Bacterial growth is a complex process involving numerous **anabolic and catabolic** reactions.

Ultimately, these biosynthetic reactions result in cell division.

In homogeneous rich culture medium, under ideal conditions, a cell can divide in as little as 10 minutes. In contrast, it is found that the cell division may occur as slowly as once in 100 years in some subsurface terrestrial environments.

Most of the information concerning the growth of microorganisms is the result of controlled laboratory studies using pure culture.

There are two approaches to the study of the growth under such controlled conditions: **Batch** culture and **Continuous** culture.
Binary Division

1. Cell wall
2. Cell membrane
3. Elongated nucleoid

Nucleoid divides; cell wall and membrane begin to form transverse septum

Transverse septum becomes complete

Daughter cells separate
Growth in Batch culture (pure culture in a flask)

The growth of a single organism or group of organisms, called a consortium, is evaluated using a defined medium to which a fixed amount of substrate is added.

several distinct phases:

– Lag phase

– Exponential growth

– Stationary phase

- Death phase
It is difficult to extend our knowledge of growth under controlled laboratory conditions to an understanding of growth in natural soil or water environments, where enhanced level of complexity are encountered such as:

1. Microbial interaction with organic and metal contaminants

2. Survival and growth or pathogens in the environment.
The number of cells present can be determined by viable plate counting (i.e., culturing), direct microscopic counting, and/or turbidity (i.e., optical density)
Growth Phases

- Lag
- Exponential
- Stationary
- Death

Log$_{10}$ viable organisms/ml vs. Time

Turbidity (optical density)
The Lag phase

- Is thought to be due to the physiological adaptation of the cell to the cultural condition.

- When microbes inoculated into fresh medium they do not start to grow immediately (lag phase)

- Length of lag phase variable – depends on history of the culture and growth conditions

  – exponentially growing culture inoculated into same media, same growth conditions – no lag phase
– old culture, same media & conditions – lag phase because cells need to replenish essential constituents to start growth & cell division cycle

– Cells damaged (heat, radiation, toxic chemicals) - lag phase as cells repair damage

– Cells transferred from rich medium to poor culture medium, lag phase as cells have to synthesize more enzymes etc. to enable synthesis of macromolecules not present in poor culture medium.
The Exponential Phase

- Each cell divides to form 2 cells; 2 cells divide to 2 cells; 2 cells divide to form 4 cells ……

- Rate of exponential growth influenced by environmental conditions (temperature, composition of culture composition of culture medium) & genetic characteristics of organism

\[
\frac{dx}{dt} = \mu x
\]

Where, \( x \) is the number or mass of cells (mass/volume), \( t \) is time and \( \mu \) is the specific growth rate constant (1/ time)

Rearrange \( \frac{dx}{x} = \mu dt \)

\[
\int_{x_0}^{x} \frac{dx}{x} = \mu \int_{0}^{t} dt
\]

For \( x \) to be doubled: \( \frac{x}{x_0} = 2 \)

Therefore, \( 2 = e^{\mu t} \) Where \( t \) is the generation time
Bacteria Undergo Exponential Growth

<table>
<thead>
<tr>
<th>Number of cells</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of generations</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Exponential value</td>
<td>$2^1$ (2×1)</td>
<td>$2^2$ (2×2)</td>
<td>$2^3$ (2×2×2)</td>
<td>$2^4$ (2×2×2×2)</td>
<td>$2^5$ (2×2×2×2×2)</td>
<td></td>
</tr>
</tbody>
</table>
The Stationary phase

• In a batch culture exponential growth cannot occur indefinitely
  – Essential nutrients in medium is used up and/or some waste product of the organism builds up to an inhibitory advice
  – Exponential growth ceases = stationary phase

• In stationary phase – no net increase or decrease in cell number

\[ \frac{dx}{dt} = 0 \]

• Many cell functions continue – energy metabolism, biosynthesis

• In some populations some slow growth may continue – some cells die and some grow – 2 processes balance out so no net change (cryptic growth)
The Death phase

• If incubation continues after stationary phase, cells may remain alive and continue to metabolize or they may die = death phase

• In some cases cell death is accompanied by lysis

• Rate of cell death generally slower than that of exponential growth

\[
\frac{dx}{dt} = -K_d x
\]

Where \( K_d \) is the specific death rate
Effect of substrate concentration on growth

Monod equation, which developed by Jacques Monod in the 1940s:

\[ \mu = \mu_m \frac{S}{K_s + S} \]

- \( \mu_m \) = maximum specific growth rate, \( T^{-1} \)
- \( S \) = concentration of the limiting substrate, mg/L
- \( K_s \) = half saturation constant, mg/L

The above equation is a hyperbolic function as shown on the figure below:
There are two constants in this equation, $\mu_m$ maximum specific growth rate and $K_s$, the half saturation constant.

Both reflect intrinsic physiological properties of particular type of microorganisms.

