ESM 219

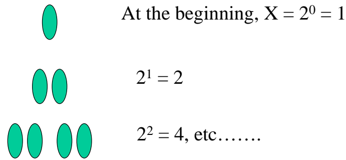
Lecture 4: Growth and Kinetics

Concepts

- Concerned with populations of cells and population numbers and characteristics
- Various ways to report population sizes
- Unrestricted cellular division is well-understood
- Mass transfer-limited growth models, including on solid media, involve more terms

Unrestricted Cell division = population growth

X = number of cells
n = number of division events (doublings)
$X = 2^n$



Population growth, $X = 2^n$ (cont.)

Let n = (# doublings / time) * t
= growth rate constant * t
= μ*t
where μ = "specific growth rate"

Notice that equation is exponential,
so can express as $X = e^{\mu t}$

Note that starting concentration is not 1,
So $X = X_o e^{\mu t}$ where X_o is the initial concentration.

Unrestricted cell division is a "first order" rate process

- Unrestricted cell division is a first order rate process.

$$\frac{d}{dt}(X) = \frac{d}{dt}(X_0 e^{\mu t})$$

So, …..

$$\frac{dX}{dt} = \mu X$$

$$\frac{dX}{dt} = \mu X$$

Now let's go the other direction…..

$$\frac{dX}{X} = \mu dt$$

Separate variables.

$$\int_{t=0}^{t=t} \frac{dX}{X} = \mu \int_{t=0}^{t=t} dt$$

Integrate over the interval 0 to t.

$$\ln \frac{X_t}{X_0} = \mu t$$

The result is a straight line equation….

$$\ln X = \ln X_0 + \mu t$$

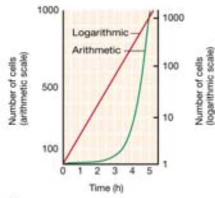
$$X_t = X_0 e^{\mu t}$$

And if we raised e to both sides, we'd be back where we started…

Time (h)	Total number of cells	Time (h)	Total number of cells
0	1	4	256
0.5	2	4.5	512
1	4	5	1,024
1.5	8	5.5	2,048
2	16	6	4,096
2.5	32	.	.
3	64	.	.
3.5	128	10	1,048,576

(a)

During exponential phase growth, a log-linear plot produces a straight line, because



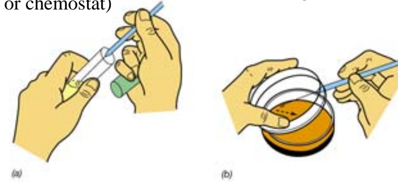
(b)

$$\ln(X) = \ln(X_0) + \mu t$$

Microbial Laboratory Culture

Well-mixed liquid (flask or culture tube or chemostat)

Solid media culture (agar or sand culture)



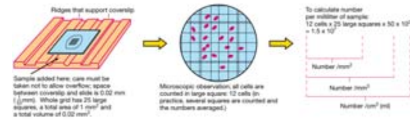
(a) (b)

In all cases

- Prepare media with nutrients, and establish conditions required for growth
- Inoculate
  - With a small amount of known cells for pure culture
  - With an environmental sample for enrichment culture
- Monitor over time
  - Quantify population size at various time points
  - Or measure / observe beginning and end.

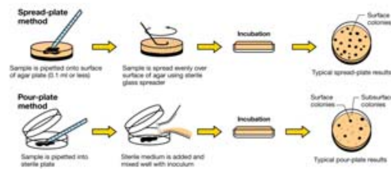
How to measure cell concentration

1. Direct counts with a microscope

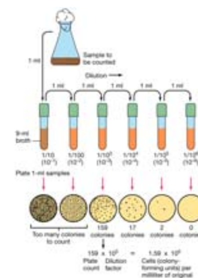


How to measure cell concentration

2. Culturable counts



With or without a dilution series.



