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1. **INTRODUCTION**

Solvent cleaning is an established process for removal of surface contaminants in a variety of industrial applications [1]. Many of the conventional solvents used for cleaning, such as hydrochlorofluorocarbons, are considered detrimental to the environment and are increasingly subject to regulations for reduction...
in their use, and eventual phaseout [2,3]. As a result, there is a continuing effort to find alternate cleaning methods to replace these solvents. One such alternative is microbial cleaning that takes advantage of naturally occurring microbes to remove a wide variety of contaminants from various surfaces. This chapter is focused on the application of microbial cleaning for removal of surface contaminants.

2. SURFACE CONTAMINATION AND CLEANLINESS LEVELS

The most common categories of surface contaminants include particles, thin film or molecular contamination that can be organic or inorganic, ionic contamination, and microbial contamination. Other contaminant categories include metals, toxic and hazardous chemicals, radioactive materials, and biological substances that are identified for surfaces employed in specific industries. Surface contamination can be in many forms and may be present in a variety of states on the surface. Common contamination sources can include machining oils and greases, hydraulic and cleaning fluids, adhesives, waxes, human contamination, and particulates. In addition, a whole host of other chemical contaminants from a variety of sources may soil a surface. Typical cleaning specifications are based on the amount of specific or characteristic contaminant remaining on the surface after it has been cleaned.

Cleanliness levels in precision technology applications are typically specified for particles, as well as for hydrocarbon contamination represented by nonvolatile residue (NVR). For example, civilian and defense space agencies worldwide specify surface cleanliness levels for space hardware for particles in the micrometer per unit area size range and for NVR in the microgram per square centimeter range [4,5]. The cleanliness levels are based on contamination levels established in industry standard IEST-STD-CC1246D for particles from Level 1 to Level 1000 and for NVR from Level AA5 (0.1 ng/cm²) to Level J (0.025 mg/cm²) [6]. In many commercial applications, the precision cleanliness level is defined as an organic contaminant level of <10 µg of contaminant/cm², although for many applications the requirement is set at 1 µg/cm² [6]. These cleanliness levels are either very desirable or are required by the function of parts such as metal devices, electronic assemblies, optical and laser components, precision mechanical parts, and computer parts.

3. BACKGROUND

Microbial cleaning is part of the broader concept of bioremediation. As the name implies, bioremediation is a natural solution to contamination mitigation. It is technically defined as the accelerated breakdown of organic compounds through the use of natural biological agents such as bacteria, enzymes, or fungi. For carbon-based contaminants (grease and oil), the end products are carbon dioxide and water. Bioremediation is a safe, environmentally friendly way to
process many kinds of hazardous waste and is supported by the Environmental Protection Agency as a viable solution for cleanup of oil spills and other contaminants, as well as an alternative to solvent cleaning.

3.1. Microbial Agents

There are six main groups of microbes [7].

1. Archaea are a group of unicellular prokaryotic cells that sometimes produce methane during their metabolism. They are specifically adapted to a wide variety of environmental conditions by means of special types of membranes and metabolism.

2. Bacteria are also unicellular prokaryotic organisms. They have a unique type of cell wall and cell membrane that distinguishes them from Archaea. They can digest hydrocarbon contaminants.

3. Fungi are nonphotosynthetic eukaryotes that absorb their nutrients directly from the environment. This group includes mushrooms, molds and yeast.

4. Protista are animal-like, nonphotosynthetic eukaryotes common in moist environments.

5. Viruses are made up of nucleic acids (DNA or RNA) and protein and have some of the characteristics of life. But they lack ribosomes (for protein synthesis), membranes, and means to generate energy, which are properties of cells.

6. Microbial Mergers refer to combinations and collaborations between different microbe species.

Of these microbes, only bacteria (commonly) and fungi (less commonly) have been used for remediation and removal of contaminants [8–14]. When activated, microbes secrete enzymes which break down the contaminants. Hence, pure enzymes manufactured from different microbial strains under aseptic conditions are also used for cleaning [15–17]. Cleaning applications include parts and components cleaning, artworks, oil spills, wastewater, and household and industrial cleaning.