They also depend on substrate being utilized and temperature of growth.

Monod equation can be expressed in terms of cell number or cell mass ($x$) as the following:

$$\frac{dx}{dt} = \mu x \quad \text{where} \quad \mu = \mu_m \frac{S}{K_s + S}$$

Thus,

$$\frac{dx}{dt} = \frac{\mu_m S x}{K_s + S}$$
The Monod equation has two limiting cases:

1. **High substrate concentration**: $S >> K_s \quad \frac{dx}{dt} = \mu_m x$
   - Under these conditions, growth will occur at the maximum growth rate

2. **Low substrate concentration**: $S << K_s \quad \frac{dx}{dt} = \frac{\mu_m S x}{K_s}$
   - This type of growth is typically found in batch flask systems at the end of the growth curve as the substrate is nearly all consumed.
   - It is also the typical growth that happened in the natural environment where substrate and nutrients are limiting.
Monod Growth Kinetics

• First-order region: $S \ll K_S$, the equation can be approximated as $\mu = \mu_{\text{max}} S / K_s$

• Center region, Monod “mixed order” kinetics must be used

• Zero-order region: $S \gg K_S$, the equation can be approximated by $\mu = \mu_{\text{max}}$
The Monod equation can also be expressed as a function of substrate utilization given that the growth is related to substrate utilization by a constant called cell yield:

\[
\frac{ds}{dt} = -\frac{1}{Y} \left[ \frac{dx}{dt} \right] \quad \rightarrow \quad \frac{ds}{dt} = -\frac{1}{Y} \left[ \frac{\mu_m S X}{K_s + S} \right]
\]

where, \( Y = \text{biomass yield} = \frac{\text{g biomass produced}}{\text{g Substrate consumed}} \)
Cells Growth in Continuous Culture

**Continuous culture**: fresh nutrient medium is continually supplied to a well-stirred culture and products and cells are simultaneously withdrawn. At steady state, concentrations of cells, products and substrates are constant.
Wastewater Treatment Plant
Cells Growth in Continuous Culture ……continue

The vessel that is used as a growth container in continuous culture is called a **bioreactor or a chemostat**.

Chemostat can produce microbial product more efficiently than batch fermentation. As the chemostat can hold a culture in the exponential phase of growth.

The combination of growth and dilution within the chemostat will ultimately determine growth. Thus, the change in biomass with time is

\[
\frac{dx}{dt} = \mu x - Dx
\]

Where, $x$ is the cell mass, $\mu$ is the specific growth rate and $D$ is the dilution rate

A steady state will be reached when $\mu = D$
\[
\frac{dx}{dt} = \mu x - Dx
\]

If \( \mu > D \), the utilization of substrate will exceed the supply of substrate, causing the growth rate to slow until it is equal to the dilution rate.

If \( \mu < D \), the amount of substrate added will exceed the amount utilized. Therefore, the growth rate will increase until it is equal to the dilution rate.

Steady state at \( \mu = D \), Such a steady state can be achieved and maintained as long as the dilution rate \( D \) does not exceed a critical rate, \( D_c \).

The critical dilution rate can be determined by substituting the value of \( \mu \) in the following equation:

\[
\mu = \mu_m \frac{S}{K_s + S}, \quad D_c = \mu \quad \rightarrow \quad D_c = \mu_m \left( \frac{S}{K_s + S} \right)
\]
Washed out: If $D$ is set at a value greater than $\mu_m$ ($D > \mu_m$), the culture cannot reproduce quickly enough to maintain itself.
Growth in the environment

Oligotroph (k-Strategists) versus Copiotrophs (r-Strategists)

“The trouble with ecology is that you never know where to start because everything affects everything else.” Robert A. Heinlein, Farmer in the sky, 1950
These terms, $r$ and $K$, are derived from standard ecological algebra, as illustrated in the simple Verhulst equation of population dynamics:

$$\frac{dn}{dt} = rn(1 - \frac{n}{k})$$

where $r$ is the growth rate of the population ($N$), and $K$ is the carrying capacity of its local environmental setting.
Oligotroph (k-Strategists):

is an organism that can live in a very low carbon concentration, less than one part per million.

Most oligotrophs are bacteria, though archaean oligotrophs also exist.

Oligotrophs are characterized by slow growth, low rates of metabolism, and generally low population density.

Long generation time.

They often use energy obtained from metabolism simply for cell maintenance.

oligotrophs may be found a wide range of environments including in deep oceanic sediments, caves, glacial and polar ice, deep subsurface soil, aquifers, and ocean water. An example of an oligotrophic bacteria, *Pelagibacter ubique.*
**Copiotrophs (r-Strategists):**

organisms tend to be found in environments which are rich in nutrients, particularly carbon, and are the opposite to oligotrophs which survive in much lower carbon concentrations.

May exhibit high rates of metabolism and perhaps exponential growth for short periods.

May be found in a dormant state (حالة السبات).