How to measure cell concentrations

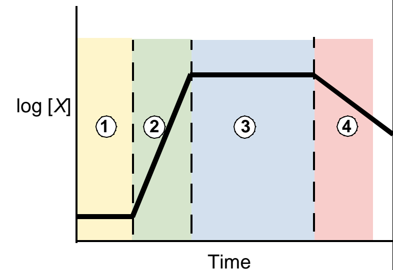
3. Flow cytometry

-----one might also measure proxies for biomass-----

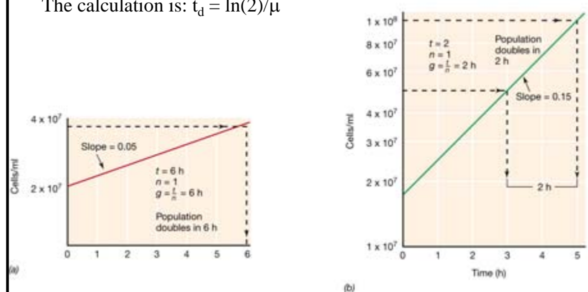
- Biochemically (DNA, protein, etc.)
- Absorbance, reported as optical density or OD (indirect based on turbidity)

Microbial Growth phases in well mixed liquid culture

- **Region 1:**
  Lag phase
  - microbes are adjusting to the new substrate (food source)
- **Region 2**
  Exponential growth phase,
  - microbes have acclimated to the conditions
- **Region 3**
  Stationary phase,
  - limiting substrate or electron acceptor limits the growth rate
- **Region 4**
  Decay phase,
  - substrate supply has been exhausted



Generation time, a.k.a. doubling time, is the time required for the population to double.
The calculation is: $t_d = \ln(2)/\mu$



Exponential Phase Growth

- Log phase growth is first order, ie
- Growth rate ∝ to population size
- So lnX vs. t is linear, slope = μ
- μ units are 1/t (i.e. hr^{-1})

$$\frac{dX}{dt} = \mu X$$

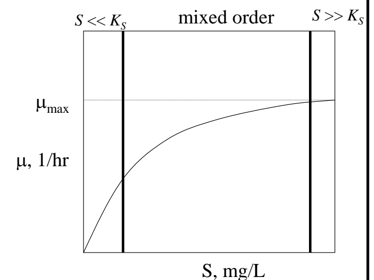
Monod Growth Kinetics

- Relates specific growth rate, μ, to substrate concentration
- Empirical---no theoretical basis—it just “fits”!
- Have to determine μ_{max} and K_s in the lab
- Each μ is determined for a different starting S

$$\mu = \frac{\mu_{max} S}{K_s + S}$$

Monod Growth Kinetics

- First-order region, $S << K_S$, the equation can be approximated as $\mu = \mu_{max} S/K_s$
- Center region, Monod “mixed order” kinetics must be used
- Zero-order region, $S >> K_S$, the equation can be approximated by $\mu = \mu_{max}$



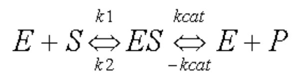
Determining Monod parameters

- Double reciprocal plot (Lineweaver Burke)
  - Commonly used
  - Caution that data spread are often insufficient
- Other linearization (Eadie Hofstee)
  - Less used, better data spread
- Non-linear curve fitting
  - More computationally intensive
- Progress-curve analysis (for substrate depletion)
  - Less lab work (1 curve), more uncertainty

Michaelis Menten Kinetics

- Used when microbe population is constant = non-growing (or short time spans)
- Derivable from first principles (enzyme-substrate binding rates and equilibria expressions)
- Parameter determination methods used for Monod calculations (i.e. Lineweaver Burke)

Michaelis-Menten Equation for Enzyme Kinetics

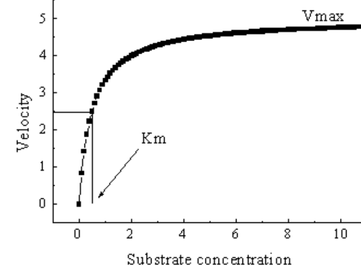


$$E + S \underset{k2}{\overset{k1}{\Leftrightarrow}} ES \underset{-kcat}{\overset{kcat}{\Leftrightarrow}} E + P$$

