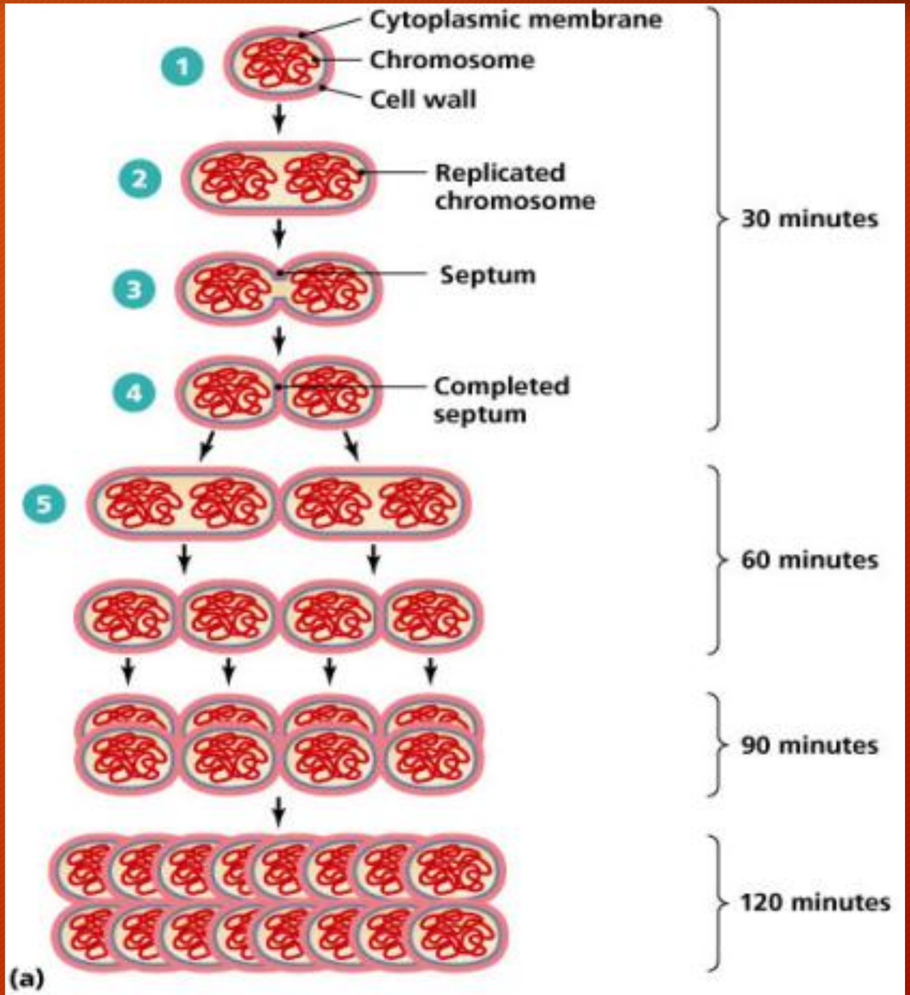
Microbes

Microbes

- In many situations microbes in the subsurface can help remediate contamination by consuming/altering contaminants.
- In order to understand this though, we need to know how microbe populations evolve in time
- We will start this chapter with a focus on microbial population dynamics

Microbial Growth

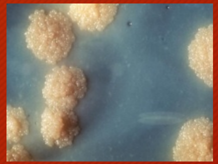
- In each generation a bacterium splits into two

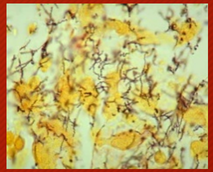


Generation Time Under Optimal Conditions

(at 37°C)

Organism	Generation Time
Bacillus cereus	28 min
Escherichia coli	12.5 min
Staphylococcus aureus (causes many types of infections)	27-30 min
Mycobacterium tuberculosis (agent of Tuberculosis)	18 – 24 hrs
Treponema pallidum (agent of Syphilis)	30 hrs





Images: *B. cereus*, *E. coli* & *S. aureus* by T. Port; TB culture, Dr. George Kubica PHIL #4428, *Treponema pallidum*, Dr. Edwin P. Ewing, Jr., PHIL #836

Thus each generation doubles in size

- Mathematically

$$N(k+1) = 2\,N(k) \quad k=1,\ldots\ldots,N$$

- Question – for *Escherichia coli*, starting from one bacterium how large a colony will exist at the end of the day

Thus each generation doubles in size

- Mathematically

$$N(k+1) = 2\,N(k) \quad k=1,\ldots\ldots,N$$

- Question – for *Escherichia coli*, starting from one bacterium how large a colony will exist at the end of the day (115 generations)

k	N(k)
1	1
2	2
3	4
…	…
n	2^{n-1}
115	2.0769e+34

1 E.Coli = 1 μm^3

2.0760e+34= 2.0769e+16 m^3

Insane

Before we deal with that….

