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Chapter 15

Application of Microbial Cleaning Technology for Removal of Surface Contamination

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1 INTRODUCTION

Solvent cleaning is a well-established process for removal of surface contaminants in a wide variety of commercial cleaning applications [1]. Many of the conventional solvents used for cleaning, such as hydrochlorofluorocarbons (HCFCs), are considered detrimental to the environment and are increasingly subject to regulations for reduction in their use, and eventual phasing out of these solvents [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. 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 endproducts are carbon dioxide and water. Bioremediation is a safe, environmentally-friendly way to process many kinds of hazardous waste and is supported by the U.S. EPA (United States Environmental Protection Agency) as a viable solution for cleanup of oil spills and other contaminants, as well as an alternative to solvent cleaning.

The use of microbial cleaning for removal of surface contaminants was reviewed recently [4]. The purpose of this chapter is to update the previously published information on applications of microbial cleaning.

2 SURFACE CONTAMINATION AND CLEANLINESS LEVELS

The most common categories of surface contaminants include: particles; organic contaminants that may be present as hydrocarbon thin films or organic residue such as oil droplets, grease, resin additives, and waxes; molecular contamination that can be organic or inorganic; metallic contaminants present as discrete particles on the surface or trace impurities in the matrix; ionic contamination; and microbial contamination. 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 can 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. Product cleanliness levels in precision technology applications are typically specified for particles by size (in the μm range) and number of particles, as well as for hydrocarbon contamination represented by nonvolatile residue (NVR) in mass per unit area for surfaces or mass per unit volume for liquids [5–7]. The surface cleanliness levels are based on contamination levels established in industry standard IEST-STD-CC1246E for particles from Level 5 to Level 1000 and for NVR from Level R1E-5 ($10 \text{ ng}/0.1 \text{ m}^2$) to Level R25 ($25 \text{ mg}/0.1 \text{ m}^2$) [7]. In many commercial applications, the precision cleanliness level is defined as an organic contaminant level of less than $10 \mu\text{g}$ of contaminant per cm^2 , although for many applications the requirement is set at $1 \mu\text{g}/\text{cm}^2$ [7]. 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. A new standard ISO 14644-13 has been published that gives guidelines for cleaning of surfaces in cleanrooms to achieve defined levels of cleanliness in terms of particles and chemical classifications [8].

3 APPLICATIONS

Some key considerations of microbial cleaning and its applications are discussed in the following sections.

3.1 Microbial Agents

There are six main groups of microbes [9]:

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 eucaryotes 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 acid (DNA or RNA) and protein and have some of the characteristics of life. However, 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 bacterial strains (commonly) and fungi (less commonly) have been used for remediation and removal of contaminants [10–18]. 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 [19–21]. Cleaning applications include parts and components cleaning, artworks, oil spills, waste water, 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 facilities¹ for handling and use. They are not subject to distribution restrictions by the ATCC, U.S. Department of Health, Public Health Service or the Toxic Substances Control Act (TSCA).

1. Four biosafety levels have been assigned by the U.S. Centers for Disease Control for activities involving microorganisms [22]. 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* [23–25]. Nitrate-reducing bacteria, such as *Pseudomonas stutzeri*, have been used to remove animal glue and nitrate salt contaminants and different strains of sulfate-reducing bacteria (SRB). *Desulfovibrio vulgaris* and *D. desulfuricans* have been employed for effective cleaning of sulfate contaminants such as calcium sulfate deposits on buildings [16]. 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.

