

Environmental Microbiology

4 Bacterial Growth and Kinetics

Textbook Chapter 3 with supplemental information



Li Tianxin

**Department of Environmental Engineering
University of Science and Technology Beijing**

Approximate Numbers of Bacteria in Culture and Environmental Samples

Source	No. per mL or g
Pure/mixed culture(shake flask)*	10^5 - 10^9 /mL
Pure/mixed culture(fermenter)*	$\geq 10^{10}$ /mL
Biosolids(activated sludge)	10^{10} /mL
Surface soils	10^9 - 10^{10} /g
Subsoils (ground water sediment)	10^6 - 10^7 /g
Fresh water(eg.lake, river)	10^3 - 10^6 /mL
Seawater	10^3 - 10^6 /mL
Drinking water	10^3 - 10^5 /mL
Raw sewage	10^6 - 10^7 /ml

*grown on culture media

Example: What is the % dry wt. bacteria in a surface soil sample if there are 10^{10} cells/g dry weight soil?

individual cell weigh= 2.8×10^{-13} g and for 10^{10} cells
 $x = 2.8 \times 10^{-3}$ g = 0.0028g cells/g dry wt. or 0.28%

Individual Cell Growth

Binary Fission (Transverse Fission)

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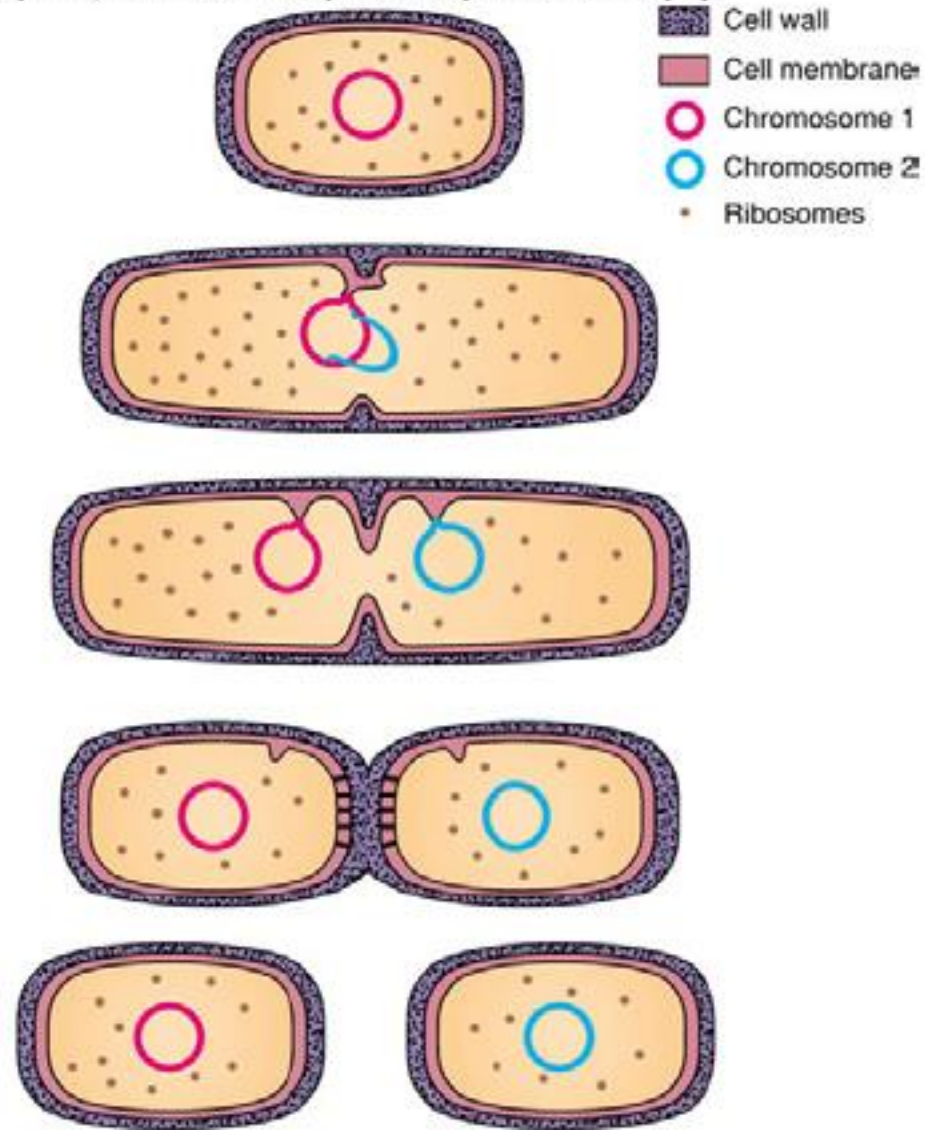
A. A young cell at early phase of cycle.

B. A parent cell prepares for division by enlarging its cell wall, cell membrane, and overall volume. Midway in the cell, the wall develops notches that will eventually form the transverse septum, and the duplicated chromosome becomes affixed to a special membrane site.

C. The septum wall grows inward, and the chromosomes are pulled toward opposite cell ends as the membrane enlarges. Other cytoplasmic components are distributed (randomly) to the two developing cells.

D. The septum is synthesized completely through the cell center, and the cell membrane patches itself so that there are two separate cell chambers.

E. At this point, the daughter cells are divided. Some species will separate completely as shown here, while others will remain attached.



Population Growth in Batch Culture



Algal culture



BOD tests

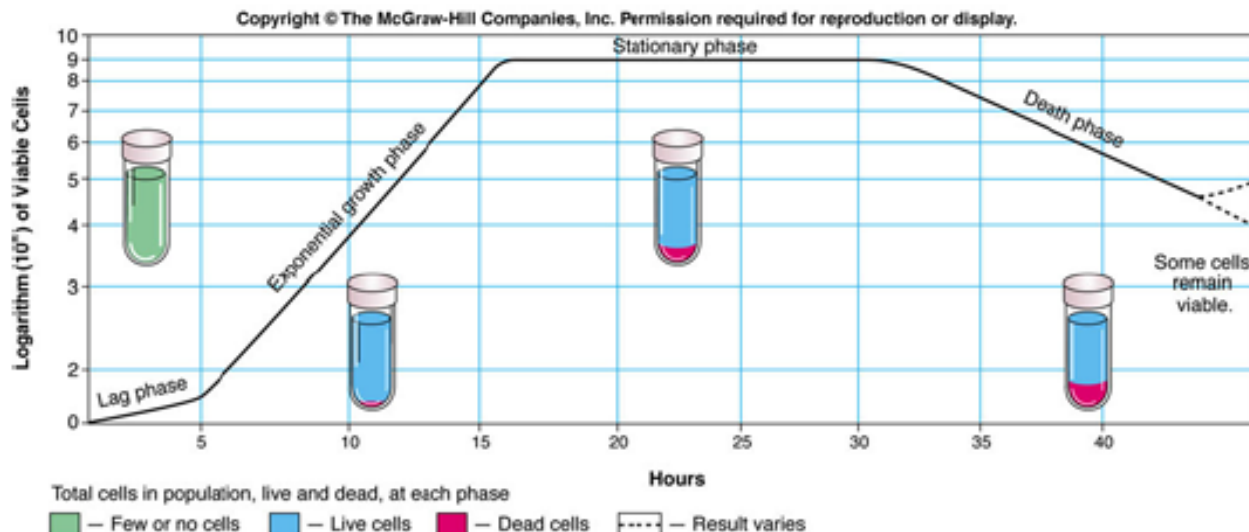
Culture media is initiated with bacterial seed to start the reaction, until the limiting substrate is exhausted

Batch bacterial population growth in a flask:

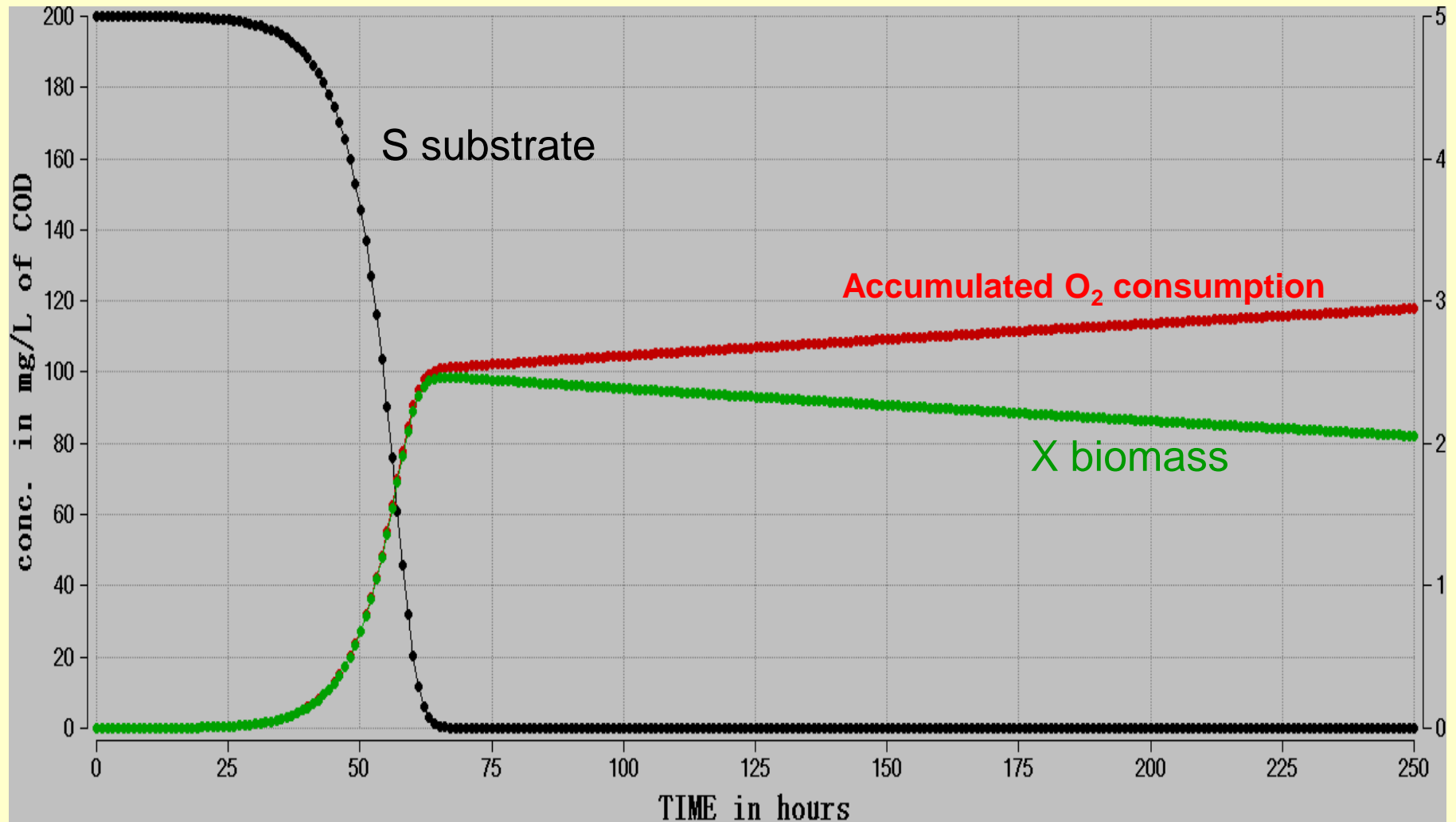
Usually there are 4 phases in a batch growth curve:

- The lag phase
- The exponential growth phase
- The station phase
- The decay or death phase.

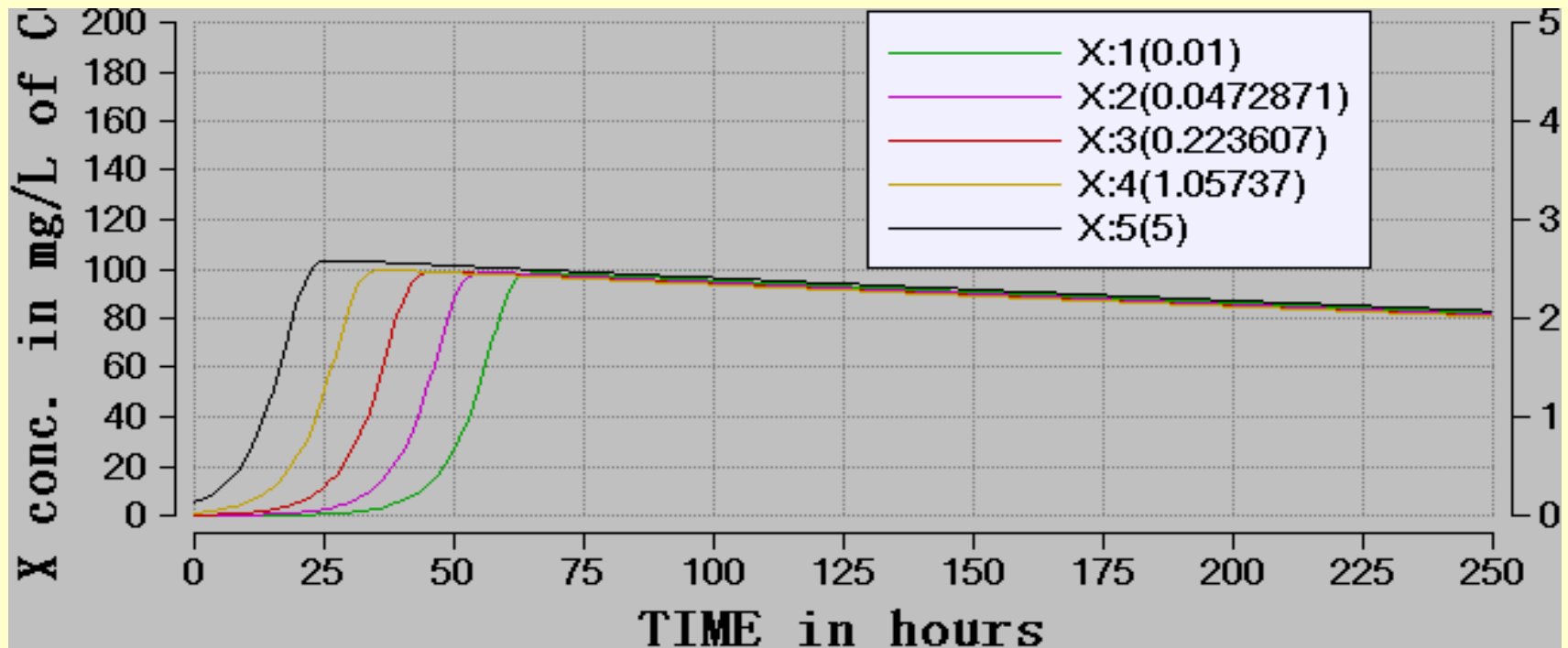
The Population Growth Curve



Batch growth curve with S, substrate concentration, being used, the bacteria, X, growth and oxygen being consumed for respiration (as e^- acceptor) for a heterotrophic bacterial culture

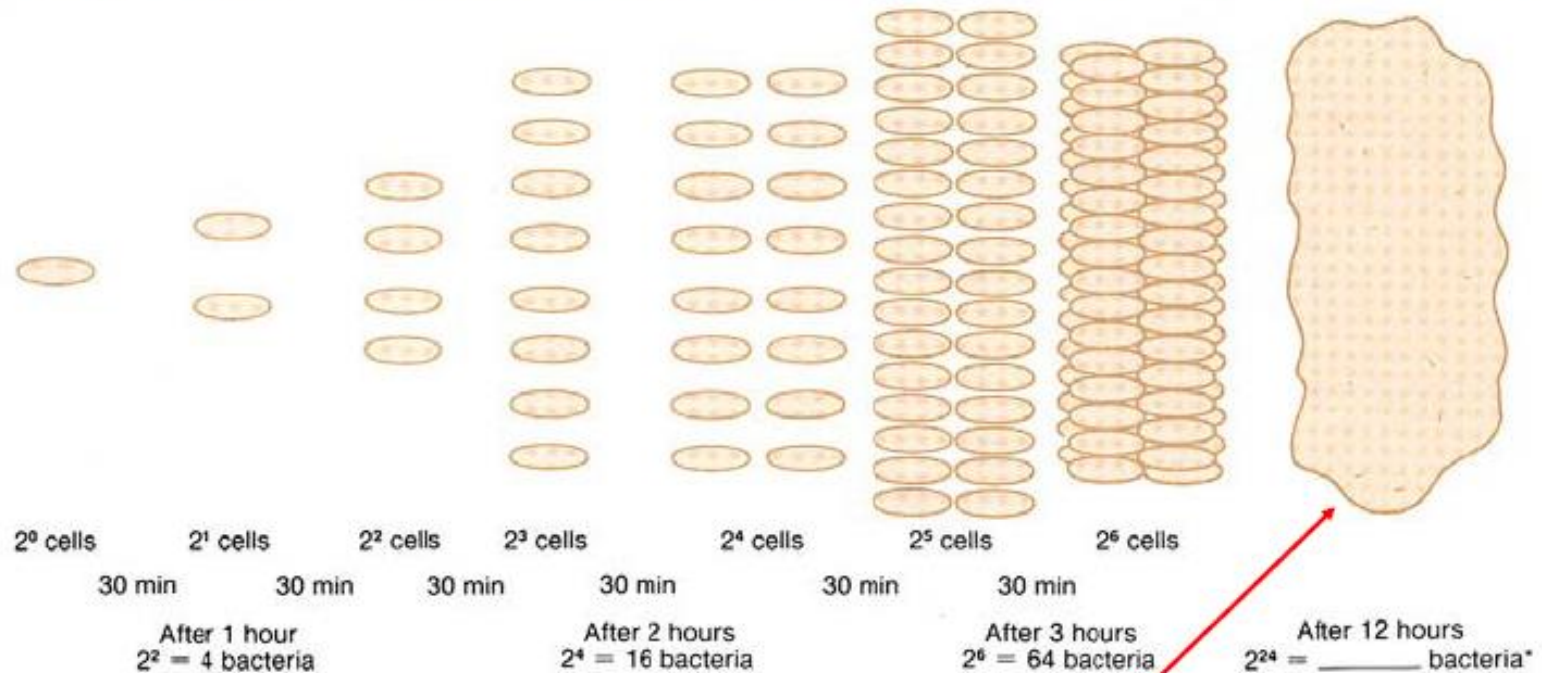


1. The lag phase: Once the substrate is added, the initial growth of the bacteria is zero or not detectable. Growth begins after a period of time. This is called lag time.
 - This may be due to the initial seed culture has to adapt to the new environment. (pH, temperature, oxygen availability, salt conc.)
 - Or the seed culture has to adapt to the new substrate
 - Or the initial seed culture numbers are too low (see next).



