

CHAPTER 12

FOOD MICROBIOLOGY AND REFRIGERATION

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**R**EFRIGERATION'S largest overall application is the prevention or retardation of microbial, physiological, and chemical changes in foods. Even at temperatures near the freezing point, foods may deteriorate through growth of microorganisms, changes caused by enzymes, or chemical reactions. Holding foods at low temperatures merely reduces the rate at which these changes take place. A few spoilage organisms can grow at or below temperatures at which food begins to freeze.

Refrigeration also plays a major role in maintaining a safe food supply. Overall, the leading factor causing foodborne illness is improper food-holding temperatures. Another important factor is improperly sanitized equipment. Engineering directly affects the safety and stability of the food supply in design of cleanable equipment and facilities, as well as maintenance of environmental conditions that inhibit microbial growth. This chapter briefly discusses the microbiology of foods and the effect of design decisions on the production of safe and wholesome foods. Methods of applying refrigeration to specific foods are discussed in [Chapters 17](#) to [29](#).

**BASIC MICROBIOLOGY**

Microorganisms play several roles in a food production facility. They can contribute to food spoilage, producing off-odors and flavors, or altering product texture or appearance through slime production and pigment formation. Some organisms cause disease; others are beneficial and are required to produce foods such as cheese, wine, and sauerkraut through fermentation.

Microorganisms fall into four categories: bacteria, yeasts, molds, and viruses. Bacteria are the most common foodborne pathogens. Bacterial growth rates, under optimum conditions, are generally faster than those of yeasts and molds, making bacteria a prime cause of spoilage, especially in refrigerated, moist foods. Bacteria have many shapes, including spheres (cocci), rods (bacilli), or spirals (spirochetes), and are usually between 0.3 and 5 to 10  $\mu\text{m}$  in size. Bacteria can grow in a wide range of environments. Some, notably *Clostridium* and *Bacillus* spp., form endospores (i.e., resting states with extensive temperature, desiccation, and chemical resistance).

Yeasts and molds become important in situations that restrict the growth of bacteria, such as in acidic or dry products. Yeasts can cause gas formation in juices and slime formation on fermented products. Mildew (black mold) on humid surfaces and mold formation on spoiled foods are also common. Some molds produce very powerful toxins (mycotoxins) that, if consumed, may be fatal.

Viruses are obligate intracellular parasites that are specific to an individual host. All viruses, including human viruses (e.g., hepatitis A), cannot multiply outside living cells or tissue. Refrigeration design features must include facilities for good employee handwashing and sanitation practices to minimize potential for product contamination. Bacterial viruses (phages), however, may contribute to

starter culture failure in bacterial fermentations if proper isolation, ventilation, and sanitation procedures are not followed. The use of commercial concentrated cultures, selected for phage resistance, has greatly reduced this problem.

**Sources of Microorganisms**

Bacteria, yeasts, and molds are widely distributed in water, soil, air, plant materials, and the skin and intestinal tracts of humans and animals. Practically all unprocessed foods are contaminated with a variety of spoilage and, sometimes, pathogenic microorganisms because foods act as excellent media for bacterial multiplication. Food processing environments that contain residual food material will naturally select for the microorganisms that are most likely to spoil the particular product.

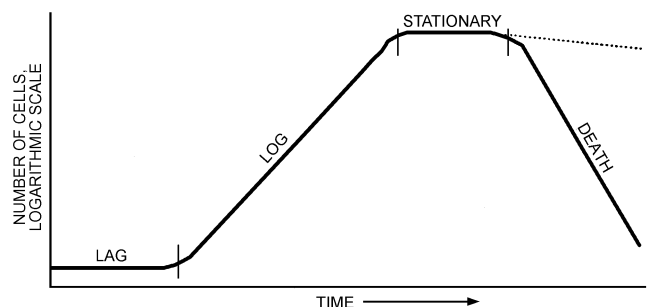
**Microbial Growth**

Changes in microbial populations follow a generalized growth curve ([Figure 1](#)). An initial lag phase occurs as organisms adapt to new environmental conditions and start to grow. The lag phase is very important because the maximum extension of shelf life and length of production runs are directly related to the length of the lag phase. After adaptation, the culture enters into the maximum (logarithmic) growth rate, and control of microbial growth is not possible without major sanitation or other drastic measures. Numbers can double as fast as every 20 to 30 min under optimum conditions.

