BIOLOGY: Laboratory Exercise No. 1

Basics of Laboratory Work and Handling Biological Material

Task 1 – Bacterial Growth Curve

Bacterial population growth is caused by repetitive binary cell division. Reproduction is influenced by environmental factors, and its course can be demonstrated through a growth curve.

The bacterial growth curve can be divided into several phases:

I. **Lag Phase** – Bacteria adapt to the new environment, and the number of cells does not increase. Cells that have not adapted to the change in environment die. The volume and mass of cells increase, and their sensitivity to physical and chemical factors grows. The amount of enzymes increases, and components necessary for cell division are synthesized.

II. **Acceleration of Growth** – The cells have adapted and begin dividing. The growth rate increases, and the generation time shortens.

III. **Exponential Phase (Log Phase)** – This is the most important phase, where bacteria divide intensively, the growth rate is at its highest, the generation time is shortest, and the population grows exponentially. This phase lasts until nutrients are depleted. Cells have a constant size, and metabolic by-products accumulate. The growth rate and duration are influenced by the availability of nutrients.

IV. **Deceleration Phase** – The growth rate slows down, the number of dying cells increases, and nutrients become depleted.

V. **Stationary Phase** – A balance between cell division and cell death is established, so the total number of cells remains relatively constant. Metabolites accumulate, and the nutrient medium is exhausted.

VI. **Death Phase** – The number of dying cells is higher than the number of new cells. Dormant stages may form, and the overall number of cells gradually decreases. The number of dying cells is higher than the number of new cells

This outlines the typical stages of bacterial population growth under ideal conditions.

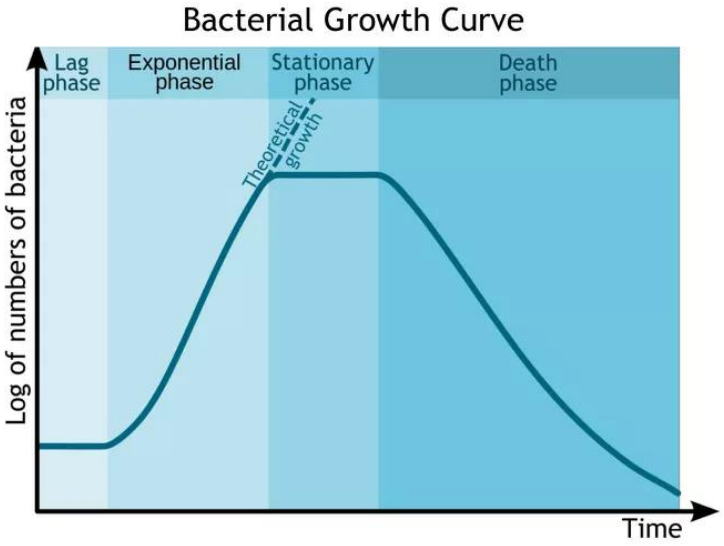
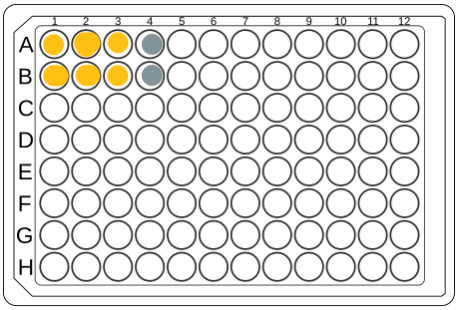


Figure 1:Bacterial Growth Curve. The bacterial growth curve represents the number of living cells in a population over time.

Procedure:

1. Prepare a bacteriological test tube with 5 ml of nutrient medium. One test tube will be placed on the workbench (6 students).
2. Using a bacteriological loop, take bacteria from a Petri dish and resuspend them in the test tube with the medium. This prepares the inoculum. Use a densitometer to determine the cell concentration in the inoculum. The required minimum concentration is 0.9 MF (McFarland).
3. Prepare a 96-well plate. Each pair of students uses 4 wells in one row and two rows below them (a total of 8 wells). Pipette 180 µl of nutrient medium into 6 wells, then add 20 µl of inoculum to these wells. Pipette 200 µl of medium into two additional wells, which will serve as blanks.



4. Measure the absorbance at a wavelength of 600 nm and place the plate in the incubator with a shaker in a plastic bag to prevent contamination. Repeat the measurements every 20 minutes until the end of the exercise. Record the measured values and then plot them on graph paper.
   On graph paper, we record the average of the 6 measured absorbance values. The value for each well is calculated as the absorbance of the well with inoculum minus the absorbance of the blank well.

Task 2 – Preparation of a Dilution Series

Copper sulfate is an inorganic compound with the chemical formula $CuSO_4$. It forms hydrates $CuSO_4 \cdot nH_2O$, where n can range from 1 to 7. The pentahydrate (n = 5), a bright blue crystal, is the most encountered hydrate of copper sulfate.

