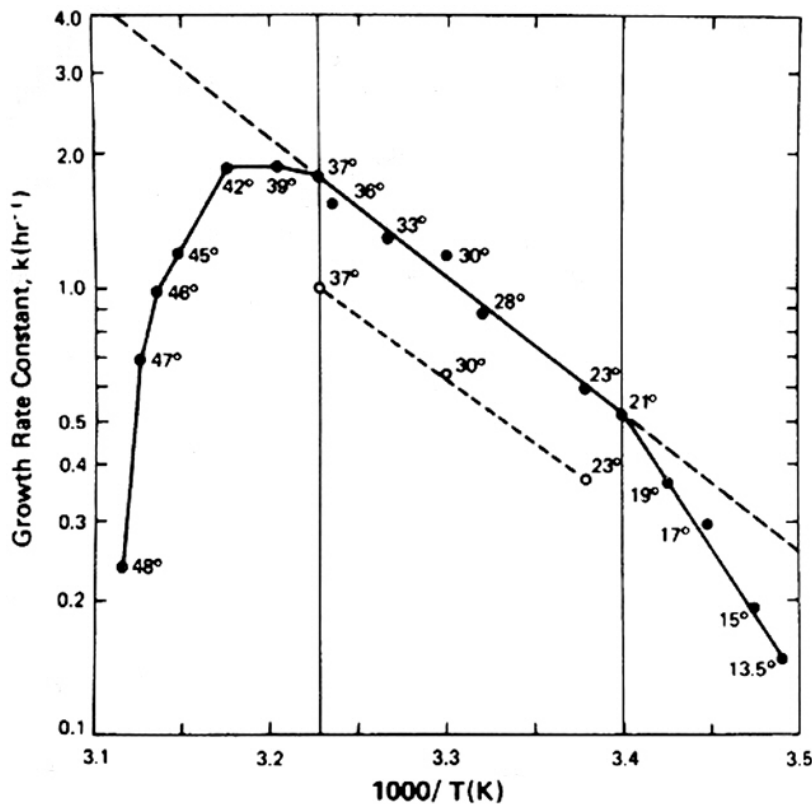


**Lab 3. Growth of *Escherichia coli* B as a Function of Temperature.**

Read Leboffe pp. 49; 230-232.

Different species of microbes have evolved to grow at various ranges of environmental conditions, such as temperature, salinity, organic concentration, and pH. We will investigate the effect of temperature on growth of *Escherichia coli* W3110 in LBK (beef yeast broth). Previously, the effect of temperature was shown for *E. coli* B growth in glucose-minimal medium:



Source: Neidhardt, F., 1996, *E. coli* and *S. typhimurium*. ASM Press, p. 1571.

This relationship between growth rate ( $k$ , doublings/hr) and absolute temperature (Kelvins,  $K$ ) approximately follows the Arrhenius relationship:

$$\log_{10} (k_2/k_1) = 3000^\circ K (1/T_1 - 1/T_2)$$

The Arrhenius law of physical chemistry predicts the same relationship between temperature and reaction rate for any chemical system, even at different growth rates under different environmental conditions. We will test the relationship between growth rate and temperature for a different nutritional condition: growth in complex beef broth medium (LB).

The SPECTRAMax PLUS UV-VIS microplate spectrophotometer is used to measure optical density of bacterial cultures. The class will obtain data together, in a single microplate. Then the data are exported into Excel, and each student independently manipulates all the data to obtain results.

## Procedure.

1. Overnight cultures of *E. coli* have been grown in a rich complex medium including beef broth and yeast extract (Luria-Bertani medium, LB).
2. Each student dilutes overnight culture 1:50 (1 ml into 50 ml) into a prewarmed baffled flask. (The "baffles" increase the rate of air mixing into the medium.) The flasks of medium are located in the four shaking incubators at these temperatures: 27°C, 32°C, 38°C, or 43°C.
3. Remove 200 µl culture twice (for duplicate samples). Incubate culture shaking; never let it sit still. Place samples in appropriate grid positions:

	1	2	3	4	5	6	7	8	9	10	11	12
A	27°		32°		38°		43°					Blank
B	"		"		"		"					
C	27°		32°		38°		43°					
D	"		"		"		"					
E	27°		32°		38°		43°					
F	"		"		"		"					
G	27°		32°		38°		43°					
H	"		"		"		"					

Place in microplate reader drawer. Take readings. **Record your cell positions.** The machine automatically subtracts the blank in cell 12A. Export to Excel; save in p:\data\biology\biol238\

4. At 20 minute intervals, remove 200 µl and record OD<sub>600</sub>. Lab partners will take turns taking readings for each other, with a new plate, **using the same cell positions each time**; then work on bacterial genetics in HIG 321.
5. Partners: Each pair plot your growth curve in Excel to obtain slope and k(per hour). Note any possible evidence of stationary phase (how can you tell?). Label the growth phases that you see.
7. For Thursday: Calculate the growth constant k (doublings per hour) for log phase growth at your temperature. Place your K values in the table on board.
8. Combine class data to plot log<sub>10</sub>k (growth rate) as a function of reciprocal temperature T, using the Arrhenius equation (above). Remember to use Kelvins (degrees C + 273).
9. Use linear regression to determine the change in growth rate as a function of reciprocal temperature. Discuss in class whether one or more temperatures "fall off" the plot (as they do in the Neidhardt figure above).

In your Discussion of results, consider:

- The strengths and limitations of your instrumentation.
- How did you manipulate your data and decide which parts of the growth curve to use?
- How was the growth rate affected by temperature? Was the error spread also affected?
- Why do the ends of the growth curve (Figure 1) deviate from the Arrhenius relationship?
- How might this experiment have to be adapted to study a different organism?