The microbes used in cleaning applications are nonpathogenic and have no recognized hazard potential under ordinary conditions of handling. They are all classified as American Type Culture Collection (ATCC) Class I, are completely safe to humans and the environment, and do not require special biosafety level facilities\(^1\) for handling and use. They are not subject to distribution restrictions by the ATCC, U.S. Department of Health, Public Health Service and the Toxic Substances Control Act (TOSCA).

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\(^1\) Four biosafety levels have been assigned by the Centers for Disease Control for activities involving microorganisms [23]. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.
For most surface cleaning applications, the microbes are a highly specialized blend of cultures specifically selected and adapted to degrade a wide range of hydrocarbons. They aggressively attach to and break down oil and grease, but will not attack other substances such as industrial grade metal or natural rubber. The most common strains of bacteria for removal of hydrocarbon contaminants are *Pseudomonas* and *Bacillus* [18–20], while different strains of sulfate-reducing bacteria (SRB) *Desulfovibrio vulgaris* and *Desulfovibrio desulfuricans* are employed for effective cleaning of sulfate contaminants such as calcium sulfate deposits on buildings [21]. In the latter case, the bacteria dissociate calcium sulfate into Ca$^{2+}$ and SO$_4^{2−}$ ions, and further reduce the SO$_4^{2−}$ ion to the S$^{2−}$ ion.

4. PRINCIPLES OF MICROBIAL CLEANING

The basic principle of microbial cleaning for removal of hydrocarbon contaminants involves the reduction of the contaminants into harmless CO$_2$ and water by the action of microbes [9,22]. Figure 4.1 shows a life cycle diagram of the cleaning process. In a typical surface cleaning application, a cleaning fluid containing a strong surfactant/degreaser contacts the contaminated surface. The surfactant reduces the interfacial tension between the contaminant and the part surface, and separates the contaminants from the surface. A combination of microbes and nutrients is released into (and now living in) the fluid. Nutrients are generally added as part of the cleaning mix to provide emerging microbes with fortification until sufficient amounts of oil and grease have been introduced as carbon sources. The microbes secrete natural enzymes (for example,
lipase [fats, oils], amylase [starches], and protease [proteins]), which cleave the molecular bonds and dissociate the hydrocarbon molecules (contaminants, like oil and grease). This action releases carbon as a source nutrient for the microbes. The microbes are activated and begin to digest the oil and grease that are subsequently absorbed through the cell wall and digested further. The contaminants are then carried by the cleaning fluid through a filtering device, where particulate matter, such as dirt, paint chips, and other items >50 µm, are retained.

In parts cleaners, the cleaning action is due to the surfactant, not the microbes. However, while the microbes do not participate in surface contaminant removal, over time they will remove any hydrocarbons in the cleaning system. In a conducive, nutrient-rich environment, the bioremediation materials continue to manufacture themselves throughout the contaminated solution, increasing the overall biomass of microbes in an exponential manner until all the available hydrocarbons are consumed, thus leaving a clean system with a hydrocarbon-free cleaning solution. Bacteria multiply very rapidly. A single cell can grow to $10^{21}$ within 24 h [22]. The clean solution can be recirculated through the system, the cleaning cycle is repeated, and there is no interruption in the cleaning process.

Enzymes released by the microbes can only attack one surface of the contaminant. This leads to slower, less effective remediation. The process can be enhanced by a catalyst. Typically, a biocatalyst contains a combination of nonionic surfactants and emulsifiers and water, as well as nutrients that are essential to microbial life. The combination of surfactants and emulsifiers acts to break up the hydrocarbon into very small globules to bring it into intimate contact with the microbes. The globules are surrounded by the enzymes, thereby increasing the rate at which they are dissociated and subsequently digested. The biocatalyst significantly increases bioavailable oxygen. This provides a catalyst for the microbes to multiply faster, resulting in more rapid, more complete bioremediation. The by-products of this process (with pure hydrocarbons) are carbon dioxide, water and soluble fatty acids.

