus niger* from carpets to the compression fabrics wrapped fingers was not significantly different from that to the bare fingers. It seems that the compression fabric shows more similarity to human skin when picking up large fungus rather than small bacteria.

Table 4.10 Mean microbial transfer from carpets to compression fabrics wrapped fingers*

Carpet		Mean CFU's of microorganisms transferred (Std. Dev.)			
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Aspergillus niger*
Cut pile	Control (No treatment)	733 (300)	15133 (2402)	0 (0)	250 (50)
	Freepel™ 1225 treated	2667 (642)	183333 (20816)	0 (0)	400 (165)
	Humectant A treated	430 (26)	550 (50)	0 (0)	100 (0)
	Sulfated 2-EH treated	30 (0)	30 (0)	0 (0)	180 (53)
Loop pile	Control (No treatment)	2900 (96)	13200 (1058)	0 (0)	500 (86)
	Freepel™ 1225 treated	8333 (654)	161333 (58011)	0 (0)	2200 (1053)
	Humectant A treated	1400 (529)	1013 (240)	0 (0)	327 (25)
	Sulfated 2-EH treated	30 (0)	30 (0)	0 (0)	150 (50)

* Transfer was performed 24 hours after microorganisms applied to carpets using airbrush method

Table 4.11 Mean microbial transfer from carpets to bare fingers (human skin)*

Carpet		Mean CFU's of microorganisms transferred (Std. Dev.)			
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Aspergillus niger*
Cut pile	Control (No treatment)	5067 (622)	5800 (2133)	0 (0)	200 (9)
	Freepel™ 1225 treated	7867 (222)	24400 (6533)	0 (0)	300 (22)
	Humectant A treated	4633 (150)	3600 (239)	0 (0)	100 (0)
	Sulfated 2-EH treated	30 (0)	0 (0)	0 (0)	220 (4)
Loop pile	Control (No treatment)	7567 (289)	10467 (832)	0 (0)	660 (106)
	Freepel™ 1225 treated	13833 (2222)	14800 (58011)	0 (0)	2800 (200)
	Humectant A treated	4833 (111)	5367 (667)	0 (0)	426 (44)
	Sulfated 2-EH treated	30 (0)	0 (0)	0 (0)	100 (0)

* Transfer was performed 24 hours after microorganisms applied to carpets using airbrush method

Microbial Transfer from Carpets to Receptor Fabrics Using Materials Evaluator by Sliding

No transfer of *Klebsiella pneumoniae* was observed from carpets to any receptor fabric. The results of the transfer of *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger* are presented in Figures 4.47-4.52.

Wilcoxon's signed rank test was completed to analyze the effect of receptor fabrics on the microbial transfer from carpets (Appendix E). The following null hypotheses were tested:

Hypothesis 20: There was no significant difference of microbial transfer from carpet to compression fabric and cotton knit fabric.

Hypothesis 21: There was no significant difference of microbial transfer from carpet to compression fabric and cotton woven fabric.

Hypothesis 22: There was no significant difference of microbial transfer from carpet to cotton woven fabric and cotton knit fabric.

All p values were less than the significance level, 0.01 (Appendix E). Therefore, the null hypotheses 20-22 were rejected and the microbial transfer from carpets to the receptor fabrics used in this study were significantly different from each other.

The transfer of *Staphylococcus aureus* from the Sulfated 2-EH treated loop pile carpets to the compression fabric was higher than that to the cotton knit (single jersey) and cotton woven fabrics (twill weave) (Figure 4.48) and the transfer of *Aspergillus niger* from the Freepel™ 1225 cut pile treated carpet to the compression fabric was higher than that to the cotton knit and cotton woven fabrics (Figure 4.51). However, for the rest of the testing conditions in this study, the transfer of microorganisms (except *Klebsiella pneumoniae*) from carpets to the cotton knit fabric was the highest, followed by the compression fabric and then the cotton woven fabric (Figures 4.47-4.52). The surfaces of the different fabrics were not completely smooth. They were upraised and depressed due to the interlacing yarns of the woven fabric and the interlocking loops of the knit fabric (Figure 4.53). This resulted in the actual contact area between the receptor fabric and the carpet surface being smaller than the fabric area (Slayton *et al*., 1997). In this study, the receptor fabrics had varying surface properties (smoothness) due to the different constructions and the actual contact areas may have been different from each other. Consistent with that, the transfer of microorganisms from both loop and cut pile carpets to different receptor fabrics through direct contact was found to be different from each other.

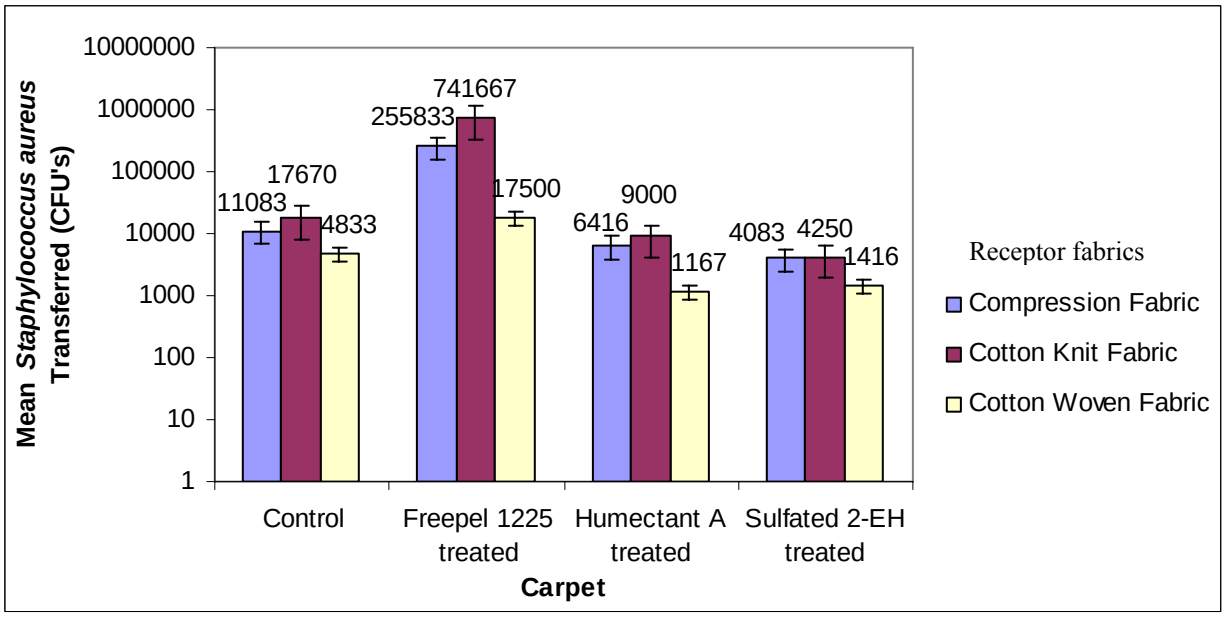


Figure 4.47 Transfer of *Staphylococcus aureus* (mean of three replications) from cut pile carpets to the receptor fabrics using materials evaluator by sliding (log scale)

