Survival of Microorganisms in the Natural Environment

The population of microorganisms in the biosphere is roughly constant: Growth is counterbalanced by death. The survival of any microbial group within its niche is determined in large part by successful competition for nutrients and by maintenance of a pool of living cells during nutritional deprivation. It is increasingly evident that many microorganisms exist in consortia formed by representatives of different genera. Other microorganisms, often characterized as single cells in the laboratory, form cohesive colonies in the natural environment.

The Meaning of Growth

Growth is the orderly increase in the sum of all the components of an organism. Thus, the increase in size that results when a cell takes up water or deposits lipid or polysaccharide is not true growth. growth leads to an increase in the number of individuals making up a population or culture.

The Measurement of Microbial Concentrations

Microbial concentrations can be measured in terms of:

- **cell concentration** :the number of viable cells per unit volume of culture.
- **biomass concentration**: dry weight of cells per unit volume of culture.

These two parameters are not always equivalent, because the average dry weight of the cell varies at different stages in the history of a culture. Nor are they of equal significance: In studies of microbial genetics or the inactivation of cells, cell concentration is the significant quantity; in studies on microbial biochemistry or nutrition, biomass concentration is the significant quantity.

Cell Concentration

The viable cell count is usually considered the measure of cell concentration. However, for many purposes the turbidity of a culture, measured by photoelectric means, may be related to the viable count in the form of a **standard curve**. A rough visual estimate is sometimes possible: A barely turbid suspension of *Escherichia coli* contains about 10^7 cells per milliliter, and a fairly turbid suspension contains about 10^8 cells per milliliter. In using turbidimetric measurements, it must be remembered that the correlation between turbidity and viable count can vary during the growth and death of a culture; cells may lose viability without producing a loss in turbidity of the culture.

Example of a Viable Count

Dilution	Plate Count[a]
Undiluted	Too crowded to count
10^{-1}	Too crowded to count
10^{-2}	510
10^{-3}	72
10^{-4}	6
10^{-5}	1

[a] Each count is the average of three replicate plates.

Biomass Density

In principle, biomass can be measured directly by determining the dry weight of a microbial culture after it has been washed with distilled water. In practice, this procedure is cumbersome, and the investigator customarily prepares a standard curve that correlates dry weight with turbidity. Alternatively, the concentration of biomass can be estimated indirectly by measuring an important cellular component such as protein or by determining the volume occupied by cells that have settled out of suspension.

The Growth Curve

If a fixed volume of liquid medium is inoculated with microbial cells taken from a culture that has previously been grown to saturation and the number of viable cells per milliliter is determined periodically and plotted.

Table 1:Phases of Microbial Growth Curve

Phase	Growth Rate
Lag	Zero
Exponential	Constant
Maximum stationary	Zero
Decline	Negative (death)

1. **The Lag Phase**

The lag phase represents a period during which the cells, depleted of metabolites and enzymes as the result of the unfavorable conditions that existed at the end of their previous culture history, adapt to their new environment. Enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume.

If the cells are taken from an entirely different medium, it often happens that they are genetically incapable of growth in the new medium. In such cases a long lag may occur, representing the period necessary for a few mutants in the inoculum to multiply sufficiently for a net increase in cell number to be apparent.

2. **The Exponential Phase**

During the exponential phase,. New cell material is being synthesized at a constant rate, but the new material is itself catalytic, and the mass increases in an exponential manner. This continues until one of two things happens: either one or more nutrients in the medium become exhausted, or toxic metabolic products accumulate and inhibit growth. For aerobic organisms, the nutrient that becomes limiting is usually oxygen. When the cell concentration exceeds about 1 x 10^7/mL (in the case of bacteria), the growth rate will decrease unless oxygen is forced into the medium by agitation or by bubbling in air. When the bacterial concentration reaches 4–5 x 10^9/mL, the rate of oxygen diffusion cannot meet the demand even in an aerated medium, and growth is progressively slowed.

3. **The Maximum Stationary Phase**

Eventually, the exhaustion of nutrients or the accumulation of toxic products causes growth to cease completely.

In most cases, however, cell turnover takes place in the stationary phase: There is a slow loss of cells through death, which is just balanced by the formation of new cells through growth and division. When this occurs, the total cell count slowly increases although the viable count stays constant.

4. The Phase of Decline: The Death Phase

After a period of time in the stationary phase, which varies with the organism and with the culture conditions, the death rate increases until it reaches a steady level. The mathematics of steady-state death is discussed below. In most cases the rate of cell death is much slower than that of exponential growth. Frequently, after the majority of cells have died, the death rate decreases drastically, so that a small number of survivors may persist for months or even years. This persistence may in some cases reflect cell turnover, a few cells growing at the expense of nutrients released from cells that die and lyse.

A phenomenon, in which cells are called **viable but not culturable (VBNC)**, is thought to be the result of a genetic response triggered in starving, stationary phase cells. Just as some bacteria form spores as a survival mechanism, others are able to become dormant without changes in morphology. Once the appropriate conditions are available (eg, passage through an animal), VNBC microbes resume growth.

