General Microbiology -- **Bacterial Growth**

Begin with increase in Cell Mass AND Number: Generation time is the time required to DOUBLE **cell number** (titer) OR **cell mass.** Check out Fig 6.1, bacterial cell cycle (how different is it from the eucaryotic cell cycle?):

1. Chromosome and cell mass doubling.
2. Septum formation and partitioning of at least one chromosome to each daughter cell.
3. Completion of septum formation (functional generation time).
4. Cleavage = cell separation.

What are the functions of the **Fts** proteins (see Fig 6.2)? The **divisome**? See peptidoglycan synthesis in Gram positives (Figs 6.3 to 6.5).

Population Growth

Arithmetic Growth: 1=>2=>3=>4=>5.... etc. where each "=>" is a generation time.

Logarithmic Growth or Exponential Growth: 1=>2=>4=>8=>16.... etc.

Mathematically this can be expressed : $N = N_o 2^n$ Number at any time = Number at time zero times 2 to the nth power where "n" = number of generations. Thus if you have data to show that in a two hour laboratory session, the number of bacteria increased from 10^5 cells/ml to 10^7 cells/ml....you could calculate the generation time:

rearrange $N = N_o 2^n$ to be: $\log N = \log N_o + n\log 2$

$n = (\log N - \log N_o)/\log 2 = (7-5)/0.301 = 6.64$ generations.

Thus there were 6.64 generations/2 hours....so, the generation time = 2hrs/6.64 = 0.30 hr or 20 minutes.

Mathematically this can also be expressed as a first order rate equation: $dN/dt = \mu N$ where "μ" is the first order growth rate constant (your text uses "k", page 143). Integration of this differential between any two times t=0 to t=1 is:

$$\ln Nt_1 - \ln Nt_o = \mu\,(t_1 - t_o)$$

so in the problem above,

$$16.12 - 11.51 = \mu\,(2\text{hrs}) \ldots \text{solve for } \mu = 2.3/\text{hr or } 2.3\text{hr}^{-1}$$

Generation time = $\ln 2/\mu$ = 0.693/2.3 = 0.30hr or 20 minutes

Why does Generation time = ln 2/μ ?? Can you derive this from the equation above inserting the appropriate assumptions?

Special Practice Problem (not in text)....suppose you took one cell of *E. coli* which weighs has a dry weight of 2.8 x 10^{-13} grams, or a whole cell weight (wet weight) of 2.5 x 10^{-12} grams...when actively growing. Now, place that ONE cell into a very large flask complete with all the nutrients required to keep this cell and its progeny in active growth...with a generation time of 22 minutes. What will be the net weight of the bacteria in this culture after 48 hours of logarithmic growth from one cell? Is this net weight bigger that is something rather large? (think geographiclly large! Answer later at end of these notes..but, first calculate it yourself!) It's easy, you are solving for Nt_1. Simple algebra!

The BATCH GROWTH CURVE or batch culture...a flask of sterile medium inoculated with a bacterial culture (inoculum). Lag Phase, Log Phase, Stationary Phase, Death Phase. What is going on during each phase? During transition periods?

Measurement of Growth:

1. *Total Cell Counts* (What are the advantages and disadvantages of total cell counts?)

1. a. Petroff-Hauser slides (Fig 6.9).
   b. Coulter Counter.

2. *Viable Cell Count* = dilutions and plating..see Figs 6.10 and 6.11 for methodology.
What are errors in these counts? Why do we have the 30-300 rule and would that apply to a larger plate? smaller plate? What is the "*great plate count anomaly"*?

3. *Measurement of Cell Mass*

2. a. Turbidity (Check out Fig 5.8) linearity falls off above OD = 0.7 and below 0.05...but why?
   b. Dry weight.
   c. Chemical measurement (protein, DNA, RNA). What assumptions are here?)

Cell mass measurements that are indirect, such as turbidity need to be standardized, but to what? This makes a great exam question.