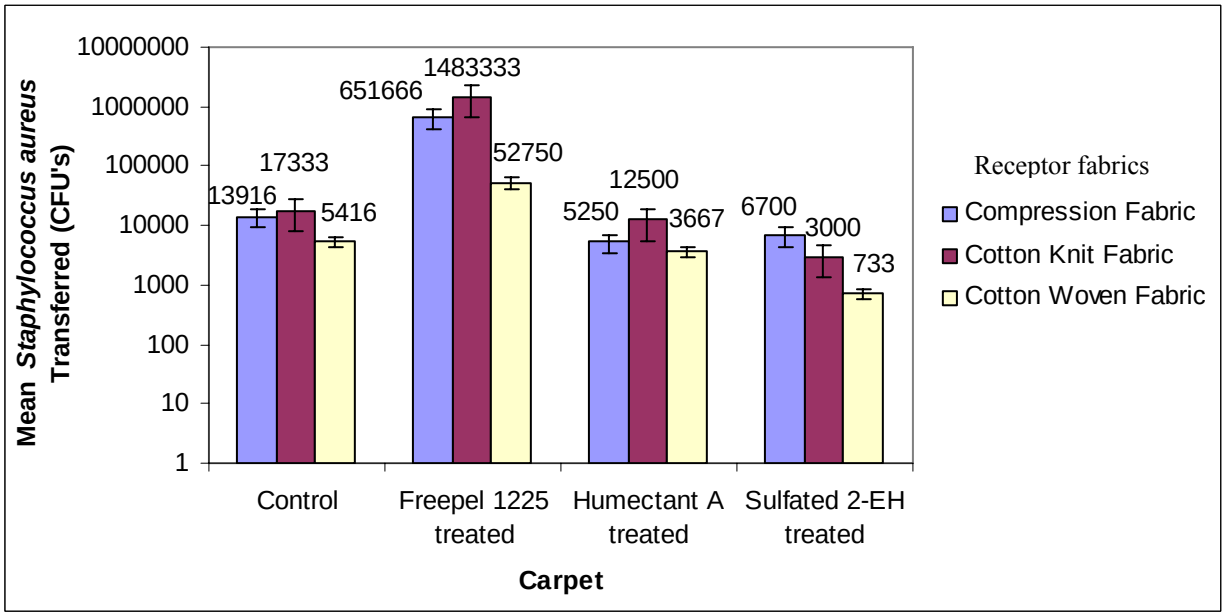


Figure 4.48 Transfer of *Staphylococcus aureus* (mean of three replications) from loop pile carpets to the receptor fabrics using materials evaluator by sliding (log scale)

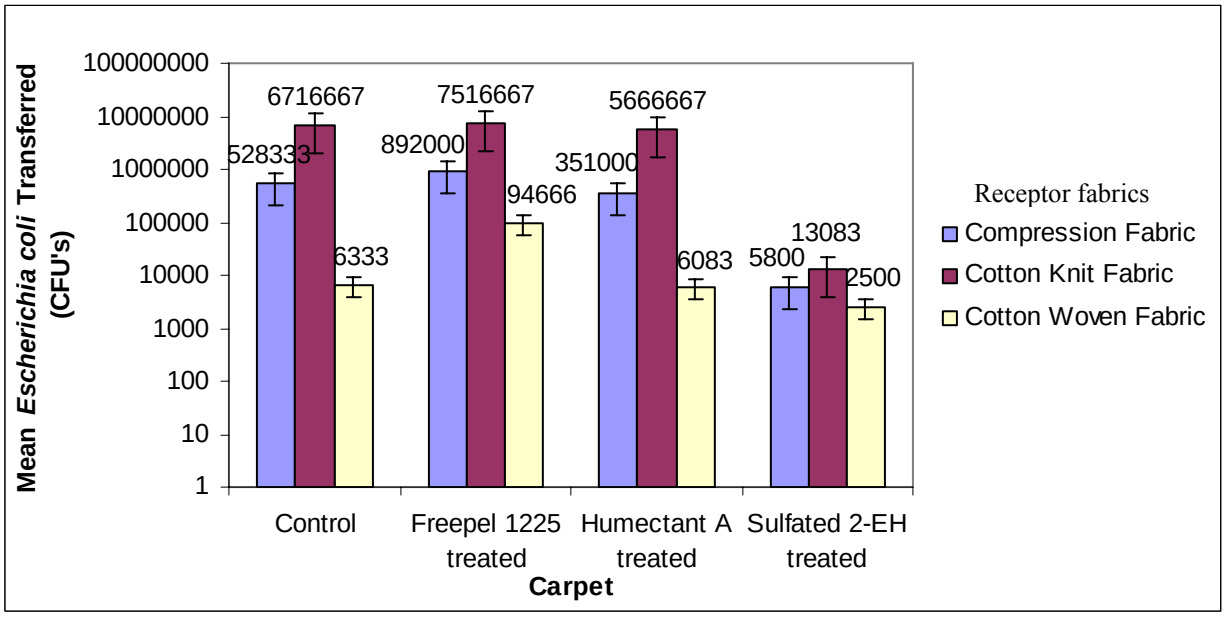


Figure 4.49 Transfer of *Escherichia coli* (mean of three replications) from cut pile carpets to the receptor fabrics using materials evaluator by sliding (log scale)

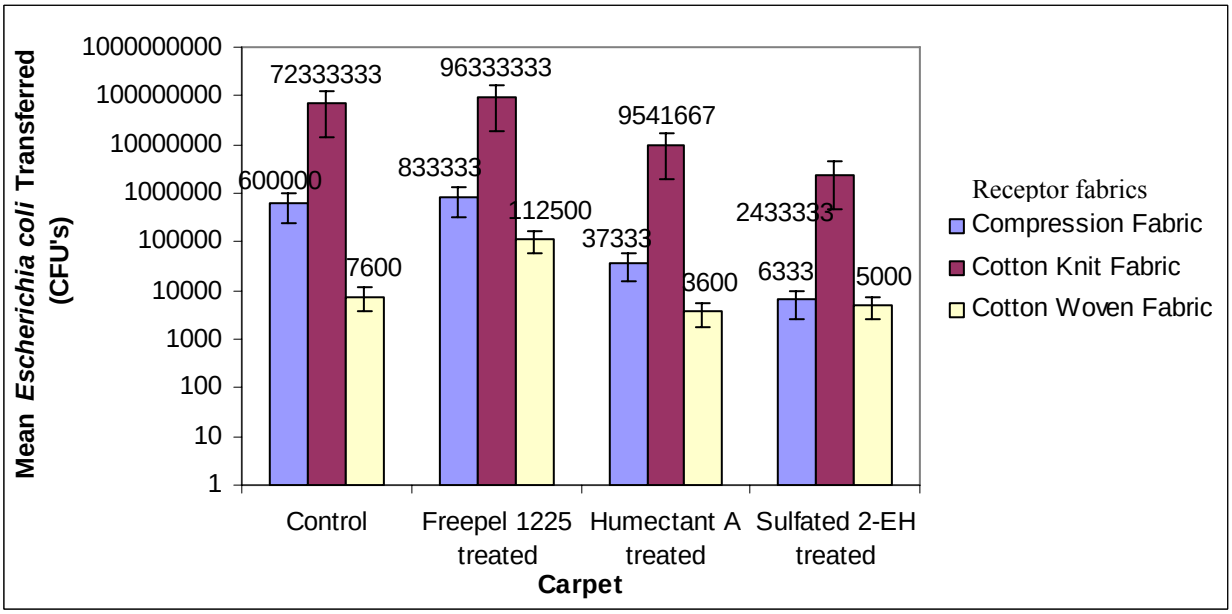


Figure 4.50 Transfer of *Escherichia coli* (mean of three replications) from loop pile carpets to the receptor fabrics using materials evaluator by sliding (log scale)

