Benefits and safety of microbiologically augmented cleaning products

This document is an executive summary of a paper entitled “Benefits and safety of microbiologically augmented cleaning products” developed by the Consumer Specialty Products Association (CSPA). CSPA is a U.S. trade association representing the interests of companies engaged in the manufacture, formulation, distribution and sale of more than $80 billion annually in the U.S. of familiar consumer products that help household and institutional customers create cleaner and healthier environments.

Introduction
Microorganisms (such as bacteria and fungi) are naturally occurring, ubiquitous (found everywhere), and necessary in order for our environment to function. The vast majority of these microorganisms are fundamentally harmless to humans, animals, and the environment. Clearly, there are several potentially harmful bacteria that do present a public health risk (e.g., *Staphylococcus*, *E. coli*, *Salmonella* and so forth), but it is easy to forget that most microorganisms are beneficial and necessary for our healthy existence and survival. It is estimated that there are between 500 and 1,000 different species of bacteria in and on our body, each hard at work digesting our food, outcompeting potentially pathogenic microbes, producing the vitamins needed to keep us healthy, and boosting our immune system to help ward off illness. In fact, over 90% of the cells in a normal healthy human are bacterial cells! This is a good example of a symbiotic relationship, whereby beneficial microorganisms thrive on the waste by-products that our bodies produce and, in return, provide essential vitamins and nutrients, as well as perform the vital functions that keep all of us fit and well.

Away from our own bodies, beneficial microorganisms are also hard at work maintaining critical life cycles in nature. They break down organic materials (e.g., in the compost heap), help plants grow and thrive, and some even degrade toxic chemicals (synthetic and/or natural). As our understanding of the immense power of microorganisms has grown, so scientists have discovered ways of harnessing the talents of these beneficial microorganisms in order, among other things, to ferment wine, break down man-made waste, and enhance the effectiveness of cleaning and odor control products. We have seen a significant increase in the inclusion of beneficial microorganisms in much of the food and other consumer products that we use on a daily basis. An excellent example of this is the use of microorganisms in foods such as yogurt, salami, and cheese. Microbiologically augmented cleaning products contain beneficial bacteria or enzymes to improve product performance. The bacteria used in cleaning products are Class 1 (or BioSafety Level 1, or Risk Group 1) organisms that are known to pose a very low risk to people and the environment. Due to the fact that bacteria are the microorganisms most commonly used in these industrial and consumer products, we will limit the rest of this discussion to bacteria and their characteristics.

Nature and life cycle of microbes used in cleaning products
Specialty chemicals and cleaning products comprised of microorganisms have been used successfully and safely for many years in a wide spectrum of industrial and household/consumer applications. These products work because the microorganisms can break down, degrade, and eventually consume a great variety of soil and other materials via the respective enzymes and metabolic pathways they utilize. At the same
time, we know these microorganisms to be low risk because, traditionally, only nonpathogenic (i.e., non-disease causing) organisms are used in cleaning product applications. In short, selected strains of microorganisms in industrial and household/consumer application and cleaning products offer beneficial characteristics that include:

- Nonpathogenic to humans and animals
- Rapid degradation, consumption, and digestion of organic wastes
- Generation of safe, innocuous digestion by-products that do not produce malodors
- Grow and reproduce quickly and readily in the environments in which these products are used.
- The incorporation of bacteria into cleaning products is commonly done with bacteria in spore form. Spores are a metabolically inactive state in the life of the cell. Spores are highly resilient due to their resistance to unfavorable environmental conditions (high heat, acidic/alkaline pH, low water content, little or no nutrients) and harsh antimicrobial chemicals (toxic chemical agents, radiation, and desiccation).
- The vegetative cell then begins to degrade and break down complex material, including organic material comprised of proteins, carbohydrates, fats, oils, greases, sugars, cellulose, starch, and so forth, via the cells enzymatic activity. Enzymatic degradation of these complex organic substrates results in smaller, simpler compounds and molecules which can be utilized by bacteria for metabolism and food source purposes. There are no free enzymes resulting from the microorganism spore in the final product.

