Chapter 6 Lecture Notes: Microbial Growth

I. The Growth Curve in batch culture
   A. Growth is an increase in cell constituents
   B. For most microbes, growth in indicated by an increase in cell # because cell division accompanies growth
   C. Batch culture = cultivation of organisms in 1 batch of liquid medium
   D. Growth curve (Fig. 6-1)
      1. Experimental design
         a) Introduce small number of microbes into new medium →
         b) monitor # of viable cells as a function of time by spectrophotometry or by diluting aliquot of culture and plate on agar plates (plate counts)
         c) plot on semilogarithmic scale: log for # of cells (or OD) and linear for time)
      2. Phases of growth of a population of cells
         a) Lag
            (1) No increase in cell # when cells are introduced into fresh media
            (2) Reasons
               (a) Cells may be depleted of a variety of factors that may need to be resynthesized
               (b) Medium may be different that previous one and thus new enzymes may be needed for growth
               (c) Cells may be injured and need time to recover
         b) Exponential phase
            (1) Microbes are growing at maximal rate possible for the particular conditions
            (2) Growth rate is constant
            (3) Growth is exponential – cell growth doubles every x min (generation time)

time	# of cells
0	1
0.5	2
1	4
1.5	8
2	16
2.5	32
3	64
3.5	128
4	256
4.5	512
5	1024
5.5	2048

c) Stationary phase
  (1) No net increase in cell #
  (2) Mostly due to cessation of cell division
      (a) depletion of nutrients
      (b) accumulation of waste
  (3) Also due to balance between cell death and cell division
  (4) For bacteria typically at 10^9 bacteria/ml

d) Death phase
  (1) Decrease in viable cell #
  (2) Causes are extended nutrient deprivation and accumulation of toxic waste

E. Generation time
  1. Generation time (g) is the time it takes a culture or microbial population to double in number (AKA doubling time)
  2. Determination g using mathematics
     a) $g = t/n$ where:
        (1) t = time of exponential growth
        (2) n = # of generations in time t as calculated:

          exponential growth = 2^n
          N_0 = # of cells in population initially
          N_t = # of cells in population at time t

          $N_t = N_0 \times 2^n$
          $\log N_t = \log N_0 + n\log 2$
 $\dfrac{\log N_t - \log N_0}{\log 2} = n$

     b) For example: What is the generation time if 100 bacterial cells growing logarithmically for 5 hours produced 1.7×10^6 cells?

          $n = \dfrac{\log(1.7 \times 10^6) - \log 100}{\log 2}$ =   14 generations in 5 hours

          g = 5 hours/14 generations = 0.357 generations/hour

3. Determination of g using growth curve data
    a) Plot time on X axis and CFU/ml on Y axis (log scale)
    b) Pick a point on the Y axis in log growth
    c) Draw a line from the Y axis point in (b) to the plotted graph and then down to the X axis to determine the time at which the population was at that cell density (b)
    d) Multiple the point that you picked in (b) by 2 (because we want to know when the population doubles)
    e) Draw a line from the Y axis point determined in (d) to the plotted graph and then down to the X axis to determine the time at which the population was at that cell density (d)
    f) The generation time is the difference between the X values from (e) and (c)
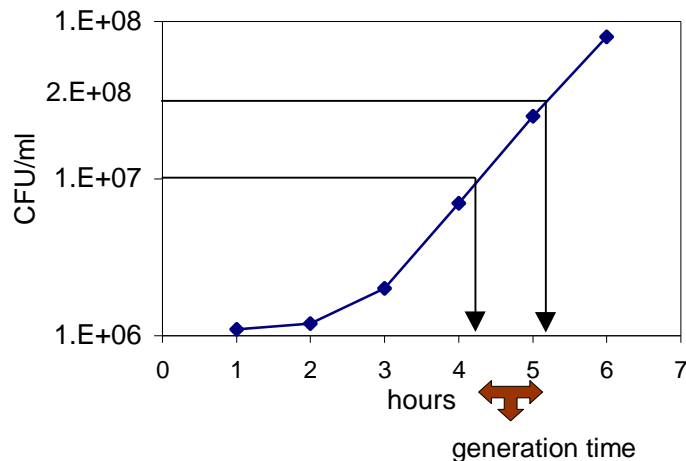
II.   Measurement of microbial growth
  A. Total cell number by direct counting
    1. Counting chambers (Fig. 6-4): Special slides with a chamber that holds a known volume and contains an etched grid in the bottom for counting → microbes are counted and normalized per ml based on the chamber volume
      a) Pros: Easy, inexpensive, and quick
      b) Cons: Cannot tell live from dead; need population $>10^6$/ml; precision is difficult to achieve due to small sample
    2. Coulter counter (measures resistance as cells are passed through orifice)
      a) Pros: Easy, inexpensive, and quick
      b) Cons: Cannot tell live from dead; only for larger microbes
    3. Filter and stain systems (Fig. 6-7, 8): Filter aliquot of a sample through a membrane filter which retains the bacteria → bacteria on filter are stained with fluorescent dye → bacteria are then counted using a fluorescence microscope (some stains can differentiate live from dead)