Toxin production and spore maturation, if possible, usually occur at the end of the exponential phase as the culture enters a stationary phase. At this time, essential nutrients are depleted and/or inhibitory by-products are accumulated. Eventually, culture viability declines; the rate depends on the organism, medium, and other environmental characteristics. Although refrigeration prolongs generation time and reduces enzyme activity and toxin production, in most cases, it will not restore lost product quality or safety.

**CRITICAL MICROBIAL GROWTH REQUIREMENTS**

Factors that influence microbial growth can be divided into two categories: (1) intrinsic factors that are a function of the food itself



**Fig. 1 Typical Microbial Growth Curve**

The preparation of this chapter is assigned to TC 10.9, Refrigeration Application for Foods and Beverages.

**Table 1 Approximate Minimum Water Activity for Growth of Microorganisms**

Organism	$a_w$	Foods
Pseudomonads	0.98	Fresh fruits, vegetables, meats
<i>Salmonella</i> spp., <i>E. coli</i>	0.95	Many processed foods
<i>Listeria monocytogenes</i>	0.93	
<i>Bacillus cereus</i>	0.92	Salted butter
<i>Staphylococcus aureus</i>	0.86	Fermented sausage
Molds	0.84	Soft, moist pet food
	0.80	Pancake syrup, jam
	0.70	Corn syrup
Xerotropic molds	0.65	Caramels
Osmophilic yeasts	0.62	
Limit of microbial growth	0.60	Wheat flour
	0.40	Nonfat dry milk

and (2) extrinsic factors that are a function of the environment in which a food is held.

**Intrinsic Factors**

Intrinsic factors affecting microbial growth include nutrients, inhibitors, biological characteristics, water activity, pH, and presence of competing microorganisms in a food. Although engineering practices have little effect on these parameters, an understanding of how intrinsic factors influence growth is useful in predicting the types of microorganisms that may be present.

**Nutrients.** Like other living organisms, microorganisms require food to grow. Carbon and energy sources are usually sugars and starches. Nitrogen requirements are met by the presence of protein. Vitamins and minerals are also necessary. Lactic acid bacteria have rather exacting nutritional requirements, but many aerobic spore formers have tremendous enzymatic capabilities that allow growth on a wide variety of substrates. Cleanable systems facilitate removal of residual food material and deprive microorganisms of the nutrients required for growth, thus preventing a buildup of organisms in the environment.

**Inhibitors.** Either naturally occurring or added as preservatives, inhibitors may be present in food. Preservatives are not substitutes for hygienic practices and, with time, microorganisms may develop resistance. A cleanable processing system is still essential in preventing development of a resistant population.

**Competing Microorganisms.** The presence of one type of microorganism affects other organisms in foods. Some organisms produce inhibiting compounds or grow faster; others are better able to use the available nutrients in a food matrix.

**Water Activity.** All life-forms require water for growth. Water activity  $a_w$  refers to the availability of water in a food system and is defined at a given temperature as