- Typically bacteria don't double at the exact same time, but at some average rate

$$\frac{dN}{dt} = \alpha N$$

$$\Longrightarrow N = N_0 e^{\alpha t}$$

Strictly α is difference between birth and death rate

- How to calculate α from doubling time data?
- So - what's wrong with this model….?

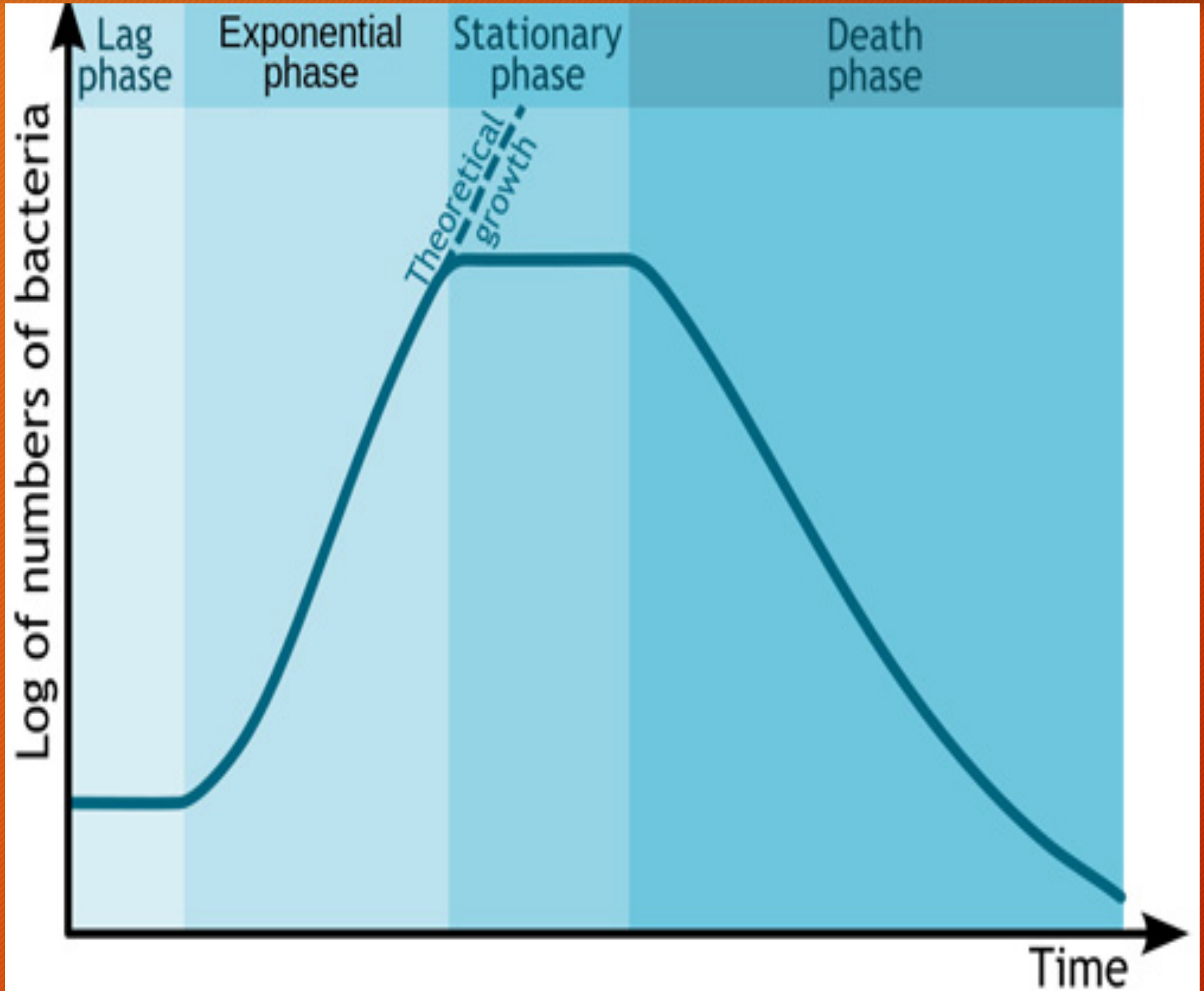
Slightly Modified- Discrete Markov chain model of bacterial growth

- We assume that at each time step (generation) a bacterium undergoes binary fission (divides into two cells) with probability p and dies with probability 1 − p, and these are independent of all the other bacteria

$$E(N_k) = (2p)^k N_0$$

- If p>1/2 – exponential growth
- If p<1/2 – death
- Again – what's the problem?

