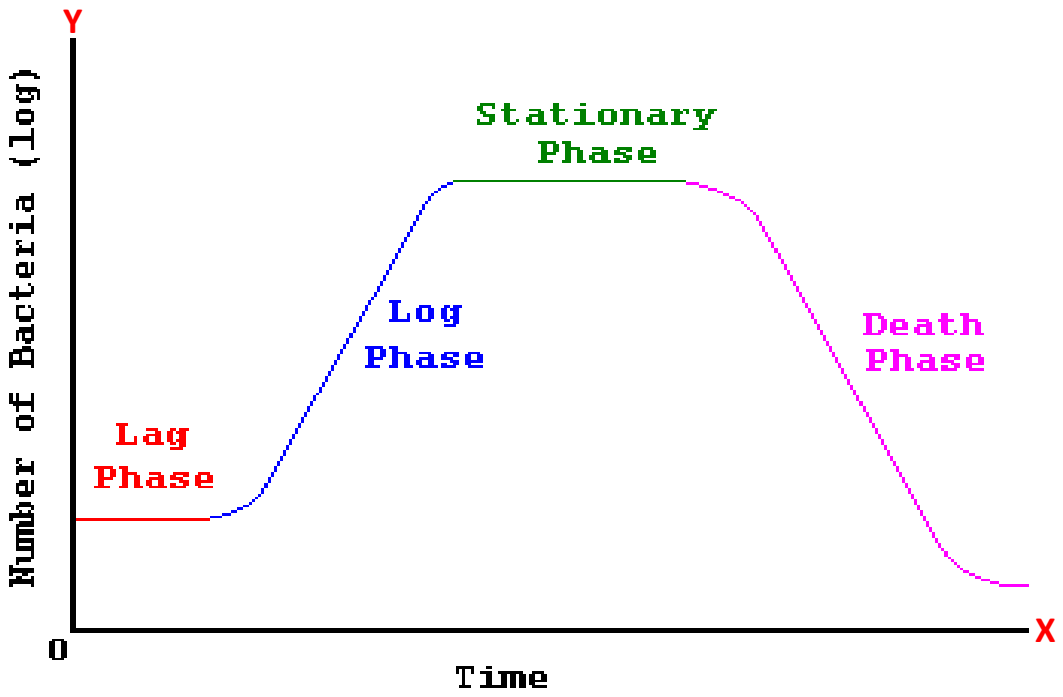
Bacterial Growth Curve

- **Bacterial Growth:** Regular increase in cell mass and number. Include replicate **DNA**, new **cell wall**, and **plasma membrane** synthesis; all cell components are double the cell division. This asexual process of reproduction is called **binary fission**.
- **Generation time:** The time required for a population of cells to double in number. A batch culture or Close culture: A closed bacterial culture system with specific **nutrients**, **temperature**, **pressure**, **aeration**, and other **Environmental conditions** to optimize growth. Because nutrients are not added or waste products removed during incubation, batch cultures can only complete a limited number of life cycles before nutrients are consumed and growth stops.
- **A batch culture passes through 4 distinct stages or phases:**

1. **Lag phase:** When a microorganism is introduced into the fresh medium, adjusting to the new environment takes some time. This phase is termed the Lag phase, in which cellular metabolism is accelerated, and cells are increasing in size, but the bacteria cannot replicate, and, therefore, there is no increase in cell mass. The organism will start synthesizing the necessary proteins, co-enzymes, and vitamins for their growth.
2. **Log phase or exponential phase:** The microorganisms rapidly grow and divide during this phase. Their metabolic activity increases and the organism begins the DNA replication by binary fission at a constant rate. Different bacterial growth medium phases are exploited maximally; the culture reaches the maximum growth rate. The number of bacteria increases logarithmically (exponentially). Finally, the single cell divides into two, replicating four, eight, sixteen, and thirty-two. The time the bacteria takes to double in number during a specified period is known as the **generation time**. The generation time tends to vary with different organisms.
   - ✓ *E.coli* divides every 20 minutes,
   - ✓ *Staphylococcus aureus*, it is 30 minutes.

3. **Stationary phase:** As the bacterial population grows, the microorganism uses all the nutrients in the growth medium to multiply rapidly. This results in the accumulation of waste materials, toxic metabolites, and inhibitory compounds, such as antibiotics, in the medium. This shifts the conditions of the medium, such as pH and temperature, thereby creating an unfavorable environment for bacterial growth. The reproduction rate will slow, the cells undergoing division will equal the number of cell deaths, and finally, the bacterium will stop its division completely. The cell number is not increased; thus, the growth rate is stabilized.
4. **Decline or Death phase:** The depletion of nutrients and the subsequent accumulation of metabolic waste products and other toxic materials in the media will facilitate the bacterium's move to the Death phase. During this, the bacterium completely loses its ability to reproduce. Individual bacteria begin to die, and the death is rapid and uniform. The number of dead cells exceeds the number of live cells. Some organisms resisting this condition can survive in the environment by producing **endospores**.



Turbidity measurements: Measuring the concentration of cells in bacterial cultures is a typical experiment in microbiology. Researchers often use OD_{600} (Optical Density at 600nm) measurements to quickly estimate bacterial concentration, which in turn helps them understand bacterial growth curves. In that case, OD_{600} measurements using a spectrophotometer will help accurately determine bacterial cell concentration and stage of growth.

