Microbial Growth and Its Control

Chapter 5

Binary Fission, Budding, and Biofilms

In microbiological terms, *growth* is defined as an *increase in the number of cells*. In most *Bacteria* and *Archaea*, cellular growth occurs as *binary fission* (Figure 5.1), in which a single cell elongates and divides down the middle along a *septum* (Figure 5.2) into two identical daughter cells. This process requires a large number of genes and gene products (proteins), as well as a large investment of energy. The amount of time required for a prokaryotic cell to undergo binary fission and develop into two daughter cells is called the *generation time*. This feature is highly variable between species, and even within a species the generation time can vary considerably, depending upon the physiochemical conditions of the environment (e.g., temperature, pH, and nutritional composition). Under optimal laboratory culture conditions, the fastest generation time among prokaryotic species is 15–20 minutes.

Q/ How a bacterium can have a generation time of 20 minutes if it takes 40 minutes for its DNA polymerase to replicate its chromosome. See Figure 7.5 for the answer to this conundrum.

Budding bacteria, often exhibit complex life cycles and unusual mechanisms of cell division. They also possess a variety of intricate morphologies, such as cells having hyphae or stalk-like appendages (Figure 5.3). Species of *Hyphomicrobium* and *Caulobacter*, respectively, are key examples of these bacteria.

Most important in microbial growth is to differentiate between *planktonic* growth (cells maintaining a suspended lifestyle in an aqueous medium) and *sessile* growth (cells maintaining a stationary lifestyle affixed to a surface). The latter condition is conducive to the formation of a *biofilm*, a thick layer of cells embedded in a polysaccharide matrix (Figure 5.4). (e.g., algae on an immersed rock near the edge of a pond, dental plaque, a contaminated urinary catheter)

Quantitative Aspects of Microbial Growth

Generation time of bacteria varies widely between species and even within a single species, depending upon both nutrient and environmental conditions. This leads to the concept of *exponential* (or *logarithmic*) growth. Semilogarithmic graphs allow the plotting of very small to very large cell numbers, and the exponential growth phase of a culture can be visualized as a straight line (in contrast to the "J-curve" slope of an arithmetic scale; Figure 5.5*b*).

Mathematics of exponential growth regarding the calculation of generation times and cell numbers (Figure 5.6) are important tools in microbial genetics and applied aspects of industrial microbiology and the biotechnology industry.

The Microbial Growth Cycle and Continuous Culture

Two types of cultures, continuous and exponential cultures. Bacterial cells cannot remain indefinitely in exponential growth phase. A bacterium that divides every 20 minutes for 48 hours

during exponential growth would produce a mass of cells that would weigh 4000 times the weight of Earth! To show this, the calculation would progress in the following manner:

48 h × 3 divisions/h = 144 divisions = 2144 cells

An average bacterial cell weighs approximately 1 × 10–12 g (a picogram).
Therefore, 2144 cells multiplied by 1 × 10–12 g/cell would equal ~2.23×1028 kg, a mass of cells that is equivalent to about 4000 times the weight of Earth.

Bacterial cells grown in closed vessels (batch cultures) go through a growth curve, as shown in Figure 5.7. as you notice that bacterial cells go through the *lag*, *exponential*, *stationary*, and *death* phases.

Most cultures we have in the lab is *batch cultures* (exponential) or (closed systems) and as such they display a typical growth curve in which the nutrient concentration affects both growth rate and growth yield (Figure 5.9). However, in a chemostat, both growth rate and cell density are controlled independently by the dilution rate and the concentration of a limiting nutrient, respectively (Figure 5.10). In nature, conditions vary dramatically from those in laboratory culture with respect to nutrient concentrations. The chemostat is thus a useful tool for looking at microbial growth rates in real-world scenarios in which both substrate concentrations and the accumulation of waste products can fluctuate dramatically.

Culturing Microbes and Measuring Their Growth

The culture of microorganisms using artificial growth media is traditionally the first step toward understanding physiological similarities and differences between various isolated strains and, ultimately, their ecological activities in nature. *E. coli* can synthesize all of its organic macromolecules using just glucose as the sole carbon and energy source, along with a few inorganic salts and trace elements. By comparison, *L. mesenteroides* lacks many of the enzymes (and therefore genes) required for the biosynthesis of diverse organic molecules solely from monosaccharides. Its growth also depends on the availability of amino acids, nucleotides, and other growth factors in its environment. Therefore, *E. coli*, having the greater metabolic potential, is the more nutritionally versatile organism. Culture media, pure cultures, and aseptic technique (Figures 5.11 and 5.12) are very important concepts in growing microbes.