Effective bioremediation systems use a combination of aerobic and anaerobic microorganisms. Aeration provided by the flow of fluid through nozzles and spigots provides adequate additional oxygen to certain strains, while other strains work below the surface in the holding reservoir to break down contaminants that may settle at the bottom of the reservoir.

5. CLEANING SYSTEMS

For parts cleaning applications, the cleaning equipment is especially designed for optimum cleaning performance. Other surface cleaning applications, such as cleaning of artworks and household cleaning, do not require any special equipment.
5.1. Parts Cleaners

Microbial parts cleaners are commercially available in several sizes and models. Figure 4.2 shows examples of free-standing microbial parts cleaning units [24–30]. Typically, these heated cleaning systems consist of an upper sink and a lower tank, filter assemblies to trap visible particulate matter (for example, sand, grit, dirt and paint chips), power module, onboard diagnostics, recirculating pumps, cleaning nozzles, and a tank aeration system that increases the effectiveness of the microbes. Higher pump pressure also improves the cleaning action. The load capacity of these systems ranges from 20 to 200 kg of parts. These units are best for light-duty manual cleaning of parts similar to conventional solvent sink-top units, although recently a larger capacity system

![Figure 4.2 Examples of bioremediation parts washers. (a) Graymills Biomatics™ Parts Washer, (b) Bio-Circle, and (c) ChemFree SmartWasher [28–30]. Source: Courtesy of Graymills Corporation, J. Walter Company, and ChemFree Corporation, respectively.](image)
has been introduced for cleaning entire bicycles [31]. This model includes an integrated bike stand with the parts washer.

5.2. Cleaning Solutions and Microbial Compositions

A wide variety of cleaning solutions and microbial compositions has been developed for many different applications. The powerful degreasing solutions used in cleaning applications are nonhazardous, noncorrosive, pH neutral, nonflammable, nontoxic, noncaustic, aqueous-based degreasing solutions. They are not known to cause damage to humans or the environment. When used in accordance with directions they do not create liquid hazardous wastes or produce cradle-to-grave liabilities. The manufacturers of parts washers offer degreasing solutions that work exclusively in their machines and are not recommended for use in other washers [32–34]. Similarly, the microbial blends are designed for the individual cleaning systems. The specific conditions for elimination of the hydrocarbon contaminants (oil and grease), such as specific temperatures, compensation for foam, aeration parameters, and flow rates, are optimized for the individual units. If the microbial blend is diluted or the cleaning solution composition is changed, it can severely impact the performance of the cleaner. Use of the solutions in other cleaners may affect the digestive effectiveness of the microbes, impair cleaning performance, or even damage the machine, and could void the warranty.

Several manufacturers offer concentrated microbial cleaning solutions that can be used in manual cleaning applications with conventional spray cleaning systems [35–43]. These solutions are used in a typical 20:1 dilution ratio.

Many enzyme-based cleaning compositions have been developed and are available commercially [44–64]. These compositions are formulated from commercially available enzymes [65–67] and are used in varied institutional and household cleaning applications. Section 7 discusses some of these applications.

5.3. Application of Microbes

Industries that perform cleaning of parts prior to rust proofing, phosphating, plating, painting, powder coating, or hot dip galvanizing or coating can benefit from microbial cleaning. Microbes have been successfully used for bioremediation in petrochemical plants, chemical plants, refineries, food processing plants, marine barges, machine shop parts washers, truck washes, wood treating plants and groundwater remediation applications. Examples of applications are discussed in Section 7.

5.4. Types of Contaminants

The cleaning solutions typically contain very strong surfactants, so they will clean a wide variety of contaminants. However, they are designed and are
recommended for cleaning biodegradable hydrocarbon contaminants, including the following:

- Crude oil
- Other oils (cutting oil and motor oil)
- Hydraulic transmission fluid
- Solvents
- BTEX (benzene, toluene, ethylbenzene, and xylene)
- Greases
- Lubricants
- Amines
- Creosote
- Phenols
- Fats
- Peptide nucleic acid.