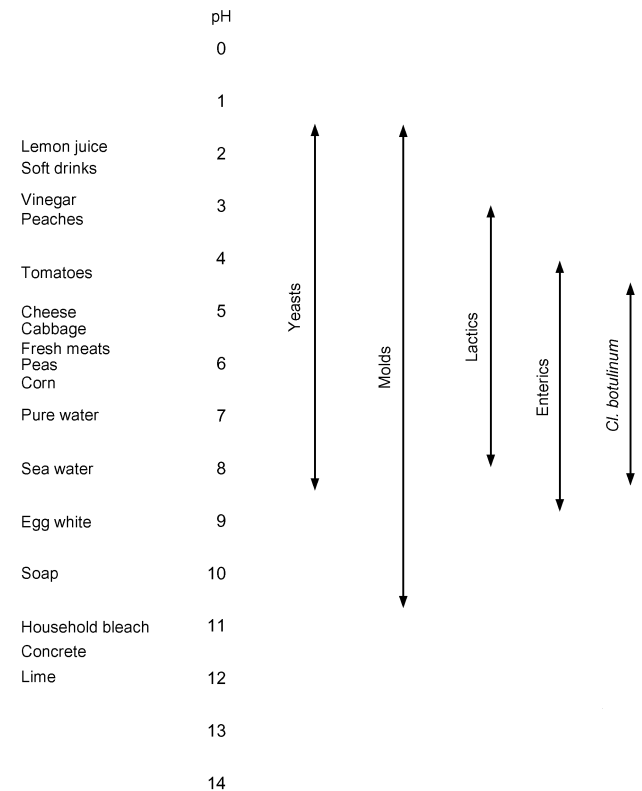
$$a_w = \frac{\text{Vapor pressure of solution (food)}}{\text{Vapor pressure of solute (water)}}$$

The minimum water activities for growth of a variety of microorganisms, along with representative foods, are listed in Table 1. These  $a_w$  minima are also factors in environmental humidity control discussed in the section on Extrinsic Factors.

When food is enclosed in airtight packaging or in a chamber with limited air circulation, an equilibrium  $a_w$  is achieved that is equal to the  $a_w$  of the food. In these situations, the  $a_w$  of the food determines which organisms can grow. If the same foods are exposed to reduced environmental relative humidity, such as meat carcasses hanging in a controlled aging room or vegetables displayed in an open case, surface dehydration acts as an inhibitor to microbial growth. Likewise, if a dry product, such as bread, is exposed to a moist environment, mold may grow on the surface as moisture is absorbed. Environmental relative humidity thus significantly affects product shelf life.

**Table 2 Minimum Growth Temperatures for Some Bacteria in Foods**

Organism	Possible Significance	Approximate Minimum Growth Temperature, °F
<i>Staphylococcus aureus</i>	Foodborne disease	50
<i>Salmonella</i> spp.	Foodborne disease	42
<i>Clostridium botulinum</i> , proteolytic	Foodborne disease	50
		38
<i>Lactobacillus</i> and <i>Leuconostoc</i>	Spoilage of fresh and cured meats	32
<i>Listeria monocytogenes</i>	Foodborne disease	34
<i>Acinetobacter</i> spp.	Spoilage of precooked foods	31
Pseudomonads	Spoilage of raw fish, meats, poultry, and dairy products	31



**Fig. 2 pH Ranges for Microbial Growth and Representative Examples**

**pH.** For most microorganisms, optimal growth occurs at neutral pH (7.0). Few organisms grow under alkaline conditions, but some, such as yeasts, molds, and lactic acid bacteria, are acid tolerant. Figure 2 depicts pH values of a variety of foods and limiting pH values for microorganisms.

**Extrinsic Factors**

Extrinsic factors that influence the growth of microorganisms include temperature, environmental relative humidity, and oxygen levels. Refrigeration and ventilation systems play a major role in controlling these factors.

**Temperature.** Microorganisms can grow over a wide range of temperatures. Minimum growth temperatures for a variety of spoilage and pathogenic bacteria of significance in foods are summarized in Table 2. Previously, 45°F was thought to be sufficient to control

growth of pathogenic organisms. However, the emergence of psychrophilic pathogens, such as *Listeria monocytogenes*, has demonstrated the need for lower temperatures. In the United States, 41°F is now recognized as the upper limit for safe refrigeration temperature, although in some cases 34°F or lower may be more appropriate. Foods that can support growth of pathogenic microorganisms should not be held between 41 and 140°F for more than 2 h.

Temperature is used to categorize microorganisms. Those capable of growth above 113°F, with optimum growth at 130 to 150°F, are **thermophiles**. Thermophilic growth can be extremely rapid, with generation times of 10 to 20 min. Thermophiles can become a problem in blanchers and other equipment that maintain food at elevated temperatures for extended periods. These organisms die or do not grow at refrigeration temperatures.