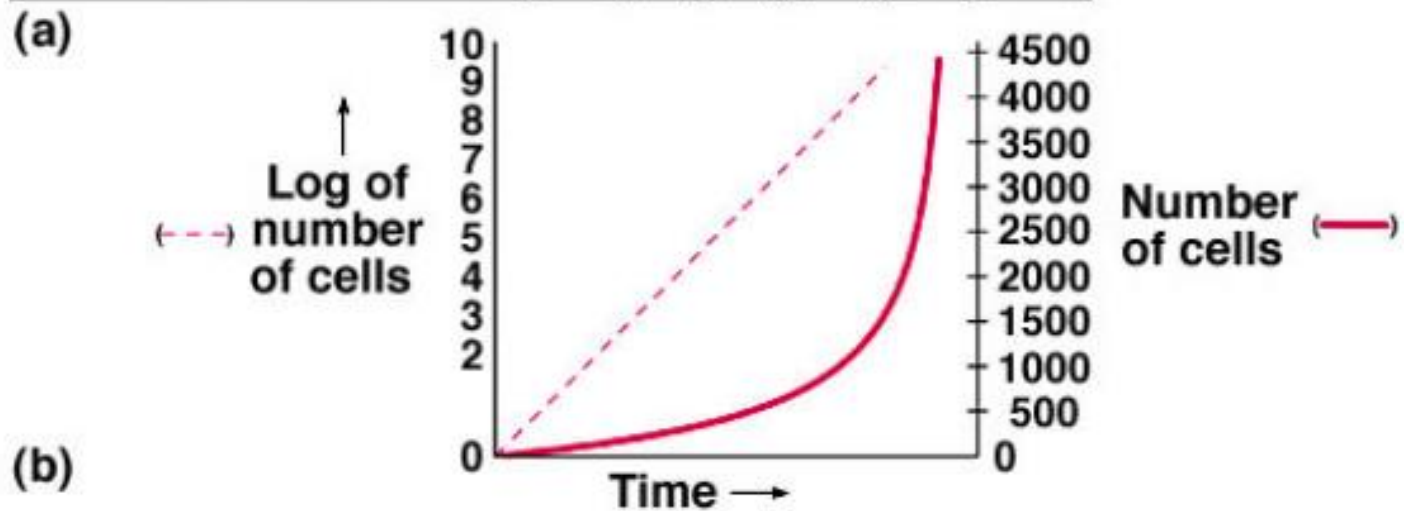
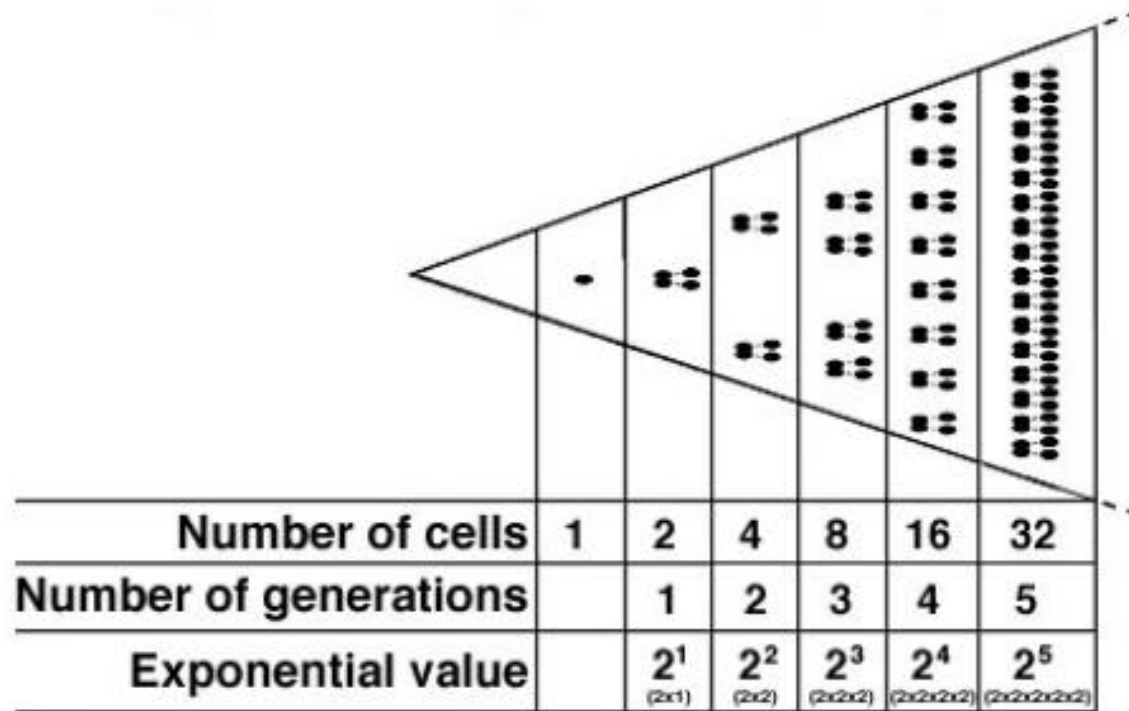
Growth - We are interested in the increase of bacterial population (less interested in the growth of individual cells) due to the infusion of substrate (food) . Under favorable conditions, the cell divides into 2, 4, 8,16.....

Growth Rate – Binary Fission and Generation Time in Bacteria



Colony $2^{24} = 16,777,216$

The Rate of Population Growth – Arithmetic #s vs. logarithms



Population growth vs. individual cell growth

Studying the growth of bacterial populations in batch or continuous cultures does not permit any conclusions about the growth behavior of individual cells, because the distribution of cell size (and hence cell age) among the members of the population is completely random.

What we are observing is averaged behavior.

Generation Time or Doubling Time - the time required to complete a fission cycle from parent cell to 2 new daughter cells.

The length of the generation time is a measure of the growth rate of an organism.

Average Generation Time for bacteria is – 30-60 minutes

Shortest averages 5-10 min.; longest in days

Pathogens like *Salmonella enteritidis* and *Staphylococcus aureus* double in 20-30 minutes

How can one relates the doubling time to a more useful growth rate?

$$X = X_0 \cdot 2^{\left(\frac{t}{t_{double}} \right)}$$

2. The exponential growth phase: The growth of bacteria cells are doubling every generation.

The growth can be represented by this equation: However, the death rate is low at the exponential growth one can ignore it. Here

$$\frac{dX}{dt} = \mu \cdot X - b \cdot X \quad \text{then} \quad \frac{dX}{dt} = \mu \cdot X$$

0

X is the concentration of bacteria or biomass, either weight or number concentration. And μ is the growth rate of the biomass, in [1/ time] unit, such as per hour, b is the decay rate constant [1 / time]

Rearrange:

$$\frac{dX}{X} = \mu \cdot dt \quad \text{And integrate} \quad \int_{X_0}^X \frac{dt}{X} = \int_0^t \mu \cdot dt$$

One gets:

$$X = X_0 \cdot e^{\mu \cdot t}$$

And since X number doubles in doubling time:

$$\frac{X}{X_0} = 2 = e^{\mu \cdot t_{double}} \quad \text{or} \quad \mu = \frac{0.693}{t_{double}}$$

Or one can also calculate μ from measurement

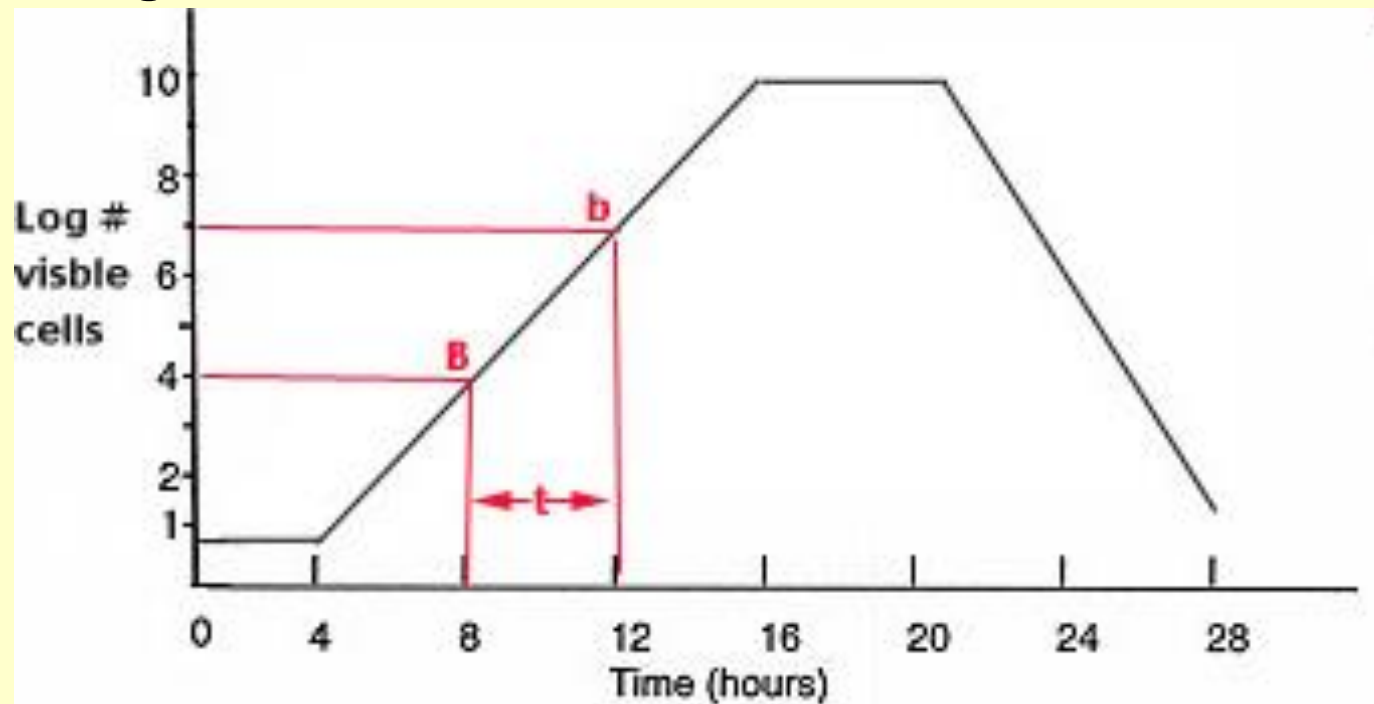
$$\mu = \frac{\ln(X_2) - \ln(X_1)}{t_2 - t_1}$$

For example, If a culture has a μ of 0.02/h, it doubles about every 34.6 h

For example. In 4 hrs a culture increases in no. from 1.5×10^5 to 5.5×10^8 , what is the growth rate(μ)?

$$\begin{aligned} \mu &= \frac{\ln(5.5 \times 10^8) - \ln(1.5 \times 10^5)}{4 \text{ [hours]}} = \frac{20.03 - 11.9}{4} \\ &= 2.05 \text{ per hour} \end{aligned}$$

Example: What is the generation time (doubling time) of a bacterial population that increases from 10,000 cells to 10,000,000 cells in four hours of growth?



$$\text{Gen time} = \frac{t}{3.3 \log b/B}$$

$$\text{Gen time} = \frac{240 \text{ minutes}}{3.3 \log 10^7/10^4}$$

$$\text{Gen time} = \frac{240 \text{ minutes}}{3.3 \times 3}$$

$$\text{Gen time} = 24 \text{ minutes}$$

$$\mu = \frac{0.693}{t_{\text{double}}}$$

$$\mu = 0.029 \text{ per minute or } 1.73 \text{ per hour}$$

Example Calculation 3.2 Calculation of Mean Generation Time

Following a dilution and plating experiment, the following data were obtained:

At the beginning of exponential growth:

$$t_0 = 0$$

$$X_0 = 1000 \text{ cells/ml}$$

At time $t = 6$ hours:

$$X = 16,000 \text{ cells/ml}$$

Using Eq. 3.3:

$$n = \frac{\ln X - \ln X_0}{0.693}$$

$$n = \frac{\ln 16,000 - \ln 1000}{0.693}$$

$$\therefore n = \frac{9.7 - 6.9}{0.693}$$

And

$$n = \frac{1.204}{0.693} = 4 \text{ generations}$$

\therefore Since there are 4 generations in 6 hours, the mean generation time $= 6/4 = 1.5$ hours.

3. Stationary phase: The number of bacteria is not increase but remain station for this period of time. This is due to the exhaust of the substrate, no food is available for growth. Normally this phase is short

$$\frac{dx}{dt} = \mu \cdot X - b \cdot X \quad \text{And soon} \quad \frac{dx}{dt} = -b \cdot X$$

4. The death or decay phase: In this phase, the bacteria are using their stored components in their cell for energy. Eventually, they have to consume the essential components and lead to their death. During this period recycle of the cellular organics to be used by other species or predation by higher animals predominant. So in this phase the e⁻ acceptor consumption continues.

$$\frac{dx}{dt} = -b \cdot X$$

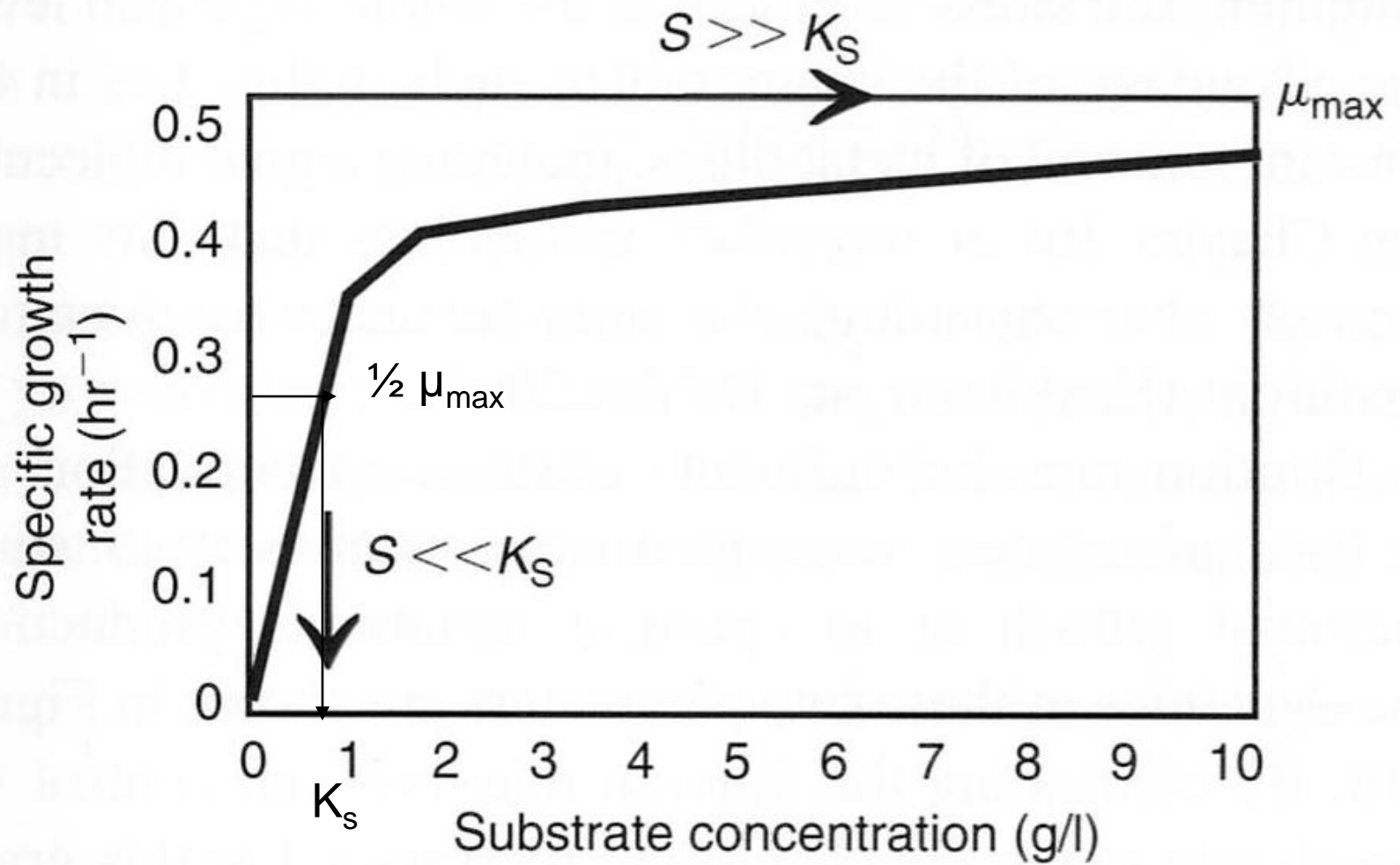
The effect of substrate concentration on the growth rate:
The Monod Kinetics equation is usually used to describe the growth of biomass relating to the LIMITING substrate concentration:

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

where μ is the specific growth rate (1/time), μ_{\max} is the maximum specific growth rate (1/time) for the culture, S is the substrate concentration (mass/volume), and K_s is the half-saturation constant (mass/volume) also known as the affinity constant.

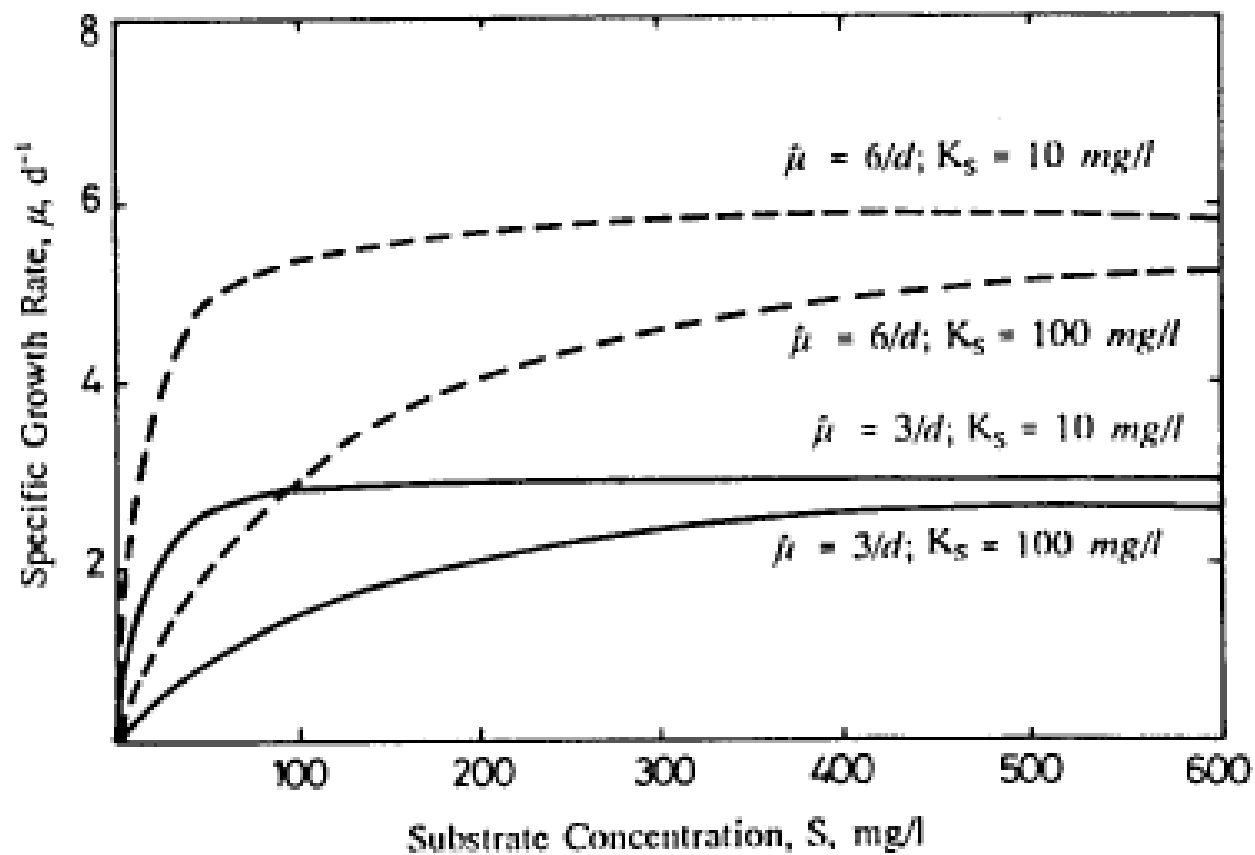
Sometimes, the kinetics can be written for double limiting substrate, For both organics and DO:

$$\mu = \frac{\mu_m \cdot S}{K_s + S} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}}$$

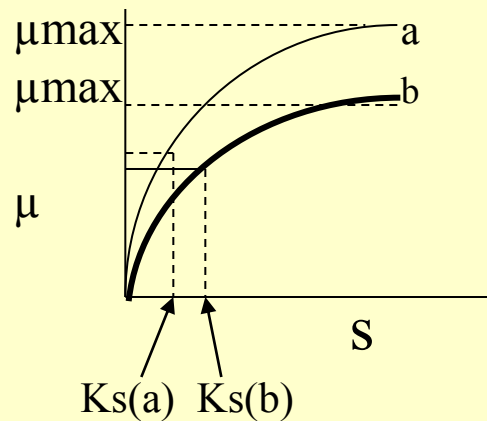


Generation Times/Growth Rates of Different Microbes

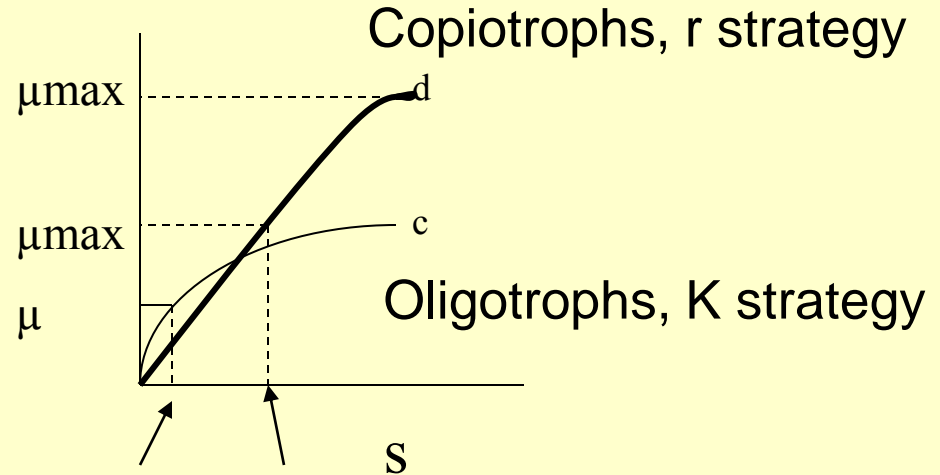
Organism*	Temp.,C**	g,min.(h)		μ_{max} , 1/h	
Habitat					
E. coli	37	20	(0.3)	2.2	Intestines
S.aureus	37	28	(0.47)	1.5	Skin
Ps. putida	30	45	(0.75)	0.9	Soil
Lactobacillus acidophilus	37	75	(1.25)	0.5	Dairy cult. stomach
Mycobacterium	37	360	(6.0)	0.1	TB lung,soil tuberculosis
Nostoc	25	570	(9.5)	0.07	Water, soil
Anabaena	25	840	(14)	0.05	Water,soil
Treponema pallidum	37	1980	(33)	0.02	Syphilitic tissues
Nitrosomonas	20	1980	(33)	0.02	Water, soil
Nitrobacter	20	1500	(25)	0.03	Water, soil
(Domestic sewage	20	300	(5)	0.1-0.2	Biosolids)



Think of K_s as affinity constants that depend upon a cell's "enzyme machinery" and environmental conditions to grow on a substrate (or chemical); low constants make utilizing compounds more efficient.