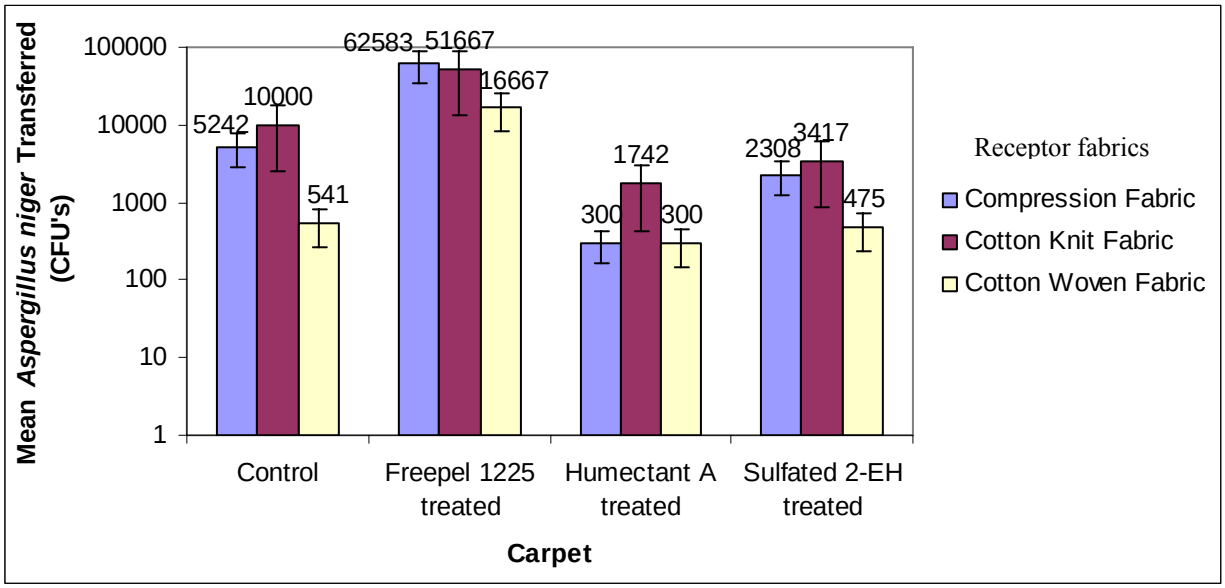


Figure 4.51 Transfer of *Aspergillus niger* (mean of three replications) from cut pile carpets to the receptor fabrics using materials evaluator by sliding (log scale)

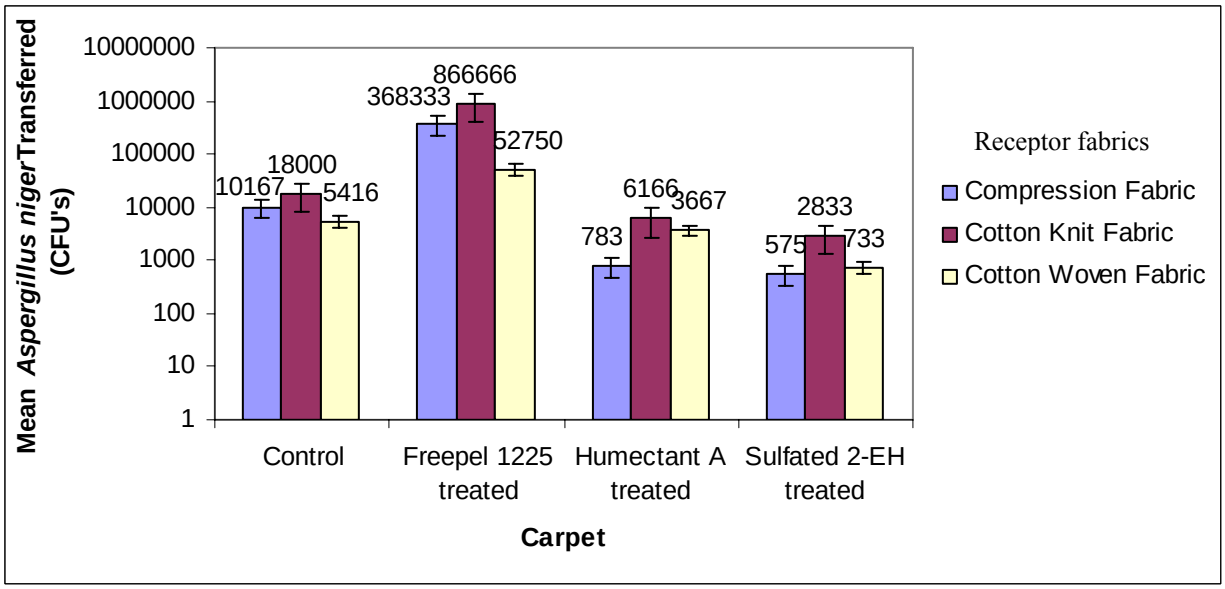


Figure 4.52 Transfer of *Aspergillus niger* (mean of three replications) from loop pile carpets to the receptor fabrics using materials evaluator by sliding (log scale)

(a) (b) (c)

Figure 4.53 Interlacing yarns and loops in the receptor fabrics; (a) compression fabric (b) cotton knit fabric, (c) cotton woven fabric

The cotton woven (twill weave) and cotton knit (single jersey) fabrics are two commonly used fabrics for pants and T-shirts respectively. In this study, the microorganisms (except *Klebsiella pneumoniae*) were transferred from the carpets to the typical pants and T-shirt fabrics as well as to the human skin. Therefore, sitting or lying on the carpets should be avoided to prevent the microbial transfer from carpets to clothes and skin.

The chemical treatment of carpets continued to have an influence on the microbial transfer from carpets to receptor fabrics. For every fabric, the transfer of microorganisms (except *Klebsiella pneumoniae*) from the Freepel^{TM} 1225 treated carpets was the highest, followed by the untreated carpets, and then the Humectant A treated carpets and the Sulfated 2-EH treated carpets. These results are like those when the bare fingers were used as the receptor material. Regardless of which receptor material was used, the higher the adhesion between the microorganisms and carpet pile yarns, the fewer the microorganisms could be transferred to the receptor materials. In this study, the Humectant A treatment and the Sulfated 2-EH treatment increased the adhesion between microorganisms and carpet pile yarns while the Freepel^{TM} 1225 treatment decreased the adhesion. Therefore, the transfer of microorganisms from the Freepel^{TM}

1225 treated carpets to the receptor fabrics was always higher than that from the untreated carpets; the transfer of microorganisms from the Humectant A treated carpets and the Sulfated 2-EH treated carpets to the receptor fabrics was always lower than transfer from the untreated carpets.

CHAPTER 5

CONCLUSIONS AND FUTURE WORK

Conclusions

The primary objective of this dissertation was to study the influence of chemical finishes, selected to alter the surface energy and electrostatic property of carpets, on the transfer of microorganisms from carpets to human skin and selected textile materials. To carry out this research, two carpets (cut pile and loop pile), four microorganisms (*Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae*, and *Aspergillus niger*), three chemicals (Humectant A, Freepel^{TM} 1225, and Sulfated 2-EH), and four receptor materials (human skin, compression fabric, cotton knit fabric, and cotton woven fabric) were used for completing the transfers. Statistical analysis was performed to determine the significance of each variable. Two methods used to apply microorganisms to carpets, pipette method and airbrush method, were investigated and compared. The distribution of GFP *Escherichia coli* in the carpet structure was studied using LSCM. The major findings of this dissertation are summarized as follows:

1. An airbrush method was developed to apply the microorganisms to the carpet in this study. The LSCM images showed that most of the microorganisms were located at the surface and near the surface of the carpet when applied to the carpet using this airbrush technique. However, microorganisms that were applied to the carpet using the pipette method were distributed through the depth of the carpet. Since only the microorganisms on the surface or near the surface of the carpet were available for the transfer through direct contact, the airbrush method showed a distinct advantage in

the application of the microorganisms for transfer when compared with the pipette method.

2. Microbial species significantly influenced the transfer of microorganisms from the carpets to fingers. There was a significant difference between the transfer of bacteria and the transfer of fungi. The number of bacteria (except *Klebsiella pneumoniae*) transferred from carpet to fingers was larger than that of fungal spores and hyphae. The bacteria species also resulted in a significant difference of bacterial transfer. *Klebsiella pneumoniae* did not transfer from the carpet to fingers once the adhesion between the bacteria and carpet pile yarns occurred. *Escherichia coli* transferred more than *Staphylococcus aureus* especially from carpets with low surface energy.
3. Chemical treatments of the carpets significantly influenced the microbial transfer. Freepel™ 1225 treatment increased the microbial transfer from the carpets to fingers and receptor fabrics by decreasing the surface energy of carpet. The Humectant A treatment reduced the microbial transfer by increasing the surface energy of the carpet. The microbial transfer was also reduced from carpets after the Sulfated 2-EH treatment. It is known that microorganisms can cause human illness. If fewer microorganisms are transferred from carpets to human skin, the risk of causing disease can be reduced. Therefore, the carpets should have a relatively high surface energy to reduce the microorganisms transferred from carpets to human skin and farbics.
4. Different transfer methods significantly influenced the microbial transfer from carpet to fingers. The transfer of microorganisms using sliding was higher than that using compression.