**Mesophiles** grow best between 68 and 113°F. Most pathogens are in this group, with optimum growth temperatures around 98.6°F (i.e., body temperature). Mesophiles also include a number of spoilage organisms. Growth of mesophiles is quite rapid, with typical generation times of 20 to 30 min. Because mesophiles grow so rapidly, perishable foods must be cooled as fast as possible to prevent spoilage or potential unsafe conditions. Also, slower cooling rates cause mesophiles to adapt and grow at lower temperatures. With mild temperature abuse, prolific growth can occur, leading to spoilage or a potential health hazard.

**Psychrophiles** can grow at 41°F; some are able to grow at temperatures as low as 23°F and are a primary cause of spoilage of perishable foods. Psychrophilic growth is slow compared to mesophilic and thermophilic growth, with maximum growth rates of 1 to 2 h or longer. However, control of psychrophilic growth is a major requirement in products with extended shelf life. Because many psychrophiles have optimum temperatures in the mesophilic range, what may seem to be an insignificant increase in temperature can have a major effect on the growth rate of spoilage organisms. Growth roughly doubles with each 5°F increase in temperature. In practice, shelf life of fresh meat is maximized at 29°F and is reduced 50% by holding at 36°F. Meat freezes at 28°F.

For all critical growth factors, the range over which growth can occur is characteristic for a given organism. The range for growth is narrower than that for survival. For example, the maximum temperature for growth is slightly above the optimum, and death usually occurs just slightly above the maximum. This is not the case at the lower end of the temperature range. Survival of psychrophilic and most mesophilic microorganisms is enhanced by low storage temperatures. Freezing is not an effective lethal process; some organisms, notably gram-negative bacteria, are damaged by freezing and may die slowly, but others are extremely resistant. In fact, freezing is used as an effective means of preserving microbial cultures at extremely low temperatures (e.g., -110°F).

**Environmental Relative Humidity.** Water, previously discussed as an essential intrinsic growth factor, is also a major extrinsic factor. Environmental water acts as a vector for transmission of microorganisms from one location to another through foot traffic or aerosols. Refrigeration drain pans and drip coils have been identified as significant contributors of *L. monocytogenes* contamination in food processing environments. Aerosols have also transmitted the agent that causes Legionnaires' disease. High relative humidity in cold rooms is a particular problem and leads to black mold build-up on walls and ceilings as well as growth of organisms in drains and other reservoirs of water. Condensation on ceilings supports microbial growth and can drip onto product contact surfaces. Inadequately drained equipment collects stagnant water and supports microbial growth that is easily transported throughout a production facility when people walk through puddles. It is extremely important to control environmental relative humidity in food production environments. Control measures are discussed further in the section on Regulations and Standards.

**Oxygen.** Microorganisms are frequently classified by their oxygen requirement. Strictly aerobic microorganisms, such as molds and pseudomonads, require oxygen for growth. Conversely, strict anaerobes, such as *Clostridium* spp., cannot grow in the presence of oxygen. Facultatively anaerobic microorganisms (e.g., coliforms) grow with or without oxygen present, and microaerophiles, such as lactobacilli, grow best in conditions with reduced oxygen levels. Controlled-atmosphere (CA) chambers for fruit storage use lower oxygen levels to prolong storage life by retarding growth of spoilage organisms as well as influencing ripening. Vacuum packing also uses this extrinsic growth factor by inhibiting the growth of strict aerobes.

### Biological Diversity

Most bacteria prefer specific environmental habitats, but some are more broadly adaptable because of their enzyme systems. Some grow well at low temperatures with or without oxygen (e.g., *Listeria monocytogenes*) or survive high temperatures (e.g., *Bacillus cereus*) and still grow at body temperature and cause illness.

### DESIGN FOR CONTROL OF MICROORGANISMS

Microorganisms can be controlled by one of three mechanisms: contamination prevention, growth prevention, or destruction of the organisms themselves. Design of refrigeration and ventilation systems can affect all these areas.

#### Contamination Prevention

To prevent the entry of microorganisms into food production areas, ventilation systems must provide adequately clean air. Because bacteria are generally transported through air on dust particles, 95% filters (as defined in ASHRAE *Standard 52.1*) are sufficient to remove most microorganisms. High-efficiency particulate air (HEPA) filters provide sterile air and are used for cleanrooms.