The cleaning performance for these types of contaminants is excellent. For example, analyses performed on samples of cleaning solutions from operating bioremediation cleaners have consistently shown hydrocarbon (oil and grease) levels in the 1400 ppm range, compared to an average of 20,000 ppm of oil and grease from nonmicrobial conventional aqueous solvent cleaning [9,11]. Other contaminants that have been successfully treated include paint, ink, glue, adhesive, sealant, wax, tar, graffiti, pen marks, rubber, and resins.

5.5. Types of Substrates

Substrates such as carbon and stainless steels, galvanized steel, brass, copper, aluminum, plastic, and ceramics, fiberglass, glass/quartz, sterling silver, nickel, titanium, plastics, and concrete have been successfully cleaned. Not only is the cleaning solution effective on metal parts, but also will not damage nonmetal components that may be attached to the parts being cleaned such as rubber or plastic fittings. As with all parts cleaners, some surfaces will be cleaned at different rates than others due to the degree and type of contamination on the surface. Because the cleaners operate at a near-neutral pH and lower temperatures, metal parts can be cleaned without etching. Metal, plastic and fiberglass parts will keep their original finish.

5.6. Parts Cleaning

Parts cleaners are simple to operate. As shown in Fig. 4.3, the degreasing solution is sprayed on the contaminated part through the nozzle located in the upper sink. The microbes and nutrients are introduced into the used degreasing solution in the lower tank where the microbes are activated and begin to digest the hydrocarbons in the solution. The clean solution is filtered to trap particulate matter and is recirculated to the upper sink where it can be used to clean additional parts. Heating elements in the lower tank maintain the operating
temperature within a range that is ideal for the microbes to thrive, generally 323–360 K. The sink itself is also maintained clean (Fig. 4.4).

In a well-maintained microbial cleaning system, the only regularly generated waste is the used filters that are replaced every 3–8 weeks. The cleaning solution is only replaced when it is no longer effective, which is usually several years. The waste is considered hazardous unless it is tested to demonstrate it is nonhazardous.

5.6.1. Operating Guidelines

Microbial parts cleaning systems are very effective and easy to use. General guidelines will help maintain optimum cleaning performance of the system.

- The cleaning fluid must be heated and aerated constantly to achieve peak cleaning performance. Most microbes require a warm environment to survive and continue to digest the hydrocarbons at an optimal level to clean the solution as quickly as possible. Also, warm solution simply cleans better than cold solution.
Developments in Surface Contamination and Cleaning

- Aggressive chemicals, such as disinfectants, bleach, solvents, acids or chlorinated substances, should not be added to the cleaning solution since they tend to kill the microbes.
- The fluid should be maintained at an optimum level with solutions designed for the unit. If the microbial blend is diluted or the cleaning solution composition is changed, it can severely impact the performance of the system.
- The microbes need time to adapt to the type of contaminants being cleaned. If the microbe solution does not clean effectively at first, cleaning performance will improve after the microbes adapt and digest the new contaminants.
- Very heavily contaminated parts with excessive greases, oils and fluids should be precleaned. Sudden loading of concentrated oils and grease may harm the microbes.
- The filters should be replaced regularly to keep solids from building up at the bottom of the unit and decreasing cleaning performance. The trapped contaminants in the filters can also reach hazardous levels. Replacing the filters can introduce fresh cleaning solution to the existing microbe colony which keeps the system working at an optimum level.
- Parts should be dried after cleaning to prevent rusting or oxidation by residual fluid on the surface. A protective film should be applied to the part before storage.
- Environmental contaminants, such as solvents from aerosols and other sources, can harm microbe populations. Cleaning operations should be performed away from solvent sources.