Prof Makemson will do a special problem in class along with introduction to graph paper types, you will get a growth curve problem on Exam 1. Please be sure you know how to do these NOW, don't wait until just before the exam. There is another at the end of this set of notes...more detailed to practice! Here is the one done in class:

A culture of bacteria was serial diluted and spread plated to get the following titers once an hour:

Time	Hours	Titer (cells/ml)
8AM	0	2×10^7
9AM	1	2.1×10^7
10AM	2	2.3×10^7
11AM	3	3.25×10^7
Noon	4	6.6×10^7
1 PM	5	1.4×10^8
2PM	6	2.92×10^8
3PM	7	6.1×10^8
4PM	8	1.1×10^9
5PM	9	1.23×10^9
6PM	10	1.3×10^9

Now...plot the data, determine where log phase is and calculate μ and the Generation Time.

What is GROWTH YIELD? What is the deal with the concentration of the limiting nutrient? Can any nutrient (macro nutrient or micro nutrient be limiting?)

CONTINUOUS CULTURE - the Chemostat (Fig 6.13). What happens at different concentrations of limiting nutrient? Check out Fig 6.14 and 6.15

Dilution rate vs Growth rate...Washout? Relationship between yield and growth rate?

Here is some data about *Salmonella enterica* growing in continuous culture set at different dilution rates to obtain different growth rates (data not in text):

Generation Time	Dry Weight of One Cell	DNA as % cell mass	RNA as % cell mass	# 70S Ribosomes/Cell
25 min	0.77 pg	3.0%	31%	69,800
50 min	0.32 pg	3.5%	22%	16,300
100 min	0.21 pg	3.7%	18%	7,100
300 min	0.16 pg	4.0%	12%	2,000

What does this mean for slow growing cells? This data came from continuous culture experiments, but what does it mean for batch culture when the bacteria make the transition from log phase to stationary phase? Do they move through this chart?

ENVIRONMENTAL FACTORS affecting Growth Rate

1. Temperature.
Why is it that all the plots of growth rate vs temperature look the same for pyschrophiles...through to thermophiles, only the cardinal temperatures change? The shape of the curve (Fig 6.16) does not change (Fig 6.17), why?

Psychrotolerant vs Psychrophilic

Psychrophily - molecular adaptions:

3. a. membrane fluidity.
   b. temperature of enzyme activity - all vital enzymes.
   c. cryoprotectants.

Thermophiles: thermophiles + hyperthermophiles molecular adaptions:

4. a. heat stable proteins: salt bridges, -S-S-, hydrophobic interiors.
   b. membrane fluidity. (Archaea ether linked phytane (isoprenes).
   c. histone like proteins.

-Ecology of thermophiles: Figs 6.20 and 6.21 and Upper temperature limits (Table 6.1).

-Biotech applications of thermophiles (Taq plus other industrial applications).

2. pH. Bacterial vs. fungal....adaption to pH and low molecular weight organic acids... maintenance of internal pH at about 7.2. Acidophiles vs alkalanophiles....check out Fig 6.22.

3. Solutes or really Water Activity (a_w) ...add one, decrease the other.

Terrestrial water act = 0.90 to almost 1.0 (1.00=pure water). Most terrestrial bacteria require a_w > 0.9. Marine conditions (average sea water is 3.5% NaCl (roughly 0.5 M Na^+), 0.05 M Mg^{++} , and 0.01 M each of K^+ and Ca^{++} , 0.025 M $SO_4^=$ with the balance in Cl^- and other anions. Marine bacteria have a specific requirement for Na^+ ...this differentiates them from terrestrial bacteria. (All life, terrestrial bacteria included, does require some Na^+ , K^+ , Mg^{++}, and trace minerals.) so marine bacteria are moderate halophiles.

Halotolerant terrestrial bacteria (Fig 6.23) can grow at NaCl concentrations above sea water (*Staphylococcus aureus* is a case in point! where does it live, other Gram positive cocci?)

Extreme halophiles such as some Archaea (*Halobacterium*) require generally >15% NaCl for growth and grow right up to NaCl saturation (25-30%). Where are these s found?

Omsophiles - microbes growing in high sugar concentrations.

Xerophiles - microbes growing in almost "dry" conditions.

Compatible Solutes - when growing in high osmotic conditions, unless the cells accumulated solutes, there would be a net osmotic flow OUT of the cells...thus, the molecular adaption here is to accumulate solutes (amino acids, sugars, KCl...see Table 5.4). OK, how does it work? What is this glycine-betaine molecule anyhow? . . . Table 6.3 lists most of the compatible solutes + molecular structures are in Fig 6.24.