$K_s(a) < K_s(b)$
 $\mu_{max}(a) > \mu_{max}(b)$
 "a" grows faster than "b"



$K_s(c) < K_s(d)$
 $\mu_{max}(c) < \mu_{max}(d)$
 "c" grows faster than "d" @ low S
But μ_{max} of "d" > "c" at high S

Information Box 3.1 The Monod Growth Constants

Both μ_{\max} and K_s are constants that reflect:

- The intrinsic properties of the degrading microorganism
- The limiting substrate
- The temperature of growth

The following table provides representative values of μ_{\max} and K_s for growth of different microorganisms on a variety of substrates at different temperatures and for oligotrophs and copiotrophs in soil.

Organism	Growth temperature (°C)	Limiting nutrient	μ_{\max} (1/h)	K_s (mg/l)
<i>Escherichia coli</i>	37	Glucose	0.8–1.4	2–4
<i>Escherichia coli</i>	37	Lactose	0.8	20
<i>Saccharomyces cerevisiae</i>	30	Glucose	0.5–0.6	25
<i>Pseudomonas</i> sp.	25	Succinate	0.38	80
<i>Pseudomonas</i> sp.	34	Succinate	0.47	13
Oligotrophs in soil			0.01	0.01
Copiotrophs in soil			0.045	3

Source: Adapted from Blanch and Clark (1996), Miller and Bartha (1989), Zelenev *et al.* (2005).

Oligotrophs, using K selection (low K_s) prefer low concentration. Maintaining slow but consistent growth in the soil environment/ Copiotrophs using r selection (higher μ_{\max}). They can take advantage of the periodical high nutrient released into their environment.

The growth rate can be related to the substrate utilization rate by the yield:

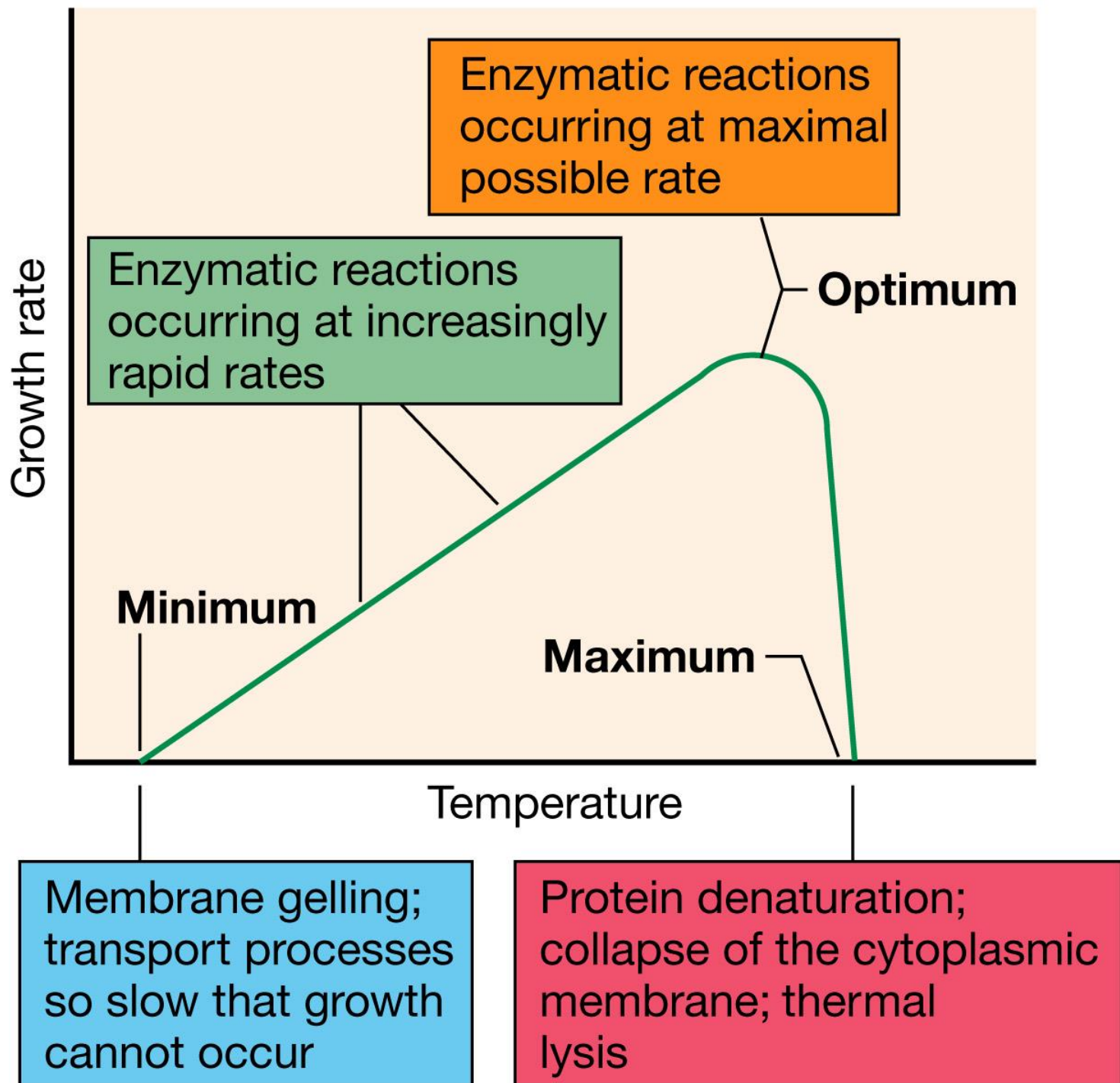
$$\frac{dS}{dt} = \frac{1}{Y} \frac{dX}{dt}$$

Here Y is the yield coefficient: mg of cell material synthesized per mg of substrate used. And

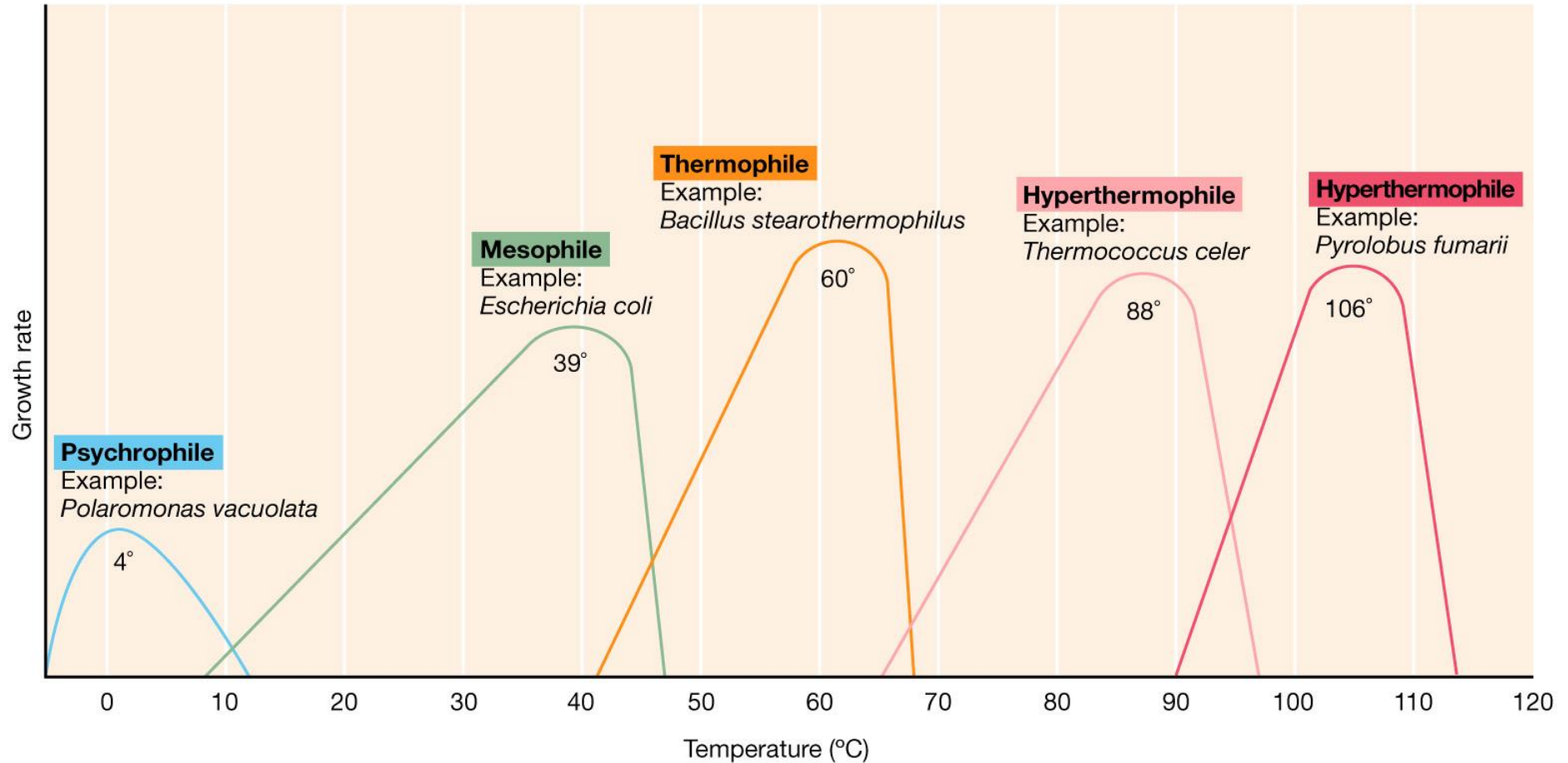
$$\frac{dS}{dt} = - \frac{1}{Y} \frac{\mu_{\max} SX}{K_s + S}$$

The environmental factors affecting the growth rate:

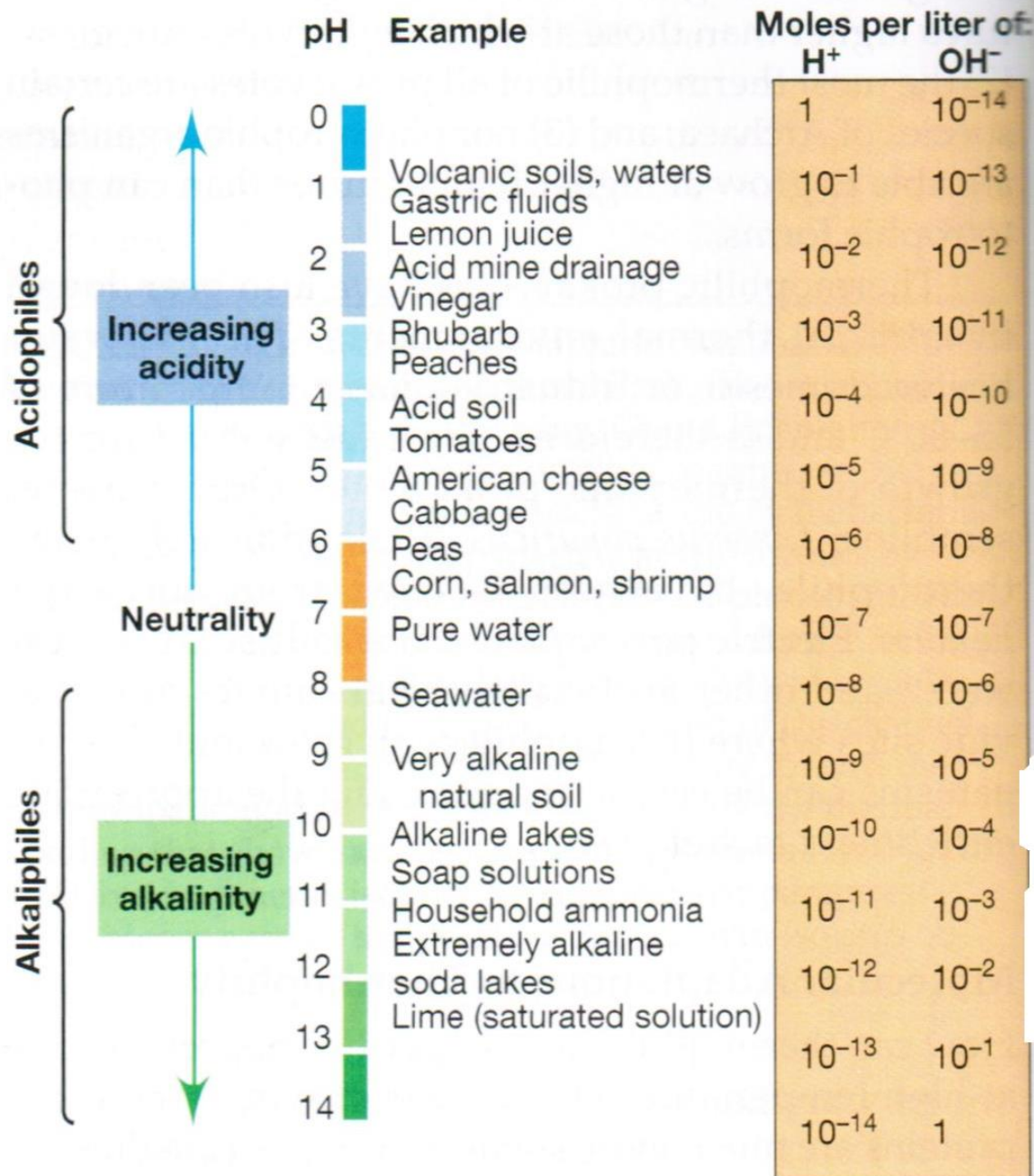
- 1 Temperature**
- 2 pH**
- 3 Dissolved oxygen concentration for aerobic bacteria**
- 4 The presence of some toxic material**



1、Temperature



- 2、 pH
 - Acidophiles:
 - Grow optimally between ~pH 0 and 5.5
 - Neutrophiles
 - Grow optimally between pH 5.5 and 8
 - Alkalophiles
 - Grow optimally between pH 8 – 11.5



3、 DO effct

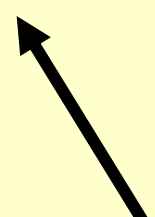
Multiple-substrate limiting kinetics:

$$\mu = \left(\frac{\mu_m \cdot S}{K_s + S} \right) \cdot \left(\frac{S_{O_2}}{K_{O_2} + S_{O_2}} \right)$$

Organics



**Dissolved
oxygen**



Environmental conditions for Biological Growth

1. Dissolved oxygen concentration or Redox condition

Table 2.4 Oxygen and microorganisms (adapted from Madigan and Martinko, 2006)

Group	Relationship to O ₂	Type of metabolism
Aerobes		
Obligate	Required (e.g. 20%)	Aerobic respiration
Facultative	Better if present, not essential	Aerobic or nitrate respiration, fermentation
Microaerophilic	Requires low levels (e.g. 1%)	Aerobic respiration
Anaerobes		
Aerotolerant	Not required, not affected by its presence	Fermentation or sulphate reduction
Obligate	O ₂ harmful or lethal	Fermentation of anaerobic fermentation

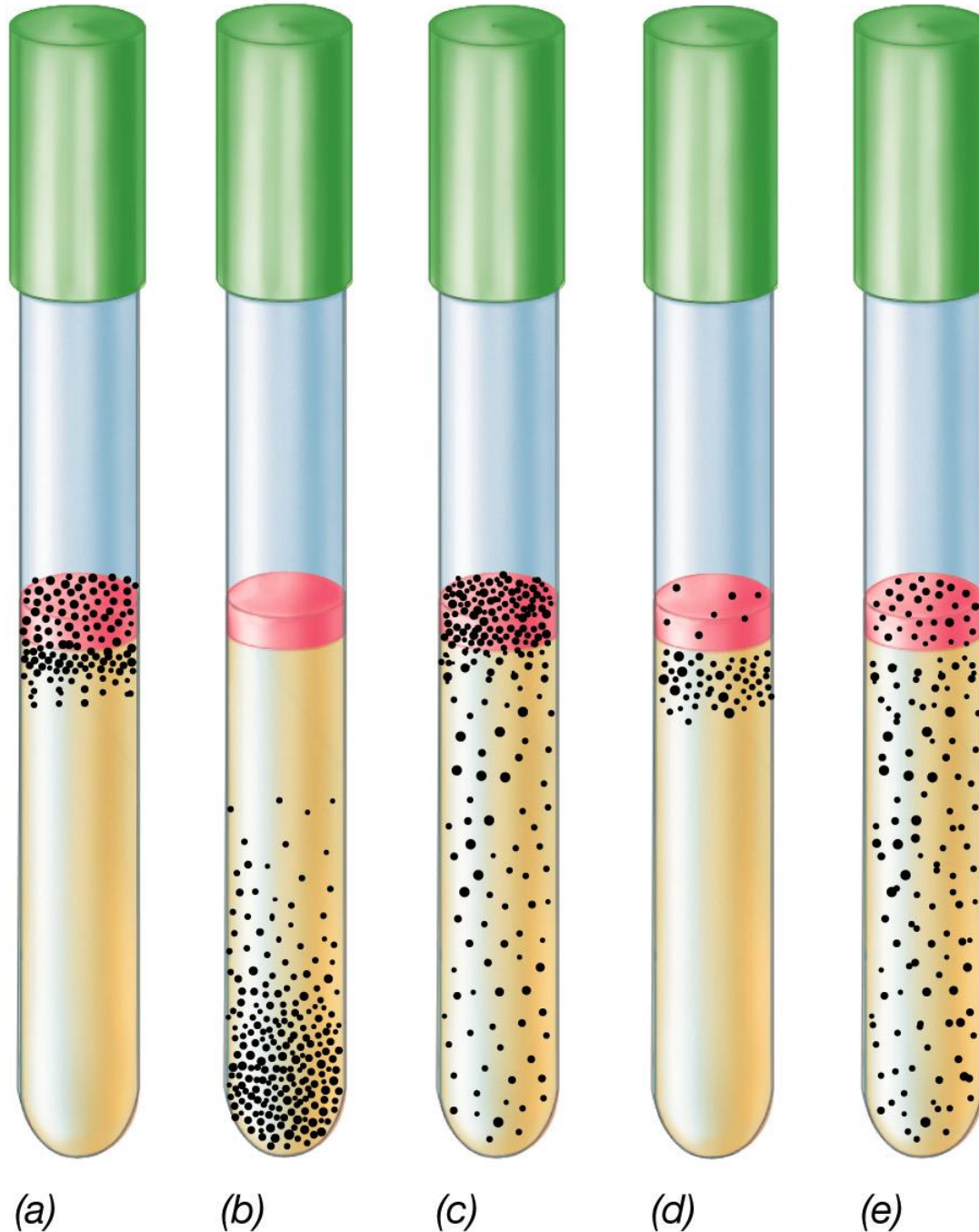
Table 2.5 Engineering definition of some environmental conditions

Condition		Electron acceptor	
		Present	Absent
Aerobic	OX	O ₂	
Anoxic	AX	NO _x	O ₂
Anaerobic	AN		O ₂ and NO _x

NO_x refers to nitrate (NO₃⁻) plus nitrite (NO₂⁻)

Classification of microbes according to their oxygen responses.