5.7. Costs

Parts washers are relatively inexpensive, costing around $2500–$8500, depending on the size and capacity of the system [28–31]. Operating costs are generally low. Consumption of cleaning chemicals is minimal since the microbes tend to clean the solution and free up the surfactants to clean and emulsify more contaminants. The premixed or in-situ activated microbial cleaning solution never needs to be replaced, rather, it is topped off in the tank on average every 8–10 weeks to cover losses due to evaporation and fluid left on parts after they are cleaned. The costs of the cleaning solution itself are around $400 for 5 gallons, but it is diluted on average in a 20:1 ratio. Power costs are minimal because there is minimal heat input into the process to maintain an operating temperature in the range 323–360 K. Some system providers offer maintenance contracts at around $600/year for bimonthly service calls [30]. Waste disposal costs for microbial cleaning are low since the primary waste stream is the filters that are replaced every 3–8 weeks. Overall, the costs of microbial cleaning are lower than solvent cleaning, as illustrated by some examples below.
5.7.1. Examples of Cost Savings

The Texas Army National Guard invested approximately $15,000 in August 1995 to purchase 10 bioremediation parts washers to replace solvent cleaners for motor pool operations. In the first year, the Guard eliminated 600 gallons of solvent waste and significantly reduced volatile organic compound emissions, saved $5130 in waste disposal manifesting requirements, and saved $4200 in yearly solvent purchase costs. The estimated payback period was about 18 months. A major aeronautical firm realized savings of more than $80,000 by reducing solvent usage by more than 900 gallons during the first year through use of 23 bioremediation parts washers to replace solvent cleaners [9].

Other studies have shown annual cost savings of nearly 40% by replacing solvent cleaning units with an aqueous cleaner and a microbial cleaning unit with an average payback period of 1.5 years, although in one case, the payback period was <3 months [10,68]. Table 4.1 compares the cost of microbial cleaning with solvent cleaning. The total costs include equipment, cleaning solutions and chemicals, and waste disposal. The subsequent yearly cost for microbial cleaning is slightly higher than the first year which can be attributed to the cost of replenishment of the cleaning solution.

As part of the Lakehurst Pollution Prevention Equipment Program of the U.S. Navy, a solvent-based cleaning system was compared with a bioremediation parts washing system [69,70]. The bioremediation system reduced the waste stream by nearly 100%, saving $1800 in waste disposal costs. In addition, the cleaning solution can be used indefinitely with only occasional replenishment. The equipment is safe to use and does not require personal protective equipment.

6. ADVANTAGES AND DISADVANTAGES OF MICROBIAL CLEANING

The advantages and disadvantages of the microbial cleaning are given in the following sections.
6.1. Advantages

1. This process completely breaks down contaminants to innocuous end products such as water, CO₂, and soluble fatty acids.
2. Microbial cleaning is a natural and safe process. It is a noncorrosive and environmentally friendly cleaning process. No hazardous wastes and emissions are generated.
3. Bioremediation eliminates the need for transportation of spent solvents and other hazardous materials.
4. Microbial cleaning is more economical than traditional solvent cleaning technologies.
5. Cleaning is performed under benign operating conditions with minimal energy input to maintain slightly warmer than ambient temperatures.
6. Microorganisms are nonpathogenic, are completely safe to use and have no recognized hazard potential under ordinary conditions of handling.
7. The rate at which microbes can digest hydrocarbons can approach 80% every 7 days.
8. Most bioremediation parts washers can handle large, tough, dirty jobs.
9. Parts are usually cleaned in the first pass. Even the tiniest crevices and tight spaces in contaminated parts are cleaned because the microbes have close and unhindered contact with the parts.
10. Parts are always exposed to clean solution because the microbes constantly clean the solution and keep the bath clean.
11. Microbes improve the cleaning ability of the cleaning solution. The bioremediation process that takes place in the solution frees the surfactants allowing them to clean and emulsify even more contaminants.
12. Microbes have been successfully used on a variety of contaminants ranging from crude oil to hydrocarbon films.
13. Energy usage is low because of low operating temperature of the process.
14. The process operating costs are low.
15. There is no downtime for maintenance of the system.
16. The cleaning system is simple to use.
17. The costs of waste disposal are low since the filters are the only waste stream generated in low volumes.
18. The cleaning solutions are pH neutral and noncaustic that will not dry, crack or irritate the skin.