Typical microbial community life cycle…



- Concentration stagnates until some Initiation time

- It then saturates and levels off at some maximum value

- Typically this maximum value is called the carrying capacity – i.e. the maximum population that a given environment can actually sustain
- Denote carrying capacity by K.

- How can we modify the governing equatin to capture such a behavior?

Let’s make some assumptions

- Population rate increases proportionally to the number of bacteria (just like before)

- Population rate slows down and stops as you approach carrying capacity.

$$\frac{dN}{dt} = \alpha N \left(1 - \frac{N}{K}\right)$$

Logistic Growth Model

- So with carrying capacity

$$\frac{dN}{dt} = \alpha N\left(1 - \frac{N}{K}\right)$$

- Solution

$$N(t) = \frac{K}{1 + \left(\frac{K}{N_0} - 1\right)\exp(-\alpha t)}$$

Great – but what is missing from this equation?

Food

- Bacteria need food and energy to actually grow
- Everything we have done so far basically assumes that they have enough of that to grow as quickly as they can.
- What happens when this becomes limited?
- Less food means slower growth; more than a certain amount means grow as efficiently as possible (just like so far)

Monod Kinetics

- Growth of bacteria is proportional to
  - Number of bacteria
  - Concentration of food S (substrate)
  - When S is high $dN/dt=\alpha N$
  - When S is zero $dN/dt=0$
  - How do I achieve this?

Monod Kinetics

- How about this?

$$\frac{dN}{dt} = \alpha N \frac{S}{G + S}$$

- S – concentration of substrate (limiting resource)
- G is the concentration of the limiting resource when the growth rate is half the maximum.
- What is missing here?

Monod Kinetics – with depletion of resource

- How about this?

$$\frac{dN}{dt} = \alpha N \frac{S}{G+S}$$

$$\frac{dN}{dt} = -\varepsilon \frac{dS}{dt}$$

- What is ε? And what can I do with this information…
- Is anything conserved in this system?

Monod Kinetics – with depletion of resource

- Is anything conserved in this system?