3.2 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 [4,11,26]. Fig. 15.1 shows a lifecycle diagram of the cleaning process. In a typical surface cleaning application, a cleaning solution 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 solution. Nutrients are generally added as part of the cleaning mix to provide emerging

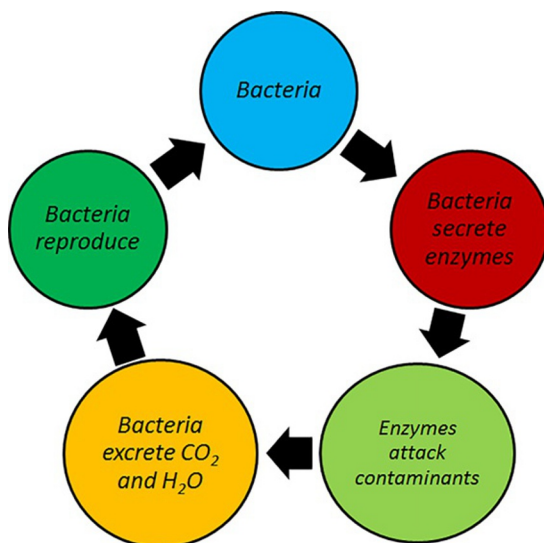


FIGURE 15.1 A typical microbial cleaning lifecycle diagram.

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 solution through a filtering device, where particulate matter, such as dirt, paint chips, and other items larger than 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 of 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 hours [26]. 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 non-ionic 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 bioremediating 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.

3.3 Parts Cleaners

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. Microbial parts cleaners are commercially available in several sizes and models [27–33]. 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 has been introduced for cleaning entire bicycles [34]. This model includes an integrated bike stand with the parts washer.

3.4 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 [35–37]. 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 [38–46]. These solutions are used in a typically 20:1 dilution ratio.

Many enzyme-based cleaning compositions have been developed and are available commercially [47–67]. These compositions are formulated from commercially available enzymes [68–70] and are used in varied institutional and household cleaning applications. Examples of cleaning applications are discussed in [Section 3.7](#).

3.5 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:

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

The cleaning performance for these types of contaminants is excellent. For example, analyses performed on samples of cleaning solutions from operating bioremediating cleaners have consistently shown hydrocarbon (oil and grease) levels in the 1400 ppm range, compared to average of 20 000 ppm of oil and grease from nonmicrobial conventional aqueous solvent cleaning [11,13].

Other contaminants that have been successfully treated or removed include salt crusts (nitrate and sulfate), paint, ink, glue, protein adhesive (animal glue), sealant, wax, tar, graffiti, pen marks, rubber, and resins.

3.6 Types of Substrates

Substrates such as carbon and stainless steels, galvanized steel, brass, copper, aluminum, plastics, ceramics, fiberglass, glass/quartz, sterling silver, nickel, titanium, and concrete have been successfully cleaned. Not only is the cleaning solution effective on metal parts, it 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 present 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.

3.7 Application Examples

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 remediation in petrochemical plants, chemical plants, refineries, food processing plants, marine barges, truck washes, wood treating plants, oil spill cleanup, soil

decontamination, and ground water remediation applications. Several surface cleaning applications have also been demonstrated including parts washing, oil and grease removal, cleaning of artworks and structures, disinfection, and household cleaning. The types of contaminants removed include biodegradable oils and greases, lubricants, bacterial contaminants, animal glue (protein adhesive) and nitrate and sulfate crusts [4]. In the following sections, previously published information on many of the cleaning applications is revised and updated since the recent review [4].

3.7.1 Parts Cleaning

This is one of the most common applications for microbial cleaning. 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.

Parts cleaners are simple to operate. As shown in Fig. 15.2, 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



FIGURE 15.2 Cleaning of parts in a wash basin [33]. (Courtesy of J. Walter Co. Ltd, Quebec, Canada).

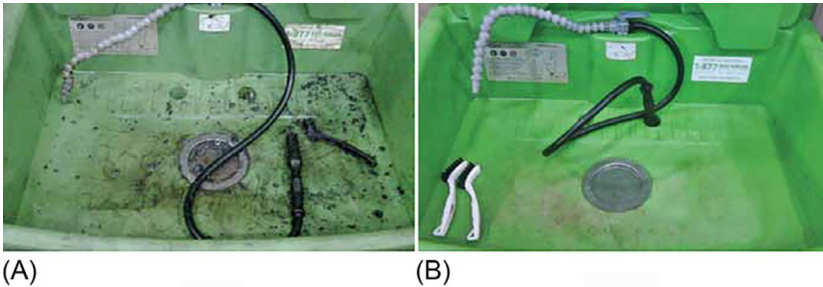


FIGURE 15.3 Parts cleaner sink (a) prior to cleaning, and (b) after cleaning [33]. (Courtesy of J. Walter Co. Ltd, Quebec, Canada).

temperature within a range that is ideal for the microbes to thrive, generally 323 to 360 K. The sink itself is also maintained clean (Fig. 15.3).