6.2. Disadvantages

1. Microbes are susceptible to any biocides designed to kill microbes, such as bleach or strong chemicals that kill living things like some strong pesticides and rat poisons.
2. Added microbes can cohabit with resident bacteria, which can work against the goal of maintaining sanitary conditions in medical and food processing industries, as well as affecting cleaning performance in other applications.
3. The process is limited mainly to removal of biodegradable hydrocarbon contaminants. Most inorganic contaminants, large particles, and other debris cannot be removed.
4. Microbial cleaning is generally not applicable for high-precision cleaning of sensitive parts.
5. The microbial fluid composition is unique to each cleaning system.
6. Filters are the principal waste stream, which must be handled and disposed as hazardous waste.
7. Cleaning may require more scrubbing effort than solvent cleaning.
8. It is difficult to clean heavy or stubborn contaminants.
9. Keeping microbes alive requires proper worker training.
10. Workers may react negatively to certain odors.
11. Cleaning times may be longer than cleaning with conventional solvents.

7. APPLICATIONS

Microbes have been successfully used for remediation in petrochemical plants, chemical plants, refineries, food processing plants, marine barges, truck washes, wood treating plants, oil spill cleanup, soil decontamination, and groundwater remediation applications. Several surface cleaning applications have also been demonstrated including parts washing, oil and grease removal, cleaning of artworks and structures, surface cleaning and disinfection, and household cleaning. The types of contaminants removed include biodegradable oils and greases, lubricants, bacterial contaminants, and sulfate crusts. Some of these cleaning applications are discussed below.

7.1. Parts Washing

This is one of the most common applications for microbial cleaning as discussed at length in Sections 5. Several thousands of parts washers have been installed worldwide and have proven to be cost-effective alternatives to conventional solvent cleaning. In most cases, cleaning effectiveness has been equivalent to, or sometimes even better than, cleaning with solvents.

7.2. Oil and Grease Removal

Industrial activity frequently leaves oil and grease stains on concrete and other floor surfaces, which can build up to a thick layer and can present a safety concern, if it is not removed. Examples are truck bays, machine shop floors, manufacturing facilities, and similar locations. Microbial cleaning has been successfully used to clean up the stains and caked on debris. Figure 4.5 shows a
truck fueling bay before and after cleaning with a microbial solution diluted in a 2:1 ratio with water [36]. The solution was sprayed on the contaminated areas (∼1670 m²) and allowed to work for approximately 4 h on the contamination, followed by power washing of the surface. The results are dramatic evidence of the effectiveness of microbial cleaning.

Many examples of heavy oil and grease removal by microbial and enzyme cleaning from drains and grease traps in manufacturing facilities, hospitals, restaurants, food processing facilities, and similar locations have been described on the web sites of the product suppliers [36–43,65–67]. Figure 4.6 shows a cleaning tank heavily contaminated with an oily sludge that was effectively cleaned by microbial solution treatment.

An innovative method of removing oil and grease on slick surfaces is to replace the microbe-enhanced surfactants with protein-enhanced surfactants [12]. This has the benefit that no bacteria are added to the local environment, thus avoiding cohabitation with resident bacteria. The proteins increase the metabolism of the resident bacteria in the wastewater during cleaning or mopping.

7.3. Sulfate-Reducing Bacteria in Oilfields

One of the deleterious consequences of SRB in oilfields is that it can lead to the onset of hydrogen sulfide generation, which can cause corrosion of
pipelines, platform structures and other equipment, and presents health risks due to the toxicity of H\textsubscript{2}S [71–73]. Several microbial processes have been proposed to control SRB contamination in oilfields, including addition of nitrate-reducing bacteria to inhibit H\textsubscript{2}S production. These methods have been reviewed recently [73].