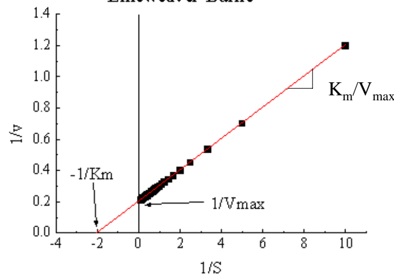
Assumptions:
1. Formation of ES complex
2. k1 and k2 >>> kcat Km = k2/k1 = [E][S]/[ES]
3. [S] >>> [E] such that [ES] formation does not change [S]
4. [P] is very small, valid for initial rates only

v/Vmax = [S]/(Km + [S]) Michaelis-Menten Equation

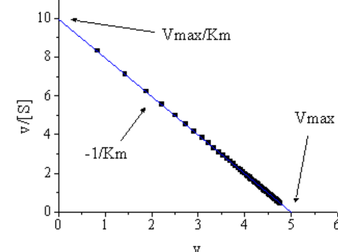
v/Vmax = [S]/(Km + [S])



1/v = (Km/Vmax)(1/[S]) + 1/Vmax



v/[S] = v(-1/Km) + Vmax/Km



Monod vs. Michaelis-Menten: recap of differences

- Monod
  - Growth
  - Empirical
  - K_s
  - μ, 1/t
- Michaelis Menten
  - No growth; constant E
  - Derived from theory
  - K_m
  - v, mg/L-t

Similarities are shape of curves, form of function, parameter estimation techniques.

Substrate Depletion Kinetics

- The rate of biodegradation or biotransformation is a focus of environmental studies
- Substrate consumption rates have often been described using 'Monod kinetics'

$$-\frac{dS}{dt} = \frac{kSX}{K_S + S}$$

- *S* is the substrate concentration [mg/L]
- *X* is the biomass concentration [mg/ L]
- *k* is the maximum substrate utilization rate [sec^{-1}]
- K_S is the half-saturation coefficient [mg/L]

Substrate Depletion Kinetics

- Since $\frac{dX}{dt} = \mu X = -Y\frac{dS}{dt}$
- And $\mu = \frac{\mu_{max} S}{K_s + S}$
- Then $-\frac{dS}{dt} = \frac{\mu X}{Y} = \frac{\mu_{max} SX}{(K_S + S)Y}$
- And $-\frac{dS}{dt} = \frac{kSX}{K_S + S}$ Where $k = \frac{\mu_{max}}{Y}$

Modeling Substrate Depletion

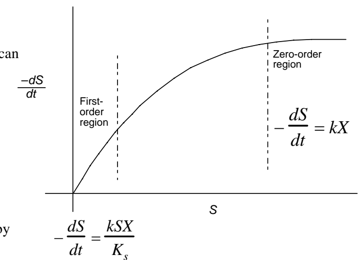
- Three main methods for modeling
  - Monod kinetics (mid range concentrations)
  - First-order decay (low concentration of S, applicable to many natural systems)
  - Zero-order decay (substrate saturated)

Modeling First-Order Decay

- *dS/dt= kS where k is a pseudo first order constant* Generally assumes nothing about limiting substrates or electron acceptors
- Degradation rate is proportional to the concentration
- Generally used as a fitting parameter, encompassing a number of uncertain parameters

Monod Kinetics

- First-order region, $S << K_S$, the equation can be approximated by exponential decay ($C = C_0 e^{-kt}$)
- Center region, Monod kinetics must be used
- Zero-order region, $S >> K_S$, the equation can be approximated by linear decay ($C = C_0 - kt$)

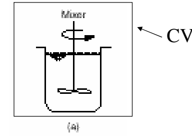


Microbial Kinetics in Modeling Fate of a Substrate

- Use mass balance framework for modeling fate of substance, S
- Choose appropriate “ideal” reactor analogy (usually batch or complete mix)
- Substitute appropriate reaction expression into the framework

