

# Chapter 6

## Microbial Growth

**Growth:** an increase in cellular constituents which may lead to a rise in cell number when MO reproduce by budding or binary binary fission:

- **Budding:** Vegetative outgrowth of yeast and some bacteria as a means of asexual reproduction. The daughter cell is smaller than the parental cell.
- **Binary fission:** Asexual reproduction in which cell or an organism separates in two cells.
  - Portions of the parental cells envelope are shared with the progeny cells.
  - Cells enlarge and divide to yield two daughter cells of equal size.

Microbiologists usually study growth by following the change **in population number**.

**Population Growth** is studied by the **Growth Curve** of a microbial culture.

### **Batch culture**

- MO are grown in a liquid medium
- **Closed system:**
  - MO incubated in a closed culture vessel with a single batch of medium where no fresh medium is introduced
  - nutrient concentration declines
  - concentrations of wastes increases.

### **Growth Curve:**

- **growth of MO reproduce by binary fission can be plotted as the logarithm of the number of viable cells verses the incubation time.**

### **Four Phases:**

#### **1. Lag Phase**

- When MOs are inoculated into fresh growth medium, there is usually no immediate increase in cell numbers.
  - Yet the cell is synthesizing new components
- **Reasoning for Lag Phase:**
  - Old cell depleted of ATP, essential cofactors and ribosomes (All necessary before cell growth)
  - Different medium, may mean new enzymes needed to use different nutrients
  - Possible injured MOs require time to recover

- Eventually, cells retool, replicate DNA, begin to increase in mass and finally divide

## 2. Exponential Phase or Log Phase

- **MOs are growing and dividing at the maximal rate possible, genetic potential, nature of medium, and conditions under which they are growing.**
  - Rate of growth is constant (MOs are dividing and doubling in number)
  - Population is most uniform in terms of chemical and physiological properties
  - **Balanced growth:** all cellular components are manufactured at constant rates relative to each other.
  - **Unbalanced growth:** if nutrient levels or other environmental conditions change
    - **growth:** rate of synthesis of cell components **varies** until a new balanced is reached.
    - **Shift-up** experiments: bacteria are transferred from a nutrient poor to a rich medium.
      - cells construct new ribosomes for protein synthesis,
      - increase in protein and DNA synthesis
      - Rise in reproductive rates
    - Unbalanced growth also results from a **Shift-down** from a rich medium to a poor one.
      - When introduced into a nutrient inadequate medium, Mos need time to make enzymes required for biosynthesis of unavailable nutrients.
      - **After Shift-down:**
        - Cell division and DNA replication continue, but net protein and RNA synthesis slow
        - Cells become smaller and reorganize metabolically until they are able to grow.

## 3. Stationary Phase

- Population growth ceases and the growth curve become horizontal
  - Bacteria at population level of  $10^9$  cells/ml
  - Protozoan and algal cultures reach population level of  $10^6$
  - Total number of viable Mos remain constant – balance between cell division and cell death or population may cease to divide though remaining metabolically active.

### **Mos enter stationary phase:**

- **Nutrient limitations:** essential nutrient depleted – growth slowed.
- **Aerobic growth:** limited for lack of Oxygen availability.
  - Oxygen is not very soluble and may be depleted quickly resulting in adequate oxygen for only the surface of the culture to grow.

- Only if culture is shaken or aerated will the cells beneath the surface grow
- **Accumulation of toxic waste products**
  - limiting factor for many anaerobic cultures.
  - *Streptococci* → lactic acid → from sugar fermentation → medium becomes acidic and growth is inhibited.
- **Critical Population is reached**
- **Response to Starvation:**
  - produce starving proteins which makes cell more resistant to damage:
    - Increase peptidoglycan cross-linkage and cell wall strength
    - Dps protein protects DNA
    - Chaperon prevents protein denaturation and renature damaged protein
    - **Resulting in the starved cell becoming harder to kill**

#### 4. Death Phase

- Nutrient depletion and buildup of toxic waste lead to the decline in the number of viable cells
  - Death of microbial population is logarithmic (constant proportion of cells die every hour).
  - Death: the irreversible loss of the ability to reproduce.