In a well-maintained microbial cleaning system, the only regularly generated waste is the used filters that are replaced every 3 to 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.

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

- The cleaning solution 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.
- Aggressive chemicals, such as disinfectants, bleach, solvents, acids or chlorinated substances, should not be added to the cleaning solution as they tend to kill the microbes.
- The liquid 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 liquids should be pre-cleaned. 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 liquid 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.

3.7.2 Surface Decontamination

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. Fig. 15.4 shows a truck fueling bay before and after cleaning with a microbial solution diluted in a 2:1 ratio with water [39]. The solution was sprayed on the contaminated areas ($\sim 1670 \text{ m}^2$) and allowed to work for approximately 4 hours 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 websites of the product suppliers [see for example 39–46, 68–70]. Fig. 15.5 shows a cleaning tank heavily contaminated with an oily sludge that was effectively cleaned by microbial solution treatment.

The major benefit to food manufacturing facilities (such as flour mills, bakeries, and meat and fish processing plants) is that when parts are cleaned with microbial solutions, they can be rinsed in potable water or sterilized through some other medium, which is difficult to accomplish when any petroleum-based compound is left on the surface of the parts.



FIGURE 15.4 Photos of a truck fueling bay before (left) and after (right) microbial cleaning [39]. (Courtesy Worldware Enterprises, Ontario, Canada).

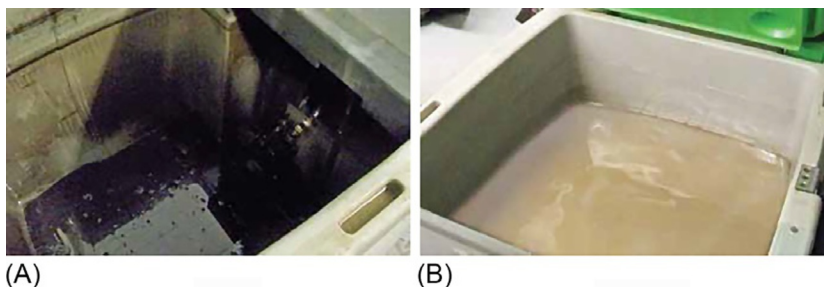


FIGURE 15.5 A cleaning tank (a) after drainage but before cleaning, and (b) after microbial treatment [37]. (Courtesy of J. Walter Co. Ltd, Quebec, Canada).

An innovative method of removing oil and grease on slick surfaces is to replace the microbe-enhanced surfactants with protein-enhanced surfactants [14]. 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.

Recently, mutagenized enzymes have been developed for decontamination of surfaces contaminated by chemical warfare agents [71–79]. In general, decontamination efficiencies on nonporous surfaces increase with increasing temperature, humidity, and interaction time, although the effects are strongly dependent on the specific enzyme formulation. Most current chemical decontamination enzyme development efforts are focused on improvements in agent specific activity, stability of the enzyme for increased shelf-life and pot-life (defined as the period of time that the enzyme is active in aqueous solution) through immobilization of the enzyme, and enhanced thermostability.

Enzymes appear to have a great potential to decontaminate surfaces because of their ease of application, negligible damage to surfaces, and relatively effective decontamination under different environmental conditions. The addition of a cosolvent, for example, to an enzyme solution (at a sufficiently low concentration to avoid degradation of the enzyme itself) might further enhance the decontamination efficiency through the improved solubility of these types of chemicals.