**Ubiquity of microorganisms**

Bacteria are found in every environment, in and on almost every available surface, thriving across wide humidity, pH and temperature ranges. They are found in and on humans, animals, and plants. While it is true that there are some bacteria that will cause disease and do harm to other living things, a significant amount of research on identified bacteria has shown that not only are bacteria beneficial to people and the environment in many ways, but they are also essential to our lives.

There has been a relationship between people and their resident bacteria since the dawn of humankind. A person’s skin can also be thought of as two square meters of ecosystem) harboring beneficial bacteria. It is widely known in the microbiological world that there are 10 times more bacterial cells than human cells in and on an average person. The majority of bacteria found on the human body are gram-positive organisms, which are better suited to living on the skin (7).

- The colonization of the resident normal flora, which is able to provide nutrients and other beneficial materials to their human host, inhibits the growth of pathogenic bacteria.
- Molecular analyses of skin samples have shown that there can be as many as 182 different known species on human skin.
- Since the late 19th century, microorganisms have also been used on an industrial scale to produce food, including bread, wine, beer, yogurt, cheese, vinegar, kimchi, and sauerkraut.

**Use of microbes as probiotics**
Probiotics are defined as dietary supplements of live bacteria or yeasts thought to be healthy for the host organism. Probiotics are being developed commercially for both human use (primarily as novel foods and dietary supplements), and in animal feeds (to prevent gastrointestinal infections). Probiotics can also be used as alternatives to antibiotics and prophylactics. Health-promoting microorganisms or probiotics are increasingly included in various food products and are used as food supplements and as treatments for several infectious diseases.

- For decades *Bacillus* bacteria and their metabolites have been used in several biotechnological applications, including enzymes, amino acids, and antibiotic production, preparation of fermented foods, and pest control.
- *Bacillus*-based preparations have only been introduced to the international market more recently and are currently used as probiotics for bacteriotherapy and bacterioprophylaxis of human gastrointestinal (GI) disorders.
- In terms of probiosis, this ability of spores to germinate in the gut is key to explaining the mode of action, and this is likely to include immunomodulation and secretion of antimicrobials.
- Stimulation of the immune system, or immunomodulation, is considered an important mechanism in supporting probiosis.
- A number of studies in humans and animal models have provided strong evidence that oral administration of spores stimulates the immune system.

In the United States, bacteria considered safe for human consumption are assigned "Generally Regarded As Safe" status (GRAS) by the Food and Drug Administration on a case-by-case basis. In the EU, the Food Safety Authority has published an opinion referred to as "Qualified Presumption of Safety" (QPS). The aim is to harmonize the safety assessment of microorganisms throughout the food chain.

**Safety of microbial products**

The microorganisms commonly used in cleaning products are all well-known, non-pathogenic and non-toxic microorganisms that meet the criteria for classification as Class 1 organisms, that is, safe for people and the environment, according to US NIH (National Institutes of Health) guidelines. The strains are among those considered to be safe host organisms for the production of food and feed enzymes, and have also long been used in the production of a variety of bioindustrial products, such as probiotics and additives, and in the production of certain foods. There have been no published reports since the early 1970's on consumer allergies caused by industrial enzymes or microorganisms in any cleaning product, or in any other consumer products containing industrial enzymes or microorganisms.

The European Food Safety Authority has listed many *Bacillus spp* on their list of Qualified Presumption of Safety (QPS) microorganisms intentionally added to food or feed. Suppliers of microbial components often run toxicity testing to determine whether the organism is non-toxic with respect to acute inhalation, oral toxicity, and dermal sensitization.

Exposure to microorganisms has repeatedly been found to be inversely related to the manifestation of atopic diseases such as asthma and hay fever. In populations with higher bacterial exposures, the prevalence of asthma and atopy has been shown to be
substantially lower. In a study comparing children living on farms who were exposed to a wider range of microbes than children in the reference group (from the city) showed an inverse relation between asthma and growing up on a farm, or increased exposure to microorganisms. Children who grow up in environments that afford them a wide range of microbial exposures, such as traditional farms, are protected from childhood asthma and atopy (Figure 1).

Prevalence of asthma and atopy among children living on farms compared with reference groups

Parisfal & Gabriela

![Figure 1. Prevalence of Asthma and Atopy among Children Living on Farms as Compared with Reference Groups.](image)

The PARSIFAL study population included 6843 school-age children 6 to 13 years of age, and the GABRIELA study population included 9668 children between 6 and 12 years of age. Calculations of prevalence in GABRIELA were weighted on the basis of the total number of children who were eligible for inclusion in the study (34,491 children).