During exponential phase each Mo is dividing at constant intervals; thus population will double in number during a specific length of time - **generation time or doubling time.**

### Measurement of Microbial Growth:

Direct microscopic counting:

Sample can be liquid or dried

#### ■ Petroff-Hausser counting chamber used for PC.

- bacteria in several of squares are counted
  - average number of bacteria in these squares is used to calculate the concentrations of cells in the original sample.
  - there are 25 squares covering an area of 1 mm<sup>2</sup>:
  - The total number of bacteria in 1mm<sup>2</sup> of the chamber is (number/square) (25 squares).
  - Chamber is 0.02 mm deep which can be converted 1/50 mm<sup>2</sup>
  - Chamber's volume and any dilutions
- $$\begin{aligned} \text{Bacteria/mm}^3 &= (\text{bacteria/square}) (25 \text{ squares}) (50)(10^3) \\ &= (28) (25) (50) (10^3) \\ &= 3.5 \times 10^7 \end{aligned}$$

**Limitations of this method:**

- (1) *Dead cells are counted b/c they are not distinguished from live cells.*
- (2) *Small cells are difficult to see under the microscope and can be missed*
- (3) *Precision is difficult*
- (4) *A phase contrast microscope is required when cells are not stained.*
- (5) *Not suitable for cell suspension of low density.*

**Membrane Filter**

- *Microbial numbers can be determined by from counts of colonies growing on special membrane filters.*
  - *Sample drawn thru a special membrane filter (with different pore sizes small enough to trap bacteria)*
  - *Filter is placed on agar medium*
  - *Incubated until each colony forming unit forms a separate colony*
  - *Colony represents the number of Mos in the filtered sample.*
  - *Used widely in aquatic samples.*

*Spread Plate*

*Pour Plate*

**Measurement of Cell Mass:**

*Increase in total cell mass accompany cell growth.*

*Most Direct Approach is Determination of:*

➤ **Microbial Dry Weight**

- *Cells growing in liquid medium*
- *Collected by centrifugation*
- *Washed*
- *Dried in an oven*
- *Weighed*
- *Used for Fungi, time consuming and is not very sensitive*

**Turbidity and Microbial Measurement**

- *More rapid and sensitive techniques – microbial cells scatter light that strikes them.*
- *B/c microbial cells in a population are constant size, the amount of scattering is directly proportional to the biomass of cells present and indirectly related to cell number.*
- *$10^7$  cells per ml population- medium appears cloudy.*

- *The extent of light scattering can be measured by a spectrophotometer and is almost linearly related to bacterial concentration at low absorbance levels.*

## ***The Continuous Culture of Microorganisms:***

### ***Continuous Culture System***

- *A microbial population of can be maintained in the exponential growth phase and at a constant biomass concentration for extended periods.*
- ***Open System:*** *system with constant environmental conditions maintained through continual provision of nutrients and removal of wastes .*

## ***Two Common Major Types of Continuous Culture Systems:***

### ***(1). Chemostats***

- *Sterile medium is fed into the culture vessel at the same rate as the media containing the Mos is removed.*
- ***Two important factors that controls the chemostat***

#### ***(1). Concentration of the limit nutrient:***

- *Medium possess an essential nutrient (AA) in limiting quantities. B/c of the limiting nutrient, the growth rate is determined by the rate at which the medium is fed into the growth chamber. The final cell density depends on the concentration of the limiting nutrient.*

#### ***(2). Dilution rate:***

- *The rate of nutrient exchange is expressed as the **dilution rate**: the rate at which medium flows through the culture vessel relative to the vessel volume.*

$$D = f/V$$

*D – Dilution rate*

*F – flow rate (ml/hr)*

*V – vessel volume*

(2). **Turbidostats**

- *Photocell that measures the absorbance or turbidity of the culture in the growth vessel.*
  - *The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity or cell density.*