Procedure:

1. Prepare 5 ml of stock solution of copper sulfate $CuSO_4$ (M_{rCuSO_4} = 159.609 g/mol) at a concentration of 1 mol/l. This stock solution will be shared at the table (6 students). ($M_{r(CuSO_4 * 5H_2O)}$ = 249.686 g/mol)
2. Each pair of students will take 1 ml of the colored stock solution into an Eppendorf tube. Prepare 4 additional Eppendorf tubes and pipette 500 µl of water into each.
3. Perform a dilution according to the dilution series. Take 500 µl of the copper sulfate solution and add it to an Eppendorf tube containing 500 µl of water. From the newly created solution, take 500 µl and add it to the next Eppendorf tube. Repeat this process until the final concentration of copper sulfate is 0.0625 mol/l.
4. Verify the dilution process using a spectrophotometer. Take 200 µl from each Eppendorf tube and pipette it into a 96-well plate.
5. Measure the absorbance at a wavelength of 635 nm. The resulting absorbance will indicate whether the dilution series was prepared correctly.

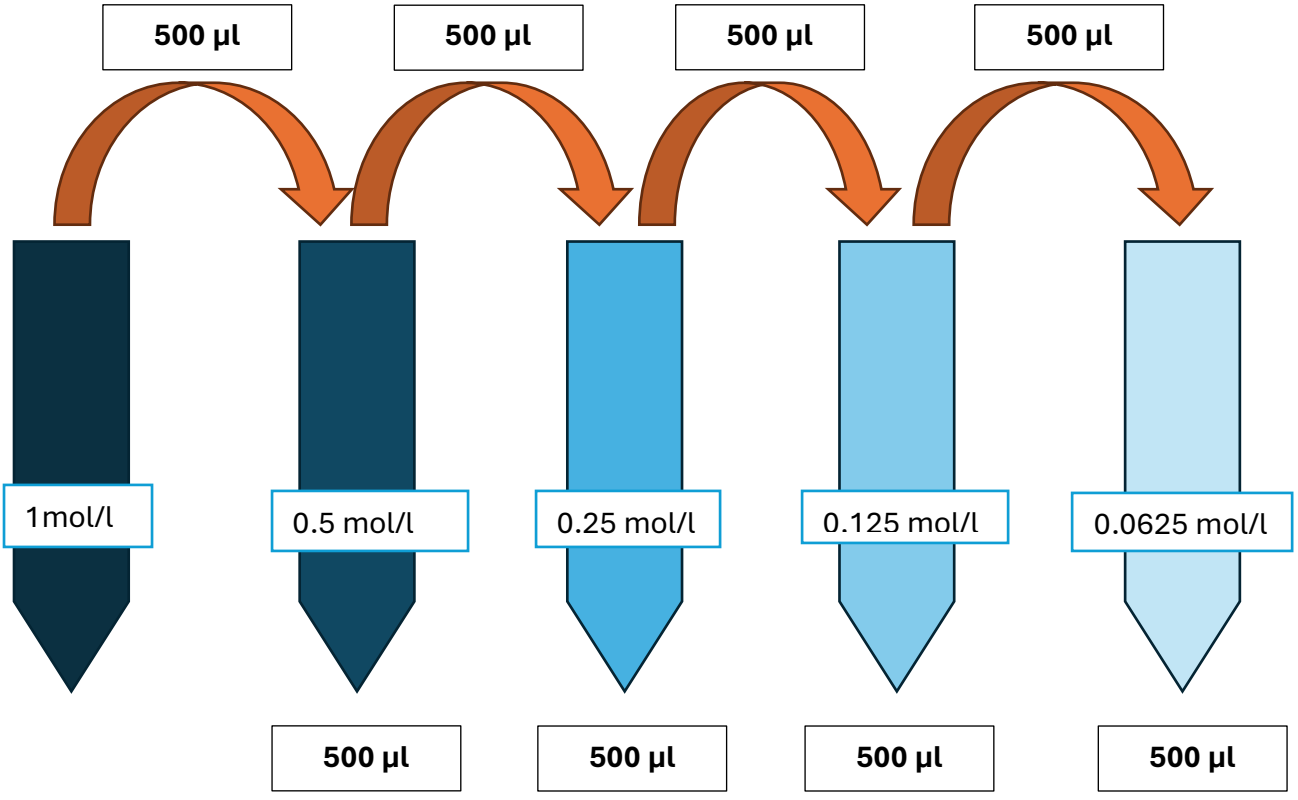


Figure 2: Preparation of a Dilution Series.

Task 3 – Preparation of Resazurin Solution

Resazurin is a blue dye that is commonly used as a viability indicator in microbiology and cell biology. Resazurin is reduced to pink resorufin by aerobic respiration of metabolically active cells.

Procedure:

1. Prepare 1 ml of 10X resazurin solution (440 µM) from the 1000X stock solution (44 mM). Phosphate-buffered saline (PBS) is used for dilution.
2. Properly label the Eppendorf tube containing the 10X resazurin solution with the initials of the pair and the lab group.
3. Place it in the storage box for freezing at -20°C.
4. The stock solution of resazurin will be used in laboratory exercise No 3.