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



4、 Presence of toxic material: organic as well as metals are usually toxic to bacteria growth. For example:

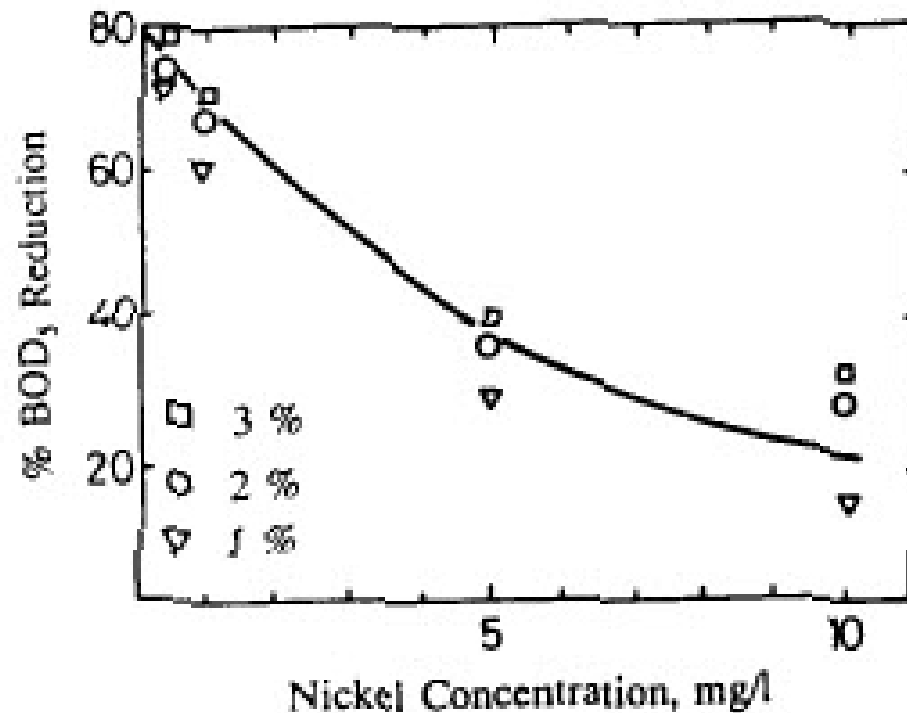
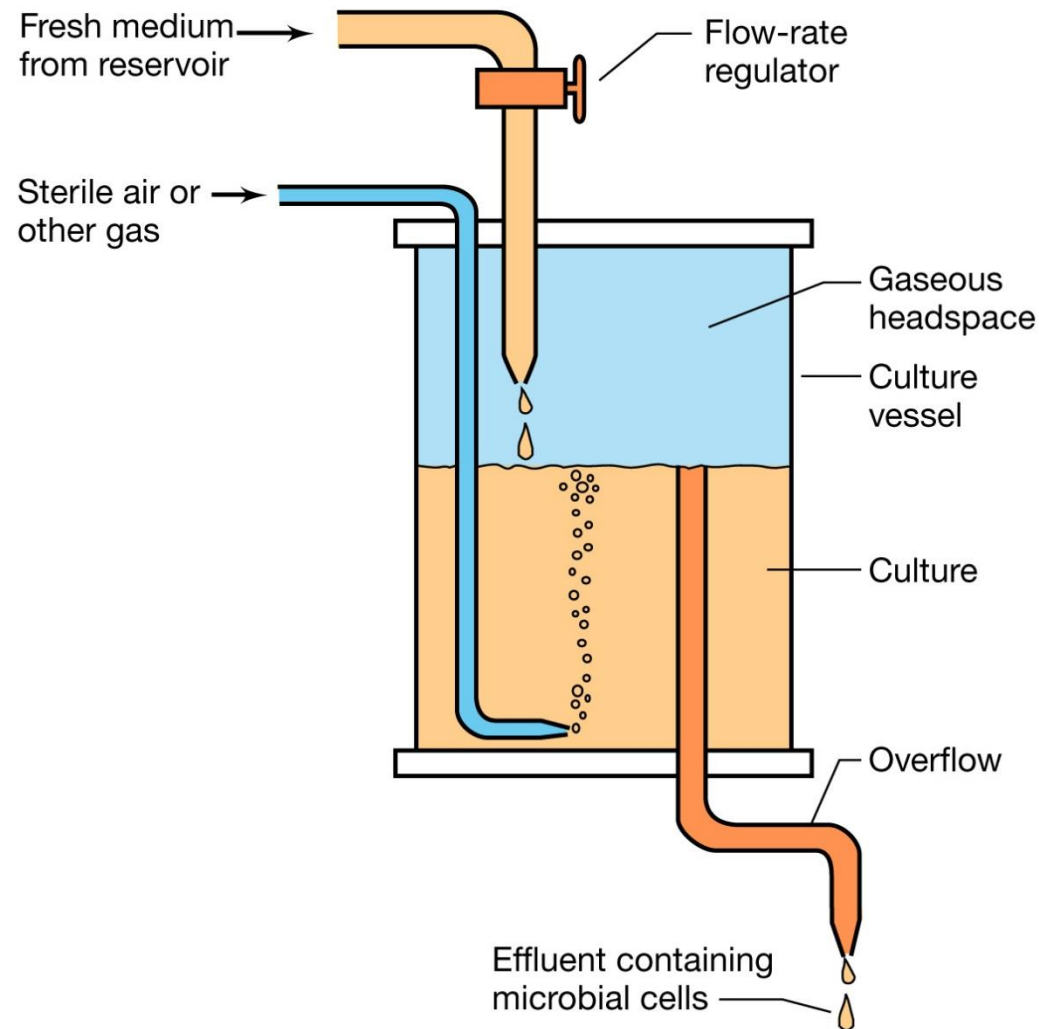


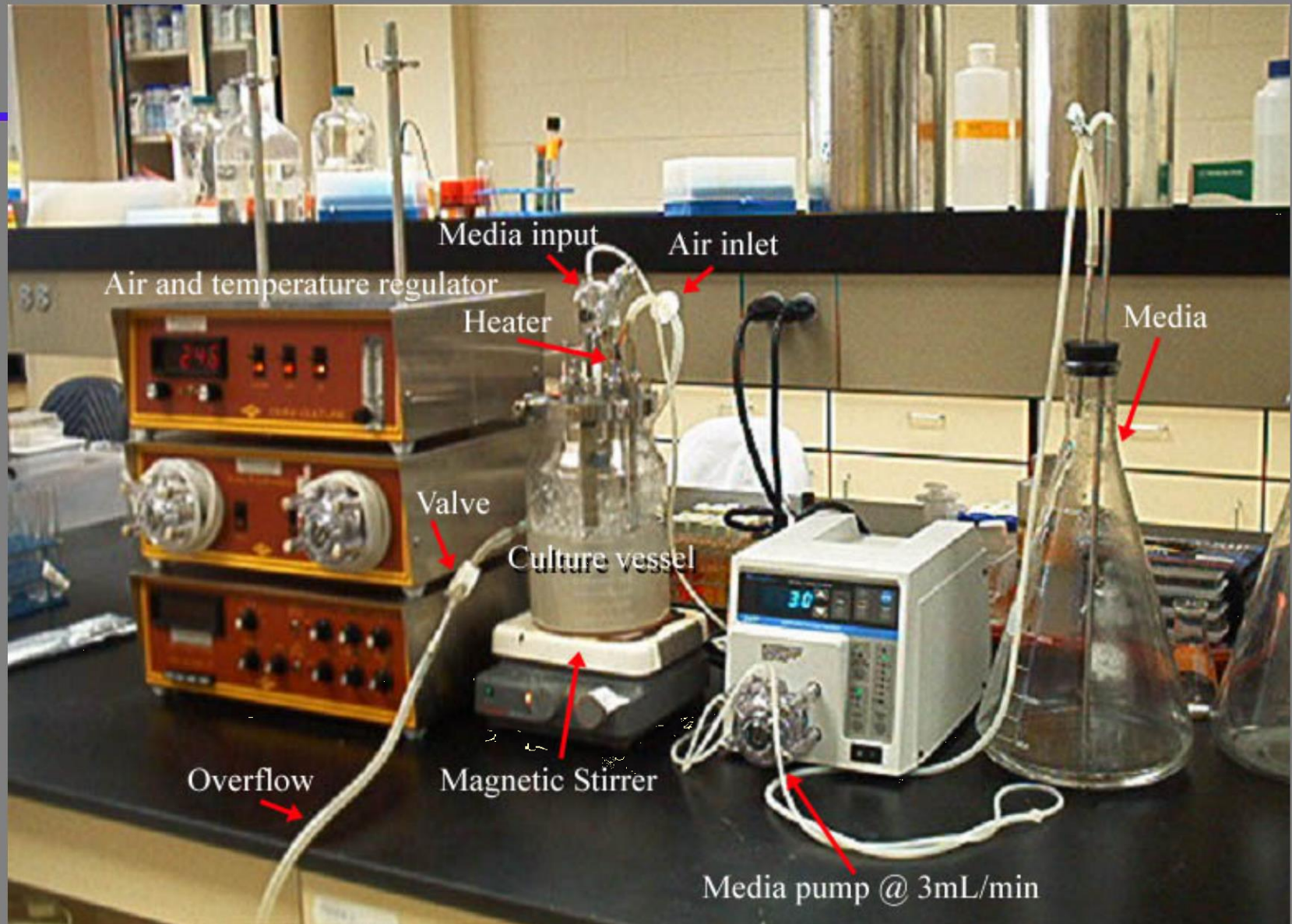
Figure 3.6 BOD reduction due to Ni addition [36].

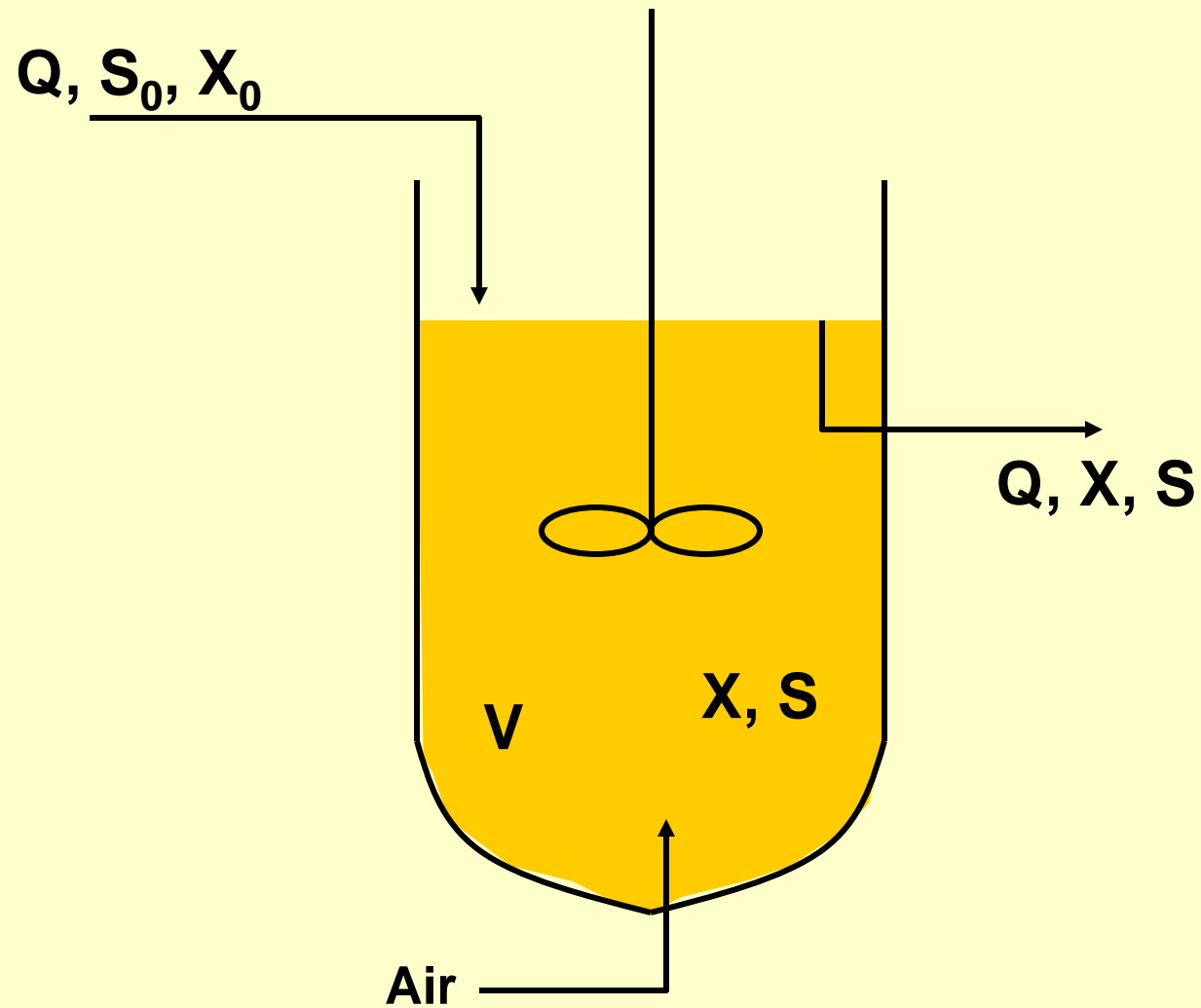
Continuous Culture

Continuous culture: with continuous feed of influent with substrate and biomass growing in the reactor is continuously discharged with the effluent



Our Chemostat System





The growth in a chemostat can best be described by a mass balance equation:

$$\text{Mass accumulation} = \text{Mass in} - \text{Mass out} + \text{Reaction}$$

For biomass: $\left(\frac{dX}{dt}\right) \cdot V = Q \cdot (X_0 - X) + r \cdot V$

$$r = (\mu - b) \cdot X$$

At steady state: $\frac{dX}{dt} = 0$ and $X_0 = 0$, then

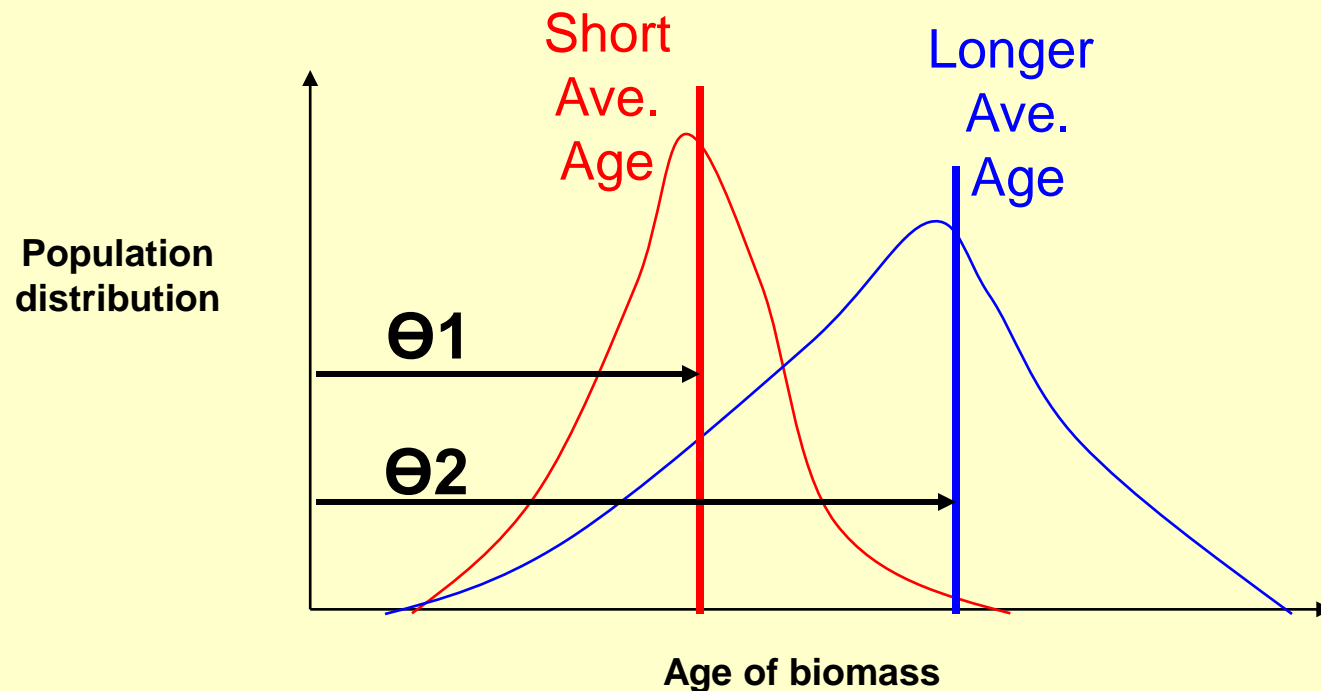
$$X \cdot \left(\frac{Q}{V}\right) = (\mu - b) \cdot X$$

$$\left(\frac{Q}{V}\right) = D = 1/\theta = \mu - b$$

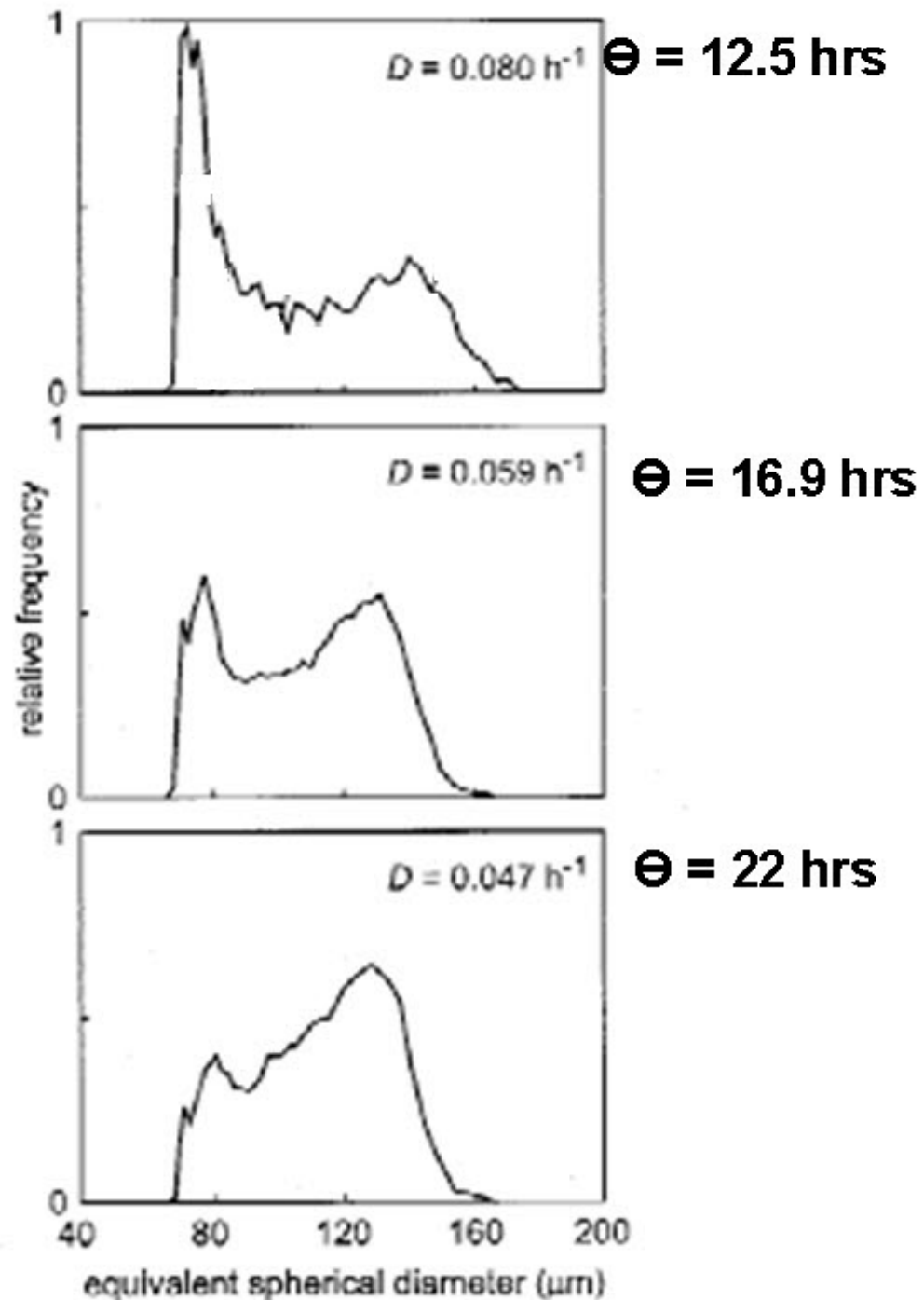
where: D is the dilution rate and θ or T is the hydraulic retention time of the system

Dilution rate is the most important parameter in controlling biological growth in a chemostat or any biological reactors

1. The dilution rate of a reactor is equivalent to the average growth rate of the biomass population in the system.
2. The detention time of the reactor is equivalent to the **AVERAGE AGE** of the population. For example:



An example of age distribution of populations at various dilution rate of a chemostat with protozoa populations.