B. Viable cell counting techniques

    1. Plate aliquot of liquid culture on solid media → count colony forming units (CFU). Assumption is that each cell in the aliquot can form one CFU on the solid media.

        a) Pros: Easy; high sensitivity

        b) Cons: Have to do several dilutions, many plates; Need correct media; clumps of cells will only give one CFU

        c) Calculation of colonies in sample:

$$\frac{\text{\# colony forming units}}{\text{(ml plated) (dilution plated)}} = \text{total CFU/ml} \sim \text{total bacteria /ml}$$

    2. Filter techniques: Filter aliquot of a sample through a membrane filter which retains the bacteria → filter is placed on agar medium → each cell grows into a colony that can be counted

        This technique is frequently used to sample water supplies; agar medium that filter is placed on can be selective for certain kinds of bacteria (Fig. 6-7, 8)

C. Measurement of cell mass

    1. Dry weight – concentrate the culture into a pellet by centrifugation and weigh; useful for larger microbes

    2. Turbidity measurement using spectrophotometer (Fig. 6-9)

        a) The spectrophotometer measures the turbidity of a sample and generates a value called optical density (OD).

        b) Turbidity is a measure of the light absorption by particles in a sample (i.e. microbes in media). Within limits, the light absorbed by a sample is proportional to the concentration of light absorbing materials.

        c) Since the OD of a culture increases as the number of organisms increases in a culture, the OD can be used to indirectly calculate the number of microbes.

        d) Pros: very easy once relationship between # of cells and OD reading is determined for an organism

        e) Cons: Samples must contain at least $10^7 – 10^9$ bacteria per ml; does not indicate whether cells are viable

    3. Measurements of cell components

        a) total protein

        b) chlorophyll

        c) ATP

III.   Continuous culture
  A. Maintenance of a culture in constant environmental conditions through continual provision of nutrients and removal of wastes.   Useful for:
    1.   Study in a certain growth phase
    2.   Study under low nutrient concentrations
    3.   Evolution studies
  B. The chemostat
    1.   Apparatus that feeds sterile media into a culture at the same rate in which it is removed
    2.   Essential nutrient is limiting so that flow rate determines growth rate
  C. Turbidostat
    1.   Flow rate into the system is adjusted to maintain preset turbidity (cell density).
    2.   No limiting nutrient

IV.   Influence of environmental factors on growth
  A. General info:
    1.   Most microbes live in moderate environments; however, some called extremophiles live in extremely harsh environments (bacteria of generation X)
    2.   Study of environmental effects on growth important for understanding ecological distribution of organisms, control of organisms
    3.   While we are addressing growth here, keep in mind that some organisms may survive (but not grow) under conditions above and below the growth conditions
  B. Water activity (and osmolarity)
    1.   Water activity
      a)   Amount of water available to an organism affected by
        (1) water interactions with solute molecules (osmotic effect)
          (a) Hypertonic solution = osmotic [] is higher outside → water exits the cell
          (b) Hypotonic solution = osmotic [] is lower outside → water enters the cell
        (2) water adsorption to solid surfaces (matric effect)
      b)   water activity of a solution = a_w
        (1) $a_w = \dfrac{\text{vapor pressure of solution}}{\text{vapor pressure of water}}$

        (2) a_w = (% relative humidity of solution) / 100
        where relative means relative to water

