

BACTERIAL GROWTH CURVE:

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Escherichia coli GROWN ON SALTS VERSUS COMPLEX MEDIA

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http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Growth_Curve/Growth_Curve.htm

Bacteria display a characteristic four-phase pattern of growth in liquid culture. The initial **Lag phase** is a period of slow growth during which the bacteria are adapting to the conditions in the fresh medium. This is followed by a **Log Phase** during which growth is exponential, doubling every replication cycle. **Stationary Phase** occurs when the nutrients become limiting, and the rate of multiplication equals the rate of death. **Logarithmic Decline Phase** occurs when cells die faster than they are replaced. (This latter occurs over a much longer period of time that the previous three.)

We will study the patterns of aerated growth in minimal salts medium (Vogel's E or Cold Spring Harbor A with 0.1% glucose) versus a complex medium (Tryptic Soy Broth = TSB). Bacterial population in the culture will be estimated by measuring its turbidity, to which it is proportional, using a spectrophotometer. Turbidity is classically measured as the absorbance at 660 nanometers of the suspension. You should record the collected data and make two graphs of it in your notebook: one on a linear scale, and one on semi-log paper. Label the phases and determine the doubling times for each medium.

EQUIPMENT AND SUPPLIES:

air pump	Complex medium: Tryptic Soy Broth (or other rich medium)
humidification flask	Minimal salts medium: CSHA medium with 0.1% glucose
manifold with spaghetti tubing	Timex "Triathlon"® watch
sterile 16x150 bubbler tubes	<i>E. coli</i> stationary culture
37°C hot block	sterile pipettes (5 & 1 mL)
	two spectrophotometers which can accept 16x150 mm tubes (one each for VE & TSB)

1. **SET UP AND ILLUSTRATE: GROWTH CURVE APPARATUS:** **air source** bubbles air through a **humidification flask**, the **humidified air** piped to a **valved manifold**, which is connected by **spaghetti tubing** to **bubbler tubes**, set in the holes of a pre-heated 37°C **hot block**. (Describe aeration mechanism of bubbler tube.)
2. Inoculate 0.025 mL of a stationary (over night) culture of *Escherichia coli* into 4.5 mL Tryptic Soy Broth. Determine the resulting A_{660} . It should read between 0.010 and 0.020. Correct if necessary with a new dilution. Repeat the inoculation into 4.5 mL Vogel's E minimal salts medium + 0.1 % glucose. Read in a second spectrophotometer.
3. Record the $T_0 A_{660}$ of the two cultures with two spectrophotometers, one blanked to the VE, the other blanked to the TSB.
4. Aerate these cultures at 37°C in a Hot Block. Read the A_{660} every 30 minutes. The intervals may be conveniently timed using a Timex Triathlon in its "CDR" mode (Count Down Repeat) set to repeat a signal at 30 minute intervals.
5. Collect the growth data on a computer for at least three hours, five if possible.
6. Make two graphs, each with the two curves of the culture A_{660} s versus time:
 - a) a standard linear-linear graph
 - b) a three cycle semi-log graph.

Label the lag and log phases, and determine the generation time. Mount these in your notebook. (Consult protocol for *Making a Graph* for details to be included.)