Once a culture has obtained a suitable cell density, total cell numbers can be determined by direct *microscopic counts* (Figure 5.13). Unfortunately, this technique does not allow one to distinguish an intact but nonviable cell from a viable cell. It is especially difficult to obtain reliable cell counts when the culture has reached stationary phase. Cell densities are high at this time, and the total cell number obtained will invariably be higher than the viable cell number. To overcome this limitation, *viable cell counts* are determined by plating liquid cell cultures, by either the spread-plate or the pour-plate method (Figure 5.14), usually after first performing a serial dilution (Figure 5.15). Because only viable cells will form colonies on agar media, an estimation of living cells/ml can be obtained using this method, assuming the solid medium employed is suitable for reliable growth of the organism.

A third technique for measuring microbial growth is the *turbidimetric method*, in which a spectrophotometer is used to estimate cell numbers based on the density of a liquid culture (Figure 5.16). Readings are given as *optical density* (*OD*) at a given wavelength, and a growth curve can be generated over time. To relate actual cell numbers to OD units, a standard curve comparing the two must be made. If another culture medium or growth condition is employed, a new standard curve should be obtained for that condition to equate OD units to actual cell numbers. As part of this discussion, you should note and discuss the limitations and sources of error in all three methods of measuring microbial growth.

Q/ How bacteria that produce pigments or grow as biofilms can cause interference when trying to obtain accurate turbidity measurements of growth.

Environmental Effects on Growth: Temperature

Every microorganism has a *cardinal temperature* profile that defines its minimum, optimum, and maximum temperature for growth (Figure 5.17). If one compares the temperature optima of microorganisms from a diversity of environments, four classes can be defined: (1) *psychrophiles*, (2) *mesophiles*, (3) *thermophiles*, and (4) *hyperthermophiles* (Figure 5.18).

Environmental Effects on Growth: pH, Osmolarity, and Oxygen

Some microorganisms have the capability to grow at or below pH 1 or at pH 10 or higher (Figure 5.23 and Table 5.3). However, most will not have considered the problems each organism faces in generating ATP and maintaining a near neutral internal pH. Challenge students to consider a couple of scenarios to investigate outside of class:
• *Alkaliphiles* live bathed in a sea of hydroxyl ions but can still generate a PMF (i.e., pump advantages and disadvantages of this environment for these organisms? protons outside the membrane). How can this charge separation be established under such conditions to drive the synthesis of ATP?
• *Acidophiles*, on the other hand, live in a sea of protons. What are the bioenergetic

Despite the antimicrobial properties of high salt concentrations, some microorganisms either tolerate or absolutely require high salinity conditions for life. Organisms that can tolerate relatively high salt concentrations but do not require them for growth are *halotolerant*, such as species of *Staphylococcus* (Figure 5.24). Organisms that require high salt conditions for survival are *halophilic* (Figure 5.24), and they deal with the osmotic stress of living in high salt environments by synthesizing compatible cytoplasmic solutes (Table 5.5).

Extremophiles often require more than one extreme condition for growth and viability. Examples would be those organisms that thrive in the following environments:
• Acidic hot springs (high temperature + low pH) = a *thermophilic acidophile*
• Deep-sea hydrothermal vents (high temperature + high pressure) = a *hyperthermophilic*

piezophile
• Saline soda lakes (high salt concentration + high pH) = a *halophilic akaliphile*
• Antarctic saline lakes (low temperature + high salt concentration) = a *psychrophilic halophile*

Most microorganisms must deal with fluctuating levels of molecular oxygen (O_2) during growth, with some organisms requiring its presence, others tolerating its presence, and some absolutely requiring its absence for survival (Figures 5.25 and 5.26; Table 5.6). For example, aerobic organisms contain the enzymes *superoxide dismutase* (SOD) and *catalase* (Figures 5.28*a*, *c*, and *d* and 5.29). However, strict anaerobes lack SOD and thus are rapidly killed in the presence of O2. Some obligately anaerobic *Archaea* contain a similar enzyme, *superoxide reductase*, which reduces superoxide to peroxide without producing O2 as a byproduct (Figure 5.28*e*). A peroxidase-like enzyme then reduces peroxide to water, again avoiding the formation of O2 (Figure 5.28*b*).

