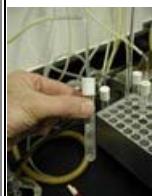


BACTERIAL GROWTH CURVE

Escherichia coli GROWN ON MINIMAL SALTS VERSUS COMPLEX MEDIA

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bubbler system to
grow bacterial cultures

This page has been accessed times since 13 July 2001.
File "growth_curve.htm" was last modified on 14 Jul 2011.
3 August 1992, rvsd 29 June '94, 9 July '95, 3 July '97, 29 June 00, 10 July 00, 17 July 04

Bubbler tube
used to aerate
growing
cultures

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 than the previous three.)

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

EQUIPMENT	SUPPLIES
compressed air source or air pump humidification flask manifold with spaghetti tubing (be sure the tubing is in good repair) sterile 16 x 150 bubbler tubes 37C hot block, to accept 16 mm tubes Timex "Triathlon"® watch sterile pipettes (5 & 1 mL) two spectrophotometers (one each for CSHA & TSB)	Nutrient Broth and/or Tryptic Soy Broth Cold Spring Harbor A minimal medium with 0.1% glucose <i>E. coli</i> stationary overnight culture

PROCEDURE

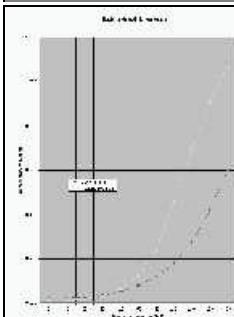
	Set up and illustrate: GROWTH CURVE APPARATUS (here is a labeled image): air pump bubbles air through a humidification flask , the humidified air is piped to a valved manifold , which is connected by spaghetti tubing to bubbler tubes , set in the holes of a pre-heated 37C hot block . To simplify readings, use two spectrophotometers, one blanked to the minimal salts medium, the other blanked to the TSB.
	Inoculate 0.02 mL of an overnight shaken culture (left image) of <i>Escherichia coli</i> into 5.0 mL Tryptic Soy Broth (right image) and into 5.0 mL of minimal salts medium (for instance, Vogel's E or Cold Spring Harbor A) + 0.1% glucose.
	Mix and determine the resulting A_{660} of the inoculated cultures, (it should be between 0.010 and 0.020. This A_{660} is the time zero reading. Insert the bubbler apparatus, place in 37 C hot block with aeration adjusted, and start the 30 minute count down repeat watch.



Read the A_{660} of the two cultures 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.

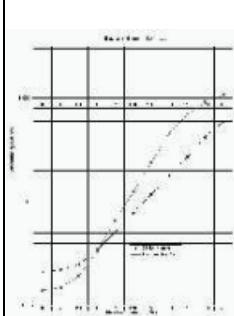
Minutes Incubation	Absorbancy at 660 nm	
	CSHA	TSB
0	0.021	0.014
3	0.022	0.015
6	0.025	0.019
9	0.037	0.033
12	0.051	0.065
15	0.078	0.121
18	0.118	0.238
21	0.179	0.460
24	0.273	0.698
27	0.420	0.910
30	0.538	1.070

Collect the growth data for at least three hours (five if possible). Use a computer for ease and ability to manipulate the data at the end of the experiment.



Make two graphs of the A_{660} of the cultures versus time in minutes:

First, a **standard linear-linear graph** of a time-course experiment, with minutes incubation on the X axis, and A_{660} along the Y axis. I have my students construct a graph by hand to develop their graphing skills. If they then would like to use a spreadsheet program to construct a graph, that is fine.



Second, produce a **semi-log graph** using three cycle semi-log graph paper. Take care to correctly label the Y axis values. (First label 0.010, 0.100 and 1.000 lines, then fill in values between.)

DOUBLING TIME: Use a ruler to draw a line through the linear phases of these curves. This represents their exponential or log phases. Pick an A_{660} value on the lower end of this straight line, note its time. Then find the point at which the A_{660} has doubled and note that time. The time elapsed between the two points is the doubling time.

Label the lag and log phases, and determine the generation time. Mount these in your notebook.