*Difference of Turbidostat and Chemostat:*

- *The dilution rate in a turbidostat varies rather than remains constant*
- *Culture medium lacks a limiting nutrient*
- *Operates best at high dilution rate where chemostat operates best at low DR.*

**Environmental Factors on Growth:**

*The growth of Mos are effected by Chemical and Physical surroundings:*

- *Procaryotes are present anywhere life can exist.*
- **Extremophiles:** *Mos grow in harsh environments  
Live 1.5 miles below the earth's surface, w/o oxygen, and below 60°C.*

**Temperature**

- *Microbial cell temperature directly reflects that of the cell's surrounding.*
- *Most bacteria can grow over a **temperature range** of about 30° or more but have a narrow range for **optimal growth**.*
- *As we decrease the temperature below the optimum, we see a decline in growth rate that is consistent with enzymatic activity, but then it becomes very steep, giving rise to a fairly well defined minimal growth temperature.*
- *Above the optimum temperature, we see the growth rate decline very steeply, which gives rise to a sharply defined maximum growth temperature*

*It is not known what sets the upper and lower temperature although they are thought to*

- *reflect properties of the membrane lipids,*
- *effects on protein conformation, and/or initiation of protein synthesis.*

***Temperature Sensitivity of Enzyme-Catalyzed Reactions:***

- *A temp rise, increases the growth rate due to the velocity of an enzyme-catalyzed reaction.*
- *Velocity will double for every 10° C rise in temperature.*
- *As rate increase, the metabolism is more active at higher temp, Mo grow faster.*
- *Example: 10 -- 30, Velocity is 15 What is the velocity of the cell at 50° C?*

***High Temperatures:***

- *Damage MOs by denaturing enzymes, transport carriers, and other proteins*
- *Membranes are disrupted, lipid bilayer simply melts and disintegrates.*

***Low Temperatures:***

- *membranes solidify and enzymes don't work properly.*

***The temperature range of an organism can be used as a classifying characteristic.***

***Cardinal Temperatures Growth Temperatures:***

- *Minimum*
- *Optimum*
- *Maximum*

*The major factor determining the growth range is water.*

*Pc can grow at much higher Temp than EC*

***Psychrophiles*** *can grow at temperatures between 0-20°, optima growth is 15°*

- *Frequently found in naturally cold waters and soils.  
Such as the Artic and Antarctic.*
- *Examples include the Pseudomonads and Bacillus,*
- *Enzymes, transport systems and protein synthetic mechanisms function well as low temp.*
- *Cell membrane have high levels of unsaturated fatty acid and remain semifluid when cold.*

**Psychrotrophs or Facultative Psychrophiles** can grow at 0 to 7 ° C.

- *Optima* 20-30 ° C
- *Maxima* 35 ° C
- *Psychrotrophic bacteria and fungi* are important in spoilage of refrigerated foods

Most bacteria are **Mesophiles** and grow between 20-45 °C.

- Those that are found in the mammalian body have an optimum temperature of 37-44 ° C, *Maxima* is 45 ° C.
- Those found in the environment have an optimum of about 30 degrees C
- Almost all human pathogens are mesophiles, *env.* is around 37°C.

**Thermophiles** grow at temperature of 55° C or higher.

*Minimum* of 45 °C and *optima* between 55 and 65 ° C.

- *Majority of prokaryotes*
- *Flourish in composts, self-heating hay stacks, hot water lines, and hot springs.*
- *More heat stable enzymes and protein synthesis systems / funct at higher temp.*
- *Membranes lipids more saturated and have higher melting points causing membrane to remain in tact at higher temp.*
- *These organisms are extremely useful in that they serve as sources for exceptionally stable forms of enzymes (i.e. bacillus stearothermophilus)*

**Hyperthermophiles** are thermophiles that can grow at 90° C or above,

- *Prokaryotes growth optima between 80 and 113 ° C.*
- *Do not grow well below 55 ° C.*
- These organisms are extremely useful in that they serve as sources for exceptionally stable forms of enzymes (i.e. bacillus stearothermophilus)