Controlling Microbial Growth

In vitro methods of controlling microbial growth can be differentiated between *physical methods* (e.g., heat, radiation, and filter sterilization; Sections 5.15 and 5.16) and *chemical methods* (e.g., antiseptics, disinfectants, and sterilants; Section 5.17), and we will consider the physical methods first. *Heat sterilization* is probably the most common method used by microbiologists to eliminate microorganisms and includes dry heat, incineration, and autoclave sterilization. A faster and more convenient method is to determine an organism's *thermal death time*. This measurement varies depending on the species, and remind them that endospores are naturally much more resistant to heat exposure. Think about how water is retained and partitioned into a gel-like state from the interactions of calcium (*Ca2)- dipicolinic acid* and *small acid-soluble spore proteins (SASPs)*. It is important to understand that *autoclaves kill cells by heat, not by pressure*. The temperature required to penetrate and kill endospores (121°C) is well above the boiling point of water at 1 atm, and therefore pressure is increased to prevent the boiling of culture media at this temperature. Also note that *the pasteurization process does not sterilize*; it is a method used to reduce the number of microorganisms in a liquid, but it does not eliminate them completely.

Many medical and laboratory products are sterilized by radiation, such as tissue grafts, antibiotics and vaccines, and prepackaged, disposable supplies (e.g., syringes, pipettes, and needles). In addition, in many countries, ionizing radiation is an accepted technology for decontaminating foods, such as fruit and poultry.

Another effective sterilization method is the use of *filters*. Filters are used to remove microorganisms from liquids and gases. If heat-sensitive liquids containing larger particles are to be sterilized, depth filters are often used as prefilters to remove larger particles, whereas *membrane filters* having tiny pores are used to remove microbial cells from the liquid (Figures 5.34*b–d*, and 5.35).

Finally, c*hemical methods* can be used in controlling microbial growth (Section 5.17), including the microbial actions of the three basic types of antimicrobial agents: *bacteriostatic*, *bactericidal*, and *bacteriolytic* (Figure 5.36). Many techniques for measuring *antimicrobial activity*, including *minimum inhibitory concentration* (MIC) and the *disc diffusion technique* (Figures 5.37 and 5.38, respectively).

Q/ What are the differences between *antiseptics* (or *germicides*), *disinfectants*, *sanitizers*, and *sterilants* (Table 5.8).

You should familiarize yourself with the following notes (Very Important):

1. The intensely interwoven nature of the structure of biofilms prevents harmful chemicals (for example, antibiotics or other toxic substances) from penetrating the microbial cells. Biofilms are also a barrier to bacterial grazing by protists and prevent cells from being washed away into a potentially less favorable habitat.
2. The generation time (g) is the time required for the formation of two cells from one. Thus, g is the time required for a population of cells to double during exponential growth, and it is expressed as t/n, where t is the duration of exponential growth (in days, hours, or minutes), and n is the number of generations. The latter is determined using the initial (N_0) and final (N) cell numbers of the exponentially growing culture (cell counts can be performed in a variety of ways, as described in Sections 5.6–5.8) in the expression $n = [3.3(\log N - \log N_0)]$. Once both t and n are known, the generation time is easily calculated as $g = t/n$.
3. A population inoculated from an old culture into a fresh medium will show a lag phase, an exponential phase, a stationary phase, and, if incubated further, a death phase.

   For a population already in mid-exponential growth, there will be no obvious lag phase, and the growth will continue into the exponential phase, stationary phase, and death phase.

4. A chemostat can regulate growth rate and cell density independently by separately adjusting the nutrient concentration and the dilution rate. Over a broad range of dilution rates, the bacterial concentration in the chemostat remains relatively constant. Thus, as the dilution rate increases, the growth rate also increases. At a constant dilution rate, increasing the limiting substrate will also increase the growth rate and cell yield (to a point).
5. A medium is said to be chemically defined when the concentrations of all of its components are exactly known. Yeast extract is an undefined mixture of chemical compounds; thus, the medium is not chemically defined. Aseptic technique is the careful execution of a series of manipulations designed to prevent contamination of sterile objects or pure cultures of microorganisms during handling. Both in microbiology research and in clinical medicine, careful observation of aseptic technique is essential to prevent contamination and infection.