Mass Balance: Batch example

→Closed
→Well-mixed
→Constant volume



CV

Verbal: In – Out + Reaction = Accumulation

Math: 0 0 $rV\,\Delta t$ $\Delta S\,V$

Units: $m/l^3\text{-}t\ l^3\ t$ $m/l^3\ l^3$

Rearrange: $r\ V = \Delta S/\Delta t\ V$

Mass Balance: Batch example

Take limits as ΔS and $\Delta t \rightarrow 0$ $r = \frac{dC}{dt}$

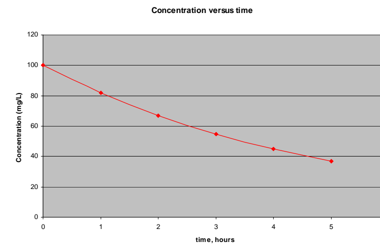
Substitute a rate equation for “r”
e.g. 1st order decay of S: -kS

So, $-kS = \frac{dS}{dt}$ Rearrange, integrate: $\int_{S_0}^{S_t} \frac{dS}{S} = -k\int_0^t t$

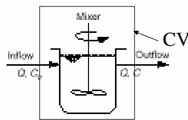
$$\left.\ln S\right|_{S_0}^{S_t} = -kt \rightarrow \frac{S_t}{S_0} = e^{-kt} \rightarrow S_t = S_0 e^{-kt}$$

Mass Balance: Batch example of exponential decay

$S_0 = 100$ mg/L, k =-0.2/hr



Mass Balance: CFSTR



Verbal: In – Out + Reaction = Accumulation

Math: $QS_0\,\Delta t$ - $QS\,\Delta t$ + $r\,V\,\Delta t$ = $\Delta S\,V$

Units: $l^3/t \bullet m/l^3 \bullet t$ $m/l^3 t \bullet t \bullet l^3$ $m/l^3 \bullet l^3$

Rearrange: $Q/V \bullet (S_0 - S) + r = \Delta S/\Delta t$

Mass Balance: CFSTR

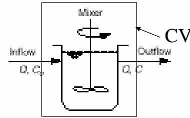
Take limits as ΔS and $\Delta t \rightarrow 0$

$$Q/V \bullet (S_0 - S) + r = \frac{dS}{dt}$$

→Substitute a rate equation for “r”
e.g. 1st order decay of S: -kS
→Make *steady state* (SS) assumption (no net accumulation or depletion: $\frac{dS}{dt} = 0$
→Rearrange

$$S = \frac{S_0}{[1 + k(V/Q)]}$$

Chemostat: CFSTR for Microbial Growth



Verbal: In – Out + Reaction = Accumulation

Math: $QX_0 \Delta t - QX \Delta t + r V \Delta t = \Delta X V$

Units: $l^3/t \cdot m/l^3 \cdot t$ $m/l^3 t \cdot t \cdot l^3$ $m/l^3 \cdot l^3$

Rearrange: $Q/V \cdot (X_0 - X) + r = \Delta X/\Delta t$

Chemostat: CFSTR for Microbial Growth

Take limits as ΔX and $\Delta t \rightarrow 0$

$$Q/V \cdot (X_0 - X) + r = \frac{dX}{dt}$$

→Substitute exponential growth equation for "r"

→Set $X_0 = 0$ (no influent cells)

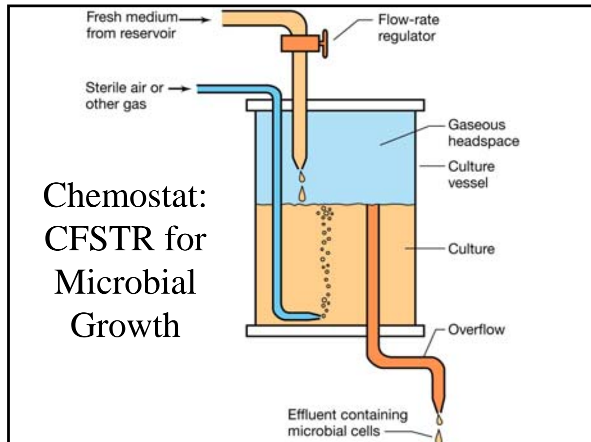
→Make *steady state* (SS) assumption (no net accumulation or depletion): $\frac{dX}{dt} = 0$