Dormant cells are often rounded and small in comparison with lab. specimens.

Dormant cells may become Viable But Non-Culturable (VBNC).

VBNC are thus difficult to culture because cell stress and damage.

In addition many environmental microbes are Viable But Difficult to Culture (VBDC)
(k-Strategists) versus (r-Strategists)
Some examples

r strategists

K strategists

Rapid colonization of organic matter by fungi

Cellulose-degrading Actinomycetes species
1. The lag phase:

The lag phase in the natural environment can be much longer than in the batch culture.

This is due to a combination of limited nutrient and suboptimal environmental conditions.

The second explanation for long lag period in the environmental samples is that the capacity for degradation of an added carbon source may not initially be present within the existing population. This situation may required a mutation or a gene transfer to introduce appropriate degradative genes into a suitable population.

One of the first documented cases of gene transfer in soil was the transfer of the plasmid pJP4 from an introduced organism to the indigenous soil population. The plasmid transfer resulted in rapid and complete degradation of the herbicide 2.4-D.

Once an environment has been exposed to a particular pesticide and developed a community for its degradation, the disappearance of succeeding pesticide application will occur with shorter lag periods. This phenomena called adaptation.
2. The Exponential phase:

In the environment the second phase of growth, exponential growth, occurs for only very brief periods following addition of substrate.

Such substrate might be crop residues, vegetative litter, root residues or contaminants added or spilled into the environment.

It is the copiotrophic cells, many of which are initially dormant.

Upon substrate addition, these dormant cells become physiologically active and briefly enter the exponential phase until the substrate is utilized.
3. The Stationary and death phase:

Stationary phase in the laboratory (batch culture) is a period where there is active cell growth that is matched by cell death.

In the environment the stationary phase is most likely of short duration if it exist at all.

Recall that most cells never achieve an exponential phase because of nutrient limitations and environmental stress.

Might they are in dormancy or in maintenance state.

Complicating the issue is the presence of bacteriophage that can infect and lyse significant portions of the living bacterial community.
Mass balance of Growth:

**Growth condition**: when the substrate utilized to increase the cell mass.

**Non growth condition**: when the substrate and some nutrients is limiting, utilization of the substrate occurs without production of new cells. The energy from substrate utilization is used to meet the maintenance of the cell.

Cell yield coefficient \((y)\) \[= \frac{\text{g Cell mass production}}{\text{g Substrate consumed}}\]

The value of cell yield is dependent on substrate being utilized.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Chemical formula</th>
<th>Cell yield coefficient ((y))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentachlorophenol</td>
<td>(C_6\text{HOCI}_5)</td>
<td>0.05</td>
</tr>
<tr>
<td>Glucose</td>
<td>(C_6\text{H}_{12}\text{O}_6)</td>
<td>0.4</td>
</tr>
<tr>
<td>Octadecane</td>
<td>(C_{18}\text{H}_{36})</td>
<td>1.49</td>
</tr>
</tbody>
</table>
**Example:** A bacterial culture is grown using glucose as a source of carbon and energy. The cell yield is 0.4. What percentage of glucose (substrate) carbon will be found as cell mass and as CO$_2$. Assume that you start with 1 mole glucose.

**Solution:**

Glucose = $C_6H_{12}O_6$ molecular weight = 180 g/mol.

Cell mass = $C_5H_7NO_2$ molecular weight = 113 g/mol.

Substrate mass x cell yield = cell mass production

$$180 \times 0.4 = 72 \text{ g}$$

Mol cell mass = $\frac{72 \text{ g cell mass}}{113 \text{ g}} = 0.64 \text{ mol cell mass}$

In terms of carbon

Cell mass: $0.64 \text{ mol cell} \times (5 \text{ mol C/mol cell mass}) \times (12 \text{ g/mol C}) = 38.4 \text{ g C}$

Substrate: $1 \text{ mol substrate} \times (6 \text{ mol C/mol substrate}) \times (12 \text{ g/mol C}) = 72 \text{ g C}$

The percentage of substrate carbon found in the cell mass = $\frac{38.4}{72} = 53\%$

Carbon release as CO$_2$ = $100\% - 53\% = 47\%$
Why there are such differences in cell yield for Pentachlorophenol, Glucose and Octadecane substrates?

As microbes have evolved, standard catabolic pathways have developed for common carbohydrate and protein containing substrate.

This translates into a cell yield of approximately 0.4 for sugar such as glucose.

Industrialization began in the late 1800s, many new molecules have been manufactured for which there are no standard catabolic pathways.

Pentachlorophenol is an example to utilize it, a microbe must alter the chemical structure to allow use of standard catabolic pathways. So microbes must expend much energy to break the strong bond carbon-halogen. So little energy is left to produce cells.

In contrast, Octadecane is a hydrocarbon found in petroleum products formed on early earth, standard catabolic pathways exist for most petroleum compounds thus the energy is stored to produce new cells.