$$\frac{dN}{dt} = \alpha N \frac{S}{G+S}$$

$$\frac{dN}{dt} = -\varepsilon \frac{dS}{dt}$$

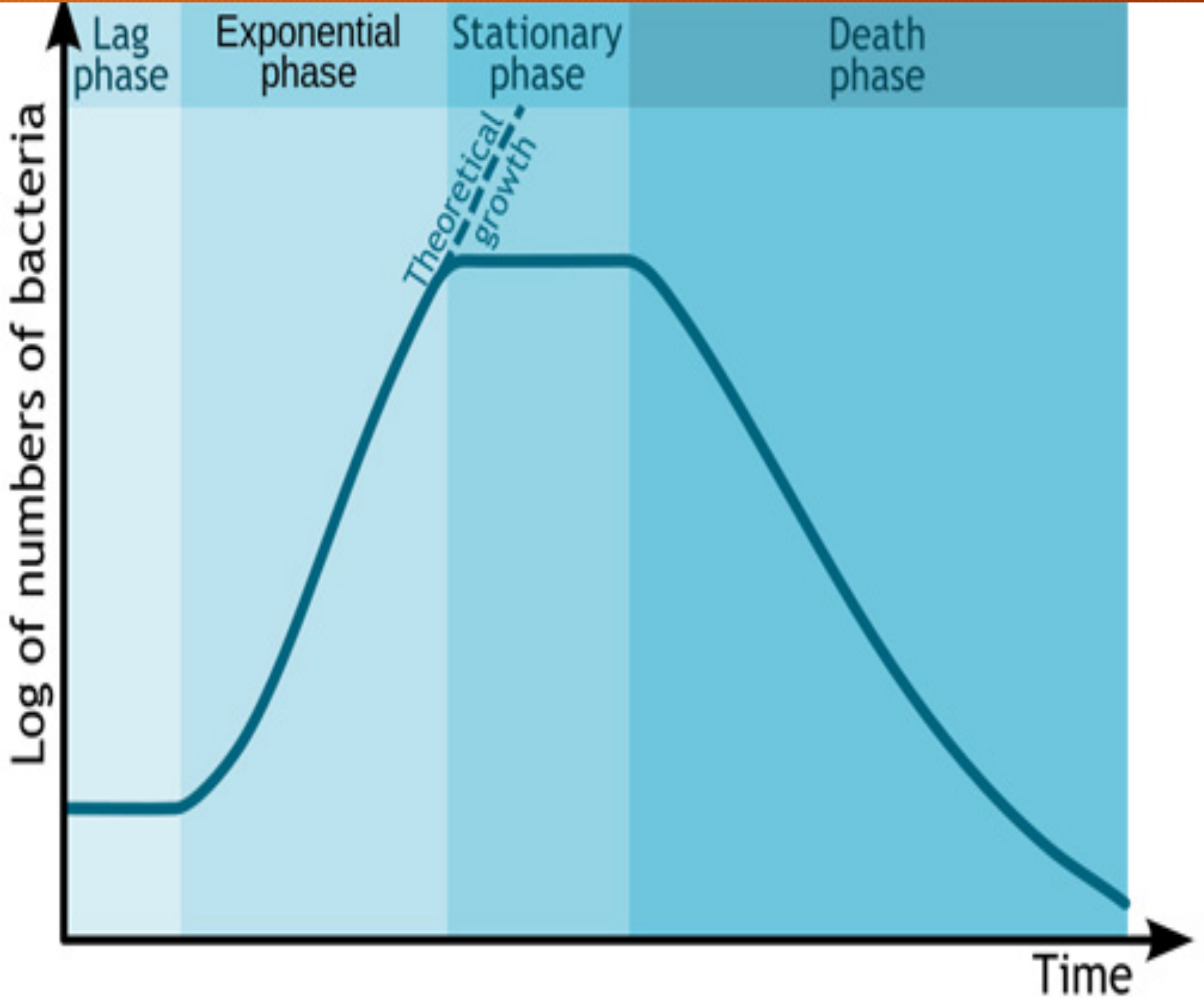
$$\frac{dN}{dt} + \varepsilon \frac{dS}{dt} = 0$$

$$N + \varepsilon S = constant = N(0) + \varepsilon S(0) = M_0$$

$$\frac{dN}{dt} = \alpha N \frac{(M_0 - N)}{\varepsilon G + (M_0 - N)}$$

A single differential equation

Typical microbial community life cycle…



- Concentration stagnates until some Initiation time
- It then saturates and levels off at some maximum value
- Typically this maximum value is called the carrying capacity – i.e. the maximum population that a given environment can actually sustain
- Denote carrying capacity by K.
- How can we modify the governing equatin to capture such a behavior?

During the final stage - death

- Death stage looks something like

$$\frac{dN}{dt} = -k_d N$$

Exponential death rate

Now I can combine everything together

- Exponential Growth, Logistic Growth, Monod Kinetics and Death

$$\frac{dN}{dt} = \left[\alpha\left(1 - \frac{N}{K}\right)\frac{S}{G + S} - k_d\right]N$$

Sample Problem – Continues over several pages..

- (i) Mathematical models are used to predict the growth of a population, i.e. population size at some future date. The simplest model is that for exponential growth. The calculation requires a knowledge of the organism's maximum specific growth rate (α). A value for this coefficient can be obtained from field observations of population size or from laboratory experiments where population size is monitored as a function of time:

Time (d)	Biomass (mg/L)
0	50
1	68
2	91
3	123
4	166
5	224

- From this table calculate α

Continued…

- (ii) Once a value for α has been obtained, the model may be used to project population size at a future time. Assuming that exponential growth is sustained, what will the population size be after 25 days?

- (iii) Exponential growth cannot be sustained forever because of constraints placed on the organism by its environment, i.e. the system's carrying capacity. This phenomenon is described using the logistic growth model. Calculate the size of the population after 25 days, assuming that logistic growth is followed and that the carrying capacity is 50,000 mg/L. What percentage of the exponentially-growing population size would this be?

Continued…

- (iv) Food limitation of population growth is described using the Monod model. Population growth is characterized by the maximum specific growth rate (α) and the half-saturation constant for growth (G). Take the population vs. time data at the start of this problem and calculate the maximum specific growth rate that would be required using the Monod model with a substrate concentration of 25 mg/L and a G of 5 mg/L. What percentage of the growth rate (μ) for an exponentially-growing population would this be? Explain why the growth rate for the Monod model is higher or lower than the growth rate for the exponential model.