7.4. Bacterial Characterization and Monitoring of Surface Cleanliness

Most bacteria are small, approximately 1 \( \mu \text{m} \) in diameter, and are not easily removed from a surface. Parts or surfaces cleaned by microbial methods may leave behind bacteria located in scratches, crevices, or similar tight spaces. In-situ visualization and characterization of the bacteria is of interest from both remediation and cleanliness monitoring perspectives. This cannot be done directly because of the large surfaces and fixed installations. Recently, a replication technique using cellulose acetate-replicating tape has been developed to characterize electron microscopically food-borne bacteria on a stainless steel surface [74]. Bacteria are clearly visible in the micrograph of the replica (Fig. 4.7).

Methods for monitoring and measuring the cleanliness of surfaces have been described in detail [75].

7.5. Mercury Bioremediation

Heavy metals, such as mercury, cannot be converted into nontoxic forms by naturally occurring bacteria, but previous attempts have been made to genetically engineer bacteria for heavy-metal remediation without success [76–78].
In a recent study, a transgenic system has been developed for mercury remediation [79]. The proposed system effectively expresses metallothionein (\textit{mt-1}) and polyphosphate kinase (\textit{ppk}) genes in bacteria in order to provide high mercury resistance and accumulation, as high as 80 µM and 120 µM of mercury. This engineered bacterial system presents a viable technology for mercury bioremediation. It may have an application in cleaning mercury-contaminated surfaces.

7.6. Wound Debridement

Several systems have been developed for wound cleansing and debridement using enzyme-based cleaning solutions [80,81 and patents and references cited therein]. Debridement is the surgical excision or enzymatic cleaning of dead, devitalized, or contaminated tissue and removal of foreign matter from a wound to enable healing [82]. These systems are based on the use of pressurized fluid jets to penetrate the skin for delivery and removal of the cleaning solution; a negative-pressure thermotherapeutic fluid delivery device attached to the wound area; pad or dressing with a single or multiple infusion and drainage tubes for continuous delivery of the cleaning solution; or a spray system based on supersonic gas–liquid technology. Most commonly, vegetable-derived proteolytic enzyme solutions are used for cleaning that can include additives, such as activators and inhibitors, to maintain optimum catalytic activity of the enzymes in the cleaning solution [81].

7.7. Disinfection and Cleaning

In the health and food sectors, bacterial and viral infections being transmitted to personnel and patients are a subject of growing concern. One reason for the spread of infection is incomplete or ineffective disinfection of surfaces. Many viruses, bacteria and other pathogens, such as severe acute respiratory syndrome or methicillin-resistant \textit{Staphylococcus aureus} (MRSA), are resistant to existing conventional surface cleaning agents/disinfectants. A new antibacterial cleaning composition has been developed that contains different enzymes (proteolytic, amylolytic, lipolytic, or cellulolytic, or their mixtures) and microbes (\textit{Bacillus} or \textit{Pseudomonas}) together with a surfactant and an aqueous carrier to maintain a minimum 95% catalytic activity at the pH range of 5.5–13.5 [19]. This solution is effective against several resistant bacterial strains, such as MRSA, vancomycin-resistant \textit{Enterococci}, and glycopeptide-intermediate \textit{S. aureus}, and can be used as a cleaning and disinfecting agent in affected areas. It can also be used for killing or inactivating bacteria, viruses, or fungi to prevent spreading of the contaminants. Variations of this composition can be used for cleaning metal, ceramic, glass, or plastic parts, concrete and tile floors, cleaning grease traps, and other household cleaning applications. The contaminants that can be removed include carbon deposits, oil, grease, carbohydrates, starch, and meat and dairy products.
Beyond cleaning and disinfection of surfaces, it is also critical to prevent the growth of microorganisms using a solution such as a quaternary ammonium and benzothiazole composition [83].

Another area of concern is inadequate cleaning and disinfecting of ocular devices such as contact lenses. Several methods have been proposed for cleaning, disinfecting and preserving contact lenses using different microbial cleaning compositions [84,85].