For substrate concentration:

Since:
$$\mu = \frac{\mu_m \cdot S}{K_s + S}$$

then

$$S = \frac{K_s \cdot (D + b)}{\mu_m - (D + b)} = \frac{K_s \cdot (\frac{1}{\theta} + b)}{\mu_m - (\frac{1}{\theta} + b)}$$

For a fixed bacteria growing on a specific substrate, S, the only control one have is D or Θ or the hydraulic retention time, HRT

Mass accumulation = Mass in – Mass out + Reaction

For substrate: $\left(\frac{dS}{dt}\right) \cdot V = Q \cdot (S_0 - S) - \frac{\mu \cdot X}{Y} \cdot V$

At steady state: $\frac{dS}{dt} = 0$ **then:** $Q \cdot (S_0 - S) = \frac{\mu \cdot X}{Y} V$

$$\frac{Q \cdot Y(S_0 - S)}{V} = \mu \cdot X \quad \text{or} \quad \frac{Y(S_0 - S)}{\theta} = \left(\frac{1}{\theta} + b\right) X$$

$$X = \frac{Y(S_0 - S)}{1 + b \cdot \theta}$$

Again, the controlling parameter is Θ or the HRT

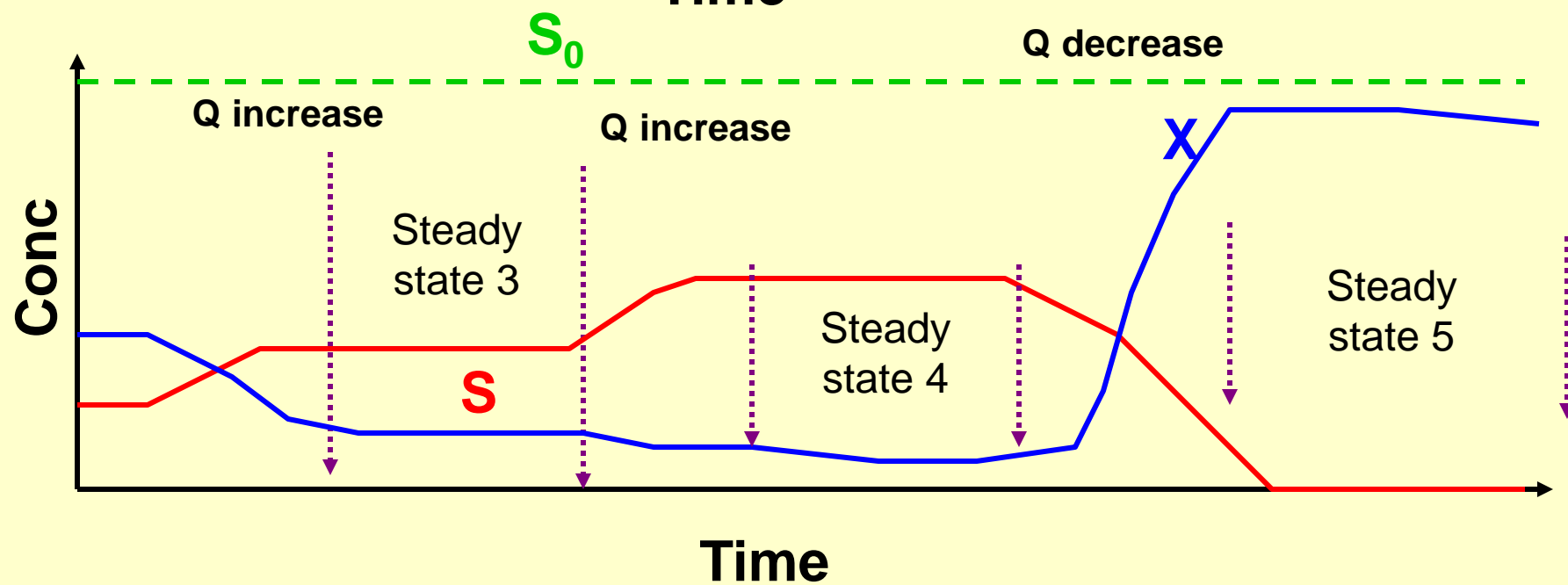
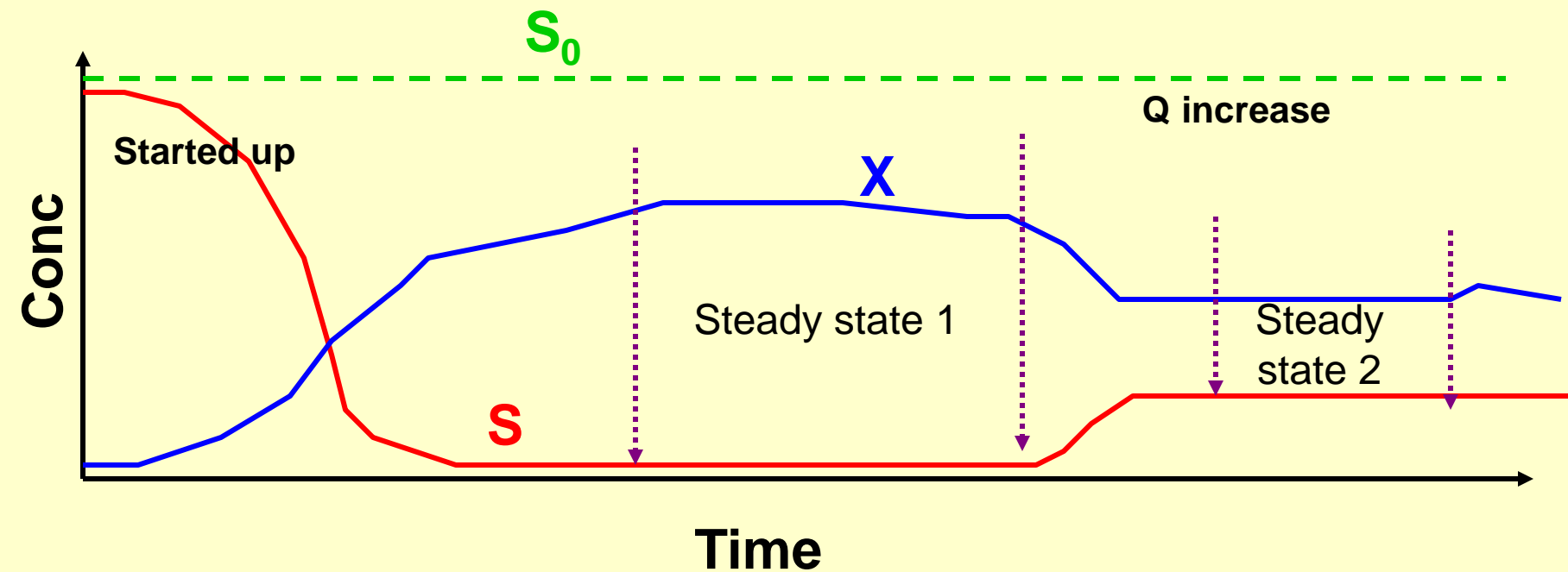
At the washout condition (see next graph), the bacteria can not grow fast enough to be diluted out by the feed rate.

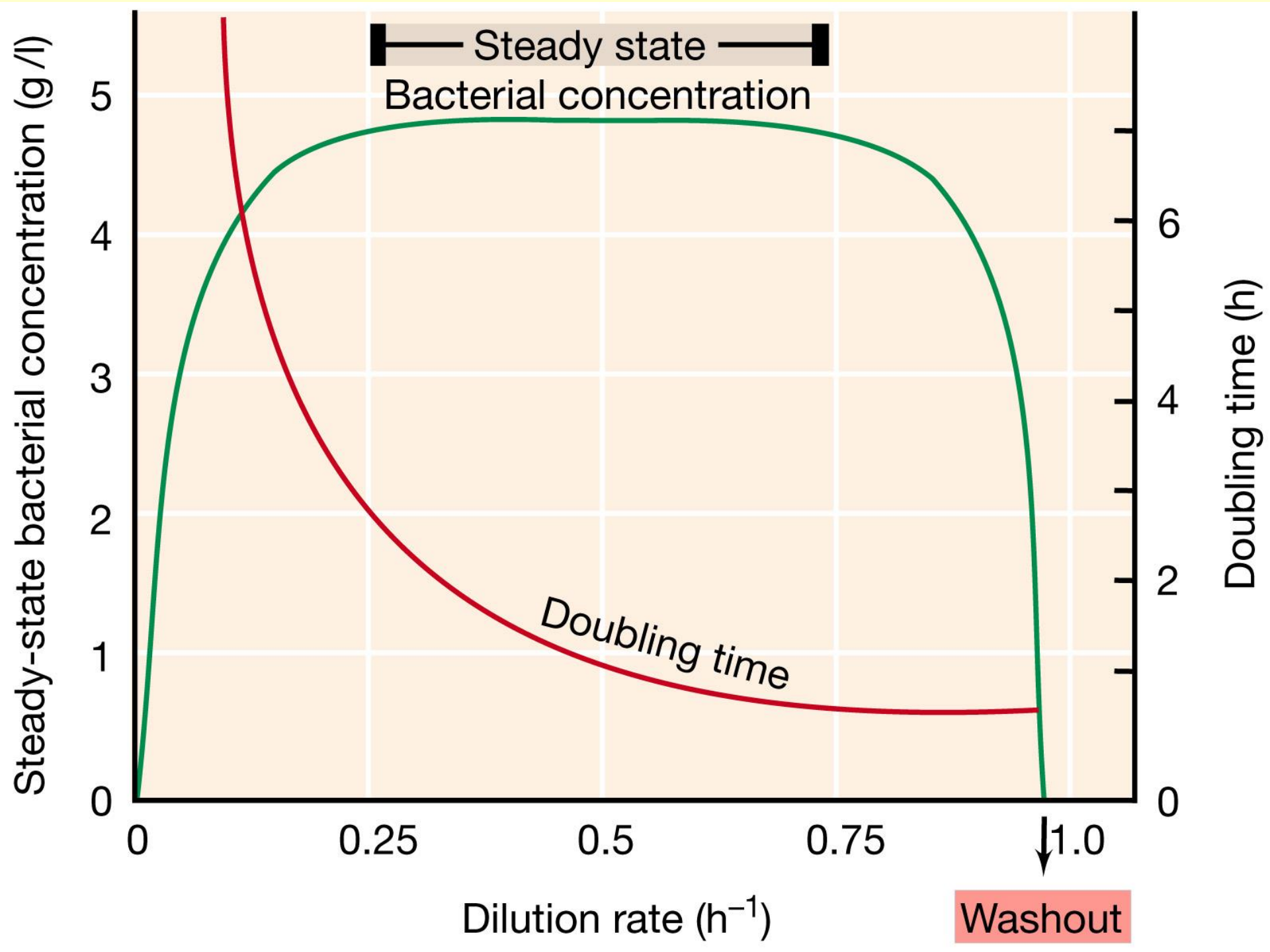
Oxygen requirement:

$$\text{Oxygen Demand input} = Q \times S_0$$

$$\text{Oxygen Demand output} = Q \times S + Q \times 1.42 \times X$$

$$\begin{aligned} \text{Oxygen Consumed in reactor} \\ = Q \times (S_0 - S - 1.42 \times X) \end{aligned}$$





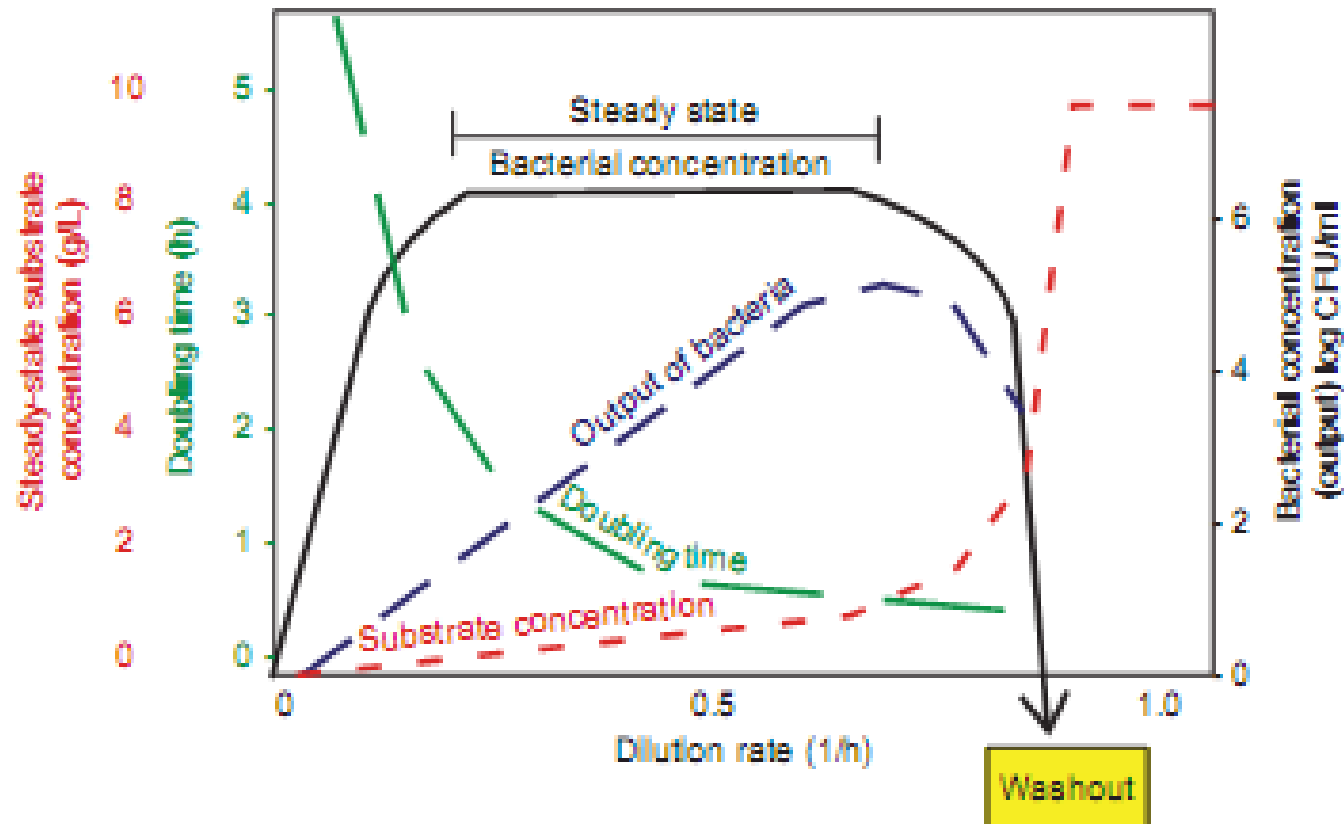
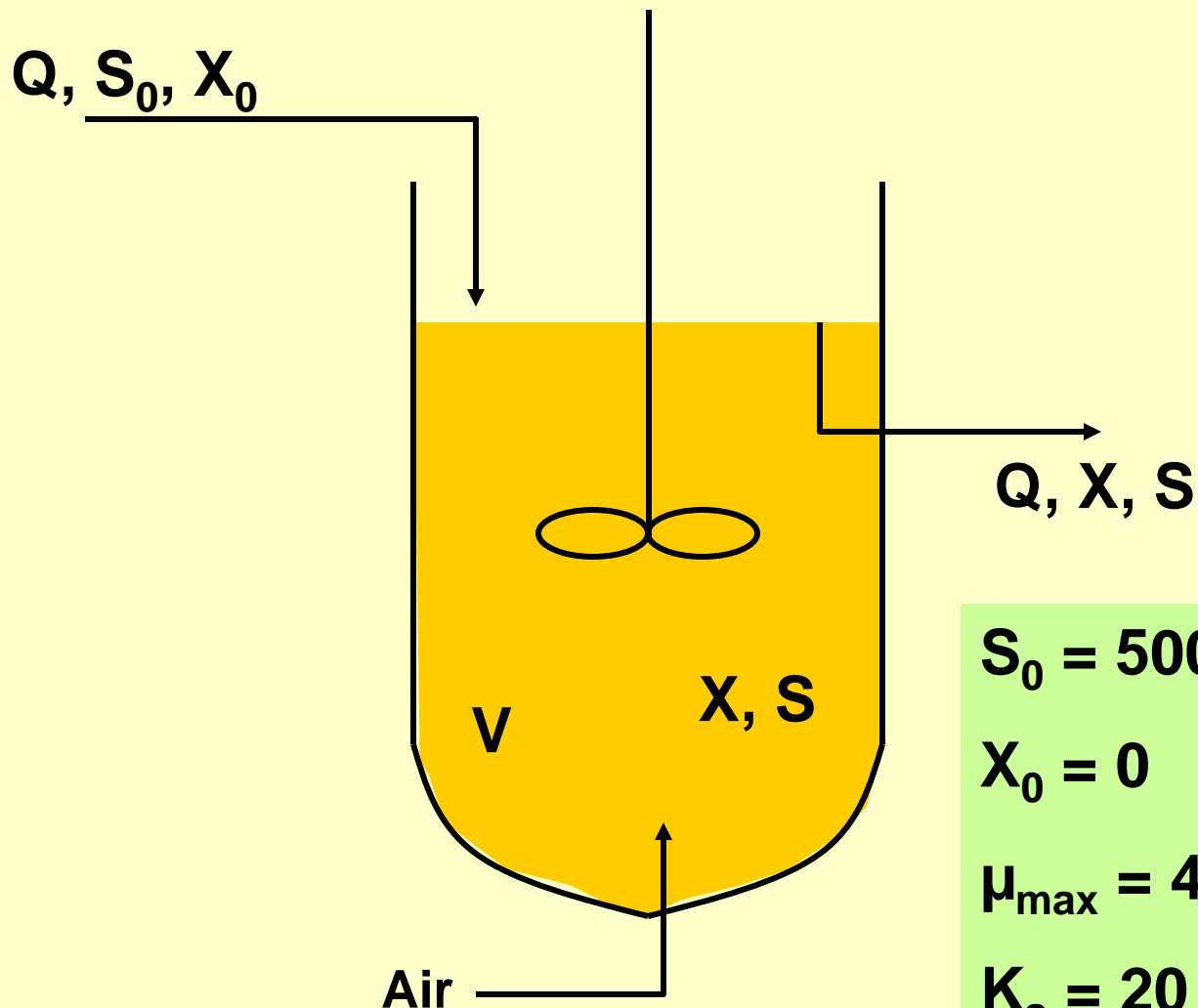


FIGURE 3.10 Steady-state relationships in the chemostat. The dilution rate is determined from the flow rate and the volume of the culture vessel. Thus, with a vessel of 1000 ml and a flow rate through the vessel of 500 ml/h, the dilution rate would be $0.5 L/h^{-1}$. Note that at high dilution rates, growth cannot balance dilution and the population washes out. Thus, the substrate concentration rises to that in the medium reservoir (because there are no bacteria to use the inflowing substrate). However, throughout most of the range of dilution rates shown, the population density remains constant and the substrate concentration remains at a very low value (that is, steady state). Note that although the population density remains constant, the growth rate (doubling time) varies over a wide range. Thus, the experimenter can obtain populations with widely varying growth rates without affecting population density. Adapted with permission from Madigan and Martinko (2006).



