FDE 328
INDUSTRIAL MICROBIOLOGY

Microbial Growth

What is microbial growth?

- **Microbial growth** can be defined as an orderly increase in cellular components, resulting in cell enlargement and eventually leading to cell division.

- This definition is not strictly accurate as it implies that a consequence of growth is always an increase in cell numbers. However, under certain conditions growth can occur without cell division, for example, when cells are synthesizing storage compounds, e.g. glycogen or poly β-hydroxybutyrate. In this situation the cell numbers remain constant, but the concentration of biomass continues to increase. This is also true for coenocytic organisms, such as some fungi, that are not divided into separate cells. Their growth results only in increased size.

- Growth kinetics of homogeneous unicellular suspension cultures can be modelled using differential equations in a continuum model.

- However, filamentous growth and growth in heterogeneous cell is much more complex.

- The growth kinetics of filamentous organisms and heterogeneous systems will not be discussed.

- **The growth model that will be examined is bacterial binary fission in homogeneous suspension cultures, where cell division produces identical daughter cells.**

- Each time a cell divides is called a **generation** and the time taken for the cell to divide is referred to as the **generation time**.

- The generation time or doubling time (t_d) is the time required for a microbial population to double.

Types of fermentations

Fermentation processes can be classified into the following three categories.

1. Batch fermentation
2. Fed batch fermentation
3. Continuous culture fermentation

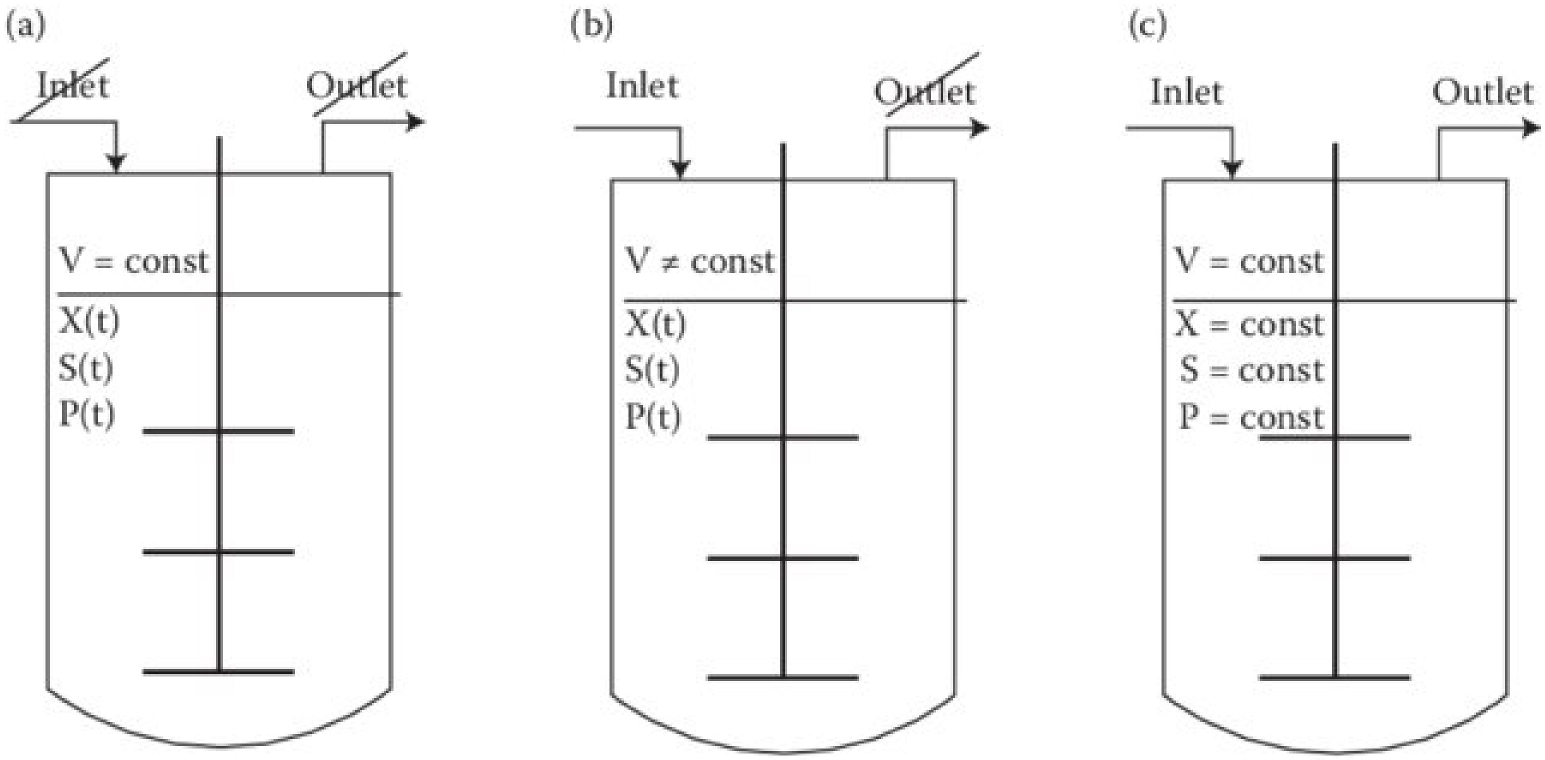
Types of fermentations

- **Batch growth** involves a closed system where all nutrients are present at the start of the fermentation within a fixed volume. The only further additions may be acids or bases for pH control, or gases (e.g. aeration, if required).

- In **fed-batch systems** fresh medium or medium components are fed continuously, intermittently or are added as a single supplement and the volume of the batch increases with time.

- **Continuous fermentations** are open systems where fresh medium is continuously fed into the fermentation vessel, but the volume remains constant as spent medium and cells are removed at the same rate.

Inlet: Substrate
Outlet: Product



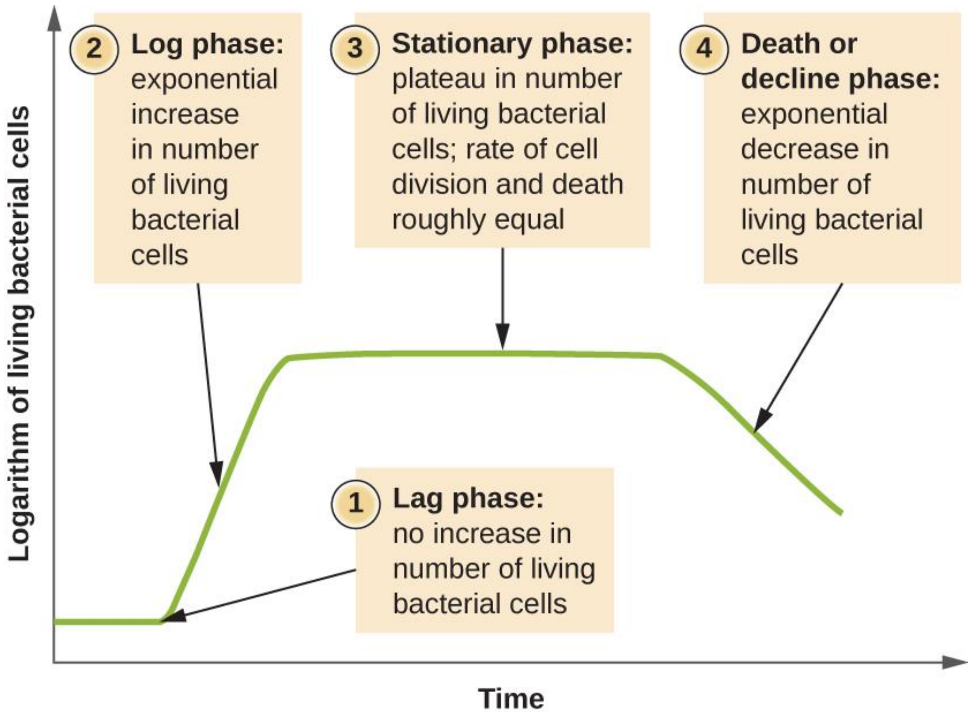
Simplified scheme of (a) batch, (b) fed-batch, and (c) continuous cultivation.

Microbial Growth Curve

The Growth Curve

Microorganisms grown in closed culture (also known as a batch culture), in which no nutrients are added and most waste is not removed, follow a reproducible growth pattern referred to as **the growth curve**.