Inadequate cleaning of surgical instruments can result in disastrous consequences for patients in health care facilities [86]. Detergents containing microbial proteases very effectively clean endoscopes and other critical and semicritical surgical instruments. Blood and protein removal during the cleaning is especially critical. Both glutaraldehyde and peracetic acid, used in the disinfection step, are known to fixate residual blood protein. Similarly, removal of body fluids, tissue, residual organic matter, and biofilm is critical to ensure proper cleaning and subsequent high-level disinfection. In general, these detergent formulations offer faster cleaning cycles at lower temperatures, cost savings by extending the lifetime of the instruments, and reduced risk of infections through in-depth cleaning prior to high-level disinfection/sterilization [65].

7.8. Cleaning of Historical Art Objects and Structures

Deterioration of historically and culturally significant monuments, stone structures and artworks is of growing concern [21]. Exposure to the outdoor environment or to uncontrolled indoor environments (temperature and relative humidity) leads to deterioration largely due to atmospheric pollution from a variety of contaminants. Deterioration is a complex process involving chemical, physical, and biological mechanisms. For example, black salt crusts form on stone surfaces as a result of the chemical and microbial interactions between the atmospheric contaminants (sulfur dioxide forming sulfuric acid), the stone (calcium carbonate reacting with sulfuric acid to form calcium sulfate), and microbes that can form calcium oxalate in the crusts. Dust and dirt combine with the calcium sulfate and oxalate, resulting in the black crust. Several microbial techniques have been proposed and successfully demonstrated for cleaning and restoration of stone buildings, frescoes, marble surfaces, and other objects [87–97]. Figure 4.8 shows the Stories of the Holy Fathers fresco at the Monumental Cemetery in Pisa, Italy before and after treatment for 2 h with Pseudomonas stutzeri bacterial strain [97]. The effects of the treatment on the restoration of the fresco are obvious.

Given the delicate and fragile nature of the surfaces, the cleaning process is almost always a manual process that must be carefully performed. Although biorestoration is promising, the risks posed by the technology have not been sufficiently addressed, as well as the advantages and limitations compared with other physical, chemical and mechanical cleaning processes [21].
7.9. Household and Institutional Applications

One of the most widespread applications of microbial cleaners is as a laundry detergent and for stain and spot removal on fabrics. Other household and institutional applications of microbial cleaning include floors and other hard surfaces in kitchens, bathrooms, locker rooms, garages, loading docks, and similar facilities, tank and equipment cleaning such as ultrafiltration membranes and heat exchangers, as well as for odor control. A wide range of enzymatic formulations have been developed as additives or blends in laundry detergents and other household and institutional applications [44–67,98]. The benefits of microbial cleaning for these applications are effective cleaning performance at lower temperatures, reduced usage of chemicals such as surfactants, increased lifetime of the equipment due to milder cleaning conditions, targeted removal of different contaminants, and lower safety and health risks.

8. SUMMARY AND CONCLUSIONS

Microbial cleaning has been shown to be an effective alternative to conventional solvent cleaning for many applications. The method is based on the affinity of

![Figure 4.8](image-url)  
**FIGURE 4.8** Effect of biocleaning with *Pseudomonas stutzeri* bacterial strain on the *Stories of the Holy Fathers* fresco before (a) and after (b) treatment [97]. A color version of this figure appears in the color plate section.
microbes for hydrocarbons which are digested, producing harmless carbon dioxide, water and soluble fatty acids. The microbes are nonpathogenic and safe to handle and dispose. The process is environmentally friendly and is less expensive than solvent cleaning, but it is not applicable to high-precision cleaning applications. Typical applications include parts washing; oil and grease removal from concrete and other floor surfaces, and from drains and grease traps in manufacturing facilities, hospitals, restaurants, food processing facilities, and similar locations; decontamination; cleaning of historical artworks and structures; cleaning and disinfection in health care facilities; wound debridement; controlling SRB in oil fields; mercury bioremediation; and household and institutional cleaning applications.

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