2. environment with low a_W = high osmotic pressure
    a) effects on cell if placed in such an environment
        (1) dehydration
        (2) plasmolysis (plasma membrane shrinks from the cell wall)
        (3) cessation of metabolism
    b) Bacterial solution to the problem: Compatible solutes - solutes that bacterium takes up to maintain a slightly hypertonic state; are compatible with metabolism and growth of the organism when at high [] inside
        (1) bacteria: choline, betaine, potassium, some amino acids
        (2) algae/fungi: sucrose and polyols
        (3) Halobaterium: potassium ions
        (4) mycoplasmas are the exception – they do not have compatible solutes; instead they maintain their cytoplasm at the same osmolarity as the environment using a Na pump
    c) Halophiles actually require high levels of NaCl in the environment to survive. Adaptations include:
        (1) modified cell wall structure that has adapted to high NaCl: acidic glycoprotein cell wall is stabilized by sodium ions binding and shielding negative charge. When NaCl is removed, acidic proteins repel each other resulting in cell lysis.
        (2) K accumulation to remain hypertonic to their high salt environment
        (3) acidic proteins that have adapted to high K ions in the cytoplasm
    d) environments with low a_W /high osmotic pressure: dried foods, salt lakes, salted foods, preserves
3. high a_W = low osmotic pressure
    a) effects on cells: cells will take up water
    b) bacterial solution to the problem
        (1) cell wall provides protection against lysis up to a point
        (2) mycoplasmas maintain their cytoplasm at the same osmolarity as the environment using a Na pump
    c) environments with high a_W /low osmotic pressure: water, blood
4. osmotolerant organisms can grow over a wide range of a_W

C. pH
1. measure of the H^+ activity in a solution (Fig. 6-13)

$$pH = -\log[H^+] = \log (1/[H^+])$$
low pH = acidic
high pH = basic/alkaline

2. Growth of microbes
a) acidophiles (0-5.5)
(1) many fungi, few bacteria
(2) locations: acid thermal springs, gastric juice, acid mine drainage, acid soils
b) neutrophiles (5.5-8)
(1) most bacteria
(2) locations: most foods except for acidic ones, rain water, milk, saliva, blood
c) alkalophiles (8.5 – 11)
(1) locations: alkaline soils and lakes
3. EXTERNAL pH ? INTERNAL pH
a) Internal pH must be near neutral to avoid harming acid or alkali labile molecules (for *E. coli* internal pH is 7.4-7.8 when grown at pH between 5 and 9)
b) Mechanism for maintaining internal pH
(1) General
(a) plasma membrane is impermeable to H^+
(b) Buffering agents in the cytoplasm
(2) Neutrophiles
Keep cytoplasm slightly basic by pumping out H^+ in exchange for K^+
(3) Acidophiles
(a) Decarboxylases take the carboxyl group off acidic amino acids to generate alkaline products to keep the cytoplasm from acidifying
(b) up regulate fermentative pathways that generate less acid
(c) pump in cations to decrease proton motive force in membrane so H^+ are less likely to flow back into the cell
(4) Alkalophiles
(a) Pump H^+ into the cell to prevent the cytoplasm from becoming too alkaline
4. Microbes themselves change the pH of their environment by producing acidic or basic waste products

D. Temperature
1. Microbes are poikilothermic – their temperature varies with the environmental temperature
2. Why does temperature matter?
    a) Enzyme activity
        (1) low temp = slow rate of enzyme reactions
        (2) high temp = denaturation of enzymes
    b) Membrane structure
        (1) low temp = not fluid enough
        (2) high temp = disrupted
3. Cardinal temperatures (Fig. 6-14)
    a) minimal
    b) optimal
    c) maximal
4. Range
    a) stenothermal = small temp range
    b) euthermal = large temp range
5. Classes based on growth at a variety of temperatures (Fig. 6-15)
    a) Psychrophiles
        (1) min = $\leq 0°C$ / opt = $\leq 15°C$ / max = $20°C$
        (2) location – mostly in deep sea
        (3) adaptations
            (a) enzymes have evolved to function best at cold temps
            (b) membranes have high levels of unsaturated fatty acids that remain fluid in the cold
    b) Psychrotrophs or facultative psychrophiles
        (1) min = $0°C$ / opt = $20-30°C$ / max = $35°C$
        (2) location – cold, temperate environments that warm up in the summer; food in fridge
    c) Mesophiles
        (1) min = $15-20°C$ / opt = $20-45°C$ / max = $45°C$
        (2) location – varied as most identified microbes fall in this category; human pathogens
    d) Thermophiles
        (1) min = $45°C$ / opt = $55-65°C$ / max = $80°C$
        (2) location – hot springs, hot water lines, compost bins, soils exposed to direct sunlight
        (3) adaptations –
            (a) heat stable proteins; evolved to function best at high temperatures
                (i) several critical amino acid changes increase stability (as opposed to drastically altered protein)
                (ii) increase in salt bridges in the proteins (bridges between negatively charged amino acids with cations)

(iii) densely packed hydrophobic interiors (less unfolding)