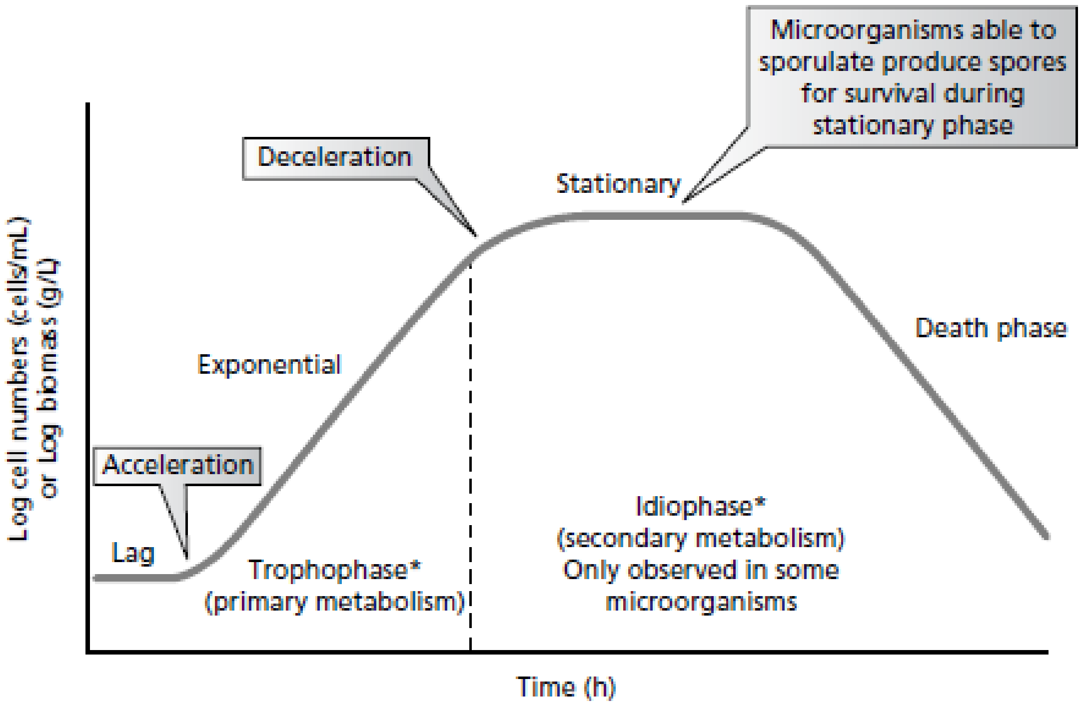
When the number of live cells is plotted against time, **four distinct growth phases** (the lag phase, the exponential or log phase, the stationary phase, and the death or decline phase) can be observed in the curve.



There are four basic phases of growth:

1. **Lag phase**
2. **Log phase**
3. **Stationary phase**
4. **Decline phase**

Microbial Growth Curve



Growth of a microorganism in a batch culture

During batch fermentations the populations of microorganisms goes through several distinct growth phases:

1. Lag
2. Acceleration
3. Exponential Growth
4. Deceleration
5. Stationary
6. Death

Microbial Growth Curve (Lag phase)

1. **The Lag Phase:**

- The beginning of the growth curve represents a small number of cells, referred to as an inoculum, that are added to a fresh culture medium, a nutritional broth that supports growth.

- The initial phase of the growth curve is called the lag phase, during which cells are gearing up for the next phase of growth.

- The lag phase is an adaptation period, where the bacteria are adjusting to their new conditions.

- The number of cells does not change during the lag phase; however, cells grow larger and are metabolically active, synthesizing proteins needed to grow within the medium.

- In other words, in the lag phase virtually no growth occurs and the microbial population remains relatively constant. Nevertheless, it is a period of intense metabolic activity as the microbial inoculum adapts to the new environment.

Microbial Growth Curve (Lag phase)

1. **The Lag Phase:**

- When cells are inoculated into fresh medium they may be deficient in essential enzymes, vitamins or cofactors, etc., that must be synthesized in order to utilize available nutrients, prior to cell division taking place.
- Typically cells in the lag period are synthesizing RNA, enzymes, and essential metabolites that might be missing from their new environment (such as growth factors or macromolecules), as well as adjusting to environmental changes such as changes in temperature, pH, or oxygen availability. They can also be undertaking any necessary repair of injured cells.
- If any cells were damaged or shocked during the transfer to the new medium, repair takes place during the lag phase.

Microbial Growth Curve (Acceleration phase)

2. The Acceleration Phase:

- Once the cells have adapted to their new environment they enter the acceleration phase.
- The acceleration phase generally involves a small period of time between the lag phase and the logarithmic phase.
- Cell division occurs with increasing frequency until the maximum growth rate (μ_{max}) for the specific conditions of the batch fermentation is reached. (At this point exponential growth begins.)

