Microbial Contamination Control in the Pharmaceutical Industry

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<table>
<thead>
<tr>
<th>Book Title</th>
<th>Editor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23. Pharmaceutical Process Validation, <em>edited by Bernard T. Loftus and Robert A. Nash</em></td>
<td></td>
</tr>
<tr>
<td>26. Drug Dynamics for Analytical, Clinical, and Biological Chemists, <em>Benjamin J. Gudzinowicz, Burrows T. Younkin, Jr., and Michael J. Gudzinowicz</em></td>
<td></td>
</tr>
<tr>
<td>27. Modern Analysis of Antibiotics, <em>edited by Adjoran Aszalos</em></td>
<td></td>
</tr>
<tr>
<td>28. Solubility and Related Properties, <em>Kenneth C. James</em></td>
<td></td>
</tr>
<tr>
<td>31. Transdermal Controlled Systemic Medications, <em>edited by Yie W. Chien</em></td>
<td></td>
</tr>
<tr>
<td>33. Pharmacokinetics: Regulatory • Industrial • Academic Perspectives, <em>edited by Peter G. Welling and Francis L. S. Tse</em></td>
<td></td>
</tr>
<tr>
<td>34. Clinical Drug Trials and Tribulations, <em>edited by Allen E. Cato</em></td>
<td></td>
</tr>
<tr>
<td>36. Aqueous Polymeric Coatings for Pharmaceutical Dosage Forms, <em>edited by James W. McGinity</em></td>
<td></td>
</tr>
<tr>
<td>37. Pharmaceutical Pelletization Technology, <em>edited by Isaac Ghebre-Sellassie</em></td>
<td></td>
</tr>
<tr>
<td>38. Good Laboratory Practice Regulations, <em>edited by Allen F. Hirsch</em></td>
<td></td>
</tr>
<tr>
<td>41. Specialized Drug Delivery Systems: Manufacturing and Production Technology, <em>edited by Praveen Tyle</em></td>
<td></td>
</tr>
<tr>
<td>42. Topical Drug Delivery Formulations, <em>edited by David W. Osborne and Anton H. Amann</em></td>
<td></td>
</tr>
</tbody>
</table>
45. Biodegradable Polymers as Drug Delivery Systems, edited by Mark Chasin and Robert Langer
46. Preclinical Drug Disposition: A Laboratory Handbook, Francis L. S. Tse and James J. Jaffe
47. HPLC in the Pharmaceutical Industry, edited by Godwin W. Fong and Stanley K. Lam
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Preface

Since the implementation of good manufacturing practices (GMPs) in the early 1970s, major improvements have been achieved in the control of microbial contamination in pharmaceutical environments. However, microbial contamination of pharmaceutical products is one of the major reasons for product recall and manufacturing problems. Knowledge of the distribution and survival of microorganisms in pharmaceutical environments is critical in the process control of nonsterile and sterile pharmaceutical products. This knowledge is somewhat limited by the ubiquitous distribution of microorganisms in manufacturing facilities, the diversity of microorganisms in environmental samples, and the flexibility of microorganisms in surviving under different environmental fluctuations. Optimization of pharmaceutical manufacturing has led to more efficient testing systems to monitor the analysts, environment, water, raw materials, and finished products that are the major sources of introduction of microorganisms into the processes. However, to avoid microbial contamination, adherence to GMP is the foundation for manufacturing safe and efficacious pharmaceutical products.

With the latest developments in computer science, automation, genomics, combinatorial chemistry, and process control, the manufacture and quality control analysis of pharmaceuticals will be changed significantly. Therefore, optimization of quality control analysis in pharmaceutical oper-
ations has become an interdisciplinary endeavor that requires communication and cooperation between microbiologists and other scientists. This book discusses major issues regarding testing and quality control in pharmaceutical manufacturing, which will ensure product and process integrity. Why is it important to control the presence of microorganisms in a manufacturing facility? What systems do we need to prevent this contamination? What tests do we perform to guarantee the safety and efficacy of the products manufactured under those conditions? What new technologies are available to optimize sample analysis and manufacturing? What regulations must be followed to provide quality products? We hope to provide answers to all these questions. This book is aimed at pharmacy students, chemists, engineers, pharmaceutical scientists, and microbiologists working in or associated with the pharmaceutical industry, with the intention of being a first step toward the understanding of microbial control in pharmaceutical environments.