5. Carpet texture (cut and loop) did not significantly influence the microbial transfer in this study. The number of microorganisms transferred from the loop pile carpet was not significantly different from that from the cut pile carpet in this study.
6. Receptor materials significantly influenced the number of microorganisms transferred from carpets in this study.
    (a) When the transfer was performed by an individual, the transfer of *Staphylococcus aureus* and *Escherichia coli* from carpets to the compression fabric wrapped fingers was significantly different from that to the bare fingers. However, the transfer of *Aspergillus niger* from carpets to the compression fabric wrapped fingers was not significantly different from that to the bare fingers.
    (b) When the transfer was completed using materials evaluator, under most testing conditions the microbial transfer from carpets to the cotton knit fabric was the highest, followed by the compression fabric, and then the cotton woven fabric. The receptor fabrics with different construction resulted in the different transfer.

Future work

Microbial transfer from carpets through direct contact is a very complicated process. To fully understand this transfer process, further study is recommended as follows;

1. The mechanism of how the Sulfated 2-EH (anionic surfactant) treatment reduced the number of microorganisms transferred from carpets to human skin is still unknown. Some variables evaluating the electrostatic properties, such as the zeta potential of

both microorganisms and the treated carpet, should be measured to investigate their influence on adhesion between the microorganisms and the treated carpet pile yarns.

2. The microbial transfer was reduced after treating the carpet with a chemical which increased the surface energy. Therefore, the optimization of this chemical treatment and the longevity of the chemical on the carpets during normal use and care should be studied.
3. It was difficult to compare the results of microbial transfer from carpets performed by the individual and the materials evaluator. The correlation between these two methods of mechanical transfer needs further study. The influence of different individuals on microbial transfer is also of interest.
4. The real contact area between the different carpets and different receptor materials should be investigated to determine how it influences the microbial transfer.

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APPENDICES

APPENDIX A: Test of Normality

R Code

```
data=read.table("bacteria.txt",h=T)
bacteria=data.frame(data)
summary(bacteria)

shapiro.test(bacteria$S.aureus)
shapiro.test(bacteria$E.coli)
shapiro.test(bacteria$K.pneumoniae)
shapiro.test(bacteria$A.niger)

data=read.table("carpet.txt",h=T)
carpet=data.frame(data)
summary(carpet)

shapiro.test(carpet$Cut)
shapiro.test(carpet$Loop)

data=read.table("chemical.txt",h=T)
chemical=data.frame(data)
summary(chemical)

shapiro.test(chemical$Control)
shapiro.test(chemical$Freepel)
shapiro.test(chemical$Humectant)
shapiro.test(chemical$Sulfated)
```

R output

Shapiro-Wilk normality test for microorganisms

```
data:  bacteria$S.aureus
W = 0.4831, p-value < 2.2e-16

data:  bacteria$E.coli
W = 0.3604, p-value < 2.2e-16

data:  bacteria$K.pneumoniae
W = 0.4457, p-value < 2.2e-16
```

```
data:  bacteria$A.niger
W = 0.68, p-value < 2.2e-16
```

Shapiro-Wilk normality test for carpet textures

```
data:  carpet$Cut
W = 0.145, p-value < 2.2e-16
```

```
data:  carpet$Loop
W = 0.2103, p-value < 2.2e-16
```

Shapiro-Wilk normality test for chemicals

```
data:  chemical$Control
W = 0.2037, p-value < 2.2e-16
```

```
data:  chemical$Freepel
W = 0.2175, p-value < 2.2e-16
```

```
data:  chemical$Humectant
W = 0.1521, p-value < 2.2e-16
```

```
data:  chemical$Sulfated
W = 0.2016, p-value < 2.2e-16
```

APPENDIX B: Generalized Linear Model

R Code

```
data=read.table("friedman.txt",h=T)
transfer=data.frame(data)
summary(transfer)
g=glm(Transfer~factor(Microbe)+factor(Carpet)+factor(Chemical),transfer,family=quasipoisson
)
summary(g)
anova(g,test="Chi")
```

R output

Summary of generalized linear model

```
Call:
glm(formula = Transfer ~ factor(Microbe) + factor(Carpet) + factor(Chemical),
    family = quasipoisson, data = transfer)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-1531.72   -581.00   -134.42    -32.85   5640.89

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)         7.6320     1.3574   5.622 2.47e-08 ***
factor(Microbe)E    5.2218     1.3474   3.876 0.000114 ***
factor(Microbe)K    3.8603     1.3578   2.843 0.004566 **
factor(Microbe)S    1.7677     1.4540   1.216 0.224381
factor(Carpet)L     0.3659     0.1765   2.073 0.038447 *
factor(Chemical)F   0.7655     0.2218   3.452 0.000581 ***
factor(Chemical)H  -0.6168     0.3094  -1.993 0.046511 *
factor(Chemical)S  -0.2691     0.2784  -0.967 0.333911
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasipoisson family taken to be 1215465)

    Null deviance: 903330993  on 959  degrees of freedom
Residual deviance: 609548576  on 952  degrees of freedom
AIC: NA

Number of Fisher Scoring iterations: 7
```

Analysis of Deviance

```
                  Df  Deviance Resid. Df Resid. Dev P(>|Chi|)
NULL                                  959  903330993
factor(Microbe)    3 242932194       956  660398799 4.506e-43
factor(Carpet)     1   5310621       955  655088177 3.659e-02
factor(Chemical)   3  45539601       952  609548576 3.665e-08
```

APPENDIX C: Wilcoxon's Rank Sum Test for Post Hoc Analysis

R Code

```
data=read.table("sa.txt",h=T)
sa=data.frame(data)
summary(sa)

data=read.table("ec.txt",h=T)
ec=data.frame(data)
summary(ec)

data=read.table("kp.txt",h=T)
kp=data.frame(data)
summary(kp)

data=read.table("an.txt",h=T)
an=data.frame(data)
summary(an)

wilcox.test(sa$Transfer,ec$Transfer,paired=TRUE,conf.int=TRUE)
wilcox.test(sa$Transfer,kp$Transfer,paired=TRUE,conf.int=TRUE)
wilcox.test(sa$Transfer,an$Transfer,paired=TRUE,conf.int=TRUE)
wilcox.test(ec$Transfer,kp$Transfer,paired=TRUE,conf.int=TRUE)
wilcox.test(ec$Transfer,an$Transfer,paired=TRUE,conf.int=TRUE)
wilcox.test(kp$Transfer,an$Transfer,paired=TRUE,conf.int=TRUE)

data=read.table("control.txt",h=T)
control=data.frame(data)
summary(control)

data=read.table("F.txt",h=T)
Freepel=data.frame(data)
summary(Freepel)

data=read.table("H.txt",h=T)
Humectant=data.frame(data)
summary(Humectant)

data=read.table("S.txt",h=T)
Sulfated=data.frame(data)
summary(Sulfated)

wilcox.test(control$Transfer,Freepel$Transfer,paired=TRUE,conf.int=TRUE)
wilcox.test(control$Transfer,Humectant$Transfer,paired=TRUE,conf.int=TRUE)
wilcox.test(control$Transfer,Sulfated$Transfer,paired=TRUE,conf.int=TRUE)
```

```
wilcox.test(Freepel$Transfer,Humectant$Transfer,paired=TRUE,conf.int=TRUE)
wilcox.test(Freepel$Transfer,Sulfated$Transfer,paired=TRUE,conf.int=TRUE)
wilcox.test(Humectant$Transfer,Sulfated$Transfer,paired=TRUE,conf.int=TRUE)
```

R output

Comparison of *Staphylococcus aureus* transfer and *Escherichia coli* transfer from carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  sa$Transfer and ec$Transfer
V = 7424, p-value = 0.0002294
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -6560  -350
sample estimates:
(pseudo)median
         -2100
```

Comparison of *Staphylococcus aureus* transfer and *Klebsiella pneumoniae* transfer from carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  sa$Transfer and kp$Transfer
V = 15330, p-value = 0.002054
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
  559.9999 1910.0001
sample estimates:
(pseudo)median
          1250
```