$S_0 = 500$ mg/L of COD

$X_0 = 0$

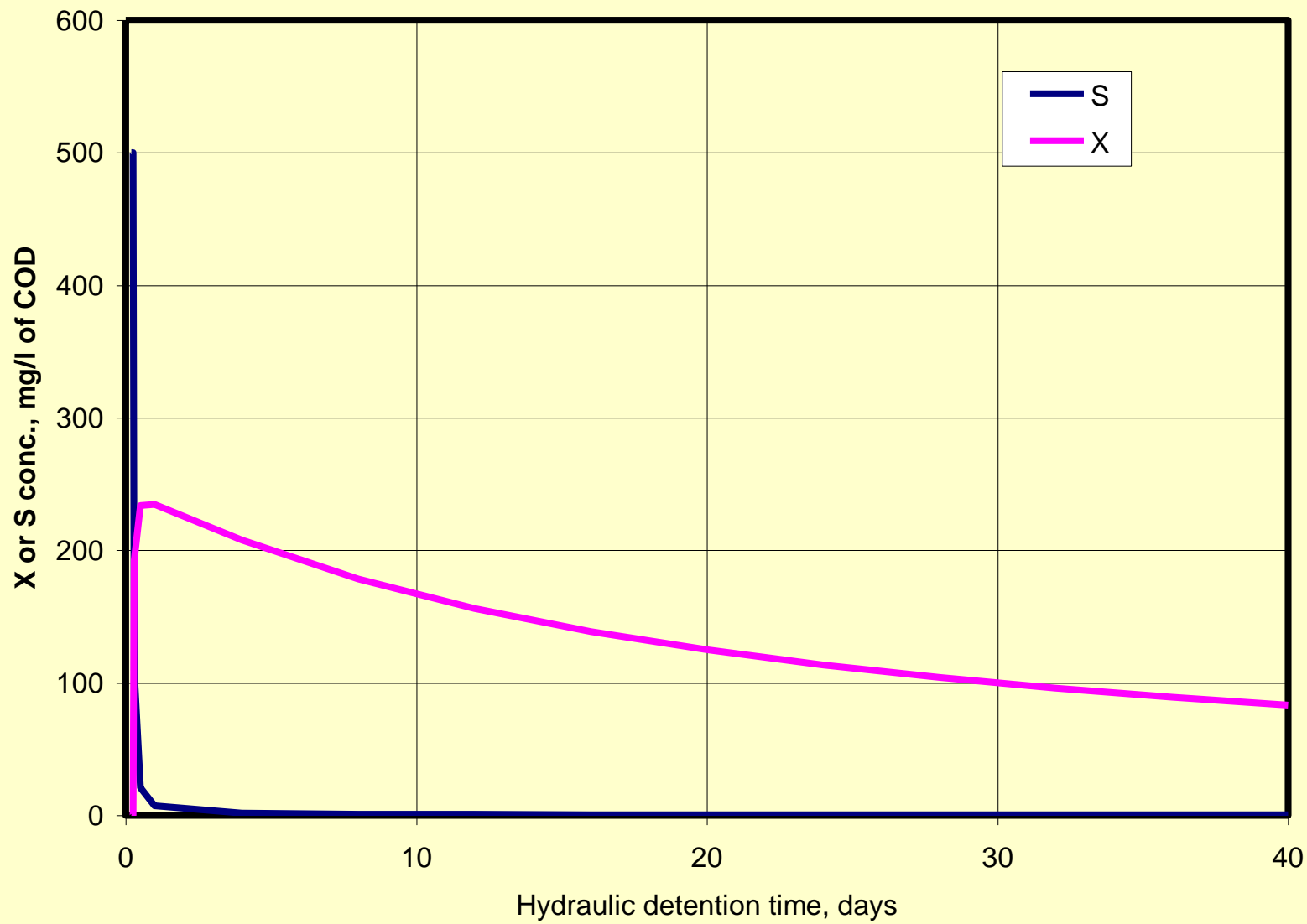
$\mu_{\max} = 4$ per day

$K_s = 20$ mg/L of COD

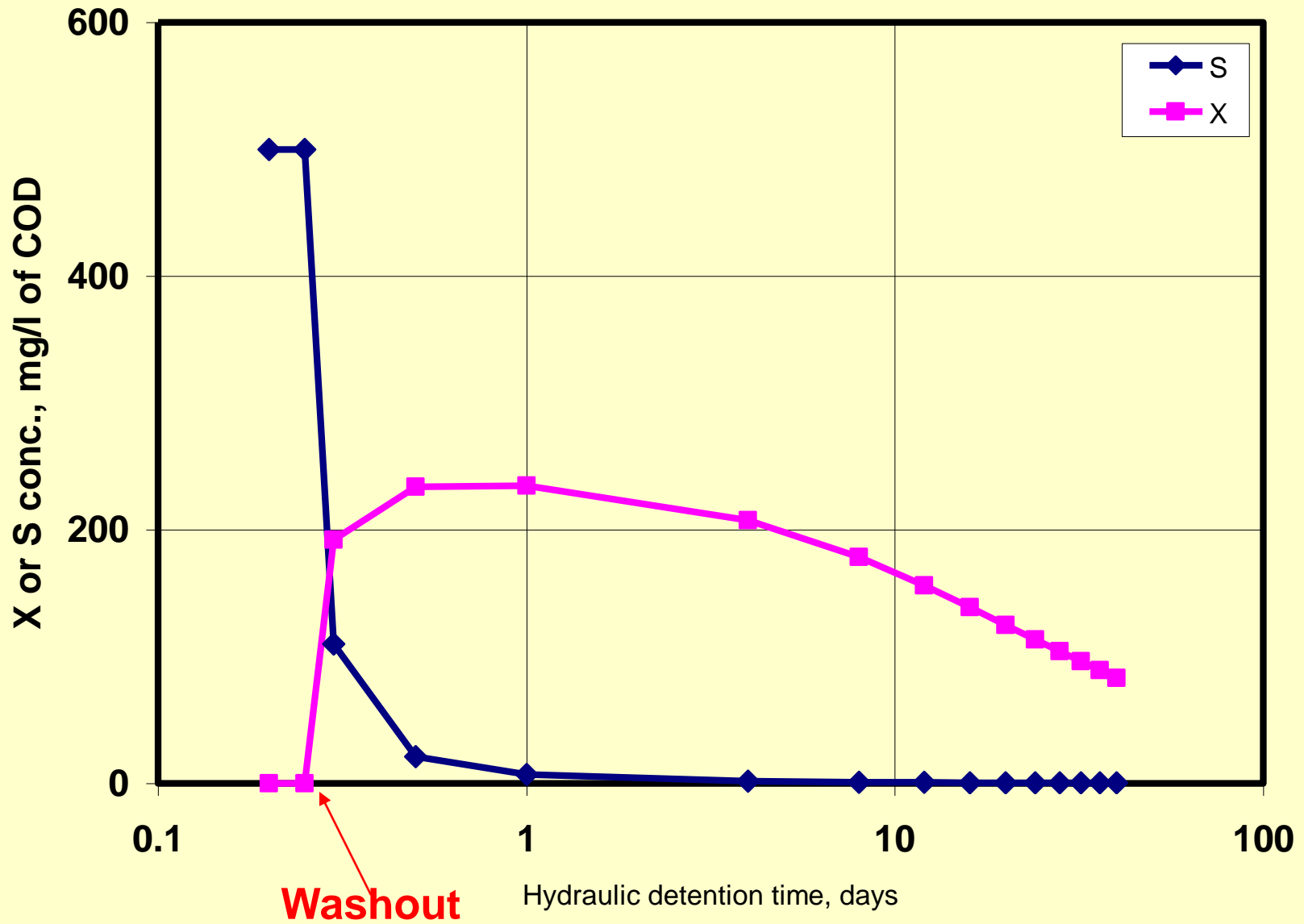
$Y = 0.5$ mg/L of VSS / mg
of COD removed

$b = 0.05$ per day

Steady state relationship in the Chemostat



Steady state relationship in the Chemostat



At wash out condition: $X = 0$ and $S = S_0$

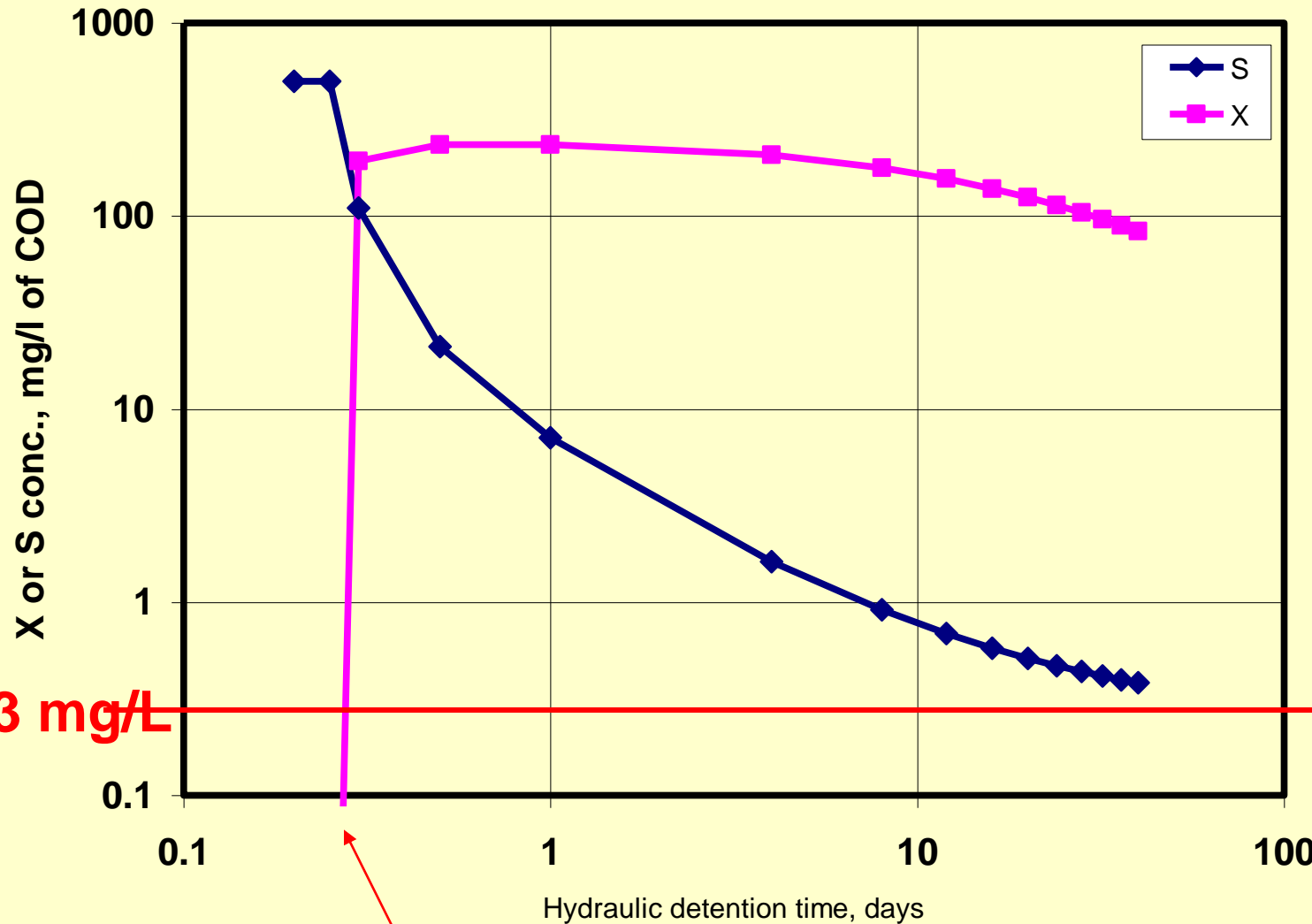
$$\Theta_{\min} = \frac{K_s + S_0}{S_0(\mu_{\max} - b) - bK_s} = 0.26 \text{ days}$$

$$\text{Ultimately } \Theta_{\min} = \frac{1}{\mu_{\max} - b} = 0.25 \text{ days}$$

At $\Theta = \infty$, S will be the lowest value

$$S_{\min} = K_s \frac{b}{\mu_{\max} - b} = 0.253 \text{ mg/L of COD}$$

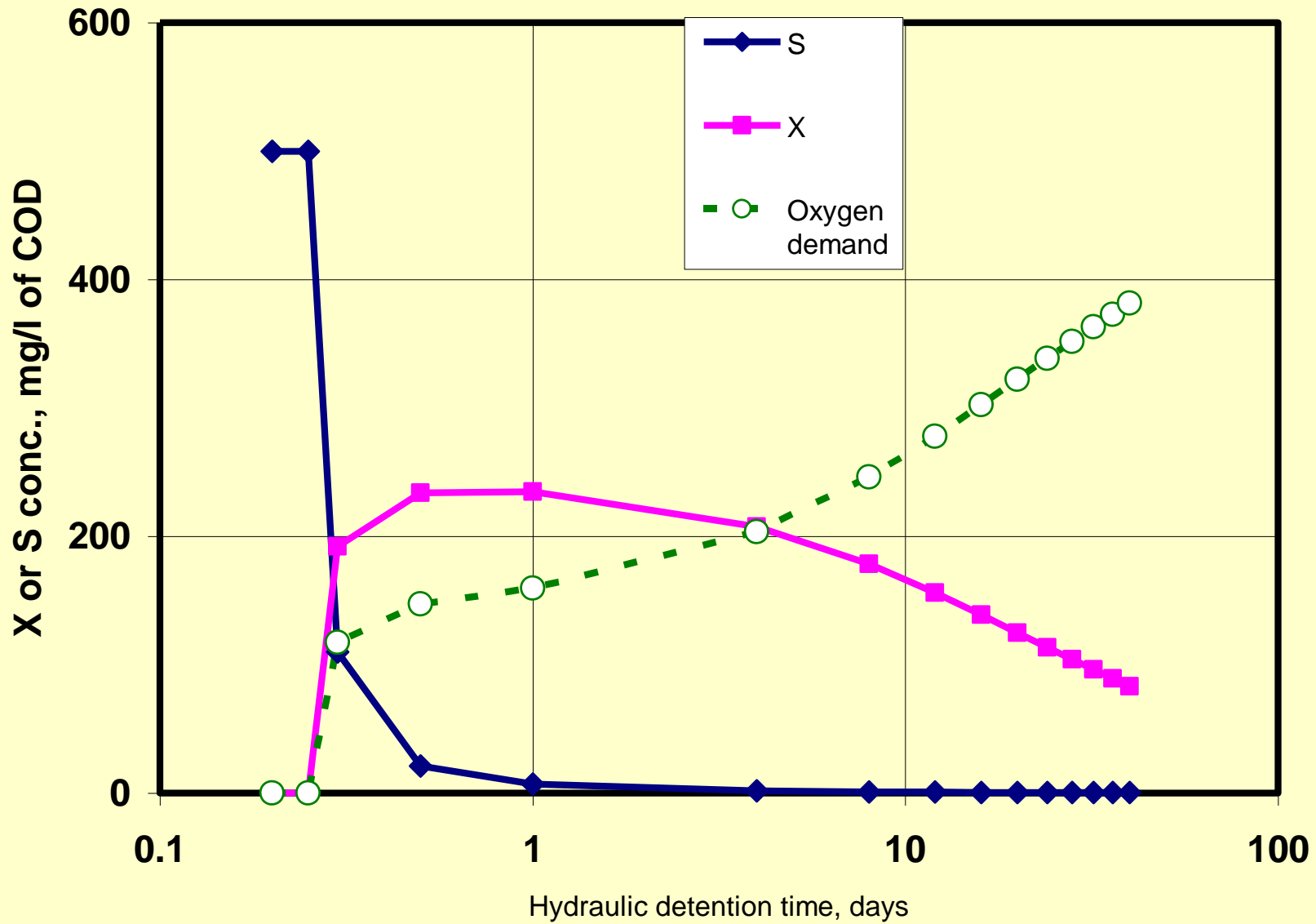
Steady state relationship in the Chemostat



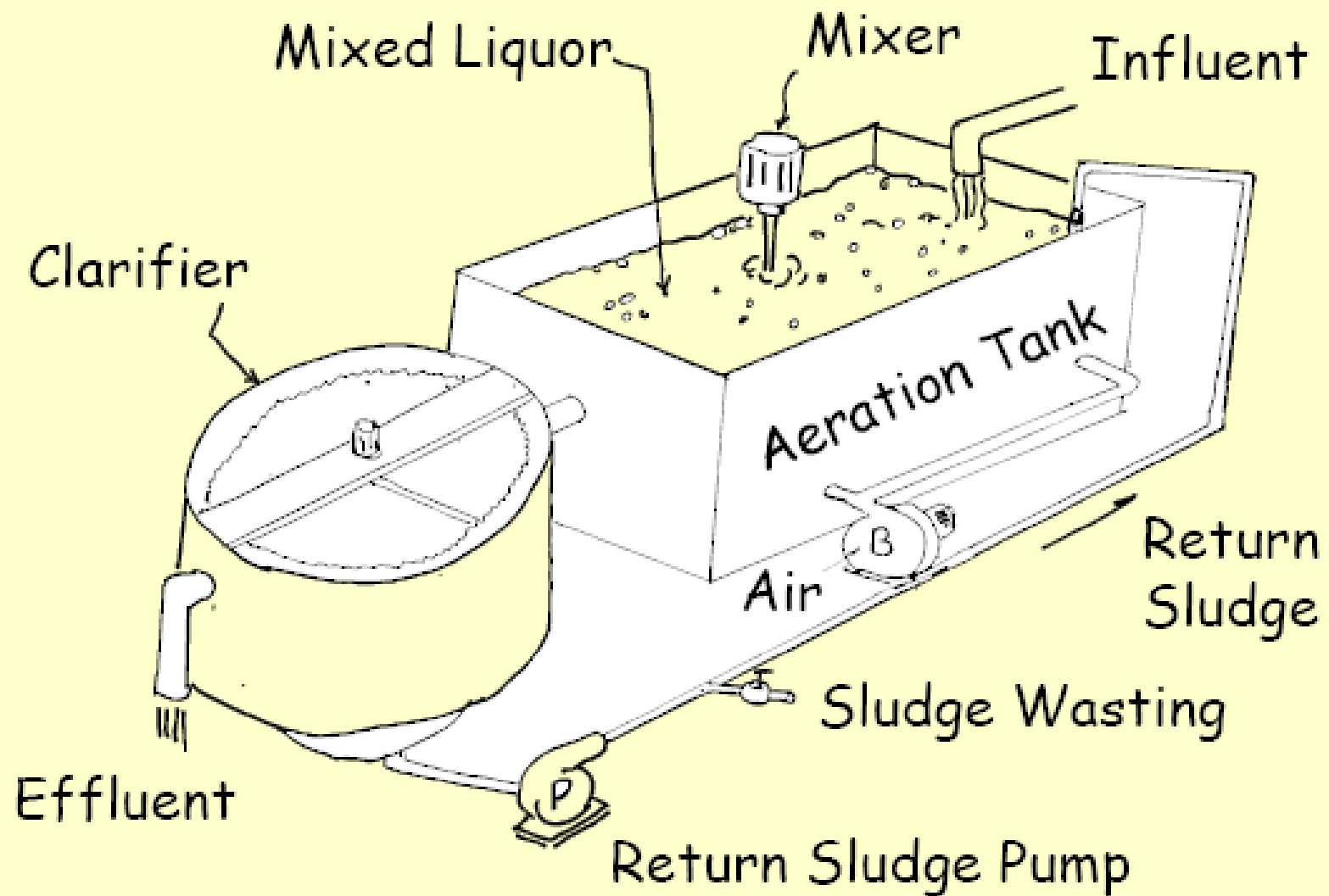
Washout @ $\theta = 0.26$ day

S_{\min}

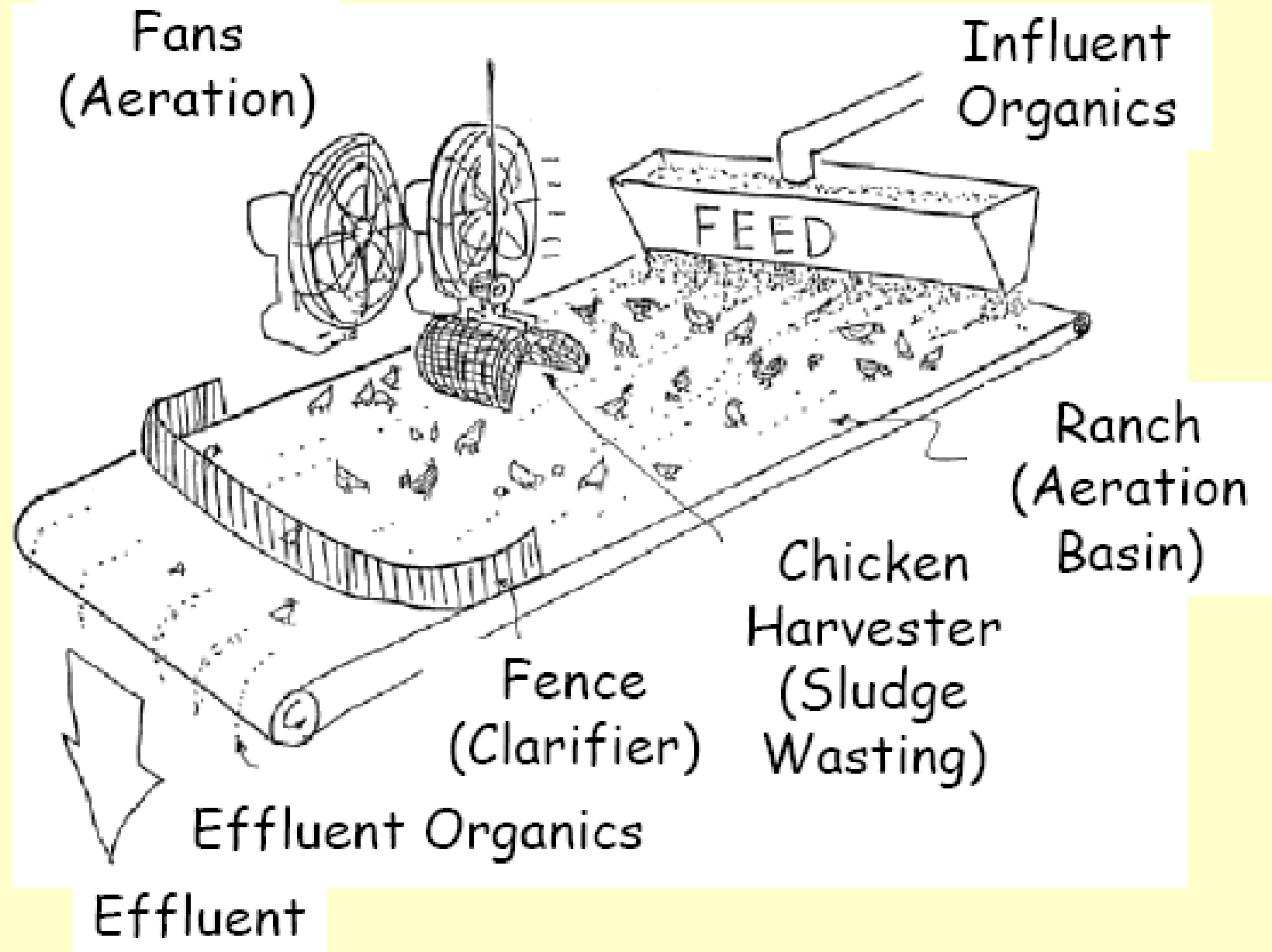
Steady state relationship in the Chemostat



Typical Activated Sludge Treatment Process



Chicken Ranch Theory of Activated Sludge





Rules of Chicken Ranch Operation

- Ranch feed rate is constant and continuous.
- The ranch is fenced in. The fence is good until it is crashed by too much weight.
- Chickens are left alone for their own survival and reproduction.
- Fans are needed for ventilation; otherwise the birds will suffocate.
- The contents of the ranch are completely mixed.
- The ranch is seeded with a small number of birds to start.
- The number of birds increases as time goes by and feed keeps coming in.
- Bird harvesting is the only way to control the population.
- The harvesting is continuous and random. A portion of the population, young and old, is taken out each month.
- After a while, the average age of the bird population is controlled by the amount of harvesting per unit time.

Chicken Age on a Chicken Ranch

If chicken harvesting is continuous and random, after a long time, the average age of the chicken population will be:

$$\text{Average age (months)} = \frac{\text{Total number of birds in ranch}}{\text{Number of birds harvested / month}}$$

- If the harvesting rate is faster than the generating rate of the birds, eventually there will be no chickens left (wash out)
- It takes ~6 months for chicken to mature and start reproducing. The most one can harvest is ~1/6 of the total chickens per month. Average age = 6 months.
- Example: If one harvests 1/10 or 10% of the population per month, the average age will be 10 months.