Luis Jimenez
Contents

Preface iii
Contributors vii

1. Microorganisms in the Environment and Their Relevance to Pharmaceutical Processes 1
Luis Jimenez

2. Microbial Limits 15
Luis Jimenez

3. Microbial Monitoring of Potable Water and Water for Pharmaceutical Purposes 45
Anthony M. Cundell

4. Sterility Test and Procedures 77
Luis Jimenez

5. Environmental Monitoring 103
Luis Jimenez
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Author</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Biological Indicator Performance Standards and Control</td>
<td>Jeanne Moldenhauer</td>
<td>133</td>
</tr>
<tr>
<td>7</td>
<td>Rapid Methods for Pharmaceutical Analysis</td>
<td>Luis Jimenez</td>
<td>147</td>
</tr>
<tr>
<td>8</td>
<td>Endotoxin: Relevance and Control in Parenteral Manufacturing</td>
<td>Kevin L. Williams</td>
<td>183</td>
</tr>
<tr>
<td>9</td>
<td>Proper Use and Validation of Disinfectants</td>
<td>Laura Valdes-Mora</td>
<td>251</td>
</tr>
<tr>
<td>10</td>
<td>Antimicrobial Effectiveness Test and Preservatives in Pharmaceutical Products</td>
<td>Luis Jimenez</td>
<td>283</td>
</tr>
</tbody>
</table>

*Index* 301
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Microorganisms in the Environment and Their Relevance to Pharmaceutical Processes

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1. INTRODUCTION

Microorganisms on Earth are widely distributed across different environmental habitats [1]. They are present in water, air, sediments, and soil. One of the reasons for the wide distribution of microorganisms in the environment is the great physiological diversity regarding the utilization of inorganic and organic compounds to sustain microbial viability, maintenance, reproduction, and growth [1]. Microbial cells degrade organic and inorganic compounds to sustain microbial metabolism. Some microbial species do not require high concentrations of organic or inorganic compounds to survive and grow. Microbial species such as *Pseudomonas* spp., *Acinetobacter* spp., *Burkholderia* spp., and *Stenotrophomonas* spp. exhibit a tremendous physiological versatility by using a wide variety of organic and inorganic compounds to support microbial metabolism.

Microorganisms carry the energy needed for metabolic processes in the phosphate energy-rich molecule called adenosine triphosphate (ATP) [2].
ATP is the most important energy compound in the microbial cell. Enzymatic reactions are an important part of the catabolic pathways used by microorganisms to generate ATP. For instance, organic compounds such as carbohydrates are converted to pyruvate through a process called glycolysis. Some microbes use glycolysis to generate ATP, in the absence of oxygen. The end products of that process (e.g., fermentation) are alcohols and acids. Other microorganisms utilize inorganic compounds such as sulfate and nitrate to generate ATP. In other cases, solar energy is utilized to generate ATP by bacterial photosynthesis. When oxygen is present in the environment, microorganisms develop metabolic reactions driven by inorganic or organic compounds to generate ATP. Furthermore, respiratory metabolism is also used. Respiratory metabolism is based upon the transfer of electrons from different types of electron donors and acceptors such as nicotinamide-adenine dinucleotide hydrogen (NADH), flavin-adenine dinucleotide hydrogen (FADH), and cytochromes. Some bacteria use oxygen as the ultimate electron acceptor (e.g., aerobic respiration) whereas others use different types of inorganic compounds (e.g., anaerobic respiration). However, other types of bacteria can live in the presence or absence of oxygen (facultative).

Although microbial populations are present in all types of habitats, there are several major limiting factors that affect microbial distribution, survival, and proliferation in the environment. These factors are:

- Temperature
- Available water
- Concentration of organic compounds
- Concentration of hydrogen ions (pH)
- Concentration of inorganic compounds
- Concentration of particulates in the air
- Redox potential (Eh)
- Pressure
- Light intensity

Because of the different environmental fluctuations encountered, natural microbial communities do not exist in a state of perpetual proliferation and growth. There are major seasonal fluctuations regarding temperature, light intensity, available water, and concentration of organic and inorganic compounds on the basis of the geographical location of a given microbial community. For instance, water habitats in tropical locations do not undergo the same temperature fluctuations observed in temperate habitats. Therefore, microorganisms in temperate habitats exhibit a higher tolerance to increased temperatures when compared to microorganisms in tropical climates. The environment is always changing and microorganisms respond to these changes by adapting and surviving. Some of these adaptations allow
microbial cells to grow very slowly or remain dormant for long periods of time.