Continued…

- (v) α and G describe an organism's ability to function in the environment. Populations with a high α grow rapidly and take up substrate very quickly. Those with a low G are able to take up substrate quite efficiently, reducing it to low levels. These are important when considering microorganisms to clean up pollution. Consider two genetically engineered organisms intended for use in a chemical spill cleanup.
  - Organism "A" has an α of 1 d^{-1} and a G of 0.1 mg/L.
  - Organism "B" has an α of 5 d^{-1} and a G of 5 mg/L.
- Chemical levels are initially about 100 mg/L and must be reduced to below 0.1 mg/L. We wish to use the organisms in sequence - first one to rapidly reduce chemical levels before they spread and second, one to reduce chemical levels to target concentrations. Which organism would be most effective in *rapidly reducing levels of pollution*? Which organism would be most effective in *reducing the pollutant to trace levels*? Back your answer up with calculations.

Continued…

- (vi) When food supplies have been exhausted, populations die away. This exponential decay is described by a simple modification of the exponential growth model. Engineers use this model to calculate the length of time for which a swimming beach must remain closed following pollution with fecal material. For a population of bacteria with an initial biomass of 90 mg/L and a kd = 0.35 d-1, calculate the time necessary to reduce the population size to 15 mg/L.

Conceptual Problem– Steady State

- There is a continuous source of a pollutant spewing from a source at x=0 that you wish to remediate. It advects downstream in an aquifer with known Darcy velocity q and porosity n. You may neglect dispersion and treat the system as one dimensional.
- You inject a bacterial community into the aquifer for remediation and let the system go to steady state.
- Write down the equations for transport and reactions and see if you can solve them.
- Imagine A grams of substrate produces B grams of biomass.

Equations

- Bacteria – we assume do not flow

$$\frac{dN}{dt} = \left[\alpha\left(1 - \frac{N}{K}\right)\frac{S}{G+S} - k_d\right]N$$

- Substrate moves by advection and reaction

$$\frac{\partial S}{\partial t} + u\frac{\partial S}{\partial x} = -r$$

What is r?

Equations

- Bacteria – we assume do not flow

$$\frac{dN}{dt} = \left[\alpha\left(1 - \frac{N}{K}\right)\frac{S}{G + S} - k_d\right]N$$

- Substrate moves by advection and reaction

$$\frac{\partial S}{\partial t} + u\frac{\partial S}{\partial x} = -\frac{1}{Y}\left[\alpha\left(1 - \frac{N}{K}\right)\frac{S}{G + S}\right]N$$

What is Y?

Steady State

- Bacteria – we assume do not flow

$$\frac{dN}{dt} = \left[\alpha\left(1-\frac{N}{K}\right)\frac{S}{G+S} - k_d\right]N \Rightarrow N = \frac{K([\alpha - k_d]S - k_d G)}{S\alpha} \quad \text{OR} \quad N = 0$$

- Substrate moves by advection and reaction

$$\frac{\partial S}{\partial t} + u\frac{\partial S}{\partial x} = -\frac{1}{Y}\left[\alpha\left(1-\frac{N}{K}\right)\frac{S}{G+S}\right]N \Rightarrow u\frac{\partial S}{\partial x} = \frac{k_d K([k_d - \alpha]S + k_d G)}{\alpha S Y}$$

Do you see any problems with these equations? What are they trelling you?

What next

- Solving these equation is a nightmare

$$0 = \left[\alpha\left(1 - \frac{N}{K}\right)\frac{S}{G+S} - k_d\right]N$$

$$0 = \left[\alpha^*\left(1 - \frac{N}{K}\right) - k_d\right]N \qquad \alpha^* = \alpha\frac{S}{G+S}$$

$$N = K\left(\frac{\alpha^* - k_d}{\alpha^*}\right) \quad \text{OR} \quad N = 0$$

Think about what this means?

What next

- Solving these equation is a nightmare

$$N = K\left(\frac{\alpha^* - k_d}{\alpha^*}\right) \quad \text{OR} \quad N = 0 \qquad \alpha^* = \alpha\frac{S}{G+S}$$

N must be >0 or =0 – cannot be negative

Therefore $\alpha^* > k_d$

$$S \geq \frac{k_d G}{\alpha - k_d}$$

Cannot remediate to a concentration below this for a continuous source