Air-filtering materials must remain dry. Wet filters in ventilation systems support microbial growth, and organisms are transported throughout the production facility in the air. All ventilation systems must also be protected from water and condensate formation to prevent mold growth. This may require increased airflow or dehumidification systems. Positive pressure in the production environment prevents the entry of airborne contamination from sources other than ventilation ducts. Air intakes for production areas must not face areas that are prone to contamination, such as puddles on roofs or nesting sites for birds. Microbial contamination from airborne sources has been recorded, but quantitative assessments are not available to specify design parameters.

Refrigeration drip pans are a significant source of *L. monocytogenes* contamination. Condensation drip pans should be plumbed directly to drain to prevent contamination of floors and subsequent transport of organisms throughout a production facility. Drip pans must be easily accessible to allow scheduled cleaning, thus preventing organism growth. Air defrost should be avoided in critical areas. Continuous glycol-sprayed evaporative coils offer advantages, because glycol has been found to trap and kill microorganisms. Being hygroscopic, glycol depresses the air's dew point, providing a drier environment.

Traffic flow through a production facility should be planned to minimize contact between raw and cooked products, as mandated in USDA regulations for plants that cook meat products. Straight-line flow of a raw product from one end of a facility to the other prevents cross contamination. Walls separating raw product from cooked (or dirty from clean), with positive pressure in the cooked area, should be considered, because this provides the best protection. Provide adequate storage facilities to allow separate storage of raw ingredients from processed products, especially in facilities that handle meat products, which are a significant source of *Salmonella*. Raw meat must not be stored with cooked meats and/or vegetables or dairy products.

## Growth Prevention

Water control is one of the most effective and most frequently overlooked means of inhibiting microbial growth. All ventilation systems, piping, equipment, and floors must be designed to drain completely. Residual standing water supports rapid microbial growth, and foot and forklift traffic transports organisms from puddles throughout the facility.

Condensation on ceilings and chilled pipes also supports microbial growth and may drip onto product contact surfaces that are not adequately protected. Preventing condensation is essential to prevent contamination. Insulation of pipe and/or dehumidifying systems may be necessary, particularly in chilled rooms. Increased airflow may also be useful in removing residual moisture. Maintaining 70% rh prevents the growth of all but the most resistant microorganisms; less than 60% rh prevents all microbial growth on facility surfaces (see [Table 1](#)).

Sanitation procedures use much water and leave the facility and surfaces wet. Adequate dehumidification should be provided to remove moisture during and after sanitation.

Limiting relative humidity is not always possible. For example, aging meat carcasses requires relative humidities of 90 to 95% to prevent excessive drying of the tissue. In these cases, a temperature of 29°F, just above product freezing point, should be used to inhibit microbial deterioration. Temperatures below 41°F inhibit the most common organisms that cause foodborne illness; however, 34°F is required to inhibit *L. monocytogenes*. Airflow, relative humidity, and temperature must be finely balanced to achieve maximum shelf life with limited deterioration of quality.

Freezing is also an effective means of microbial control. Limited death may occur during freezing, especially during slow freezing of gram-negative bacteria. However, freezing is not a reliable way to kill microorganisms. Because no microbial growth occurs in frozen foods, as long as a product remains well below its freezing point, microbial safety issues are nonexistent. Frozen foods must be stored below 0°F for legal and quality reasons.

## Destruction of Organisms

High temperature is an effective means of inactivating microorganisms and is used extensively in blanching, pasteurization, and canning. Moist heat is far more effective than dry heat. High temperatures (170°F) may also be used for sanitation when chemicals are not used. Although hot-water sanitation is effective against vegetative forms of bacteria, spores are not affected.

In addition to heat, high pressure, pulsed electric fields, high-energy white light, irradiation, ultraviolet light, hydrogen peroxide, ozone, and sanitation chemicals are effective in destroying microorganisms.