2. STRATEGIES FOR MICROBIAL SURVIVAL IN THE ENVIRONMENT

How do microorganisms respond to different environmental fluctuations in the environment? They respond to these fluctuations by adopting different survival strategies [3]. These strategies are based upon the minimal utilization of energy to support microbial metabolism and growth. Growth is defined as an increase in the number of cells over time. However, microbial populations do not grow continuously because of the fluctuations in the amount of available water, food, etc. When laboratory cultures are prepared, microbial cells are inoculated into rich growth media with high concentrations of carbon, nitrogen, and phosphate. After inoculation and a brief phase, where microbes do not grow (lag phase), microorganisms grow exponentially until they utilize all available food sources (log phase). At that time, the numbers of cells stabilize. This is called stationary phase. If the culture media is not replenished with fresh growth media, the number of cells decreases due to the lack of nutrients and cell death. For instance, laboratory cultures of *Escherichia coli* double every 20 min when grown in rich nutrient media. However, when cell suspensions of the same microorganism are introduced into growth chambers immersed in a low-nutrient environment, doubling time is significantly slower [4]. Evidently, different growth dynamics are found between a high-nutrient and low-nutrient environment.

Some of the survival strategies are based upon the formation of bacterial spores as a response to nutrient deficiency and high temperature. *Bacillus* spp. and *Clostridium* spp. are commonly known as spore formers. These bacterial species are widely distributed in air, water, and soil samples. Germination of the spores is triggered by environmental factors indicating the presence of optimal conditions for microbial growth.

Other microorganisms respond to environmental fluctuations by changes in the enzymatic and protein profiles [3]. These changes are generally found in a wide variety of microbial species such as *Acinetobacter* spp., *Arthrobacter* spp., *Agrobacterium* spp., *Pseudomonas* spp., and the family Enterobacteriaceae. When low-nutrient concentration environments are encountered, microbial cells produce new types of enzymes and proteins, which are essential for microbial survival and maintenance.

Another survival strategy is when microbial cells reduce their size and metabolism. Along with size reduction, there is a decrease in respiration and cell numbers. In some cases, an increased adhesion to surfaces has been
reported. This results in the formation of biofilms. Biofilm formation concentrates the cells on a surface and creates a microenvironment where nutrient utilization is optimized.

To support microbial metabolism under low-nutrient concentration conditions, four classes of carbon, phosphate, and nitrogen compounds are used for potential storage of food sources. These compounds are:

- Carbohydrates
- Lipids (poly-β-hydroxybutyrate and polyalkanoates)
- Polyphosphates
- Cyanophycin/phycocyanin.

These compounds are degraded by microorganisms under stress-induced conditions to provide endogenous sources of energy to maintain microbial viability and growth.

*Arthrobacter* spp. are a good example of these types of bacterial populations. They are pleomorphic bacteria undergoing different cell morphologies under different nutritional conditions. A transition from rods to cocci is observed when cultures go from exponential growth phase to stationary growth phase. The cells are capable of long-term survival under hostile environmental conditions by utilizing endogenous sources of energy.

Gram-negative bacterial species undergo a viable but nonculturable stage [3]. When microbial cells enter this stage, several changes take place. It has been reported that cell size, enzymatic profile, membrane proteins, and microbial metabolism are dramatically reduced. New enzymes and proteins are produced as a response to the environmental fluctuations encountered. This response is commonly triggered by the lack of carbon, nitrogen, and phosphate sources. Furthermore, microorganisms undergoing this transitional stage do not grow on regular growth media (e.g., uncultured). However, they have been enumerated and proven to be physiologically viable by alternative methods with increasing sensitivity and resolution.

When microbial populations adopt some of these survival strategies, identification by standard methods is difficult and might lead to erroneous conclusions. This is because standard methods are based upon the phenotypical analysis of microorganisms. Macroscopical and microscopical analyses are based upon colony morphology, cell size, enzymatic profiles, and carbon utilization profiles.