Comparison of *Staphylococcus aureus* transfer and *Aspergillus niger* transfer from carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  sa$Transfer and an$Transfer
V = 21585.5, p-value = 3.646e-11
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 1335 3160
sample estimates:
(pseudo)median
          2185
```

Comparison of *Escherichia coli* transfer and *Klebsiella pneumoniae* transfer from carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  ec$Transfer and kp$Transfer
V = 15592, p-value = 2.220e-16
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
  4120 15300
sample estimates:
(pseudo)median
          7200
```

Comparison of *Escherichia coli* transfer and *Aspergillus niger* transfer from carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  ec$Transfer and an$Transfer
V = 24301.5, p-value < 2.2e-16
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
  4820 23385
sample estimates:
(pseudo)median
      7515.481
```

Comparison of *Klebsiella pneumoniae* transfer and *Aspergillus niger* transfer from carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  kp$Transfer and an$Transfer
V = 10505.5, p-value = 0.00445
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -360  -95
sample estimates:
(pseudo)median
     -230.0000
```

Comparison of microbial transfer from untreated carpet and Freepel™ 1225 water repellent treated carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  control$Transfer and Freepel$Transfer
V = 809.5, p-value < 2.2e-16
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -12780  -3500
sample estimates:
(pseudo)median
         -6300
```

Comparison of microbial transfer from untreated carpet and Humectant A treated carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  control$Transfer and Humectant$Transfer
V = 14733, p-value = 4.885e-15
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 1100 3600
sample estimates:
(pseudo)median
          1770
```

Comparison of microbial transfer from untreated carpet and Sulfated 2-EH treated carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  control$Transfer and Sulfated$Transfer
V = 15896.5, p-value < 2.2e-16
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 1780 5640
sample estimates:
(pseudo)median
      2923.668
```

Comparison of microbial transfer from Freepel™ 1225 water repellent carpet and Humectant A treated carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  Freepel$Transfer and Humectant$Transfer
V = 19227, p-value < 2.2e-16
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
  5250 16400
sample estimates:
(pseudo)median
          8500
```

Comparison of microbial transfer from Freepel™ 1225 water repellent carpet and Sulfated 2-EH treated carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  Freepel$Transfer and Sulfated$Transfer
V = 19040.5, p-value < 2.2e-16
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
  6370 24990
sample estimates:
(pseudo)median
         10250
```

Comparison of microbial transfer from Humectant A carpet and Sulfated 2-EH treated carpet to fingers

```
        Wilcoxon signed rank test with continuity correction

data:  Humectant$Transfer and Sulfated$Transfer
V = 11166, p-value = 1.596e-05
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
  300.0000 1699.9999
sample estimates:
(pseudo)median
           800
```

APPENDIX D: Wilcoxon's Signed Rank Test for comparison of microbial transfer from carpet to compression fabric wrapped on fingers and human skin

R Code

```
data=read.table("sa.txt",h=T)
sa=data.frame(data)
summary(sa)

data=read.table("ec.txt",h=T)
ec=data.frame(data)
summary(ec)

data=read.table("an.txt",h=T)
an=data.frame(data)
summary(kp)

wilcox.exact(sa$fabric,sa$skin,paired=TRUE)
wilcox.exact(ec$fabric,ec$skin,paired=TRUE)
wilcox.exact(an$fabric,an$skin,paired=TRUE)
```

R output

Comparison of *Staphylococcus aureus* transfer from carpet to compression fabric wrapped on fingers and human skin

```
        Exact Wilcoxon signed rank test

data:  sa$fabric and sa$skin
V = 0, p-value = 7.63e-06
alternative hypothesis: true mu is not equal to 0
```

Comparison of *Escherichia coli* transfer from carpet to compression fabric wrapped on fingers and human skin

```
        Exact Wilcoxon signed rank test

data:  ec$fabric and ec$skin
V = 293, p-value = 2.742e-06
alternative hypothesis: true mu is not equal to 0
```

Comparison of *Aspergillus niger* transfer from carpet to compression fabric wrapped on fingers and human skin

```
        Exact Wilcoxon signed rank test

data:  an$fabric and an$skin
V = 162.5, p-value = 0.2511
alternative hypothesis: true mu is not equal to 0
```

APPENDIX E: Wilcoxon's Signed Rank Test for comparison of microbial transfer from carpet to different fabrics (compression fabric, cotton knit fabric, and cotton woven fabric)

R code

```
data=read.table("fabric.txt",h=T)
fabric=data.frame(data)
summary(fabric)

wilcox.exact(fabric$c,fabric$knit,paired=TRUE)
wilcox.exact(fabric$c,fabric$woven,paired=TRUE)
wilcox.exact(fabric$woven,fabric$knit,paired=TRUE)
```

R output

Comparison of microbial transfer from carpet to compression fabric and cotton knit fabric

```
        Asymptotic Wilcoxon signed rank test

data:  fabric$c and fabric$knit
V = 384, p-value = 8.49e-07
alternative hypothesis: true mu is not equal to 0
```

Comparison of microbial transfer from carpet to compression fabric and cotton woven fabric

```
        Asymptotic Wilcoxon signed rank test

data:  fabric$c and fabric$woven
V = 1925, p-value = 1.787e-05
alternative hypothesis: true mu is not equal to 0
```

Comparison of microbial transfer from carpet to cotton knit fabric and cotton woven fabric