Types of cleaning products and their uses

Today, beneficial bacteria and chemical components can better co-exist in a formulation providing a cleaning product that is both stable and more effective. Due to the fact that, in the majority of bioaugmented cleaning products, the biological components are usually *Bacillus* spores, formulators have to make sure that the product chemistries are not sporicidal or have detrimental effects on the spores over the shelf life. Cleaning product types that are often bioaugmented include, but are not limited to:

- Multipurpose cleaners
- Floor cleaners
- Carpet cleaners
- Drain treatments
- Grease trap/interceptor treatments
- Wastewater treatments
- Septic tank treatments
- Malodor neutralizers
In products like multipurpose cleaners, floor cleaners, and carpet cleaners, the chemical components do the cleaning, degreasing, destaining, and deodorizing in the application, but the biological component offers further degradation of organic residue in the same areas, and also in areas that cannot be reached or are not exposed to the cleaning component as they need to be. These areas include the spaces between tiles, grout, carpet padding, pores in cement, and so forth. The advantage that the biological component in bioaugmented products has over conventional formulations, is the degradation and reduction of the organic load, such as grease, oils, urine, food residues (protein, starches, and fats), and sources of bad odor.

Bio-augmented products used as drain treatments, septic treatments, and municipal wastewater treatments, digest and reduce organic waste, and control odor in a way that conventional products cannot. Establishing and increasing specific microbial populations in drains, septic tanks, lagoons, aeration basins and so forth offer a biological means of degradation that keeps loads at levels that mean less potential problems for the systems. Odor control is achieved by reducing the organic load and simply because the by-products of the metabolism of *Bacillus* used in these types of bioaugmented products are simply water and carbon dioxide.

Biological treatments of grease traps/interceptors help reduce the amount of fats, oils, and greases (FOGs) that need to be pumped out of them due to the fact that they are actually degraded and broken up and not emulsified. Properly formulated products for these applications do not simply reduce the FOGs by emulsification, but rather use bacterial action to degrade and break down the accumulated FOGs. Properly formulated products do not cause the emulsification of FOG.

F. de los Reyes III of North Carolina State University carried out a grease interceptor bio-augmentation field trial (64). In this field trial, the grease interceptors of two facilities went through a series of blind bio-augmented and control cycles. The results of this study did show that depending on the facility, the FOG (fat, oil, & grease) cap of the interceptor was thinner than during the untreated cycle.
In addition to this finding, effluent FOG levels were lower in certain treated cycles. While there were differences in data from the two grease interceptors, effluent COD and effluent BOD were generally lower in treated cycles, but in no case were effluent levels higher in bio-augmented cycles compared to untreated cycles. The conclusion from this research was that biological products did not result in the moving of FOG or volatile fatty acids out of the grease interceptors into the municipal collection systems.

The basis of bio-augmentation of cleaning products is that it offers a better product with the ability to clean one step further than conventional products. If the chemistry is efficient and comparable, then bio-augmentation improves the cleaning profile, since it contributes an extra biological component which will further degrade soils and substrates after the chemical components have done their job. The establishment of microbial colonies that will provide constant degradation of organic residue on hard surfaces (kitchen and bathroom floors), carpets, drain pipes, etc., definitely offers a competitive edge over traditional cleaning products.

**Identifying the benefits of microbial-based products over conventional cleaning products**

If a product contains bacterial spores, it is considered bacterial based, regardless of whether or not it also contains enzymes as a formulation constituent. It should also be noted that bacterial-based products are based on bacterial spores rather than vegetative bacteria. This is necessary for the purposes of product stability and shelf life. There are two primary distinguishing benefits of microbial-based products over conventional and enzymatic cleaning products. The first is digestion of soil rather than simple removal of soil, and the second is residual activity.