Standard methods are used in clinical, environmental, pharmaceutical, and food microbiology to diagnose microbial pathogenesis and contamination [5]. However, the development of better analytical methods has provided an accurate and sensitive representation of the distribution and activity of microorganisms in the environment. This new information has
supplemented the knowledge obtained using traditional culture and enrichment methods.

3. ISOLATION, ENUMERATION, AND IDENTIFICATION OF MICROORGANISMS

In the beginning of the field of microbiology, microbial isolation and identification were based upon the phenotypical analysis of microbial cells by microscopical analysis of water, fermentation products, and clinical samples by Leeuwenhoek [6], Koch [7], and Pasteur [8]. After several years, the plate count was invented in the laboratory of Koch [7]. Up to that point, most of the works were basically concentrated on infectious disease analysis for diagnosis and prognosis. It was not until the significant contributions of Winogradsky and Beijerinck that the enrichment culture technique was developed to isolate microorganisms from environmental samples. Optimizing the enrichment media to enhance the growth of microorganisms with specific metabolic activity leads to the isolation of specific microbes present in low numbers. The role of microorganisms in the cycling of materials in the environment and the common metabolic reactions between microorganisms and macroorganisms was demonstrated by the works of Kluyver, van Niel, and Stainer [2].

Further developments in microbial methodology lead to selective agar media for pathogen isolation from clinical samples. Membrane filtration analysis was introduced after the Second World War. The development of membrane filtration allowed the concentration of large volumes of liquid on a filter. Larger sample volumes were analyzed by optimizing assay sensitivity and resolution. In some cases, water samples contain low numbers of microorganisms, which would not be detected unless large volumes (e.g., 100 mL) are analyzed.

Up to that point, all analyses were based upon enumeration and detection of colonies based on morphology, color, differential staining, cell morphology, and biochemical reactions of isolated colonies. For instance, macroscopical and microscopical analyses of microbial communities from clinical and environmental samples relied on the above characteristics.

During the late 20th century, molecular biology techniques provided a clearer picture of the distribution and complexity of microbial communities in environmental and clinical samples [9–13]. Some of the techniques used are:

- Gene probes
- Polymerase chain reaction (PCR) technology
- DNA sequencing
- Nucleic acid extractions from environmental matrices.
Further studies also demonstrated the use of specific biochemical indicators for the presence of microorganisms. These analyses provide information on the microbial community, microbial population, and individual cells. For instance, microbial biomass can be determined by:

- Direct microbial counts
- ATP and total adenylate
- Cell wall components (lipids and muramic acid)
- Bacteriochlorophyll and other pigments
- DNA
- Proteins.

The application of these molecular biology techniques and biomass measurements to environmental and clinical analysis demonstrated that the majority of microorganisms in the environment are unculturable but viable.

Studies demonstrated that when individual cells are counted and analyzed by direct microscopy, different growth dynamics are observed. For example, direct microbial counts using epifluorescence microscopy yield higher counts than standard plating techniques [2]. However, overestimation of the numbers is a result of the inability to distinguish between living and dead microorganisms. Direct microscopy with fluorochromes (dyes) such as acridine orange (AODC), 4',6-diamidino-2-phenyl-indole (DAPI), Hoechst 33258, and fluorescein isothiocyanate (FITC) provided an alternative to the plate count. However, it was difficult to determine cell viability. Are these cells viable? Are we just counting dead cells?

Several modifications of the direct count method allow the determination of the numbers of viable cells [e.g., combining the direct count method with INT (2-\([p\text{-iodophenyl}]-3-\([p\text{-nitrophenyl}]-5\text{-phenyl tetrazolium chloride}\) staining). Respiring microorganisms reduce INT to INT-formazan by accumulating intracellular dark red spots visible through a microscope. Other methods rely on the inhibition of cell division by nalidixic acid (DVC) where microscopical observations show elongated cells [3]. Another method counts the numbers of cells dividing actively [14].