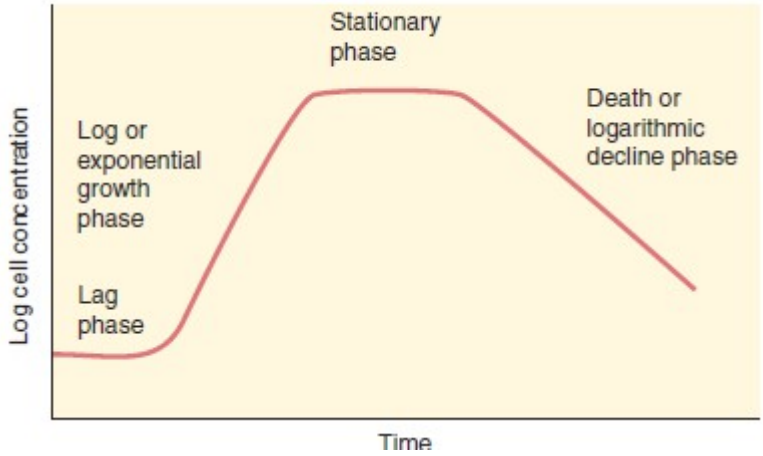

Figure 1:Bacterial growth curve

Maintenance of Cells in the Exponential Phase

Cells can be maintained in the exponential phase by transferring them repeatedly into fresh medium of identical composition while they are still growing exponentially. This is referred to as **continuous culture**; the most common type of continuous culture device used is a chemostat.

The Chemostat

This device consists of a culture vessel equipped with an overflow siphon and a mechanism for dripping in fresh medium from a reservoir at a regulated rate. The medium in the culture vessel is stirred by a stream of sterile air; each drop of fresh medium that enters causes a drop of culture to siphon out.

The medium is prepared so that one nutrient limits growth yield. The vessel is

inoculated, and the cells grow until the limiting nutrient is exhausted; fresh medium from the reservoir is then allowed to flow in at such a rate that the cells use up the limiting nutrient as fast as it is supplied. Under these conditions, the cell concentration remains constant and the growth rate is directly proportionate to the flow rate of the medium.

AEROBIC & ANAEROBIC GROWTH

For most organisms, an adequate supply of oxygen enhances metabolism and growth. The oxygen acts as the hydrogen acceptor in the final steps of energy production catalyzed by the flavoproteins and cytochromes. Because the use of oxygen generates two toxic molecules, hydrogen peroxide (HO) and the free radical superoxide (O2), bacteria require two enzymes to utilize oxygen. The first is **superoxide dismutase,** and the second is **catalase,** which catalyzes the reaction The response to oxygen is an important criterion for classifying bacteria and has great practical significance because specimens from patients must be incubated in the proper atmosphere for the bacteria to grow.

1. Some bacteria, such as *M. tuberculosis,* are **obligate aerobes;** that is they require oxygen to grow because their ATP-generating system is dependent on oxygen as the hydrogen acceptor.

2.Other bacteria, such as *E. coli,* are **facultative anaerobes;** they utilize oxygen to generate energy by respiration if it is present, but they can use the fermentation pathway to synthesize ATP in the absence of sufficient oxygen.

3. The third group of bacteria consists of the **obligate anaerobes,** such as *Clostridium tetani,* which cannot grow in the presence of oxygen because they lack either superoxide dismutase or catalase, or both. Obligate anaerobes vary in their response to oxygen exposure; some can survive but are not able to grow, whereas others are killed rapidly

Definition & Measurement of Death

The Meaning of Death

For a microbial cell, death means the irreversible loss of the ability to reproduce (grow and divide). The empirical test of death is the culture of cells on solid media: A cell is considered dead if it fails to give rise to a colony on any medium. Obviously, then, the reliability of the test depends upon choice of medium and conditions: A culture in which 99% of the cells appear "dead" in terms of ability to form colonies on one medium may prove to be 100% viable if tested on another medium. Furthermore, the detection of a few viable cells in a large clinical specimen may not be possible by directly plating a sample, as the sample fluid itself may be inhibitory to microbial growth. In such cases, the sample may have to be diluted first into liquid medium, permitting the outgrowth of viable cells before plating.

The conditions of incubation in the first hour following treatment are also critical in the determination of "killing." For example, if bacterial cells are irradiated with ultraviolet light and plated immediately on any medium, it may appear that 99.99% of

the cells have been killed. If such irradiated cells are first incubated in a suitable buffer for 20 minutes, however, plating will indicate only 10% killing. In other words, irradiation determines that a cell will "die" if plated immediately but will live if allowed to repair radiation damage before plating.

A microbial cell that is not physically disrupted is thus "dead" only in terms of the conditions used to test viability.

The Measurement of Death

When dealing with microorganisms, one does not customarily measure the death of an individual cell, but the death of a population. This is a statistical problem: Under any condition that may lead to cell death, the probability of a given cell's dying is constant per unit time. For example, if a condition is employed that causes 90% of the cells to die in the first 10 minutes, the probability of any one cell dying in a 10-minute interval is 0.9. Thus, it may be expected that 90% of the surviving cells will die in each succeeding 10-minute interval .

The number of cells dying in each time interval is thus a function of the number of survivors present, so that death of a population proceeds as an exponential process.