Microbial Growth Curve (Log/exponential phase)

3. The Logarithmic/Exponential Phase:

- In the logarithmic (log) growth phase, sometimes called exponential growth phase, the cells are actively dividing by binary fission and their number increases exponentially.
- In this phase, it is assumed that the microorganisms are active , young and vigorous.
- There are lots of nutrients and **no growth-inhibiting substances** are present in the environment.
- Since cell death is almost negligible during the log phase, this factor is not considered in kinetic calculations.
- The increase in cell numbers is similar with the increase in biomass concentration.

Microbial Growth Curve (Log/exponential phase)

3. **The Logarithmic/Exponential Phase:**

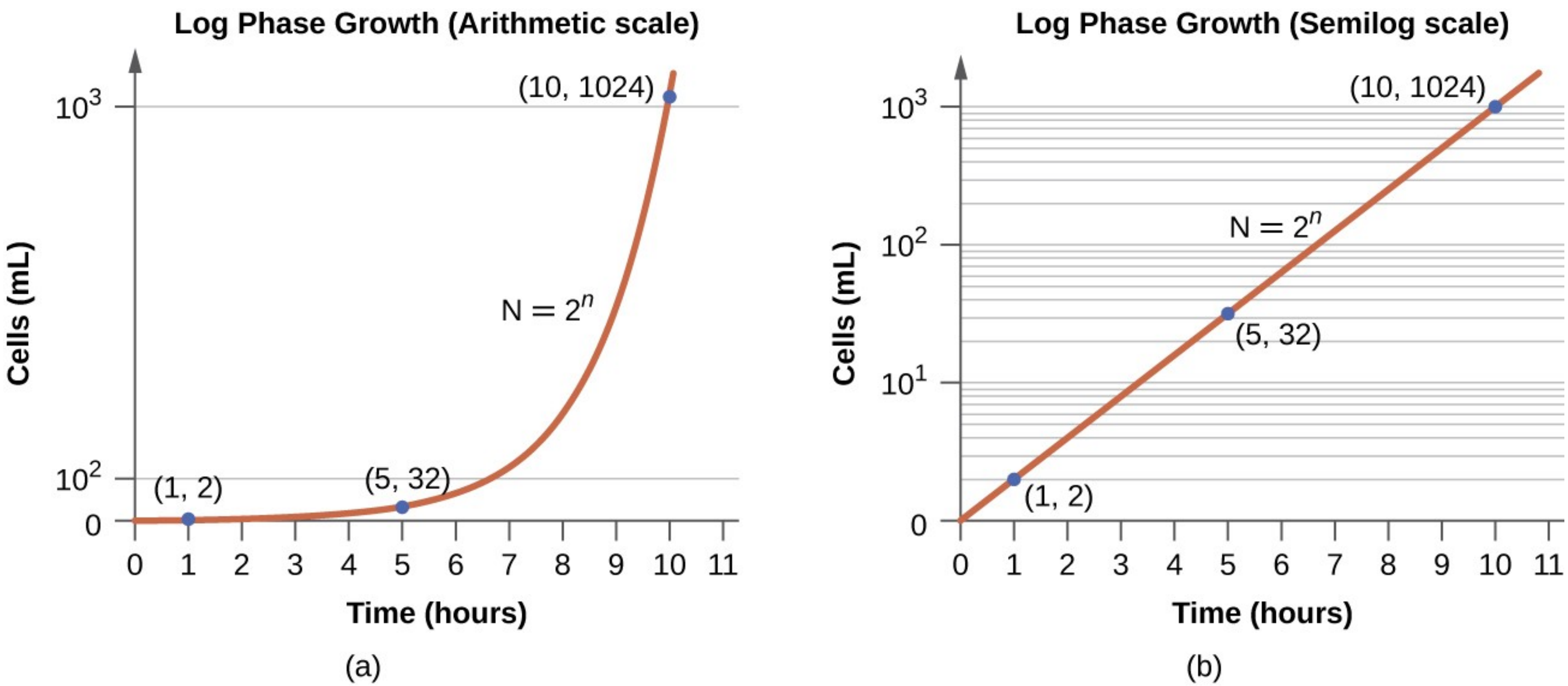
- In this phase, the microorganisms start dividing rapidly at its highest possible rate. The cell numbers/biomass increase at a constant rate. The rate of growth is constant during the exponential phase.

- **The specific growth rate is maximum and constant** during the exponential growth phase **(μ= constant= μ_{max})**

- Cells in the log phase show constant growth rate and uniform metabolic activity. For this reason, cells in the log phase are preferentially used for industrial applications and research work.