Combining direct microscopy with radioactive substrates to analyze incubated microorganisms was also used [15]. Specific types of microorganisms can be also be detected by fluorescent antibody techniques [3]. All these studies consistently indicated that a high percentage of the microorganisms in a sample did not grow on standard plate media but were viable. Up to that point, viability was understood as the capacity of a microorganism to grow on plate media. Growth on plate media requires duplication of microbial cells to a stage where colonies are visually detected. The minimum numbers of cells required for a colony to be visible ranges from $1 \times 10^6$ to...
5×10^6 cells. However, on the basis of these and other studies, viability was defined as an indication of bacterial activity, not growth [3].

Direct extraction of DNA and RNA from environmental and clinical samples further confirmed that the great majority of the microbial community in a given sample do not grow on standard plate media [16–18]. Furthermore, important microbial populations were detected and characterized using molecular biology techniques. It seems that because of the extensive physiology of microbial populations, no single medium or defined set of growth conditions can provide all the requirements for most of the organisms present in a given environmental sample. It seems that many of the microbial species dominating natural environments are not adapted to grow in media containing high concentrations of organic compounds. However, when low-nutrient media is used, a higher microbial recovery is found in some environmental samples [19,20]. Different types of low-nutrient media have recovered a previously unculturable segment of microorganisms from water, soil, and clinical samples. These populations do not grow on blood agar, soybean casein digest agar, soybean casein digest broth, nutrient broth, and nutrient agar, but have been shown to be metabolically active.

4. ANALYSIS AND CONTROL OF PHARMACEUTICAL ENVIRONMENTS TO MINIMIZE MICROBIAL SURVIVAL

One of the most important areas in pharmaceutical process control is the development of systems to control the numbers, survival, and proliferation of microorganisms during manufacturing of nonsterile and sterile pharmaceutical products. The facility where products are manufactured is basically a closed environment where people and materials will move in and out to carry out different processes.

Microorganisms, as previously mentioned, have a great catabolic capacity to derive energy from any type of organic or inorganic compounds. Therefore, having microorganisms in a product can cause spoilage of the formula by breaking down active ingredients and excipients. This might compromise the potency and efficacy of the drug. Furthermore, the presence of high numbers of microorganisms and pathogens represents a serious health threat to consumers because products will be ingested, injected, or applied to human skin. Pharmaceutical products are commonly used after a pathological condition (e.g., disease) is diagnosed. The disease can be based upon microbial infection or metabolic disorders.

Therefore, minimizing the numbers or preventing the introduction of significant numbers of microorganisms into pharmaceutical facilities and processes becomes the most important aspect of process control during
pharmaceutical manufacturing [21]. What are the critical areas where microorganisms can be introduced?

First, some of the raw materials utilized for the development of pharmaceutical formulations are based upon natural products that contain a high microbial load. The production processes for these raw materials do not eliminate all microorganisms. Therefore, they are not sterile. Testing must be performed to determine the quality of these materials. The absence of *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium* is required before raw materials can be used in pharmaceutical products. However, some of the manufacturing processes are designed to significantly reduce the number of microorganisms. Different types of bacteria commonly found in pharmaceutical raw materials are *Lactobacillus* spp., *Pseudomonas* spp., *Bacillus* spp., *Escherichia* spp., *Streptococcus* spp., *Clostridium* spp., *Agrobacterium* spp., etc. and molds such as *Cladosporium* spp. and *Fusarium* spp.

A second critical area is the air in the facility. Air ventilation systems in manufacturing facilities are built to minimize the survival, distribution, reproduction, and growth of microbes. This facility is provided with humidity, ventilation, and air conditioning units (HVAC), which control these parameters. The air is filtered through a 0.5-μm filter to prevent the introduction into the facility of any particle higher than 0.5 μm. Microorganisms are commonly associated with particles in the air. Therefore, the exclusion of these particles in the facility minimizes the chances of microbial distribution and contamination by air. Air flow and pressure are controlled to exclude any nonviable and viable particle from entering critical areas. Humidity also controls the number of microorganisms in a room. The more humid is the room, the more chances there are for microorganisms to be carried by droplets of moisture. Therefore, a dry room provides a more hostile condition for microbes to grow than a humid room. A general practice in pharmaceutical environments is to apply ultraviolet light (UV) to reduce microbial contamination by air. Some of the microbial species commonly found in air samples in pharmaceutical environments are bacteria such as *Bacillus* spp., *Staphylococcus* spp., *Corynebacterium* spp. Common mold species are *Aspergillus* spp. and *Penicillium* spp.