Spectrophotometer: This instrument measures the amount of light absorbed by a sample. Techniques are used to measure the concentration of solutes in solution by measuring the amount of light absorbed by the solution in a cuvette placed in the spectrophotometer.

1. Prepare sterile broth for inoculation.
2. Set the spectrophotometer's wavelength to 600 nm (or another appropriate setting). Add (1 mL) of uninoculated sterile media to a clean cuvette and blank the machine by setting it to 0 ABS with this sample.
3. Take a 1 mL sample of the inoculated media and pipette it into a clean cuvette. Please place it in the blank spectrophotometer and record the OD reading.
4. Plot the readings on a graph with Time as the **X-axis** and OD as the **Y-axis**.

○Results:

The values obtained provide valuable information about cell growth. For instance,

- ✓ The OD_{600} value between 0.6 and 0.8 indicates that the culture is in the logarithmic growth phase of rapid growth.
- ✓ When a bacterial culture's OD_{600} value exceeds 0.8, proliferation has probably stopped, and transfer to a fresh culture medium is necessary to maintain normal cell growth.
- ✓ An OD_{600} value of more than 1.0 suggests that the bacteria have reached saturation and are ready for subculture.

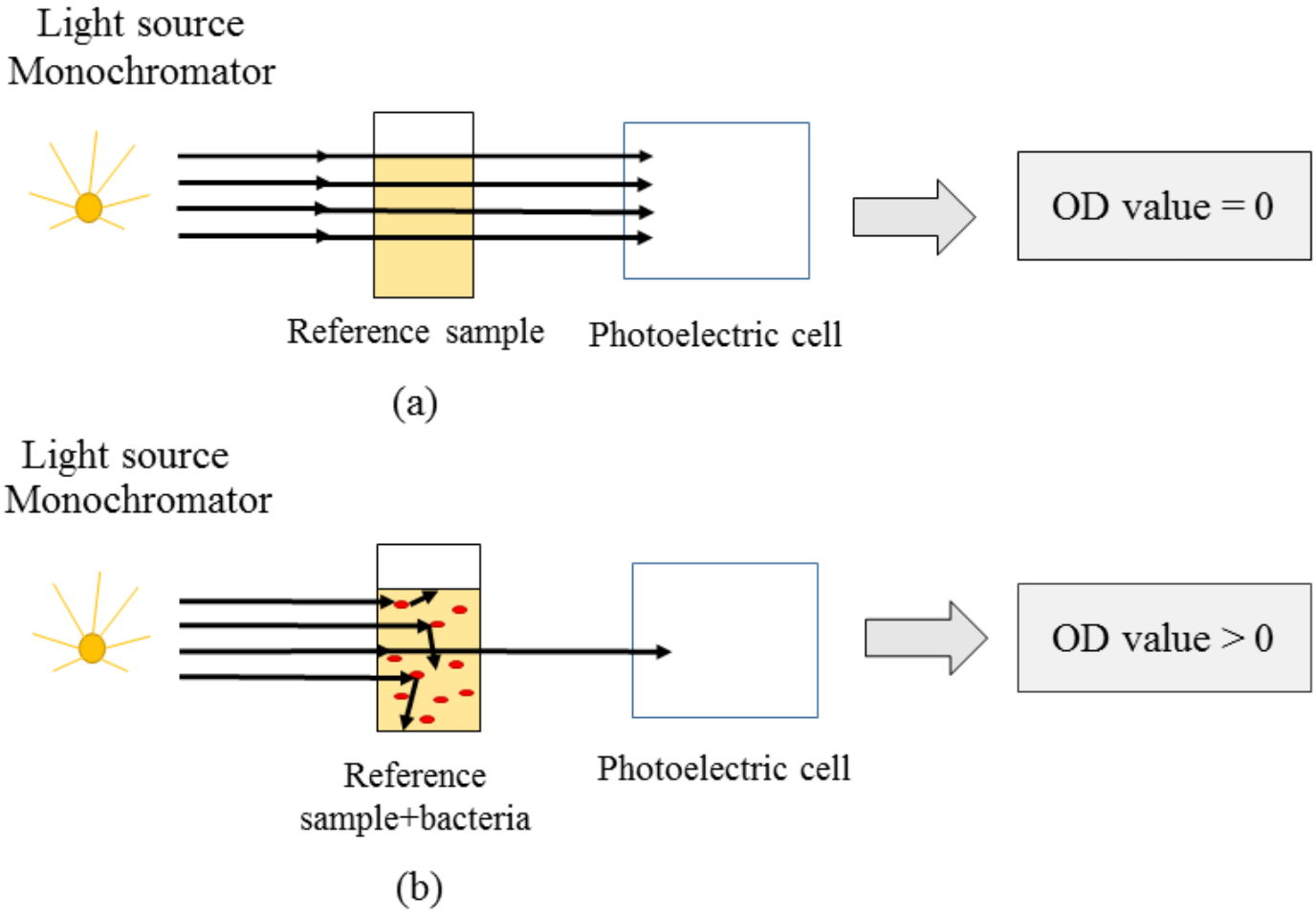


Figure: Schematic illustration of the basis of a spectrophotometer.