## THE ROLE OF HACCP

Many of the procedures for control of microorganisms are managed by the Hazard Analysis and Critical Control Point (HACCP) system of food safety. Developed in the food industry since the 1960s, HACCP is a preventive system that builds safety control features into the food product's design and production. The HACCP system is used to manage physical, chemical, and biological hazards. The approach is described in the seven principles developed by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF 1998):

1. Conduct a hazard analysis and identify control measures.
2. Identify critical control points.
3. Establish critical limits.
4. Establish monitoring procedures.
5. Establish corrective actions.
6. Establish verification procedures.
7. Establish record-keeping and documentation procedures.

Each food manufacturing site should have an HACCP team to develop and implement its HACCP plan. The team is multidisciplinary, with members experienced in plant operations, product development, food microbiology, etc. Because of their knowledge of the manufacturing facility and the process equipment, engineers are important members of the HACCP team. They help identify potential hazards and respective control measures, implement the HACCP plan, and verify its effectiveness.

## SANITATION

Cleaning and sanitation are key elements that incorporate all three strategies for control of microorganisms. Cleaning controls microbial growth by removing the residual food material required for proliferation. Sanitizing kills most of the bacteria that remain on surfaces, preventing subsequent contamination of food being produced. Most microbial issues that occur in food processing environments are caused by unclean equipment, sometimes because of design. Therefore, equipment and facilities designed with cleaning and sanitizing in mind maximize the effectiveness of control.

Products that are frozen before packaging are particularly vulnerable to contamination. Many freezing tunnels in food processing facilities are difficult or impossible to clean because of limited access and poor drainage. Although freezing temperatures control microbial growth, proliferation of organisms does occur during downtime, such as on weekends. The following points should be considered during design to minimize potential problems:

- Provide good access for the cleaning crew to facilitate cleaning and adequate lighting (50 fc) to allow inspection of all surfaces.
- Eliminate inaccessible parts and features that allow product accumulation.
- Design equipment that is easy to dismantle using few tools, especially for areas that are difficult to clean. Design air-handling ducts for easy cleaning. Provide removable spools or access doors. Washable fabric ducts designed and approved for such use are another option.
- Use smooth and nonporous construction materials to prevent product accumulation. Materials must tolerate common cleaning and sanitizing chemicals listed in [Table 3](#). Consult sanitation personnel to identify chemicals likely to be used. Give special attention to insulation materials, many of which are porous. Insulation must be protected from water to avoid saturation and resultant microbial growth. An effective method is a well-sealed PVC or stainless steel cover. Avoid using fiberglass batts in food processing plants.
- All equipment must drain completely.
- Consult references and regulations on sanitary design principles.

Innovation is needed in the area of drying after cleaning is complete. Providing adequately sloped surfaces and sufficient drains to handle water is important. Dehumidification systems and/or increased airflow in new and existing systems could greatly reduce problems associated with water, especially in cleaning chilled production environments.

Standard water washing procedures are not appropriate for some food production facilities, such as dry mix, chocolate, peanut butter, or flour milling operations. Refrigeration or ventilation systems for these plants must be made to facilitate dry cleaning, reduce condensation, and restrict water to a very confined area if it is absolutely necessary.

**Table 3 Common Cleaning and Sanitizing Chemicals**

Cleaning Compounds	Sanitizers
Caustic	Chlorine
Chlorinated alkaline detergents	Iodophors
Acid cleaners	Quaternary ammonium compounds
	Acid sanitizers

### REGULATIONS AND STANDARDS

Facilities and equipment should be designed and installed to minimize microbial growth and maximize ease of sanitation. Take care to use material that can withstand moisture and chemicals.

The food industry has many equipment materials, fabrication, and installation standards. In the United States, examples include those by the International Association for Food Protection (IAFP), which publishes the *3-A Dairy Standards*; the Baking Industry Sanitation Standards Committee (BISSC); and the U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS), which ensures that the meat, poultry, and egg product food supply is safe, wholesome, and properly labeled.

Chapter VII, Section 701(A) of the Federal Food, Drug and Cosmetic Act as amended establishes current Good Manufacturing Practices (GMPs) in manufacturing, processing, packaging, or holding human food. These GMPs are listed in 21CFR110.

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### [Related Commercial Resources](#)