```
        Asymptotic Wilcoxon signed rank test

data:  fabric$knit and fabric$woven
V = 1889.5, p-value = 1.197e-05
alternative hypothesis: true mu is not equal to 0
```

APPENDIX F: Raw data of microbial transfer

Table 1. *Staphylococcus aureus* transfer from carpets to fingers using compression method

Carpet		Rep. Number	Colony Forming Units (CFU's)				
			0h	6h	12h	24h	48h
Cut Pile	Control (No Treatment)	1	7000	3000	1920	1680	980
		2	4000	2200	2080	1600	1020
		3	6400	2500	1980	1640	760
		Mean	5800	2566.67	1993.33	1640	920
		S.D.	1587.45079	404.145	80.829	40	140
	FreepelTM 1225 Treated	1	13600	4400	4000	3280	2600
		2	8600	4180	3680	3060	1900
		3	11400	4300	3800	3100	2400
		Mean	11200	4293.33	3826.67	3146.67	2300
		S.D.	2505.99282	110.151	161.658	117.189	360.555
	Humectant A Treated	1	3600	500	400	200	30
		2	4400	600	300	240	30
		3	3800	640	440	300	30
		Mean	3933.33333	580	380	246.667	30
		S.D.	416.3332	72.111	72.111	50.3322	0
	Sulfated 2-EH Treated	1	3200	1340	400	30	30
		2	2000	2040	500	30	30
		3	4000	960	480	30	30
		Mean	3066.66667	1446.67	460	30	30
		S.D.	1006.64459	547.844	52.915	0	0
Loop Pile	Control (No Treatment)	1	4200	2260	1240	1320	420
		2	3000	2400	1500	1160	380
		3	5600	1500	1200	980	370
		Mean	4266.66667	2053.33	1313.33	1153.33	390
		S.D.	1301.28142	484.286	162.891	170.098	26.4575
	Freepel™ 1225 Treated	1	5600	3320	2640	2660	1500
		2	7000	3000	2800	2700	1420
		3	8600	3100	2700	2500	1560
		Mean	7066.66667	3140	2713.33	2620	1493.33
		S.D.	1501.1107	163.707	80.829	105.83	70.2377
	Humectant A Treated	1	2600	500	260	260	30
		2	3000	720	280	400	30
		3	3900	800	400	240	30
		Mean	3166.66667	673.333	313.333	300	30
		S.D.	665.832812	155.349	75.7188	87.178	0
	Sulfated 2-EH Treated	1	2040	1340	560	30	30
		2	1900	1040	400	30	30
		3	2800	860	480	30	30
		Mean	2246.66667	1080	480	30	30
		S.D.	484.286417	242.487	80	0	0

Table 2. *Staphylococcus aureus* transfer from carpets to fingers using sliding method

Carpet		Rep. Number	Colony Forming Units (CFU's)				
			0h	6h	12h	24h	48h
Cut Pile	Control (No Treatment)	1	64000	84000	5800	6200	2300
		2	60000	42000	9600	4200	1900
		3	84000	60000	8000	5000	2400
		Mean	69333.3333	62000	7800	5133.33	2200
		S.D.	12858.201	21071.3	1907.88	1006.64	264.575
	Freepel™ 1225 treated	1	155600	142000	76000	7600	5000
		2	157000	116000	66000	7800	4700
		3	168600	144000	71000	8200	4300
		Mean	160400	134000	71000	7866.67	4666.67
		S.D.	7135.82511	15620.5	5000	305.505	351.188
	Humectant A Treated	1	8000	7400	6000	4400	2000
		2	8000	7800	5000	4800	1800
		3	7800	6000	5000	4700	2500
		Mean	7933.33333	7066.67	5333.33	4633.33	2100
		S.D.	115.470054	945.163	577.35	208.167	360.555
	Sulfated 2-EH Treated	1	5000	1460	440	30	40
		2	5000	1360	320	30	30
		3	4000	1160	700	30	40
		Mean	4666.66667	1326.67	486.667	30	36.6667
		S.D.	577.350269	152.753	194.251	0	5.7735
Loop Pile	Control (No Treatment)	1	172000	54000	16000	8000	3200
		2	128000	66000	15800	7300	2200
		3	140000	60000	15900	7400	2000
		Mean	146666.667	60000	15900	7566.67	2466.67
		S.D.	22744.9628	6000	100	378.594	642.91
	Freepel™ 1225 treated	1	156000	142000	30800	15600	8000
		2	148000	84000	76000	10500	7100
		3	162000	52000	34000	15400	7300
		Mean	155333.333	92666.7	46933.3	13833.3	7466.67
		S.D.	7023.76917	45621.6	25223.3	2888.48	472.582
	Humectant A Treated	1	64000	20600	8600	4800	2100
		2	98000	17600	9000	4700	1900
		3	74000	32000	7500	5000	2300
		Mean	78666.6667	23400	8366.67	4833.33	2100
		S.D.	17473.7899	7597.37	776.745	152.753	200
	Sulfated 2-EH Treated	1	4000	1400	220	30	30
		2	6500	1200	400	30	30
		3	5800	1360	160	30	30
		Mean	5433.33333	1320	260	30	30
		S.D.	1289.70281	105.83	124.9	0	0

Table 3. *Escherichia coli* transfer from carpets to fingers using compression method

Carpet		Rep. Number	Colony Forming Units (CFU's)				
			0h	6h	12h	24h	48h
Cut Pile	Control (No Treatment)	1	15600	2400	1620	1440	900
		2	11400	1900	1680	1900	800
		3	22000	2600	2060	1460	780
		Mean	16333.3333	2300	1786.67	1600	826.667
		S.D.	5337.9147	360.555	238.607	260	64.291
	Freepel™ 1225 Treated	1	100400	3280	2680	2500	1680
		2	83600	3500	2900	2400	1660
		3	87200	3400	2800	2400	1900
		Mean	90400	3393.33	2793.33	2433.33	1746.67
		S.D.	8845.33775	110.151	110.151	57.735	133.167
	Humectant A Treated	1	3840	500	400	200	100
		2	2740	600	300	240	100
		3	3440	640	440	300	100
		Mean	3340	580	380	246.667	100
		S.D.	556.776436	72.111	72.111	50.3322	0
	Sulfated 2-EH Treated	1	2840	0	0	0	0
		2	2280	0	0	0	0
		3	3560	0	0	0	0
		Mean	2893.33333	0	0	0	0
		S.D.	641.664502	0	0	0	0
Loop Pile	Control (No Treatment)	1	52800	2120	1500	1420	760
		2	54400	1880	1700	1480	680
		3	46200	2060	1280	1220	800
		Mean	51133.3333	2020	1493.33	1373.33	746.667
		S.D.	4346.64622	124.9	210.079	136.137	61.101
	Freepel™ 1225 Treated	1	61800	3560	3040	2720	1560
		2	78400	3540	3000	2580	1800
		3	56000	3640	3200	2560	1840
		Mean	65400	3580	3080	2620	1733.33
		S.D.	11625.8333	52.915	105.83	87.178	151.438
	Humectant A Treated	1	4700	500	260	260	100
		2	4020	720	280	400	100
		3	3880	800	400	240	100
		Mean	4200	673.333	313.333	300	100
		S.D.	438.634244	155.349	75.7188	87.178	0
	Sulfated 2-EH Treated	1	3700	0	0	0	0
		2	4620	0	0	0	0
		3	4180	0	0	0	0
		Mean	4166.66667	0	0	0	0
		S.D.	460.144905	0	0	0	0

Table 4. *Escherichia coli* transfer from carpets to fingers using sliding method

Carpet		Rep. Number	Colony Forming Units (CFU's)				
			0h	6h	12h	24h	48h
Cut Pile	Control (No Treatment)	1	156800	41000	60000	3200	2500
		2	162000	92000	55000	5000	2200
		3	157000	66000	50000	9000	2800
		Mean	158600	66333.3	55000	5733.33	2500
		S.D.	2946.18397	25501.6	5000	2968.73	300
	Freepel™ 1225 Treated	1	1234000	110400	47200	21000	5000
		2	1192000	120000	98400	18000	6200
		3	1104000	120000	48000	34200	5600
		Mean	1176666.67	116800	64533.3	24400	5600
		S.D.	66342.5454	5542.56	29332.1	8618.58	600
	Humectant A Treated	1	9400	12000	7400	4600	1200
		2	10200	9200	6600	3200	1500
		3	10800	8000	5800	3000	2000
		Mean	10133.3333	9733.33	6600	3600	1566.67
		S.D.	702.376917	2052.64	800	871.78	404.145
	Sulfated 2-EH Treated	1	11800	0	0	0	0
		2	16000	0	0	0	0
		3	14200	0	0	0	0
		Mean	14000	0	0	0	0
		S.D.	2107.13075	0	0	0	0
Loop Pile	Control (No Treatment)	1	520000	28000	15600	10200	2500