A third critical area is the personnel in the plant and testing laboratories. Microorganisms are part of the normal flora of the human skin and body. Therefore, operators and laboratory analysts are the major sources of contamination during manufacturing and testing [22]. Some of the species living in the human skin are *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus hominis*, *Propionibacterium* spp., *Propionibacterium acnes*, *Micrococcus* spp., etc. The normal flora for the human oral cavity is comprised of *Streptococcus salivarius*, *Streptococcus mutans*, etc. Molds can also be possible contaminants. Common molds from human flora are *Tricho*
phyton spp., *Epidermophyton* spp., *Microsporon* spp., etc. To protect critical areas from human microbial flora, personnel wear gowns, hair covers, hoods, shoe covers, laboratory coats, face masks, gloves, boots, etc.

A fourth area of concern is water. Water is the most common raw material in pharmaceutical manufacturing. Drinking water is physically and chemically treated to reduce microbial numbers and pathogenic microorganisms. Water for pharmaceutical processes is further treated to minimize microbial numbers, endotoxin substances, and organic and inorganic compounds. The less organic compounds there are in the water, the fewer microorganisms will be found. Bacterial species such *Pseudomonas* spp., *Alcaligenes* spp., *Stenotrophomonas* spp., *Burkholderia cepacia*, *Burkholderia picketti*, *Serratia* spp., and *Flavobacterium* spp. are commonly found in water samples. Other types of bacteria can also be present but when found, they indicate fecal sources of contamination. These bacteria are *E. coli*, *Enterobacter* spp., *Salmonella* spp., *Shigella* spp., *Clostridium perfringes*, and *Enterococcus* spp. Recent studies using 16S ribosomal analysis, PCR amplification, and denaturing gradient gel electrophoresis (DGGE) testing demonstrated the presence of the following culturable bacterial species: *Bradyrhizobium* spp., *Xanthomonas* spp., and *Stenotrophomonas* spp. However, the predominant bacterial type in the water system could not be detected on culture media.

A fifth area of concern is the equipment and building areas. Unless equipment is cleaned and sanitized, there is always the risk of microbial contamination. However, cleaning and sanitization of the equipment must provide a hostile environment for microorganisms to survive and grow. Bacteria such as *Pseudomonas* spp., *S. epidermidis*, *Bacillus* spp., etc. are commonly found in equipment. Molds are commonly found in walls and ceilings. Continuous sanitization and disinfection of floors, drains, walls, and ceilings are advised to avoid the microbial colonization of these areas. Some of the mold species are *Aspergillus* spp., *Penicillium* spp., and *Aureobasidium* spp., etc. Using 16S ribosomal DNA analysis and sequencing, other microbial species found are *Taxeobacter* spp., *Flexibacter* spp., *Cytophaga* spp., *Ultradimicrobacterium* spp., *Stenotrophomonas* spp., *Streptococcus* spp., *Sphingomonas* spp., and *Comamonas* spp.