Sludge Age (SRT) in Activated Sludge

If sludge wasting is continuous and random, after a long time, the average age of the microbial population will be:

$$\text{Average age (days)} = \frac{\text{Total biomass content (aeration tank + clarifier)}}{\text{Mass of biomass wasted / day}}$$

- If the sludge wasting rate is faster than the bacterial growth rate, eventually there will be no biosolids left in the aeration tank and no BOD removal will occur
- Sludge age is controlled by wasting
- Example: If one wastes 20% of the biomass per day, the sludge age would be 5 days

Effect of Bird Age on a Chicken Ranch

Young Population: If one harvests a lot, but below washout rate:

- Bird yield is high (or more bird per unit feed)
- Low number of birds in ranch
- Average age is low (young birds)
- Feed not completely used - lots of feed left on conveyor.

Old Population: If one harvests at low rate

- Bird yield is low (or less bird per unit feed)
- Large number of birds in ranch.
- Less food available per bird.
- Food picked clean by hungry birds; very little food left on belt.
- The starving birds will eat each other

Population Explosion: If one never harvests the birds

- Large number of birds in ranch.
- Birds are starving.
- Birds may eventually overwhelm the fence.

Reduce the Living Space: If one reduces the size of the ranch

- High density of birds per volume
- Birds may suffocate
- Birds may overwhelm the fence

Effect of Sludge Age on Activated Sludge

Young Population: If one wastes a lot of sludge daily (but below washout rate)

- A lot of sludge to dewater and dispose of
- Low concentration of MLVSS
- Low sludge age, high food to mass (F/M) ratio
- Biomass won't attack less degradable organics
- Organics in feed not completely used - higher effluent BOD concentration

Old Population: If one wastes less sludge daily

- Less sludge to dispose of (within limits)
- High MLVSS concentration in aeration basin
- Low food to biomass (F/M) ratio
- Bugs more likely to attack harder to degrade compounds
- Low effluent BOD concentration
- Bacteria will decay (endogenous respiration)

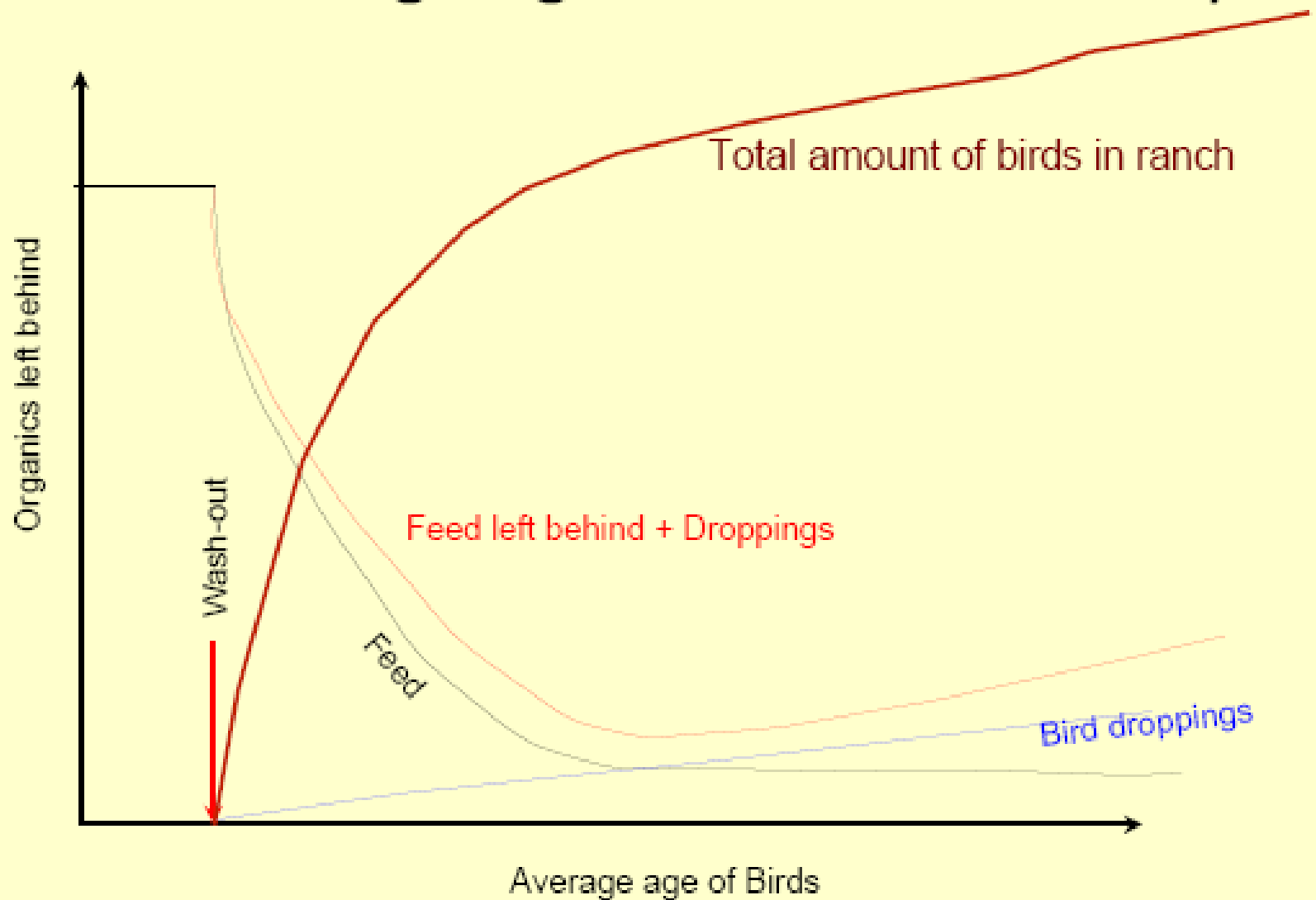
Population Explosion: If one does not waste for a long period of time

- Higher MLVSS concentration in aeration tank
- Most of biomass in decaying condition
- Sludge blanket in clarifier will overflow

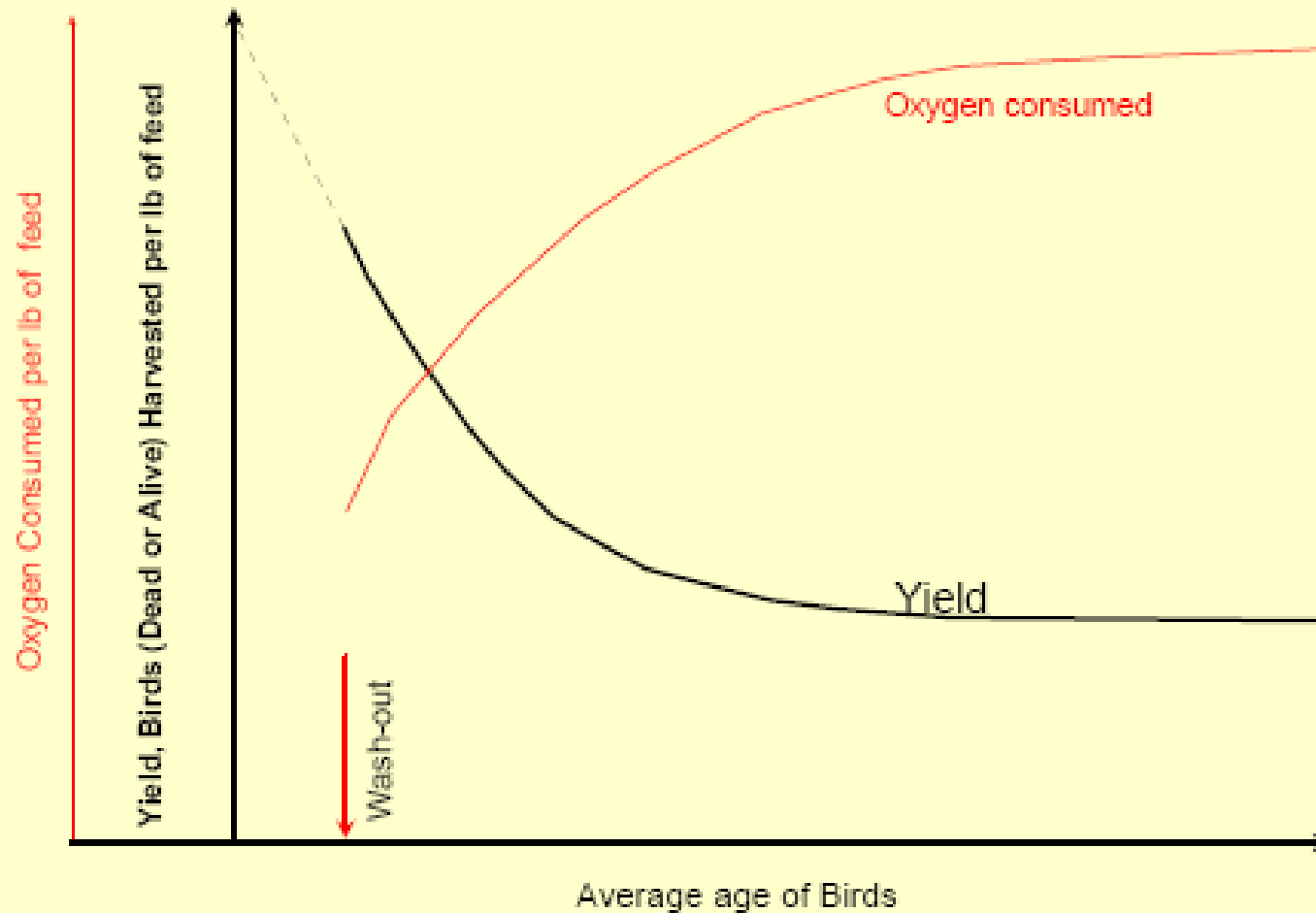
Reduce the Living Space: If one reduces the size of the aeration tank

- High MLVSS concentration
- System may be oxygen limited
- Sludge blanket in clarifier may overflow

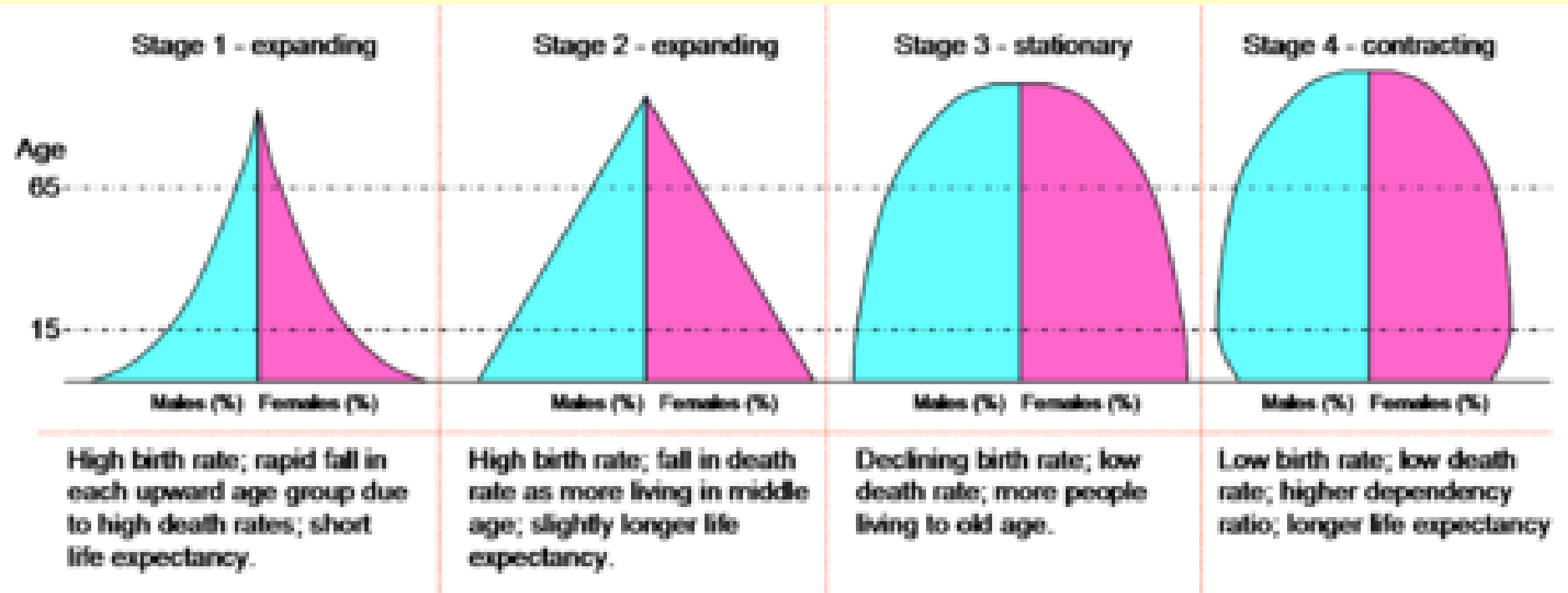
Effect of Sludge Age on Effluent Quality

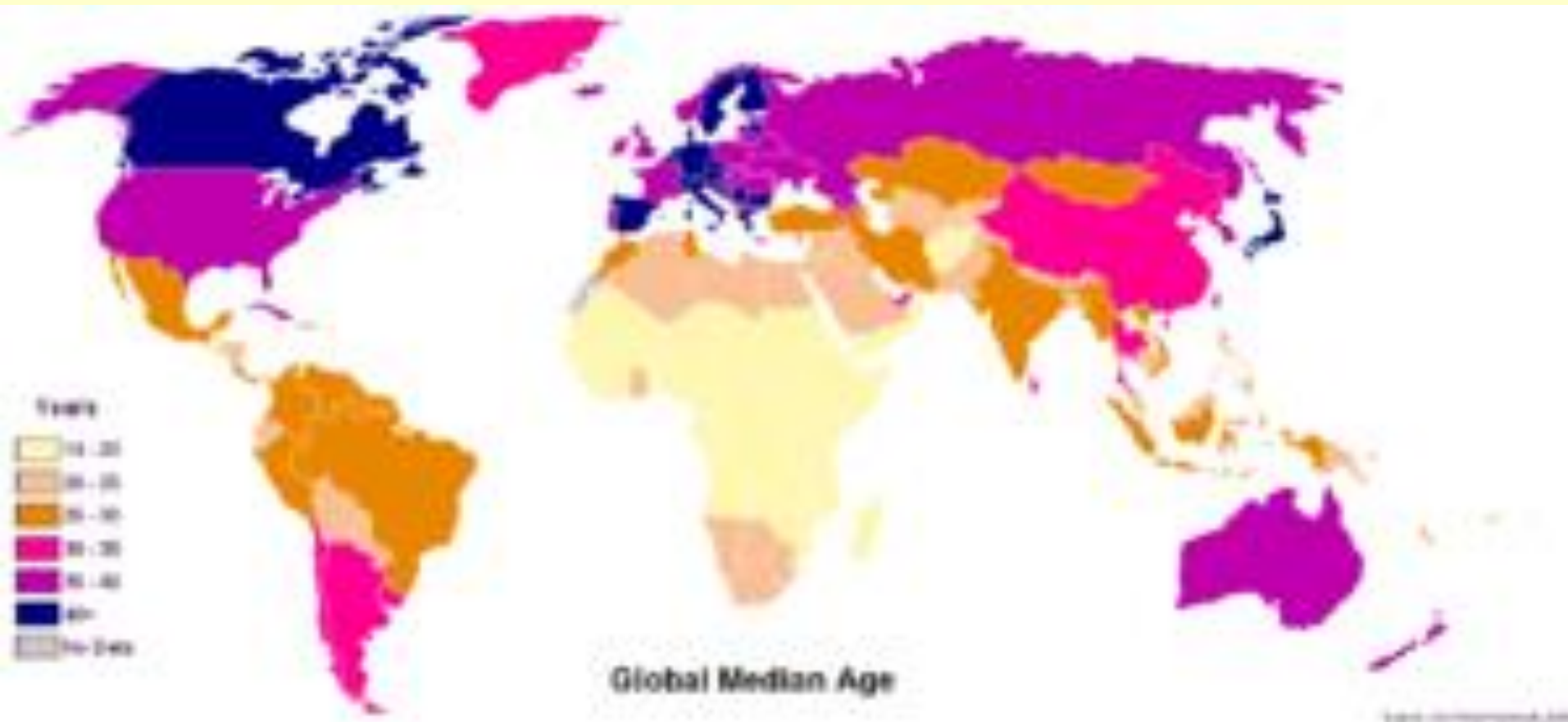


Yield vs. Age



Population pyramid – human population study



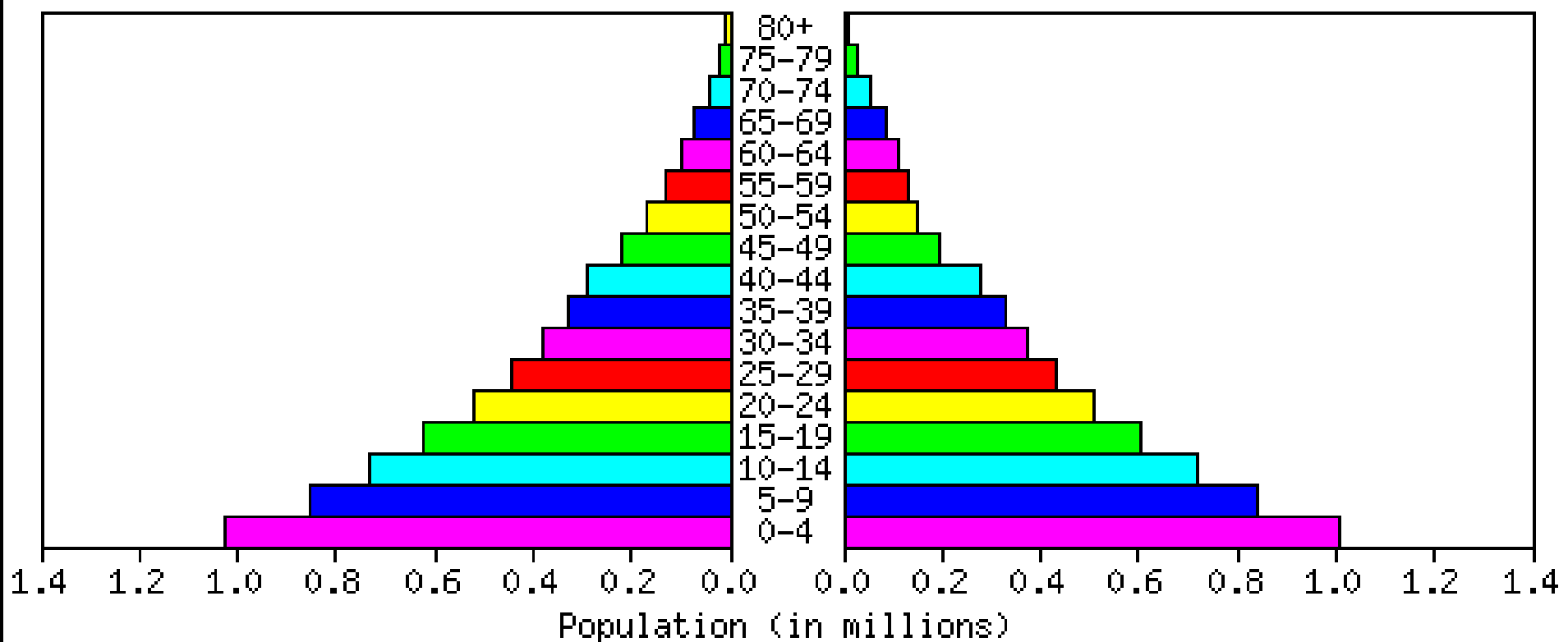


Angola

Angola: 2005

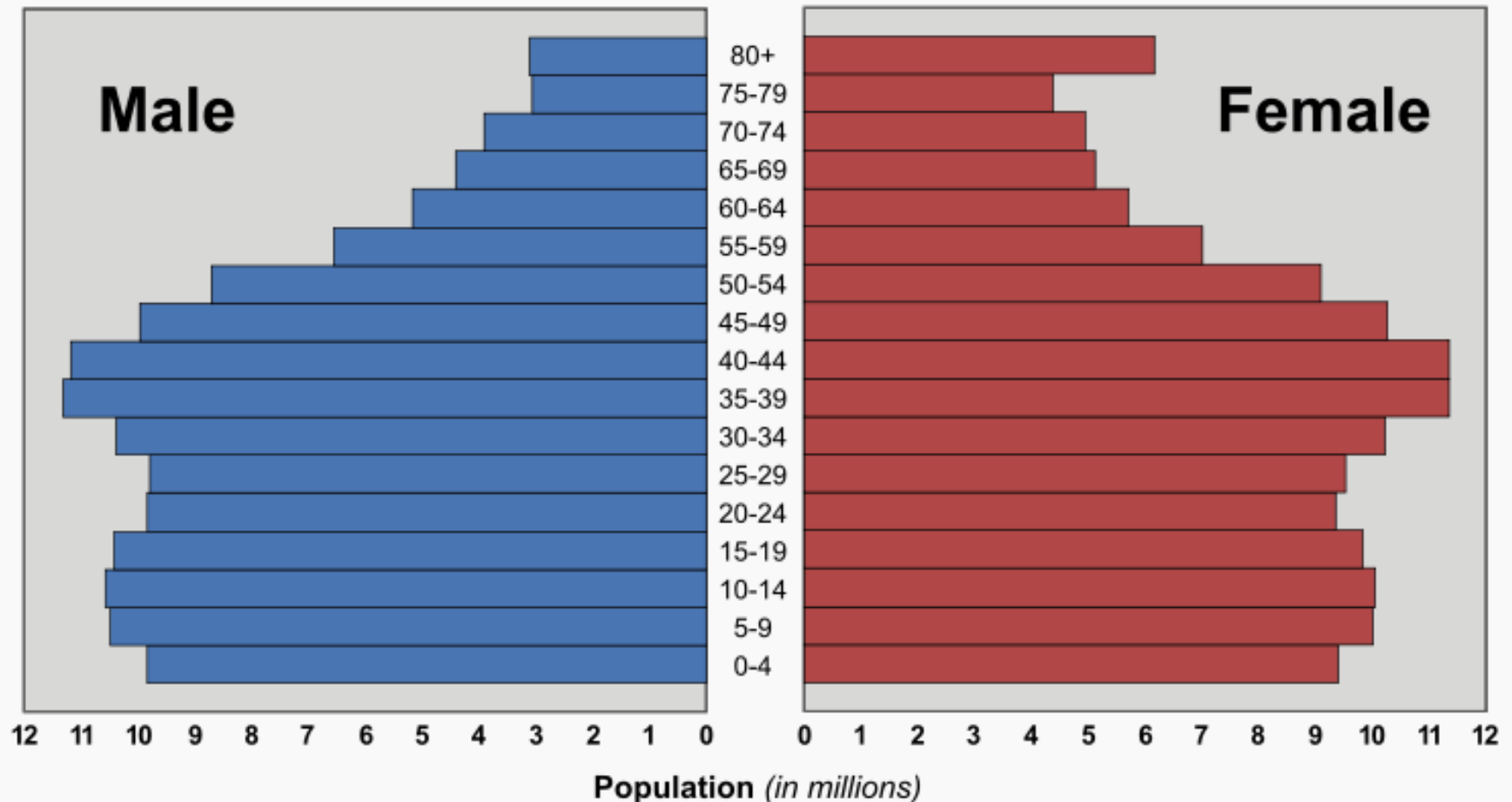
MALE

FEMALE



Source: U.S. Census Bureau, International Data Base.