		2	676000	60000	10200	11000	2000
		3	840000	56000	15800	10200	2200
		Mean	678666.667	48000	13866.7	10466.7	2233.33
		S.D.	160016.666	17435.6	3177	461.88	251.661
	Freepel™ 1225 Treated	1	700000	70000	19600	17400	5600
		2	1000000	82000	16000	14000	4800
		3	1060000	66000	36000	13000	5500
		Mean	920000	72666.7	23866.7	14800	5300
		S.D.	192873.015	8326.66	10660.8	2306.51	435.89
	Humectant A Treated	1	47800	11000	8800	6400	1600
		2	56400	13800	7000	4200	1900
		3	68000	13800	9000	5200	1820
		Mean	57400	12866.7	8266.67	5266.67	1773.33
		S.D.	10137.0607	1616.58	1101.51	1101.51	155.349
	Sulfated 2-EH Treated	1	102000	0	0	0	0
		2	60000	0	0	0	0
		3	57400	0	0	0	0
		Mean	73133.3333	0	0	0	0
		S.D.	25033.0448	0	0	0	0

Table 5. *Klebsiella pneumoniae* transfer from carpets to fingers using compression method

Carpet		Rep. Number	Colony Forming Units (CFU's)				
			0h	6h	12h	24h	48h
Cut Pile	Control (No Treatment)	1	10200	0	0	0	0
		2	8000	0	0	0	0
		3	13600	0	0	0	0
		Mean	10600	0	0	0	0
		S.D.	2821.3472	0	0	0	0
	Freepel™ 1225 Treated	1	20400	0	0	0	0
		2	15600	0	0	0	0
		3	18600	0	0	0	0
		Mean	18200	0	0	0	0
		S.D.	2424.87113	0	0	0	0
	Humectant A Treated	1	8420	0	0	0	0
		2	10600	0	0	0	0
		3	9800	0	0	0	0
		Mean	9606.66667	0	0	0	0
		S.D.	1102.78435	0	0	0	0
	Sulfated 2-EH Treated	1	7000	0	0	0	0
		2	6500	0	0	0	0
		3	8400	0	0	0	0
		Mean	7300	0	0	0	0
		S.D.	984.88578	0	0	0	0
Loop Pile	Control (No Treatment)	1	62800	0	0	0	0
		2	54600	0	0	0	0
		3	84000	0	0	0	0
		Mean	67133.3333	0	0	0	0
		S.D.	15171.4644	0	0	0	0
	Freepel™ 1225 Treated	1	54200	0	0	0	0
		2	62800	0	0	0	0
		3	48000	0	0	0	0
		Mean	55000	0	0	0	0
		S.D.	7432.36167	0	0	0	0
	Humectant A Treated	1	9000	0	0	0	0
		2	9500	0	0	0	0
		3	9600	0	0	0	0
		Mean	9366.66667	0	0	0	0
		S.D.	321.455025	0	0	0	0
	Sulfated 2-EH Treated	1	7800	0	0	0	0
		2	6800	0	0	0	0
		3	7000	0	0	0	0
		Mean	7200	0	0	0	0
		S.D.	529.150262	0	0	0	0

Table 6. *Klebsiella pneumoniae* transfer from carpets to fingers using sliding method

Carpet		Rep. Number	Colony Forming Units (CFU's)				
			0h	6h	12h	24h	48h
Cut Pile	Control (No Treatment)	1	500000	0	0	0	0
		2	626000	0	0	0	0
		3	574000	0	0	0	0
		Mean	566666.667	0	0	0	0
		S.D.	63319.2967	0	0	0	0
	Freepel™ 1225 Treated	1	1180000	0	0	0	0
		2	840000	0	0	0	0
		3	1400000	0	0	0	0
		Mean	1140000	0	0	0	0
		S.D.	282134.72	0	0	0	0
	Humectant A Treated	1	92600	0	0	0	0
		2	62000	0	0	0	0
		3	80000	0	0	0	0
		Mean	78200	0	0	0	0
		S.D.	15379.2067	0	0	0	0
	Sulfated 2-EH Treated	1	152000	0	0	0	0
		2	106000	0	0	0	0
		3	200000	0	0	0	0
		Mean	152666.667	0	0	0	0
		S.D.	47003.546	0	0	0	0
Loop Pile	Control (No Treatment)	1	826000	0	0	0	0
		2	900000	0	0	0	0
		3	640000	0	0	0	0
		Mean	788666.667	0	0	0	0
		S.D.	133960.193	0	0	0	0
	Freepel™ 1225 Treated	1	910000	0	0	0	0
		2	1020000	0	0	0	0
		3	980000	0	0	0	0
		Mean	970000	0	0	0	0
		S.D.	55677.6436	0	0	0	0
	Humectant A Treated	1	70000	0	0	0	0
		2	80000	0	0	0	0
		3	80000	0	0	0	0
		Mean	970000	0	0	0	0
		S.D.	55677.6436	0	0	0	0
	Sulfated 2-EH Treated	1	660000	0	0	0	0
		2	766000	0	0	0	0
		3	738000	0	0	0	0
		Mean	721333.333	0	0	0	0
		S.D.	54930.2588	0	0	0	0

Table 7. *Aspergillus niger* transfer from carpets to fingers using compression method

Carpet		Rep. Number	Colony Forming Units (CFU's)				
			0h	6h	12h	24h	48h
Cut Pile	Control (No Treatment)	1	6600	1600	480	180	100
		2	7800	1740	680	100	120
		3	5600	1040	800	100	30
		Mean	6666.66667	1460	653.333	126.667	83.3333
		S.D.	1101.51411	370.405	161.658	46.188	47.2582
	Freepel™ 1225 Treated	1	8000	5000	1420	640	180
		2	7800	4600	800	220	300
		3	8200	4800	1500	600	250
		Mean	8000	4800	1240	486.667	243.333
		S.D.	200	200	383.145	231.805	60.2771
	Humectant A Treated	1	2800	520	300	100	30
		2	3600	760	480	80	30
		3	3000	720	480	260	30
		Mean	3133.33333	666.667	420	146.667	30
		S.D.	416.3332	128.582	103.923	98.6577	0
	Sulfated 2-EH Treated	1	7000	2000	500	200	60
		2	8000	1820	620	280	60
		3	6600	1080	440	180	60
		Mean	7200	1633.33	520	220	60
		S.D.	721.110255	487.579	91.6515	52.915	0
Loop Pile	Control (No Treatment)	1	7000	2060	1860	360	150
		2	5400	2200	640	680	200
		3	7200	2600	760	460	170
		Mean	6533.33333	2286.67	1086.67	500	173.333
		S.D.	986.576572	280.238	672.409	163.707	25.1661
	Freepel™ 1225 Treated	1	8400	4400	1860	1020	320
		2	7600	3000	1640	880	350
		3	6400	4160	1760	660	280
		Mean	7466.66667	3853.33	1753.33	853.333	316.667
		S.D.	1006.64459	748.688	110.151	181.475	35.1188
	Humectant A Treated	1	3200	800	300	200	30
		2	1600	500	300	100	30
		3	2700	500	300	150	30
		Mean	2500	600	300	150	30
		S.D.	818.535277	173.205	0	50	0
	Sulfated 2-EH Treated	1	6600	3000	680	340	100
		2	6200	2200	920	280	100
		3	6400	2000	800	400	100
		Mean	6400	2400	800	340	100
		S.D.	200	529.15	120	60	0

Table 8. *Aspergillus niger* transfer from carpets to fingers using sliding method

Carpet		Rep. Number	Colony Forming Units (CFU's)				
			0h	6h	12h	24h	48h
Cut Pile	Control (No Treatment)	1	16000	6600	1000	300	60
		2	12000	6000	800	200	60
		3	14800	4000	1200	210	60
		Mean	14266.6667	5533.33	1000	236.667	60
		S.D.	2052.64058	1361.37	200	55.0757	0
	Freepel™ 1225 Treated	1	18000	8000	2000	300	120
		2	15600	8000	1800	340	100
		3	16500	8000	1200	260	80
		Mean	16700	8000	1666.67	300	100
		S.D.	1212.43557	0	416.333	40	20
	Humectant A Treated	1	20000	5500	800	220	30
		2	14000	4500	700	250	30
		3	14100	4000	600	220	30
		Mean	16700	8000	1666.67	300	100
		S.D.	1212.43557	0	416.333	40	20
	Sulfated 2-EH Treated	1	12000	1980	780	120	60
		2	16000	1660	940	120	60
		3	14000	1420	600	140	60
		Mean	14000	1686.67	773.333	126.667	60
		S.D.	2000	280.951	170.098	11.547	0
Loop Pile	Control (No Treatment)	1	17200	3000	1200	680	200
		2	12200	4000	1500	380	180
		3	13600	3600	1540	500	250
		Mean	14333.3333	3533.33	1413.33	520	210
		S.D.	2579.40562	503.322	185.831	150.997	36.0555
	Freepel™ 1225 Treated	1	13400	8600	4700	3000	900
		2	15400	5200	2900	2500	820
		3	10600	3340	1240	2900	780
		Mean	13133.3333	5713.33	2946.67	2800	833.333
		S.D.	2411.08551	2667.31	1730.47	264.575	61.101
	Humectant A Treated	1	15000	4600	1200	300	100
		2	14000	1200	1000	480	100
		3	12000	3000	1200	440	200
		Mean	13666.6667	2933.33	1133.33	406.667	133.333
		S.D.	1527.52523	1700.98	115.47	94.5163	57.735
	Sulfated 2-EH Treated	1	14200	4000	800	500	100