3.7.3 Cleaning of Historical Art Objects and Structures

Deterioration of historically and culturally significant monuments, stone structures, documents, and artworks is of growing concern [16]. 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, including nitrates, sulfates, black crusts, organic matter, and microorganisms [16,80–83]. 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 [84–112]. Fig. 15.6 shows the *Stories of the Holy Fathers* fresco at the Camposanto Monumental Cemetery in Pisa, Italy before and after



FIGURE 15.6 Effect of biocleaning with *Pseudomonas stutzeri* bacterial strain on the Stories of the Holy Fathers fresco before (top) and after (bottom) treatment [99].

treatment for two hours with *Pseudomonas stutzeri* bacterial strain [99]. The effects of the treatment on the restoration of the fresco are obvious.

Other bioremediated examples include the Cathedrals of Milan, Florence, and Matera in Italy; the base of Michelangelo's Pietà Rondanini in Milan; the loggias (exterior galleries) of the Casina Farnese on the Palatine Hill in Rome, Italy; sculptures in the Buonconsiglio Castle courtyard in Trento and the Monumental Cemetery in Milan; a nineteenth-century building in Riga, Latvia; the Epidauro Theatre in Greece; marble bas relief in Palero, Italy; and biocleaned frescoes in the Santos Juanes Church in Valencia, Spain [86,88,90,92,93,96–112].

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 [16,104].

3.7.4 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 (SARS) or methicillin-resistant *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 (*Bacillus* or *Pseudomonas*) together with a surfactant and an aqueous carrier to maintain a minimum 95% catalytic activity at pH range of 5.5 to 13.5 [24]. This solution is effective against several resistant bacterial strains, such as MRSA, VRE (vancomycin-resistant *Enterococci*), and GISA (glycopeptide intermediate *Staphylococcus 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 and plastic parts, concrete and tile floors, cleaning grease traps, and other household 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 [113].

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 [114,115].

Inadequate cleaning of surgical instruments can result in disastrous consequences for patients in health care facilities [116]. Detergents containing microbial proteases very effectively clean endoscopes and other critical and semi-critical 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 and/or sterilization [68].

3.7.5 *Bacterial Characterization and Monitoring of Surface Cleanliness*

Most bacteria are small, approximately 1 μm 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 both from remediation and cleanliness monitoring perspectives. This cannot be done directly because of the large surfaces and fixed installations. Recently, a technique using cellulose acetate replicating tape has been developed to characterize food-borne bacteria on a stainless steel surface by an electron microscope [117]. Bacteria are clearly visible in the micrograph of the replica (Fig. 15.7).

Methods for monitoring and measuring the cleanliness of surfaces have been described in detail [118].

3.7.6 *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 [119–121]. In a recent study, a transgenic system has been developed for mercury remediation [122]. The proposed system effectively expresses metallothionein (*mt-1*) and polyphosphate kinase (*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 application in cleaning mercury-contaminated surfaces.

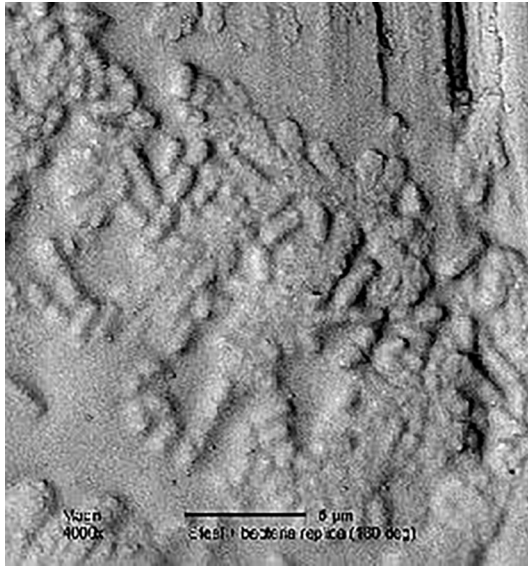


FIGURE 15.7 Inverted image of a cellulose acetate replica of bacteria in a vegetable marinade [117]. The scale bar is 5 μm .

3.7.7 Wound Debridement

Several systems have been developed for wound cleansing and debridement using enzyme-based cleaning solutions [123,124 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 [125]. 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 [123].