United States population (2000)

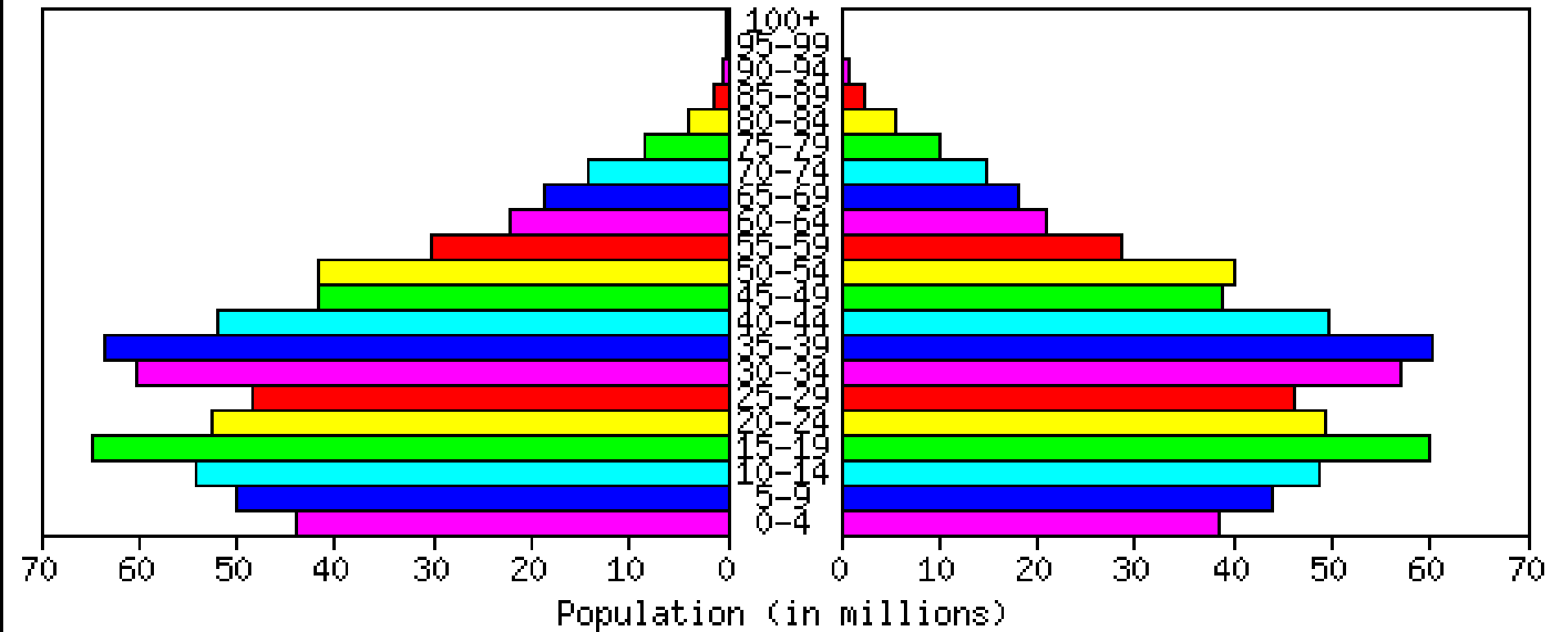


Data source: U.S. Census Bureau, International Data Base (IDB), <http://www.census.gov/ipc/www/idbnew.html>

China: 2005

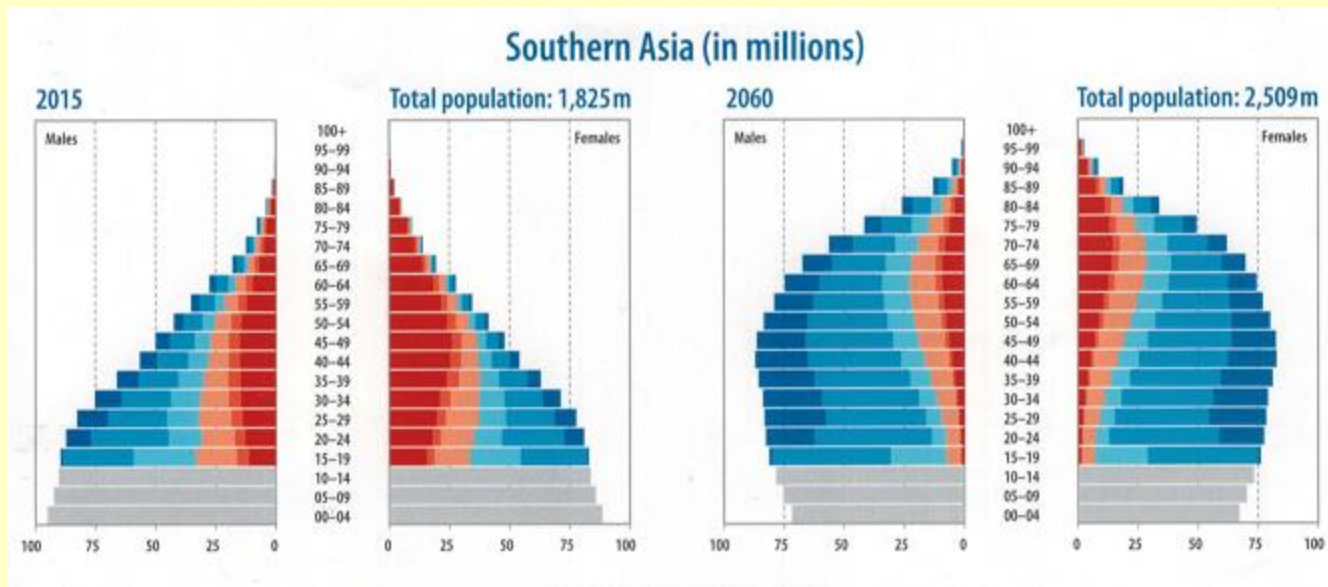
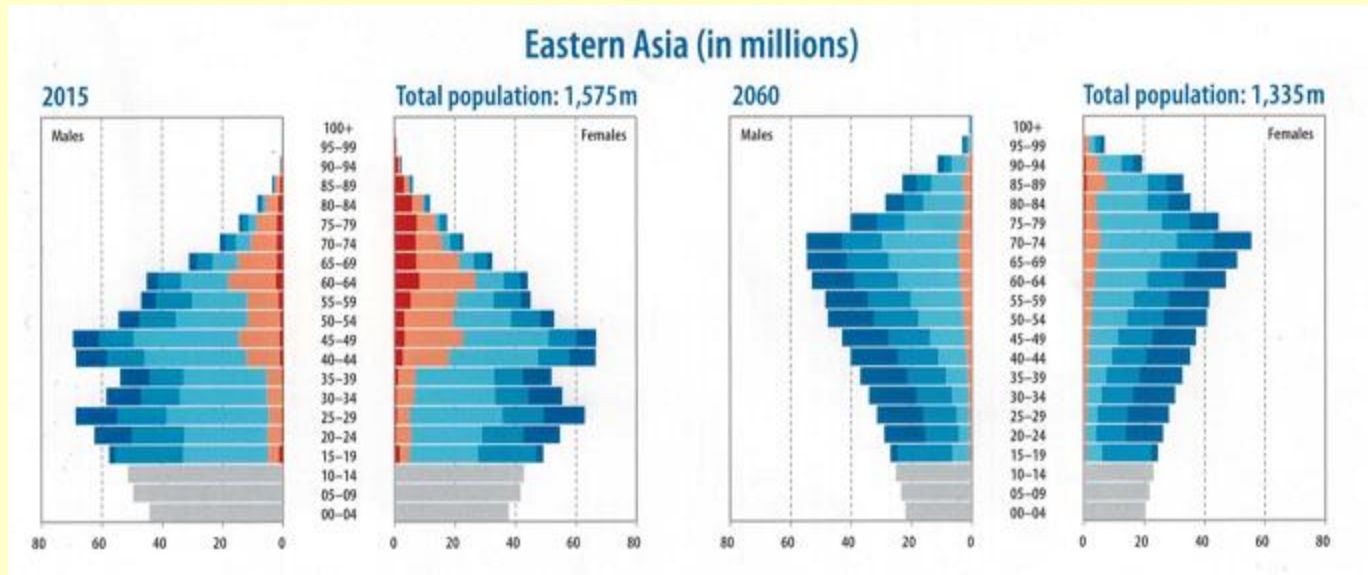
MALE

FEMALE

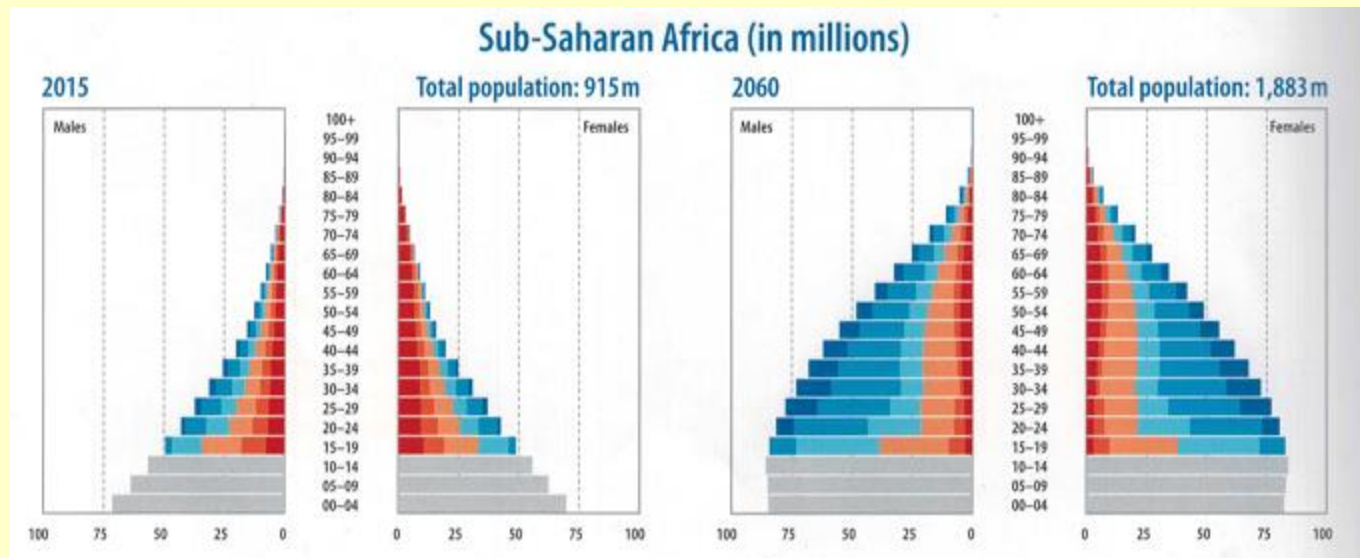
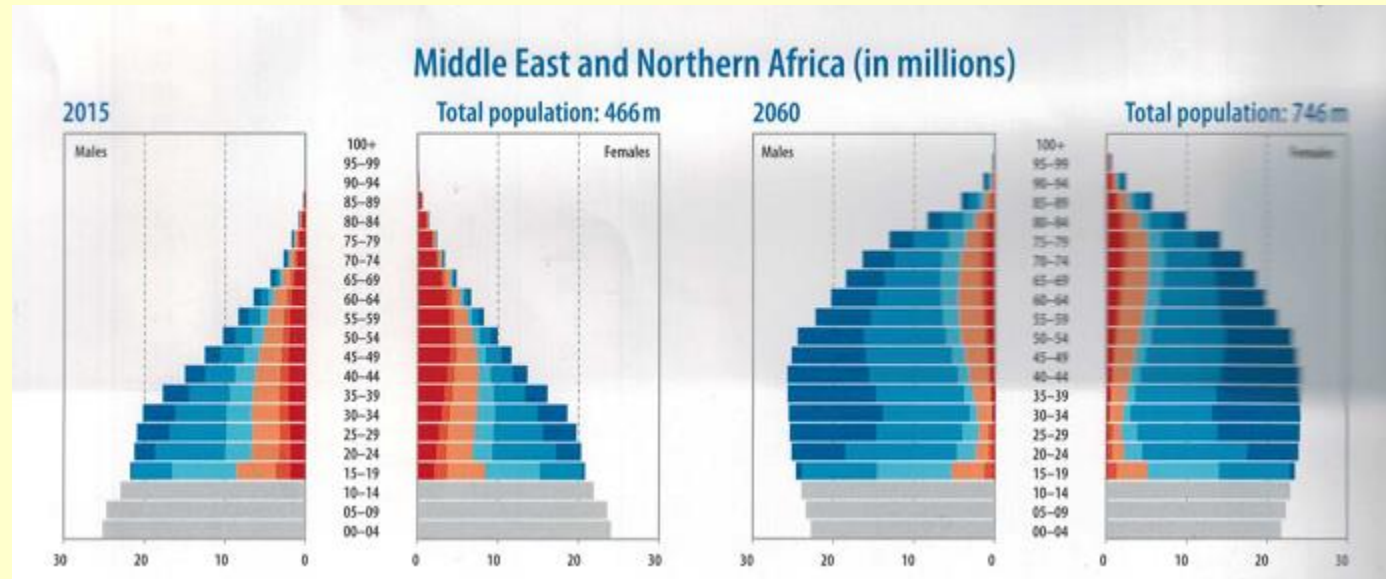


Source: U.S. Census Bureau, International Data Base.

China Population Change



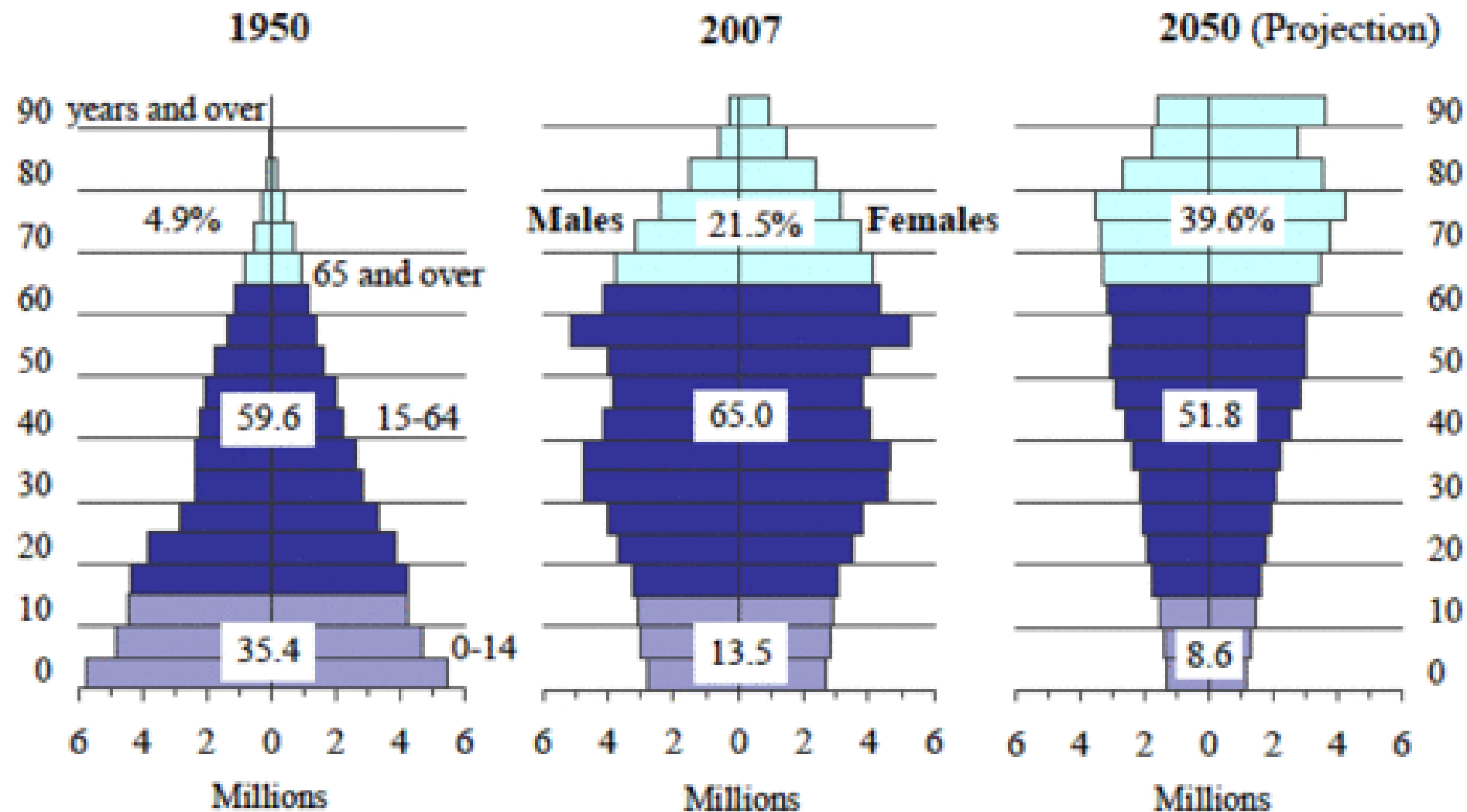
China Population Change



Japan

Figure 2.3

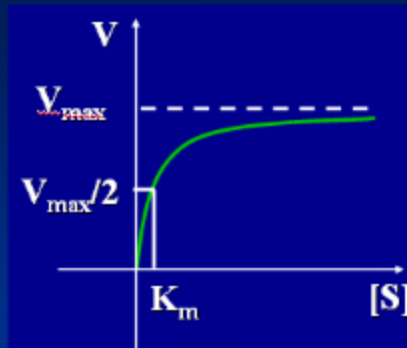
Changes in the Population Pyramid



Source: Statistics Bureau, MIC; Ministry of Health, Labour and Welfare.

2. Meaning of K_m and V_m

◇ K_m equal to $[S]$
when the velocity
is half of the V_{max} .
(mol/L)



$$\frac{V_{max}}{2} = \frac{V_{max} [S]}{K_m + [S]}$$

$$\Rightarrow K_m = [S]$$

◇ K_m 的大小反映该酶对底物亲和力的大小 the value of K_m weigh the affinity of enzyme with substrate.

$$K_m = \frac{K_2 + K_3}{K_1} \quad \text{If } K_2 \gg K_3, \quad K_m = \frac{K_2}{K_1} = K_S = \frac{[E][S]}{[ES]}$$

◇ K_m 值是酶的特性常数，只与酶的结构、底物和反应环境有关，与酶的浓度无关。在 $10^{-6} \sim 10^{-2} \text{ mmol/L}$ 之间。

V_m , V_{max} , 最大反应速度, 酶完全被底物饱和时的反应速度.

K_3 , 转换数 turnover number:

当酶被底物充分饱和时, 单位时间内每个酶分子催化底物转变为产物的分子数. 大多数在 $1 \sim 10^4/s$ 之间.

$$K_3 = \frac{V_m}{[E]}$$

3. Determination of K_m and V_m

$$\frac{1}{V} = \frac{K_m}{V_m} \cdot \frac{1}{[S]} + \frac{1}{V_m}$$

$$V = \frac{V_{\max}[S]}{K_m + [S]}$$