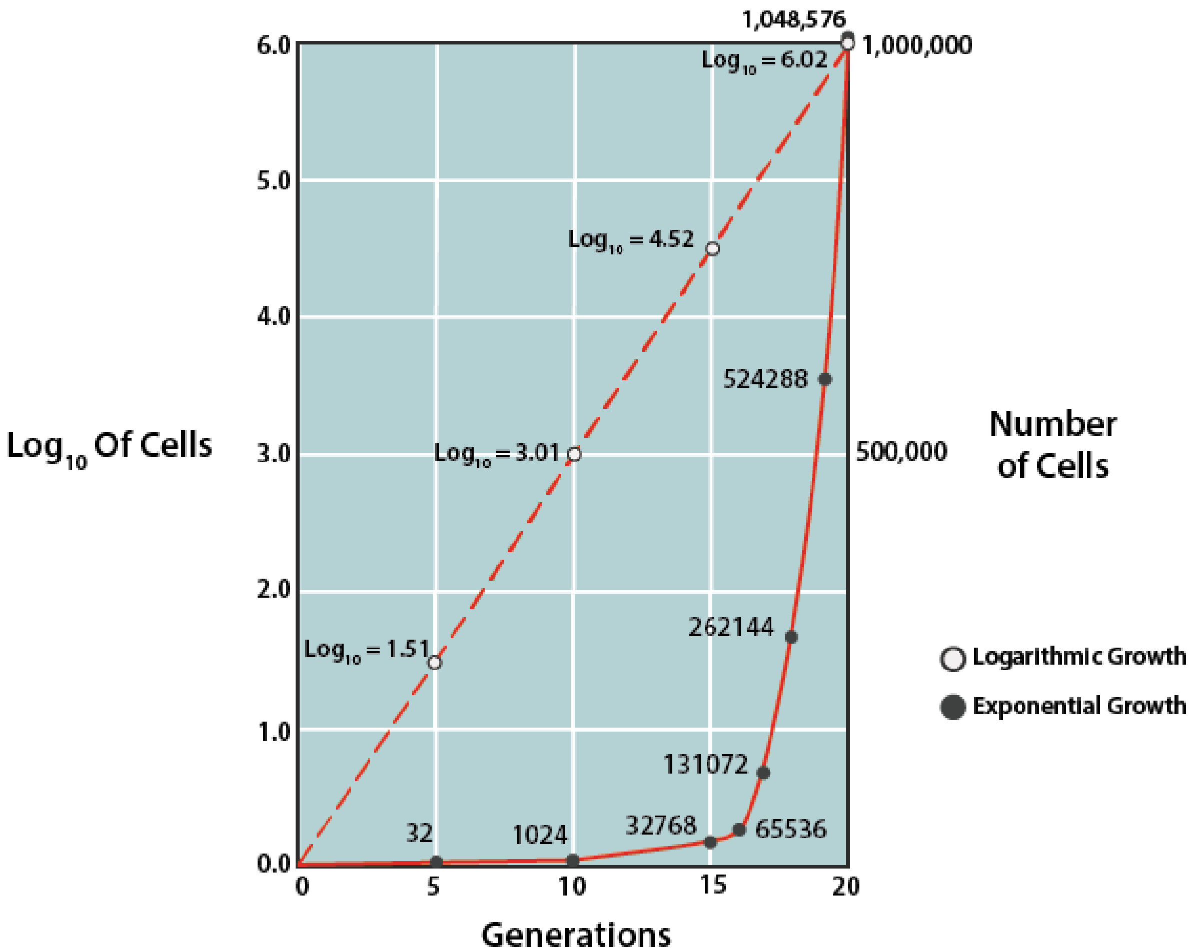
- During the log phase, the relationship between time and number of cells is not linear but exponential; however, the growth curve is often plotted on a semilogarithmic graph, which gives the appearance of a linear relationship.

Microbial Growth Curve (Log/exponential phase)



Both graphs illustrate population growth <u>during the log phase</u> for a bacterial sample with an initial population of one cell and a doubling time of 1 hour.
(a) When plotted on an arithmetic scale, the growth rate resembles a **curve.**
(b) When plotted on a semilogarithmic scale (meaning the values on the y-axis are logarithmic), the growth rate appears **linear**.

Microbial Growth Curve (Log/exponential phase)



Microbial Growth Curve (Log/exponential phase)

3. The Logarithmic/Exponential Phase:

- The **exponential or log phase** of growth is marked by predictable doublings of the population, where 1 cell become 2 cells, becomes 4, becomes 8 etc.

- Conditions that are optimal for the cells will result in very rapid growth (and a steeper slope on the growth curve), while less than ideal conditions will result in slower growth.