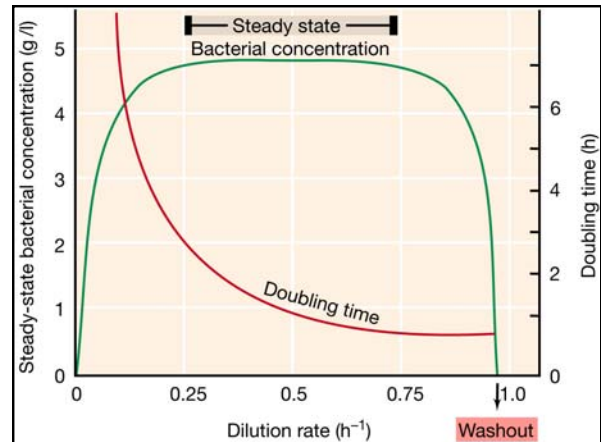
→ Let Q/V = D = dilution rate

→Rearrange:

$$-\frac{Q}{V}X = \mu X \rightarrow -\frac{Q}{V} = \mu \rightarrow \mathbf{D = \mu}$$

Chemostat: CFSTR for Microbial Growth





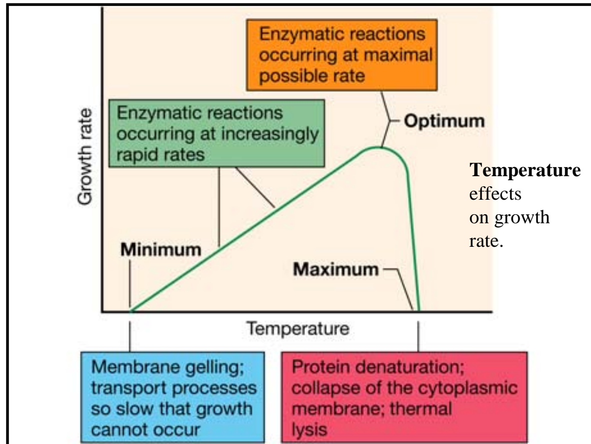
Environmental Factors

- Temperature
- pH
- Salinity
- Oxygen Concentration

Environmental Factors

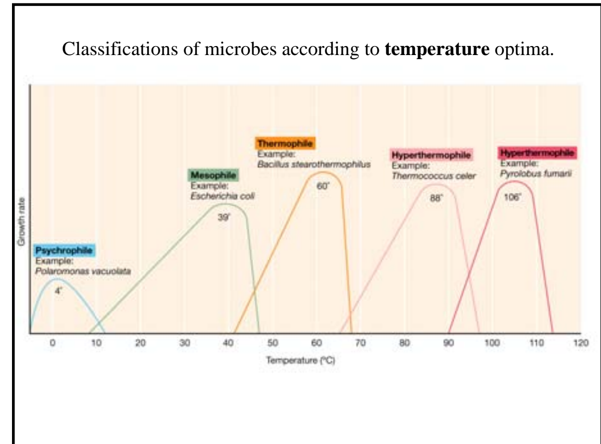
Extremophiles can tolerate or perhaps require extreme conditions in any of the above.

Cellular compensation outside of their optima can reduce **growth rate** and **yield**.



Temperature effects on growth rate.

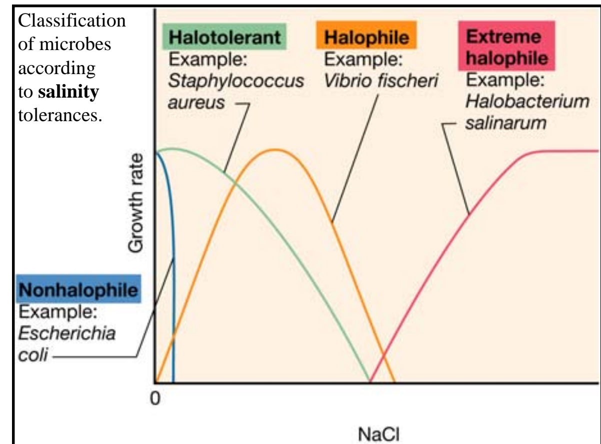
Classifications of microbes according to **temperature** optima.