4. Oxygen (concept started in Nutrition Metabolism lecture). Check out Table 6.4, Fig 6.25, which tubes look almost, but not quite similar?

Anaerobic culture methods: jars, hoods (glove boxes) (Fig 6.26).
Microaerophiles: candle jars, requirements for low O_2 and some CO_2.
Review SOD, peroxidases, catalase.
Where do anaerobic conditions occur on the planet?

Be sure to visit the text book's web site for this chapter (http://www.prenhall.com/madigan).

ANSWER to the Growth Question Above: 5.7×10^{27} grams or 5.7×10^{24} kg. Be careful, if you round out some numbers and are dealing with logs, you won't get exactly 5.7...but that is OK as long as it is times 10^{27} grams! What weighs that much = the Planet Earth! The total weight of our planet is 6×10^{24} kg. Thus, clearly we can't do this experiment, there is no flask large enough!! But, this mental exercise shows the impressive growth potential of bacteria: what they do best is grow fast. No other form of life can grow as fast as bacteria, thus given nutrients, bacteria are the first to exploit any environment.

ANSWER to the Text's Application Questions:

1. Beginning with 4 cells. After 1 hour = 32 cells/ml. So in 1 l, then 3.2×10^4 cells. After 2 hours 256 cells/ml, and in 1 liter of this culture, then 2.56×10^5 cells. If one cell was dead (of the 4 cells/ml inoculated, then there would be 192 cells/ml.

This is gotten at by: $\ln Nt_1 - \ln Nt_o = \mu\ (t_1 - t_o)$. Generation time of 20 minutes is a growth rate constant of 0.03465/min. This is gotten from $\mu = \ln 2/\text{Gen time}$. $\mu = 0.693/20\text{min}$. So for the last example (starting with 3 cells/ml growing for 2 hours): $\ln Nt_1 - \ln 3 = 0.03465\ (120\ \text{min})$. Thus $\ln Nt_1 - 1.099 = 0.03465\ (120\ \text{min})$. $\ln Nt_1 = 5.257$ so $Nt_1 = 192$ cells/ml. 2. 0.496 hr or just under

30 min.

2. This assumes that no growth took place during the lag. Thus,

$$\ln(5.4 \times 10^9) - \ln(5 \times 10^6) = \mu\ (5\text{hrs})$$

$$(22.4097 - 15.4249)/5\ \text{hr} = \mu$$

$$\mu = 1.397/\text{hr}$$

$$\text{Generation time} = \ln 2/\mu = 0.693/1.397\ \text{hr} = 0.496\ \text{hr or } 29.8\ \text{min.}$$

3. For *Escherichia coli* 80ºC will heat denature its enzymes (it's a mesophile) and it's cell membrane would be totally disorganized, the cells will die. For *Pyrolobus fumarii* (it's an extreme thermophile) and this temperature, 80ºC, is too cold and **below** the minimum temperature of growth (their cell membrane is a crystalline solid at this temperature that is at the same time too hot for *E. coli.*

4. No, psychrophiles will be killed by high temperatures (see #3 above), but thermophiles get into cold water their polymers "freeze", but since ice is not formed, they are not killed.

GROWTH CURVE QUESTION (Similar to one you could have on the exam)
Suppose you were *doing* a growth curve. In the lab you collected the following data from a culture inoculated by an overnight growth of *E. coli* in Nutrient Broth at 37C wherein you measured both the titer and optical density at 660 nm:

Time	Titer	OD-660nm
8:30 AM	3×10^7	0.050
9:00 AM	2.9×10^7	0.051
9:30 AM	3.5×10^7	0.065

10:00 AM	6×10^7	0.12
10:30 AM	2.4×10^8	0.51
11:00 AM	1.01×10^9	2.0
11:30 AM	2.3×10^9	3.4
noon	3.1×10^9	4.3
12:30 PM	3.5×10^9	4.4

What is the generation time of *E. coli* growing in Nutrient Broth at this temperature? To get at this, plot BOTH titer and OD on semi-log graph paper. This needs to be done to find out where log phase is and to see if this is a normal growth curve. This takes only one piece of paper...are the data consistent? Do you get the same value using different measurements of growth?