(b) membranes have high levels of saturated fatty acids and thus have higher melting temperatures

e) Hyperthermophiles

(1) min = 55°C / opt = 80-113°C / max = ?°C

(2) location - hydrothermal vents on the ocean floor, hot springs

(3) adaptations – as above except that membranes are Archeal

(4) no eukaryotes – Why? internal membranes of organelles must remain porous for transport of large macromolecules and thus would not stand high heat

(5) interests

(a) protein and membrane stability

(b) industrial uses

(c) Taq polymerase for PCR

E. Oxygen concentration

1. Classification based on oxygen use/sensitivity (Fig. 6-16)

a) Obligate aerobes – require atmospheric oxygen for growth

b) Facultative anaerobes – do not require oxygen for growth but grow better in its presence

c) Microaerophiles – require oxygen at levels below that in the atmosphere

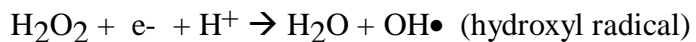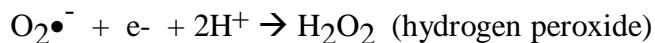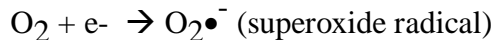d) Aerotolerant anaerobes – can tolerate oxygen but do not use it for growth

e) Obligate anaerobes – killed by oxygen and obviously do not use it for growth

2. Sensitivity to oxygen

a) Inactivation of proteins that are sensitive to O_2 (generally because of sulfhydryl groups or bound cofactors)

b) Inability to detoxify toxic O_2 derivatives produced by interaction with cell components and radiation

(1) Toxic products (powerful oxidizing agents):

$$O_2 + e\text{-} \rightarrow O_2\bullet^- \text{ (superoxide radical)}$$

$$O_2\bullet^- + e\text{-} + 2H^+ \rightarrow H_2O_2 \text{ (hydrogen peroxide)}$$

$$H_2O_2 + e\text{-} + H^+ \rightarrow H_2O + OH\bullet \text{ (hydroxyl radical)}$$

(2) Usually detoxified by superoxide dismutase (SOD) then catalase:

$$2O_2\bullet^- + 2H^+ \rightarrow O_2 + H_2O_2 \quad \text{by SOD}$$
$$2H_2O_2 \rightarrow 2H_2O + O_2 \qquad \text{by catalase}$$
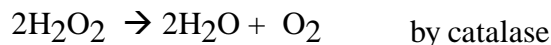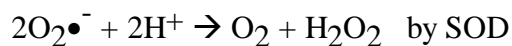
3. Cultivation of anaerobes
   a) "Anaerobic media" which contains reducing agents such as thioglycollate or cysteine to eliminate any O_2 by conversion to H_2O
   b) Elimination of O_2 in container or work area (glove box)
      (1) by flushing with nitrogen
      (2) applying vacuum
      (3) GasPak jar (Fig. 6-18): O_2 consuming gas removes O_2
4. Cultivation of aerobes
   a) Vigorous shaking
   b) Bubbling in sterile air
5. Location of organisms depends on the O_2 available and the presence of other organisms that consume or generate O_2

F. Pressure
   1. Most organisms exist at 1 atm
   2. In deep sea → higher pressures
      a) barotolerant organisms – survive at high pressures but grows better at 1 atm
      b) moderate barophiles – grow at high pressures but still grow at 1 atm
      c) extreme (obligate) barophiles – only grows at high pressures
   3. Adaptations
      a) Because water flows into bacteria, hydrostatic force will not crush them
      b) Enzymes are adapted so that the molecular volume of the activated state of a reaction complex is smaller than the reactants
      c) Membranes are composed of unsaturated fatty acids that are less likely to gel at high pressures

G. Radiation
1. Ionizing radiation (radiation that is very short wavelength/high energy)
    a) Types
        (1) Gamma
        (2) X-rays – artificial
    b) Effects that damage cell components and DNA
        (1) break H bonds
        (2) oxidize double bonds
        (3) destroy rings
        (4) polymerizes some molecules
        (5) formation of hydroxyl radical
2. UV radiation
    a) Far UV (260 nm) - DNA damage via formation of T-T dimers
    b) Near UV (325-400nm) – Tryptophan breakdown to toxic products which introduce breaks in DNA
3. visible
    a) interaction of light with certain photosensitizer pigments excites the pigment → excited pigment transfers energy to O_2 to generate singlet O_2 → singlet O_2 is a higher energy form of O_2 in which outer shell electrons become highly reactive
    b) carotenoid pigments absorb energy to convert singlet oxygen back into the unexcited state