		2	13000	4800	1000	600	60
		3	13800	4200	800	500	120
		Mean	13666.6667	4333.33	866.667	533.333	93.3333
		S.D.	611.010093	416.333	115.47	57.735	30.5505

Table 9. Microbial transfer from carpet to compression fabrics wrapped on the fingers using sliding 24 hours after the microorganisms were applied to the carpets

Microbe	Carpet	Rep. Number	Colony Forming Units (CFU's)			
			Control (No Treatment)	Freepel™ 1225 treated	Humectant A Treated	Sulfated 2-EH Treated
Staphylococcus aureus	Cut Pile	1	800	2400	400	30
		2	1000	3400	450	30
		3	400	2200	440	30
		Mean	733.333333	2666.67	430	30
		S.D.	305.505046	642.91	26.4575	0
	Loop Pile	1	1900	7000	1000	30
		2	3800	9000	2000	30
		3	3000	9000	1200	30
		Mean	2900	8333.33	1400	30
		S.D.	953.939201	1154.7	529.15	0
Escherichia coli	Cut Pile	1	17600	200000	600	30
		2	12800	160000	500	30
		3	15600	190000	550	30
		Mean	15333.3333	183333	550	30
		S.D.	2411.08551	20816.7	50	0
	Loop Pile	1	14000	104000	740	30
		2	12000	220000	1100	30
		3	13600	160000	1200	30
		Mean	13200	161333	1013.33	30
		S.D.	1058.30052	58011.5	241.937	0
Klebsiella pneumoniae	Cut Pile	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		Mean	0	0	0	0
		S.D.	0	0	0	0
	Loop Pile	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		Mean	0	0	0	0
		S.D.	0	0	0	0
Aspergillus niger	Cut Pile	1	300	580	100	120
		2	200	200	100	220
		3	250	350	100	200
		Mean	250	376.667	100	180
		S.D.	50	191.398	0	52.915
	Loop Pile	1	450	3300	350	100
		2	600	2100	300	150
		3	450	1200	330	200
		Mean	500	2200	326.667	150
		S.D.	86.6025404	1053.57	25.1661	50

Table 10. Microbial transfer from carpet to compression fabrics using materials evaluator 24 hours after the microorganisms were applied to the carpets

Microbe	Carpet	Rep. Number	Colony Forming Units (CFU's)			
			Control (No Treatment)	Freepel™ 1225 treated	Humectant A Treated	Sulfated 2-EH Treated
Staphylococcus aureus	Cut Pile	1	14250	262500	6500	2750
		2	10250	205000	8000	5250
		3	8750	300000	4750	4250
		Mean	11083.3333	255833	6416.67	4083.33
		S.D.	2843.12035	47849.6	1626.6	1258.31
	Loop Pile	1	14500	642500	5000	7000
		2	12250	577500	7500	6500
		3	15000	735000	3250	6600
		Mean	13916.6667	651667	5250	6700
		S.D.	1464.86632	79149.1	2136	264.575
Escherichia coli	Cut Pile	1	425000	956000	375000	5000
		2	560000	880000	380000	4600
		3	600000	840000	298000	7800
		Mean	528333.333	892000	351000	5800
		S.D.	91696.9647	58923.7	45967.4	1743.56
	Loop Pile	1	325000	825000	38250	4500
		2	650000	975000	30250	8000
		3	600000	700000	43500	6500
		Mean	525000	833333	37333.3	6333.33
		S.D.	175000	137689	6672.39	1755.94
Klebsiella pneumoniae	Cut Pile	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		Mean	0	0	0	0
		S.D.	0	0	0	0
	Loop Pile	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		Mean	0	0	0	0
		S.D.	0	0	0	0
Aspergillus niger	Cut Pile	1	8000	73250	300	2000
		2	3250	69250	300	2325
		3	4475	45250	300	2600
		Mean	5241.66667	62583.3	300	2308.33
		S.D.	2466.0613	15143.8	0	300.347
	Loop Pile	1	9500	355000	875	500
		2	12250	447500	1000	425
		3	8750	302500	475	800
		Mean	10166.6667	368333	783.333	575
		S.D.	1842.7787	73413.8	274.241	198.431

Table 11. Microbial transfer from carpet to cotton knit fabrics using materials evaluator 24 hours after the microorganisms were applied to the carpets

Microbe	Carpet	Rep. Number	Colony Forming Units (CFU's)			
			Control (No Treatment)	Freepel™ 1225 treated	Humectant A Treated	Sulfated 2-EH Treated
Staphylococcus aureus	Cut Pile	1	20000	72500	10000	5000
		2	17500	82500	8000	4250
		3	15500	67500	9000	3500
		Mean	17666.6667	74166.7	9000	4250
		S.D.	2254.62488	7637.63	1000	750
	Loop Pile	1	22000	1475000	17000	3000
		2	15750	1950000	9250	3000
		3	14250	1025000	11250	3000
		Mean	17333.3333	1483333	12500	3000
		S.D.	4110.45415	462556	4023.37	0
Escherichia coli	Cut Pile	1	10000000	9500000	6900000	7500
		2	4250000	7000000	5150000	12000
		3	5900000	6050000	4950000	19750
		Mean	6716666.67	7516667	5666667	13083.3
		S.D.	2960715	1782087	1072769	6196.44
	Loop Pile	1	70000000	121500000	10875000	2315000
		2	80250000	92500000	8000000	1950000
		3	66750000	75000000	9750000	3025000
		Mean	72333333.3	9.6E+07	9541667	2430000
		S.D.	7045979.94	2.3E+07	1448778	546649
Klebsiella pneumoniae	Cut Pile	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		Mean	0	0	0	0
		S.D.	0	0	0	0
	Loop Pile	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		Mean	0	0	0	0
		S.D.	0	0	0	0
Aspergillus niger	Cut Pile	1	9000	52500	2000	3000
		2	9750	42500	1400	3250
		3	11250	60000	1825	4000
		Mean	10000	51666.7	1741.67	3416.67
		S.D.	1145.64392	8779.71	308.558	520.416
	Loop Pile	1	18250	850000	4750	1000
		2	21500	1025000	6250	4750
		3	14250	725000	7500	2750
		Mean	18000	866667	6166.67	2833.33
		S.D.	3631.45976	150693	1376.89	1876.39

Table 12. Microbial transfer from carpet to cotton woven fabrics using materials evaluator

Microbe	Carpet	Rep. Number	Colony Forming Units (CFU's)			
			Control (No Treatment)	Freepel™ 1225 treated	Humectant A Treated	Sulfated 2-EH Treated
Staphylococcus aureus	Cut Pile	1	3500	15250	1000	1500
		2	6250	20000	1250	1250
		3	4750	17250	1250	1500
		Mean	4833.33333	17500	1166.67	1416.67
		S.D.	1376.89264	2384.85	144.338	144.338
	Loop Pile	1	4250	65000	3500	600
		2	5750	43000	3250	625
		3	6250	50250	4250	775
		Mean	5416.66667	52750	3666.67	666.667
		S.D.	1040.833	11211	520.416	94.6485
Escherichia coli	Cut Pile	1	7500	112500	6500	2500
		2	4500	92500	3750	2750
		3	7000	79000	8000	2200
		Mean	6333.33333	94666.7	6083.33	2483.33
		S.D.	1607.27513	16854.8	2155.42	275.379
	Loop Pile	1	8300	112500	3500	8000
		2	7500	100000	4000	3000
		3	7000	125000	3300	4000
		Mean	7600	112500	3600	5000
		S.D.	655.743852	12500	360.555	2645.75
Klebsiella pneumoniae	Cut Pile	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		Mean	0	0	0	0
		S.D.	0	0	0	0
	Loop Pile	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		Mean	0	0	0	0
		S.D.	0	0	0	0
Aspergillus niger	Cut Pile	1	500	24250	300	500
		2	775	14000	300	300
		3	350	11750	300	625
		Mean	7600	112500	3600	5000
		S.D.	655.743852	12500	360.555	2645.75
	Loop Pile	1	6000	32500	2500	750
		2	5000	42500	3250	925
		3	5250	42450	5250	525
		Mean	5416.66667	39150	3666.67	733.333
		S.D.	520.4165	5759.12	1421.56	200.52