6. When a culture is in the exponential growth phase, total cell counts are a reasonable method for assessing the culture's performance. However, when a culture is in the stationary or death phase, then knowing the viable cell count becomes necessary.
7. Total microbial cell counts, such as those obtained from microscopic methods of enumeration, include both viable (living) and intact but nonviable (dead) cells, whereas viable cell counts include only live cells.
8. Turbidity, as measured by a spectrophotometer, can beused to estimate cell numbers because cells scatter light. An increase in cell density results in an increase in light scattered (to a point). The amount of light absorbed (or transmitted) will be directly proportional to cell number during exponential growth.
9. Basic chemical concepts tell us that the rate of chemical reactions (excluding photochemical zero-order reactions) increases with increasing temperature. This also applies to enzymatic reactions that occur in cells until increasing temperature has an adverse effect on the most heat-labile enzymes controlling cellular functions. At this point, the optimum temperature is exceeded and the growth rate falls precipitously. The maximum temperature is exceeded when essential proteins denature and membranes collapse. There is only a small temperature range beyond the optimum in which damage control mechanisms can repair the adverse effects sufficiently to ensure cell viability.
10. Comparing with mesophiles, the cells of psychrophiles possess more α- helix, less β sheet; and more unsaturated bonds at the phospholipids that form the membranes.
11. Enzymes from thermophiles and hyperthermophiles have significant commercial uses. Heat-stable enzymes catalyze biochemical reactions at high temperatures and are in general more stable than enzymes from mesophiles, thus prolonging the shelf life of commercial enzyme preparations and lowering the cost in biotechnology applications. For example, the enzyme pullulanase from *Pyrococcus* (*Archaea*) is even stable above the boiling point which is stabilized by Ca^{2+}.
12. In general, fungi tend to be more acid-tolerant than bacteria, although several species of bacteria are acidophiles. By contrast, some prokaryotes are alkaliphilic, surviving under conditions of high pH. The cytoplasm of both acidophiles and alkaliphiles tends to be near neutrality, and both may find environmental conditions outside of their normal pH range to be toxic, usually due to damage to the cell membranes. Both are faced with the problems of generating a membrane potential for ATP synthesis and maintaining a physiologically stable intracellular environment.
13. A halophilic microorganism must increase its internal solute concentration either by pumping ions into the cell from the external environment or by producing an organic compatible solute internally. For example, species of *Halobacterium* concentrate potassium (K^+) within the cell, using it as the compatible solute. In any case, work must be done to ensure that the cell cytoplasm contains more dissolved solutes than the external milieu, thus making the cell interior suitable for water to enter by osmosis.
14. An obligate anaerobe will not usually grow in the presence of even traces of molecular oxygen because it lacks the ability to detoxify toxic forms of oxygen, especially superoxide (O_2^-) and the hydroxyl radical (OH•). In contrast, aerotolerant anaerobes, such as lactic acid bacteria, are able to grow by fermentation in the presence of molecular oxygen because these organisms can detoxify both O2– using superoxide dismutase and hydrogen peroxide (H_2O_2) using an NADH-dependent peroxidase.

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15. *Thermal death time* is the time required to kill all cells in a culture at a given temperature. *Decimal reduction time* is the time required for a tenfold reduction in the viability of a microbial population at a given temperature. The presence of bacterial endospores would greatly affect both of these values. Because of the heat stability of endospores, both the decimal reduction time and the thermal death time would increase for endospores, and it may be necessary to increase the temperatures used to obtain these values. For example, the decimal reduction time for vegetative cells of *Clostridium botulinum* may be one minute at 105°C, but this temperature would be insufficient to kill endospores of this bacterium. To ensure sterility in the autoclave, it is necessary to maintain a temperature of 121°C for 15 minutes.
16. Ionizing radiation can produce electrons, hydroxyl radicals, and hydride radicals, all of which can degrade and/or permanently alter the macromolecules essential for life, including nucleic acids, proteins, and lipids. Membrane filters mounted to a syringe, pump, or vacuum apparatus are commonly used to sterilize heat-sensitive liquids.
17. A series of broth tubes containing increasing concentrations of the bactericidal substance is prepared; the concentrations should run from 0% to considerably above the concentration causing complete inhibition of growth. All tubes are inoculated with a uniform quantity of the chosen *E. coli* strain. After incubation, often overnight, the series is examined for growth. The minimum inhibitory concentration (MIC) of the bactericide is the concentration in the tube containing the lowest concentration of bactericide that shows no growth of the organism, as determined by the turbidity of the culture. Disinfectants are bactericidal (or fungicidal/viricidal) agents used only on inanimate objects; antiseptics kill or inhibit the growth of microorganisms on living tissue. Because of their caustic/toxic nature, disinfectants would cause damage to living tissues; antiseptics are formulated to minimize these toxic effects.