Conventional cleaning products and enzymatic-based cleaning products both promote removal of a soil from a substrate and disperse and stabilize the removed soil in the cleaning solution. Soil removal is accomplished by a combination of true dissolution of
the soil, dispersion of the insoluble components of the soil in the cleaning solution, and
emulsification of the soil by surfactants in the cleaning solution. Many conventional
cleaning products contain components such as alkalis, acids, oxidizers, and chelants
which chemically attack the soil, breaking the larger, relatively difficult to solubilize
particles, into smaller, more easily solubilized particles. Enzymatic cleaning products
may also contain these same components, but only at reduced levels to avoid
inactivation of the enzymes.

Bacterial-based cleaning products work by the bacteria digesting the soil as a food
source. Bacterial digestion of soil involves an initial attack by extracellular enzymes.
These extracellular enzymes (exoenzymes) may be produced by the bacteria as a part of
the soil digestion process, or they may be a separate component of the cleaning product
formulation. Regardless of their source, these exoenzymes break down the larger
particles of soil into the nutrients necessary for the bacteria’s survival. These nutrients
are absorbed by the bacteria and used to fuel bacterial metabolism. The by-products of
bacterial metabolism are generally water-soluble short-chain organic acids and alcohols,
CO₂, and reduced electron acceptors.

All bacterial-based products contain a trace amount of a component which inhibits
germination of the bacterial spores in the concentrated product. Once the bacterial-
based product is diluted to use strength, the concentration of the inhibitor is diminished
to the point where it is ineffective and the spores germinate.

Bacterial-based products may be relatively simple formulations containing only the
bacterial spores, the inhibitor, and water. Bacterial spores may also be added to a
conventional cleaning product to help break down and digest soils that conventional
cleaning products have a difficult time removing. This addition of bacterial spores to a
conventional cleaning product is referred to as bio-augmentation. There are some
restrictions on the conventional products that can be bio-augmented, specifically the
spores must be able to survive in the concentrated product and to germinate in the use
solution in the presence of a food source. As such, only those products with relatively
low levels of alkalinity or acidity in the use solution are candidates for bio-
augmentation.

Bioaugmented cleaners offer the advantages of the fast action of a conventional cleaning
product, while still delivering the enhanced soil digestion and residual activity benefits
of a microbial-based cleaner.

**Microbial product production and formulation quality control requirements**

Quality control practices in the production of microorganisms are critical to the safety
and performance of the products manufactured. Use of validated, consistent assays for
critical parameters, for example microbial counts and, in some cases, metabolite (e.g.,
enzyme) concentrations, is required to ensure product performance. Microbial
identifications are used to ensure that the organism(s) contained in the product is (are)
the correct strains. This is of particular importance, since the fermentations used to
produce microorganisms are inherently susceptible to contamination, and in extreme
cases even to producing a material that contains none of the intended species. Such a
situation would, of course, have major implications for product performance but, even
more importantly, for the safe use of the product. Additional quality control checks are also required during post-fermentation product manufacturing, such as during downstream processing, formulation, and so forth. Due to the importance of these practices, a system of monitoring, training, product tracking, and compliance is used, such as is required under a quality management system such as ISO certification (i.e. ISO 9001:2008).

The identification of microorganism strains is of great importance to industry, academia, and regulatory agencies. Microorganism taxonomy has changed significantly over the past 20 years. Today, the most generally accepted microbial taxonomy procedure is via 16S rDNA sequencing. From an academic standpoint, a multiphasic approach using 16S rDNA sequencing as the core is the preferred method to accurately identify a microorganism.

Many microorganism-based products are comprised of multiple microorganism species or strains. In general, each microorganism strain must be fermented independently. Production of multiple microorganisms in the same fermentation is not permitted due to the extreme difficulty of maintaining consistent product quality using this method. Use of independent fermentations enables the manufacturer to ensure that each target microorganism has been produced successfully and without contamination. In the formulation process, the individual strains can be blended to achieve the specific microorganism requirements and activity rate (colony-forming units or cfu) for the product.

It is necessary to ensure sterility in critical equipment. Fermentors must be properly designed with appropriate valves, o-rings, and filtered air supply, and must be pressurizable to withstand sterilization conditions. Fermentors and other equipment must be scrupulously cleaned between batches. Fermentors and growth media are sterilized at 121 °C for the appropriate time. Fermentation and formulation areas of the plant are sanitized on a regular basis with various disinfectants to minimize the risk of contamination.