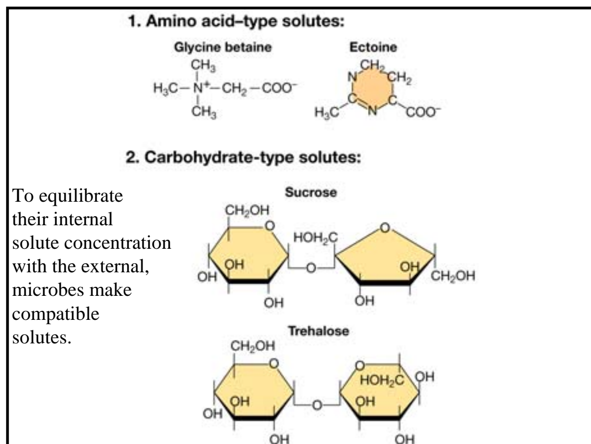


Classification of microbes according to tolerance of **pH** extremes

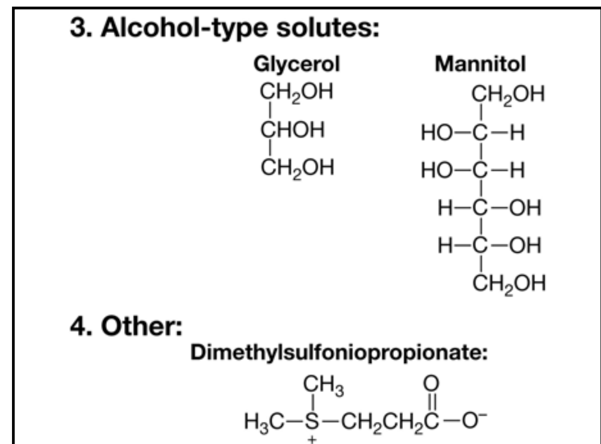
	pH	Example	Moles per liter of: H^+	OH^-
Acidophiles / Increasing acidity	0		1	10^{-14}
	1	Volcanic soils, waters; Gastric fluids	10^{-1}	10^{-13}
	2	Lemon juice; Acid mine drainage	10^{-2}	10^{-12}
	3	Vinegar; Rhubarb; Peaches	10^{-3}	10^{-11}
	4	Acid soil; Tomatoes	10^{-4}	10^{-10}
	5	American cheese; Cabbage	10^{-5}	10^{-9}
	6	Peas; Corn, salmon, shrimp	10^{-6}	10^{-8}
Neutrality	7	Pure water	10^{-7}	10^{-7}
Alkaliphiles / Increasing alkalinity	8	Seawater	10^{-8}	10^{-6}
	9	Very alkaline natural soil	10^{-9}	10^{-5}
	10	Alkaline lakes; Soap solutions	10^{-10}	10^{-4}
	11	Household ammonia; Extremely alkaline soda lakes	10^{-11}	10^{-3}
	12	Lime (saturated solution)	10^{-12}	10^{-2}
	13		10^{-13}	10^{-1}
	14		10^{-14}	1

Classification of microbes according to **salinity** tolerances.



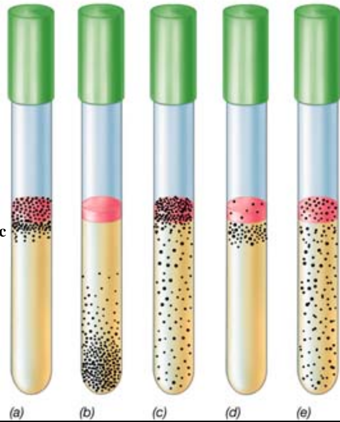


To equilibrate their internal solute concentration with the external, microbes make compatible solutes.



Classification of microbes according to their oxygen responses.

a. **Aerobic**
b. **Anaerobic**
c. **Facultative**
d. **Microaerobic**
e. **aerotolerant**



Oxygen tolerance is conferred by enzymes that scavenge and scrub toxic free radicals. Enzymes include superoxide dismutase, catalase and peroxidase.