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Chapter 15

Quantitative analysis of bacterial growth: Methods for measuring growth curves and kinetic parameters

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1. Introduction:

To comprehensively investigate bacterial population dynamics, Viable cells must be inoculated into a sterile broth medium, and the culture must be carefully maintained at ideal pH, temperature, and gas composition. (Stanier et al., 1986; Atlas, 2010; Yadav et al., 2021). These controlled parameters facilitate swift cellular proliferation, enabling the monitoring of microbial growth progression through a population growth curve (Monod, 1949; Reddy et al., 2019; Tripathi et al., 2018). This curve is delineated by plotting the rise in cell numbers during the incubation period. Understanding the phases of a typical growth curve is fundamental:

1. **Lag Phase:** Cells acclimate to their environment, with heightened metabolic activity leading to the rapid production of cellular components, particularly enzymes. Despite cellular enlargement, there's no division, thus no increase in cell count.
2. **Logarithmic (Log) Phase:** Under ideal conditions, cells replicate swiftly and evenly via binary fission, resulting in exponential population growth until reaching carrying capacity. The generation time, marking the doubling period, varies depending on the organism and medium composition, typically lasting 6 to 12 hours.
3. **Stationary Phase:** Growth plateaus as cell division balances with cell death, maintaining a stable population due to diminishing nutrients and accumulation of metabolic byproducts.
4. **Decline or Death Phase:** Population dwindles due to mortality, mirroring the exponential growth pattern. Though theoretically, the entire population would perish within a timeframe akin to the log phase, resilient organisms prolong survival.

To construct a comprehensive bacterial growth curve, periodic measurements of population size from a 24-hour shake flask culture are essential. Tracking turbidity changes over time helps monitor cellular mass growth. Extrapolating from the log phase

facilitates generation time determination. Employing dry cell weight per volume as a biomass measure is often more practical than cell count. Batch experiments during rapid growth phase follow this expression (Tripathi et al 2013a, b; Yadav et al., 2016):

$$dX/dt = X$$

where, X is the specific growth rate of the cells.

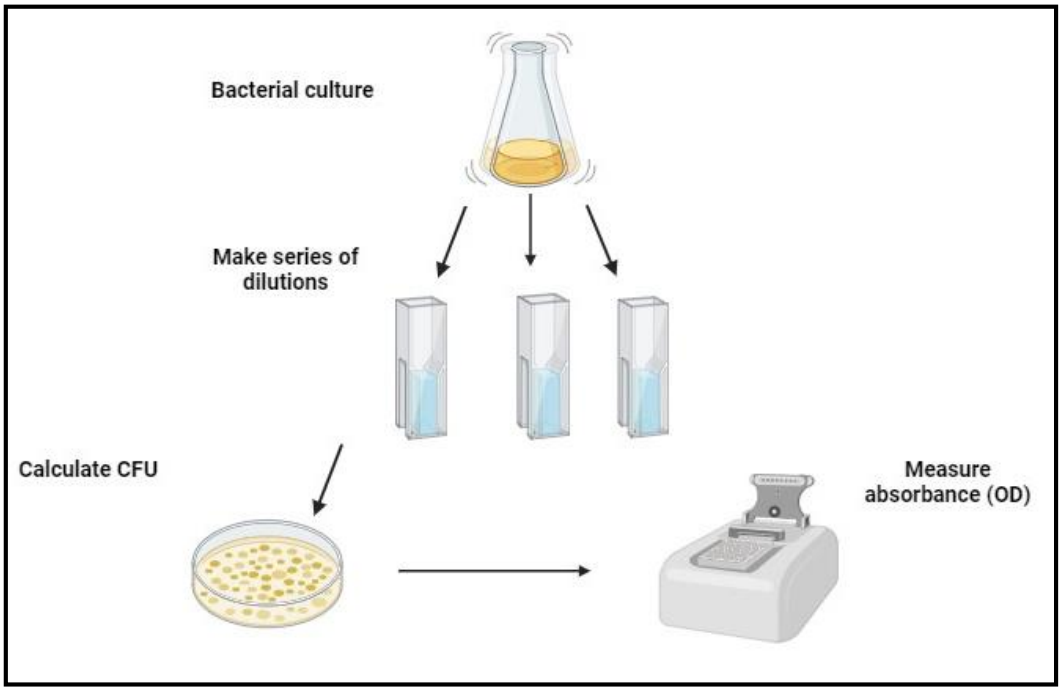

Figure 1: Measurement of bacterial growth by spectrophotometer

2. Material Required:

1. Autoclave
2. Laminar flow hood
3. Orbital shaking incubator
4. 250 ml conical flasks
5. 15 ml test tubes
6. Glassware marker
7. Sterile disposable tips (1.0 and 0.2 ml)
8. Micropipettes
9. 12–18-hour nutrient broth culture of *E. coli* DH5α

3. Media Preparation:

1. Prepare nutrient broth media (given previously)
2. The aforementioned components were dissolved in distilled water as required.
3. Subsequently, the media underwent sterilization via autoclaving.

4. Procedure:

1. Prepare a culture of *E. coli* DH5α by inoculating 100 ml of nutrient broth in a 250 ml conical flask with an overnight culture at a 1% ratio.
2. Place the flask containing the culture in an orbital shaker and incubate at 37°C with a rotation speed of 180 rpm.
3. Collect samples of the culture at regular intervals and measure turbidity using a spectrophotometer at 600 nm, with nutrient broth as a reference.
4. Measure the optical density ($O.D._{600}$) of the samples over a 24-hour period to monitor growth.
5. Plot $O.D._{600}$ values against time on semi-logarithmic paper to generate the growth curve.
6. Determine the bacteria's generation time by extrapolating from the growth curve.
7. Plot the growth curve and calculate the generation time accordingly.
8. Utilize a previously obtained calibration curve to determine biomass concentration in various samples.
9. Plot a graph illustrating the relationship between biomass concentrations and time. Calculating specific growth involves analyzing the linear portion of the graph, representing the exponential growth phase.

5. Observations and Results:

Table 1: Observation table

Incubation Time (h)	Optical Density (600 nm)
0	
1	
2	
3	
4	
5	
6	
8	
12	
18	
24	

6. Precautions:

1. Ensure all equipment, including spectrophotometers or colony counters, is properly calibrated.
2. Use sterile culture media and equipment to avoid contamination.
3. Standardize inoculum size and density for consistent results.
4. Incubate cultures at the appropriate temperature and conditions suitable for the specific bacteria.
5. Avoid prolonged exposure of cultures to light or temperature fluctuations during measurements.
6. Handle cultures gently to prevent disruption or clumping.
7. Record measurements promptly to minimize error and ensure accuracy.
8. Clean and sterilize equipment thoroughly after each use to prevent cross-contamination.
9. Verify results with multiple measurements or replicate experiments to ensure reliability.
10. Dispose of cultures and contaminated materials properly according to biohazard guidelines.

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