3.7.8 Sulfate-Reducing Bacteria in Oilfields

One of the deleterious consequences of sulfate-reducing bacteria (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_2S [126–128]. Several microbial

processes have been proposed to control SRB contamination in oilfields, including addition of nitrate-reducing bacteria to inhibit H_2S production. These methods have been reviewed recently [128].

3.7.9 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 [47–70,129–134]. 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.

4 OTHER CONSIDERATIONS

Some other considerations need to be addressed in the application of the microbial approach for surface cleaning.

4.1 Costs

Parts cleaners are relatively inexpensive, costing around US\$2500 to \$8500, depending on the size and capacity of the system [31–34]. Operating costs are generally low. Consumption of cleaning chemicals is minimal, as 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 to 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 US\$400 for 5 gallons, but it is diluted on average in 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 (50–77°C). Some system providers offer maintenance contracts at around US\$600 per year for bimonthly service calls [33]. Waste disposal costs for microbial cleaning are low since the primary waste stream is the filters that are replaced every 3 to 8 weeks. Overall, the costs of microbial cleaning are lower than solvent cleaning, as illustrated by some examples below.

4.1.1 Examples of Cost Savings

The Texas Army National Guard invested approximately US\$15000 in August 1995 to purchase 10 bioremediating parts cleaners to replace solvent cleaners for motor pool operations. In the first year, the Guard eliminated 600 gallons of solvent waste and significantly reduced VOC emissions, saved US\$5130 in waste disposal manifest requirements, and saved US\$4200 in yearly solvent purchase costs. The estimated payback period was about 18 months. A major aeronautical firm realized savings of more than US\$80 000 by reducing solvent usage by more than 900 gallons during the first year through use of 23 bioremediating parts washers to replace solvent cleaners [11].

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 less than 3 months [12,135]. Table 15.1 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 bioremediating parts cleaning system [136,137]. The bioremediating system reduced the waste stream by nearly 100%, saving US\$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.

For remediation of cultural heritage, an analysis of the costs of the fast bio-cleaning process for organic substances (animal glue and casein) showed the total cost was about 90 Euro/l for bacterial culture with 2 l of the bacterial suspension used for cleaning about 1 m² of fresco surface. Comparing the costs with the use of enzymes (which is a comparable technique in terms of cleaning

TABLE 15.1 Comparison of the Costs of Conventional Solvent Process With Microbial Cleaning [135]

	Total First Year Cost (US\$)	Total Subsequent Yearly Cost (US\$)
Solvent Cleaning	\$5050	\$3450
Microbial Cleaning System	\$1820	\$1850
Savings	64%	46%

efficiency and lack of damage to artworks), biocleaning is clearly far less costly because the cost of protease is about 150 Euro/l and the cost of collagenase is about 500 Euro/l [96,104]. The costs of the microbial cleaning process are also generally comparable with the usual chemical-physical techniques, but the microbial bacterial approach is more convenient in terms of both the short time needed for the biocleaning and the number of treatments (a single treatment of 2 h), as well as the environmental safety of the process (no hazardous chemical waste disposal). The relatively low-cost of microbial cleaning means that its application represents a highly competitive, cost-effective remediation solution for cultural heritage.

4.2 Advantages and Disadvantages of Microbial Cleaning

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

4.2.1 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 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, 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 seven days.
8. Most bioremediating parts cleaners 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, because the filters are the only waste stream generated in low volumes.
18. The cleaning solutions are pH neutral, non-caustic that will not dry, crack or irritate the skin.
19. For cultural heritage, microbial cleaning is highly effective in removing all types of surface contaminants without damage to the surface.

4.2.2 Disadvantages

1. Microbes are susceptible to any biocides designed to kill microbes, such as bleach or strong chemicals that kill living organisms such as 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.

5 SUMMARY

Microbial cleaning has been shown to be an effective alternative to conventional solvent cleaning for many applications. The method is based on the affinity for microbes for hydrocarbons which are digested, producing harmless carbon dioxide, water and soluble fatty acids. The microbes are nonpathogenic and are 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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