Quality control analysis in the pharmaceutical industry relies on standard enrichment and/or plating of the different types of pharmaceutical raw materials and finished products [23–28]. Environmental monitoring of all critical areas also relies on standard microbiological assays [21]. When microorganisms contaminate pharmaceutical products, standard methods are performed to quantify, detect, and identify the numbers and types of microorganisms present in a given pharmaceutical batch. Standard, compendial methods are based upon the enrichment, incubation, and isolation of micro-
organisms from pharmaceutical samples. Because of the long incubation
times, continuous manipulation, and time-consuming procedures, results are
normally obtained within 6–8 days for nonsterile products and 14 days for
sterile products. It has been recently reported that standard methods, as found
in environmental samples, underestimate the numbers and diversity of mi-
crobial communities present in pharmaceutical environments [29–33]. This has
been demonstrated in samples of water, contact plates, and air from different
pharmaceutical manufacturing facilities and clean room environments. ATP
bioluminescence, flow cytometry, direct viable counts, DNA, and PCR
technology have demonstrated that a nonculturable portion of the microbial
community in pharmaceutical environments is viable and not detected by
standard methods. Therefore, these new technologies complement standard
methods by providing higher resolution and discrimination between microbial
species. Accurate information of the types and numbers of microorganisms in
pharmaceutical environments will lead to the optimization of processes that
minimize microbial distribution, viability, growth, and proliferation.
Furthermore, identification of several environmental isolates from
pharmaceutical environments using standard identification procedures is
proven to be incorrect [34]. When identification is performed by biochemical,
lipids, and DNA analyses, DNA analysis provides the best reproducibility,
sensitivity, accuracy, and resolution. To develop the proper corrective action
when out-of-specification (OOS) results are obtained, accurate microbial
identification is needed if the contamination source has to be determined and
tracked. A corrective action is not effective if wrong information is used to
develop a proper solution to a given problem.
On the basis of these studies, it is evident that in some cases, standard
methods are not accurate and precise to optimize process control, leading to
faster releasing time, sample analysis, and high-throughput screening of
samples. Standard methods must be complemented by other technologies
that can provide additional information on the processes and systems used in
pharmaceutical manufacturing. Although standard methods are valuable
and do provide information on the numbers, microbial genera, and species,
they were developed as previously stated for the identification of micro-
organisms from clinical samples. Most clinical samples originate from human
fluids or tissues, which are rich in nutrients and exhibit temperatures of 35–
37°C. Environmental samples (e.g., raw materials, finished products, air,
water, equipment swabs, and contact plates) taken from production facilities
are not rich in nutrients (oligotrophic) and temperature fluctuates below and
above ambient temperature. Low water activity and dramatical changes in
pH also contribute to microbial stress. Furthermore, manufacturing of
pharmaceutical products comprises physical processes such as blending,
compression, filtration, heating, encapsulation, shearing, tableting, granu-
lation, coating, and drying. These processes expose microbial cells to extensive environmental stresses. The facility where manufacturing takes place is designed to create an environment where microorganisms will not survive. Air flow, temperature, pressure, air particulates, etc. are optimized to reduce the numbers of microorganisms.

Microorganisms, as previously stated, survive under those conditions by adapting to the lack of nutrients and other environmental fluctuations by undertaking different survival strategies. Furthermore, bacterial cells that do not grow on plate media but retain their viability by going through the viable but culturable stage are still capable of causing severe infections to humans. Several studies have shown that microbial cells in pharmaceutical environments have changed the cell size and enzymatic and physiological profiles as a response to environmental fluctuations [35,36]. Similar responses have been reported by bacteria exposed to drug solutions where significant morphological and size changes are observed. Bacterial cells spiked into different types of injectable products have shown different changes in their metabolism, enzymatic profiles, and structural changes, which interfered with their identification using standard biochemical assays [35]. Furthermore, bacteria undergoing starvation survival periods are capable of penetrating 0.2/0.22 μm rated filters, which are supposed to retain all bacterial species [36].

Therefore, using enzymatic and carbon assimilation profiles (e.g., biochemical identification) to discriminate and identify microorganisms from pharmaceutical samples might, in some cases, yield unknown profiles that will not provide any significant information on the microbial genera and species. In pharmaceutical environments, information on the genera and species of a microbial contaminant will provide valuable information on the possible sources of the contamination, allowing the implementation of effective corrective actions.

It has been also shown that the recovery of microorganisms from environmental samples in pharmaceutical clean room environments is enhanced by using low-nutrient media [31,33]. The recovery of microorganisms from pharmaceutical water samples has been shown to be increased by the use of a low-nutrient media [30]. Similar results are observed for other environmental samples when low-nutrient media is used. The need for a stress recovery phase is demonstrated by longer incubation times and low-nutrient media.

Evidently, pharmaceutical environments are subjected to microorganisms originating from air, water, personnel, and materials introduced into the different facilities where products are manufactured and tested. New methods and additional information on the distribution, survival, and growth of microorganisms in pharmaceutical facilities provide additional information to enhance our understanding of the factors controlling the presence of microbial communities in pharmaceutical environments.
5. CONCLUSION

The progress and development of new analytical technologies to enumerate, isolate, and characterize microorganisms from the environment have provided a greater resolution and sensitivity to describe the composition, distribution, and biomass of microorganisms on Earth. The great majority of microbes in nature do not grow on plate media. Similar results have been observed in pharmaceutical environments. New information on the distribution, survival, growth, and reproduction of microorganisms in pharmaceutical environments will lead to the optimization of process control by optimizing the systems used for controlling microbial contamination.

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